ABSORPTION OF AMINO ACIDS AND B VITAMINS

FROM THE RUMEN

by

FREDERICK DABELL SMITH

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ABSTRACT

The purpose of this investigation was to provide direct experimental evidence for or against the suggestion that amino acids and B vitamins are among the nutrients absorbed from the rumen of the ruminant.

The investigation was subdivided into two parts: (1) six ruminal fistula experiments and (2) nine blood-sampling experiments. The goat (Capra) was the experimental animal. In the ruminal fistula experiments, solutions of amino acids, B vitamins, and usually propionic acid were added to an empty rumen for 120 or 150 minutes. Propionic acid, a substance known to be absorbed from the rumen, was included in most of the solutions to provide a positive control for ruminal absorption. In addition, in all but the first two experiments, polyethylene glycol was added to the solutions in order to provide a marker substance for differential water movement into or out of the rumen. Controls for the experiments included the taking of a sample from the solution in the rumen at zero time and incubating the sample for the duration of the experiment in a water-bath held at 37°C. The ruminal and control solutions were sampled at periodic intervals during the experiment. The results demonstrated not only marked decreases in the concentrations of propionic acid in the ruminal solutions but also, in most cases, marked decreases in the concentrations of the amino acids and the B vitamins assayed: tryptophan, methionine, tyrosine, glycine, lysine, riboflavin, nicotinic acid, and pantothenic acid. In contrast, the concentrations of these constituents in the control solutions usually demonstrated either no decrease or a small increase. In the first three blood-sampling experiments, the concentrations of amino acids

and nicotinic acid in the plasma draining the rumen were compared with
the concentrations in the peripheral plasma. The results demonstrated
that seldom were the concentrations of an amino acid or nicotinic acid
higher in the plasma draining the rumen than in the peripheral plasma.
The results did suggest, however, that there had been slight increases
in the plasma concentrations of the amino acids with time. Accordingly,
the next six blood-sampling experiments were designed to determine if
time-related increases in the blood concentration of alpha amino nitrogen
occurred after the addition of amino acids to the rumen. Blood samples
were removed periodically from anesthetized goats, both before and after
the addition of a solution of amino acids to an empty rumen. The results
demonstrated time-related increases in the blood concentrations of alpha
amino nitrogen. These results and those obtained for the ruminal fistula
experiments are interpreted as supporting the suggestion that amino acids
and B vitamins are absorbed from the rumen.

In the General Discussion, a preliminary attempt is made to answer
three questions:

1) What is the manner of the passage of amino acids and B vitamins
across the ruminal epithelium?

2) Does this passage of amino acids and B vitamins occur under
normal feeding conditions?

3) How does this passage of amino acids and B vitamins across the
ruminal epithelium fit into the scheme pictured for the metabolism and
final fate of these compounds in the ruminal contents?
As a part of the attempt to answer the first question, literature reviews
are presented on the manner of absorption of amino acids and B vitamins in

the small intestine, kidney, placenta, and other tissues. Then, after a literature review on the manner of absorption of substances other than amino acids and B vitamins from the rumen, the data of the present investigation are examined for evidence as to the manner of absorption of amino acids and B vitamins from the rumen. The examination led to the following statement: the movement of amino acids across the ruminal wall of the goat is determined by a summation of the effects of chemical, electrical, and possibly metabolic potentials. Unfortunately, other than to suggest that the process of simple diffusion is involved, the nature of the data for the B vitamins was not such as to yield much information on their manner of absorption. In the attempt to answer the second question, the fulfillment or non-fulfillment of the following conditions is considered: (1) the demonstration of the presence of amino acids and B vitamins in the rumen, (2) the demonstration of the relation of the concentrations of individual amino acids and B vitamins in the ruminal liquor to their concentrations in the blood plasma, and (3) the demonstration of the absorption of individual amino acids and B vitamins from an ingesta-filled rumen. Although fulfillment of the third condition was not obtained, fulfillment of the first two conditions was obtained and led to the statement that a strong possibility exists that the absorption of amino acids and B vitamins occurs from the rumen under normal feeding conditions. In the attempt to answer the third question, a number of factors are discussed in turn: (1) the environmental conditions of the rumen, (2) the influence of the diet on the composition of the population of microorganisms in the rumen (3) the physical and chemical nature of the dietary constituents, (4) the relative stability of

amino acids and B vitamins in the ruminal ingesta, and (5) the physiological state of the animal. Evidence is presented to demonstrate that each of these factors probably exerts an important influence on the content of amino acids and B vitamins in the rumen. It is suggested that, when the interaction of the many factors is such as to produce higher concentrations of amino acids and B vitamins in the rumen, the proportions of amino acids and B vitamins that pass from the rumen by absorption through its wall will be an important pathway for these nutrients.

To conclude the General Discussion, the possible nutritional implications of the absorption of amino acids and B vitamins from the rumen are considered. One suggestion made is that a general absorption of nutrients from the rumen would permit the simultaneous arrival at the liver, or elsewhere in the body, of volatile fatty acids, amino acids, B vitamins, and inorganic ions. This should promote more efficient utilization of each of the nutrients. Another suggestion made is that ruminal absorption of amino acids and B vitamins places the host ruminant in a better competitive position relative to its contained microbial population in the competition for nutrients. Finally, a suggestion is made that the action of the ruminal wall in absorbing amino acids helps to explain the results that have been obtained for certain nitrogen retention experiments in ruminants.

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Faculty of Graduate Studies

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DOCTOR OF PHILOSOPHY

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FREDERICK DABELL SMITH

B. A., B. S. A., British Columbia, 1947
M. A., British Columbia, 1951

IN ROOM 3332, BIOLOGICAL SCIENCES BUILDING

SATURDAY, SEPTEMBER 26th, 1959 AT 9:30 A. M.

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The investigation was subdivided into two parts: (1) six ruminal fistula experiments and (2) nine blood-sampling experiments. The goat *(Capra)* was the experimental animal.

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To conclude the General Discussion, the possible nutritional implications of the absorption of amino acids and B vitamins from the rumen are considered.

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Yours sincerely,

Frederick D. Smith
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FDS:jh

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been accepted and is in your possession. Unfortunately,
in going through my own copy of the thesis, I now find
that there are still three small errors:

1) page 196, line 6 from the bottom should have
    "or" replaced by "over" and should read as

    ---case the force favouring glycine
       over tryptophan, tyrosine, or lysine---;

2) page 247, footnote C should have the table
    and page number changed from "35" to "34"
    and "196" to "241", respectively, and
    should read as

    ---CThese data are taken from Table 34 on
       page 241.;

3) page 303, line 6 from bottom should have the
    word "his" inserted before the last two words
    in the sentence and should read as

    ---perhaps the growth obtained by Barton-
       Wright in his supposedly biotin- ---.

Could you please have these errors corrected for me.
I do not know what procedure you follow in these cases
and, therefore, I give to you the right to make the
appropriate changes in whatever manner that you think
is the most suitable.

Yours sincerely,

Frederick D. Smith
Assistant Professor.

FDS:jh

GENERAL INTRODUCTION

The bovids provide the only satisfactory intermediary by
which grass can be used as a contributor to human life. This,
with their peaceful and gregarious disposition, has made them
become our most important commensal. If there were no Bovidae
there would be fewer human beings in the world, and our social
organization would be very different.
J. Z. Young in The Life of Vertebrates (419).

The term ruminant is a common name for a group of mammals designated

by Simpson (341) as Pecora, an infraorder within the order Artiodactyla.

The infraorder Pecora is further subdivided by Simpson into three super-

families: Cervoidea, Giraffoidea, and Bovoidea. These superfamilies are

typified by the deer, the giraffe, and the ox, respectively. The goat,

the animal used in the present investigation, belongs to the superfamily

Bovoidea, family Bovidae, subfamily Caprinae, tribe Caprini, genus Capra.

The ruminants are characterised by possessing a stomach of four com-

partments. The compartments are designated, in the order of their

anatomical sequence, as rumen, reticulum, omasum, and abomasum. The

mucosal surface of the first three compartments is lined by stratified

squamous epithelium and is devoid of glands. The fourth compartment,

the abomasum, is glandular and is homologous to the simple glandular

stomach of most non-ruminants. Sisson (347) gives the average capacity

of the stomach as being 30 to 40 gallons in the ox and about 4 gallons in

the sheep.[1] The total volume of the stomach in the adult ox is partition-

ed among the four compartments according to the approximate percentages

---

[1]By way of comparison, the capacity of the stomach in adult man is
between 2 to 3 pints or about 1/4 gallon (149).

that follow (347, 227):

rumen 80%

reticulum 5%

omasum 7%

abomasum 8%

The relative capacities of the stomach compartments in the sheep and goat

approximate to the relationship described for the ox. Figures 1 and 2

outline schematically the anatomy of the ruminant stomach (see also

Fig. 14, p. 103).

The Activities of the Microbial Population
in the Ruminant Stomach: A Brief Review

Microbial Population

During the growth of the ruminant, the rumen acquires a population of

microorganisms from the wide range of microbial species consumed with

grass, hay, soil, and other materials that normally constitute or are

associated with the diet of the young ruminant (49, 86, 174, 193, 195,

305). This acquired population, consisting of a complex mixture of many

species of bacteria and protozoa (23, 154, 176, 282, 386), usually is

large. The protozoa exhibit counts as high as 2×10^6 cells per milli-

liter of rumen contents (387); the bacteria exhibit counts as high as

billions (10^9) of cells per milliliter (154). Hastings (154) has stated

that "the protozoa and bacteria represent a density of population that is

never met in vitro and probably not elsewhere in nature." This large

population of microorganisms not only exhibits interdependence among its

members but exists in a symbiotic relationship with its ruminant host

(280, 311, 376).[2]

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[2]As lambs and goats that have been freed of protozoa do not demon-
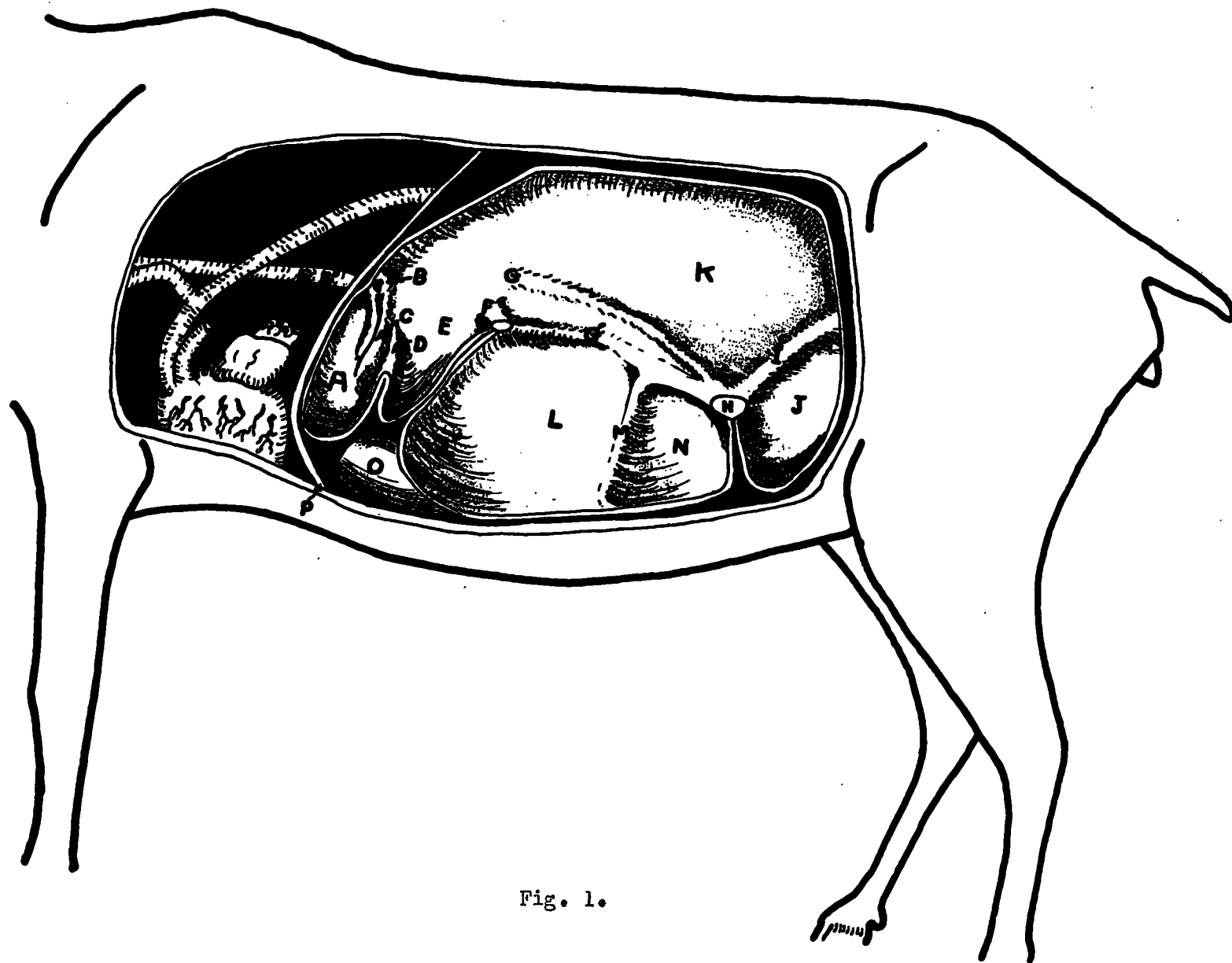
Fig. 1.

Fig. 1. - The ruminal and reticular cavities, viewed from the left (adapted from figure 121 of Martin and Schauder, Lehrbuch der Anatomie der Haustiere, vol. 3, third edition, 1938)

The left walls of the rumen and the reticulum have been removed. A, reticulum; B, posterior orifice of the oesophagus; B', eosophagus; C, reticular groove connecting posterior orifice of the oesophagus with reticuloomasal orifice; D, ruminoreticular fold; E, anterior dorsal sac; F, anterior pillar; G, G', right longitudinal pillar split into two parts; H, cross-section of posterior pillar; I, dorsal coronary pillar; J, posterior dorsal blind sac; K, dorsal sac; L, ventral sac; L', anterior ventral blind sac; M, ventral coronary pillar; N, posterior ventral blind sac; O, abomasum; P, diaphragm.
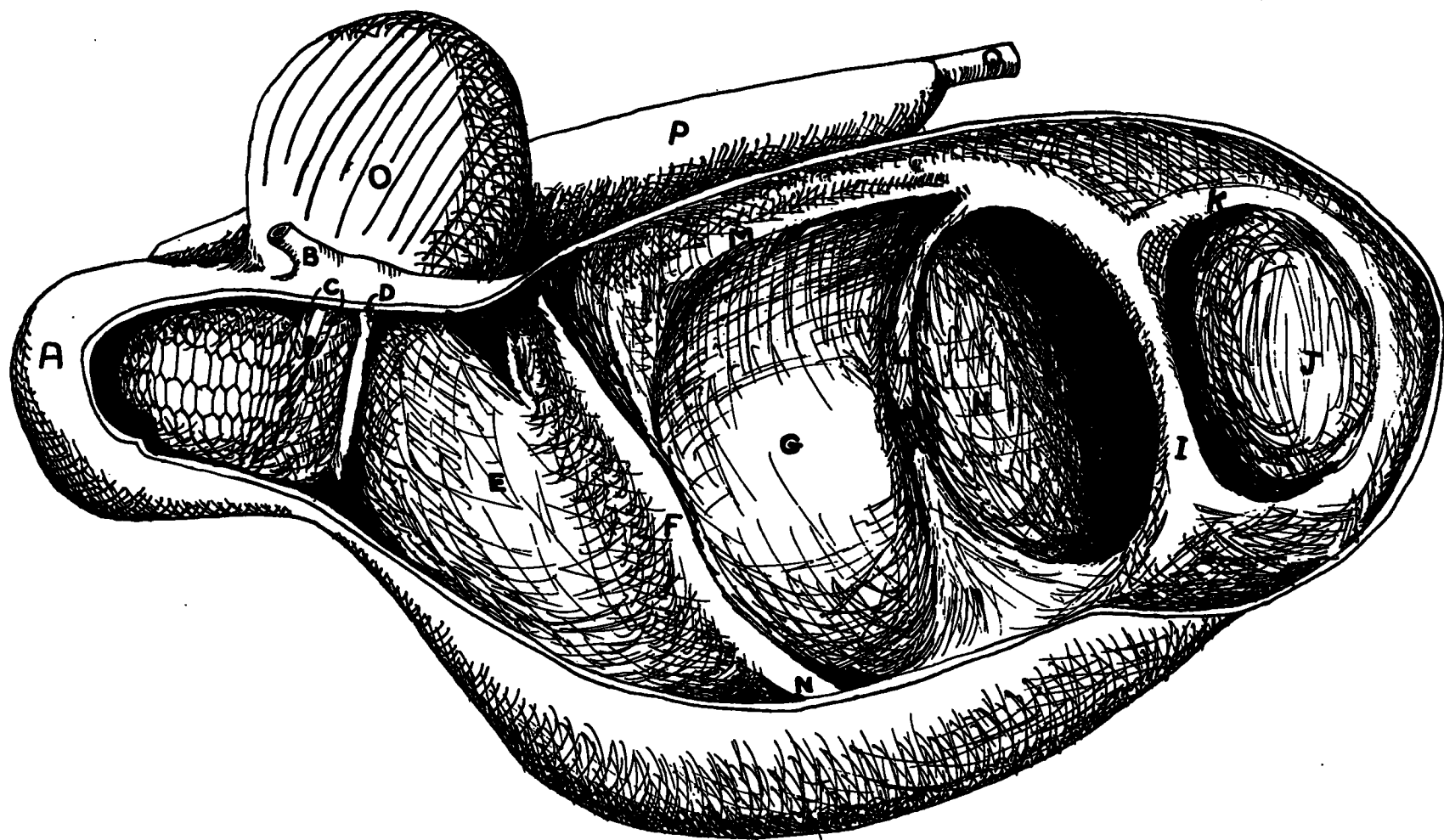
Fig. 2.

Fig. 2. - Sheep stomach, viewed dorsally and partly from the left (adapted from figure 771 of Ellenberger-Baum, Handbuch der Vergleichenden Anatomie der Haustiere, eighteenth edition, 1943)

The stomach has been opened by removal of parts of the walls of the rumen and reticulum. A, reticulum; B, oesophagus; C, the two lips of the reticular groove; D, ruminoreticular fold; E, anterior dorsal sac; F, anterior pillar; G, ventral sac; H, posterior ventral blind sac; I, posterior pillar; J, posterior dorsal blind sac; K, dorsal coronary pillar; L, ventral coronary pillar; M, M', right longitudinal pillar split into two parts; N, beginning of left longitudinal pillar; O, omasum; P, abomasum; Q, beginning of duodenum.

Symbiosis between the Ruminant and its Microbial Population

The Ruminant.- The beneficial activities of the ruminant host toward the members of its microbial population are:

1) to provide living space, water, and food in the form of grasses, hays, leaves, and other fibrous materials;

2) to provide a temperature of approximately 39°C. and a carbon dioxide-rich atmosphere of anaerobiosis (50, 51, 401), conditions which are favourable for microbial fermentation;

3) to accelerate the microbial fermentation by:

a) masticating the food, both when the food is ingested and when it is regurgitated, thereby increasing enormously the surface area of the food that is accessible to enzymic attack by the ruminal microorganisms;

b) mixing the ruminal ingesta through strong, rhythmic contractions of the ruminal pillars and sacs;

4) to maintain a range of hydrogen ion concentration that is favourable for microbial fermentation by:

a) buffering the ruminal contents with a constant inflow of a bicarbonate-phosphate-rich saliva (102, 242) and a secretion by the ruminal wall of large amounts of phosphate and bicarbonate buffers (106, 214, 284, 349, 350);

b) increasing the rate of ruminal absorption of volatile fatty acids when the pH of the ruminal contents begins to decrease (88, 138, 230, 294);

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strate signs of nutritional deficiency, the protozoa are considered to be commensals rather than symbionts (57, 282, 311).

5) to remove the gaseous and dissolved end products of the micro-
bial fermentation by eructation, absorption through the wall of the
stomach (139, 234, 235, 299), and passage to the more distal parts of the
digestive tract (102).

<u>Microbial Population</u>.- The beneficial activities of the microorgan-
isms toward the ruminant host are the result of their fermentation of the
cellulose-containing plant material that is ingested by the host. The
ruminant itself does not possess a cellulase (154, 229, 311). Were it not
for the presence in the rumen of cellulase-possessing microorganisms
(175, 311), the ruminant would be unable to utilize the cellulose of the
plant cells. Further, as the cellulose forms a part of the cell wall of
the plant cells, the ruminant also would be unable to utilize the carbo-
hydrates, proteins, amino acids, vitamins, and other nutrients contained
within the cells (23, 374). The great benefit to the ruminant of its
microbial population is that, by their fermentation of cellulose, they
permit the ruminant to utilize plant material that otherwise would be
unavailable.

Specific examples of the beneficial activities of the ruminal micro-
organisms are:

1) to ferment cellulose and other carbohydrates to form volatile
fatty acids--mainly acetic, propionic, and butyric (23, 108, 141, 142,
295, 301, 374, 386)--which, on being absorbed from the stomach, provide a
major contribution to the energy requirement of the ruminant (59, 295,
335); the magnitude of this fermentation is indicated by Phillipson's
(295) suggestion that 40 to 50 per cent of the carbohydrate in common
rations may be converted to fatty acids in the rumen;

2) to produce a dynamic system of nitrogen compounds (55, 62, 92, 240, 281, 287), in which protein is being both hydrolyzed and synthesized, by:

a) attacking proteins in the ruminal contents with protease (92, 367), deaminase (110, 400), and decarboxylase (326) enzymes-- the possible value of this hydrolysis to the host ruminant will be presented in the general discussion (see p. 267);

b) utilizing the nitrogen of compounds such as ammonium salts and urea (33, 147, 151, 152, 220, 250, 351, 352) and, in some cases, the sulfur of compounds of inorganic sulfate (13, 42, 43, 103, 220) to synthesize amino acids[3] or protein--because of its microbial utilization, urea can serve as a source material for approximately one third of the amino acid requirements of the ruminant (102, 250, 287, 319);

3) to synthesize vitamin K and most if not all the B-complex group of vitamins, including vitamin B_{12} (1, 31, 76, 78, 102, 135, 199, 200, 201, 226)--because of this synthesis, the ruminant can be independent of exogenous sources of vitamin K and the B vitamins (201; see also 102).

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[3]Loosli et al (220) conducted an experiment in which sheep and goats were kept in positive nitrogen balance and gained weight on a diet containing no source of nitrogen other than urea or glycine. Assay of the hydrolyzed ruminal contents for the presence of amino acids demonstrated that the ten amino acids "essential" (an "essential amino acid" is one that must be supplied to an animal in its diet because the animal cannot synthesize the amino acid or cannot synthesize it rapidly enough to meet the total requirement of the tissues of the animal for the amino acid) for the rat were synthesized in large amounts (see also 5, 47, 103, 114, 170, 241, 248, 283). Black et al (35) have presented evidence that the requirements of the ruminant for the essential amino acids are the same as for the dog and rat (see also 38).

Summary. - The ruminant apprehends, masticates, and swallows plant material. This material passes from the oesophagus into the warm, moist environment of the rumen. Here, the strong contractions of the ruminal walls rhythmically mix the ingesta and the enzyme-rich microorganisms of the rumen actively attack the fragments of plant material.[4] At intervals, the ruminant regurgitates portions of the fermenting ingesta for remastication and then reswallows these portions for renewed fermentation. A constant inflow into the rumen of large volumes of bicarbonate-phosphate-rich saliva and ruminal secretion helps to maintain a pH favourable for fermentation, as well as to provide a basal medium favourable for micro-biological activity. This activity results in the formation of volatile fatty acids from cellulose, amino acids and other nitrogenous compounds from proteins, amino acids and proteins from urea or ammonium salts, and the syntheses of vitamin K and the B-complex of vitamins. The volatile fatty acids are absorbed from the first three compartments of the rumen--this absorption not only makes readily available to the ruminant a major source of its energy, but also assists in maintaining a favourable pH in the rumen for fermentation. The synthesized proteins, amino acids, and vitamins are assumed to pass along to the small intestine where they are digested and absorbed (18, 23, 92, 154, 200, 201, 228, 317). The flow of these and other nutrients out of the rumen and reticulum occurs when the ingesta becomes fine enough to pass from the reticulum to the omasum. The omasum absorbs water, as well as bicarbonate and phosphate ions, from the ingesta before it passes on to the abomasum (106, 129). Beyond this

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[4]Recently, Holtenius (171) has obtained evidence which indicates that the enzyme systems of the ingested plant material can contribute to the fermentation process in the rumen.

point, the action of the digestive tract on the ingesta usually is assumed to be similar to the action of the digestive tract in many non-ruminant herbivores (62, 102, 343).[5]

The above brief review has attempted to point up the salient feature of ruminant nutrition that is illustrated aptly in the following statement (176):

> The first step in ruminant nutrition is microbial nutrition. Microorganisms are first fed and they in turn feed the host.

Absorption from the Rumen: a Review

General

For a long time, absorption studies on the rumen were discouraged because of its luminal lining of stratified squamous epithelium (see the review on this in 377). The same type of epithelium forms the outer layer of the epidermis--a structure considered to be relatively impermeable to water and water-soluble substances. The inference was that the rumen likewise would be impermeable to water and water-soluble substances. Comparative studies (27, 99, 377) on the epithelia of the epidermis and the rumen have demonstrated, however, that important structural differences exist between the two epithelia. In contrast to the epidermal epithelium, the ruminal epithelium lacks in many areas a stratum granulosum, has a relatively thin stratum corneum, and is not covered with sebaceous secretion. Further, the ruminal epithelium possesses a rich blood supply, an

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[5]The results of Larson and Stoddard (209), however, do not appear to support this statement. They placed a complete non-ruminant ration (a hog ration) into the abomasum of yearling cattle. After six to ten days on this diet, the animals were near death. In contrast, if the same ration was placed in the rumen, the animals did not sicken.

extensive network of capillaries being intimately associated with the basal epithelial cells. These results prompted one group of workers (27) to state that it is not surprising that the rumen absorbs soluble nutrients that are continually in contact with its epithelium. Indeed, much experimental evidence now has been accumulated to support the belief that the ruminal absorption of water and of many water-soluble substances does occur.

Substances Absorbed

Trautmann reported in 1933 (377) on absorption experiments in which drugs such as pilocarpine and atropine were introduced into the stomach compartments of goats and sheep. From the results of these experiments, Trautmann proposed that the rumen, as well as the reticulum and omasum, rapidly absorbs water and substances dissolved in water. Rankin (315, 316) demonstrated in 1940 the absorption of glucose, potassium iodide, pilocarpine, strychnine, and sodium cyanide from the rumen. Beginning in 1942, Barcroft et al (26, 27, 234, 301) published a series of papers that demonstrated the absorption of the lower volatile fatty acids--acetic, propionic, and butyric--from the rumen, as well as from the reticulum and omasum, of sheep. More recently, higher volatile fatty acids--isobutyrate, n-valerate, isovalerate, and alpha-methyl butyrate--also have been reported to be absorbed from the rumen (297). Some of the other substances which have been reported to pass across the ruminal wall into the portal blood are sodium (88, 286, 298), potassium (286, 359), magnesium (361), chloride (97, 284, 359), bromide (359), sulfide (13), phosphate (214, 285, 330, 359, 418), lactate (172), bicarbonate (299), and different gases--e.g., ammonia (47, 95, 237, 238), carbon dioxide (102), and methane (102). Some of these substances also have been demonstrated to pass from the

blood across the ruminal wall into the rumen. This is true for sodium (286), chloride (230, 286), bicarbonate (230, 286, 298), and phosphate (215, 285, 330, 349, 350). There exists, then, considerable evidence for the permeability of the ruminal epithelium and the ability of the rumen to absorb many of the constituents of the ruminal contents.

Comment

The foregoing experimental results substantiate Trautmann's original suggestion that the rumen absorbs water and water-soluble substances (377). More recently, Phillipson and Cuthbertson (299) have stated "that anything in solution in the rumen probably penetrates the rumen epithelium unless the mass is too great." As amino acids and B vitamins are in solution in the rumen and as the range of their masses is of the same order of magnitude as of the masses of substances known to be absorbed from the rumen, it is possible that also amino acids and B vitamins are absorbed from the rumen. A few experiments and several suggestions have been reported in the literature on the absorption from the rumen of amino acids and B vitamins. A brief review of these experiments and suggestions is presented in the next two sections.

Amino Acids

As far as this author is aware, no one has obtained direct evidence as to the permeability of the ruminal epithelium to amino acids. Several workers (216, 319, 375, 411) have suggested that amino acids are absorbed from the rumen. Chalmers and Synge even have suggested (62) that amino acids may pass from the blood into the ruminal contents:[6]

It is ... quite possible that free amino acids and urea of the blood may pass into the rumen through its wall. As far as

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[6]Akkada and El-Shazly (7) recently have stated that they are utilizing radioactive isotopes to determine if amino acids can pass into the

we are aware, this possibility has not been investigated
experimentally. The concentration gradients between blood
and rumen liquor are [sometimes⁷] such that substantial
quantities could pass by simple diffusion.

The entry of nonprotein   [including amino acids]
into the rumen from the animal's own body with subsequent
conversion to protein could assume importance under star-
vation conditions, and may be decisive for the survival
of wild ruminants during winter and drought conditions
when the only available food is the equivalent of badly
leached hay.

Annison (15), in two experiments, unsuccessfully attempted to demonstrate

the absorption of amino acids from the rumen. The premise underlying the

procedures in these two experiments was that if amino acids are absorbed

into the blood draining the rumen, that because of the removal by the

liver of amino acids from the portal blood (389), the concentration of

amino acids would be higher in the blood of the portal vein than in the

blood of the jugular vein or common carotid artery. In the first experi-

ment, Annison added by way of a ruminal fistula 100 grams of casein hydro-

lysate to the ingesta-filled rumen of a sheep. The animal immediately was

anesthetized with sodium pentobarbitone. Ninety minutes after the addi-

tion of the hydrolysate, Annison removed blood samples from the portal and

jugular veins. As the concentration of alpha amino nitrogen in the blood

of the portal vein was lower than that in the blood of the jugular vein,

he did not obtain evidence in this first experiment for ruminal absorp-

tion of amino acids. In the second experiment, Annison added by way of a

ruminal fistula 70 grams of casein hydrolysate to the ingesta-filled rumen

of a sheep. The animal was not anesthetized. Annison removed blood

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rumen through its wall.

[7]See page 209 where it is demonstrated that the concentration of
alpha amino nitrogen in the ruminal liquor can be higher than in the
blood.

samples from the sheep by using a portal vein cannula and an exteriorized

common carotid artery.[8] The results of the experiment are given in

Table 1. As for the results of the first experiment, the results record-

ed in Table 1 do not demonstrate a greater concentration of amino acids

in the portal blood than in the carotid blood; hence, Annison was unable

to obtain evidence in either of his two experiments to support a

hypothesis that amino acid absorption occurs from the rumen.

Vitamins

A few experiments and some comments have been in the literature on

the question of ruminal absorption of B vitamins. In 1941 Hunt et al

(179) suggested the possibility of riboflavin absorption from the rumen

of cattle. In 1944, Iardinois et al (208) made the same suggestion for

thiamine. Both suggestions were made on the basis of indirect evidence,

the evidence being dependent upon a comparison of the concentration of

the vitamin in the feed with the concentration of the vitamin in the rumen.

Unfortunately, the method of experimentation did not exclude the observed

decrease of concentratiom being the result of vitamin destruction. In

1947 Kon and Porter (201) discussed the absorption of B vitamins from the

rumen:

> Further work on vitamin absorption also is required. It has
> usually been assumed that absorption is mainly from the
> abomasum and small intestine, but since the demonstration by
> Marshall and Phillipson (1945) that volatile fatty acids are
> absorbed from the rumen, the possibility exists that vita-
> mins also may be absorbed from it.

Contrary to the expectation of the above quotation, French workers (322,

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[8]See the appendix for a description of the techniques for the
cannulation of veins (p. 290) and the exteriorization of the common
carotid artery (p. 286).

TABLE 1. - The results of an experiment by Annison (15) to
determine if, after the addition of casein hydrolysate to
the ingesta-filled rumen of a sheep, there was a greater
concentration of alpha amino nitrogen in the blood of the
portal vein than in the blood of the common carotid artery

Time after adding amino acids (hr.)	Alpha amino N (mg./100 ml.)		
	Ruminal contents	Portal blood	Carotid blood
0	0.86	3.84	3.04
1	83.7	3.18	2.94
1.5	. . .	2.54	2.91
4.5	18.8	2.02	1.93

373) were unable to demonstrate ruminal absorption of B vitamins. They did obtain indirect evidence that nicotinic acid was absorbed from the reticulum and that some of the other B vitamins were absorbed from the omasum. On the other hand, a personal communication to Phillipson and Reid (302) by Kon and Porter states that they have demonstrated thiamine to be absorbed from the rumen. Although meager and even conflicting, the evidence to date suggests that B vitamin absorption may occur from the rumen.

Comment

The previous review of the literature presents the ruminant as an animal that possesses a stomach with a highly developed capacity for absorbing certain nutrients. Direct experimental evidence exists for the function of the rumen in the absorption of volatile fatty acids and many inorganic ions. Indirect evidence has led several authors to suggest that the rumen functions in the absorption of amino acids and B vitamins. The present investigation, divided into two parts, was designed to provide direct experimental evidence for or against the suggestion that amino acids and B vitamins are among the nutrients absorbed from the rumen of the ruminant.

EXPERIMENTATION

One aspect of ... this type of work which is not generally
appreciated, ... , is the physical difficulty of carrying out
experimental work on ruminants: the difficulty of anesthesia,
difficulties due to their peculiar anatomy, and many others.
A physiologist working with experimental animals such as
sheep and goats has problems which a person working on small
laboratory animals never encounters.

                                            Dr. F. Alexander (10).

Part I: Ruminal Fistula Experiments

Introduction

The first approach to the detection of ruminal absorption of amino

acids and B vitamins was to demonstrate a decrease in the concentration of

these substances when they were added in solution to an empty rumen. The

use of goats with ruminal fistulas not only enabled the rumen to be

emptied of its contents, but also facilitated the addition to the rumen of

an experimental solution and the sequential sampling of this same solution.

Pectin or polyethylene glycol was added to the experimental solutions. As

neither of these substances is absorbed from the rumen (138, 181, 360),

their addition provided a marker substance for any water movement into the

rumen through the ruminal wall or by saliva inflow and for water movement

out of the rumen through the ruminal wall. The measurement of water move-

ment was considered necessary in order that concentration changes of amino

acids and vitamins due to water movement could be differentiated from

concentration changes of these substances due to ruminal absorption.

Propionic acid also was added to the experimental solutions of nearly all

the experiments. The addition of this substance, known to be absorbed

from the rumen, was made to provide a positive control for ruminal

absorption.

The selection of the particular amino acids and B vitamins to be studied and the selection of the method of their analysis were determined by several considerations. The amino acids and B vitamins were chosen on the basis of: (1) their susceptibility to microbiological assay, (2) their diversity of structure, (3) their variation of molecular volume. The method of assay, the microbiological assay, was chosen on the basis of: (1) the existence of excellent facilities for this type of assay, (2) the relative simplicity and accuracy of the assay when it is carried out by an individual experienced in microbiological procedures, (3) the applicability of this one method of assay to a large number of amino acids and B vitamins. All these considerations, together with the limitations of time and money, determined the choice of the particular amino acids and B vitamins to be studied and the method of their assay.

Method

Experimental Procedure

General.- This series of experiments was carried out using goats which previously had been provided with a ruminal fistula (see p. 279).[9] The first step of the experimental procedure was to immobilize the goat on a milking stand. The contents of the rumen and reticulum were removed manually via the fistula. The rumen and reticulum then were rinsed with warm tap water. This rinsing procedure was repeated several times until the ruminal wall appeared to be clean. In the first two experiments, some difficulty was experienced in rinsing the reticulum adequately. In

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[9]The details of the surgical and analytical procedures utilized in the present investigation are presented in the appendix.

the other experiments, a one-third horsepower vacuum pump facilitated the

rinsing procedure by drawing most of the rinsing solution into a large

suction flask that was inserted in the intake line of the pump. The

thoroughness of the rinsing could be checked by observing the cloudiness

of the solution in the suction flask.

The solution that was added to the rumen was prepared on the day

previous to the experiment. After first warming this solution to a

temperature of 37° C., a sample was taken from it and labelled experi-

mental solution control. In the first two experiments, the experimental

solution control was subdivided. One part was placed for the duration of

the experiment in a hot air oven that was kept at approximately 37° C.

The other part of the solution was placed in a refrigerator that was held

at 4 to 10° C.[10] In the rest of the experiments, the experimental solu-

tion control was not subdivided, all of it being placed in a refrigerator.[11]

Two to four liters of the experimental solution now were added through

the fistula to the rumen. The amount of solution added was enough to fill

the rumen to within one or two inches of the level of the anterior pillar

(see Figs. 1 and 2, pp. 3 and 5). This restriction on the fill was

imposed, not only in order to confine the absorption studies to the rumen,

but also to reduce the possibility of contamination of the experimental

solution in the rumen—now called the ruminal solution—by ingesta from

the reticulum or the omasum. This reduction of the possibility of contamin-

ation was the reason for also removing the ingesta of the reticulum.

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[10]In the first two experiments (W1 and W2), the experimental solution
controls served to measure the effect of temperature on the concentration
of the constituents when they were contained together in solution and held
at 37° C. for two hours.

[11]In these experiments (W3 to W6), the experimental solution controls

Immediately after the addition of the solution to the rumen, the experi-
mentalist passed his hand through the fistula and vigorously mixed the
contents. This period of mixing was judged to be about 30 seconds.
At the end of the mixing period, samples were taken from the solution in
the rumen. The pH of the samples was determined electrometrically using
a glass electrode.[12] The samples then were filtered through four layers
of cheesecloth. In the first two experiments, the filtered samples were
labelled zero minute samples and placed in the refrigerator. In the rest
of the experiments, these samples first were subdivided. One part,
labelled zero minute sample, was placed in the refrigerator and the other
part, labelled ruminal solution control,[13] was placed in a water-bath that
was held at 37° C. Immediately after the solution in the rumen had been
sampled, the fistula either was draped with a heavy towel or closed with
a fistula-plug.

At periodic intervals, more samples were taken from the solution in
the rumen. After pH measurements of the samples had been made, they were
placed in a refrigerator. Each time the solution in the rumen was
sampled, the ruminal solution control in the water-bath also was sampled.
The temperature of the solution in the rumen was checked at frequent
intervals by inserting a glass thermometer through the fistula. At the
end of the experiment, as much as possible of the solution was collected

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served to provide samples for the determination of the concentration of the
experimental solution constituents before they were placed in the rumen.

[12]A Beckman pH meter was used.

[13]In these experiments (W3 to W6), the ruminal solution controls
served to measure not only the effect of temperature, but also the effect
of possible contamination by ruminal ingesta on the concentration of the
constituents when they were contained together in solution and held at
37° C. for two and one half hours.

from the rumen and the volume measured. All samples were frozen at about -17° C. In the first two experiments, the samples were kept frozen until their assay. In the other experiments, the samples were kept frozen for two to four weeks and then were transferred by car five hundred miles to another institution. For the transfer, the frozen samples were packed in ice; as, however, the journey occurred during warm weather and lasted about twelve hours, the samples lacked refrigeration for a part of the journey. At the end of the journey, the samples were re-frozen at about -13° C.

More specific details for each of the experiments now are presented.

Experiment W1.-

1. Animal history: A young adult female goat weighing 98 pounds and of mixed breeding was used. Seven months prior to the experiment, the left common carotid artery was exteriorized to form a "carotid loop" (see p.286). Four months prior to the experiment, the goat was provided with a ruminal fistula. The operation was carried out in two stages, the second stage taking place about 10 days after the first. At the time of the experiment, the animal apparently was in good health.

2. Composition of the experimental solution: Table 2 presents the composition of the experimental solution.

3. Sampling procedure: Two liters of the experimental solution were placed in the rumen of the goat. Single samples were removed from the solution in the rumen. The sampling times were 0, 40, 60, 95, and 120 minutes after the initial 30 second period of mixing. Single samples were removed from the experimental solution control at the 0 and 120 minute sampling times. The total volume of solution removed by the sampling procedure from the solution in the rumen was 570 milliliters. The

TABLE 2. - Composition of the experimental solution
for experiment W1

Constituent[a]	Milligrams per Liter
Calcium-d-pantothenate...................	20
L-glutamic acid..........................	441
Glycine.................................	225
L-leucine...............................	394
L-lysine................................	439
L-methionine............................	448
Nicotinic acid..........................	20
Riboflavin..............................	20
L-tryptophan............................	613

[a]In addition, 3.75 grams per liter were added
of pectin (Nutritional Biochemical Corporation,
Cleveland, Ohio: Pectin, N.F.). The pH of the
solution was adjusted to 6.75 by making it 0.067
molal in sodium orthophosphate, mono-hydrogen
(Na_2HPO_4).

volume of solution recovered from the rumen at the end of the experiment was 1200 milliliters. This volume together with the volume removed by the sampling procedure accounts for 1770 of the original 2000 milliliters placed in the rumen. At least some of the volume not accounted for was lost as a small but unknown volume of spillage through the fistula during restless movements of the goat. Also, not recoverable at the end of the experiment was a small but unknown volume of the solution lost in the folds and depressions of the ruminal wall. This loss occurred in the other experiments as well.

Experiment W2.-

1. Animal history: An adult male goat weighing 100 pounds and of mixed breeding was used. About 9 months prior to the experiment, the spermatic cords were crushed to cause a reduction in the odour of the animal. Eight months prior to the experiment, the goat was provided with a ruminal fistula in the same manner as for the goat of experiment W1. About 7 months prior to the experiment, the left common carotid artery was exteriorized. At the time of the experiment, the animal apparently was in good health.

2. Composition of the experimental solution: The amino acids were supplied in the form of a casein acid-hydrolysate solution.[14] One hundred milliliters were used in each liter of total solution. Riboflavin, pectin, sodium orthophosphate (mono-hydrogen), and potassium orthophosphate (di-hydrogen) were added to the experimental solution in the same concentration as in the solution for experiment W1.

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[14]Nutritional Biochemical Corporation, Cleveland, Ohio: "Vitamin-Free Casein Hydrolysate-Acid (10%) solution."

3. Sampling procedure: Three and one half liters of the experimental solution were placed in the rumen of the goat. Single samples were removed from the solution in the rumen. The sampling times were 0, 65, 95, and 125 minutes after the initial 30 second period of mixing. Single samples were removed from the experimental solution control at the 0 and 120 minute sampling times. The total volume of solution removed by the sampling procedure was 522 milliliters. The volume of solution recovered from the rumen at the end of the experiment was 600 milliliters. This volume together with the volume removed by the sampling procedure accounts for only 1122 of the original 3500 milliliters placed in the rumen. At least some of the volume of 2300 milliliters not accounted for was lost as a large but unknown volume of spillage through the fistula. Most of this spillage occurred during the later part of the experiment and was caused by unexpected jumping-movements of the goat.

Experiment W3.-

1. Animal history: A young adult female goat weighing 110 pounds and of Saanen breeding was used. Two and one half months prior to the experiment, the left common carotid artery was exteriorized and the first stage of the ruminal fistula operation was performed. One month prior to the experiment, the second stage of the fistula operation was performed. Three weeks prior to the experiment, the goat aborted. After the abortion, the goat ceased to eat and exhibited an elevation of body temperature. Treatment in the form of ruminal ingesta transplants[15] and

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[15]The contents of the rumen and reticulum in the sick goat had lost their normal smell, colour, and consistency: being foul-smelling, brown, and watery. After first removing the foul contents and rinsing the compartments, large transplants of ingesta--enough to half-fill the rumen--were transferred from a healthy fistulated goat to the sick goat. Such a

antibiotic therapy was begun and maintained for four days. The animal resumed eating and the body temperature returned to normal.

2. Composition of the experimental solution: The amino acids were supplied in the form of a casein acid-hydrolysate solution. Twenty milliliters were used in each liter of total solution. For each liter of solution, there also were added 40 milligrams of glycine, 50 milligrams of nicotinic acid, 5 grams of sodium propionate, 1.625 grams of sodium ortho-phosphate (di-hydrogen), and 2.5 grams of polyethylene glycol.[16] The pH of the solution was adjusted to 6.5 with sodium hydroxide.

3. Sampling procedure: Three liters of the experimental solution were placed in the rumen of the goat. Single samples were removed from the solution in the rumen. The sampling times were 0, 10, 30, and 70 minutes after the initial 30 second period of mixing. Single samples were removed from the ruminal solution control at the 0 and 70 minute sampling times. The total volume of solution removed by the sampling procedure was 565 milliliters. The volume of solution recovered from the rumen at the end of the experiment was 2320 milliliters. This volume together with the volume removed by the sampling procedure accounts for 2885 of the original 3000 milliliters placed in the rumen. Spillage of the solution through the fistula was not observed to occur.

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procedure not only supplied the sick goat with a new population of actively fermenting ruminal microorganisms but also probably supplied the goat with pre-formed volatile fatty acids and other nutrients. The author's personal experience was that the use of such large transplants usually proved to be beneficial to fistulated goats that had "gone off feed". In a series of papers (77, 161, 305, 307, 308, 309), Pounden, Hibbs, and Conrad have demonstrated the nutritional benefits that result from the successful transplantation of ruminal microorganisms into calves by means of cud inoculations from adult cows.

[16]Carbide and Carbon Chemicals Company, New York, N.Y.: Carbowax 4000.

Experiment W4.-

1. Animal history: The goat of experiment W3 was used also for this experiment. At the time of the experiment, the goat still appeared in a weakened, emaciated condition and had required several ruminal ingesta transplants to keep it eating. There was not, however, any elevation of body temperature.

2. Composition of the experimental solution: Most of the amino acids were supplied in the form of a casein acid-hydrolysate solution. Forty milliliters were used in each liter of total solution. Table 3 presents the constituents that were added to complete the composition of the experimental solution.

3. Sampling procedure: Three and seven tenths liters of the experimental solution were placed in the rumen of the goat. Duplicate samples were removed from the solution in the rumen. The sampling times were 0, 10, 30, 70, and 150 minutes after the initial 30 second period of mixing. Duplicate samples were removed from the ruminal solution control at the same times as for the solution in the rumen. The total volume of solution removed by the sampling procedure was 1300 milliliters. The volume of the solution recovered from the rumen at the end of the experiment was 2130 milliliters. This volume together with the volume removed by the sampling procedure accounts for 3430 of the original 3700 milliliters placed in the rumen. At least some of the volume of 270 milliliters not accounted for was lost as an unknown volume of spillage through the fistula.

Experiment W5.-

1. Animal history: A young adult female goat weighing 90 pounds and of Saanen breeding was used. Three months prior to the experi-

TABLE 3. - Composition of the experimental solution
for experiment W4

Constituent[a]	Milligrams per Liter
L-cystine....................................	100
Calcium-d-pantothenate....................	100
Glycine......................................	100
Nicotinic acid...............................	100
Riboflavin...................................	100
DL-serine...................................	100
L-tryptophan................................	100

[a]Most of the amino acids were supplied in the
form of casein acid-hydrolysate solution (10%), 40
milliliters in 1 liter. Other constituents that
were added to each liter of solution were 5 grams
of sodium propionate, 2.5 grams of polyethylene
glycol, and 1.625 grams of sodium orthophosphate,
di-hydrogen (NaH_2PO_4). The pH of the solution
was adjusted to 6.5 with sodium hydroxide.

ment, the left common carotid artery was exteriorized and the first stage of the ruminal fistula operation was performed. One and one half months prior to the experiment, the second stage of the fistula operation was performed. At the time of the experiment, the animal apparently was in good health.

2. Composition of the experimental solution: Most of the amino acids were supplied in the form of a casein acid-hydrolysate solution. Twenty milliliters were used in each liter of total solution. Table 4 presents the constituents that were added to complete the composition of the experimental solution.

3. Sampling procedure: Three liters of the experimental solution were placed in the rumen of the goat. The number of samples removed and the times of sampling were the same as for experiment W4. The total volume of solution removed by the sampling procedure was 1300 milliliters. The volume of the solution recovered from the rumen at the end of the experiment was 800 milliliters. There was, however, still fluid left in the arterior ventral blind sac of the rumen (see Fig. 1, p. 3) which could not be recovered. The volume recovered together with the volume removed by the sampling procedure accounts for 2100 of the original 3000 milliliters placed in the rumen. In addition to the unknown volume of solution left in the rumen, at least some of the volume of 900 milliliters not accounted for was lost as an unknown volume of spillage through the fistula.

Experiment W6.-

1. Animal history: The goat of experiment W5 was used also for this experiment. At the time of the experiment, the animal apparently was in good health.

TABLE 4. - Composition of the experimental solution
for experiment W5

Constituent[a]	Milligrams per Liter
L-cystine	50
Calcium-d-pantothenate	50
Glycine	50
Nicotinic acid	50
Riboflavin	50
DL-serine	50
L-tryptophan	50

[a]Most of the amino acids were supplied in the form of a casein acid-hydrolysate solution (10%), 20 milliliters in 1 liter. Other constituents that were added to each liter of solution were 5 grams of sodium propionate, 2.5 grams of polyethylene glycol, and 1.625 grams of sodium orthophosphate, dihydrogen (NaH_2PO_4). The pH of the solution was adjusted to 6.5 with sodium hydroxide.

2. Composition of the experimental solution: The solution for this experiment was prepared in the same way as for the experimental solution of experiment W4.

3. Sampling procedure: Three liters of the experimental solution were placed in the rumen of the goat. The number of samples removed and the times of sampling were the same as for experiment W4. The total volume of solution removed by the sampling procedure was 1300 milliliters. The volume of solution recovered from the rumen at the end of the experiment was 880 milliliters. There was, however, fluid still left in the rumen which could not be recovered. The volume recovered together with the volume removed by the sampling procedure accounts for 2180 of the original 3000 milliliters placed in the rumen. In addition to the unknown volume of solution left in the rumen (estimated as 300 milliliters), at least some of the volume of 820 milliliters not accounted for was lost as an unknown volume of spillage through the fistula.

Listing of Individual Constituents for Experiments W1 to W6.— Table 5 presents, for experiments W1 to W6, the concentrations of the individual constituents in the experimental solutions.

Assay Procedure

The details of these procedures are presented in the appendix. Listed here are the substances assayed, with a brief statement as to the method of their assay.

Pectin.— The method of pectin assay was adapted from the procedure of Nanji and Norman (269). Basically the method involves precipitating the pectin as calcium pectate and weighing the precipitate.

Polyethylene Glycol.— The gravimetric procedure of Sperber et al (360) was used.

TABLE 5. - Complete listing of the constituents present in the experimental solutions, the concentrations[a] being expressed as milligram-moles per liter

Constituent	Experiment				
	W1	W2	W3	W5	W4 and W6
Amino acids					
Alanine	. . .	2.13	0.43	0.43	0.85
Arginine	. . .	2.18	0.44	0.44	0.87
Aspartic acid	. . .	3.08	0.62	0.62	1.23
Cystine	. . .	0.13	0.03	0.24	0.47
Glutamic acid	3.00	14.83	2.97	2.97	5.93
Glycine	3.00	0.67	0.80	0.80	1.60
Histidine	. . .	1.23	0.25	0.25	0.49
Hydroxyproline	. . .	0.15	0.03	0.03	0.06
Isoleucine	. . .	3.90	0.78	0.78	1.56
Leucine	3.00	11.28	2.26	2.26	4.51
Lysine	3.00	4.05	0.81	0.81	1.62
Methionine	3.00	2.00	0.40	0.40	0.80

TABLE 5 - Continued

Constituent	Experiment				
	W1	W2	W3	W5	W4 and W6
Phenylalanine	. . .	2.38	0.48	0.48	0.95
Proline	. . .	7.30	1.46	1.46	2.92
Serine	. . .	0.38	0.08	0.56	1.11
Threonine	. . .	3.88	0.78	0.78	1.55
Tryptophan	3.00	. . .	. . .	0.25	0.49
Tyrosine	. . .	2.88	0.58	0.58	1.15
Valine	. . .	4.45	0.89	0.89	1.78
Vitamins					
Nicotinic acid	0.16	. . .	0.41	0.41	0.81
Pantothenic acid	0.04	. . .	. . .	0.11	0.21
Riboflavin	0.05	0.05	. . .	0.14	0.27
Other					
Pectin[b]	?	?	. . .	. . .	. . .
Polyethylene glycol	. . .	. . .	0.63	0.63	0.63

TABLE 5 - <u>continued</u>

Constituent	Experiment				
	W1	W2	W3	W5	W4 and W6
Potassium orthophosphate, di-hydrogen	0.067	0.067	. . .	. . .	. . .
Sodium hydroxide[c]	. . .	. . .	?	?	?
Sodium orthophosphate, di-hydrogen	. . .	. . .	11.77	11.77	11.77
Sodium orthophosphate, mono-hydrogen	0.067	0.067	. . .	. . .	. . .
Sodium propionate	. . .	. . .	52.04	52.04	52.04
Total	18.384	67.084	78.94	80.13	95.67

[a]These concentration data are constituted of composite figures based on:

1. The manufacturer's (Nutritional Biochemical Corporation) estimate of the concentrations of the constituents in the casein acid-hydrolysate solution;
2. the weighed-out amounts of the constituents added in making up the experimental solutions.

[b]It is not possible to calculate a molal concentration for pectin, as pectin is a mucilagenous vegetable carbohydrate of unknown molecular weight (323).

[c]A record was not kept of the amount of sodium hydroxide added to the experimental solutions; however, enough was added to adjust the pH to 6.5.

34.

Propionic Acid.- An adaptation of the steam distillation procedure of Nish (271) was utilized.

Amino Acids.- The amino acids were assayed by use of two procedures: the microbiological procedures of Barton-Wright (30) and the Van Slyke manometric determinations of alpha amino nitrogen (148, 391).

B Vitamins.- The microbiological procedures of Barton-Wright (30) were used.

RESULTS

Pectin

This substance was added to the experimental solutions of experiments W1 and W2 to serve as a marker for water movement. Unfortunately, the assay for pectin was carried out improperly. There is not a record, therefore, of the water movements that may have occurred during these two experiments. The osmotic pressure for each of the two solutions, however, can be calculated:[17] for experiment W1, 0.5 atmospheres; for experiment W2, 1.7 atmospheres. The osmotic pressure of goat's blood averages about 8 atmospheres at 37° C. (9). At the time of being added to the rumen, the solutions of experiments W1 and W2 were, therefore, strongly hypotonic relative to the blood draining the rumen. Parthasarathy and Phillipson (286), working with sheep, found that the rumen absorbed 70 to 305 milliliters of water in one hour from solutions only slightly hypotonic to the blood draining the rumen. This should mean that water was absorbed when the hypotonic solutions for experiments W1 and W2 were

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[17] $\Pi = mRT$, where Π is the osmotic pressure in atmospheres, m is the molality, R is the gas constant (0.08205 liter-atmospheres per degree per mole), and T is the absolute temperature.

placed in the rumen of the experimental goats.

Polyethylene Glycol

This substance was added to the experimental solutions of experiments W3 to W6 to serve as a marker for water movement. The results are presented in Table 6 and Figure 3. The data were analyzed statistically by applying analysis of variance to determine the significance for the divergence from a zero gradient of the slopes of linear "best-fit" lines. Although the best-fit lines are not drawn in Figure 3, the statements of significance for the best-fit lines are entered in the figure. In experiment W5, for example, there is a statement of significance given for a concentration change in the rumen that reads as: .005 (0 30). This statement means that the data of the 0, 10, and 30 minute samples were used to calculate a best-fit line; further, that the divergence from a zero gradient of the slope of this best-fit line is significant at or at less than the 0.5 per cent level. When a statement of significance is not recorded on the figure for a particular part of the data, this means that the divergence of the slope of the best-fit line for this part of the data was not significant at even the 10 per cent level. This method of presenting the statements of significance is used also for the data that are recorded in Figures 4 to 20.

Figure 3 indicates that only in experiment W5 was there in the rumen a change of concentration that was significant at the 10 per cent level or less. In experiment W5, the data for the 0 to 30 minute interval yield a best-fit line whose positive slope is significant at the 0.5 per cent level or less. These results can be interpreted to indicate that only in experiment W5 did demonstrable water movement occur. In this experiment, the increase of polyethylene glycol concentration should

TABLE 6. - Ruminal fistula experiments W3, W4, W5, and W6: changes in the weights (milligrams from 3-milliliter samples) of <u>polyethylene glycol precipitates</u> (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Time (min.)	Experiment							
	W3		W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	51.5 ± 2.1 (2)	. . . .	55.5 ± 2.0 (6)	. . . .	. . . .	. . . .	. . . .
0	54.0 ± 4.2 (2)	54.0 ± 4.2 (2)	49.8 ± 2.7 (4)	49.8 ± 2.7 (4)	40.5 ± 2.4 (4)	40.5 ± 2.4 (4)	51.4 ± 3.0 (5)	51.4 ± 3.0 (5)
10	50.5 ± 4.9 (2)	. . . .	52.5 ± 3.3 (4)	49.8 ± 3.8 (4)	46.5 ± 2.9 (4)	45.5 ± 1.7 (4)	51.5 ± 3.9 (4)	52.8 ± 3.5 (4)
30	52.5 ± 2.1 (2)	. . . .	50.0 ± 4.6 (4)	48.7 ± 2.9 (3)	50.0 ± 3.2 (4)	45.8 ± 2.1 (4)	50.5 ± 2.1 (4)	47.5 ± 4.8 (4)
70	49.0 ± 4.2 (2)	57.5 ± 3.4 (4)	48.9 ± 4.3 (4)	53.3 ± 4.0 (4)	49.0 ± 4.3 (4)	49.3 ± 2.9 (4)	51.0 ± 2.9 (4)	54.7 ± 1.3 (3)
150	. . . .	. . . .	48.9 ± 4.7 (4)	. . . .	50.5 ± 2.4 (4)	. . . .	52.6 ± 2.9 (5)	. . . .

[a]Except for the data of the experimental solution, the word "control" here designates the data of the ruminal solution control (see p. 21).

[b]Analysis of the ruminal solution before it was placed in the rumen.
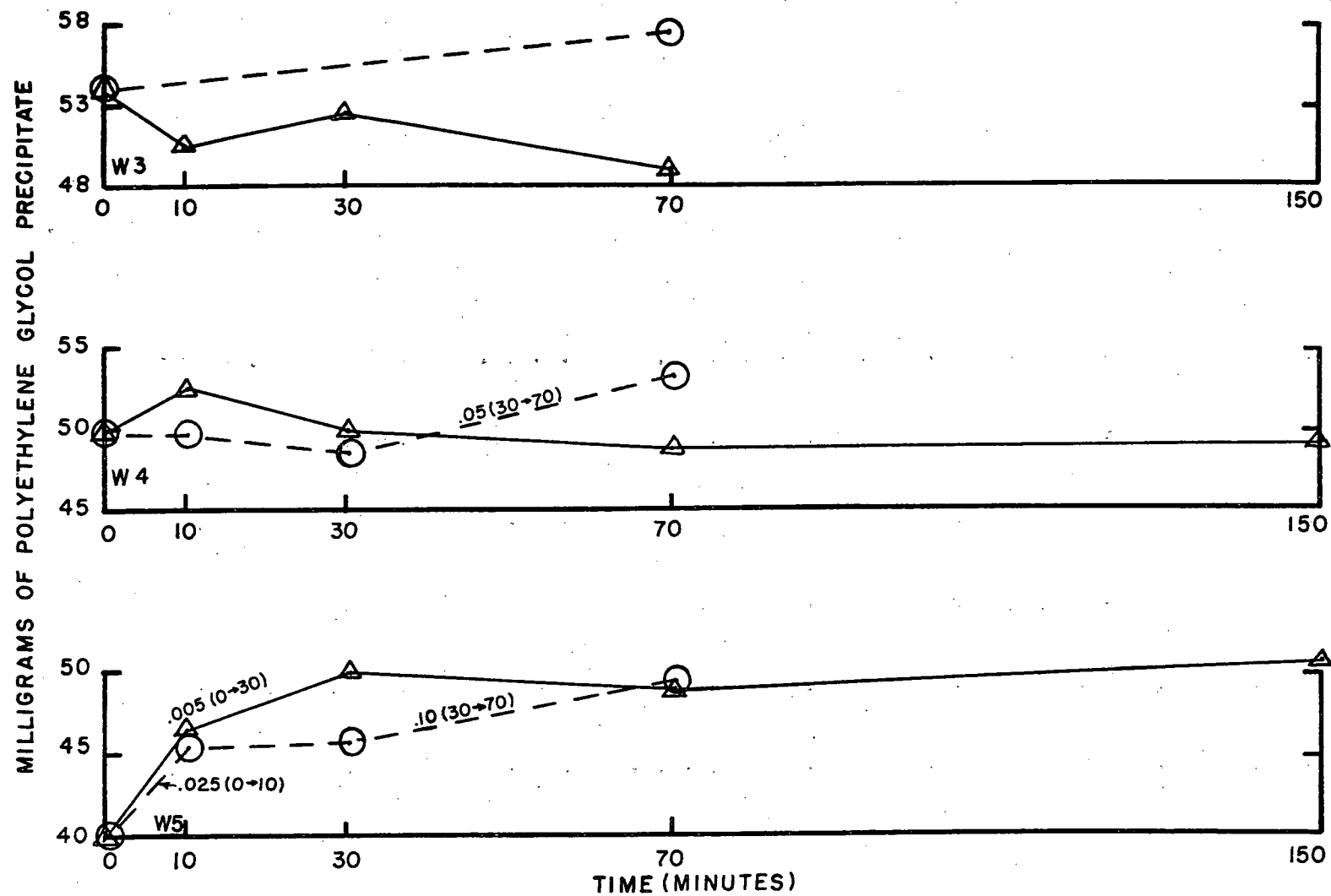
Fig. 3 (First part)
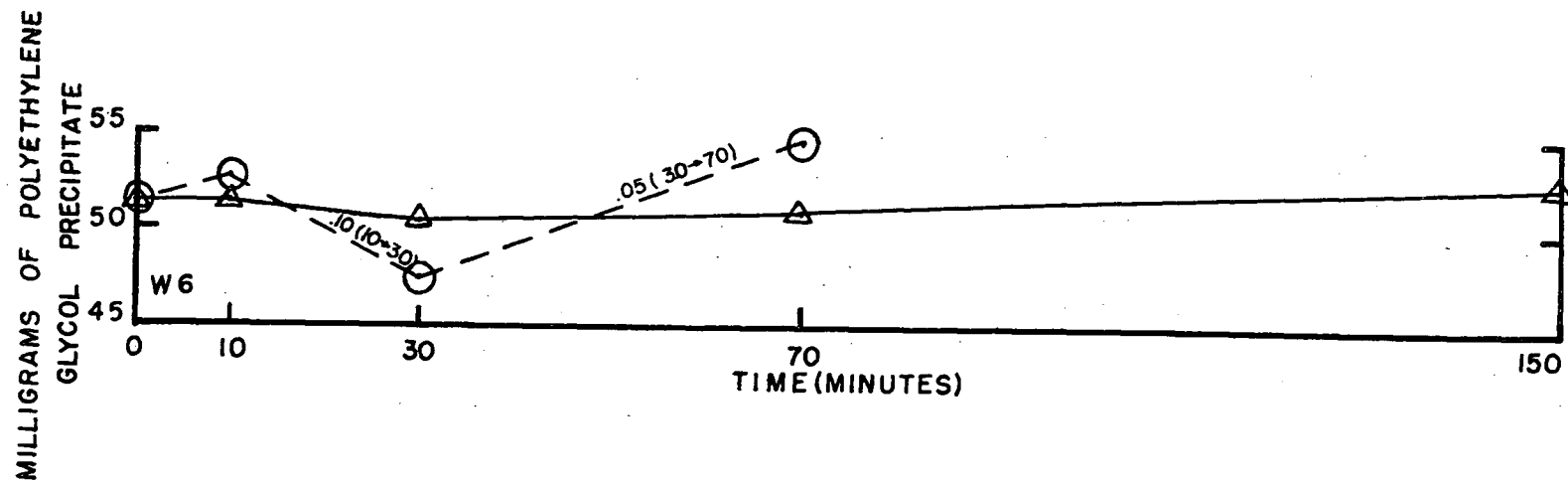
Fig. 3 (Second part)

Fig. 3. - Ruminal fistula experiments W3, W4, W5, and W6: changes in weights of polyethylene glycol in the ruminal solution (△————△) and in the ruminal solution control (⊖— — —⊖). The inscriptions such as .10 (10 ——→ 30) are statistical statements of significance, the explanation of which is given in the text.

mean that water was absorbed from the rumen. Ordinarily, this would lead

to correcting the data of the amino acids, B vitamins, and propionic acid

for the concentrating effect of the water movement. The polyethylene

glycol concentration changes in the control, however, complicate the

interpretation of the polyethylene glycol concentration changes in the

rumen. Between 0 and 10 minutes, there is in the control a concentration

increase that is significant at less than the 2.5 per cent level. Between

30 and 70 minutes, there is another concentration increase that is signif-

icant at less than the 10 per cent level. Although these concentration

increases in the control do not match those in the rumen, it can not be

said with certainty that all the increase of polyethylene glycol concen-

tration in the rumen is due to water absorption. If the polyethylene

glycol concentration changes in the control are real changes and not just

reflections of sampling error, then the same factor that effected the change

of concentration in the control also may have effected a corresponding change

of concentration in the rumen. If, indeed, such a change of concentration

was effected in the rumen, then the use of the polyethylene glycol concen-

tration changes to estimate the absorption of water from the rumen would

result in an overestimation. As there appears to be no way to determine

if the control changes were the result of the effect of some factor or

merely sampling error, the data for the constituents in experiment W5

will be presented both corrected[18] and uncorrected for water movement.

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[18]To correct the data for the constituents--i.e., propionic acid or
any one of the amino acids or B vitamins that was determined--, each
measured concentration was multiplied by its own correction factor. Each
correction factor was the ratio of the polyethylene glycol concentration
in the 0 minute sample to the polyethylene glycol concentration in one of
the subsequent samples; thus, the correction factor for the measured
concentration of a constituent in the 10 minute sample from the rumen is
$40.5/46.5 = 0.8709$.

Tables 7 to 25--tables that present the results for propionic acid, the amino acids, and the B vitamins--have two rows of data for each sampling time in the columns for experiment W5. The top row presents the data uncorrected for water movement; the bottom row presents the data corrected for water movement. Likewise, Figures 4 to 12 present the data of experiment W5 as both uncorrected (W5-U) and corrected (W5-C) concentrations. Except for the 0 minute sample concentration, the corrected concentrations of the different constituents are all lower than the uncorrected concentrations. The corrected data indicate a greater decrease of concentration than do the uncorrected data. The uncorrected data of experiment W5 represent, therefore, a more conservative estimate of the constituents' concentration changes. By utilizing both sets of data, it is hoped that the true concentration changes will be bracketed within upper (uncorrected data) and lower (corrected data) limits. Actually, comparison of the uncorrected and corrected data for the different constituents reveals that only for nicotinic acid, pantothenic acid, and tyrosine does the interpretation differ much between the two sets of data. Even for these three constituents, the differences in interpretation are minor.

The polyethylene glycol results of experiments W3, W4, and W6 require further discussion. In none of these experiments was there in the rumen a concentration change that showed significance at the 10 per cent level or less. These results were interpreted as indicating that demonstrable water movement did not occur. These results do not mean necessarily, however, that there was no water movement; only that, according to the methods employed, water movement did not occur that showed significance at the 10 per cent level. A visual method was

devised to check on the possibility that perhaps real but undetected (undetected by significance testing) water movements could produce concentration changes of the amino acids and B vitamins. This method consists of plotting the data for polyethylene glycol on the graphs of the data for each the other constituents (e.g., see Fig. 4, p. 46). In each graph, the scale of concentration changes of polyethylene glycol is adjusted[19] to the scale of concentration changes of the constituent concerned. In this way, a constituent's concentration changes that result only from water movement will be indicated in the figures; thus, if the slope of the line drawn between the 0 and 10 minute concentrations for polyethylene glycol is parallel to the slope of the line drawn between the 0 and 10 minute concentrations for an amino acid, then the concentration change of the amino acid in this time interval is probably the result of water movement alone. Conversely, any significant divergence of the slopes of two such lines should be an indication that the concentration change of the amino acid is the result of more than just the effects of water movement. This type of comparison between the concentration changes for polyethylene glycol and the concentration changes for a constituent is made for propionic acid and each the amino acids and B vitamins that was measured.

Propionic Acid

The results of the steam-distillation assay for propionic acid are

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[19]To adjust the scale of concentration changes of polyethylene glycol to the scale of concentration changes of a constituent, each of the polyethylene glycol concentrations was multiplied by a conversion factor. Each conversion factor is the ratio of the constituent's concentration in the 0 minute sample to the polyethylene glycol concentration in the same sample; thus, the conversion factor to be used to convert the polyethylene glycol concentrations for plotting on the graph of propionic acid in experiment W3 is 50.6/54.0 = 0.9370.

presented in Table 7 and Figure 4. Examination of Figure 4, taking note
of the statements of significance, reveals that in all the experiments
there was a strong decrease of propionic acid concentration in the ruminal
solution during at least a part of the experiment. At the same time, the
concentration in the control solution either remained constant or increas-
ed. In addition, a comparison of the slopes of the lines for the adjusted
polyethylene glycol data and the propionic acid data demonstrates that,
with one possible exception, the concentration changes of propionic acid
in the ruminal solution were not a reflection of a dominant influence of
water movement. The one possible exception is in experiment W3. In this
experiment, it appears that at least a part of the concentration changes
could have resulted from the effects of water movement. With the possible
exception of these data for experiment W3, the results of the propionic
acid experiments indicate ruminal absorption of propionic acid. As the
rumen already is known to absorb propionic acid, the results of the
propionic acid experiments provide controls which indicate that the rumens
of the goats in experiments W3 to W6 were exhibiting absorption.

Amino Acids: Tryptophan, Methionine, Tyrosine, Glycine, and Lysine

The results of the microbiological assay for these compounds are
presented in Tables 8 to 12 and Figures 5 to 9. Examination of the
figures, taking note of the statements of significance, reveals that in
all the experiments, except one, there were strong decreases in the concen-
trations of the amino acids in the ruminal solution during at least a
part of the experiment. The one exception is the uncorrected data for
tyrosine. Even in this experiment, however, there were decreases of
concentration that were significant at the 10 per cent level. At the same
time, the concentrations of the amino acids in the control solution

TABLE 7. - Ruminal fistula experiments W3, W4, W5, and W6: changes in concentration (milligram-moles per liter) of propionic acid (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given: this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment							
	W3		W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	47±8 (2)	. . . .	69±9 (4)	. . . .	72±13 (4)	. . . .	77±16 (4)
0	51±3 (2)	51±3 (2)	68±1 (4)	68±1 (4)	53±4 53±4 (4)	53±4 53±4 (4)	65±8 (4)	65±8 (4)
10	46±6 (2)	. . . .	63±5 (4)	. . . .	70±4 61±3 (4)	. . . .	66±2 (4)	. . . .
30	45±7 (2)	. . . .	61±5 (4)	. . . .	59±4 48±3 (4)	. . . .	58±5 (4)	. . . .
70	41±2 (2)	54±0 (2)	51±5 (4)	. . . .	51±3 42±3 (4)	. . . .	41±11 (4)	. . . .
150	. . . .	. . . .	39±3 (4)	68±1 (2)	35±2 28±2 (4)	73±11 . . . . (2)	28±3 (4)	69±5 (2)

a,b The same as for Table 6.

Fig. 4 (First part)

Fig. 4 (Second part)

Fig. 4. - Ruminal fistula experiments W3, W4, W5, and W6: changes in concentration of propionic acid in the ruminal solution (△————△) and in the ruminal solution control (⊖————⊖). The symbols W5-U and W5-C designate the data of experiment W5, uncorrected for water movement and corrected for water movement, respectively. The dotted lines (■·····■) in the figure demonstrate the concentration changes of polyethylene glycol. The reason for including the polyethylene glycol data in the figure is presented in the text.

TABLE 8. - Ruminal fistula experiments W4, W5, and W6: changes in concentration (microgram-moles per 100 milliliters) of tryptophan (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

| | Experiment | | | | | |
| | W4 | | W5 | | W6 | |
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	• • • •	18.5±0.8 (3)	• • • •	5.4±0.7 (3)	• • • •	9.7±1.6 (3)
0	13.5±1.1 (12)	13.5±1.1 (12)	1.2±0.2 1.2±0.2 (12)	1.2±0.2 1.2±0.2 (12)	7.9±0.9 (12)	7.9±0.9 (12)
10	13.9±1.3 (11)	12.4±0.9 (11)	0 0 (12)	1.4±0.2 1.3±0.2 (12)	0.8±0.2 (12)	13.4±1.4 (12)
30	12.6±0.7 (12)	12.3±0.8 (12)	0 0 (12)	1.6±0.2 1.4±0.2 (12)	0.8±0.1 (12)	12.9±1.4 (12)
70	8.1±0.9 (12)	11.2±0.7 (12)	0 0 (12)	1.8±0.2 1.5±0.2 (6)	0.2±0.2 (12)	12.3±1.0 (12)
150	3.9±1.5 (12)	9.5±1.6 (12)	0 0 (12)	• • • • • • • •	1.5±0.3 (12)	11.0±0.5 (6)

[a],[b]The same as for Table 6.
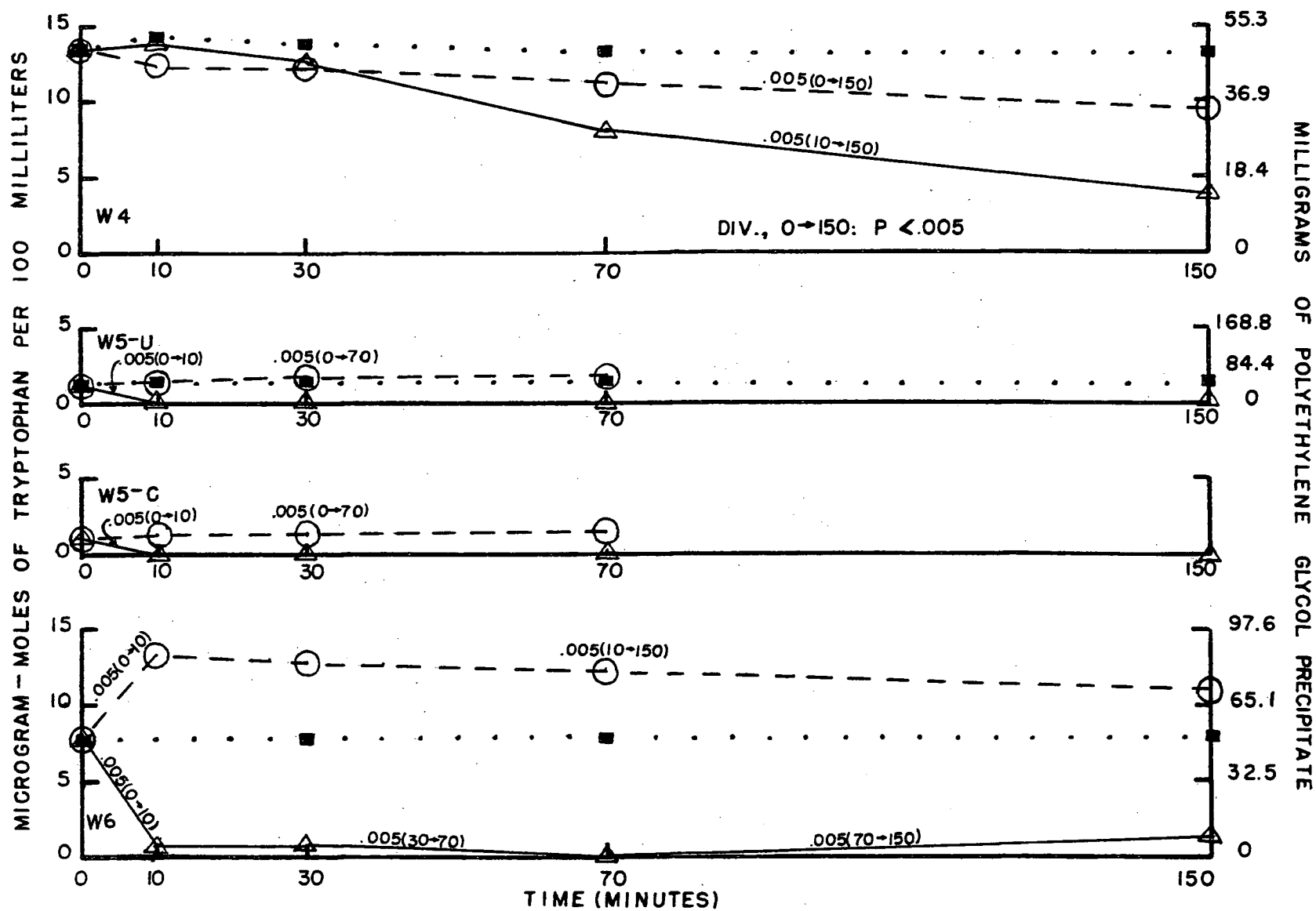
Fig. 5.

Fig. 5. - Ruminal fistula experiments W4, W5, and W6: changes in concentration of tryptophan in the ruminal solution (A————A) and in the ruminal solution control (O— — —O), plus changes in concentration of polyethylene glycol (■ · · · ·■) in the ruminal solution. In the graph for experiment W4, there is recorded the inscription, "Div., 0——→150: P < .005." This type of inscription is entered in those graphs for which any doubt exists that a negative gradient of the best-fit line for the data of the ruminal solution control might not diverge significantly from the negative gradient of the best-fit line for the data of the ruminal solution. The inscription recorded in the graph for experiment W4 indicates that the divergence of those two best-fit lines (not drawn in the graph) which are based on the 0 to 150 minute data, inclusive, is significant at the 0.5 per cent level or less. The rest of the symbols in this figure have the same meaning as previously described in Figures 3 and 4.

TABLE 9. — Ruminal fistula experiments W4, W5, and W6: changes in concentration (microgram-moles per 100 milliliters) of <u>methionine</u> (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment					
	W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .
0	21.3±2.0 (8)	21.3±2.0 (8)	7.4±1.2 7.4±1.2 (8)	7.4±1.2 7.4±1.2 (8)	18.8±1.5 (8)	18.8±1.5 (8)
10	20.5±1.7 (8)	22.2±1.8 (8)	8.9±1.9 7.7±1.6 (8)	10.8±2.5 9.6±2.2 (8)	15.4±1.3 (8)	20.9±2.1 (8)
30	19.0±0.9 (8)	22.5±2.3 (8)	5.2±0.7 4.2±0.6 (5)	10.5±2.1 9.3±1.9 (7)	15.8±0.5 (8)	21.5±3.1 (8)
70	16.6±1.1 (8)	21.6±1.7 (8)	4.4±1.2 3.6±1.0 (8)	10.8±1.2 8.9±1.0 (7)	8.9±2.5 (8)	20.9±2.9 (8)
150	13.5±0.9 (8)	. . . .	4.0±1.1 3.2±0.9 (8)	. . . .	8.0±1.3 (8)	. . . .

a,b The same as for Table 6.
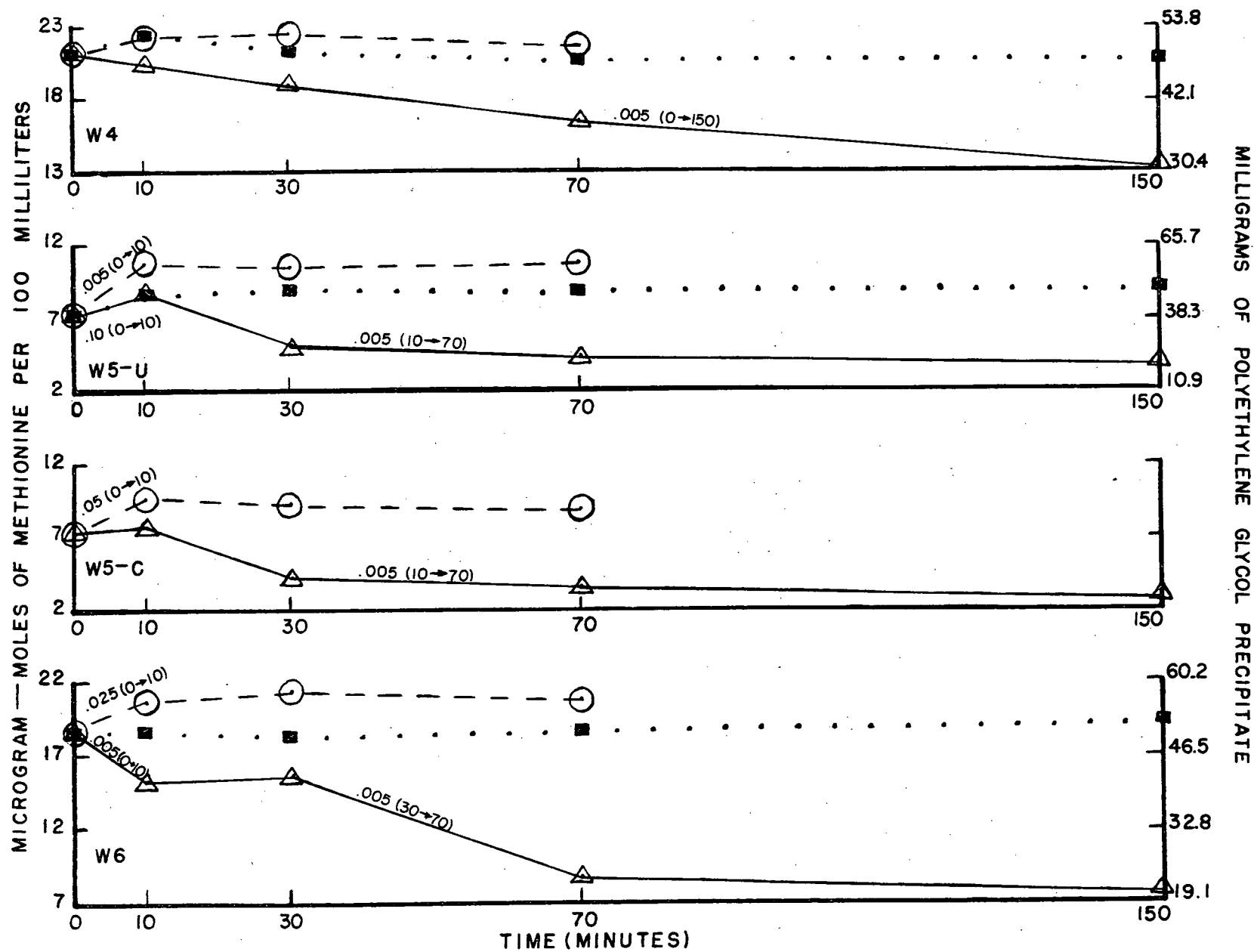
Fig. 6.

Fig. 6. - Ruminal fistula experiments W4, W5, and W6: changes in concentration of <u>methionine</u> in the ruminal solution (△————△) and in the ruminal solution control (⊖— — —⊖), plus changes in concentration of polyethylene glycol (■ · · · · ■) in the ruminal solution. The symbols in this figure have the same meanings as previously described in Figures 3 and 4.

TABLE 10. - Ruminal fistula experiments W4, W5, and W6: changes in concentration (microgram-moles per 100 milliliters) of tyrosine (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment					
	W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	21.5±1.0 (2)	. . . .	. . . .	. . . .	18.2±2.9 (2)
0	21.3±2.7 (12)	21.3±2.7 (12)	3.6±0.7 3.6±0.7 (8)	3.6±0.7 3.6±0.7 (8)	14.0±1.6 (12)	14.0±1.6 (12)
10	22.4±1.8 (12)	20.5±1.5 (12)	2.9±0.8 2.5±0.7 (8)	5.2±0.3 4.6±0.3 (5)	10.8±1.8 (12)	16.9±1.5 (12)
30	21.3±2.3 (12)	21.0±1.9 (12)	3.6±0.4 2.9±0.3 (4)	5.0±0.8 4.4±0.7 (6)	13.0±2.2 (12)	18.3±2.0 (10)
70	19.7±1.9 (12)	20.2±1.8 (12)	3.2±0.2 2.6±0.2 (8)	5.3±0.6 4.4±0.5 (3)	11.3±1.7 (12)	16.5±1.2 (12)
150	16.7±1.6 (12)	. . . .	3.0±0.3 2.4±0.2 (8)	. . . .	11.0±1.9 (12)	. . . .

[a],[b]The same as for Table 6.
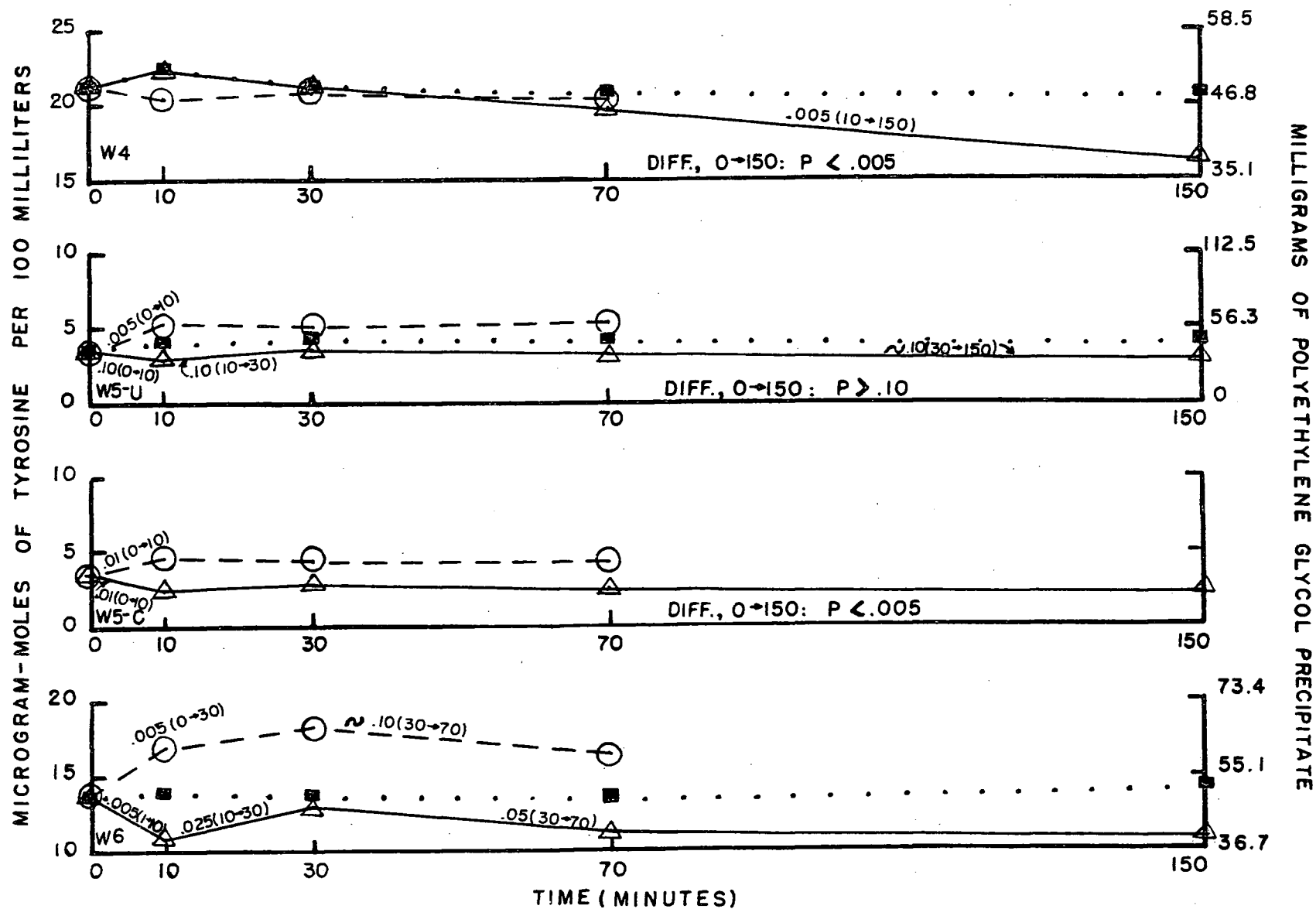
Fig. 7.

Fig. 7. — Ruminal fistula experiments W4, W5, and W6: changes in concentration of <u>tyrosine</u> in the ruminal solution (△——————△) and in the ruminal solution control (⊖— — —⊖), plus changes in concentration of polyethylene glycol (■ · · · · ■) in the ruminal solution. In the graphs for experiment W4, there is recorded the inscription, "Diff., 0 ——→150: P < .005." This type of inscription is entered in those graphs for which any doubt exists that there is not a significant difference between the initial (0 minute) and final (150 minute) concentrations of a constituent in the ruminal solution. To make the test of significance, the slope of a line joining the mean concentration of the 0 minute sample to the mean concentration of the 150 minute sample was tested for its divergence from a zero gradient. The inscription recorded in the graph for experiment W4 indicates that the difference between the initial and final concentrations is significant at the 0.5 per cent level or less. In the graph for experiment W5-U, there is added to one of the statements of significance the sign, ∿ . This sign is used in this case to indicate that the level of significance is only slightly higher than the 10 per cent level. The sign is used again with the same type of meaning in the graph for experiment W6. The rest of the symbols in the figure have the same meaning as previously described in Figures 3 and 4.

TABLE 11. - Ruminal fistula experiments W4, W5, and W6: changes in concentration (microgram-moles per 100 milliliters) of <u>glycine</u> (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment					
	W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	189.2±22.4 (8)	. . . .	86.4±16.9 (8)	. . . .	201.4±13.1 (7)
0	172.5±18.9 (8)	172.5±18.9 (8)	60.1±9.7 60.1±9.7 (8)	60.1±9.7 60.1±9.7 (8)	193.6±18.4 (8)	193.6±18.4 (8)
10	185.8±15.2 (8)	180.5±20.6 (8)	82.2±11.5 71.3±10.0 (8)	84.6±15.1 75.5±13.5 (8)	172.9±14.0 (8)	193.6±14.5 (8)
30	183.0±5.9 (8)	188.0±11.7 (8)	78.9±6.6 63.8±5.3 (8)	92.2±5.9 81.7±5.2 (8)	178.0±5.9 (8)	193.2±9.7 (8)
70	176.4±10.3 (8)	193.8±6.7 (8)	72.2±9.4 59.2±7.7 (8)	91.2±8.8 75.1±7.2 (8)	153.2±21.0 (8)	196.7±5.9 (8)
150	156.5±17.9 (8)	190.2±25.6 (8)	69.7±11.9 55.8±9.5 (8)	85.7±13.7 . . . . (8)	130.2±12.5 (8)	200.5±9.7 (8)

a,b The same as for Table 6.
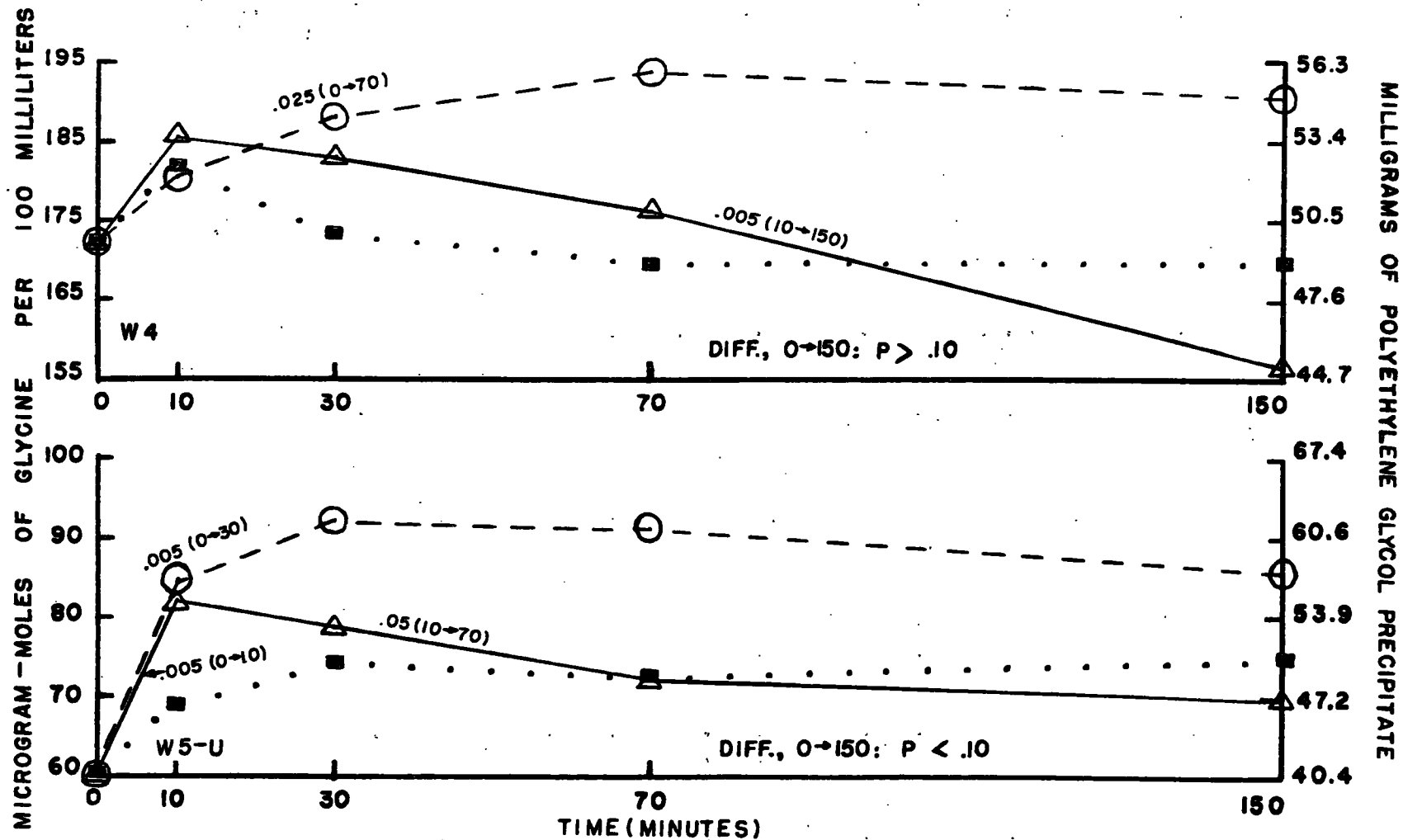
Fig. 8. (First part)
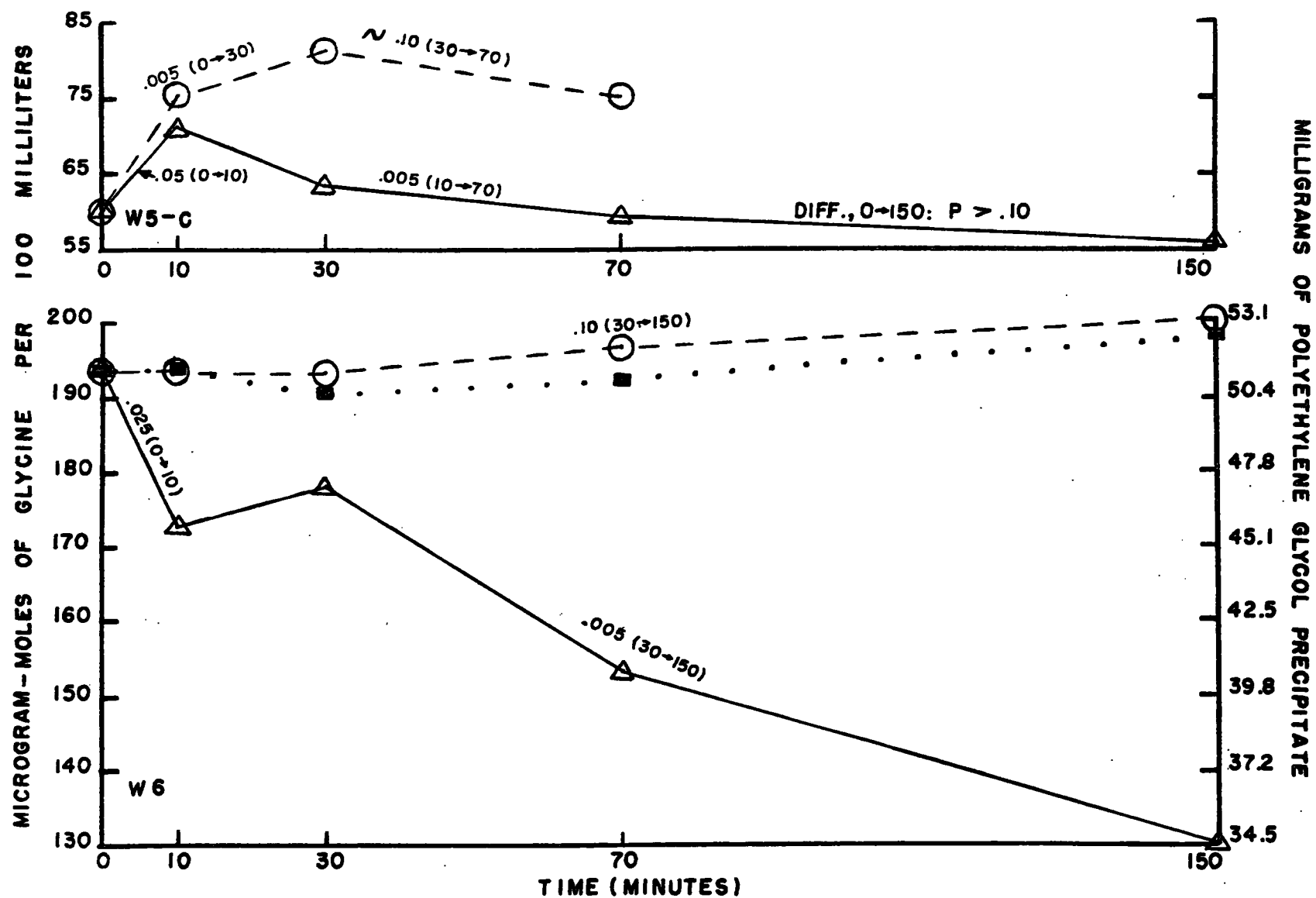
Fig. 8. (Second part)

Fig. 8. - Ruminal fistula experiments W4, W5, and W6: changes in concentration of <u>glycine</u> in the ruminal solution (△————△) and in the ruminal solution control (○————○), plus changes in concentration of polyethylene glycol (■·····■) in the ruminal solution. The symbols in the figure have the same meaning as previously described in Figures 3, 4, and 7.

TABLE 12. - Ruminal fistula experiments W4, W5, and W6: change in concentration (microgram-moles per 100 milliliters) of lysine (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment					
	W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	• • • •	187.6±5.5 (3)	• • • •	62.0±3.5 (3)	• • • •	177.8±4.4 (3)
0	185.9±8.4 (12)	185.9±8.4 (12)	59.9±11.3 59.9±11.3 (12)	59.9±11.3 59.9±11.3 (12)	176.4±14.9 (8)	176.4±14.9 (8)
10	174.0±12.5 (12)	183.5±14.0 (12)	72.6±4.8 63.0±4.2 (12)	76.3±6.5 68.1±5.8 (12)	163.1±19.8 (8)	175.8±10.7 (8)
30	170.5±13.4 (12)	192.3±14.8 (12)	68.9±3.6 55.7±2.9 (12)	77.7±4.3 68.9±3.8 (12)	156.6±4.3 (8)	180.7±13.3 (8)
70	166.2±12.3 (12)	184.2±10.7 (12)	64.2±3.4 52.6±2.8 (12)	79.0±4.7 65.1±3.9 (12)	147.3±8.3 (8)	181.7±13.2 (8)
150	144.2±10.3 (12)	183.7±16.3 (12)	56.3±5.6 45.1±4.5 (12)	78.1±5.4 • • • • (11)	128.1±10.2 (8)	184.0±12.0 (8)

a,b The same as for Table 6.

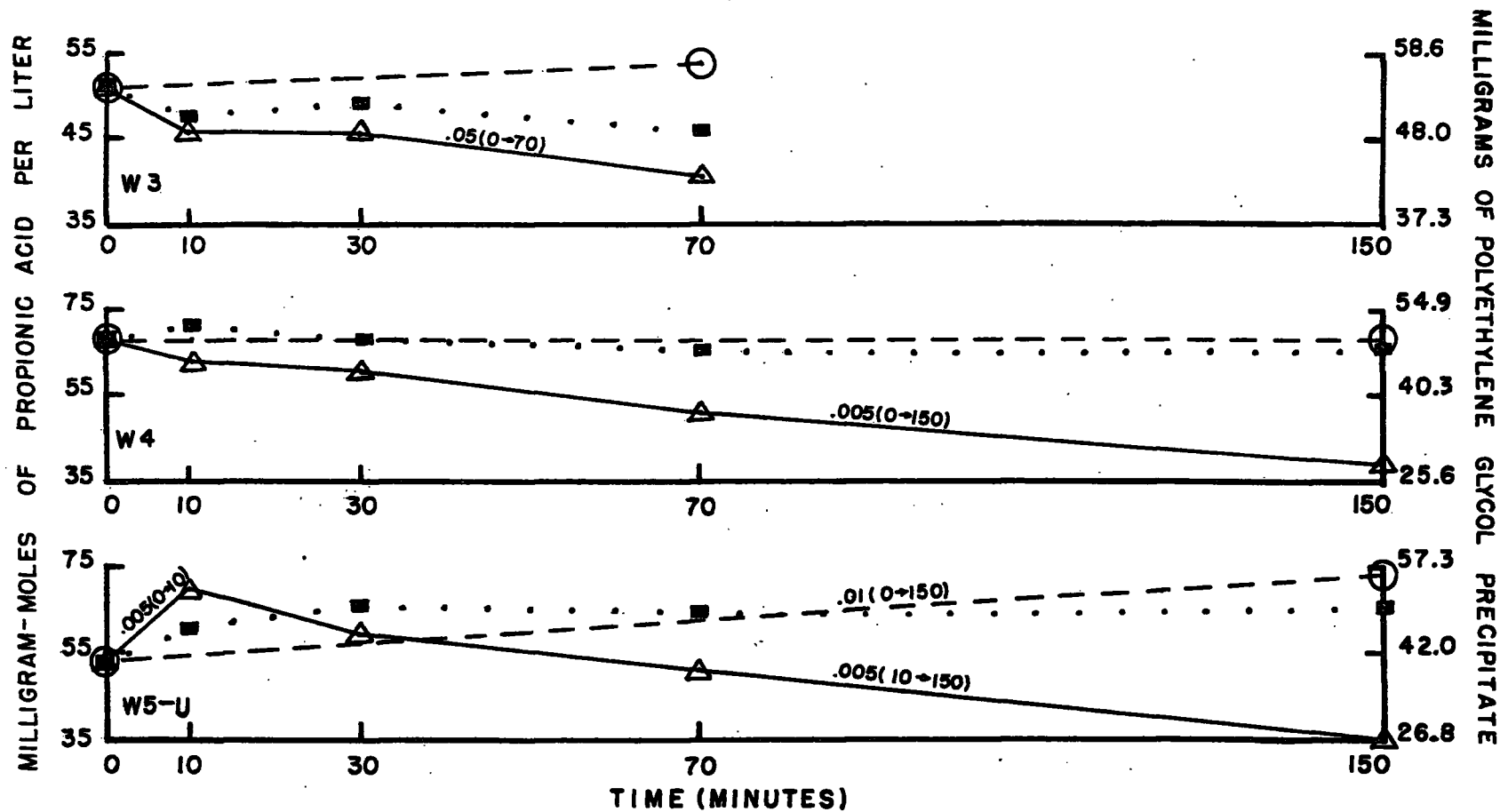Fig. 9 (First part)
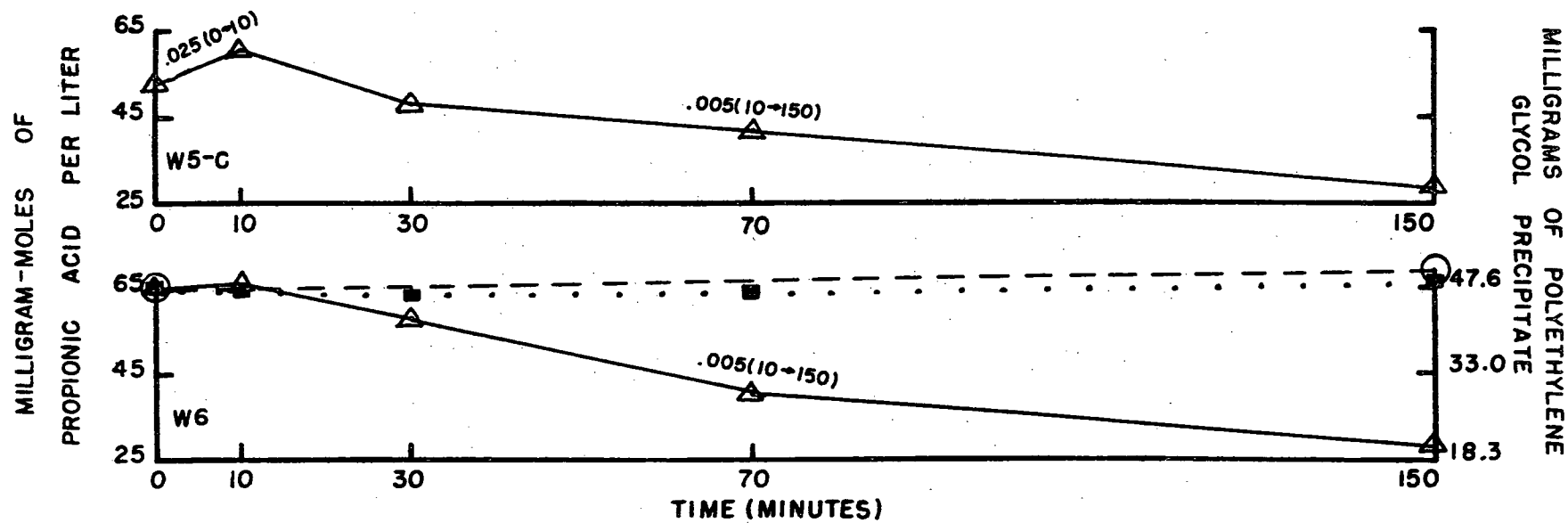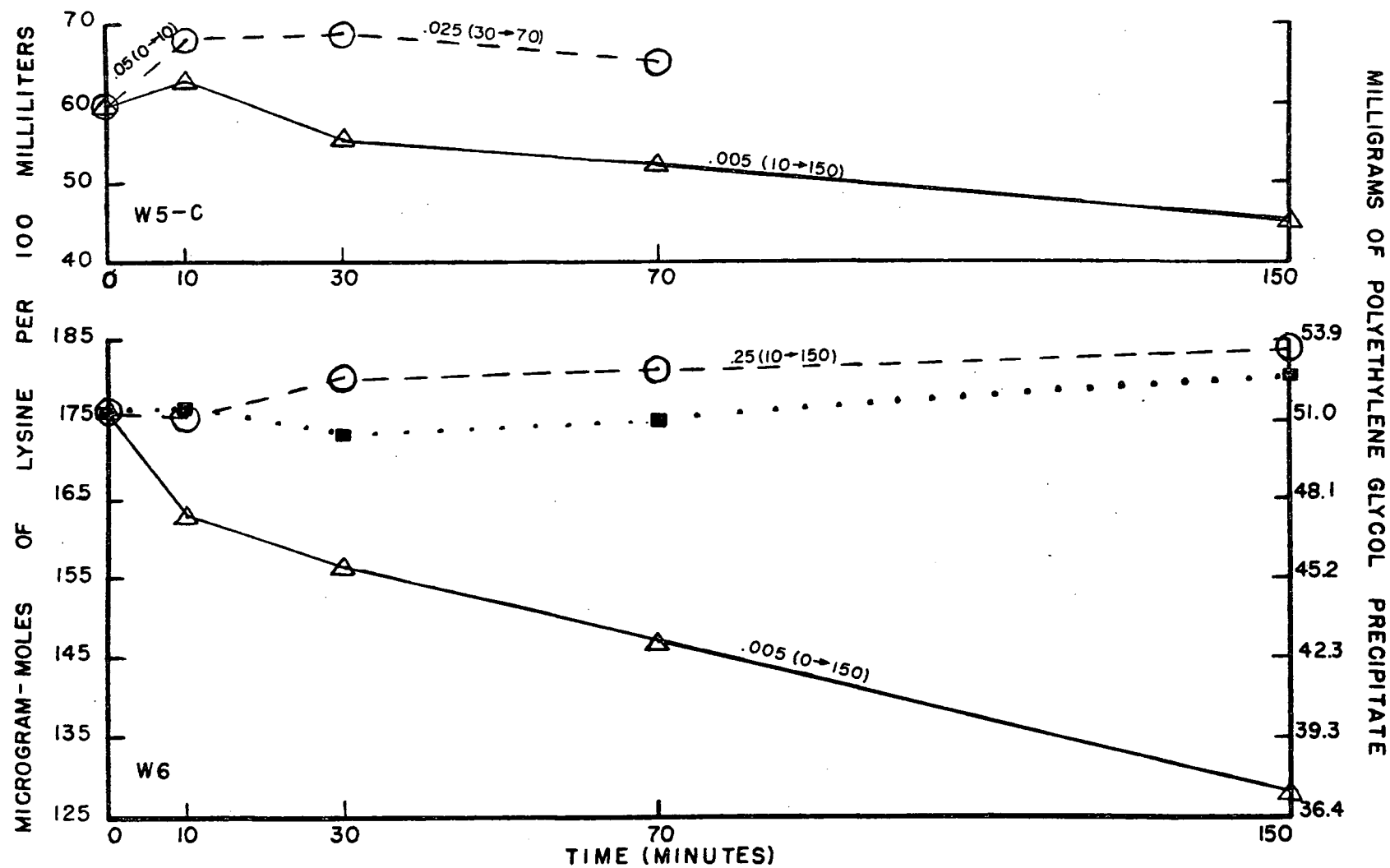
Fig. 9. (Second part)

Fig. 9. - Ruminal fistula experiments W4, W5, and W6: changes in concentration of lysine in the ruminal solution (△————△) and in the ruminal solution control (⊖ — — —⊙), plus changes in concentration of polyethylene glycol (■ · · · · ·■) in the ruminal solution. The symbols in the figure have the same meaning as previously described in Figures 3, 4, and 7.

usually increased or remained constant. The data of the control solution

for tryptophan in experiment W5 (Fig. 5), however, exhibited a progressive

decrease of concentration. The decreases of tryptophan concentration in

the ruminal solution were, nevertheless, still greater than those in the

control solutions; and, the best-fit lines for the concentrations in the

ruminal and control solutions exhibit a divergence that is significant at

the 0.5 per cent level or less. A comparison of the slopes of the lines

for the adjusted polyethylene glycol data and the amino acid data demon-

strates that, with one exception, the concentration changes of the amino

acids in the ruminal solution were not a reflection of a dominant influence

of water movement. The one exception is for lysine in experiment W4 (Fig.

9). In this experiment, a large part of the concentration changes could

have resulted from the effects of water movement. There is still a part of

the concentration decrease, however, for which water movement can not

account. These results support the hypothesis that the amino acids can

be absorbed from the rumen.

Alpha Amino Nitrogen

The results of the Van Slyke assay for alpha amino nitrogen in

experiment W6 are presented in Table 13 and Figure 10. Examination of

Figure 10, taking note of the statements of significance, reveals a

large decrease in the concentration of alpha amino nitrogen in the ruminal

solution. At the same time, there is an over-all increase in the concen-

tration of alpha amino nitrogen in the control solution. A comparison

of the slopes of the lines for the adjusted polyethylene glycol data and

the alpha amino nitrogen data demonstrates that the concentration changes

of alpha amino nitrogen in the ruminal solution were not a reflection of

a dominant influence of water movement. These results for alpha amino

nitrogen support the hypothesis that all or some of the amino acids can

TABLE 13. - Ruminal fistula experiment W6: changes
in concentration (milligrams per 100 milliliters)
of alpha amino nitrogen (the data recorded are
means and standard deviations, with numbers of
assays in parentheses)

Time (min.)	Experiment W6	
	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	. . . .
0	37.1 ± 0.9 (4)	37.1 ± 0.9 (4)
10	34.7 ± 1.3 (3)	41.5 ± 1.6 (4)
30	34.1 ± 2.0 (4)	39.2 ± 3.1 (3)
70	32.2 ± 1.9 (4)	37.9 ± 0.6 (4)
150	29.1 ± 1.5 (4)	40.1 ± 2.0 (4)

[a,b]The same as for Table 6.
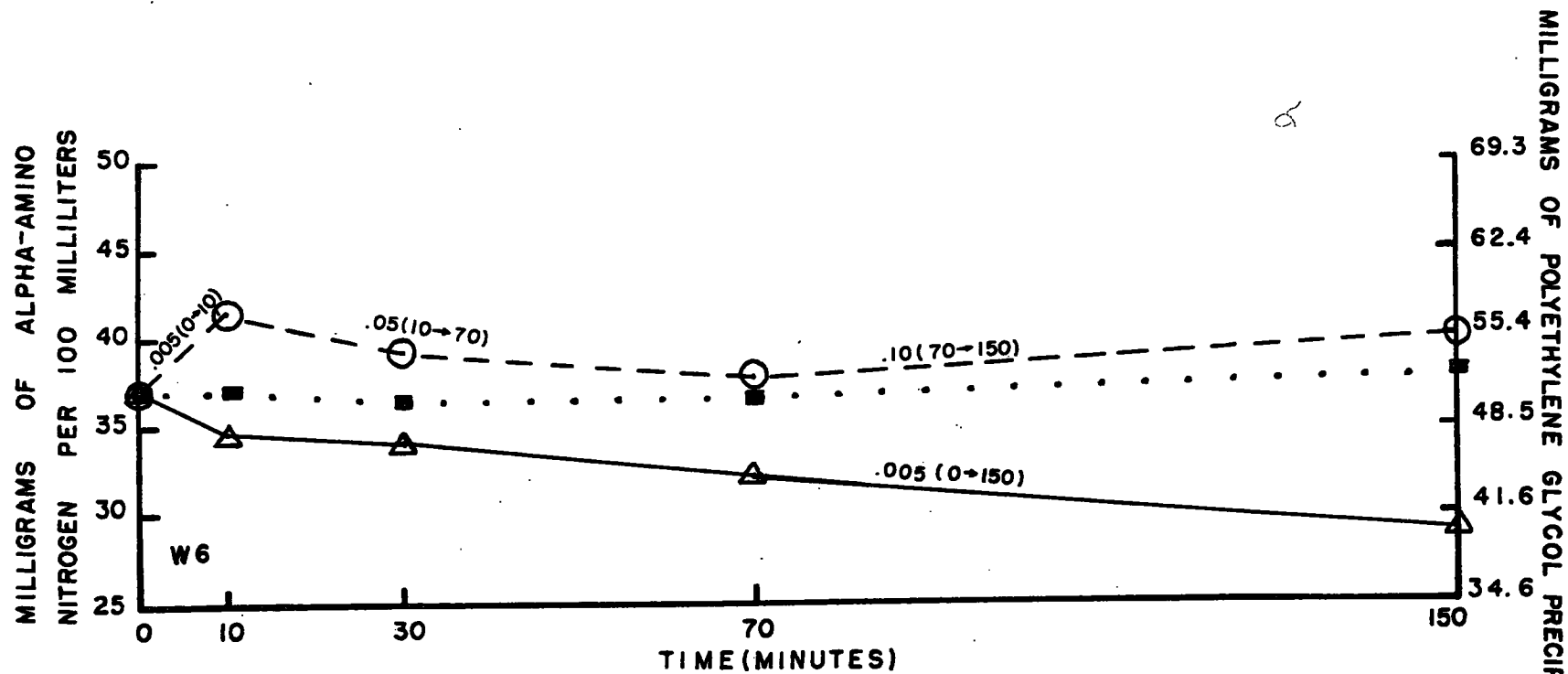
Fig. 10.

Fig. 10. - Ruminal fistula experiment W6: changes in concentration of alpha amino nitrogen in the ruminal solution (△———△) and in the ruminal solution control (⊖———⊙), plus changes in concentration of polyethylene glycol (■·····■) in the ruminal solution. The symbols in the figure have the same meaning as previously described in Figures 3 and 4.

be absorbed from the rumen.

B Vitamins: Riboflavin, Nicotinic Acid, and Pantothenic Acid

The results of the microbiological assays for these substances are

presented in Tables 14 to 16 and in Figures 11 to 13. Examination of

the figures, taking note of the statements of significance, reveals that

in all the experiments, except one, there were decreases in the concen-

trations of the B vitamins in the ruminal solution during at least a

part of the experiment. At the same time, the concentrations of the

B vitamins in the control solution either exhibited increases, or remained

relatively constant, or exhibited slight decreases. A slight concen-

tration decrease occurred in the control solution for riboflavin in

experiment W5 (Fig. 11). The decrease of riboflavin concentration in

the ruminal solution was still greater than that in the control solution;

and, the best-fit lines for the concentrations in the ruminal and control

solutions exhibit a divergence that is significant at the 1 per cent

level or less. The one exception to the previous statement--that is,

about the decreases in the concentrations of the B vitamins in the

ruminal solution--are the results for pantothenic acid in experiment W5

(Fig. 13). In this experiment, there was not in the ruminal solution a

concentration decrease that was significant at the 10 per cent level or

less. In experiments W3 to W6, a comparison of the slopes of the lines

for the adjusted polyethylene glycol data and the B vitamin data demon-

strates that the concentration changes in the ruminal solution were not

a reflection of a dominant influence of water movement. In experiments

W1 and W2, the absence of polyethylene glycol or pectin data does not

permit this type of comparison to be made. As, however, the solutions in

these two experiments were strongly hypotonic (see p. 35), it is likely

TABLE 14a. - Ruminal fistula experiments W1 and W2: changes in concentration (microgram-moles per 100 milliliters) of riboflavin (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Time (min.)	Experiment			
	W1		W2	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	5.9 ± 1.8 (8)	. . . .	4.5 ± 1.1 (9)
0	5.6 ± 1.6 (9)	5.6 ± 1.6 (9)	3.5 ± 0.7 (8)	3.5 ± 0.7 (8)
40	3.2 ± 0.9 (9)	. . . .	. . . .	. . . .
60	4.5 ± 1.0 (8)	. . . .	. . . .	. . . .
65	. . . .	. . . .	2.3 ± 0.6 (9)	. . . .
95	2.7 ± 0.8 (9)	. . . .	2.7 ± 0.6 (8)	. . . .
120	3.1 ± 0.6 (8)	5.0 ± 0.7 (9)	. . . .	. . . .

TABLE 14a – continued

Time (min.)	Experiment			
	W1		W2	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]
125	. . . .	. . . .	2.8±0.7 (9)	4.3±1.4 (9)

[a]The word "control" here designates the data of the experimental solution control. Data for the ruminal solution control are not available for these two experiments (see p. 20).

[b]The same as for Table 6.

TABLE 14b. - Ruminal fistula experiments W4, W5, and W6: changes in concentration (microgram-moles per 100 milliliters) of <u>riboflavin</u> (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment					
	W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	18.0±4.2 (8)	. . . .	7.9±2.0 (8)	. . . .	10.4±2.1 (4)
0	14.2±3.1 (8)	14.2±3.1 (8)	5.9±0.8 5.9±0.8 (8)	5.9±0.8 5.9±0.8 (8)	. . . .	. . . .
10	18.0±2.6 (8)	19.2±2.8 (8)	7.7±1.0 6.6±0.9 (8)	7.7±1.4 6.9±1.3 (8)	12.4±1.5 (8)	11.4±1.5 (8)
30	16.9±2.9 (8)	20.1±3.4 (8)	7.6±1.1 6.1±0.9 (8)	8.9±0.8 7.9±0.7 (8)	13.1±2.1 (8)	11.8±1.8 (8)
70	14.9±2.7 (8)	19.4±3.5 (8)	7.4±0.7 6.0±0.6 (8)	8.5±1.0 7.0±0.9 (8)	12.8±1.6 (8)	13.5±1.1 (8)
150	15.2±1.9 (8)	19.6±3.5 (8)	5.7±0.9 4.5±0.7	8.1±0.9 . . . . (8)	10.7±2.0 (8)	14.8±1.8 (8)

[a,b]The same as for Table 6.

73.
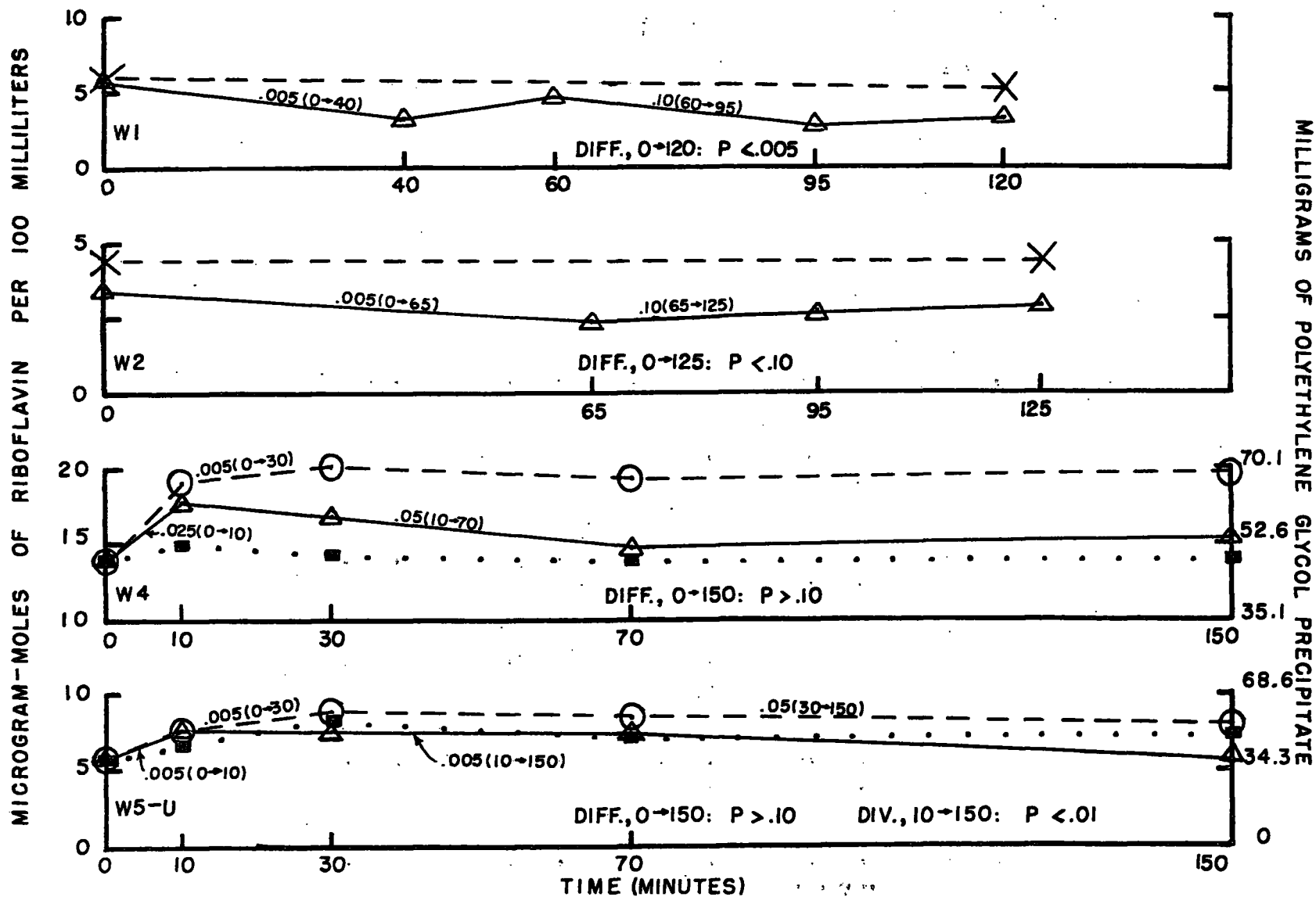
Fig. 11. (First part)
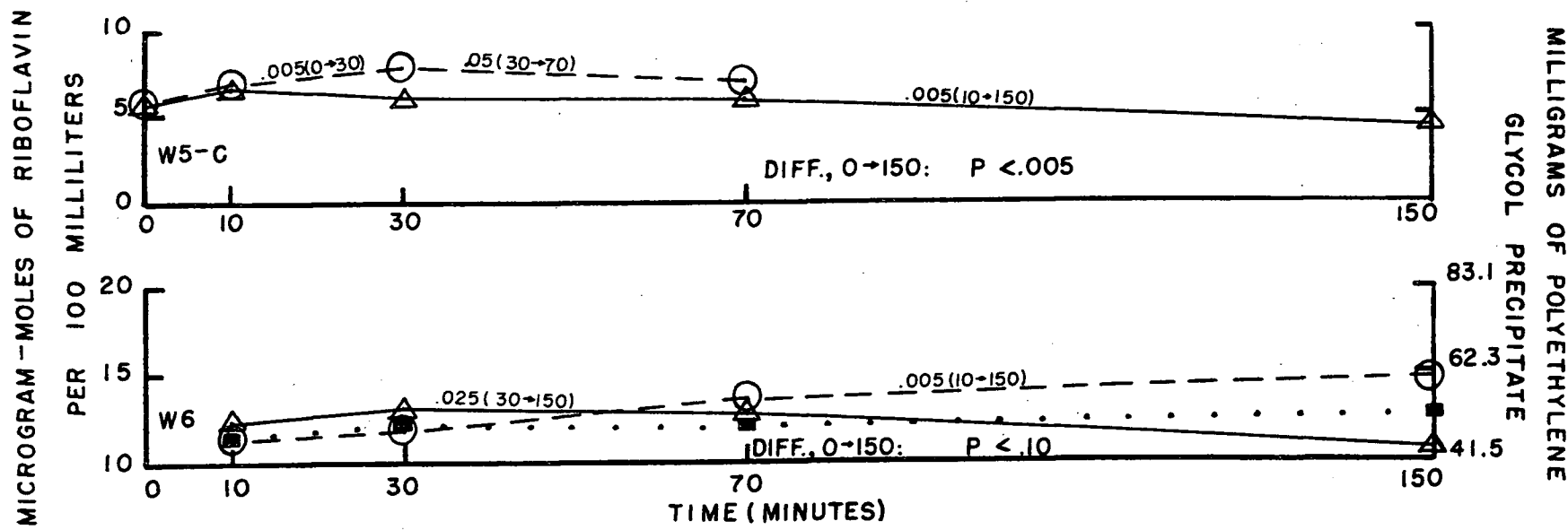
Fig. 11. (Second part)

Fig. 11. - Ruminal fistula experiments W1, W2, W4, and W6: changes in concentration of riboflavin in the ruminal solution ($\triangle$———$\triangle$) and in the ruminal solution control ($\ominus$— — —$\ominus$), plus changes in concentration of polyethylene glycol ($\blacksquare$ · · · · ·$\blacksquare$) in the ruminal solution. The symbols in the figure have the same meaning as previously described in Figures 3, 4, 5, and 7.

TABLE 15a. - Ruminal fistula experiment W1:
changes in concentration (microgram-moles per 100
milliliters) of nicotinic acid (the data recorded
are means and standard deviations, with numbers
of assays in parentheses)

Time (min.)	Experiment W1	
	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	14.6±2.4 (3)
0	12.2±2.2 (3)	12.2±2.2 (3)
40	8.3±0.5 (3)	. . . .
60	8.2±0.8 (3)	. . . .
95	6.9±0.2 (3)	. . . .
120	5.7±0.2 (2)	13.7±2.5 (3)

[a]The same as for Table 14a.

[b]The same as for Table 6.

TABLE 15b. - Ruminal fistula experiment W5 and W6: changes in concentration (microgram-moles per 100 milliliters) of <u>nicotinic acid</u> (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5 a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment			
	W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	29.9±7.2 (12)	. . . .	77.9±7.5 (12)
0	24.7±3.6 24.7±3.6 (12)	24.7±3.6 24.7±3.6 (12)	. . . .	. . . .
10	34.3±6.2 29.8±5.4 (12)	32.9±3.2 29.3±2.8 (12)	71.3±5.0 (12)	76.9±5.5 (12)
30	33.6±6.3 27.1±5.1 (10)	33.4±2.3 29.6±2.0 (12)	72.6±11.1 (12)	72.6±5.2 (12)
70	29.5±6.1 24.3±4.9 (12)	35.8±6.1 29.5±5.3 (12)	64.3±5.4 (12)	73.3±5.4 (12)
150	30.0±2.1 24.0±1.7 (12)	36.7±7.3 . . . . (12)	58.1±5.9 (12)	73.6±5.4 (12)

[a,b]The same as for Table 6.
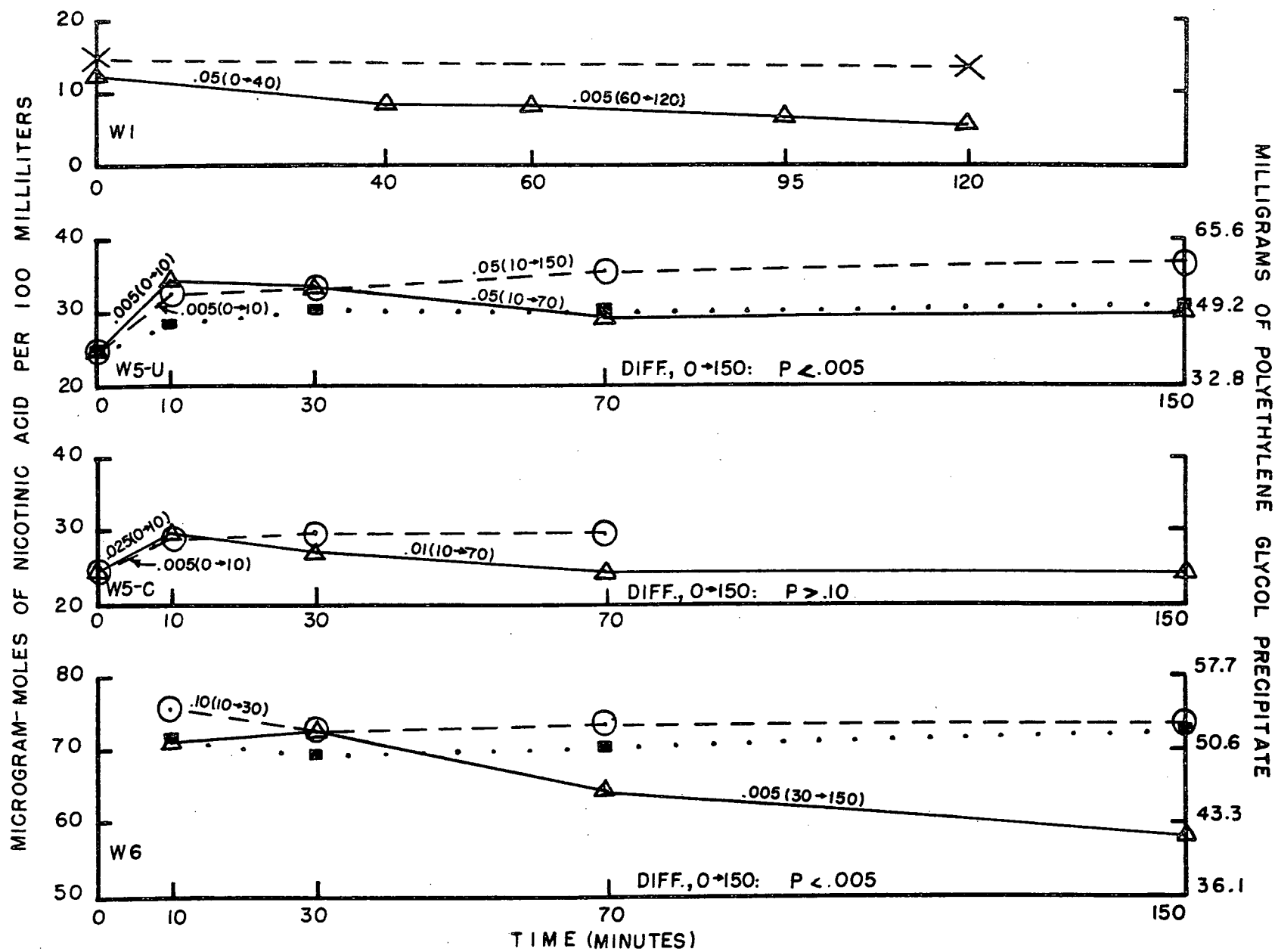
Fig. 12.

Fig. 12. - Ruminal fistula experiments W1, W5, and W6: changes in concentration of nicotinic acid in the ruminal solution (△————△) and in the ruminal solution control (⊝— — —⊙), plus changes in concentration of polyethylene glycol (■·· · ·■) in the ruminal solution. The symbols in the figure have the same meaning as previously described in Figures 3, 4, and 7.

TABLE 16a. - Ruminal fistula experiment W1:
changes in concentration (microgram-moles per 100
milliliters) of pantothenic acid (the data recorded
are means and standard deviations, with numbers of
assays in parentheses)

Time (min.)	Experiment W1	
	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	5.8±0.2 (2)
0	5.7±0.6 (3)	5.7±0.6 (3)
40	3.9±0.1 (3)	. . . .
60	3.9±0.5 (3)	. . . .
95	3.8±0.1 (3)	. . . .
120	4.1±0.2 (3)	5.7±0.4 (3)

[a]The same as for Table 14a.

[b]The same as for Table 6.

TABLE 16b. - Ruminal fistula experiments W4, W5, and W6: changes in concentration (microgram-moles per 100 milliliters) of <u>pantothenic acid</u> (the data recorded are means and standard deviations, with numbers of assays in parentheses; in experiment W5, a second set of data is given : this set results from multiplying the first set of data by polyethylene glycol correction factors)

Time (min.)	Experiment					
	W4		W5		W6	
	Ruminal solution	Control[a]	Ruminal solution	Control[a]	Ruminal solution	Control[a]
Experimental solution[b]	. . . .	17.4 ± 2.9 (12)	. . . .	7.7 ± 1.4 (12)	. . . .	16.1 ± 4.1 (12)
0	20.6 ± 7.2 (12)	20.6 ± 7.2 (12)	7.2 ± 0.8 7.2 ± 0.8 (12)	7.2 ± 0.8 7.2 ± 0.8 (12)	10.8 ± 1.3 (12)	10.8 ± 1.3 (12)
10	20.8 ± 5.6 (12)	18.3 ± 3.7 (12)	8.9 ± 0.9 7.7 ± 0.8 (12)	8.9 ± 0.9 7.9 ± 0.8 (12)	10.0 ± 2.0 (12)	16.8 ± 2.4 (9)
30	16.2 ± 4.0 (12)	16.2 ± 2.0 (12)	9.9 ± 2.4 8.0 ± 1.9 (12)	9.9 ± 1.4 8.8 ± 1.2 (12)	9.1 ± 1.3 (12)	10.6 ± 2.8 (12)
70	13.8 ± 2.3 (9)	19.7 ± 3.9 (12)	9.0 ± 1.7 7.4 ± 1.4 (12)	9.5 ± 1.2 7.8 ± 1.0 (12)	8.4 ± 0.7 (12)	11.4 ± 2.6 (12)
150	13.6 ± 3.4 (12)	18.5 ± 4.0 (12)	8.8 ± 1.6 7.0 ± 1.3 (12)	9.6 ± 1.6 . . . . (12)	. . . .	9.9 ± 2.3 (12)

[a,b]The same as for Table 6.

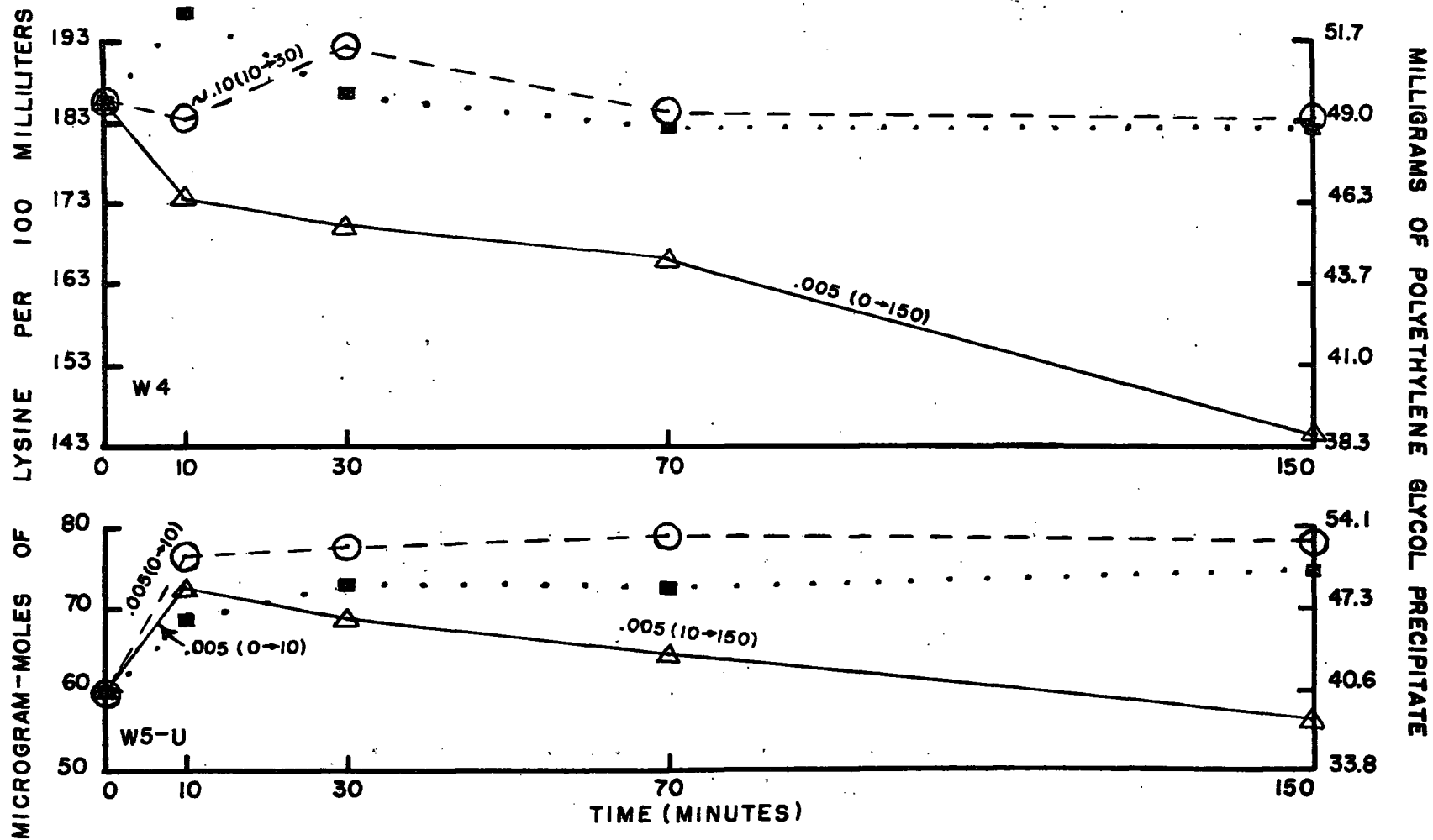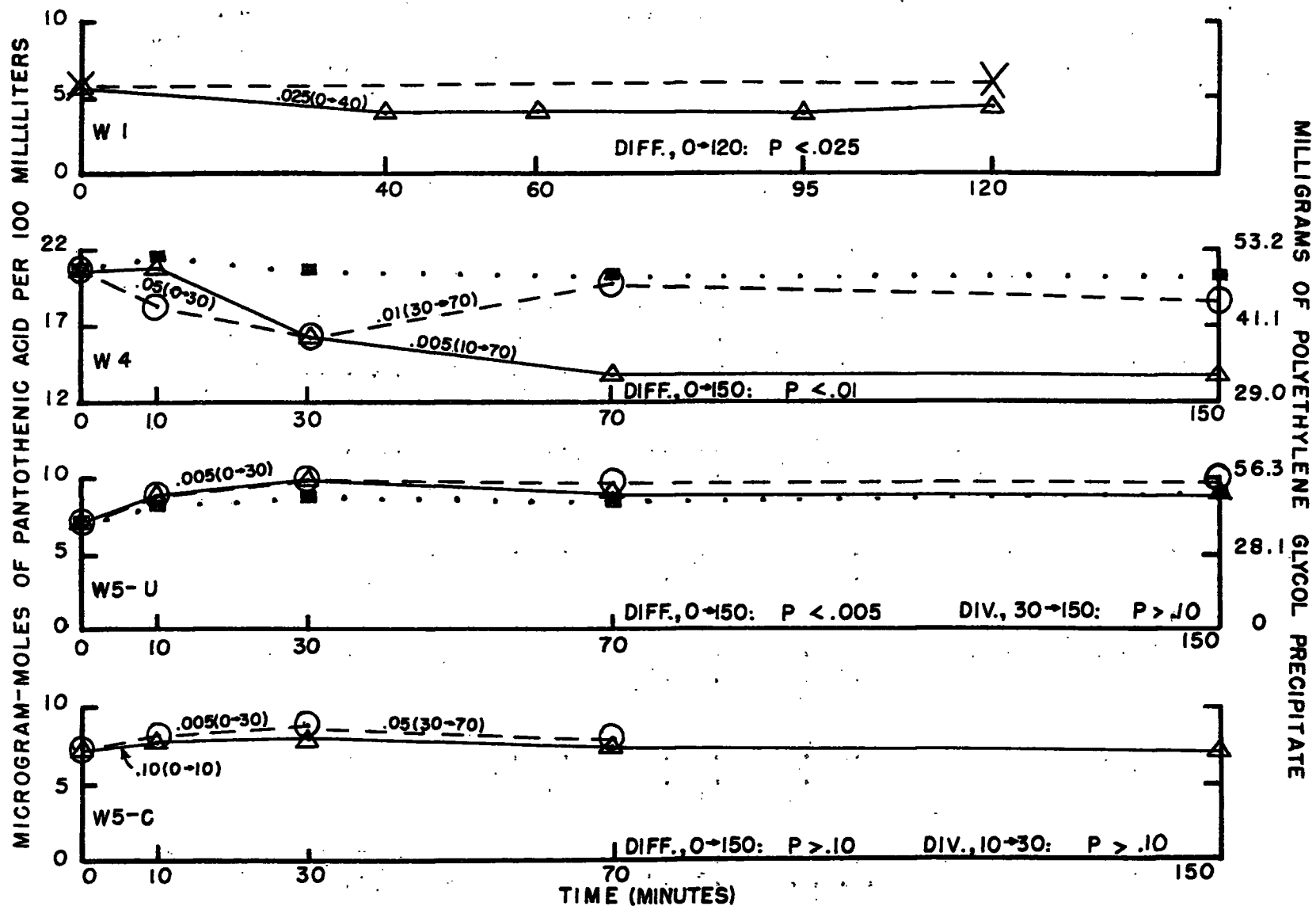Fig. 13. (First part)
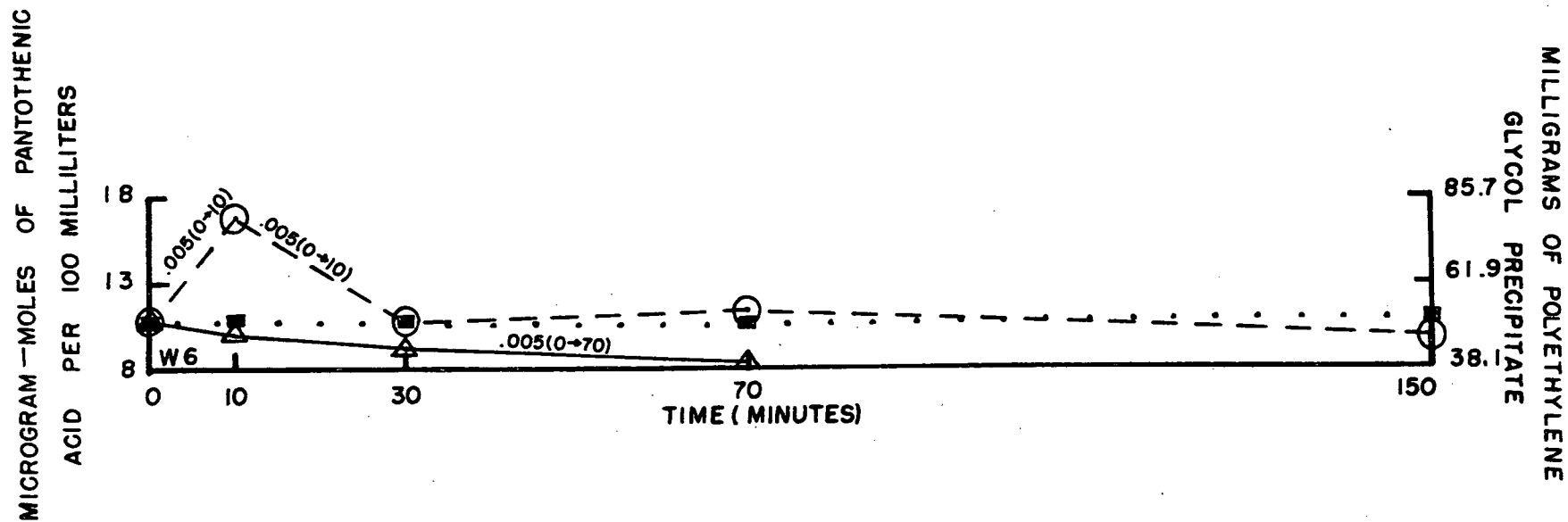
Fig. 13. (Second part)

Fig. 13. - Ruminal fistula experiments W1, W4, W5, and W6: changes in concentration of pantothenic acid in the ruminal solution (△————△) and in the ruminal solution control (O— — —O), plus changes in concentration of polyethylene glycol (■·····■) in the ruminal solution. The symbols in the figure have the same meaning as previously described in Figures 3, 4, and 7.

that water was absorbed from the rumen. Such absorption, if not accompanied by a more rapid absorption of the other constituents in the ruminal solution, would produce increased concentrations of these other constituents. The fact that the decreases in the concentrations of the B vitamins occurred in spite of the concentrating effect of the water absorption indicates that the actual decreases were probably greater than the recorded decreases. These results for the B vitamins, excluding the results for pantothenic acid in experiment W5, support the hypothesis that the B vitamins can be absorbed from the rumen.

Temperature and pH

Table 17 presents the temperature and pH measurements made for the six ruminal fistula experiments: experiments W1 to W6. Examination of the table reveals that, throughout experiments W1 and W2, the temperature of the solutions in the rumen approximated to the body temperature of the goat.[20] The examination also reveals that the pH of the solutions remained slightly acidic. The same two statements apply to experiment W3, except that the temperature of the solution was several degrees lower than the body temperature during the first part of the experiment. In experiments W4 to W6, the temperatures of the solutions in the rumen were several degrees below body temperature throughout the experiment. The pH of the solutions for these experiments did not remain acidic, but became basic (pH 7.3 to 7.6) between 30 to 60 minutes after the beginning of the experiments. The following quotation (344; see also 88, 230) is pertinent

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[20]Rectal temperatures for the goat have been recorded within the range of 38.7 to 40.7° C., with an average temperature of 39.9° C. (102).

TABLE 17. - Ruminal fistula experiments W1, W2, W3, W4, W5, and W6: temperature (°C.) and pH measurements for the ruminal solutions (the data in the upper left-hand corner of the squares are pH measurements; the data in the lower right-hand corner are temperature measurements)

Time (min.)	Experiment					
	W1	W2	W3	W4	W5	W6
Experimental solution[a]			6.5	6.5	6.5 / 35	6.5 / 32
0	6.5	6.4	6.5 / 34	6.8	6.4 / 32	6.4 / 31
10			/ 35	6.8		6.8 / 32
30		/ 39	6.6	6.9 / 34	7.1 / 34	7.0 / 34
40	6.7 / 37					
60	6.6 / 37		6.7			
65		6.6 / 39				
70			/ 37	7.0 / 35	7.4 / 35	7.3 / 35
95	6.8 / 38	6.8 / 39				
120	/ 39					
125		/ 39				
150				7.5 / 35	7.6 / 35	7.6 / 35

[a]This is the ruminal solution before being placed in the rumen.

to the pH changes that occurred in experiments W4 to W6:

> Any solution in the rumen tends to change to a pH of
> 7.2 - 7.4, whether acid or alkaline originally, and
> this is due to a differential passage of ions across
> the epithelium in both directions.

Discussion

The results of the experiments for propionic acid, the amino acids, and the B vitamins may be interpreted to support the hypothesis that these constituents can be absorbed from the rumen. There remain, however, several details that require discussion.

Stability of Amino Acids and B Vitamins During Freezing Storage

As there was a relatively long period of storage[21] before the samples of the ruminal fistula experiments were assayed, the question can be raised as to the stability of the constituents during this period of storage of the samples. In each of the experiments, before the experimental solution was added to the rumen, a sample (designated as experimental solution control) of the experimental solution was removed. Unfortunately, this control sample was not assayed at the time of its removal; rather, it was frozen and stored along with the other samples taken during the experiments. The assays of the control sample were performed at the end of the storage period, along with the assays of the other samples. There are not, therefore, any control data for the original concentrations--i.e., the concentrations at the beginning of the storage period--of the constituents in the experimental solution. The availability of such control data, together with the data of the assays at the end of the storage period, would have permitted

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[21]All the samples, including those for the experimental solution controls, were frozen and stored together. Between one and six weeks after the beginning of the storage period, the samples were thawed periodically to permit subsamples to be taken for vitamin assays. Between two and six months after the beginning of the storage period, the samples again were thawed periodically, this time to permit subsamples to be taken for amino acid assays. In addition to these periods of thawing to permit subsampling, the samples also thawed out during their transfer five hundred miles by car from the University of British Columbia to the Washington State University (see p. 22).

a determination of the stability of the amino acid and vitamin molecules during their storage at freezing temperatures. In place of the missing control data, the calculated concentrations recorded in Table 5 for the constituents of the experimental solutions can be utilized. Table 18, listing only those constituents that were assayed, presents a comparison between their calculated concentrations at the beginning of the storage period and their analytically-determined concentrations at the end of the storage period. By the use of this table, evidence may be gained as to the stability of the constituents between the time of their use in the experiments and the time of their assay.

Evidence of Instability.- Examination of Table 18 reveals that for some of the constituents--tryptophan, tyrosine, probably methionine, and riboflavin in experiments W4 to W6--the calculated concentrations do not lie within or close to the 95 per cent confidence intervals for the analytically-determined concentrations. These results may indicate poor stability of these constituents under the storage conditions to which they were exposed in the present investigation. There are, however, several other suggestions that can be made to explain these significant discrepancies between the calculated and the analytically-determined concentrations:

1. The calculated concentrations of the constituents listed in Table 18 consist of composite figures that are based on: (a) the manufacturer's estimate of the concentrations of the amino acids in the casein acid-hydrolysate solution, (b) the weighed-out amounts of those constituents that were used in addition to the casein hydrolysate in preparing the experimental solutions. The manufacturer's estimate of the amino acid concentrations did not include confidence intervals. There is a possibility that at least some of the 95 per cent confidence intervals of the calcu-

TABLE 18. - Ruminal fistula experiments W1 to W6: molecular compounds that were examined for absorption from the rumen; a comparison between their calculated[a] and analytically-determined[b] concentrations in the original experimental solutions[c]

Molecular compound	Experiment											
	W1		W2		W3		W4		W5		W6	
	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined
Amino acids[d]												
Glycine	...	...	...	...	...	...	1.60	1.89 $\pm$ 0.19	0.80	0.86 $\pm$ 0.14	1.60	2.01 $\pm$ 0.12
Lysine	...	...	...	...	...	...	1.62	1.88 $\pm$ 0.14	0.81	0.62 $\pm$ 0.09	1.62	1.78 $\pm$ 0.11
Methionine[e]	...	...	...	...	...	...	0.80	0.21 $\pm$ 0.02	0.40	0.07 $\pm$ 0.01	0.80	0.19 $\pm$ 0.02
Tryptophan	...	...	...	...	...	...	0.49	0.19 $\pm$ 0.02	0.25	0.05 $\pm$ 0.02	0.49	0.10 $\pm$ 0.04
Tyrosine	...	...	...	...	...	...	1.15	0.22 $\pm$ 0.09	0.58	...	1.15	0.18 $\pm$ 0.26

TABLE 18 — continued

Molecular compound	Experiment											
	W1		W2		W3		W4		W5		W6	
	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined	Calculated	Analytically-determined
Vitamins												
Nicotinic acid	0.16	0.15 ± 0.06	...	...	...	...	0.81	...	0.41	0.30 ± 0.05	0.81	0.78 ± 0.05
Pantothenic acid	0.04	0.06 ± 0.02	...	...	...	...	0.21	0.17 ± 0.02	0.11	0.08 ± 0.01	0.21	0.16 ± 0.03
Riboflavin	0.05	0.06 ± 0.02	0.05	0.05 ± 0.01	...	...	0.27	0.18 ± 0.04	0.14	0.08 ± 0.02	0.27	0.10 ± 0.03
Other												
Sodium propionate	...	...	...	...	52	47 ± 68	52	69 ± 14	52	72 ± 21	52	77 ± 26

[a] The calculated data are based on:

1. the manufacturer's estimate of the concentrations of the amino acids in the casein acid-hydrolysate solution;

2. the weighed-out amounts of the constituents added in preparing the experimental solutions.

[b]The data for the analytically-determined concentrations are presented as means with their 95 per cent confidence intervals designated.

[c]The ruminal solution before it was placed in the rumen.

[d]Comparative data for alpha amino nitrogen exist only in experiment W6. In this experiment, the calculated concentration of alpha amino nitrogen in the experimental solution is 42.6 milligrams per 100 milliliters (from Table 13, p. 67). Unfortunately, the experimental solution was not analyzed for its content of alpha amino nitrogen; however, the analytically-determined concentration of alpha amino nitrogen in the 0 minute sample of the ruminal solution--that is, the experimental solution after being placed in the rumen--is 37.1 ± 1.4 milligrams per 100 milliliters. This concentration is expressed as the mean with its 95 per cent confidence interval.

[e]The experimental solutions of experiments W4, W5, and W6 were not assayed for their methionine concentrations; therefore, the methionine concentrations in the 0 minute samples of the ruminal solutions for these experiments are substituted as possible approximations.

lated concentration means may have overlapped the 95 per cent confidence interval s of the analytically-determined concentration means. This suggestion can not apply to tryptophan, as supposedly the only tryptophan present in each of the experimental solutions was that which was added during the preparation of the solutions.

2. The manufacturer's casein acid-hydrolysate solutions were stored at room temperature on an open shelf for an unknown length of time. Decreases in the concentrations of some of the amino acids may have occurred during this time of holding because of molecular destruction or precipitation.

3. The effect of poor stability in the period of freezing storage may have been combined with the effects of one or both the factors in the above two suggestions.
Unfortunately, there is not enough evidence available to decide whether poor stability of some of the constituents during their storage period or one of the other factors discussed is responsible for the observed discrepancies.

Fortunately, the decision as to which of the factors it was that caused the significant discrepancies between the calculated and analytically-determined means is not critical for the previously-made proposal that the results of the present ruminal fistula experiments support the hypothesis of ruminal absorption of amino acids and B vitamins; that is, the existence of the discrepancies does not alter the fact that concentration decreases of all the assayed constituents occurred in the rumen. Knowledge of the cause of the discrepancies becomes important, however, when an attempt is made to compare the concentrations of the constituents in the ruminal solution with their concentrations in the blood (see p. 203).

If the discrepancies are in part or wholly the result of the effect of poor stability of the constituents during the storage period of the samples, then such a comparison may be meaningless--because the concentrations of the constituents in the ruminal solution samples at the end of the storage period would be less than the concentrations of the constituents in the ruminal solution at the time of the experiments. If the discrepancies are the result of the effects of the factors in the first two suggestions, then a comparison between the concentrations in the ruminal solution samples and the concentrations in the blood samples probably would be valid--because the discrepancies would have been effected before the experiment began. As a comparison will be made in the General Discussion between the concentrations of the experimental constituents in the ruminal solutions and the concentrations of the same constituents in the blood, the question of the discrepancies will be discussed again.

Evidence for Stability.- In contrast to the constituents that were discussed in the previous section, the calculated concentrations of the other constituents--glycine, lysine, nicotinic acid, pantothenic acid, sodium propionate, and riboflavin in experiments W1 and W2--usually lie within or just outside the 95 per cent confidence intervals for the analytically-determined concentration means. There also is evidence (see footnote d to Table 18) that many of the other amino acids in the experimental solution controls would exhibit, upon assay, the same close agreement between the calculated and the analytically-determined concentration means. This evidence derives from a comparison in experiment W6 of the calculated concentration of alpha amino nitrogen for the experimental solution control with the analytically-determined concentration mean of

alpha amino nitrogen for the 0 minute sample[22] from the rumen. The calcu-
lated concentration of 42.6 lies not far above the upper limit, 38.5, of
the 95 per cent confidence interval for the analytically-determined
concentration of 37.1. The relative closeness of these two concentra-
tions probably indicates that the calculated concentrations for most the
individual amino acids in the experimental solution controls would lie
close to, or within, the 95 per cent confidence intervals for the
analytically-determined concentrations. These results can be interpreted
to mean that, in addition to the apparent stability of nicotinic, panto-
thenic, and propionic acids, stability also was a property of glycine,
lysine, and most of the other amino acids.

Fluctuations of Constituents' Concentrations

Examination of the figures (Figs. 5 to 13) for the ruminal fistula
experiments reveal that concentration fluctuations occurred for the
constituents in both the ruminal solutions and the ruminal solution
controls. The concentration decreases of the constituents in the
ruminal solutions can be explained as resulting from the absorption of
the constituents through the ruminal wall. There may be, however, addition-
al factors whose effects cause concentration decreases or increases of the
constituents. Suggestions are made below as to the possible identity of
these factors.

Factors Causing Concentration Increases.- The increase of a
constituent's concentrations in the ruminal solution and in the ruminal

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[22]Unfortunately, the experimental solution control was not assayed
for alpha amino nitrogen. The analytically-determined concentration for
the 0 minute sample of the solution in the rumen is substituted, there-
fore, as an approximation.

solution and in the ruminal solution control may be the result of micro-

bial activity--fermentation, synthesis, autolysis. Although the rumen and

reticulum were rinsed with warm tap water prior to the addition of the

experimental solution to the rumen, this rinsing procedure would still

leave an "inoculum" of microorganisms and ingesta on the walls of these

compartments.[23] Upon the addition of the experimental solution to the

rumen, the microorganisms conceivably could have begun to grow and in-

crease in number. Growth and multiplication of these microorganisms,

with their attendant metabolic activities, might account for all or some

of the constituents' concentration increases in the ruminal solution and

in the ruminal solution control. A concentration increase that results

from microbial activity in the rumen might mask partly, or even completely,

a concentration decrease that is occurring because of ruminal absorption.

The present ruminal fistula experiments may present examples of such a

masking effect. Figures 7, 8, 11, 12, and 13 record, for the 150 minutes

of the experiments, statements of significance for the net decreases of

concentration (recorded as, for example, Diff., 0 —→150: P < .01) in

the rumen.[24] Some of these net decreases do not demonstrate significance

at the 10 per cent level or less. The graph for experiment W5-U in

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[23]Gross contamination apparently occurred in experiments W2, W4, and
W5. The 70 or 150 minute samples from the ruminal solutions in these
experiments exhibited either a content of brown sediment or a darker,
greener colour than did the samples of the original experimental solutions.

[24]The slope of the line joining the mean concentration of the 0
minute sample to the mean concentration of the 150 minute sample was
tested for the significance of its divergence from a zero gradient. This
significance test was made whenever any doubt existed that the concentra-
tion means for the 0 and 150 minute samples might not exhibit a difference
significant at the 10 per cent level or less.

Figure 13 demonstrates that there was even a net increase in the concentration of pantothenic acid in the rumen and that this net increase was significant at less than the 0.5 per cent level. This failure of some of the constituents in some of the ruminal solutions to exhibit, for the 150 minutes of the experiment, net decreases of concentration that were significant at the 10 per cent level or less occurred in spite of the fact that decreases of concentration that were significant at the 10 per cent level or less did occur during a part of the experiment. This apparent contradiction could be a reflection of the simultaneous occurrence of microbial activity producing concentration increases and ruminal absorption producing concentration decreases. This suggestion is supported by the fact that concentration increases occurred in many of the ruminal solution controls. The results of these experiments that do not exhibit significant net decreases of concentration still can be interpreted, then, as supporting the hypothesis of ruminal absorption of amino acids and B vitamins. It is unfortunate that size determinations of the microbial population in the samples were not made. Such determinations, if revealing a continual increase in the size of the microbial population, could provide further evidence to support the validity of the suggestion that microbial activity produced increased concentrations of the constituents in the rumen.

Factors Causing Concentration Decreases.- Two suggestions can be made to explain the decreases of constituent concentrations in the ruminal solution controls. The first suggestion is that decreases of concentration may result because of an uptake of constituents by microorganisms. The second suggestion is that decreases of concentration may result because of microbial or physical-chemical breakdown of the constituent

molecules. Moreover, decreases of concentration may be the result of the simultaneous action of both microbial uptake and molecular breakdown of the constituent molecules.

If these factors that produce decreases of concentration were operative in the ruminal solution controls, then the same factors also should have been operative in the ruminal solutions. An effectuation of a concentration decrease of a constituent in the ruminal solutions by microbial uptake or molecular breakdown would have supplemented any decrease in concentration of the same constituent effected by ruminal absorption. The value of the ruminal solution controls, then, is to provide a comparison between the concentration changes occurring in the control solutions and the concentration changes occurring in the ruminal solutions. By means of this comparison, the concentration decreases effected by ruminal absorption can be identified among the concentration decreases effected by microbial uptake or physical-chemical breakdown of the constituent molecules. In each the figures of the present experiments (Figs. 5 to 13), this identification can be made by noting a more negative slope for the lines joining the means of the ruminal solution data as contrasted to the slope of the lines joining the means of the ruminal solution control data.

Comment.-- In the previous discussion, the factors that may produce increases of constituent concentration have been considered separately from those that may produce decreases of constituent concentration. This method of presentation was adhered to in order to facilitate the analysis of the effects that might be produced by the various factors. Actually, both sets of factors may be operative simultaneously. In fact, the assumption made in the present investigation was that, with one exception, all the factors effecting increases or decreases of concentration were acting

both in the ruminal solutions and in the ruminal solution controls. The

excepted factor[25] is ruminal absorption, whose effect is confined to the

rumen. It is, however, the solutions in the rumen, not the control solu-

tions, that almost consistently exhibited the marked decreases of constit-

uent concentration. It is proposed, therefore, that the differential

decrease in the concentration of the assayed constituents in the rumen

reflects the action of ruminal absorption. Acceptance of this proposal

means that, even although the possible effects on constituent concentra-

tion by factors other than ruminal absorption be admitted, the results of

the present ruminal fistula experiments still can be interpreted as

supporting the hypothesis of ruminal absorption of amino acids and B vita-

mins.

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[25]To be accurate, also the factor of water movement acted only in
the ruminal solutions and not in the ruminal solution controls. In experi-
ments W1 and W2, there were not any corrections made for water movement.
It is known, however, that the solutions in the rumen were strongly
hypotonic to the blood (see p. 35). This means that the concentration
decreases which took place in these experiments occurred in spite of
probable water absorption, a process whose effect would be to concentrate
the constituents of the ruminal solutions. In experiments W3 to W6, the
effect of water movement was corrected for by the use of the polyethylene
glycol data.

Part II: Blood-Sampling Experiments

The second approach to the detection of ruminal absorption of amino
acids and B vitamins was to demonstrate the passage of these substances
into the blood that drains the rumen. There are two phases to this approach.

Phase A: Comparison of the Concentrations of Amino Acids and
Nicotinic Acid in Peripheral and Portal Blood

Introduction

Expected higher concentrations of amino acids and nicotinic acid in
the venous blood that drains the rumen were to be compared with expected
lower concentrations of amino acids and nicotinic acid in peripheral venous
or arterial blood. The lower concentrations of these substances in the
peripheral blood would result supposedly by a partial removal of the
absorbed amino acids and nicotinic acid from the ruminal venous blood as
it filtered through the liver. This experimental approach has been used
by several workers to demonstrate the ruminal absorption of volatile
fatty acids (e.g. 225, 230, 234) and ammonia (238).

The selection of the particular amino acids and B vitamin to be
studied and the method of their assay were determined by the same con-
siderations as given for the ruminal fistula experiments (see p. 19);
however, in contrast to the ruminal fistula experiments, the Van Slyke
manometric method for alpha amino nitrogen was used more extensively
than the microbiological assay. This was done because, although the Van
Slyke method only measures the collective concentration of amino acids,
it is more sensitive than the microbiological assay for detecting small
differences in concentration.

Method

Experimental Procedure

General.— As the procedures for the three experiments of phase A differed somewhat from one another, a general description of the experimental procedures is not given here; rather, the complete experimental procedure for each experiment will be described separately.

Experiment Bl.— In this experiment, blood samples were removed from an anesthetized goat.[26] The blood vessels that were sampled were the left jugular vein and the right ruminal vein (see Fig. 14). Appropriate surgical intervention[26] was made to enable the removal of blood samples from the right ruminal vein of the anesthetized goat.

1. Animal history: An adult female goat weighing 103 pounds and of mixed breeding was used. The goat had not been subjected to previous surgical intervention. Food but not water was withheld from the animal for the 24 hour period that preceded surgical intervention. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: An experimental solution was not added to the rumen in this experiment.

3. Sampling procedure: Ten milliliter samples of blood were withdrawn from the blood vessels. The sequence of sampling of the blood vessels is indicated in Table 19 (p. 109). Each blood vessel was sampled as soon as possible after the preceding vessel had been sampled.

Experiment B2.— In this experiment, blood samples were removed from a goat, not only while it was anesthetized, but also on several days

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[26]The details of the method of anesthezia and of the surgical procedures for all the experiments of Part II are given in the appendix.
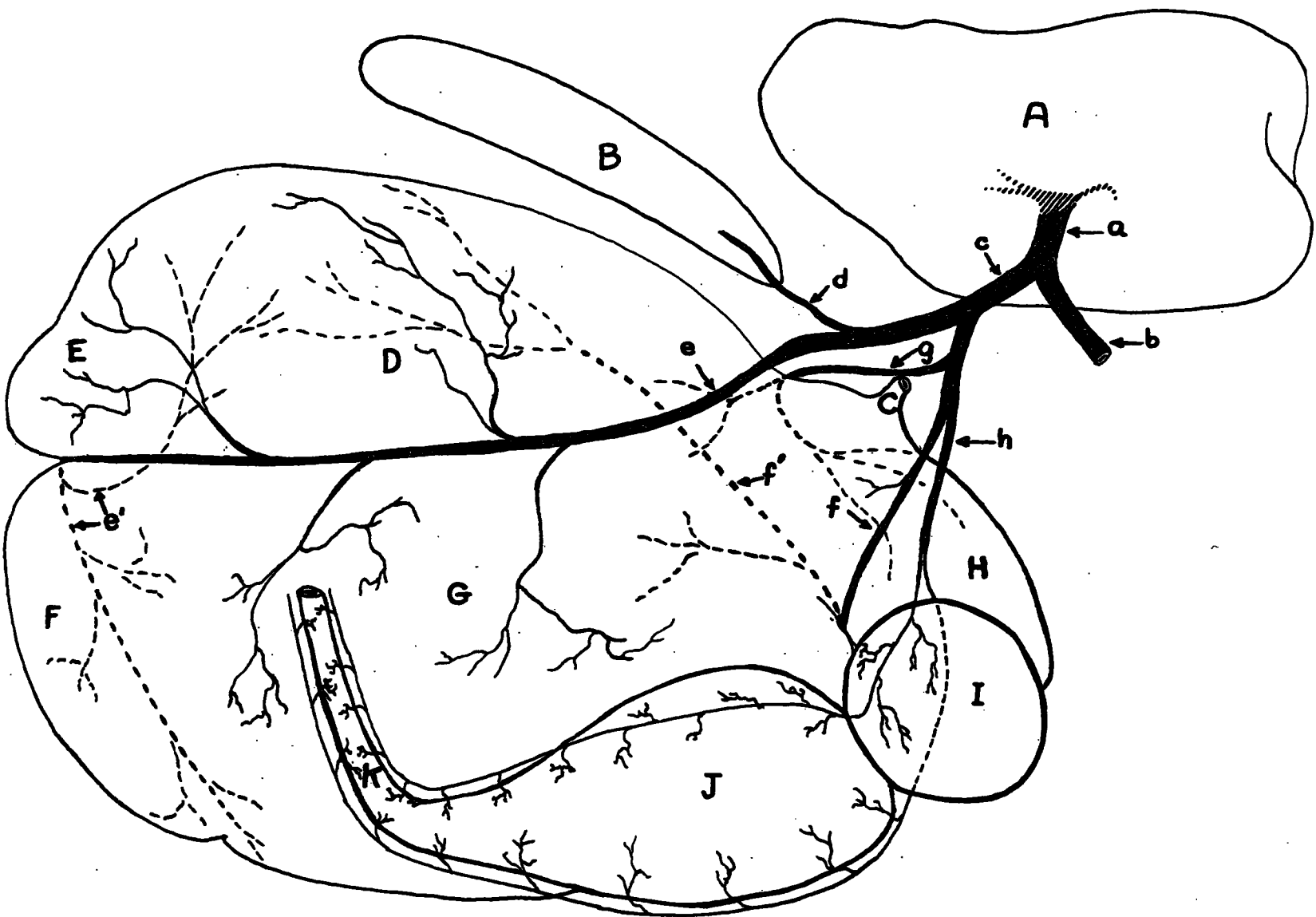
Fig. 14. - Venous drainage of the ruminant stomach, viewed from the right (adapted from figure 166 of Martin and Schauder, Lehrbuch der Anatomie der Haustiere, vol. 3, third edition, 1938). The present author traced out the venous drainage of the ruminant stomachs in several sheep and goats and found it to agree with the description presented by Martin and Schauder (see also 334).

Normally the liver is related ventrally to the reticulum, omasum, and abomasum, but in the illustration the liver has been reflected dorsocraniad. Also, the spleen has been elevated from its normal position of lying on the left face of the rumen. A, liver; B, spleen; C, oesophagus; D, dorsal sac of rumen; E, posterior dorsal blind sac; F, posterior ventral blind sac; G, ventral sac of the rumen; H, reticulum; I, omasum; J, abomasum; K, duodenum; a, portal vein; b, mesenteric vein; c, gastro-splenic vein; d, splenic vein; e, right ruminal vein; e', branches of right ruminal vein on left face of rumen; f, left ruminal vein; f', left ruminal vein on left face of rumen; g, reticular vein; h, left gastric vein.

following the first day of sampling. The blood vessels sampled were several peripheral veins and the right and left ruminal veins (see Fig. 14). To enable the removal of blood samples from the ruminal veins of the anesthetized goat to be made, appropriate surgical intervention was performed. To enable these same veins to be sampled in the non-anesthetized goat on the days that followed the day of surgical intervention, polyethylene cannulas were inserted in the veins and the free ends of the cannulas were passed through the dorsolateral part of the body wall in the lumbar region. The insertion of the polyethylene cannulas was carried out as a part of the procedure of surgical intervention (see p. 290).

1. Animal history: An adult made goat weighing 137 pounds and of mixed breeding was used. The goat had not been subjected to previous surgical intervention. Food but not water was withheld from the animal for the 24 hour period that preceded surgical intervention. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: An experimental solution as such was not used in the present experiment. Usually, however, concentrate pellets were fed to the goat prior to the sampling periods. On one occasion, casein hydrolysate powder also was fed. The details as to the quantity of feed given and the time of feeding are presented in the footnotes of Table 20 (see p. 110). The composition of the concentrate pellets is as follows:[27]

Alfalfa meal...................... 460

Whole corn...................... 640

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[27]Deer Pellets U.B.C. 15-55, 1/4 inch pellets.

Wheat bran........................ 280

Beet pulp......................... 200

Molasses.......................... 140

Soyabean meal..................... 140

Calcium carbonate................. 20

Iodized salt...................... 20

Linseed meal...................... 100

2000

3. Sampling procedure: Ten milliliters samples of blood were withdrawn from the blood vessels. The days of sampling and the sequence of sampling of the blood vessels are indicated in Table 20. Each blood vessel was sampled as soon as possible after the preceding vessel had been sampled. The time required to take each day's series of samples ranged from 15 to 30 minutes.

Experiment B3.- In this experiment, blood samples were removed periodically from an anesthetized goat after the addition to the rumen of a solution of amino acids. The solution, previously warmed to 37° C., was added to the rumen by means of a stomach tube. The addition was made just after the goat had been anesthetized. The blood vessels that were sampled were the common carotid artery and the jugular, portal, and right ruminal veins. To facilitate the removal of blood samples during the experiment, appropriate incisions were made to allow cannulation of the blood vessels (see p. 294). Upon completion of the cannulation, the incisions were closed with temporary sutures for the remainder of the experiment.

1. Animal history: A young adult male goat weighing 90 pounds and of mixed breeding was used. About one year prior to the

experiment, the goat had a section, about three inches in diameter, of the wall of the dorsal sac of the rumen removed.[29] The goat was on a diet of alfalfa hay and oats prior to the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The amino acids were supplied in the form of a 10 per cent casein acid-hydrolysate solution. The pH of the solution was adjusted to 7.0 with sodium hydroxide.

3. Sampling procedure: One hundred milliliters of the experimental solution were added to the rumen of the goat. Duplicate 10 milliliter samples of blood were removed from the blood vessels, the first sample being removed from the common carotid artery 15 minutes after the addition of the amino acids to the rumen. The sampling times for each of the blood vessels are recorded in Table 21.

Assay Procedure

Plasma was obtained from the heparinized blood samples[30] of the three experiments. The substances for whose content each of the plasma samples was assayed are indicated in the tables that record the results of each experiment (see below). Lysine, glycine, and nicotinic acid were assayed by the microbiological procedures of Barton-Wright (30). Alpha amino nitrogen was assayed by the Van Slyke manometric method (148, 391).

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[29]This section of rumen wall was removed for the purposes of another investigation that was not connected with the present investigation.

[30]The plasma samples were prepared on completion of the experiment and then frozen until their assay was begun.

Results

The results of experiments B1, B2, and B3 are presented in Tables 19, 20, and 21, respectively. Figure 15 is a graph of the results for experiment B3. Examination of the data in Tables 19 to 21[31] reveals that seldom were the concentrations of a constituent higher in the venous plasma that drained the rumen than in the peripheral plasma. In fact, the concentrations were often higher in the peripheral plasma than in the plasma that drained the rumen. These results of experiments B1 to B3 do not provide evidence, then, for ruminal absorption of amino acids or B vitamins.

Discussion

Perhaps applicable to the results of the present experiments is the explanation that was given by Fisher (117) for the apparently contradictory results that were obtained by different groups of research people in their attempts to demonstrate amino acid absorption from the small intestine. Some workers (e.g., see 119 and 393) could not demonstrate an increase of amino acid concentration in the portal blood during protein digestion in the small intestine. Other workers (118, 119, 392) readily were able to demonstrate such an increase, both during protein digestion and after the placement of a relatively large amount of an amino acid in an intestinal loop. Fisher (117; see also 392, 393)

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[31]The alpha amino nitrogen concentrations that are recorded in these tables for goat plasma approximate to the concentrations that are recorded by Annison (15) for sheep plasma (see p. 209).

TABLE 19. - Blood-sampling experiment B1: concentration (microgram-moles per 100 milliliters) of <u>lysine</u> in plasma samples taken from a goat during surgical intervention (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Blood vessel sampled[a]	Lysine
Left jugular vein	13.5±0.1 (3)
Right ruminal vein	11.6±3.0 (3)
Left jugular vein	9.1±1.1 (3)

[a]The blood vessels are listed in the order that they were sampled.

TABLE 20. - Blood-sampling experiment B2: concentration of alpha amino nitrogen, nicotinic acid, and glycine in plasma samples taken from a goat during and on days subsequent to the day of surgical intervention (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Blood vessel sampled[a]	Alpha amino nitrogen (milligrams per 100 milliliters)		Nicotinic acid (microgram-moles per 100 milliliters)		Glycine (microgram-moles per 100 milliliters)		
	b	c	d	e	e	f	g
Left jugular vein	3.73 (1)	4.18 (1)	0.69±0.03 (3)	0.36±0.01 (3)	63.9±19.3 (3)	73.7±26.9 (3)	. . . .
Left jugular vein	. . . .	4.30±0.45 (2)	. . . .	. . . .	. . . .	. . . .	. . . .
Left lateral saphenous vein	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	46.2±17.3 (3)
Right ruminal vein	3.66 (1)	3.74±0.48 (2)	0.58±0.03 (3)	. . . .	. . . .	. . . .	. . . .
Right ruminal vein	3.59 (1)	4.29±0.28 (2)	. . . .	. . . .	. . . .	. . . .	. . . .
Left ruminal vein	. . . .	. . . .	0.58±0.04 (3)	0.34±0.00 (3)	72.3±8.9 (3)	64.9±19.3 (3)	43.6±17.2 (3)
Left ruminal vein	. . . .	. . . .	0.63±0.07 (3)	0.37±0.01 (3)	63.0±17.7 (3)	61.3±13.9 (3)	. . . .
Left jugular vein	4.57 (1)	3.79±0.69 (2)	0.72±0.09 (3)	0.39±0.03 (3)	75.5±18.4 (3)	. . . .	. . . .

TABLE 20 - continued

Blood vessel sampled[a]	Alpha amino nitrogen (milligrams per 100 milliliters)		Nicotinic acid (microgram-moles per 100 milliliters)		Glycine (microgram-moles per 100 milliliters)		
	b	c	d	e	e	f	g
Left jugular vein	. . . .	3.63 (1)	. . . .	. . . .	. . . .	. . . .	. . . .
Left lateral saphenous vein	. . . .	. . . .	. . . .	. . . .	. . . .	74.2±16.9 (3)	. . . .
Left cephalic vein	. . . .	. . . .	. . . .	. . . .	. . . .	. . . .	61.7±15.5 (3)

[a]The blood vessels are listed in the order that they were sampled.

[b]The blood samples were taken the first day after the day of surgery. The animal was fed 200 grams of concentrate pellets 15 minutes prior to the taking of the first sample.

[c]The blood samples were taken the second day after the day of surgery. The animal was fed 200 grams of concentrate pellets 15 minutes prior to the taking of the first sample.

[d]The blood samples were taken during surgery. The animal had all food but not water withheld for 28 hours prior to surgery.

[e]The blood samples were taken the third day after the day of surgery. The animal had been fed 200 grams of concentrate pellets 15 minutes prior to the taking of the first sample.

TABLE 20 - <u>continued</u>

[f]The blood samples were taken the sixth day after the day of surgery. The animal was fed 200 grams of concentrate pellets 15 minutes prior to the taking of the first sample.

[g]The blood samples were taken the seventh day after the day of surgery. The animal was fed 240 grams of concentrate pellets and 30 grams of casein hydrolysate powder 10 minutes prior to the taking of the first sample.

TABLE 21. - Blood-sampling experiment B3: concentration (milligrams per 100 milliliters) of <u>alpha</u> <u>amino</u> <u>nitrogen</u> in plasma samples taken from a goat after the addition at 0 minute time of an amino acid solution[a] to the ingesta-filled rumen (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Right common carotid artery		Right jugular vein		Right ruminal vein		Portal vein	
Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)
15	3.40±0.29 (4)	19	3.67±0.12 (3)	22	3.40±0.08 (4)	25	3.50±0.14 (4)
34	3.50±0.27 (3)	42	3.40±0.14 (4)	....	. . . .	....	. . . .
59	3.78±0.17 (4)	62	3.55±0.25 (4)	50	3.43±0.32 (3)	55	3.70±0.14 (4)

[a]One hundred milliliters of a 10 per cent casein acid-hydrolysate solution were added.
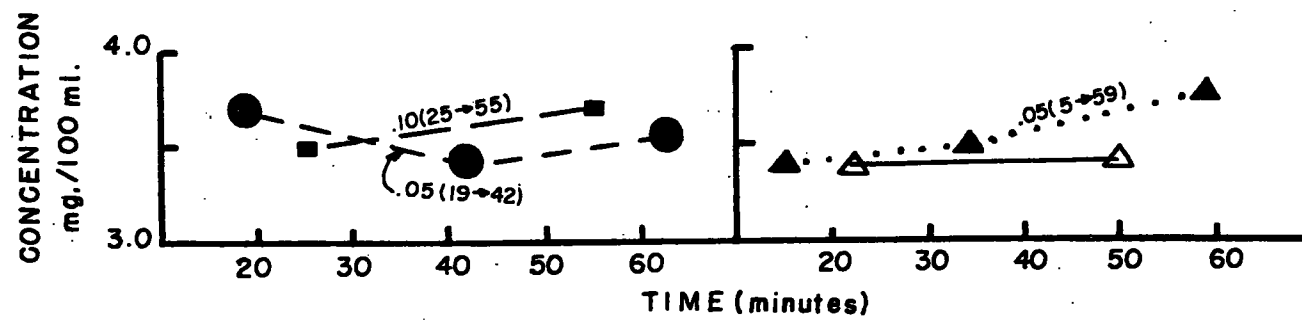
Fig. 15.

Fig. 15. - Blood-sampling experiment B3: changes in concentration of alpha amino nitrogen in goat plasma samples taken from the jugular vein (●— — —●), common carotid artery (▲·····▲), portal vein (■— — —■), and right ruminal vein (△————△). The plasma samples were taken after the addition (0 minute time) of an amino acid solution to the ingesta-filled rumen of the goat

comments on this situation as follows:[32]

> If there is good reason to suppose that a substance of a
> particular class is being added to the blood at a particular
> time, and if the concentration in the blood of this class of
> substance rises at this time, the rise may be interpreted as
> a consequence of the known process of addition. But the ab-
> sence of a detectable rise in such circumstances could not be
> taken as meaning that there was absence of addition [underlining
> mine]. When King and Rapport (1933) administered as much as
> 5 g. of tyrosine to a dog by intravenous injection, little or
> none of the tyrosine could be found in the blood five minutes
> later, in any of the forms of amino-nitrogen, phenol, non-
> protein-nitrogen or urea. In order to obtain this amount of
> tyrosine from a protein meal it would be necessary to absorb
> the products of digestion of 100-240 g. of protein.
> A change in concentration in the blood merely signalizes
> a change in relation between the totality of processes adding
> the substance to the blood and the totality of processes re-
> moving it.

Apparently, under certain feeding conditions, the quantity of amino acids

being absorbed per unit time from the small intestine is quite small

relative to the large volume of blood that carries the amino acids away

per unit time (also see 91, 397). An actual increase in the concentra-

tion of the amino acids in the portal blood may be too small to be de-

tected by the classical assay methods. The unsuccessful attempt in

experiments B1 to B3 to demonstrate increased concentrations of amino

acids and nicotinic acid in the blood draining the rumen might be a

reflection of the same type of relationship that was found in the work

with the small intestine; that is, ruminal absorption of amino acids and

nicotinic acid actually may have occurred, but the quantities absorbed

may have been so small, relative to the volume of blood passing through

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[32]Cummins (85) has expressed the same concept more briefly:

> Studies of systemic blood concentrations of in-
> gested materials reflect utilization, rate of
> storage, excretion, and circulatory competency as
> well as absorption.

the ruminal veins, that increased concentrations were not detected by the assay methods that were employed.[33]

Re-examination of Tables 19 to 21 leads to the suspicion that, although differences between the concentrations of the amino acids and nicotinic acid in the peripheral blood and their concentrations in the portal blood may not have been demonstrated, there may have been slight increases in their concentrations with time. Accordingly, the data of experiment B3 (the only one of these three experiments for which a record of the sampling times was kept) were analyzed statistically for the significances of the concentration increases with time. The method of statistical analysis already has been explained (see p. 36). Figure 15 (see p. 114), a graph of the data for experiment B3, records the results of the statistical analysis. Except for the plasma of the jugular vein, the plasmas of the different blood vessels exhibit a slight increase with time in the concentration of alpha amino nitrogen. The increase is significant at the 5 per cent level for the common carotid artery, non-significant at the 10 per cent level for the ruminal vein, and significant at the 10 per cent level for the portal vein. These results, indicating slight support for ruminal absorption of amino acids, led the author to attempt to demonstrate larger, time-related increases in the blood concentration of alpha amino nitrogen. This attempt yielded the experiments of phase B.

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[33]A group of French workers (322) have suggested a similar explanation for their failure to demonstrate directly B vitamin absorption from various parts of the alimentary tract of sheep. The method that was employed by these workers was to compare the blood concentrations of the individual B vitamins in the appropriate branches of the portal system with the blood concentrations in the jugular vein. They were unable to demonstrate significantly higher concentrations of the B vitamins in the blood of the portal system.

Phase B: Increase in the Concentrations of Amino
Acids in Peripheral and Portal Blood
with Time

Introduction

In this phase of the second part of the experimentation, time-related
increases in the blood concentrations of amino acids were demonstrated
after their addition to the rumen of a goat.[34] These increases were
contrasted with the blood concentrations that were recorded before the
addition of the amino acids. A similar experimental approach has been
used by many workers to demonstrate the intestinal absorption of amino
acids.

The selection of the method of assay for this phase of the experi-
mentation was based upon considerations of sensitivity and upon availability
of equipment and facilities. Especially applicable to the experiments of
this phase is a method of assay that utilizes amino acids tagged with
radioactive carbon. The primary reason for not utilizing such a method of
assay was the lack of equipment and facilities for handling radioactive
isotopes. As an alternative method of assay, the Van Slyke mano-
metric method for alpha amino nitrogen was selected. Although this
method measures only the collective concentration of amino acids, it
was chosen because of its sensitivity in the detection of small changes of
total amino acid concentration in the blood. It readily is acknowledged,
however, that the use of a method of assay that utilized isotope-tagged
amino acids (and B vitamins), either in addition to or in place of the
methods used, would have facilitated the experimentation, not only in

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[34]This procedure was not attempted with the B vitamins.

119.

this part, but in all parts of the present investigation.

<p align="center">Method</p>

Experimental Procedure

General.- In this series of experiments, the blood samples were removed periodically from anaesthetized goats, both before and after the addition of a solution of amino acids to the empty rumen. Appropriate incisions (see p. 294) were made to enable the contents of the rumen and reticulum to be removed. These two compartments were rinsed in the same manner as for the ruminal fistula experiments (see p. 19). After placing a tight-fitting cotton plug in the reticulo-omasal orifice, a ligature was tied around the reticulo-omasal sphincter, care being taken not to obstruct the large blood vessels that occur in the area of the sphincter. This ligating procedure was performed in order to isolate the rumen and the reticulum from the remainder of the digestive tract.[35] The addition of the experimental solution, previously warmed to 37°C., was made to the rumen [36] through the incised opening in its wall. After this addition, the opening to the rumen was sutured closed. The blood vessels that were sampled are indicated in the tables and figures that record the results for each of the experiments (see Tables 22 to 27 and Figures 16 to 21). To facilitate the removal of blood samples during the experiment, appropriate incisions were made in the body wall of the goat in order to allow

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[35]In experiment B4, additional ligatures were tied around the pyloris of the abomasum and around the cervical part of the oesophagus.

[36]As, in this series of experiments, each goat lay on its right side during the experiment, it was not possible to ensure confinement of the experimental solution to the rumen. It is possible, therefore, that a small amount of the experimental solution may have occupied the reticulum during the experiment.

cannulation of the blood vessels (see p. 294). Upon completion of the cannulation, the incisions were closed with temporary[37] sutures for the remainder of the experiment.

Experiment B4.-

1. Animal history: A young adult female goat weighing 67 pounds and of mixed breeding was used. About one year prior to the experiment, the goat had a section, about four inches in diameter, of the wall of the dorsal sac of the rumen removed.[38] Food but not water was withheld from the goat for the 72 hour period that preceded the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The amino acids were supplied in the form of a solution whose composition was 40 milliliters of casein acid-hydrolysate solution (10 per cent); 1.63 grams of sodium orthophosphate, dihydrogen (NaH_2PO_4); 2.5 grams of polyethylene glycol[39]; enough sodium hydroxide to adjust the pH of the solution to 6.5; and enough water to make a final volume of one liter.

3. Sampling procedure: One liter of the experimental solution was placed in the rumen of the goat. Five milliliter samples of blood were withdrawn from the blood vessels. The times of sampling are indicated in Table 22 (see p. 125). The volume of the experimental solution left in the rumen at the end of the experiment was not measured.

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[37]As the goats were euthanized with an overdose of sodium pentobarbital at the end of the experiments, only temporary sutures were used.

[38]See footnote 29 on page 107.

[39]This substance for checking on water movement was added to the experimental solutions because, originally, it was intended that the concentration changes of the constituents in the experimental solutions be measured.

Experiment B5.-

1. Animal history: A young adult male goat weighing 60 pounds and of Saanen breeding was used. The goat had not been subjected to previous surgical intervention. Food but not water was withheld from the goat for the 48 hour period that preceded the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The composition of the solution was the same as the composition of the solution for experiment B4.

3. Sampling procedure: One liter of the experimental solution was placed in the rumen of the goat. Duplicate 10 milliliter samples of blood were withdrawn from the blood vessels. The times of sampling are indicated in Table 23 (see p. 128). The volume of the experimental solution recovered from the rumen at the end of the experiment was 940 milliliters.

Experiment B6.-

1. Animal history: A young adult male goat weighing 90 pounds and of mixed breeding was used. About 18 months prior to the experiment, the spermatic cords were crushed to cause a reduction in the odour of the animal. Food but not water was withheld from the goat for the 96 hour period that preceded the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The amino acids were supplied in the form of a solution whose composition was 500 milliliters of casein acid-hydrolysate solution (10 per cent); 1.8 grams of sodium orthophosphate, dihydrogen (NaH_2PO_4); 2.5 grams of polyethylene glycol; enough sodium hydroxide to adjust the pH of the solution to 6.7;

and enough water to make a final volume of one liter.

3. Sampling procedure: One liter of the experimental solution was placed in the rumen of the goat. Duplicate 10 milliliter samples of blood were withdrawn from the blood vessels. The times of sampling are indicated in Table 24 (see p. 131). The volume of the experimental solution recovered from the rumen at the end of the experiment was 800 milliliters.

Experiment B7.-

1. Animal history: A young adult female goat weighing 66 pounds and of mixed breeding was used. The goat had not been subjected to previous surgical intervention. Food but not water was withheld from the goat for the 96 hour period that preceded the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The composition of the solution was the same as the composition of the solution for experiment B6, except that polyethylene glycol was omitted.

3. Sampling procedure: One liter of the experimental solution was placed in the rumen of the goat. Duplicate 10 milliliter samples of blood were withdrawn from the gastrosplenic vein (see Fig. 14, p. 103). The times of sampling are indicated in Table 25 (see p. 134). The volume of the experimental solution recoverable from the rumen at the end of the experiment was not measured.

Experiment B8.-

1. Animal history: A young adult female goat of mixed breeding was used. Through negligence, the weight of this goat was not recorded. The goat had not been subjected to previous surgical intervention. Food but not water was withheld from the goat for the 96 hour period that

preceded the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The composition of the solution was the same as the composition of the solution for experiment B6, except that polyethylene glycol was omitted.

3. Sampling procedure: The description for experiment B7 also applied here. The sampling times for the gastrosplenic vein are indicated in Table 26 (see p. 137).

Experiment B9.—

1. Animal history: A young adult male goat weighing 62 pounds and of mixed breeding was used. The goat had not been subjected to previous surgical intervention. Food but not water was withheld from the goat for the 96 hour period that preceded the experiment. At the time of the experiment, the animal appeared to be healthy.

2. Composition of the experimental solution: The composition of the solution was the same as the composition of the solution for experiment B6.

3. Sampling procedure: Nine hundred milliliters of the experimental solution were placed in the rumen of the goat. Duplicate 10 milliliter samples were withdrawn from the blood vessels. The times of sampling are indicated in Table 27 (see p. 140). The volume of the experimental solution recovered at the end of the experiment was 825 milliliters.

Assay Procedure

The heparinized blood samples were assayed for alpha amino nitrogen by the Van Slyke manometric method (148, 391). The assays were begun

within an hour of the completion of the experiments.

Results

The results for experiments B4 to B9 are recorded in Tables 22 to 27[40] and Figures 16 to 21. The data were analyzed statistically for the significances of the concentration increases. The method of statistical analysis already has been explained (see p. 36). The results of this analysis are recorded as a part of Figures 16 to 21. Increases in the concentration of alpha amino nitrogen, significant at the 10 per cent level or less, occur in most of the blood vessels.[41] The increases of concentration are large in experiments B6 to B9. In these experiments,

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[40]The alpha amino nitrogen concentrations that are recorded in these tables for the whole blood of the goats at zero or pre-zero sampling times (5.2 to 7.8 milligrams per 100 milliliters) fall within the range of concentrations that are recorded in Spector's Handbook of Biological Data (358) for the whole blood of sheep (4.6 to 8.0 milligrams per 100 milliliters). Although the goats in these experiments were fasted for 72 or 96 hours prior to the experiment, the fact that their blood concentrations fall within the range of concentrations for non-starved sheep is in agreement with the results of White et al (406). These authors found no decrease in blood amino acid nitrogen for sheep that had been subjected to decreasing levels of feed intake followed by starvation. It also is of interest to note here the effect of anesthesia on the concentration of amino acids in the blood. Folin and Lyman (120) found no change in the concentration of non-protein nitrogen in the blood of cats that had been anesthetized for as long as three hours with ether. Peters and Van Slyke (290) cite work which indicates that the blood amino acids fall slightly during anesthesia with pentobarbital or ether. Since in the present experiments anesthesia was induced with pentobarbital and maintained with ether, it is unlikely that the observed increases in the concentration of alpha amino nitrogen in the present experiments were the result of the anesthesia.

[41]It is to be noted that these increases in concentration of alpha amino nitrogen occurred in spite of the fact that the goats had been starved for 72 to 96 hours (48 hours in experiment B5) prior to the experiments. Although no measurements were made, it is probable that these animals were in the state of negative nitrogen balance at the time of the experiments (11, 131, 366). In such a state it might be expected that the tissues would take up more rapidly from the blood the amino

TABLE 22. - Blood-sampling experiment B4:
changes in concentration (milligrams per 100
milliliters) of alpha amino nitrogen in blood
samples taken from a goat before and after the
addition (0 minute time) of an amino acid solu-
tion[a] to the empty rumen (the data recorded are
means and standard deviations, with numbers of
assays in parentheses)

Time (min.)	Right jugular vein (mg.)	Right common carotid artery (mg.)
−142	6.13 ± 0.55 (4)	. . . .
−132	5.85 ± 0.15 (4)	. . . .
− 3	. . . .	5.95 ± 1.15 (4)
+ 30	. . . .	5.68 ± 0.73 (4)
+ 51	. . . .	7.60 ± 1.27 (4)

[a]The estimated concentration of alpha amino
nitrogen in the solution was 37 milligrams per
100 milliliters.
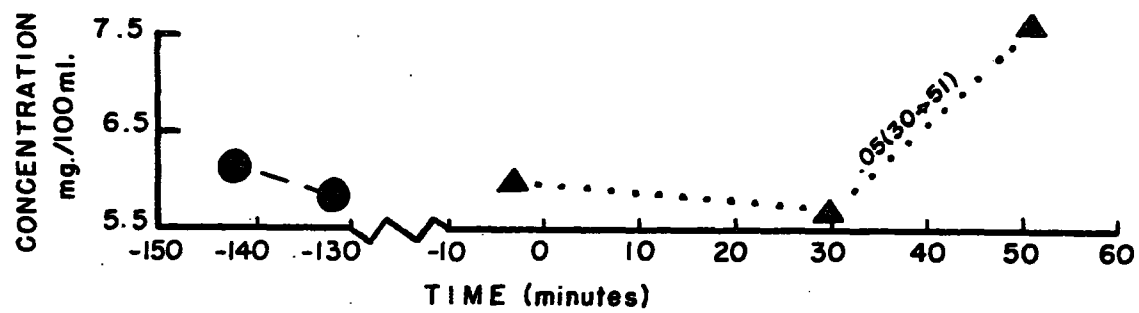
Fig. 16.

Fig. 16. - Blood-sampling experiment B4: changes in concentration of alpha amino nitrogen in goat blood samples taken from the jugular vein (● — — —●) and common carotid artery (▲ - - - - ▲). The blood samples were taken before and after the addition (0 minute time) of an amino acid solution (estimated concentration of alpha amino nitrogen: 37 milligrams per 100 milliliters) to the empty rumen.

TABLE 23. - Blood-sampling experiment B5: changes in concentration (milligrams per 100 milliliters) of alpha amino nitrogen in blood samples taken from a goat before and after the addition (0 minute time) of an amino acid solution[a] to the empty rumen (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Right jugular vein		Right common carotid artery		Portal vein	
Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)
−145	5.15±0.64 (4)	. . .	. . . .	. . .	. . . .
. . .	. . . .	− 3	5.73±0.51 (4)	− 6	5.23±0.89 (4)
. . .	. . . .	+ 37	6.08±0.52 (4)	+ 32	6.03±0.27 (4)
. . .	. . . .	+ 64	5.63±0.62 (4)	+ 60	5.98±0.31 (4)
. . .	. . . .	+ 97	6.53±0.40 (4)	+ 92	6.18±0.68 (4)

[a]The estimated concentration of alpha amino nitrogen in the solution was 37 milligrams per 100 milliliters.
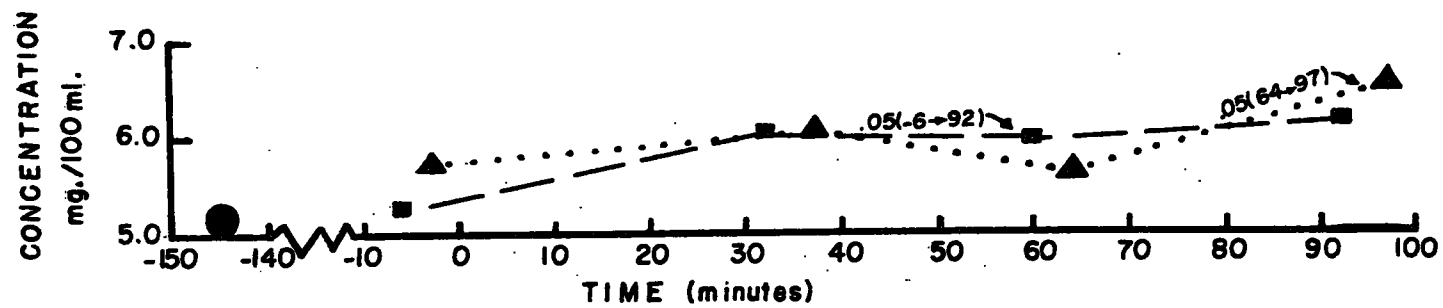
Fig. 17.

Fig. 17. - Blood-sampling experiment B5: changes in concentration of alpha amino nitrogen in goat blood samples taken from the jugular vein (●   ), common carotid artery (▲· · · · ·▲), and portal vein (■—— —— ——■). The blood samples were taken before and after the addition (0 minute time) of an amino acid solution (estimated concentration of alpha amino nitrogen: 37 milligrams per 100 milliliters) to the empty rumen.

TABLE 24. - Blood-sampling experiment B6: changes in concentration (milligrams per 100 milliliters) of alpha amino nitrogen in blood samples taken from a goat before and after the addition (0 minute time) of an amino acid solution[a] to the empty rumen (the data recorded are means and standard deviations, with numbers of assays in parentheses)

Right jugular vein		Right common carotid artery		Gastrosplenic vein	
Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)
− 133	5.48±0.61 (4)	. . .	. . . .	. . .	. . . .
. . .	. . . .	− 106	5.63±0.81 (4)	. . .	. . . .
. . .	. . . .	− 52	5.28±0.95 (4)	− 55	5.65±0.79 (4)
. . .	. . . .	− 3	5.90±0.72 (4)	− 6	6.90±0.54 (4)
. . .	. . . .	+ 10	6.68±0.61 (4)	+ 7	7.33±0.56 (4)
. . .	. . . .	+ 34	7.38±0.86 (4)	+ 31	9.50±0.22 (3)
. . .	. . . .	+ 63	8.45±0.78 (4)	+ 61	9.70±0.71 (2)
. . .	. . . .	+ 92	8.50±0.53 (4)	+ 90	11.00±0.86 (4)
. . .	. . . .	+ 124	10.43±0.63 (3)	+121	11.45±0.66 (4)

[a]The estimated concentration of alpha amino nitrogen in the solution was 462 milligrams per 100 milliliters.
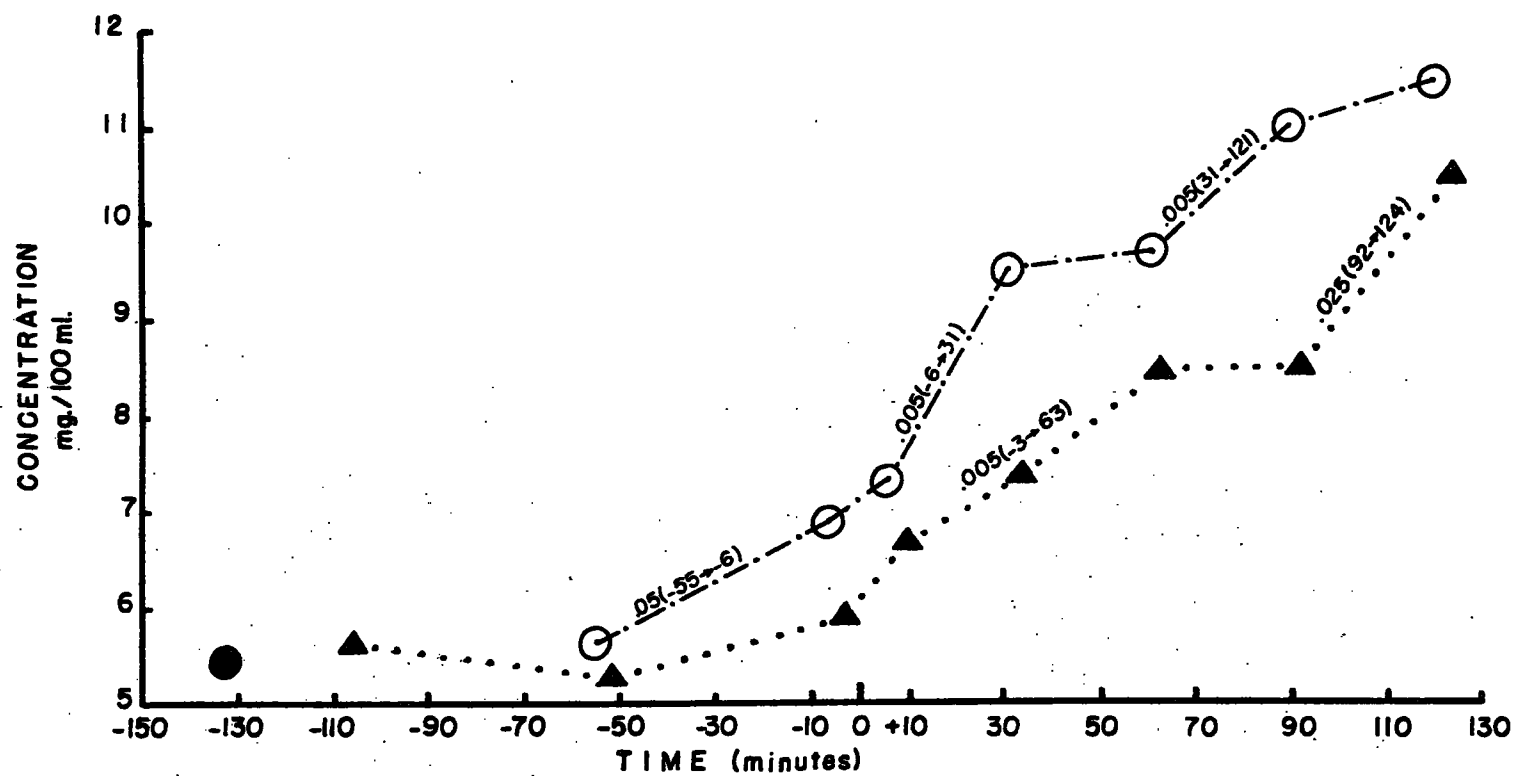
Fig. 18.

Fig. 18. - Blood-sampling experiment B6: changes in concentration of alpha amino nitrogen in goat blood samples taken from the jugular vein (●), common carotid artery (▲ · - · · - ·▲), and gastrosplenic vein (⊖ · - — · — ·⊖). The blood samples were taken before and after the addition (0 minute time) of an amino acid solution (estimated concentration of alpha amino nitrogen: 462 milligrams per 100 milliliters) to the empty rumen.

TABLE 25. - Blood-sampling experiment B7:
changes in concentration (milligrams per 100
milliliters) of alpha amino nitrogen in blood
samples taken from a goat before and after the
addition (0 minute time) of an amino acid solu-
tion[a] to the empty rumen (the data recorded are
means and standard deviations, with numbers of
assays in parentheses)

<div align="center">Gastrosplenic vein</div>

Time (min.)	Nitrogen (mg.)
− 4	6.98±0.93 (4)
+ 2	6.73±1.18 (4)
+ 31	8.20±0.87 (4)
+ 63	8.40±0.80 (4)
+ 93	9.05±0.64 (4)
+122	13.53±0.25 (4)

[a]The estimated concentration of alpha
amino nitrogen in the solution was 462 milli-
grams per 100 milliliters.
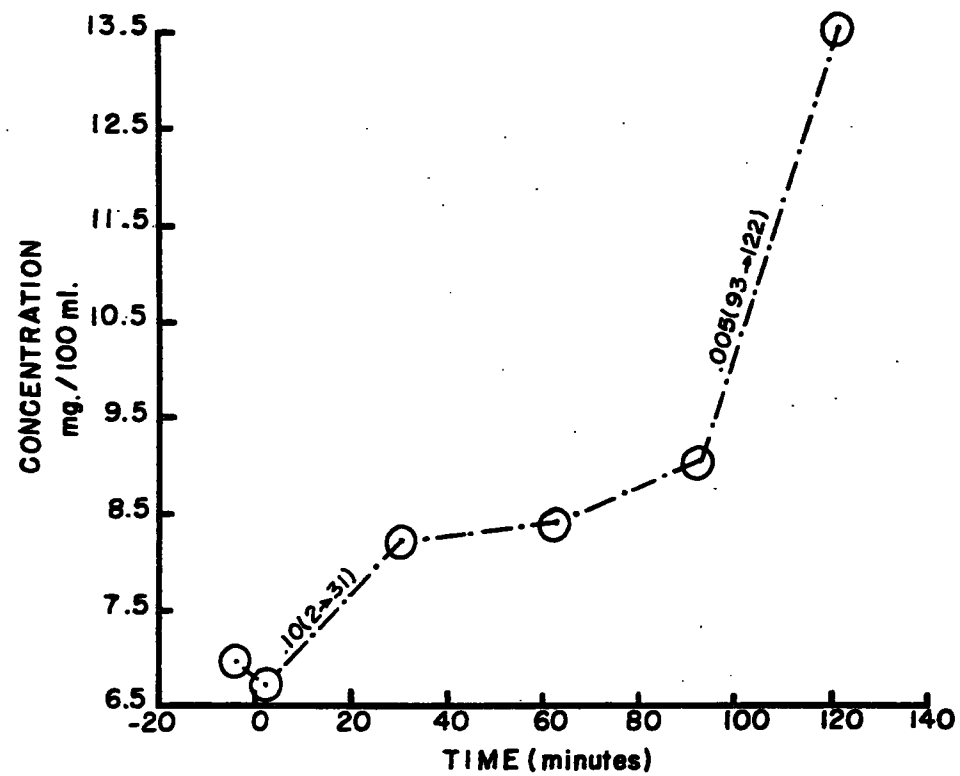
Fig. 19.

Fig. 19. - Blood-sampling experiment B7: changes in concentration of alpha amino nitrogen in goat blood samples taken from the gastrosplenic vein. The blood samples were taken before and after the addition (0 minute time) of an amino acid solution (estimated concentration of alpha amino nitrogen: 462 milligrams per 100 milliliters) to the empty rumen.

TABLE 26. - Blood-sampling experiment B8:
changes in concentration (milligrams per 100
milliliters) of <u>alpha</u> <u>amino</u> <u>nitrogen</u> in blood
samples taken from a goat before and after the
addition (0 minute time) of an amino acid
solution[a] to the empty rumen (the data record-
ed are means and standard deviations, with
numbers of assays in parentheses)

Gastrosplenic vein

Time (min.)	Nitrogen (mg.)
− 4	6.03±0.83 (4)
+ 1	8.50±0.46 (4)
+ 38	7.73±0.75 (4)
+ 61	7.58±0.51 (4)
+ 90	8.48±0.47 (4)
+120	7.50±0.80 (4)

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[a]The estimated concentration of alpha
amino nitrogen in the solution was 462 milli-
grams per 100 milliliters.
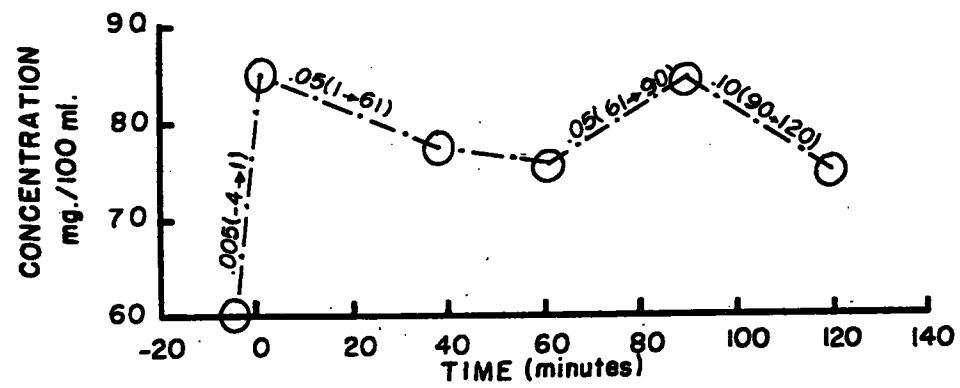
Fig. 20.

Fig. 20. - Blood-sampling experiment B8: changes in concentration of alpha amino nitrogen in goat blood samples taken from the gastrosplenic vein. The blood samples were taken before and after the addition (0 minute time) of an amino acid solution (estimated concentration of alpha amino nitrogen: 462 milligrams per 100 milliliters) to the empty rumen.

TABLE 27. - Blood-sampling experiment B9: changes in concentration (milligrams per 100 milliliters) of alpha amino nitrogen in blood samples taken from a goat before and after the addition (0 minute time) of an amino acid solution to the empty rumen (the data record-ed are means and standard deviations, with numbers of assays in parentheses)

Right common carotid artery		Gastrosplenic vein		Common mesen-teric vein	
Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)	Time (min.)	Nitrogen (mg.)
−96	6.38±0.47 (4)	. . .	. . . .	. . .	. . . .
−81	7.20±0.96 (4)	. . .	. . . .	. . .	. . . .
−47	6.50±0.74 (4)	. . .	. . . .	. . .	. . . .
. . .	. . . .	−29	5.50±0.83 (4)	. . .	. . . .
. . .	. . . .	. . .	. . . .	−11	6.53±0.82 (4)
− 5	6.93±1.18 (4)	− 3	7.80±1.62 (4)	− 0.5	6.53±0.22 (4)
+19	7.00±1.25 (4)	+16	9.68±0.52 (4)	. . .	. . . .
+33	6.78±1.43 (4)	+30	9.13±0.64 (4)	. . .	. . . .
+53	8.33±0.75 (4)	+51	11.43±0.83 (4)	+48	9.08±0.67 (4)
+68	7.73±0.47 (4)	+63	10.73±0.17 (4)	+66	10.10±0.52 (4)
+96	8.88±1.42 (4)	+90	10.63±0.44 (4)	+93	9.83±0.79 (4)

[a]The estimated concentration of alpha amino nitrogen in the solutions was 462 milligrams per 100 milliliters.
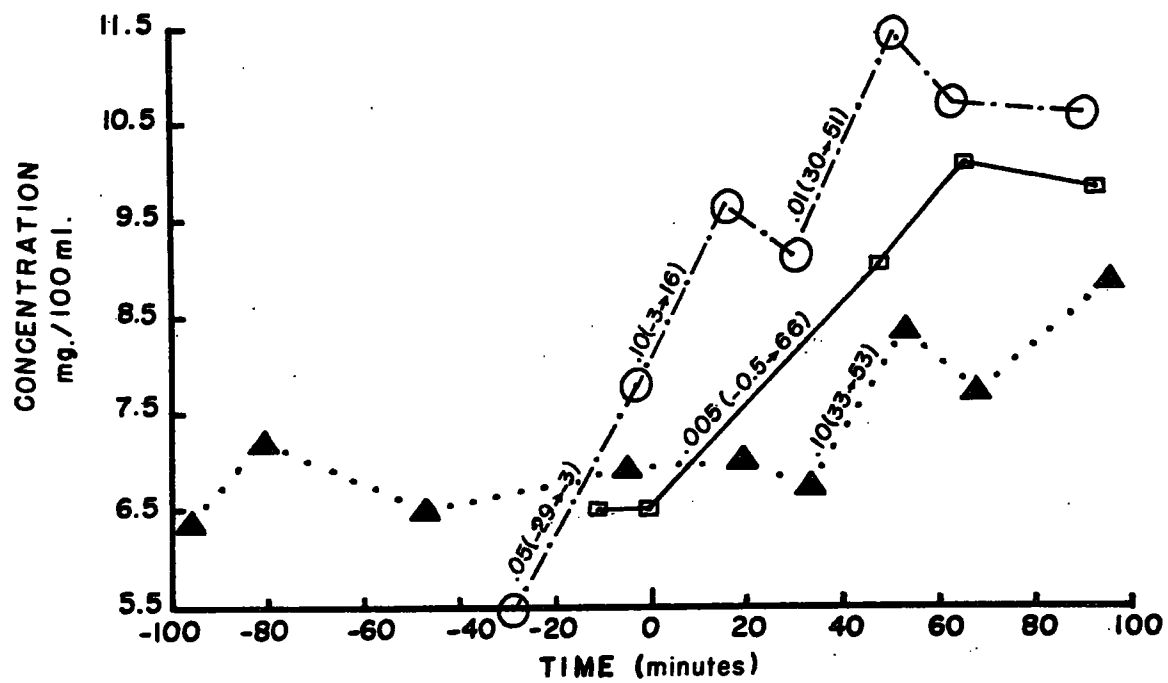
Fig. 21.

Fig. 21. - Blood-sampling experiment B9: changes in concentration of alpha amino nitrogen in goat blood samples taken from the common carotid artery (▲ · · · · ·▲), gastrosplenic vein (⊖·· — ·· —⊖), and mesenteric vein (⊟————⊟). The blood samples were taken before and after the addition (O minute time) of an amino acid solution (estimated concentration of alpha amino nitrogen: 464 milligrams per 100 milliliters) to the empty rumen.

the concentrations of the amino acids in the experimental solutions were 12.5 times the concentrations of the amino acids in the solutions for experiments B4 and B5. The heights attained by the concentrations of alpha amino nitrogen in the blood of the different vessels that were sampled in experiments B6 to B9, especially when contrasted to the pre-zero time concentrations in the blood of these vessels, appear to support the hypothesis of ruminal absorption of amino acids.

Discussion

Although the results of experiments B4 to B9 provide evidence for the ruminal absorption of amino acids, two topics remain that require discussion.

Fluctuations in the Concentrations of Amino Acids in the Blood

One striking feature of the post-zero time data of this series of experiments is that, even although there may have been a general increase in the blood concentrations of alpha amino nitrogen, there were still strong fluctuations in these blood concentrations. There also were some fluctuations in the pre-zero time concentrations; and, in experiments B6 and B9, the blood of the gastrosplenic vein even exhibited concentration fluctuations that were significant at the 5 per cent level. These fluctuations may represent merely sampling error; the fluctuations are,

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acids that were absorbed from the alimentary tract (11, 389). It is suggested, therefore, that the increases in the blood concentrations of alpha amino nitrogen that did occur in these experiments may have been more dramatic if the goats had been in positive nitrogen balance (see also the quotation of Fisher on p. 116 and footnote 32 on the same page, both of which point out that the rate of uptake of a constituent from the blood is one of several factors which determine the constituent's concentration in the blood.

however, similar to fluctuations of amino acid concentrations observed by Hartmann et al (153). These workers carried out experiments on the intestinal absorption of individual amino acids from a mixture of amino acids that were placed in intestinal loops of 16 dogs. Detection of amino acid absorption was made by noting the increase in the concentration of individual amino acids in the mesenteric blood draining the loops of intestine. By plotting the "mean value curves" (Mittelwertskurven) of the concentration data for each of the amino acids investigated, Hartmann and co-workers observed periodical maxima and minima in the curves. For some of the amino acids, the minimal concentrations were often almost as low as the initial concentrations. In experiments of about two hours duration, they observed three Resorptionsmaxima: the first maxima occurred at about 10 minutes from the beginning of the experiment, the second between 30 and 35 minutes, and the third between 50 and 60 minutes. The authors of the paper reporting on this investigation were unable to state the cause of this periodicity of maxima and minima in the "mean value curves". They did suggest, however, that the rhythm of the amino acid concentrations in the mesenteric venous blood may reflect a periodicity in the activity of an intestinal wall mechanism that is concerned in the absorption of amino acids from the intestinal lumen. The same type of suggestion can be made to explain the post-zero time fluctuations of alpha amino nitrogen concentrations in the present blood-sampling experiments for the goat.[42].

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[42]Scarisbrick and Ewer (330) found the amount of absorption of inorganic phosphate from the rumen of sheep to be quite variable, "varying considerably from time to time." These results may be a reflection of the same phenomenon that is described by Hartmann et al (see above in text) for the small intestine.

Comparison between the Results of Annison and Those of the Present Investigation

In contrast to the blood-sampling experiments of Annison (see p. 14), the results of the blood-sampling experiments in the present investigation support the hypothesis that amino acids are absorbed from the rumen. A comparison of some of the conditions in the two sets of investigations might help to explain the difference in the results obtained. Table 28, a record of some of the conditions in the two investigations, has been constructed in order to facilitate such a comparison.

One important point that is not brought out by the information in Table 28 is the criterion that was used in each experiment to judge whether or not ruminal absorption of amino acids had occurred. The criterion used by Annison was based upon a comparison between the concentration of alpha amino nitrogen in the blood of the portal vein and the concentration of the same substance in the blood of the jugular vein or common carotid artery. A concentration of alpha amino nitrogen in the blood of the portal vein that was higher than the concentration in the blood of the jugular vein or common carotid artery would be considered evidence of ruminal absorption of amino acids.[43] This criterion of Annison also was used for experiments B1 to B3 of the present blood-sampling experiments. In experiments B4 to B9, another criterion was adopted as the basis for detecting ruminal absorption of amino acids. Measurements were made, both before and after the addition of amino acids to the rumen, of the concentration of alpha amino nitrogen in the blood of a vein or artery. The ruminal absorption of amino acids was

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[43]See p.101 for the deduction of this statement.

TABLE 28. - Comparison between the conditions and results for the blood-sampling experiments of Annison (15) and the conditions and results for the blood-sampling experiments of the present investigation

Experiment[a]	Feeding prior to experiment	Ruminal contents	Initial concentration of casein hydrolysate[b] (gm. per liter)	Blood vessels sampled	Absorption of amino acids from the rumen
(1)	(2)	(3)	(4)	(5)	(6)
1-Annison	?	present	($\sim$33)	portal vein	none demonstrated
2-Annison	?	present	($\sim$23)	portal vein	none demonstrated
B 3	oats given 30 min. prior	present	($\sim$ 3)	portal and right ruminal veins	none demonstrated
B 4	72 hr. fast	removed	4	common carotid artery	questionable
B 5	48 hr. fast	removed	4	portal vein	questionable
B 6	96 hr. fast	removed	50	gastro-splenic vein	demonstrated
B 7	96 hr. fast	removed	50	gastro-splenic vein	demonstrated

TABLE 28 - continued

Experiment[a] (1)	Feeding prior to experiment (2)	Ruminal contents (3)	Initial concentration of casein hydrolysate[b] (gm. per liter) (4)	Blood vessels sampled (5)	Absorption of amino acids from the rumen (6)
B 8	96 hr. fast	removed	50	gastro-splenic vein	demonstrated
B 9	96 hr. fast	removed	50	gastro-splenic vein	demonstrated

[a]Sheep were used for the experiments of Annison; goats were used for the experiments of the present investigation.

[b]The data that are recorded in parentheses for the first three experiments listed in the table are only approximations. In these experiments, the casein hydrolysates were added to ruminal ingestas of unmeasured volumes. From the data that are presented in Ellenberger-Baum's Vergleichenden Anatomie der Haustiere (420) for the capacity of the sheep and goat rumens, volumes of 3 liters were assumed for the ruminal ingestas. The assumption at least should allow the correct order of magnitude of the casein hydrolysate concentrations to be calculated.

considered to have been demonstrated if, after the addition of amino acids to the rumen, a concentration increase occurred which was significant at the 10 per cent level or less and which was maintained. It should be noted that the design of the second experiment of Annison (see p. 14), in which he sampled the portal vein at intervals after the addition of amino acids to the rumen, allows the criterion applied to experiments B4 to B9 also to be applied to his data. By this criterion, Annison's results are still negative for the demonstration of ruminal absorption of amino acids. Finally, in experiments B6 and B9, the use of a more concentrated experimental solution in the rumen apparently has provided, even by the criterion of Annison, positive results—a higher concentration of alpha amino nitrogen in the blood that was draining the rumen via the gastrosplenic vein than in the peripheral blood of the common carotid artery (see Figs. 18 and 21).

Examination of the experimental conditions recorded in Table 28, together with a consideration of the two criteria just discussed, can lead to the conclusion that the demonstration in the present investigation of ruminal absorption of amino acids depended on all or some of the following conditions:

1. The removal of the ruminal contents prior to the addition of the amino acids to the rumen—this procedure would reduce or eliminate competition for the amino acids by the microbial population.

2. The use in experiments B6 to B9 of higher initial concentrations of amino acids in the rumen—this would produce a higher, downward concentration gradient in the direction from the ruminal solution in the rumen to the blood in the ruminal veins; such an increased gradient would accelerate the rate of diffusion of amino acids across the wall of the

rumen and into the blood.

3. The choice of the criterion to be used for the detection of ruminal absorption of amino acids: an increase in the concentration of alpha amino nitrogen in the blood of an artery or vein after the addition of amino acids to the rumen--this criterion is probably a more sensitive test than the criterion of Annison.

4. The use of the gastrosplenic vein rather than any other blood vessel for the application of the criterion just cited--this use of the gastrosplenic vein[44] enabled samples to be taken of the blood that was draining from the ruminant stomach before this blood had mixed with the blood that was draining from the intestines and other organs; this avoidance of a large dilution effect provided a more sensitive test for the detection of any increase in the concentration of alpha amino nitrogen in the blood that was draining from the rumen.

All four of the above conditions, conditions that stand in contrast to those of the experiments of Annison, were a part of the experimental design in experiments B6 to B9. These are the experiments that provide strong evidence for the ruminal absorption of amino acids. It is suggested that the difference between the design of the experiments of Annison's investigation and the design of the experiments of the present investigation accounts for the difference between the results of the two investigations.

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[44]Most of the blood in the gastrosplenic vein comes from the ruminant stomach, with a small amount coming from the spleen (see Fig. 14, p.103).

GENERAL DISCUSSION

Just as chemistry could not have developed without test
tubes to hold reacting substances, so organisms could not
have evolved without relatively impermeable membranes to
surround the cell constituents. This barrier between the
inside and the outside, the inner and external world of each
living unit, has been and always must be considered one of
the fundamental structures of a cell. No one can fail to be
impressed with the great difference in properties of living
and dead cells. The dead are completely permeable to dif-
fusible substances, while the living retain one material and
pass another. This difference, selective permeability
[underlining mine] , is so marked that it becomes the surest
test to distinguish the living from the dead, holding where
all other methods fail. It can truly be said of living cells,
that by their membranes ye shall know them.

E. Newton Harvey in Permeability of Natural Membranes by
Davson and Danielli (90).

If we can assume, on the basis of the results of the present investi-

gation, that at least some of the amino acids and B vitamins can pass

across the epithelial lining of the rumen, then several questions on this

phenomenon naturally arise.

1) What is the manner of this passage of amino acids and B vitamins

across the ruminal epithelium?

2) Does this passage of amino acids and B vitamins across the

ruminal epithelium occur under ordinary feeding conditions?

3) How does this passage of amino acids and B vitamins across the

ruminal epithelium fit into the scheme pictured for the metabolism and

final fate of these compounds in the ruminal contents?

An attempt will be made to answer each of these questions in turn. To

simplify the presentation, however, the discussions for the amino acids

and B vitamins will be presented separately. To conclude the General

Discussion, the passage of amino acids and B vitamins across the ruminal

epithelium will be discussed from the point of view of the nutritional

implications.

Part A: Amino Acids

Manner of Absorption of Amino Acids from the Rumen

In recent times, considerable work has been carried out on the
passage of amino acids across the epithelial linings of the small intestine
and kidney tubule. From such studies, theories have been proposed on
the manner of absorption of amino acids. It is not unreasonable to assume
that the passage of amino acids across the epithelial lining of the rumen
may occur in the same manner as the passage of amino acids across the
epithelial lining of the small intestine or kidney tubule. A review
will be made, therefore, of the literature dealing with the manner of
absorption of amino acids in these other structures.

Literature Review on the Manner of Absorption of Amino Acids

Small Intestine.— For many years, it was undecided as to whether
the absorption of amino acids from the small intestine was by means of
simple diffusion[45] or by some other process. The investigations of
Höber and Höber in 1936 and 1937 (165, 166) provided strong support
that more than simple diffusion was concerned in the absorption of amino
acids. Previously, it had been shown that the diffusion coefficients of
molecules tend to vary inversely as the molecular volumes (164). The

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[45]The term "simple diffusion" is used here to designate that net
transfer of molecules from one site to another that results because of
a difference in the chemical potential of the molecules in the two sites.
Some authors write of a difference in the concentrations of the molecules
in the two sites and this is adequate if the other components (e.g.,
temperature) that help to make up the chemical potential are equal in
the two sites.

results of Höber and Höber, however, demonstrated that amino acids were absorbed from the small intestine of the rat far more rapidly than many other compounds of comparable molecular volume. This behaviour of amino acids in the small intestine was the more remarkable in that earlier work had shown amino acids to possess abnormally-low permeation rates for diffusion through certain natural and artificial porous-type membranes. The following quotation (164) describes the results of this earlier work and contrasts them with the results of work on the small intestine.

> It is conspicuous from old observations of Overton concerning the osmotic properties of frog muscles, that amino-acids, like glycine or alanine, enter with great slowness, if at all, although, because of their smaller molecular size, one could expect them to exceed, for instance, erythritol. Still more unexpected is their inertia in penetrating the cell surface of the sulfur alga Beggiatoa, which is remarkable for its outstanding behaviour as a molecular sieve and in which the permeation rates of several amino-acids have been found to be abnormally low. The reason for this slowness is not of a physiological nature, since the slowness is evident also in diffusion experiments with collodion membranes, and can be explained as being due to the ampholyte character of the amino-acids, which, probably due to the formation of a shell of water dipoles around the ampholyte ions, brings about an enlargement of the molecular volume. Consequently, a porous membrane, such as the intestinal wall, would be expected to be passed comparatively slowly also. But the contrary is true. In comparison with acid amides, with erythritol, with xylose, the amino-acids actually pass the intestinal wall much faster than was anticipated from their diffusion rates.

The results of Höber and Höber also did not agree with the results to be expected if the rate of amino acid absorption by the rumen is in accordance with Fick's law of diffusion. Höber (164) has stated this law in terms that are relative to the absorption of substances from the small intestine:[46]

According to Fick's law, when a substance is administered in a

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[46]This statement can be derived from the following equation which

loop experiment at different concentrations, the absolute
amount entering by passive penetrations [that is, by
diffusion] would bear a linear relationship [sic.,
direct proportionality] to the concentration, and the
relative amount would be constant, • • • •

Such substances as polyhydric alcohols, aliphatic acid amides and certain

"non-physiological" sugars—e.g., mannose, xylose and arabinose—are

absorbed from the small intestine in accordance with Fick's law (164,

166). In contrast, the amino acids do not disappear from the lumen of the

small intestine in accordance with Fick's law. For initial amino acid

concentrations ranging between 6.5 and 71 millimoles per milliliter of

solution in the intestinal lumen, Höber and Höber (164, 166) found that,

with increasing initial concentrations, the absolute rate of disappearance

of the amino acids did not increase linearly but remained constant and the

relative rate did not remain constant but decreased. Significantly,

these results of Höber and Höber for the amino acids are the same type

of results that have been obtained for the "physiological sugars"—glu-

cose, galactose, and fructose—, sugars whose absorption is known to

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states Fick's law (162):

$$dn = -Da\left(\frac{dc}{dx}\right)_t \cdot dt,$$

where dn is the amount of substance which passes in time dt across an area
a, dc/dx is the concentration gradient, and where the constant of
proportionality, D, is named the diffusion coefficient. It is to be
noted, however, that, in the statement of Höber, the concentration of the
substance in the intestinal loop apparently is assumed to approximate to
the concentration difference (dc) or concentration gradient (dc/dx) that
existed across the intestinal wall. Such as assumption is valid when the
concentration of a substance in the lumen of the intestine is very much
larger than the concentration of the substance in the plasma. As the range
of concentration of the amino acids added by Höber and Höber (166) to the
intestinal loops was between 5.5 to 71 milligram-moles per milliliter and
as the magnitude of the concentration of amino acids in the plasma is of
the order of 10^{-5} to 10^{-3} milligram-moles per milliliter, the intestinal
concentrations of the amino acids in these loop experiments closely
approximated to the concentration differences or gradients that existed
across the wall of the small intestine.

involve more than simple diffusion (164). Höber and Höber (166) con-
cluded that the absorption of amino acids from the small intestine like-
wise involves more than simple diffusion: "Their absorption is not like
a diffusion, but is a process complicated by the presence of an acceler-
ating factor, the effect of which becomes more visible with lower than
with higher concentrations." Stated again, the conclusion of these
workers is that the intestinal absorption of amino acids is the result of
the effects of both an accelerating factor and the process of simple
diffusion, the latter being of more importance at higher concentrations
(see also the modern work of Fridhandler and Quastel (123)).

In more recent times evidence has been accumulating as to the possible
nature of the accelerating factor that was described by Höber and Höber
as being concerned in the absorption of amino acids. Several groups of
workers (2, 71, 133, 207, 232, 270, 338, 414, 415) have demonstrated a
stereochemical specificity on the part of this factor in the absorption
of amino acids from the small intestine. The L-isomer, as compared to
the D-isomer, disappears from the intestinal lumen more rapidly and
appears in greater concentration in the blood vessels draining the small
intestine. Although not necessarily so, this stereochemical specificity
on the part of the accelerating factor suggests that one or more enzymes
are involved in its action. The L-isomers of the neutral amino acids[47],
in contrast to the D-isomers that have been examined, even can be absorbed

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[47]The neutral amino acids are the mono-amino, mono-carboxylic amino
acids such as glycine and phenylalanine, in contrast to the dicarboxylic
amino acids such as aspartic acid and glutamic acid or the diamino amino
acids such as lysine and arginine.

from the small intestine against a concentration gradient (2, 414, 415,
416). This indicates that the accelerating factor is one which can
utilize metabolic energy to perform work. When the absorption experi-
ments have been conducted using solutions containing equimolar amounts
of the L-isomers of several of the neutral amino acids, the individual
amino acids have demonstrated marked differences among themselves in
their rates of disappearance from the lumen of the small intestine (4,
153, 190, 276, 277, 303, 416). The rank order of these different rates
of disappearance is not in accordance with the molecular volumes or
concentration gradients of the amino acids[48]; rather, the rank order for
the rates of transfer of the individual neutral amino acids from a mixture
of neutral amino acids generally is the reverse of the rank order found
when the rates of transfer are measured for solutions containing single
amino acids[49]. This reversal of rank order for the rates of absorption
of the neutral amino acids from a mixture of amino acids is believed to
be a reflection of competitive inhibition among these acids for a common
cellular mechanism that brings about their absorption (416, see also 190).
The preferential transfer of L-isomers also has been demonstrated to be
inhibited by potassium cyanide (2, 3), oxygen lack (123, 273, 413), cell
cooling (231, 232), 2:4 dinitrophenol (2, 3, 123, 340), but not by

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[48]Only by the use of a very high concentration of a particular amino
acid were Hartmann et al (153) able to demonstrate any consistency in the
effect of concentration gradient or molecular volume on the rate of
transfer of the amino acid from a mixture of amino acids in the small
intestine.

[49]The results of Wiseman (416) provide an example for illustration.
He found that the rapid transfer against a concentration gradient of either
glycine, L-proline, or L-histidine was inhibited completely in the presence
of the more slowly absorbed L-methionine.

phlorhizin (123, 222; see also 166).[50] Since, except for 2:4 dinitro-
phenol[51] and phlorhizin, all these compounds or conditions are known to
inhibit cellular respiratory (aerobic) metabolism, it appears that this
is further evidence that the action of the accelerating factor has a
requirement for metabolic energy;also, that the requirement is relatively
large, since the energy yield of a non-respiratory (anaerobic) metabolism
apparently is too small to meet this requirement. Further, since 2:4
dinitrophenol inhibits the utilization of the energy of the respiratory
oxidative reactions for the generation of the high-energy phosphate bonds
of adenosine triphosphate (219), the inhibition of the preferential
transfer of the L-isomers of certain amino acids by 2:4 dinitrophenol

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[50]Since phlorhizin has been demonstrated to exert an inhibiting
action on glucose transfer (122, 166, 222), this non-inhibiting action of
phlorhizin on amino acid absorption has led several authors to state that
the mechanism for the preferential absorption of amino acids is not the
same as that for glucose. Singer et al (342) have obtained results,
however, that the authors suggest may be indirect evidence of competition
between amino acids and glucose for the use of a phosphorylation mechanism
present in the intestine wall. They found that when gelatin was fed with
glucose the blood amino acid and blood glucose curves were depressed, as
compared to the curves when gelatin or glucose was fed alone. On the other
hand, Agar et al (2) found no evidence for the existence of any competition
between glucose and amino acids during their selective absorptions from
the small intestine. Furthermore, Fridhandler and Quastel (123) found
that only relatively high concentrations of glucose would inhibit the rate
of transfer of L-alanine from the small intestine. Since similar high
concentrations of sorbose or sodium chloride also had a similar retarding
effect on the rate of transfer of L-alanine, these authors suggest that
the inhibition of the absorption of L-alanine by glucose merely is due to
an osmotic effect and not due to their competing for a common mechanism
of transfer. Yet these same authors have found the presence of 0.01 M
deoxypyridoxine to inhibit the absorption of D-L alanine, glucose, and
fructose from the small intestine. They suggest that deoxypyridoxine, a
pyridoxine analogue which can compete with pyridoxine for sites of meta-
bolic action, exerts its action by the "inhibition of a mechanism con-
trolling active absorption in general."

[51]Dinitrophenol does not inhibit respiration except in high concen-
trations (219).

indicates that adenosine triphosphate (ATP) is concerned directly or indirectly in the absorption of these amino acids.[52]   Just how ATP is concerned in the absorption process remains, however, unknown. Tri-antaphyllopoulos and Tuba (380) have made one suggestion. It has been demonstrated that a soluble cell fraction from rat liver contains enzymes which, in the presence of ATP, catalyze a carboxyl activation of specific amino acids (163). The compound that is formed is suggested as being an amino acid∿adenosine monophosphate (AMP) complex, wherein extra bond energy is retained in the linkage between the carboxyl group of the amino acid and the phosphate group of the AMP. Triantaphyllopoulos and Tuba suggest that this same carboxyl activation may occur in the preferential absorption of the L-isomers of amino acids from the small intestine. The function of ATP, then, would be to aid in this carboxyl activation of the amino acids. There remains, however, another possibility, that of phosphorylation of amino acids by transphosphorylation. This follows from the demonstration that rich amounts of alkaline phosphatase are present in the epithelium of the small intestine (396; see also 124, 379), especially in the striated border which faces on the lumen (233, 262). The feeding of casein (212, 382) or of the L-isomers of individual neutral amino acids (380) causes an increase in the alkaline phosphatase concen-tration in the intestinal cells. Now this intestinal enzyme has been demonstrated to be capable not only of hydrolyzing and synthesizing phosphomonoesters (124, 254) but also of transphosphorylating ability (124,

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[52]Perhaps in confirmation of this is the recent work of Shishova (340). This worker found greater absorption of amino acids when inorganic phosphorous and ATP were included in the mixture of amino acids added to the small intestine.

144, 255, 264). Meyerhof and Green (255), for example, have demonstrated that, when phosphate compounds of higher bond energies than that of the acceptor phosphate compound were present, intestinal alkaline phosphatase was able to tring about transphosphorylation to the common biological alcohols (e.g., glucose, glycerol). It may be that in the preferential absorption of the amino acid L-isomers that a transphosphorylation occurs between ATP and the L-isomers. Lipmann (213a; see also 124) has suggested that just such a reaction may be the initial step in the synthesis of protein: ". . . the cell might first prepare the acyl phosphate with adenyl pyrophosphate [ATP] as the source of energy-rich phosphate groups." There remain other types of amino acid-phosphate compounds that also might be formed: in the laboratory, the amino and the hydroxyl groups of amino acids are known to form compounds with phosphate (134a,413a, 419a); in the animal, the amino group in the guanidino part of arginine and the hydroxyl group of serine are known to form compounds with phosphate (124). Finally, of considerable interest to the general problem under discussion are the recent results of Shishova (340). This worker has demonstrated the presence of "labile phosphorous compounds of amino acids" in the intestinal wall during the absorption of amino acids from a solution containing amino acids, inorganic phosphate, and ATP. Dinitrophenol prevented the formation of these phosphorous compounds. Shishova suggests that most, if not all, amino acids are phosphorylated during their absorption. The results do not indicate, however, the type of chemical reaction that brought about the formation of the amino acid-phosphorous compound. Although the nature of the postulated amino acid-phosphorous compound remains unknown, it does appear as possible that the accelerating factor of the Höbers is actually the cellular arrangement of enzymes that brings

about the formation of some type of phosphorous compound with the L-isomers of at least some of the amino acids. Since diffusion is a relatively slow means of conveying substances from the surrounding fluid into the interior of a cell (70), the phosphorous-linking of the amino acids would provide a chemical mechanism for increasing the rate of transfer of the amino acids from the lumen of the small intestine.

Complementary to the previous description of the Höbers' accelerating factor is the work of Agar et al (3, 4). They have published results that may be of great significance for an understanding of the manner of transfer of amino acids across an epithelial lining. Working with intestinal epithelium, Agar et al found that the uptake or accumulation of L-histidine and L-phenylalanine by the cells of the intestinal epithelium could occur against a concentration gradient. The uptake occurred even when the concentration of the amino acid in the epithelial cells was ten or more times the concentration of the amino acid in the solution bathing the cells. Very low concentrations of cyanide or 2:4 dinitrophenol inhibited the uptake of L-histidine. Equimolar concentrations of the L-isomers of amino acids with large non-polar side chains--members of the neutral amino acids--inhibited the uptake of L-histidine strongly; equimolar concentrations of the L-isomers of amino acids with polar side chains--not members of the neutral amino acids--inhibited the uptake of L-histidine weakly or not at all. Now these features for the uptake of amino acids by the cells of the intestinal epithelium are strikingly similar to those features cited previously (p. 154) for the transfer of amino acids across the intestinal epithelium. From this similarity, Agar et al (4) propose that the concentrative uptake of amino acids by the cells of the intestinal epithelium is the first step in the intestinal

transfer of amino acids against a concentration gradient. As Agar et al found the loss of amino acids from the cells apparently not to require energy of respiration—that is, the loss was not inhibited by cyanide or 2:4 dinitrophenol—, they suggested the second step to be the diffusion of the accumulated amino acids "down a concentration gradient" to the subepithelial tissues. The diffusion of the amino acids from the sub-epithelial tissues to the venous blood which drains from the intestinal wall would be the third step (44). Thus, the continual accumulation of amino acids in the cells of an epithelial lining by means of the Hobers' accelerating factor and the continual removal of amino acids by blood draining the area of the epithelial lining provide an overall mechanism for the concentrative transfer of amino acids across the epithelial lining.[53]

Kidney and Placenta.— Besides the small intestine, evidence exists for the concentrative transfer of amino acids across two other epithelial membranes. It is well known that the tubules of the kidney transfer amino acids against a concentration gradient, from the tubular lumen to the blood of the renal capillaries (164, 189). Recently, Kamin and Handler (189) have demonstrated that individual amino acids compete with one

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[53]Perhaps it should be emphasized here that the above discussion has been concerned with the concentrative transfer of amino acids from the small intestine. As it was indicated previously (see p.154), when the concentration gradient is favourable there also is a transfer of amino acids from the small intestine by the non-selective action of simple diffusion. Although apparently not mentioned in modern work, there also exists the possibility that the transfer of amino acids across the intestinal wall may be accelerated by the presence of a favourable electrical gradient (see footnote 62 on p.176).

another for the cellular mechanism of tubular absorption. The strongest evidence for this competition was among the neutral amino acids. These results suggests the probable presence of a cellular concentrative mechanism in the epithelial cells of the kidney tubules. The results of Crampton et al (81, 82) indicate that this cellular mechanism is a sterochemically specific mechanism for the reabsorption of L-amino acids. In the guinea pig, rabbit, and human, Christensen and Streicher (68) have found that amino acids are transferred across the placenta, from the maternal circulation to the foetal circulation, and this transfer occurs even against a concentration gradient. Crumpler et al (84) have confirmed these results in the human and have demonstrated the results to be the same for all the amino acids commonly found in plasma. Both groups of workers proposed that the placenta acts as an amino acid "pump".

Other Tissues.— If the concept—that a concentrative uptake of amino acids by the individual cells of the intestinal epithelium is the first step in the concentrative transfer of amino acids across the intestinal epithelium—proposed by Agar et al is true, then the results of the large amount of work that has been conducted on the uptake of amino acids by the cells of non-epithelial tissues may be applicable to the uptake of amino acids by the cells of epithelial tissues. If the results of this other work are applicable, then they should contribute to a better understanding of the manner of transfer of amino acids across epithelial membranes. A brief summary of some of this other work is presented below.

The amino acid concentrative process has been studied in greatest detail by Christensen and co-workers, who used the isolated cells of mouse ascites tumors. These cells possess a high concentrative activity for amino acids. Strong evidence was found for the existence of a special

cellular "carrier-like" mechanism that transports amino acids into the cells. This carrier mechanism for the uptake of amino acids is characterized, in part, by the following:

1. The pH of the solution influences the magnitude of the uptake of at least some of the amino acids. For glycine and histidine, the maximum concentrative uptake occurs between pH 7.3 and 7.9 (63, 65).

2. The uptake of amino acids by the cells is reduced by cyanide, 2:4 dinitrophenol, malonate, iodoacetate, fluoride, physostigmine, and anaerobiosis (64, 157). Other work (67, 324) indicates that these substances or conditions do not inhibit directly the carrier mechanism; rather, they appear to act upon that part of the cellular metabolism which supplies the energy requirements for the mechanism.

3. The carrier mechanism has a limited capacity. The existence of this limitation was deduced from an analysis of the relation between the rate of amino acid uptake by the cells and the initial concentration of amino acids in the solution bathing the cells. By use of the Michaelis-Menton type of analysis (124, 256), a rate-limiting step was demonstrated. The occurrence of this rate-limiting step is proposed as reflecting a limited capacity of the carrier mechanism (156, 157).

4. The cells concentrate both the L- and the D-isomers, the L-form far more strongly than the D-form. D-isomers can compete to some extent with the L-isomers for the carrier mechanism (65). Christensen (63) states: "A carrier which will combine with either isomer is visualized with the advantage of the L-form still to be explained."

5. The cellular absorption of both the L- and the D-isomers of any of the neutral amino acids may be inhibited competitively by the presence of one or more of the other amino acids (65, 69). Christensen (63, 65)

makes the suggestion that the inhibition among the neutral amino acids probably indicates the existence of one or a very few carriers of relatively low specificity. This supposition of low specificity is supported by the fact that many synthetic amino acids of abnormal structure can be concentrated in the cells (65, 66, 325).

6. The magnitude of the concentration gradient that is attainable for the straight-chained amino acids is found to decrease as the length of the aliphatic side chain increases. Illustrative distribution ratios for the concentration gradient between the cell and the bathing solution are: glycine, 7; alanine, 4; valine, 1.4; leucine, 1.1 (63).

7. The presence or absence of certain chemical groupings on particular sites of the amino acid molecule produce some striking effects on the rate of amino acid uptake by the cells. Two examples of this effect of chemical structure are the inhibitory action of a second carboxyl group (63, 65) and the strong stimulatory effect of a second amino group attached to the beta or gamma carbon atom of an amino acid (65, 66, 325).[54] On the basis of such results, Christensen and co-workers make the following general statement about the relation between the cellular carrier-mechanism

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[54]Hetényi and Winter (160) have found the selective absorption of glycine by the small intestine to be abolished by the loss of either the carboxyl or amino group, as neither glycine-amide nor sarcosine was absorbed selectively. Each of these latter substances was absorbed by simple diffusion alone. Also, proline, which lacks a free amino group, was absorbed by simple diffusion alone. On the other hand, beta-alanine was absorbed selectively and the authors suggest that this may mean that the selective mechanism is independent of the intramolecular position of the carboxyl and amino groups. The results of Schofield and Lewis (338), however, indicate that the rate of this selective absorption from the small intestine is dependent on the intramolecular position of the carboxyl and amino groups.

and the structure of the amino acids (65):

  The concentration which a given acid undergoes probably
is a consequence of the compositional, steric and configura-
tional details of its structure which determine its reactiv-
ity with the carrier. Leucine presents the poorest, alpha,
gamma-diaminobutryate the best approach, so far, to the
structure most favorable for concentration.

Other mammalian tissues have been demonstrated to contain amino acids

in cellular concentrations that are many times the concentrations in the

plasma. The ratio of the concentrations of amino nitrogen in several

mammalian tissues to the concentrations of amino nitrogen in the plasma

was found by Van Slyke and Meyer (393, 394) to range between five and ten

(see also the modern work of Astrup et al (20)). Similar results were

obtained by Christensen and coworkers and, in addition, these workers

found that competitive inhibition occurred among the amino acids during

their concentrative uptake by the tissues (63). The uptake of glycine

by liver and muscle cells, for example, could be inhibited by the presence

of any one of a large number of amino acids. The strongest inhibition was

among the neutral amino acids (69). The parallelism of these results

to those obtained for the small intestine and the ascites cells suggests

the existence of the same type of "carrier mechanism" in a variety of

mammalian tissues.

  Summary and Comment.- Among the previously discussed structures--epi-

thelial membranes of the small intestine, of the kidney tubule, and of

the placenta; normal tissue cells; and neoplastic cells of mouse ascites

tumors--, there are several common features in their behaviour toward

the amino acids. All the structures concentrate amino acids, presumably

by means of a cellular mechanism that is present close to the surface of

the cell. The concentrating mechanism usually has been demonstrated to

either favour the L- to the D-isomer or not measurably concentrate the D-isomer at all. The concentrative ratio is affected by temperature, pH, and respiratory inhibitors. The pattern of competitive inhibition among the amino acids during their uptake is similar for the different types of tissues. In particular, the widespread occurrence of mutual interference among the neutral amino acids is to be noted. Christensen, on the basis of this similarity of behaviour between the epithelial membranes and the non-membranous tissues, suggests that the concentrative transfer of amino acids across epithelial membranes is brought about by a "carrier mechanism". He comments on this suggestion as follows (63):

> All that is required to permit a cell-layer [i.e., an epithelial membrane] to act in this way is that the concentrative process be weaker on one surface than on another. Conceivably such cells instead may have their own distinct transfer apparatus, but a priori this appears less likely.

Although some yet-to-be-explained differences occur among some of the different types of mammalian cells in their behaviour toward amino acids (123, 417), the above review of the literature indicates a developing concept: the ubiquity among different types of mammalian cells of the same or similar mechanisms for the concentrative accumulation of amino acids. With such a concept developing, it is to be wondered if also the epithelial cells of the rumen possess a similar mechanism for concentrating amino acids. The structural arrangement of these cells in forming an epithelial membrane between the ruminal contents and the ruminal blood could favour the concentrative transfer of amino acids across the wall of the rumen. Some of the experiments reported in the literature will be cited to demonstrate that, in fact, the ruminal epithelium does possess an ability to transport at least some substances against a concentration gradient.

Literature Review on the Manner of Absorption of Substances from the Rumen

General.- Although simple diffusion is probably the manner of transfer of the gases, ammonia and methane (74, 100), the passage of most molecules across the ruminal epithelium does not appear to be by simple diffusion alone. It might be suggested, for example, that the three lower volatile fatty acids--acetic, propionic, and butyric--, because their concentrations in the ruminal contents of normally feeding sheep are always much higher than their concentrations in the blood, are absorbed from the rumen by simple diffusion alone. Parthasarathy and Phillipson (286) have found, however, that not only is the ruminal absorption of acetate ions reduced in the presence of "0.002 M-mercuric chloride", but also that this ruminal absorption of acetate ions is more efficient at low concentrations than at high concentrations. These results are not to be expected if the absorption of acetate ions occurs only by simple diffusion. The authors themselves suggest that these "are good reasons for believing that the absorption of acetate . . . is influenced by the activity of the epithelial cells lining the rumen." Although the situation for propionic acid and butyric acid may be the same as for acetic acid, this point has not been investigated.[55] Moreover, there is a complication in the study of the manner of absorption of propionic and butyric acids: the cells of the ruminal epithelium metabolize

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[55]It is perhaps of significance for the ruminal studies of fatty acid absorption that Smyth and Taylor (354, 355) have found the intestinal mucosa to be capable of transferring short-chain fatty acids against a concentrative gradient. The transfer was "greatly reduced anaerobically, in the absence of glucose or in the presence of 10^{-4} M 2:4 dinitrophenol."

appreciable amounts of the two acids, especially of butyric (288, 289).

It is, however, for the ruminal absorption of the simple ions of chloride and sodium that strong evidence has been accumulated for more than simple diffusion being involved. Both ions have been shown to be absorbed from the rumen against a concentration gradient (96, 286, 359). In seeking the mechanism of movement of chloride and sodium ions against a concentration gradient, Dobson and Phillipson (96, 97, 98) found, in normally feeding sheep, the existence of two forces. One of these forces was an electrical potential difference across the ruminal wall. The potential differences ranged between +25.4 and +44.0 millivolts, with the ruminal contents being negative with respect to the blood of the ruminal veins. The other force, that which is exerted on molecules by a concentration difference, also can be expressed in millivolts. The algebraic sum of the measured number of millivolts for the electrical and the chemical potential differences gives an estimate of the electrochemical potential difference acting on a single ion to transfer it across the ruminal epithelium (384). This relation is expressed more concisely in the following equation:[56]

$$ e\text{-}c.p.d. = \frac{RT}{F} \ln \frac{c_1 f_1}{c_2 f_2} + z\left(V_1 - V_2\right), $$

where $e\text{-}c.p.d.$ is the electrochemical potential acting on a single ion and is expressed in millivolts, R is the gas constant, T is the absolute temperature, F is the faraday, c_1 and c_2 are the respective concentrations of the ion in two solutions which are separated by a membrane and which are

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[56]This is the equation used by Dobson and Phillipson (98), but with a slight rearrangement in order to contrast more effectively the contribution of each the chemical and electrical potentials to the total potential of the ion.

denoted by the subscripts 1 and 2 , f_1 and f_2 are the respective activity

coefficients of the ion in the two solutions, Z is the ion charge, and

V_1 and V_2 are the respective potentials in the two solutions. The first

term on the right-hand side of the equation represents the chemical

potential of the ion, the second term represents the electrical potential

of the ion.

Dobson and Phillipson (97, 98, 298) found that the net[57] flux of

chloride ions across the ruminal epithelium always corresponded with that

to be expected from the effect of the estimated electrochemical potential

difference. For sodium ions, however, Dobson (96) found that the net[57]

flux was against the electrochemical potential difference. The move-

ment of sodium ions against the electrochemical gradient occurred even

when it was as high as 80 millivolts. These results indicate the existence

of another force which can move sodium ions against an electrochemical

gradient whose magnitude can be larger than 80 millivolts. Dobson

suggests that this force is energy supplied by the ruminal epithelium;

and, that it is this metabolically-supplied energy that "drives" the

sodium ions from the ruminal contents into the ruminal blood.[58] Such a

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[57]The ions of chloride, bicarbonate, sodium, and phosphate have
been demonstrated to pass in both directions across the ruminal epithelium
(see p. 12 and 13).

[58]It is interesting to note that also the kidney (28) absorbs
sodium ions against a concentration gradient. Both the epithelial cells
of the kidney tubules and the basal columnar cells of the ruminal
epithelium have the same arrangement and concentration of mitochondria:
densely packed mitochondria which often are orientated in the long axis
of the cells (99). As Bartley and Davis (28) have suggested that the
mitochondria play a significant role in the transport of sodium in
the kidney tubules, this parallelism of arrangement and concentration of
mitochondria in the two epithelial membranes provides indirect evidence
that the mitochondria of the ruminal epithelial cells are concerned in
the transport of sodium ions across the ruminal epithelium. A comment by
Phillipson (298) is of interest here: ". . . the animal has to work hard
to obtain sodium and also to reabsorb much of the sodium which pours into

force of metabolic energy also appears to be involved in the movement of bicarbonate and inorganic phosphate ions from the ruminal blood to the ruminal contents. The movement of these ions has been shown to be against a strong electrochemical gradient (230, 298, 299). In the case of potassium ions, however, no evidence for any force other than that of chemical potential has been found to explain their transfer across the ruminal epithelium. In spite of an unfavourable electrical gradient for these positively charged ions, their concentration in the ruminal contents is high enough to move them into the blood by simple diffusion. Phillipson (298) comments on the absorption of potassium ions as follows: "The quantities absorbed ... are small compared with sodium. There is no evidence yet to suppose that any 'active' mechanism is concerned."

Finally, reference now can be made to the statement previously made (p. 166) that the absorption of acetate ions is more efficient at lower than at higher concentrations of these ions in the rumen and, therefore, "is influenced by the activity of the epithelial cells lining the rumen." The electrical gradient that exists across the ruminal wall may be a manifestation of this "activity". Such a gradient, with the ruminal blood positive with respect to the ruminal contents, would accelerate the transfer of acetate ions and all other negative ions across the ruminal wall (299). For acetic and the other volatile fatty acids, the effect of the favourable electrical gradient would be added to the effect of the favourable concentration gradient. It is, however, from the equation presented on page 167, obvious that the effect of an electrical gradient on the rate of transfer of ions across a membrane will be more apparent

the rumen in the saliva in a similar manner to the kidney which has to work hard to reabsorb sodium to maintain the high concentration of sodium in the plasma."

at lower than at higher concentrations of the ions. This follows from
the fact that at the lower concentrations of the ions the electrical
potential force would constitute a greater percentage of the total force
acting on the ions; therefore, when the rate of transfer of the ions is
compared at two different concentrations, the rate of transfer will appear
to be more efficient at the lower concentrations--if only the effect of
concentration gradient is considered. Although the phenomenon of more
efficient absorption of the acetate ions at lower concentrations can be
explained as an effect of the electrical gradient, this does not rule out
additional forces acting on the ions, with the effect of these additional
forces also being more apparent at lower than at higher concentrations of
the ions. Such an additional force might be, for example, metabolic
energy supplied by the cells of the membrane being traversed. The
evidence available, however, does not permit any conclusion as to whether
metabolic energy also is a force that moves the acetate ions across the
ruminal wall. In conclusion, it can be stated that, on the basis of the
evidence available to date, the movement of molecules across the ruminal
epithelium is usually not by simple diffusion alone; rather, the movement
usually involves the interaction of the effect of at least two of the
following forces:[59] concentration gradient, electrical gradient (if the
molecules are charged), and metabolic energy.

Comment.- It has been demonstrated for many different types of
tissues, including epithelial membranes, that amino acids can be absorbed

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[59]If the ruminal solution is not isoosmotic with the plasma of the
ruminal capillaries, then the effect of an additional force may be present:
the frictional force of migrating solvent molecules of water on the solute
molecules (385).

or transferred against a concentration gradient. It also has been demonstrated that the ruminal epithelium possesses the ability to transfer at least some substances against a concentration gradient. It is proposed, teleologically, that a lack of ruminal absorption of amino acids against a concentration gradient would be an unusual exception. The question of whether or not such a movement of amino acids across the ruminal epithelium would be the result of the force of an electrical gradient, or of the force of metabolic energy, or both can not be answered as yet. Of special interest to this question, however, are the results of Lofgreen et al (214), especially if it is remembered that considerable evidence has been accumulated to indicate that amino acids are phosphorylated in their absorption from the small intestine (see p. 157 and on). Lofgreen et al, upon determining the tissue distribution of injected P^{32} into calves, found the rumen, omasum, and jejunum to have high phosphate exchange rates as compared with the other sections of the gastrointestinal tract. The authors suggest that the rapid rate of exchange of phosphates in these organs may be a reflection of the presence of a rapid phosphorylating-dephosphorylating mechanism[60] which could be important in the absorption of certain nutrients. Thus, if selective absorption--i.e., absorption that requires metabolic energy in order to

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[60]Such a mechanism would require a source of metabolic energy. With respect to possible sources of this energy, a suggestion of Pennington (288) is of interest. He found ruminal, reticular, and omasal epithelial tissues to be capable of metabolizing considerable amounts of propionic and butyric acids. From these results Pennington states:
> These epithelia are not glandular and hence would not require the energy of oxidation of the fatty acids for secretory purposes. It is possible, however, that such energy may be utilized in connexion with 'active absorption' of substances from the rumen.

occur--of amino acids from the rumen be demonstrated, perhaps this suggested phosphorylating-dephosphorylating mechanism (or transphosphory- lating!) would be the energy-requiring mechanism concerned in this selective absorption of the amino acids from the rumen. First, however, it must be determined if amino acids are absorbed selectively from the rumen. The data of the present investigation now will be analyzed, therefore, for evidence as to the manner of absorption of amino acids from the rumen.

Evidence from the Present Experiments as to the Manner of Absorption of Amino Acids from the Rumen

Introduction.- From the information provided in the literature review on the manner of absorption of amino acids from the small intestine (p.151), kidney (p. 160), and placenta (p. 160), it is evident that several criteria have been used in the attempts to elucidate the manner of transfer of amino acids across epithelial membranes. In order both to summarize the previous review and to provide an introduction to the present discussion, some of the more important criteria are listed below.

1. The effect of varying the initial concentration of the amino acid on its absolute and relative rates of transfer--if the absolute rates are directly proportional to the initial concentrations and the relative rates are constant, these relations are considered evidence for simple diffusion being the only manner of transfer; if the relative rates are inversely proportional to the initial concentration, this relation is considered evidence for simple diffusion not being the only manner of transfer: that there also is involved a cellular accelerating factor.

2. The effect of stereoisomerism of the amino acid on its rate of transfer--the more rapid rate of transfer of the L-isomer in contrast

to the rate of transfer of the D-isomer is considered evidence for simple diffusion not being the only manner of transfer; that there also is involved a cellular accelerating mechanism which demonstrates a specificity for the L-isomer.

3. The transfer of the amino acids against a concentration gradient—such a transfer is considered evidence for simple diffusion not being the only manner of transfer; that there also is involved a cellular mechanism which is able to do work.

4. The effect of the stereoisomerism of an amino acid on its transfer against a concentration gradient—the transfer of the L-isomer but not the D-isomer against a concentration gradient is considered evidence for simple diffusion not being the only manner of transfer; that there also is involved a cellular mechanism which is not only able to do work but which also demonstrates a specificity for the L-isomer.

5. The effect of various metabolic poisons or metabolically-unfavourable conditions on the transfer of amino acids against a concentration gradient—the partial or complete inhibiting action by these substances or conditions on the transfer is considered evidence for simple diffusion not being the only manner of transfer; that there also is involved a cellular mechanism which is dependent on the energy of respiration.

6. The competitive inhibition that exists among the L-isomers of the neutral amino acids when their transfer is from a mixture of amino acids—such competitive inhibition is considered evidence for simple diffusion not being the only manner of transfer; that there also is involved a cellular mechanism which, although demonstrating a relative specificity toward the L-isomers of amino acids, demonstrates a relative non-specificity toward the L-isomers of the neutral amino acids.

7. Although apparently not as yet applied to amino acid transport across epithelial membranes, a rate-limiting step has been found to occur in the uptake of amino acids by the isolated cells of the mouse ascites tumors--if the validity be assumed of the proposition of Agar et al (4) that the concentrative uptake of amino acids by the cells of an epithelial membrane is the first step in the transfer of amino acids across an epithelial membrane, then the demonstration of the existence of a rate-limiting step in the uptake of amino acids by the cells of the epithelial lining would be considered evidence for simple diffusion not being the only manner of transfer; that there also would be involved a cellular mechanism with a limited capacity for the uptake of amino acids and, therefore, also a limited capacity for the transfer of amino acids across an epithelial membrane.

The design of the ruminal fistula experiments in the present investigation was not such as to permit all the just-listed criteria to be applied to them. Actually, the original intent in conducting the experiments that are reported herein was to demonstrate that ruminal absorption of amino acids did or did not occur, not to demonstrate the manner of absorption. At the beginning of the investigation, the author had hoped, if ruminal absorption of amino acids could be demonstrated to occur, to conduct further experiments designed to elucidate the manner of this ruminal absorption of amino acids. The author, however, underestimated the time required to perform and analyze the experiments which were designed to demonstrate ruminal absorption of amino acids and, therefore, the investigation of the manner of absorption was not conducted. It is apparent, however, that the results of those experiments that were executed provide preliminary-type data which can be subjected to some of the criteria used

by various authors to determine the manner of absorption of amino acids by epithelial membranes.

In applying many of the above criteria to the ruminal absorption of amino acids, there also must be considered the effect on the amino acid molecules of the electrical gradient that has been demonstrated to exist across the ruminal wall. In the present experiments—i.e., in experiments W4, W5, and W6—the pH of the ruminal solutions ranged between 6.5 and 7.6 (see Table 17, p. 87). Within this range of pH, all the amino acids present in the ruminal solutions, with the exception of arginine, histidine, and lysine, should each exist as an equilibrium mixture of dipolar ions (Zwitterions) and anions. The excepted amino acids should each exist as an equilibrium mixture of dipolar ions and cations. This follows from a consideration of the isoionic points (pI) of the different amino acids: arginine, pI = 10.76; histidine, pI = 7.64; lysine, pI = 9.47; and for the rest of the amino acids, pI's = 2.98 to 6.3 (167).[61] As the electrical gradient across the ruminal wall is orientated with the blood side positive and the ruminal side negative, the movement of anions or cations in either direction across the ruminal wall will be accelerated or deaccelerated accordingly as the charge on the ions is orientated with or against the electrical gradient. Of necessity, then, the effect of the electrical gradient across the ruminal wall must be considered in any analysis of the manner of transfer of amino acids across the ruminal wall.

Effect of Initial Electrochemical Gradient on Rate of Absorption.—
Although Hober and Hober determined the effect of the initial concentra-

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[61]The pI numbers for the five amino acids that are discussed in the present experiments are (167): tryptophan, 5.88; methionine, 5.74; tyrosine, 5.63; glycine, 6.06; and lysine, 9.47.

tions of the amino acids on their rates of absorption from the small
intestine, the existence of an electrical gradient across the ruminal wall
should indicate that, in the case of ruminal absorption,[62] not only is a
force of concentration difference operative upon the ions of amino acids,
but also the parallel force of an electrical potential difference. As
the rate of simple diffusion of ions should be proportional to the total
force acting upon them (385), it would seem more logical to determine the
relation between the absolute rate of amino acid absorption and the
algerbraic sum of the parallel forces of chemical and electrical gradients,
rather than between the absolute rate of absorption and the chemical
gradient alone. The appropriate relation can be written:

$$ n \sim e-c.p.d. = \frac{RT}{F} \ln \frac{c_1 f_1}{c_2 f_2} + z \left(V_1 - V_2 \right), $$

where n is the amount of amino acid absorbed in a given time and the other
symbols have the same meaning as given on p. 167. This means that if no
force other than those of the chemical and electrical gradients are
acting upon the amino acid ions, the absolute rate of absorption should
be directly proportional to the electrochemical potential difference. If

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[62] Modern workers who have studied the absorption of amino acids from
the small intestine seem to have ignored the question of an electrical
gradient. Although no direct experimental evidence appears to be avail-
able for the existence of an electrical gradient across the wall of the
mammalian intestine, there is some indirect evidence in the older litera-
ture that such a gradient may exist. Verzár and McDougall (397), for
example, cite the early work of Rosenthall who found the serosal side of
the frog's intestine to be positive with respect to the mucosal or
luminal side of the intestine. More recently, Phillipson (298) cites the
work of Dobson who found a potential difference to exist across the caecal
wall of the sheep. This potential difference was of the same magnitude
and direction as the potential difference across the ruminal wall. The
existence of these just-cited electrical gradients are strongly suggestive
that a similar gradient may exist across the wall of the mammalian small
intestine. Such a gradient would influence the intestinal absorption of
the ions of amino acids.

direct proportionality is not obtained, then the results may[63] indicate

the presence of one or more other forces which also are acting on the amino

acid ions. One of these other forces could be the frictional force that

is exerted upon solute molecules by water movements (385). Such water

movements occur when there is a difference in the osmotic or hydrostatic

pressures of two solutions separated by a membrane permeable to water.

Another force could be that resulting from the active participation of

the enzymes of the cells that constitute the ruminal epithelium in the

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[63]The word "may" is used advisedly for several reasons. In the
first place, the equational calculation of the effect of concentration
difference is based upon the premise of the applicability of the van't
Hoff equation that $P = RTC$, where P is the osmotic pressure, R is the gas
constant, T is the absolute temperature, and C is the concentration of a
solution. As this equation of van't Hoff is a limiting one, that is, the
equation is strictly true only for solutions of infinite dilution, it can
be of applicability only to very dilute solutions. The closeness of the
approximation to the true data of data which are obtained by use of the
equation diminishes as the finite concentration in the solution increases.
Jacobs (183) has pointed out that the effects of frictional resistance
and of attractive forces between molecules or ions will change as the
concentration increases, thereby lessening the applicability of the
equation for solutions of stronger concentrations. Another reason for the
use of the word "may" is exemplified by a comment of Höber (164). This
author, in writing of the applicability of Fick's law to absorption by
diffusion from intestinal loops, makes the following comment:

> . . ., it should be anticipated that the method described does
> not permit an exact confirmation of Fick's law to be obtained,
> Impairment of the vitality of the delicate epithelium, through
> mechanical insults following the repeated filling and emptying
> of the loop, uncontrollable changes of the blood circulation,
> the effect of narcotics, and other factors are unavoidable
> causes of irregularity.

Höber's comment also would be true for absorption by diffusion from the
rumen. Schambye (333, 334, 335), for example, found in acute experiments
on anesthetized sheep a decrease of cardiac output and a slowing of the
blood circulation as compared with chronic experiments on non-anesthetized
sheep: the average portal blood flow for the anesthetized sheep (334) was
about 29 milliliters per minute per kilogram of body weight and the aver-
age portal blood flow for the non-anesthetized sheep (335) was about 37
milliliters per minute per kilogram of body weight (see also 230, 292).
Again, Shaw and Peterson (337) have demonstrated large blood volume

uptake of amino acids. Such enzyme participation usually involves the
utilization of chemical energy of the epithelial cells to do the "work"
of moving nutrients from one solution to another. If it is assumed that
no forces other than those previously discussed are involved in the
absorption of amino acids and if conditions in the two solutions are such
as to preclude any water movement, then a lack of direct proportionality
for the effect of the electrochemical potential differences on the absolute
rates of absorption may[64] indicate the participation of cellular enzymes
and metabolic energy in the transfer of amino acids across the ruminal
wall.

The data of the present experiments now will be examined to see if
they can be analyzed according to the terms of the above discussion. One
point of question immediately becomes apparent: Höber and Höber (166),
in their determination of the effect of initial concentrations on the
rates of absorption of an amino acid, considered the absorption of the
individual amino acid only from a pure solution of the amino acid. In
the present investigation, all the studies of amino acid absorption were
made using a mixture of amino acids. This means that, because of the
known existence of interionic forces of attraction and repulsion, the
diffusion of each amino acid could not have been an entirely free diffu-
sion. In the application of Fick's law to the effect of concentration on
rate of diffusion, one of the assumptions that is made is that there is
free diffusion of the molecules. This is a necessary assumption, as the

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changes to occur in the mammary gland of the cow during its excitation.
It is possible that a similar blood volume change occurs in the ruminant
stomach if the non-anesthetized animal is excited by the experimental
procedure.

[64]See footnote 63 on the previous page.

forces of intermolecular or interionic attraction increase as the concentration of the molecules increases. This would mean that, as the concentration increased, the attraction of a slower moving particle for a faster moving particle also would increase and, thereby, slow down the faster moving particle from its free diffusion rate and speed up the slower moving particle from its free diffusion rate. Such an effect would add to or subtract from the effect of an increased concentration difference on the rate of diffusion of a molecule or ion, thus masking any possible relation of direct proportionality between the initial concentration difference and the rate of diffusion of the molecule or ion. This effect of the intermolecular or interionic forces, plus the points discussed in footnote 63 (p. 177), make it doubtful, therefore, that a deviation of the results in the present experiments from direct proportionality between the electrochemical potential difference and the rate of absorption can be taken as evidence for the effect of forces other than the electrical and chemical potential differences acting upon the amino acids.

In spite of the doubtful significance—for the reasons just given—of any such calculation, a calculation of the effect of the initial electrochemical potential differences on the rates of absorption has been made for the data of experiments W4, W5, and W6. Table 29 presents, for each the five amino acids whose concentrations were measured, estimates of the initial electrochemical potential differences,[65] the absolute decreases

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[65]In contrast to the procedure of Höber and Höber (see discussion on page 153, as well as footnote 46 on the same page), who used in their calculations the initial concentrations of the amino acids, the initial concentration differences were calculated for the present experiments. The initial concentrations used by Höber and Höber were so high, relative to the plasma concentrations, that the initial concentrations actually approximated very closely the initial concentration differences. In the

TABLE 29. — Ruminal fistula experiments W4, W5, and W6: a tabulation, for each the five amino acids whose concentrations were measured, of estimates of the initial electrochemical potential differences (millivolts), of the absolute decreases of concentration (microgram-moles per 100 milliliters), and of the decreases of concentration relative to the initial electrochemical potential differences (per cent)

Experiment	Glycine			Lysine			Methionine			Tyrosine			Tryptophan		
	Initial e-c. p.d.	Concentration decrease[a]		Initial e-c. p.d.	Concentration decrease[a]		Initial e-c. p.d.	Concentration decrease[a]		Initial e-c. p.d.	Concentration decrease[a]		Initial e-c. p.d.	Concentration decrease[a]	
		Absolute	Relative		Absolute	Relative		Absolute	Relative		Absolute	Relative		Absolute	Relative
W5	21.0 to 35.8	15.9	75.7 to 44.4	2.1 to 18.0	12.5	600 to 69.4	45.2 to 63.9	5.3	11.7 to 8.2	6.6 to 17.7	1.8	27.3 to 10.2	- 8.1 to - 2.0	1.5	..
W6	51.3 to 66.1	43.5	84.8 to 65.8	30.0 to 45.9	34.4	114 to 74.9	69.4 to 88.1	12.0	17.3 to 13.6	42.0 to 53.1	5.2	12.3 to 9.8	41.3 to 57.4	12.1	29.3 to 21.1
W4	49.6 to 64.4	17.4	35.1 to 27.0	32.8 to 48.6	18.0	54.9 to 37.0	73.5 to 92.2	5.0	6.8 to 5.4	53.8 to 64.9	0.5	0.9 to 0.8	56.0 to 62.1	3.1	5.5 to 5.0

[a]The concentration decreases that are recorded for each experiment are those decreases which occurred in the first 70 minutes of the experiment.

of concentration, and the decreases of concentration relative to the
initial electrochemical potential differences. Unfortunately, neither the
concentrations of the amino acids in the plasma nor the electrical
gradients across the ruminal wall were measured. Lacking such data for
the calculation of the initial electrochemical potential difference, it
was decided to use data that are available in the literature. For the
calculation of the electrical component of the electrochemical gradients,
the data of Dobson and Phillipson (98) were utilized. These authors
found, both in conscious sheep with normal rumen contents and in anesthet-
ized sheep with artificial solutions in the rumen, electrical potential
differences which averaged about 30 millivolts to exist across the ruminal
wall. This number was utilized in the calculation of the electrochemical
gradient. An obvious weakness of such a procedure is that the measured
electrical potential differences will vary, not only in the same animal
from time to time, but also among different animals. Further, the
assumption is being made that the electrical potential differences across
the ruminal wall of the goat are the same as those found for the sheep.
For the calculation of the chemical component of the estimated initial
electrochemical gradients, there were utilized the initial ruminal solu-
tion concentrations of the amino acids recorded in Tables 8 to 12 (pp. 49,
52, 55, 58 and 62) and the plasma concentrations of the same amino acids
recorded in Table 30. For tyrosine, glycine, and lysine, the plasma
data given for the goat were used; for tryptophan, the plasma data for
the sheep were used; and for methionine, the plasma data for man were

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present experiments, however, the initial concentrations were of the same
order of magnitude as the plasma concentrations and, therefore, the
initial concentrations could not be used as approximations of the concen-
tration differences.

TABLE 30. — Concentration (microgram-moles per 100 milliliters) of five amino acids in the plasma of ruminants and man

Amino acid	Goat	Sheep	Cow	Man
Tryptophan	. . . .	3.9 to 4.9[a]	3.9 to 5.9[a]	3.4 to 7.4[a]
Methionine	. . . .	. . . .	. . . .	2.0 to 4.0[a]
Tyrosine	5.5 to 8.3[a]	. . . .	. . . .	4.4 to 12.1[a]
Glycine	43.6 to 75.5[b]	. . . .	. . . .	17.3 to 30.6[a]
Lysine	8.9 to 16.4[a] 9.1 to 13.5[c]	. . . .	. . . .	14.4 to 26.0[a]

[a]These data, from Albritton's Standard Values in Blood (8), are the lower and upper limits of the 95 per cent range of concentrations that have been reported in the literature.

[b]These data are the lower and upper limits of the data that are recorded in Table 20 (p.110).

[c]These data are the lower and upper limits of the data that are recorded in Table 19 (p.109).

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used.[66] In each case, both the lowest- and the highest-found plasma concentrations were utilized in order to calculate the upper and lower limits of possible concentration differences; hence, in Table 29 two numbers are given for the electrochemical gradient acting on each amino acid. The assumption made, then, is that the actual initial electrochemical gradient that occurred during the experiments was within the range limited by the two numbers.[67]

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[66]As the plasma concentrations of the amino acids might differ considerably between ruminants and non-ruminants, it is realized that the use of plasma concentrations found in man for those found in the goat may be hazardous. Nevertheless, the procedure is followed in the belief that at least the probable order of magnitude of the amino acid concentrations in the goat plasma will be indicated. Although not for glycine, such a belief proves to be valid in the case of tryptophan, tyrosine, and lysine; thus, comparison in Table 30 of the plasma concentrations for these three amino acids in man and the ruminant animals reveals a close approximation of the concentration ranges.

[67]It must be noted that an assumption was made in calculating the chemical component of the electrochemical gradient; that is, that:

$$c_1/c_2 \approx c_1 \cdot f_1 / c_2 \cdot f_2$$

where is the concentration and is the activity coefficient. The validity of this approximation rests upon the following considerations:

1) The osmotic pressure of the ruminal solution in each of the experiments W4 to W6 (see Table 6, p. 37) apparently was close to the osmotic pressure of the plasma;

2) Most of the osmotic pressure in the ruminal solutions (see Table 5, p. 32) or in the plasma was due to the presence of uni-univalent salts-- predominantly sodium propionate for the ruminal solutions and sodium chloride for the plasma--; therefore, the approximation of the osmotic pressure in the ruminal solutions to the osmotic pressure in the plasma means approximate equivalence in the total numbers of univalent ions for the two solutions;

3) The ionic strength of a solution is calculated from the following equation (70):

$$\Gamma/2 = \sum C z^2,$$

where $\Gamma/2$ is the ionic strength, C is the molar concentration of each ion

The data in Table 29 for the absolute rates of decrease of amino

acid concentrations were derived from the 0 to 70 minute time interval

results[68] that are presented in Tables 8 to 12, (pp. 49, 52, 55, 58, and

62). In order that the rates of absorption recorded in Table 29 would be

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in the solution, Z is the ion's charge number, and the sign of the
summation ($\sum$) indicates that all the products CZ^2 are to be added;
therefore, the approximation of the total number of ions (mostly univalent)
in the ruminal solutions to the total number of ions (mostly univalent) in
the plasma signifies that the ionic strengths of the two solutions also
must approximate to one another;

4) The activity coefficient is proportional to the ionic strength
of a solution, other conditions being held constant, because (70):

$$-\log f_i = K_1 z_i^2 \sqrt{\Gamma/2}$$

where f_i is the activity coefficient of an ion, z_i is its charge number,
and $\Gamma/2$ is the ionic strength of the solution; therefore, the approxima-
tion of the ionic strengths of the two solutions should mean that the
activity coefficient of each the amino acids in the ruminal solutions
approximated to the activity coefficient for the same amino acid in the
plasma;

5) In consequence of the statement under 4), the ratio (f_1/f_2) of
the activity coefficient of an amino acid in the ruminal solution (f_1)
to the activity coefficient of the amino acid in the plasma (f_2) was
assumed to be unity; therefore, in calculating the chemical component of
the electrochemical gradient, it was considered valid to substitute the
ratio, C_1/C_2, for the ratio, $C_1 \cdot f_1/C_2 \cdot f_2$.

Finally, in addition to the above considerations, there is the fact that
the activity coefficients of the amino acids change only slightly, even
when relatively large changes in the molal concentration occur. Mehl and
Schmidt (252), for example, found the activity coefficient of glycine at
30° C. to change from 0.97 for 0.2 molal concentration to 0.86 for 1.0
molal concentration. This means that the relatively small differences
that existed between the concentrations of each amino acid in the ruminal
solution and in the plasma of the ruminal capillaries were of little
consequence in the previously-made assumption that the ratio of the activity
coefficients is unity.

[68] The longest possible time interval was selected in order to
magnify any differences in the data caused by differences in rates of
absorption. The data of the 0 to 150 minute time interval could not be
used for the present calculations, however, as some of the necessary
control data were missing. The data of the 0 to 70 minute time interval,
with none of the control data missing, were used.

more likely to reflect only those changes of concentration that resulted from ruminal absorption, the data from Tables 8 to 12 were corrected for the concentration changes that occurred in the control solutions. In making the correction, the concentration changes in the ruminal and control solutions were calculated for the 0 to 70 minute time interval. Positive or negative signs were attached to the concentration changes obtained: positive for a concentration increase and negative for a concentration decrease. The concentration change for each control solution then was subtracted algebraically from the concentration change for the corresponding ruminal solution. The difference, always a negative quantity, was considered to be the concentration change that resulted because of ruminal absorption. This correction procedure assumes, of course, that the concentration changes that occurred in control solutions also occurred in the ruminal solution, and to the same extent.

Examination of the data in Table 29 for experiments W5 and W6 reveals that the absolute rates of absorption were proportional to the initial electrochemical potential differences. It is not possible to determine if the proportionality is direct or not, as the initial concentrations only can be presented as being within a given range of concentrations. If the ranges for the relative rates of absorption between the two experiments are compared for each amino acid, it is found that, for each the amino acids tyrosine, glycine, and lysine, there are overlappings of some of these ranges with one another. This means that direct proportionality between the absolute rates of absorption and the initial electrochemical potential differences could have occurred for these three amino acids, but does not mean that it did occur. For methionine, there is no overlapping of the ranges for relative rates of absorption in experiments W5

and W6. The upper limit of the range in experiment W5 is quite close, however, to the lower limit of the range in experiment W6; therefore, the possibility of direct proportionality between the absolute rates of absorption and the initial electrochemical potential differences does exist for methionine also. For tryptophan, no comparison can be made because the absorption of tryptophan in experiment W5 was apparently against the electrochemical gradient.

The data of Table 29 also can be examined for the relation between the relative rates of absorption and the initial electrochemical gradients. A comparison between the ranges for the relative rates of absorption in experiments W5 and W6 reveals that, for each the amino acids tyrosine, glycine, and lysine, the upper limit of the range in experiment W5 is higher than the lower limit of the range in experiment W6. For these three amino acids, then, the possibility does exist for their relative rates of absorption being inversely proportional to the initial electrochemical potential differences.

Up to this point, the data for experiment W4 in Table 29 purposely have been ignored. This has been done because a comparison between the data for the absolute rates of absorption in experiments W4 and W6 reveals a strong possibility of the existence of an effect of animal variability. It is suggested that the manifestation of this effect is the much smaller absolute rates of absorption for all five amino acids in experiment W4 as contrasted with the rates in experiment W6. For tryptophan and tyrosine, the smaller absolute rates of absorption in experiment W4 occurred in spite of the fact that the estimated initial electrochemical gradients for these amino acids in experiment W4 were either the same as or larger than the estimated initial electrochemical

gradients in experiments W6. For the other three amino acids, methionine, glycine, and lysine, as the ranges of their estimated initial electrochemical gradients in experiments W4 and W6 overlap, the possibility does exist that the initial electrochemical potential differences were higher in experiment W6 than in W4. For all five amino acids, however, a comparison of the relative rate of absorption in the two experiments reveals that the relative rates of absorption were higher in experiment W6 than in experiment W4. This indicates that, even for the three amino acids methionine, glycine, and lysine, any possible higher initial electrochemical potential differences in experiment W6 were not enough to account for the much larger rates of absolute absorption in experiment W6.[69]

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[69]In the calculation of the data for Table 29, a number of 30 millivolts was assumed, in all three experiments, for the electrical component of the electrochemical potential difference. Now it is true that the electrical gradient can vary in the same animal and among animals (see p. 181). It might be suggested, therefore, that the abnormally low relative rates of absorption in experiment W4--that is, considering the relation of the initial electrochemical gradients in experiment W4 to those in experiment W6, abnormally low relative to the rates of absorption found to occur in experiment W6--might not appear abnormally low if the previous assumption of the existence of an electrical gradient in experiment W4 of 30 millivolts be changed to an assumption of the existence of a much smaller electrical gradient. This latter assumption would yield, for all the amino acids except lysine, an initial electrochemical gradient for experiment W4 that was much lower than the initial electrochemical gradient for experiment W6; therefore, the relative rates of absorption in experiment W4 might no longer appear to be abnormally low. The data for lysine, however, appear to rule out the assumption of a much lower electrical gradient in experiment W4. The use of a much lower number for the electrical gradient in experiment W4 would make the initial electrochemical gradient for lysine much higher than that which is recorded in Table 29--because the electrical gradient is orientated to oppose the movement of the positively charged lysine ions and a reduction in the electrical gradient would increase the effect of the chemical gradient--; yet, the absolute rate of absorption of lysine in experiment W4 is only about one-half the absolute rate of absorption of lysine in experiment W6.

Table 31[70] reveals a similar situation for propionic acid, the substance
that was added as a positive control for ruminal absorption. The abso-
lute decrease in the concentration of propionic acid in experiment W4 was
much less than that in experiment W6, this in spite of the fact that the
initial concentration[71] in experiment W4 was slightly higher than that in
experiment W6. Noting that experiments W5 and W6 were carried out with
one goat (see p. 29) and experiment W4 was carried out with another goat
(see p. 27), it is suggested that the results of these experiments for
the absorption of amino acids and propionic acid indicate the presence
of the effect of animal variability on the rate of absorption from the
rumen. It was in order to eliminate this apparent effect of animal
variability that the previous examination of the effect of initial electro-
chemical potential difference was restricted to the data of experiments
W5 and W6--both carried out on the same goat.

It now can be asked what interpretation can be placed upon the
findings of the analyses just carried out. The following interpretations
are suggested:

    1. As, from the data of experiments W5 and W6, the absolute rates of

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[70]The data for this table were corrected in the same manner as for
the initial concentration data of Table 29 (p. 180): in calculating the
absolute rates of absorption, the data from Table 7 (p. 45) were corrected
for the concentration changes that occurred in the controls. It was
necessary, however, to use the 0 to 150 minute time interval, as the
concentrations of propionic acid in the control solutions were measured
only in the 0 and 150 minute control samples.

[71]The initial concentrations can be used in this case in place of the
calculated initial concentration differences. This could be done because
the concentrations of propionic acid in the ruminal solutions were of such
a high order of magnitude relative to the range of concentrations normally
found in the blood draining the rumen: 0.7 milligram-moles per liter
(calculated from data that are presented in 196). See also footnote 46,
p.152.

TABLE 31. — Ruminal fistula experi-
ments W4 and W6: a tabulation, for
propionic acid, of the initial concen-
trations (milligram-moles per liter),
of the absolute decreases of concen-
tration, and of the decreases of con-
centration relative to the initial
concentrations (per cent)

Experiment	Initial concentration	Concentration decrease[a]	
		Absolute	Relative
W6	65	41	63.0
W4	68	29	42.6

[a]The concentration decreases that
are recorded for each experiment are
those decreases which occurred during
the 150 minutes of the experiment.

absorption of the amino acids were proportional to their estimated initial
electrochemical potential differences and as the electrical component of
the initial electrochemical potential differences is assumed to have been
approximately the same in the two experiments—i.e., 30 millivolts—, the
findings obtained are evidence that the ruminal absorption of these amino
acids is at least partly due to the effect of the force of chemical
potential difference and that, therefore, simple diffusion forms at least
a part of the mechanism concerned in this absorption.

2. As, from the data of experiments W5 and W6, the overlappings or
approximations of the ranges for the relative rates of absorption of all
the amino acids, except tryptophan, admit of the possibility of direct
proportionality existing between the initial electrochemical gradients
and the absolute rates of absorption, there must be admitted, in turn, the
possibility that the ruminal absorption of the four amino acids entirely
was due to the effect of the forces of chemical and electrical potential
differences. On the other hand, as the upper limits of the ranges for
the relative rates of absorption in experiment W5 for each the amino
acids tyrosine, glycine, and lysine are higher than the lower limits of
the ranges for each these same three amino acids in experiment W6, there
also must be admitted the possibility that the relative rates of
absorption were inversely proportional to the initial electrochemical
gradients. This latter possibility would mean, in turn, that the absorp-
tion of the three amino acids was more efficient at the lower concentra-
tions. If such a relation occurred, then it would be similar, not only
to the relation found by Höber and Höber for the intestinal absorption of
amino acids (see p. 154), but also to the results obtained by Parthasarathy
and Phillipson for the ruminal absorption of acetic acid (see p. 166).

These two groups of workers considered such a relation as possibly indicative of the effect of cellular energy that is participating in the absorption of amino acids or acetic acid, with the effect of the cellular energy relative to the effect of the chemical gradient being more noticeable at the lower chemical gradients.

3. As, from the data of experiment W5, there is movement of tryptophan against the electrochemical gradient, this is evidence for the effect of a force other than the chemical or the electrical gradients being concerned in the movement of tryptophan across the ruminal wall. It can be suggested that this other force may have been that of metabolic energy which is supplied by the cells of the ruminal epithelium.

The above interpretations indicate, then, that the movement of the five amino acids across the ruminal wall is at least in part the result of the process of simple diffusion, plus the effect of the electrical gradient. In addition, for tyrosine, glycine, and lysine, the possibility does exist that another force, cellular energy, may have been involved in their movement. For tryptophan, it appears, indeed, that this other force of cellular energy was involved (but see page 94 for a comment on the validity of the recorded concentrations of tryptophan).

Comparison Among the Five Amino Acids of their Rates of Absorption.- There is another way that the data of the ruminal fistula experiments can be examined in this attempt to determine the forces concerned in the absorption of amino acids from the rumen. The data of Table 29 have been rearranged in Table 32. Table 32 also includes for each amino acid its molecular weight, its apparent molecular volume and the square root of

TABLE 32. — Ruminal fistula experiments W4, W5, and W6: a tabulation of data that are utilized in the text (p.194) to make a prediction of the rank-order for the rates of ruminal absorption of the five amino acids listed

Experiment	Amino acid	Molecular weight[a]	Apparent molecular volume[b]	$\sqrt{}$	Diffusion coefficient[c] $(10^{-7}\ cm.^2/sec.)$	Estimated initial electrochemical potential difference[d] (millivolts)	Absolute concentration decrease[d,e] (microgram-moles per 100. milliliters)
	Methionine	149.2	110	10.5	68	73.5 to 92.2	5.0
	Tryptophan	204.2	144	12.0	61.4	56.0 to 62.1	3.1
W4	Tyrosine	181.2	123	11.1	62	53.8 to 64.9	0.5
	Glycine	75.1	43	6.6	95.0	49.6 to 64.4	17.4
	Lysine	146.2	109	10.4	69	32.8 to 48.6	18.0
	Methionine	149.2	110	10.5	68	45.2 to 63.9	5.3
	Glycine	75.1	43	6.6	95.0	21.0 to 35.8	15.9
W5	Tyrosine	181.2	123	11.1	62	6.6 to 17.7	1.8
	Lysine	146.2	109	10.4	69	2.1 to 18.0	12.5
	Tryptophan	204.2	144	12.0	61.4	- 8.1 to -2.0	1.5

TABLE 32 - continued

Experiment	Amino acid	Molecular weight[a]	Apparent molecular volume[b]		Diffusion coefficient[c] (10^{-7} c.m.^{2}/sec.)	Estimated initial electrochemical potential difference[d] (millivolts)	Absolute concentration decrease[d,e] (microgram-moles per 100 milliliters)
				$\sqrt{}$			
	Methionine	149.2	110	10.5	68	69.4 to 88.1	12.0
	Glycine	75.1	43	6.6	95.0	51.3 to 66.1	43.5
W6	Tryptophan	204.2	144	12.0	61.4	41.3 to 57.4	12.1
	Tyrosine	181.2	123	11.1	62	42.0 to 53.1	5.2
	Lysine	146.2	109	10.4	69	30.0 to 45.9	34.4

[a]These data, rounded off to one decimal place, are from the Handbook of Chemistry and Physics (167).

[b]These data are from references (73) and (204).

[c]See footnote 72 on page 194.

[d]These data are taken from Table 29 on page 180.

[e]The concentration decreases that are recorded for each experiment are those decreases which occurred in the first 70 minutes of the experiment.

this volume, and its diffusion coefficient.[72] This information is included because it is known that the rate of free movement of a molecular species is not only directly proportional to the electrochemical gradient that exists for it, but also is dependent upon its inherent rate of free diffusion. The information will be utilized in a further attempt to elucidate the manner of absorption of the amino acids from the rumen.

Examination of the descriptive data for each of the amino acids listed in Table 32 enables a prediction to be made of the rank-order of their rates of absorption--if the effects of the forces here considered are the only ones concerned in the absorption of amino acids from the rumen. The effect of the initial electrochemical gradients and the effect of the inherent diffusion rates (as expressed by the diffusion

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[72]The diffusion coefficients for glycine and tryptophan were experimentally determined (310), those for the other amino acids were derived by the present author from a figure (Fig. 4, p. 1911) of Polson (310) that demonstrates the relation between the diffusion coefficients of amino acids and their molecular weights. These derived data are at best only approximate data, because it has been demonstrated that the diffusion coefficient of a molecule more properly is related to its volume and shape than to its molecular weight (252). It is for this reason that the square roots of the molecular volumes for the amino acids also are produced in Table 32. It is interesting to note that the diffusion coefficients that are presented in Table 32 do approximate to a direct inverse proportion with respect to the square roots of the molecular volumes. This is not an unexpected relation, since the molecular volume of a compound tends to vary as its molecular weight. It is probable that the variations in the shapes of the five amino acids that are presented in Table 32 account for at least part of the deviation from an exact proportionality. Polson (310), for example, has demonstrated that the more stretched a molecule is, the lower is its diffusion coefficient. He illustrated this by comparing the diffusion coefficients of tryptophan and arginine. Arginine, a stretched molecule, has a molecular weight of 174, a molecular volume of about 117, and a diffusion coefficient of 57.6×10^{-7} cm.^{2}/sec. Tryptophan, a more nearly spherical molecule, has a molecular weight of 204, a molecular volume of 144, and a diffusion coefficient of 61.4×10^{-7} cm.^{2}/sec.

coefficients) will be compared to determine the net effect upon the flow rate of the individual amino acids. To demonstrate the method of analysis employed, the data of experiment W4 will be considered in detail.

On comparing the ranges of electrochemical potential differences for the five amino acids in experiment W4, it is seen that the lower limit of the range for methionine exceeds the upper limits of the ranges for the other four amino acids. On this basis alone, then, a preliminary rank-order can be predicted for the absolute rates of absorption:

$$\text{methionine} > \text{tryptophan, tyrosine, glycine, lysine} \text{------------(1).}$$

Now, as the diffusion coefficient for methionine, 68, is larger than the coefficients for tryptophan, 61.4, and tyrosine, 62, the position of methionine in the rank-order, relative to the positions of tryptophan and tyrosine, is strengthened. Also, as the diffusion coefficient of methionine, 68, is almost the same as that for lysine, 69, the position of methionine in the rank-order, relative to the position of lysine, is not altered. The diffusion coefficient of methionine, 68, is smaller, however, than that of glycine, 95.0. In order, therefore, to determine the rank-order of these two amino acids, relative to one another, the ratio of the force favouring glycine over methionine will be compared with the various possible ratios of the force favouring methionine over glycine (the subscripts G and M refer to glycine and methionine,

respectively):

rates of diffusion				initial electrochemical gradients		
95_G	:	68_M	: :	92.2_M	:	49.6_G,
or 1.40_G	:	1_M	: :	1.86_M	:	1_G----(a);
95_G	:	68_M	: :	92.2_M	:	64.4_G,
or 1.40_G	:	1_M	: :	1.43_M	:	1_G----(b);
95_G	:	68_M	: :	73.5_M	:	49.6_G,
or 1.40_G	:	1_M	: :	1.48_M	:	1_G----(c);
95_G	:	68_M	: :	73.5_M	:	64.4_G,
or 1.40_G	:	1_M	: :	1.14_M	:	1_G----(d).

In (a) and (c), the force favouring methionine over glycine exceeds the

force favouring glycine over methionine. In (b), the forces are approxim-

ately equal and, in (d), the force favouring glycine over methionine

exceeds that favouring methionine over glycine. The rank-order of these

two amino acids is expressed, therefore, as:

$$\text{methionine} \Big\rangle \quad \text{or} \quad \Big\langle \text{ glycine---------------- (2)}.$$

Utilizing this same method of analysis, it next is found that in every

case the force favouring glycine or tryptophan, tyrosine, or lysine

exceeds the force favouring any one of these amino acids over glycine.

Taking into account these relations and the relation expressed by rank-

order (2), rank-order (1) now can be rewritten as:

$$\text{methionine} \Big\rangle \quad \text{or} \quad \Big\langle \text{ glycine} \Big\rangle \text{ tryptophan, tyrosine, lysine-(3),}$$

where the larger sign, $\Big\rangle$ signifies[73] that, although methionine or glycine

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[73]The larger sign indicates that all the amino acids which come before
it always will be in a higher position in the rank-order than all the
amino acids which come after it.

may be lower than one another in the rank-order, the lower members of

these two still will be higher than any of the other three amino acids

in the rank-order. Finally, ratio comparisons made among the remaining

three amino acids, in the same manner as just outlined, permit rank-order

(3) to be rewritten as follows:

$$\text{experiment W4} = \text{glycine} \rangle \text{ or } \langle \text{methionine} \rangle \text{tryptophan} \rangle \text{ or } \langle \text{tyrosine} \rangle \text{or} \approx$$
$$\text{lysine}\text{------------------------------------}(4).$$

This rank-order listing is to be read in the following manner. For the

amino acids, methionine and glycine, two possibilities exist: the first

position will be occupied by methionine and the second position by

glycine, or the first position by glycine and the second position by

methionine, but neither of these two positions can be occupied by any

of the other three amino acids. For the remaining three amino acids,

several possibilities exist: the third position will be occupied by

tryptophan, the fourth position by tyrosine, and the fifth position

by lysine; or, the third position will be occupied by tyrosine, the fourth

position by tryptophan, and the fifth position by lysine; or, the third

position will be occupied by tryptophan and the fourth position by both

tyrosine and lysine; or, the third position will be occupied by tyrosine

and the fourth position by both tryptophan and lysine; or, tryptophan,

tyrosine, and lysine can occupy together the third position.

Utilizing the same type of analysis as just given for experiment W4,

the data for experiment W5 and W6 yielded, upon analysis, the following

rank-orders:

$$\text{experiment W5: glycine} \rangle \text{ or} \langle \text{methionine} \rangle \text{tyrosine} \rangle \text{ or} \langle \text{lysine} \rangle \text{tryptophan-(5)};$$

$$\text{experiment W6: glycine} \rangle \text{ or} \langle \text{methionine} \rangle \text{tryptophan} \rangle \text{ or} \langle \text{tyrosine} \rangle \text{or} \langle \text{lysine(6)}.$$

The rank-order listing for experiment W5 is to be read in the following

manner. For methionine and glycine, the same two possibilities exist

as outlined previously for these two amino acids in experiment W4: the

first position will be occupied by methionine and the second position

by glycine, or the first position by glycine and the second position by

methionine, but neither of these two positions can be occupied by any of

the other three amino acids. For tyrosine and lysine, two possibilities

also exist: the third position will be occupied by tyrosine and the

fourth position by lysine, or the third position will be occupied by

lysine and the fourth position by tyrosine, but neither of these two

positions will be occupied by tryptophan. For tryptophan, there is

only one possibility: the fifth position in the rank-order is occupied

by tryptophan. The rank-order listing for experiment W6 likewise is to

be read in the following manner. For methionine and glycine, the same

possibilities exist as outlined previously for these two amino acids in

experiments W4 and W5 and, as before, neither of the first two positions

can be occupied by any of the other three amino acids. For tryptophan,

tryosine, and lysine, $\lfloor 3$ or 6 possibilities exist: the third position

can be occupied by any one of the three amino acids, the fourth position

by either one of the two remaining amino acids, and the fifth position

by the one remaining amino acid.

Examination of the data of Table 32 reveals the rank-orders of the

absolute rates of absorption actually to be:[74]

experiment W4 = lysine≈glycine⟩ methionine⟩ tryptophan⟩ tyrosine---(7);

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[74]The rank-orders listed are based on data for the 0 to 70 minute
time interval. The use of this time interval biases, however, tryptophan
downward in its placement in the rank-orders for experiments W5 and W6.

experiment W5 = glycine ⟩ lysine ⟩ methionine ⟩ tyrosine ⟩ tryptophan---(8);

experiment W6 = glycine ⟩ lysine ⟩ tryptophan≈methionine)tyrosine----(9).

A comparison now will be made, for each experiment, between the experimentally-found rank-order and the predicted possible rank-orders. A comparison of rank-order (7), the experimentally-found rank-order for experiment W4, with rank-order listing (4) reveals that, except for the position of lysine, rank-order (7) fits one of the predicted possible rank-orders. A comparison of rank-order (8), the experimentally-found rank-order for experiment W5, with the rank-order listing (5) reveals that, except for the position of lysine, there is a close matching of rank-order (8) to one of the predicted possible rank-orders. Finally, a comparison of rank-order (9), the experimentally-found rank-order for experiment W6, with the rank-order listing (6) reveals that, except for the positions of lysine and tryptophan, there is a close matching of rank-order (9) to one of the predicted possible rank-orders. Now, as the predicted possible rank-orders were set up upon the basis that the absolute rates of absorption of the amino acids were dependent only on the action of the electrochemical potential differences, the above comparisons can be interpreted to indicate that the force of the electro-chemical potential gradient plays a predominating role in the absorption of amino acids from the rumen. These results can not be interpreted, however, as ruling out that the absorption of the amino acids could have been due to the effect of other forces--e.g., cellular energy--as well.

This is so because, in these two experiments, all or nearly all the tryptophan was absorbed in the first 10 minutes of the experiments (see Table 8, p. 49). On making a comparison, however, of 0 to 10 minute interval data, it was found that there was no change in the position of tryptophan in the rank-orders.

<u>Transfer of Amino Acids Against an Electrochemical Gradient.</u>- A
comparison of the individual amino acid concentrations in the ruminal
solutions with the concentrations present in the plasma of the ruminal
veins, as well as a measurement of the electrical gradient existing across
the ruminal wall, would enable a calculation to be made of the magnitude
and direction of the electrochemical gradient between the ruminal solution
and the plasma of the ruminal veins. One value in making such a
calculation is that the demonstration of a net movement of an amino acid
against an electrochemical gradient would be considered as strong
evidence for the existence of one or more other forces being concerned
in the movement of the amino acid. Unfortunately, in the present experi-
ments, there is no record of the plasma concentrations for the individual
amino acids or of the electrical gradient. The same kind of procedure
will be followed here, therefore, as was carried out in the calculation of
the initial electrochemical potential differences (see p. 181). For
the calculation of the chemical component of the electrochemical gradient,
there were utilized the lowest-found ruminal solution concentrations of
the amino acids recorded in Tables 8 to 12 (pp. 49, 52, 55, 58 and 62)
and the plasma concentrations recorded in Table 30 (p.182). The lowest-
found amino acid concentrations in the ruminal solutions were used for
this calculation in order to determine if downward, plasma to ruminal
solution concentration gradients existed during any part of the ruminal
fistula experiments. Table 33 has been set up to record the calculated
amino acid concentration gradients that existed between the ruminal
solution and the plasma of the ruminal veins when the concentrations
in the ruminal solution were at their lowest. Except for lysine in
experiment W5, Table 33 records quantitatively only those concentration

Amino acid	Experiment					
	W4		W5		W6	
	Chemical gradient	Electro-chemical gradient	Chemical gradient	Electro-chemical gradient	Chemical gradient	Electro-chemical gradient
Tryptophan	+	+	-80.1 to -86.3[a]	-30.6 to -78.3[a]	-80.1 to -86.3	-30.6 to 78.3
Methionine	+	+	+ to - 6.0	+	+	+
Tyrosine	+	+	-22.4 to -35.5	+ to -27.5	+	+
Glycine	+	+	+ to - 8.2	+ to - 0.2	+	+
Lysine	+	+	+27.3 to +43.8	+ to -22.2	+	+

[a]As the lowest-found concentration for tryptophan in the ruminal solution of experiment W5 was less than the concentration detectable by the microbiological assay method, the lowest-found concentration was recorded in Table 8 (p. 49) as a zero concentration. It is unlikely, however, that a zero concentration actually was reached. In the absence of a known concentration, the lowest-found concentration for tryptophan in the ruminal solution of experiment W6, 0.2 microgram-moles per 100 milliliters, is assumed for the lowest-found concentration in experiment W5. This assumption enables a calculation to be made of the chemical gradient in terms of millivolts. This is done, however, only with the understanding that the actual chemical gradient was probably larger than indicated by the data which are recorded in the above table.

gradients that were orientated downward from plasma to ruminal solution--
these gradients are designated in the table by negative signs. This proced-
ure was adhered to because the only interest at this point is for the
detection of the ruminal absorption of amino acids that occurred <u>against</u>
an electrochemical gradient. The chemical gradients that were orientated
downward from ruminal solution to plasma are recorded in Table 33 quali-
tatively only--these data are designated in the table by positive signs.
The one exception to this procedure is for lysine in experiment W5: here,
the gradient, even although it is positive, is recorded quantitatively.
This is done because the lowest-found lysine concentrations were low
enough that they may have been overbalanced by the oppositely orientated
electrical gradients that existed for lysine. Next, the data of Dobson and
Phillipson (98) for electrical potential differences were utilized in
conjunction with the calculated chemical gradients to calculate the electro-
chemical gradients. The range found by these authors for the electrical
potential differences between ruminal contents and blood in conscious
sheep was 8 to 49.5 millivolts. It is assumed that the range for the
electrical potential differences in the goat is similar to that for
the sheep.[75] By adding algebraically the chemical and electrical[76] com-
ponents, the lower and upper limits were determined for the range of
possible electrochemical gradients. These limits are recorded as a part of

[75]Dobson (98) found the electrical potential differences for a red
poll, non-lactating heifer to be within the range found for the sheep.

[76]The electrical potential difference across the ruminal wall is
orientated with the ruminal vein blood negative with respect to the ruminal
contents (see p. 167). From the discussion that previously was given on
page 175, this means that the electrical component is orientated positively
for all the five amino acids except lysine and is orientated negatively
for lysine.

Table 33, qualitatively only when they are positive, quantitatively when they are negative. Examination of the data in the table reveals that negatively orientated electrochemical gradients existed against the movement of tryptophan in experiments W5 and W6 and may have existed against the movement of tyrosine and lysine in experiment W5. This does not mean, however, that reversals of the positively orientated electrochemical gradients could not have occurred, especially if there had been longer time intervals for absorption or lower initial concentrations of the amino acids in the ruminal solutions. This demonstration of the movement of tryptophan against an electrochemical gradient should mean that another force was involved in the movement of tryptophan across the ruminal wall. Likewise, another force also may have been involved in the movement of tyrosine and lysine. It is suggested that this other force may be that of the metabolic energy which is supplied by the epithelial cells of the ruminal wall.

Unfortunately, there exists some doubt as to the validity of the above analysis, at least for tryptophan, tyrosine, and probably methionine. In a previous discussion on the stability of the experimental solution constituents (see p. 94), it was pointed out that molecular deterioration of the aforementioned amino acids may have occurred during the storage time that occurred between the time of the experiment and the time of assay. Such molecular deterioration, if large enough, could convert an actual concentration gradient that favoured a constituent's passage from ruminal solution to plasma into an apparent concentration gradient that opposed a constituent's passage from ruminal solution to plasma. If this did happen, then the chemical and electrochemical gradients that are recorded in Table 33 would not be valid. Unfortunately,

there is no way to decide if such molecular deterioration did occur during the storage of the samples. The validity of the deductions made for tryptophan and tyrosine from the data in Table 33 remain, therefore, in doubt. Only in the case of lysine, for which no evidence was found for molecular deterioration, are the deductions not affected by this doubt of the stability of the amino acids in their storage period.

Summary and Conclusions.- The purpose of the previous discussion was to determine if the data of the ruminal fistula experiments provided any evidence which was relative to an elucidation of the manner of transfer of amino acids across the ruminal wall. The examination of the data for the effect of the initial electrochemical gradients on the absolute and relative rates of absorption led to interpretations that indicated that the forces of chemical and electrical gradients were involved in the movement of the five amino acids--tryptophan, tyrosine, methionine, glycine, and lysine--across the ruminal wall. The same examination also indicated that, for tyrosine, glycine, lysine, and tryptophan, a possibility exists for their movement being assisted by the force of metabolic energy as well. An examination of the data by comparing experimentally-found rank-orders of the absolute rates of absorption for the five amino acids with their predicted possible rank-orders led to the conclusion that the forces of the chemical and electrical gradients play a predominating role in the movement of the five amino acids across the ruminal wall. It also was noted, however, that this conclusion did not exclude the possibility of the force of metabolic energy being involved as well. Finally, an examination of the data for evidence of transfer of the amino acids against an electrochemical gradient led to the suggestion that the force of metabolic energy was

involved in the movement of tryptophan across the ruminal wall, and that

this also might be true for tyrosine and lysine. This suggestion was

qualified, however, for tryptophan and tyrosine because of the uncertainty

as to the cause of a suspected molecular deterioration of these two

amino acids in the assayed samples. Taking into consideration all these

various factors, plus a necessary prudence because of the many assump-

tions made in the discussions that led to the above interpretations, it

appears from the evidence available that the following statement can be

made with some assurance of validity: the movement of the five amino

acids--tryptophan, tyrosine, methionine, glycine, and lysine--across the

ruminal wall of the goat is determined by a summation of the effects of

the forces of chemical, electrical, and possibly metabolic potentials.

The question raised at the end of the review section (p. 171) on the

manner of absorption of substances by the ruminal epithelium--whether the

movement of amino acids across the ruminal wall against a concentration

gradient would be the result of the effect of the force of an electrical

gradient or of the force of metabolic energy or both--can not be answered

from the data that are available in the present ruminal fistula experi-

ments. All that can be said is that the data do not provide proof against

the force of metabolic energy being involved with the force of the electri-

cal gradient, but neither do the data provide conclusive evidence for the

involvement of the metabolic force. Of necessity, then, further experi-

mentation is required, especially of the type that has been carried out

on the absorption of amino acids from the small intestine (see p. 151 to

p. 160). It is hoped that the data of the present ruminal fistula experi-

ments and the examination of that data in the present discussion may at

least serve the purpose of indicating some of the assumptions and considera-

tions to be checked and taken into account in any future research on the manner of absorption of the amino acids from the rumen.[77]

Amino Acid Absorption from the Rumen under Normal Feeding Conditions

Introduction

The question naturally arises as to whether the absorption of amino acids occurs in the rumen under normal feeding conditions. To be able to answer such a question, there is required a knowledge of the fulfillment or non-fulfillment of the following conditions:

1. the demonstration of the presence of amino acids in the rumen;

2. the demonstration of the relation of the concentrations of the individual amino acids in the ruminal liquor to their concentrations in the blood plasma: a rumen to plasma, downward electrochemical gradient would permit the possibility of amino acid absorption without requiring direct participation of metabolic energy, but would not rule out the possibility of metabolic energy also participating; a plasma to rumen, downward electrochemical gradient would limit any amino acid absorption to that accomplished directly or indirectly by the force of metabolic energy;

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[77]One point that was not considered in the above discussion is the possible influence of anesthezia, shock, or poor general condition (of the experimental animal) on any special selective mechanism that would accelerate the ruminal absorption of amino acids. Several authors (46, 79, 134, 146) have presented evidence to demonstrate that these experimental conditions can reduce the selective absorption of glucose from the small intestine. Shock (146) has been demonstrated to reduce also the absorption of amino acids from the small intestine. The influence of these factors should be considered in any future research directed at determining if amino acids are absorbed selectively from the rumen.

3. the demonstration of the absorption of individual amino acids

from an ingesta-filled rumen, the concentrations of the individual amino

acids at which this absorption occurs being of the same order of magnitude

as those found in the ingesta fluid ordinarily.

Each of these points will be discussed in turn.

Condition 1: Demonstration of Amino Acids in the Rumen

Although originally McDonald (237, 240) was unable to detect the

presence of free amino acids in the ruminal liquor of sheep fed on a hay

diet, several authors (15, 62, 213) since have demonstrated the presence

of appreciable amounts of amino acids in the ruminal liquor of sheep.

These positive results for the presence of free amino acids in the ruminal

liquor are not unexpected, especially as leaves, stems, tubers, roots,

cane and beet molasses, and "fish solubles" are known to contain apprecia-

ble amounts of free amino acids (see the review by Chalmers and Synge, 62).

Annison (15), for example, has reported for sheep alpha amino nitrogen

concentrations that ranged between 10 and 15 milligrams per 100 milliliters

of ruminal liquor.[78] These concentrations occurred during and after

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[78]"These amounts are large enough to be interesting but give no indi-
cation of the daily turnover." This statement was made by Elsden and
Phillipson (109) about the measured concentrations of volatile fatty acids
in the rumens of ox and sheep. Elsden and Phillipson's statement also
applies to the data that are reported in the literature for the concentra-
tions of amino acids in the ruminal liquor. This point is emphasized here
because too often the low concentrations of the amino acids in the ruminal
ingesta are cited to support the implication that the ruminal absorption
of amino acids, if it does occur, must be of little nutritional significance.
Such an implication takes no account of the possibility that the low con-
centrations of amino acids in the ruminal liquor may be, in part at least,
a reflection of the rapid absorption of amino acids by the rumen. For
example, the concentrations that were found by Blaizot and Raynaud (see
p. 208) are relatively low; however, it is to be noted that the measure-
ments were made on ruminal contents that had been removed from the animals
24 hours after the last feeding. Of particular interest at this point is
the following statement of Almquist (12):

In the digestion and assimilation of whole proteins it is

the feeding of dried grass.[79] In addition, Blaizot and Raynaud (37)

have demonstrated chromatographically that the majority of the amino

acids are present as free acids in the ruminal liquor of the ox. They

estimated that the concentrations of eight of the amino acids were between

0.08 to 1 milligram per 100 grams of the initial fresh ruminal contents.[78]

> probable that enzymatic digestion is a relatively slow proc-
> ess and that absorption across the intestinal walls is
> relatively fast. Therefore, in disregard of known large
> differences in solubility, molecular diffusion rates, etc.,
> of the individual amino acids, they are absorbed almost as
> soon as released from food protein and enter the circula-
> tion in proportions closely related to those in the diet
> [underlining mine].

Now it must be admitted that the distance between the wall of the rumen
and the center of the ruminal contents is much greater than the distance
between the intestinal wall and the center of the intestinal contents.
This means that all the ruminal contents may not be as readily exposed
to the absorbing action of the ruminal wall as the intestinal contents
are to the absorbing action of the intestinal wall. Nevertheless, there
probably is exposure of much of the ruminal contents to the absorbing
action of the ruminal wall because of the active churning of the ruminal
contents by the contractions of the ruminal wall; and this exposure also
is over a much longer time period than it is the case in the small
intestine because the rate of passage of the ingesta through the rumen is
relatively slow as compared with the rate of passage of the ingesta
through the small intestine (102). Trautmann (377) has pointed out that
these two conditions--i.e., the churning of the contents and its relatively
slow passage--for the ruminal contents especially favour the absorption
of the diffusible constituents that are present in the rumen:

> Zunächst sind die Bedingungen zur Resorption günstig, denn der
> Inhalt verweilt hier lange und wird auch so durchmischt, dass
> immer wieder neues Material mit den Schleimhäuten in Berührung
> kommt.

With the action of these conditions just discussed to ensure a continuous
contact of new ruminal material against the ruminal wall, it again is
suggested that the existence of a rapid rate of absorption of amino acids
from the rumen, especially relative to the rate of formation of new amino
acids by proteolysis, could be one of the main reasons for the finding of
relatively low concentrations of amino acids in the ruminal liquor.

[79]Synge (369) states that "the making of silage results in conversion

Condition 2: Demonstration of an Electrochemical Gradient·

Annison (15), commenting upon the fact that young grasses contain

appreciable quantities of free amino acids,[80] suggested that "it is likely

that when ruminants graze on lush pastures the concentration of free

amino nitrogen in the rumen becomes appreciably greater than in the blood."

Such concentrations of amino acids in the ruminal liquor as just cited on

the previous page—that is, between 10 and 15 milligrams of alpha amino

nitrogen per 100 milliliters—are considerably higher than the concentra-

tions reported for the whole blood or plasma of sheep. In one sheep

anesthetized with sodium pentobarbotone, Annison (15) found plasma

concentrations of 4.11 in the portal vein and 4.27 in the jugular vein. In

another sheep, conscious rather than anesthetized, Annison found plasma

concentrations of 3.84 in the portal vein and 3.04 in the common carotid

artery. All these concentrations are milligrams of alpha amino nitrogen

per 100 milliliters of plasma. Spector's Handbook of Biological Data (358)

reports amino acid nitrogen concentrations for the whole blood of sheep as

4.6 to 8.0 milligrams per 100 milliliters. From these data, it appears

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of much of the protein of grass into soluble nitrogenous compounds of
lower molecular weight." It is to be wondered, therefore, how high the
concentration of amino acids in the rumen would be after the feeding of
grass silage.

[80]Annison cited the work of Waite and Boyd (398, 399) in support of
this statement. The present author could not find, however, any mention
in the cited work to the concentration of free amino acids in grasses.
Other work (see 173, 368, 369, 373) does exist to support the state-
ment that young grasses contain appreciable quantities of free amino
acids.

that, under certain conditions of feeding, a rumen to plasma, downward concentration gradient for amino acids can exist in the normal-feeding animal. This, in turn, means that a rumen to plasma, downward electrochemical gradient can exist, at least for those amino acids whose pI numbers are such that the amino acids form equilibrium mixtures of dipolar ions and anions in the normal ruminal contents (see previous discussion on p. 175).

Condition 3: Demonstration of Absorption from an Ingesta-Filled Rumen

The existence of such rumen to plasma, downward concentration gradients as just described suggests the possibility of amino acid absorption from the rumen by at least simple diffusion in the normal-feeding animal. Annison, however, even when he had a concentration of 83.7 milligrams of alpha amino nitrogen per 100 milliliters in the rumen liquor (see Table 1, p. 16), was unable to demonstrate the absorption of amino acids from the ingesta-filled rumen of a sheep. Likewise, the ruminal absorption of amino acids from ingesta-filled rumens was not demonstrated in the present blood sampling experiments (see Table 28, p. 146). It is to be noted, however, both in Annison's and the present experiments, that the method of detection of amino acid absorption was by the making of a comparison between the concentrations of alpha amino nitrogen in the portal and systemic plasmas. The lack of sensitivity of this procedure for the detection of substances that are absorbed continuously and in small amounts has been commented upon previously (see p. 116). Only when relatively high concentrations of amino acids were placed in empty rumens was the method of comparing blood concentrations of alpha amino nitrogen successful in demonstrating amino acid absorption. By contrast, when there was utilized the more sensitive procedure of sampling at periodic inter-

211.

vals a solution that was placed in an empty rumen--that is, as it was done

in the present ruminal fistula experiments--, the absorption of amino

acids was demonstrated at relatively low concentrations. In experiment

W6, this demonstration of ruminal absorption was made when the concentra-

tion of alpha amino nitrogen in the ruminal solution was as low as 30

milligrams per 100 milliliters (Table 13, p. 67). Such a concentration

is of the same order of magnitude as that found by Annison (15)--that is,

10 to 15 milligrams of alpha amino nitrogen per 100 milliliters--to be

present in the ruminal liquor of sheep during and after their ingestion

of dried grass.

Comment

The previous discussion indicates that the amino acids are present

in the rumen in appreciable concentrations. Further, these concentrations

often are large enough that rumen to plasma, downward electrochemical

gradients can exist for many of the individual amino acids. These grad-

ients, when they occur, should allow amino acids to be absorbed from the

rumen even if the ruminal epithelium does not possess the ability to

absorb amino acids against an electrochemical gradient. On the other

hand, when the concentrations of the amino acids in the ruminal liquor

are small enough that plasma to rumen, downward electrochemical gradients

exist, then these gradients should allow amino acids to pass from the

blood of the portal circulation into the liquor of the rumen, provided

that the ruminal epithelium does not possess the ability to absorb amino

acids against such gradients. The existence of such an ability for the

ruminal epithelium would, in accordance with the capacity of this ability,

either reduce or even eliminate any net passage of amino acids into the

rumen—even under starvation conditions.[81] Finally, although the previous

discussion does not present direct evidence which demonstrates the occurrence

of ruminal absorption of amino acids from an ingesta-filled rumen,

evidence is presented which suggests that such a demonstration could be

made if suitable methods of experimentation were utilized: perhaps, by

the use of radioactive-labelled amino acids, the ruminal absorption of

amino acids from the ingesta-filled rumen into the portal circulation

could be demonstrated.

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[81]See the quotation from Chalmers and Synge on page 13.

The Effect of Ruminal Absorption on the Fate of

Amino Acids Present in the Rumen

Introduction

The question can be raised as to how the ruminal absorption of amino acids fits into the pattern of the dynamic nitrogen metabolism that is occurring continuously in the ruminal ingesta. The discussion of the present section will attempt to answer to this question.

Chalmers and Synge (62), as well as Phillipson and Cuthbertson (299), have presented a synoptic figure of the principal reactions of nitrogenous compounds in the rumen. Figure 22 is a reproduction of the figure of these authors, but with the addition of one more arrow to indicate the ruminal absorption of amino acids. Figure 22 reveals that the fate of amino acids that enter or are formed in the ruminal ingesta is manifold:

1. the amino acids are utilized in the synthesis of microbial protein, or they are deaminated to form ammonia and nitrogen-free compounds;

2. the amino acids pass with the rest of the constituents of the ruminal ingesta into the more distal parts of the alimentary tract;

3. the amino acids are absorbed from the ruminal ingesta by passage through the ruminal wall into the portal circulation.

It is to be expected that the distribution of each amino acid among the various possible pathways just described will be influenced by the particular set of environmental conditions prevailing in the rumen, by the known influence of the diet on the composition of the population of microorganisms in the rumen, by the physical and chemical nature of the dietary constituents, by the relative stability of the amino acids in the

In saliva, from body fluids

In food

Entering rumen

Protein   Nitrate   Free amino acids   Urea
                           etc.

In rumen

Food protein ——→ Free amino acids ⇌ NH_3 ——→ Absorbed through
                    etc.   rumen wall

N-free
compounds

Absorbed through
rumen wall

Protein of
microorganisms

**Leaving rumen to
abomasum and
intestines**

Food protein   Protein of   Nonprotein
                    microorganisms   N
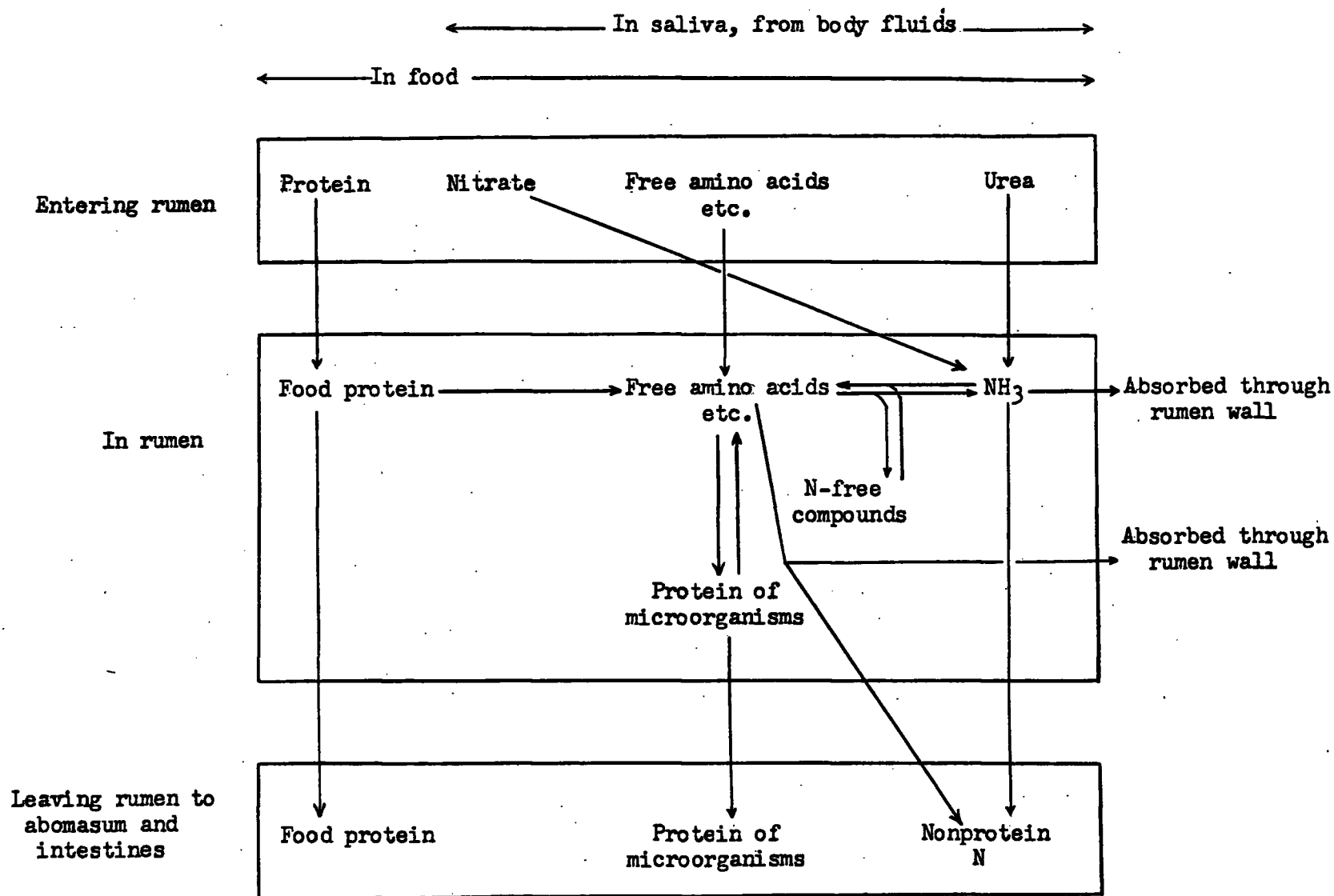
Fig. 22.

Fig. 22. - The principal reactions of nitrogenous compounds in the rumen (adapted from figures of Chalmers and Synge (62) and Phillipson and Cuthbertson (299).

ruminal ingesta, and by the physiological state of the animal. In order
to acquire some concept of the distribution of the amino acids among the
pathways which are presented in Fig. 22, each of the influencing
factors briefly will be discussed in turn.

Influence of Environmental Conditions in the Rumen on the Fate of Amino Acids in the Rumen

Variations in the environmental conditions of the rumen will have an
influence upon the fate of amino acids in the rumen. This follows from
the considerable evidence that has been accumulated relative to the
influence of temperature, pH, and so forth on the activities of micro-
organisms. Several examples will be given of the extent to which the
environmental conditions of the rumen are known to vary. In each example,
an attempt will be made to demonstrate how the variation of an environ-
mental condition probably can influence the fate of the amino acids in
the rumen.

Temperature.- Several workers (52, 87, 94, 205, 268, 378) have
measured temperature variations in the rumen, both for different positions
within the cavity of the rumen and for the effect of feed and water
ingestion. During active fermentation in the rumen, the temperature in
the rumen can increase as much as 2°C. (205, 378). After the drinking
of water by sheep, Nangeroni (268) found the temperature of the ruminal
ingesta to fall from about 40° down to about 37° in one experiment, down
to about 33° in a second experiment, and down to about 30° in a third
experiment. Similar temperature changes have been recorded in the cow
after the ingestion of water by this animal (94). Sometimes a period of
time as long as two hours is required for the ruminal ingesta temperatures

to return to their original level. Smaller temperature changes, of the order of magnitude of 1 to 2^0 C., have been recorded after the ingestion by ruminants of various quantities and types of feed (52, 87, 378). It is likely that such temperature changes in the ruminal ingesta will influence the fate of the amino acids. This statement follows from the demonstrated effect that even small changes in the incubation temperature of microorganisms can have on their synthetic ability. Borek and Waelsch (45), for example, found that a temperature change of 2^0 C. can determine whether or not a _Lactobacillus_ can synthesize its own requirement of phenylalanine. Similar results were obtained for tyrosine and aspartic acid. Borek and Waelsch also cite several authors who have found in other microorganisms this same type of temperature influence on their ability to synthesize amino acid requirements. It is significant to note that these temperature effects found by Borek and Waelsch were obtained using temperatures that lie within the range of the temperatures which are found in the rumen. This implies, then, that the demonstrated variations of temperature that occur in the ruminal ingesta likely will influence the amino acid requirements and the amino acid synthesis of the ruminal microorganisms and, therefore, will influence the fate of the amino acids in the rumen.

pH.-- It amply has been demonstrated that marked variations in the pH of the ruminal ingesta occur. Recordings as low as pH 5.5 and as high as pH 7.5 or greater have been reported (88, 102, 113, 188, 259, 261, 265, 294, 296, 320, 353; see also the citations in 139). The more usual range of pH is 6.0 to 7.0 (102). Now, Gale (126) states that many of the bacterial enzymes which are involved in amino acid catabolism are

adaptive enzymes. The production of these enzymes will be influenced by the conditions of the growth medium and, apparently, the pH of the medium is the main factor[82] determining whether the enzymes of deamination or decarboxylation will be produced. In general, microorganisms cultivated under acid conditions will produce decarboxylation systems. Gale suggests that the effective stimulus for the formation of the different enzymes may be the ionic form of the amino acid[83]; the cationic form of an amino acid stimulating production of decarboxylase enzymes and the anionic form stimulating the production of deaminase enzymes. At a pH of about 5.5, a culture of <u>Escherichia</u> <u>coli</u> demonstrated no effective decarboxylase or deaminase activity. On the acid side of this pH, the effective decarboxylase activity rapidly increased with increasing acidity. On the alkaline side, the effective deaminase activity increased slowly with increasing alkalinity and reached a near-to-maximum activity at a pH of about 7.5. These results imply, then, that the demonstrated variations of pH that occur in the ruminal ingesta[84] likely will influence

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[82]Temperature apparently is another factor that may have an influence on the formation of the adaptive enzymes of bacteria. Gale (126) states that, although little work has been done on this effect of temperature, it is known that often decarboxylases are formed in greater amount when growth takes place at low temperatures than when growth takes place at 37° C.

[83]See page 175 for a discussion of the influence of the pH on the ionic form of amino acids.

[84]Two examples are presented here to illustrate how the pH of the ruminal contents can vary, especially as it is influenced by the diet. Elsden and Phillipson (109), in writing of the influence of diet on the pH in the rumen, state: "The acidity is greatest . . . when sheep graze young summer grass; then the average pH is approximately 6, in contrast to an average figure of 6.5 with animals that are stall fed." Reid et al (320) found that the pH in the rumen declined below 5.0 when sheep were fed on rations which contained a high proportion of wheat starch.

the degree of decarboxylation and deamination of amino acids in the

ruminal ingesta and, therefore, will influence the fate of the amino

acids in the rumen.[85]

Carbon Dioxide and Oxygen.- The percentage distribution of the

ruminal gases, mostly carbon dioxide and methane, varies little with

different diets (102). The composition and total volume of the gases

in the rumen can vary considerably, however, with the time that has

elapsed after feeding (74, 313, 401). During the first few hours after

feeding, the percentage volume of carbon dioxide increases in the rumen:

in one experiment, from approximately 48 per cent up to 65 per cent. The

percentage volume of carbon dioxide then gradually falls off until a

relatively low level is reached: down to approximately 22 per cent at

20 hours after feeding. One of the gases which demonstrated a percentage

volume increase in this period of carbon dioxide decrease was oxygen:

from approximately 0 per cent at about 7 hours to approximately 7 per

cent at about 21 hours. Broberg (50, 51) states that not only does

oxygen enter the rumen during rumination, but that large quantities of

the gas enter the rumen during the consumption of feed. Now it is likely

that these variations in the tensions of carbon dioxide and oxygen will

have an influence upon the metabolic activities of the ruminal micro-

[85]Just recently, Reis and Reid (321) have obtained results which
appear to confirm this statement. They found, on incubating casein
hydrolysate in ruminal liquor for four hours, that there was an optimum
pH for the production of ammonia from the amino acids of the hydrolysate.
The optimum pH varied between 6.0 and 6.7, the optimum being dependent
upon the diet of the animal from which the ruminal liquor was obtained.
On the acid side of the optimum pH, Reis and Reid found the production of
ammonia to fall off rapidly. The authors suggest that these results are
a reflection of the effect of pH on the production and activity of the
microbial deaminase. As the present author has done (see p. 218), they
cite work which has demonstrated that pH does affect the production and
activity of microbial deaminases.

organisms. For example, carbon dioxide has been demonstrated to affect the aforementioned (see p. 217) influence of temperature on the ability of microorganisms to synthesize their amino acid requirements. Borek and Waelsch (45) found that the incubation of the microorganisms in an atmosphere rich in carbon dioxide could, in many cases, reverse the effect that a temperature rise has in increasing the amino acid requirements of microorganisms. In the case of oxygen, the tension of this gas has been demonstrated to affect bacterial deaminase production (126): the production of those enzymes that utilize oxygen in their actions are suppressed by anaerobic conditions; the production of those enzymes that do not utilize oxygen in their actions is enhanced in anaerobic conditions. Further, increased oxygen tension has been demonstrated to inhibit amino acid decarboxylase synthesis in fecal cultures from rats (253). These results suggest that the demonstrated variations in the carbon dioxide and oxygen tensions in the rumen will influence the fate of the amino acids in the rumen.

Influence of Diet on the Composition of the Population of Microorganisms in the Rumen and, therefore, on the Fate of Amino Acids in the Rumen

It is suggested that there are at least three mechanisms by which variations in the diet of the ruminant can affect the population of ruminal microorganisms:

1) by producing, within each species of ruminal microorganisms, the "discontinuous variations" of Dubos (101);

2) by influencing, within each species of ruminal microorganism, the production of the "adaptive enzymes" (101);

3) by producing, within the population of ruminal microorganisms, qualitative and quantitative variations.

Discontinuous variations.- The "discontinuous variations" of Dubos refer to the property of a single strain of a microorganism to "adapt" or "train" to a new set of environmental conditions. Dubos cites as an example E. coli mutabile, "a coliform organism which does not at first attack lactose when seeded into a lactose broth medium, but which begins to ferment the sugar only after a lapse of days or even weeks." This type of adaptation is considered to be the result of the new lactose environment bringing about the natural selection of lactose-fermenting variants which occur under normal conditions in the parent culture. It is to be suspected that qualitative changes in the diet of the ruminant would produce such "discontinuous variations" within at least some of the members of the ruminal population of microorganisms.

Adaptive enzymes.- The "adaptive" enzymes of a microorganism, in contrast to "constitutive" enzymes, depend for their production upon the presence of homologous substrate in the medium. "They appear without delay when the cells of the proper microbial species start multiplying in a medium containing the specific substrate; the specific enzymic activities reach their maximum development during growth of the very first transfer into the specific media, and the enzymes again fail to accumulate as soon as the cultures are transferred to media lacking the specific substrates" (101). It is to be suspected that qualitative changes in the diet of the ruminant would bring about the initiation or the cessation of adaptive enzyme formation among at least some of the members of the ruminal population of microorganisms.

Population variations.- Many workers have presented evidence to demonstrate that the qualitative and quantitative distributions of the different kinds of ruminal microorganisms can vary with the type of diet

(24, 53, 56, 127, 128, 168, 188, 228, 258, 306, 320, 373, 386, 412).

Wan der Wath (386), for example, has demonstrated that different bacteria preferentially attack different substrates in the rumen at the same time; therefore, if the ratio of the substrates in the diet is altered, the ruminal facies also should change. Moir (258) noted a similar influence of diet on the character of the ruminal population. He attributed this influence to variations occurring in the chemical composition of graze material--especially, the variations of the protein content (see also 260). Williams et al (412) have demonstrated that the concentration and types of microorganisms in the rumen of sheep are affected by the levels of dietary protein and dietary starch, as well as by the proportion of these two dietary constituents to one another. On low-protein diets, the addition of more starch to the diet was found to cause a reduction in the number of ruminal microorganisms. There also was a change in the types of microorganisms present: from a predominance of gram-negative microorganisms to an increased proportion of gram-positive microorganisms[86]. When the protein content of the diet was high no such effect was obtained. In diets containing starch, the addition of more protein caused the count of ruminal microorganisms to increase. Raising the nitrogen intake from 2.3 to 9.5 grams per day more than doubled the bacterial count: from 21 x 10^6 cells to 44 x 10^6 cells per cubic millimeter. In antelope, Van der Wath and Myburgh (387) found eight times as many infusoria in the ruminal ingesta when the animal browsed as when it grazed. The protein and carbohydrate content of the browse material was relatively high

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[86]Van der Wath (386) has demonstrated the starch-digesting bacteria to be gram-positive microorganisms.

compared to the protein and carbohydrate content in the graze material. Although other examples could be cited, the previous material demonstrates the effect that variations in the diet of the ruminant can have on the qualitative and quantitative distributions of ruminal microorganisms.

Comment.-[87] The previous discussion suggests that qualitative and quantitative variations in the diet of a ruminant can affect, by at least three mechanisms, the population of ruminal microorganisms. The resulting alterations of this population should cause, in turn, alterations in the metabolism or fate of the amino acids in the rumen. Warner (400), for example, has demonstrated that the power of suspensions of ruminal microorganisms to produce deaminases varies with the composition of the diet; that the presence of deaminases is dependent on the presence of readily-attacked protein in the diet of the animal from which the microorganisms were taken (see also 110, 213). Since Gale (126) suggests that deaminases are adaptive enzymes, this experimental finding of Warner could be an example of diet variation affecting the population of micro-organisms by the second mechanism: by influencing the production of adaptive enzymes. Actually, however, either one or both of the other two mechanisms also could have been operative in his experiment. The.

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[87]The three mechanisms that have been discussed in the above text probably account for the demonstrated requirement of ruminants for a period of adaptation when their diet is changed. Sheep, for example, need a week of adaptation to a diet that contains a relatively high proportion of starch in order to be able to digest it with maximal efficiency (386; see also 168, 320). Sheep also require time to become adapted to a diet that contains a relatively high proportion of protein (168). Further, lambs have been demonstrated to require two to three weeks to become adapted to the maximum utilization of diets that contain various non-protein nitrogen compounds (145). See also the discussion by Hoflund et al (168).

relationship that was observed may have been the result of the action of all three mechanisms: discontinuous variation, increased production of an adaptive enzyme, and qualitative and quantitative population variations.

Influence of the Physical and Chemical Nature of the Dietary Constituents on the Fate of Amino Acids in the Rumen

The physical and chemical nature of the dietary constituents should have an influence on the nitrogen metabolism of the ruminal ingesta. The solubility,[88] the surface area, and the type of the proteins in the diet will help to determine, for example, what proportion of the protein will be converted into amino acids and what proportion into ammonia. To illustrate, McDonald (240) found an increase in the ammonia concentration of the ruminal liquor when plant protein, in the form of meadow hay, and readily-soluble animal proteins, in the form of casein or gelatin, were fed. Similarly, El-Shazly (110) found that the ability of a washed-suspension of a mixed population of ruminal microorganisms to decompose a mixture of amino acids was "strikingly increased" by increasing the content of soluble protein in the diet of the animal from which the micro-organisms were taken (see also 213, 400). On the other hand, McDonald (240) found that the ammonia concentration in the ruminal liquor did not increase[89] when relatively-insoluble animal protein, in the form of zein,

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[88]Of interest here is the fact that the solubilities of the proteins in leaves probably are affected by the drying of hay-making. Likewise, the solubilities of the proteins in blood, meat, and fish meals probably are affected by the heating procedure to which these substances often are subjected. In both cases the effect on solubility apparently is the result of the proteins undergoing denaturation (60, 62).

[89]In fact, there actually was a decrease in the ammonia concentration. McDonald interpreted this result as indicating that the rate of proteolysis of zein by the ruminal population is slower than the capacity

was fed. These examples (see also 60, 61, 111, 128, 168, 287) indicate
that the physical and chemical nature of the dietary constituents play
a role in determining the fate of amino acids in the rumen.

Influence of the Relative Stability of the Amino Acids on their Fate in the Rumen

Gale (126) states that the ability of microorganisms enzymatically
to attack amino acids varies widely with the species. He notes that many
gram-positive microorganisms, such as lactobacillus, streptococcus, and
staphylococcus, are quite limited in their ability to attack or synthesize
amino acids. In contrast, many of the gram-negative microorganisms, such
as proteus, pseudomonas, aerobacter, and escherichia, are able to attack
a wide range of amino acids. Even these latter organisms, however, do
not always attack amino acids. In the light of these results, the
question can be raised as to the stability of the different amino acids
when they are present in a mixed population of ruminal microorganisms.
Such knowledge should help in determining what is the fate of the amino
acids in the rumen.

Several groups of workers (110, 213, 279, 345, 346) have investi-
gated the stability of amino acids in the presence of mixed cultures of
ruminal microorganisms. Sirotnak and co-workers (345, 346) studied the
degradation of amino acids, each amino acid being incubated separately
for 72 hours in vitro with mixed suspensions of ruminal microorganisms.
The temperature and pH of incubation were 39° C. and 6.9, respectively.

of the population to utilize the products of proteolysis and that already-
available ammonia was used by the microorganisms as an additional source
of nitrogen for their growth.

Of the 22 amino acids tested, only aspartic acid, glutamic acid, serine,

arginine, cystein, and cystine were deaminated or decarboxylated. The

rate of dissolution of aspartic acid was five times greater than for

the others. In the 72 hour period, the ammonia yields of aspartate, gluta-

mate, serine, and cystein approximated to the theoretical possible

yields. Arginine and cystine each demonstrated an ammonia production of

only about 50 per cent of the theoretical. Sirotnak and co-workers

further found that 5 micromoles was the maximum amount of aspartic acid

that could be broken down in two hours; that is, there was a maximum

dissolution rate. Work of perhaps greater applicability to the conditions

found in the rumen are the in vitro experiments of El-Shazly (110). This

experimenter determined the dissolution activity of washed-suspensions of

a mixed population of ruminal microorganisms on a mixture of amino acids

that was in the form of casein hydrolysate. Such a procedure allows for

the occurrence of interactions between the amino acids in the presence

of microorganisms (124, 362), a definite possibility in the ruminal

fermentations. The results demonstrated that at least some of the amino

acids are susceptible to deamination and decarboxylation by a population

of ruminal microorganisms. For incubation periods of 1 3/4 hours,

El-Shazly found the dissolution of the amino acids to be between 16 and

22 per cent of the total alpha amino nitrogen present. For incubation

periods of 3 hours, he found the dissolution to be about 70 per cent.

In further experiments of the same type as just described, except that the

amino acid mixture was incubated with ruminal liquor[90] instead of a

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[90]The term ruminal liquor--i.e., the 'rumen liquor' of Pearson and
Smith (286a)--designates a filtrate of ruminal contents that is obtained
by the use of muslin or cheesecloth. The filtrate contains microorganisms
and the smaller plant fragments.

washed suspension of microorganisms, El-Shazly determined chromatograph-
ically the disappearance or reduction in concentration of different amino
acids over a period of 3 hours. He found tyrosine, phenylalanine, proline,
alanine, threonine, glycine, serine, and aspartic acid to disappear or
decrease in concentration quite rapidly; glutamic acid was "considerably
weakened while the remaining spots were relatively strong, although
obviously weaker than the corresponding spots from C [control not
incubated] ." These results, as well as the similar results of Lewis
(213) and Otagaki et al (279), demonstrate that at least some of the amino
acids are susceptible to deamination and decarboxylation by a population
of ruminal microorganisms. This means that some amino acids are less
stable than others in the presence of ruminal microorganisms and that
this stability or lack of stability of the amino acids will influence
their fate in the rumen.[91]

Special Comment.- Of the amino acids used in the present ruminal
fistula experiments--tryptophan, methionine, tyrosine, glycine, and
lysine--, only tyrosine and glycine have been demonstrated to be utilized
extensively by a mixed population of ruminal microorganisms. Of particu-
lar significance here is the fact that the other three amino acids--trypto-
phan, methionine, and lysine--were stable in the presence of ruminal
microorganisms for at least as long as three hours. Further, the results
of El-Shazly demonstrated only between 16 and 22 per cent dissolution of

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[91]Perhaps it is appropriate at this point to emphasize that the
power of the ruminal microorganisms to cause the dissolution of amino
acids probably will vary with the environmental conditions in the rumen
(see discussion beginning on p. 216), as well as with the qualitative and
quantitative nature of the diet (see discussions beginning on p. 220 and
on p. 224).

a mixture of amino acids in 1 3/4 hours. These results mean that many of the amino acids present in casein hydrolysate demonstrate stability in the presence of ruminal microorganisms--at least, for a sufficiently long period of time to allow part of the amino acids to pass out of the rumen, either with the ingesta as it passes to more distal parts of the alimentary tract or by absorption through the ruminal wall.[92] This interpretation lends support to the prediction that was made previously (p. 212): that, with suitable methods of experimentation, the absorption of amino acids from the ingesta-filled rumen will be demonstrated.

Influence of the Physiological State of Activity of the Animal on the Fate of Amino Acids in the Rumen

No attempt will be made here to present a detailed analysis of the influence of the physiological state of an animal on the fate of amino acids in the rumen. Rather, a brief delineation will be presented to indicate the many factors involved:

1. the state of activity of the animal in the formation of its highly buffered saliva and in the secretion of bicarbonate and phosphate into the rumen--the products of these functions help to determine the pH of the ruminal ingesta and, therefore, the state of activity of the animal in these functions influences indirectly the fermentative actions of the ruminal microorganisms on the amino acids;

2. the state of activity of the animal in the absorption from the rumen of the volatile fatty acids--this function helps to determine the

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[92]Lofgreen et al (216) also have suggested that varying amounts of amino acids may be absorbed from the rumen before they can be acted upon by the ruminal microorganisms. See also footnote 78 on p. 207.

pH of the ruminal ingesta and, therefore, the state of activity of the

animal in this function influences indirectly the fermentative actions

of the ruminal microorganisms on the amino acids;

3. the state of activity of the animal in moving the ruminal ingesta

from the rumen to the more distal parts of the digestive tract--this

function serves to remove the amino acids from the fermentative and

absorptive processes of the rumen and, therefore, the state of activity

of the animal in this function influences the rate of passage of the

ingesta from the rumen;

4. the state of activity of the animal in the ruminal wall contrac-

tions--this function serves to mix and churn the ruminal ingesta and,

therefore, the state of activity of the animal in this function, by

influencing the rate and vigor of the ruminal wall contractions, influences

indirectly the fermentative actions of the ruminal microorganisms on the

amino acids;

5. the state of activity of the animal in the blood and lymph flow

through the capillaries of the ruminal wall--this function serves to remove

the absorbed amino acids from the ruminal wall and, therefore, the state

of activity of the animal in this function influences the rate of absorp-

tion of amino acids from the rumen.

The states of activity just presented are all influenced, in turn, by the

physiological age[93] of the animal, by the state of activity of the

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[93]Phillips and Gilder (293) have demonstrated in the rat that the
rate of absorption of glucose from the small intestine decreases with
increasing age of the animal.

endocrine organs,[94] and by the state of activity of the nervous system.
These and other factors, each factor playing an important role in determining the physiological state of activity of the animal, in consequence influence directly or indirectly the fate of the amino acids in the rumen.

Comment.

In the previous presentation, an attempt has been made to demonstrate that the distribution of the amino acids in the rumen among the various possible pathways will depend upon the influences of many factors. Although, for ease of presentation, the influence of each factor was considered separately, in actual fact, it is the summation of the interactions of the many factors that determines the distribution of the amino acids. Even if the previous presentation does not permit precise quantitative predictions to be made of the amino acids' distribution in the rumen, the presentation does make it evident that the proportions of the individual amino acids that are absorbed from the rumen will be greater when the interactions of the many factors are such as to produce higher concentrations of the amino acids in the ruminal ingesta. Such higher concentrations of amino acids, occurring together with a metabolically-active

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[94] Desoxycorticosterone appears to influence the membrane permeability not only of the renal tubules but of tissues in general (34). Verzár (396) has reviewed the considerable evidence which exists in support of his contention that hormones of the adrenal cortex play a role in phosphorylating mechanisms, including those that may be concerned in the absorption of glucose from the small intestines and the kidney tubules. The administration of thyroxine to an animal increases the rates of absorption of the monosaccharides from the small intestine (124). Finally, as insulin is known to promote the cellular synthesis of protein from the free amino acids in the blood (221), it is likely that insulin, especially in conjunction with the action of the growth hormone of the anterior pituitary gland, will have an effect on the rate of absorption of amino acids from the small intestine.

ruminal wall and a high rate of flow of blood and lymph through the

ruminal wall, should lead to an increased amount of amino acid absorption.

This increased absorption of the amino acids would be of great nutritional

significance to the ruminant (see p. 267 and on).

Part B: B Vitamins

Manner of Absorption of B Vitamins from the Rumen

If, on the basis of the present results, it can be assumed that one

or more of the B vitamins are absorbed from the rumen, then the question

of the manner of this absorption naturally arises. Following the same

approach utilized for the discussion of amino acid absorption, it is not

unreasonable to assume that the passage of B vitamins across the ruminal

wall might occur in the same manner as their passage across the intestinal

wall. A brief review will be made, therefore, of the literature on

B vitamin absorption from the small intestine.

Literature Review on the Manner of Absorption of B Vitamins from the
Small Intestine

Introduction.— Although it generally is assumed that the B vitamins

are absorbed from the small intestine (32, 201), little evidence is

available as to the manner of absorption of most these vitamins. Appar-

ently, this lack of evidence is, in part, the result of the fact that many

of the B vitamins have been discouvered and synthesized relatively recently.

Only for riboflavin has there been accumulated any considerable evidence as

to its manner of absorption. The evidence available for the other

B vitamins is scanty and inconclusive. Accordingly, the following review

will be confined largely to an examination of the literature that pertains

to the manner of absorption of riboflavin from the small intestine.

Riboflavin.- The early work on the manner of absorption of riboflavin adhered to the same approach that had been utilized to study the manner of absorption of the different sugars. Wilbrandt and Laszt (210, 408) had demonstrated indirectly the ability of a glycerin extract of the mucous membrane of the rat's small intestine to phosphorylate the sugars glucose, galactose, and fructose, but not mannose and xylose. The phosphorylation was shown to be inhibited by the addition of sodium monoiodoacetate.[95] Of special significance was the fact that each of the sugars that was capable of being phosphorylated also had been demonstrated in earlier work to be selectively absorbed from the small intestine, this selective absorption being inhibited by sodium moniodoacetate. Each of the two sugars that was not capable of being phosphorylated had been demonstrated to be absorbed only by the process of simple diffusion, this diffusion-type absorption being uninhibited by sodium monoiodoacetate. Utilizing this same approach for the study of riboflavin, Rudy and Kuhn (206, 328) demonstrated the phosphorylation of lactoflavin (riboflavin). Two other flavins, both closely related chemically to lactoflavin but in-effective as growth stimulants, were not phosphorylated. The implication of these results was that, perhaps, as for the selectively absorbed sugars, the lining epithelial cells of the small intestine were concerned in the selective absorption of riboflavin--and, by means of a phosphoryla-

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[95]Many enzymes are inhibited irreversibly by iodoacetate, "and it is widely assumed that the inhibition involves a combination of the reagent with essential sulfhydryl groups on the enzyme protein" (124).

tion process. In support of this implication, Laszt and Verzár (211) found that, although the addition of sodium monoiodoacetate to the food of young rats inhibited their growth, this inhibition could be prevented by the daily addition to the food of 20 micrograms of phosphorylated lactoflavin. Verzár and McDougall (397), taking cognisance of all these results, stated that the parallelism of the behaviours of glucose and riboflavin strongly suggested that riboflavin, like glucose, was phosphory-lated in the intestinal mucosa. Such a phosphorylation would require the metabolic intervention of the lining epithelial cells--that is, more than the process of simple diffusion would be involved in the absorption of riboflavin by the small intestine.[96]

Although not concerned directly with the manner of riboflavin absorption, the results of recent work on the manner of glucose absorption (89, 116, 158, 159, 191, 266, 278, 381; see also 396) not only provide indirect support for the concept that riboflavin is phosphorylated in its absorption from the small intestine, but also indicate promising future lines of investigation on the manner of riboflavin absorption. To indicate the nature and scope of this recent work that has helped to elucidate the manner of absorption of glucose, some of the results of the work are reviewed briefly here. It has been demonstrated that the intestinal epithelium is the richest source of alkaline phosphatase in the body (396; see also 379). Tuba and co-workers (93, 380, 381, 383) have demonstrated that the concentration of this intestinal alkaline phosphatase is decreas-

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[96]Lust et al (223) have reported that fetal blood (at term) has about four times as much free riboflavin as maternal blood. From their results, they suggest that an active metabolic process is involved in the transfer of riboflavin from maternal to fetal blood by the human placenta.

ed significantly after fasting and is increased significantly after the feeding of carbohydrates, fats and fatty acids, and amino acids. As alkaline phosphatase is known to function in a number of tissues either as a dephosphorylating (124), phosphorylating (254), or transphosphorylating (124, 144, 255, 264) enzyme, Tuba and co-workers suggest that these changes in the concentrations of intestinal phosphatase reflect its participation in a phosphorylation-dephosphorylation mechanism that is concerned with the selective absorption of certain substances by the small intestine. As it is conceived by several workers (155, 397; see also previous discussion on p. 156) for the absorption of glucose, this phosphorylation-dephosphorylation mechanism would function in the following manner: glucose first is phosphorylated by an enzyme-catalyzed reaction as the glucose diffuses into the epithelial cells and, then, the glucose-phosphate compound is dephosphorylated before it diffuses from the epithelial cells into the portal blood. The recent work of Ota and Shibata (278) provides experimental support for this concept. Their results suggest that the phosphorylation of the selectively absorbed sugars occurs on the mucosal surface of the intestinal epithelial cells and that dephosphorylation occurs before the sugars pass from the cells into the portal blood. Further experimental support is provided by the results of Fehér et al (116). They found that the administration of enzyme poisons sharply reduced the concentration of adenosine triphosphate (ATP) in the intestinal mucosa if, at the same time, glucose was being absorbed from the small intestine. As the enzyme poisons that were utilized--iodoacetic acid, sodium fluoride, or 2:4 dinitrophenol--are those known to prevent directly or indirectly the resynthesis of ATP (see 89, 124), the implication of these results is that the decrease in the

concentration of ATP was the result of its being utilized in the phosphory-
lation of glucose. These results, and other results not cited, provide
strong support to indicate the presence of a phosphorylation-dephosphoryla-
tion mechanism in the lining epithelial cells of the small intestine. In
view of the previously demonstrated similarities between the mechanisms
of absorption for glucose and riboflavin, there is a possibility that these
additional results obtained on the manner of glucose absorption also may
be of value for gaining an understanding of the manner of absorption of
riboflavin. There does not appear to have been any attempt, however, to
extend these sugar experiments to include the study of riboflavin absorp-
tion. The one exception to this latter statement is the work of Bourne
(48). This author demonstrated, by histochemical methods, the dephos-
phorylation of riboflavin and pyridoxal phosphates in various tissues,
including the epithelial cells in the jejunal part of the small intestine.
Bourne suggests:

> It seems likely that we are dealing here with a phosphorylating/
> dephosphorylating or transphosphorylating system which may be
> concerned with the process of synthesis of the phosphates of
> these vitamins and that under the conditions described in this
> paper the system dephosphorylates.

This evidence of Bourne supports the concept that riboflavin is absorbed
from the lumen of the small intestine by a phosphorylation process.

Thiamine.-- Very little work has been carried out on the manner of
intestinal absorption of thiamine. Verzár (395) proposed that thiamine
is phosphorylated in its absorption from the small intestine. Several
workers (327, 370, 371) have demonstrated the in vitro phosphorylation of
thiamine when they utilized dried powdered intestinal mucosa or glycerol
extracts of the intestines. Ochoa (274), however, was unable to demon-

strate such phosphorylation when he utilized an acetone preparation of the intestinal mucosa. More recently, Magyar (224) has reported that the absorption of thiamine from the intestine greatly was impaired if unphysiologically large doses of the other B vitamins or glucose were administrated subcutaneously before the absorption of thiamine was tested. Magyar's explanation of these results was that the prior administration of the B vitamins or glucose in such large amounts produced exhaustion of the phosphorylating apparatus in the bodily tissues--including the epithelium of the small intestine. Such an effect on the phosphorylating apparatus in the intestinal epithelial cells probably would reduce the intestinal absorption of thiamine, if the latter is absorbed in part or wholly by being phosphorylated. As, under the conditions of Magyar's experiments, the intestinal absorption of thiamine was reduced, his results support the concept that thiamine is phosphorylated during its absorption from the small intestine. Although scanty, most of the results obtained to-date do suggest, then, that more than the process of simple diffusion is involved in the absorption of thiamine from the small intestine.[97]

Other B Vitamins.-- Although it generally is assumed that the other B vitamins gain entry into the body by being absorbed from the small intestine (32, 327), almost no work has been done on the manner of absorption of these vitamins from the small intestine. For pyridoxine, the work of Bourne (48), on the histochemical demonstration of the dephosphorylation of pyridoxine phosphate in the epithelial cells of the

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[97]Slobody et al (348) have reported that fetal blood (at term) has about twice as much thiamine as maternal blood. The authors suggest that perhaps the placenta selectively transfers thiamine to maintain this concentration gradient.

jejunum, provides suggestive evidence for the manner of absorption of

pyridoxine. For pantothenic acid, results have been obtained (80) which

demonstrate the in vitro action of an intestine phosphatase preparation

in the reversible synthesis of co-enzyme A and intermediate compounds.

These results may be indirect evidence that also pantothenic acid is

phosphorylated during its absorption from the small intestine. In fact,

these results for pyridoxine and pantothenic acid fit in with the

suggestion that has been made by Beerstecher (32) as to the manner of

absorption of the B vitamins other than riboflavin and thiamine. By

analogy with the manner of absorption proposed for riboflavin and

thiamine—i.e., primarily by means of a phosphorylation mechanism—,

Beerstecher suggests that possibly those other B vitamins that can form

phosphoric acid esters will undergo phosphorylation as a part of their

absorption from the small intestine. This suggestion at least could apply

to pantothenic acid, nicotinic acid, and the B_6 group of vitamins—that is,

these vitamins or compounds containing them are known to be phosphorylated

in the cells of the animal body (124).

Comment.— The above review points up the scarcity of evidence that

is available in the literature on the manner of absorption of the B vita-

mins from the small intestine. The fragmentary evidence available does

provide, however, some support for a gradually developing concept: the

involvement of a phosphorylation-dephosphorylation mechanism in the

absorption of the B vitamins from the lumen of the small intestine. The

following statement by Beerstecher (32) can serve as a comment upon

the present state of knowledge of the absorption of B vitamins from the

small intestine:

> The state of knowledge of the processes involved in the absorption of the individual B-vitamins is to a large extent a function of the time that the B-vitamin in question has been well recognized. The more explicit information which is available concerning thiamine and riboflavin absorption indicates clearly that the absorption of the B-vitamins cannot be regarded as a simple process, even though it is frequently assumed to be for the more recently discovered vitamins. Passage of a metabolite across a living membrane seldom is a matter of passive transfer or simple diffusion. This is certainly a good generalization for the B-vitamins, even in view of the lack of much experimental data to verify the assertion.

It is to be wondered if the manner of absorption of some or all the B vitamins may not be the same in the rumen as in the small intestine. The data of the present ruminal fistula experiments will be examined, therefore, to see if there is any evidence to indicate that the absorption of the B vitamins from the rumen involves more than the process of simple diffusion.

Evidence from the Present Experiments as to the Mechanism of Absorption of B Vitamins from the Rumen

Introduction.- From the information provided in the literature review on the manner of absorption of the B vitamins from the small intestine, it appears that metabolic intervention by the lining epithelial cells of the intestinal wall may occur in the absorption of the B vitamins. Although the present experiments were not designed to elucidate the manner of absorption of the B vitamins from the rumen, nevertheless the data will be examined to determine if any evidence exists for a similar metabolic intervention by the lining epithelial cells of the ruminal wall occurring in the absorption of the B vitamins.

The same procedure of examination that was applied previously to the data for the amino acids (see p. 175 and on) will be applied to the data for the B vitamins. It is necessary, however, first to determine what

effect the demonstrated electrical gradient of the ruminal wall likely

will have on the ruminal absorption of the B vitamins. In the present

experiments--that is, in experiments W1 to W6--the pH of the ruminal

solutions ranged between 6.5 and 7.6 (see Table 17, p. 87). Within this

range of pH, the B vitamins riboflavin, nicotinic acid, and pantothenic

acid should exist, at least in part, as negatively charged ions. In the

case of riboflavin, this statement follows from the fact that its iso-

electric point is at pH 6 (327). For nicotinic acid and pantothenic acids,

the above statement follows from the fact that the carboxyl groups of

these two acids form negatively charged prosthetic groups by ionization--

and the degree of ionization becomes greater with increasing pH.[98] As

the electrical gradient across the ruminal wall is orientated with the

blood side positive and the ruminal side negative, the movement of the

B vitamin anions across the ruminal wall should be accelerated by the force

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[98]The ionization constants of nicotinic and pantothenic acids are
1.4×10^{-5} (167) and approximately 3.9×10^{-5} (409, 410), respectively.
Such ionization constants signify that, at the range of pH used in the
present experiments--pH 6.5 to 7.5--, nearly all the nicotinic or pantothen-
ic acid molecules would exist in the ionized state. This follows from a
consideration of the equation for the ionization constant of an acid:

$$\frac{[H^+]\ [A^-]}{[HA] - [A^-]} = K$$

This equation can be rewritten as:

$$\frac{[A^-]}{[HA]} = \frac{K}{[H^+] + K}$$

At pH 7, an acid with an ionization constant of 2×10^{-5} has
$2.00 \times 10^{-5}/2.01 \times 10^{-5}$ or 0.995 of its molecules in the ionized form.

of the electrical gradient. The analyses of the B vitamin data to be made will consider, therefore, the effect of the electrical gradient—that is; as it was done in the analyses of the amino acid data.

Effect of Initial Electrochemical Gradient on Rate of Absorption.—
It was pointed out previously for the data of the amino acids (pp. 178 and 179) that, for several reasons, any interpretation of the relation found for an amino acid between its initial electrochemical gradient and its rate of absorption might be of doubtful significance. The same doubt exists in the present analyses of the data for the B vitamins—and for the same reasons. The analyses will be made, nevertheless, in the hope of gaining some information and to point out the way for future experimentation. Table 34 presents, for each the three B vitamins measured, the estimated initial electrochemical potential differences, the absolute decreases of concentration, and the decreases of concentration relative to the estimated initial electrochemical potential differences. The calculation of the electrochemical gradients for the B vitamins was made in the same manner as for the amino acids: the 30 millivolt number of Dobson and Phillipson (98) was utilized for the electrical gradient; the initial ruminal solution concentrations of the B vitamins that are recorded in Tables 14a to 16b (pp. 71, 73 , 77 , 78, 81, and 82) and the plasma concentrations of the same B vitamins that are recorded as a part of Table 35 were utilized in the calculation of the chemical gradient. For riboflavin and nicotinic acid, the plasma data given for man[99] were

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[99]Unfortunately, plasma concentrations of riboflavin and nicotinic acid are not available for the goat or other ruminants. As a substitute, the plasma concentrations of riboflavin and nicotinic acid in man are used. The similarity of the blood concentrations of each these two B vitamins in man and sheep (see the last two columns of Table 35) suggest that the use of the plasma concentrations of man as a substitute for the plasma concentrations of goat is probably valid.

TABLE 34. - Ruminal fistula experiments W1, W2, W4, W5, and W6: a tabulation, for each the three B-vitamins whose concentrations were measured, of estimates of the initial electrochemical potential differences (millivolts), of the absolute decreases of concentration (microgram-moles per 100 milliliters), and of the decreases of concentration relative to the initial electrochemical potential differences (per cent)

Experiment	Riboflavin					Nicotinic acid					Pantothenic acid				
	Initial e-c p.d.	Concentration decrease				Initial e-c p.d.	Concentration decrease				Initial e-c p.d.	Concentration decrease			
		0 to 70 minutes[a]		Variable[b]			0 to 70 minutes[a]		Variable[b]			0 to 70 minutes[a]		Variable[b]	
		Absolute	Relative	Absolute	Relative		Absolute	Relative	Absolute	Relative		Absolute	Relative	Absolute	Relative
W2	188	...	...	1.5 (0 to 125 minutes)	0.8	...	...	...	...	...	...	...	...	...	...
W1	199	...	...	1.9 (0 to 120 minutes)	1.0	90 to 139	...	...	8.0	5.8 to 8.9 (0 to 120 minutes)	128 to 140	...	...	1.6	1.1 to 1.3 (0 to 120 minutes)
W5	199	1.0	0.5	...	...	107 to 156	5.1	3.3 to 4.8	...	...	133 to 145	0.4	0.0	...	...
W6	218	1.7	0.8	5.1 (0 to 150 minutes)	2.3	135 to 184	3.4	1.9 to 2.5	9.9	5.4 to 7.3 (0 to 150 minutes)	144 to 155	3.0	1.9 to 2.1	...	...

TABLE 34 - continued

Experiment	Riboflavin					Nicotinic acid					Pantothenic acid				
	Initial e-c. p.d.	Concentration decrease				Initial e-c. p.d.	Concentration decrease				Initial e-c. p.d.	Concentration decrease			
		0 to 70 minutes[a]		Variable[b]			0 to 70 minutes[a]		Variable[b]			0 to 70 minutes[a]		Variable[b]	
		Absolute	Relative	Absolute	Relative		Absolute	Relative	Absolute	Relative		Absolute	Relative	Absolute	Relative
W4	222	4.5	2.0	4.0 (0 to 150 minutes)	1.8	...	...	...	...	...	161 to 173	5.9	3.4 to 3.7	4.9	2.8 to 3.0 (0 to 150 minutes)

[a]The concentration decreases that are recorded for each experiment are those decreases which occurred in the first 70 minutes of the experiment.

[b]The concentration decreases that are recorded for each experiment are those decreases which occurred during the experimental time intervals that are indicated by the data in the parentheses.

TABLE 35. - Ruminal fistula experiments W1, W2, W4, W5, and W6: a comparison, for each the three B vitamins whose concentrations were measured, between the lowest-found concentrations in the ruminal solutions and the concentrations in the plasma and blood (all concentrations are expressed as microgram-moles per 100 milliliters of solution)

Vitamin	Ruminal solution in experiment[a]					Plasma[b]		Blood[b]	
	W1	W2	W4	W5	W6	Sheep	Man	Sheep	Man
Riboflavin	2.7	2.3	14.9	4.5	10.7	...	0.007 to 0.01	0.06 to 0.08	0.04 to 0.16
Nicotinic acid	5.7	...	...	24.0	58.1	...	0.20 to 1.22	3.25 to 11.37	1.62 to 7.31
Pantothenic acid	3.8	...	13.6	7.0	8.4	0.09 to 0.14	0.03 to 0.16	0.09 to 0.23	0.07 to 0.21

[a]These data, the lowest-found concentrations, are taken from Tables 14a to 16b (pp. 71, 73, 77, 78, 81, and 82).

[b]These data, from Albritton's Standard Values in Blood (8), are the lower and upper limits of the 95 per cent range of concentrations that have reported in the literature.

used; for pantothenic acid, the plasma data given for sheep were used. In the case of riboflavin, because of range of concentrations of this vitamin in the plasma is so narrow and so close to one unit, a concentration of 1×10^{-2} micromoles per 100 milliliters was assumed for the plasma concentration. For pantothenic and nicotinic acids, the lowest- and the highest-found plasma concentrations were utilized in order to calculate the upper and lower limits of the possible concentration differences; hence, in Table 34 two numbers are given for the electrochemical gradients acting on these two vitamins. The data in Table 35 for the absolute rates of decrease of B vitamin concentrations were corrected for the concentration changes that occurred in the control solutions. This correction procedure was done in the same manner as for the amino acids (see p. 185).

Examination of the riboflavin data in Table 34 does not reveal any uniform effect of the initial electrochemical potentials on the rates of absorption of riboflavin. The lack of a consistent effect in these data in part may be due to animal variability, but also to the fact that the differences among the initial electrochemical potentials were not large enough to produce a striking difference among the rates of absorption. The nicotinic acid data in Table 34 likewise do not permit any definite conclusion to be made about the effect of initial electrochemical potentials on the rates of absorption. In these data, there are over-lappings of the ranges of the possible initial electrochemical potentials. These overlappings make it difficult to draw any conclusions. On the other hand, the pantothenic acid data in Table 34 demonstrate the following relation between the initial electrochemical potentials and the rates of absorption of pantothenic acid: the absolute rates of

absorption are proportional to the initial electrochemical potential differences. These just-described results for the three B vitamins do not yield too much information. The data for riboflavin and nicotinic acid do not provide any evidence to elucidate their manner of absorption from the rumen. The pantothenic acid data do indicate, however, that the ruminal absorption of this vitamin is at least partly due to the effect of the chemical potential difference--assuming the electrical potentials to have been approximately the same in each the experiments--and that, therefore, simple diffusion forms at least a part of the mechanism concerned in this absorption. Other than this, the present analysis permits nothing more to be stated as to the manner of absorption of B vitamins from the rumen.

Comparison Among the Three B Vitamins of their Rates of Absorption.-
The data of Table 34 have been rearranged in Table 36. Table 36 also includes the molecular weights of the three vitamins. Examination of the data in the table indicates a probable influence of the molecular size of the molecules on their rates of absorption: in most the experiments, the smaller molecules usually demonstrate more rapid absorption, even although the initial electrochemical gradients are often such as to favour the larger molecules. These results suggest that the process of simple diffusion plays an important role in the absorption of the B vitamins from the rumen. Whether or not there also is metabolic assistance by the lining epithelial cells of the ruminal wall can not be stated from the present data. Further experimentation, with more appropriately designed experiments, is required.

Transfer of the B Vitamins Against an Electrochemical Gradient.- As it already has been pointed out for the amino acids (see p. 200), the

TABLE 36. - Ruminal fistula experiments W1, W4, W5, and W6: a tabulation of data that are utilized in the text (p.245) to determine the influence of the molecular size of each the three vitamins on their rates of ruminal absorption

Experiment[a]	B-vitamin	Molecular weight[b]	Estimated initial electro-chemical potential difference (millivolts)	Concentration decrease[c]	
				Absolute (microgram-moles per 100 milliliters)	Relative (per cent)
W1 (120 min.)	Riboflavin	376.4	199	1.9	1.0
	Pantothenic acid	219.2	128 to 140	1.6	1.1 to 1.3
	Nicotinic acid	123.1	90 to 139	8.0	5.8 to 8.9
W4 (70 min.)	Riboflavin	376.4	222	4.5	2.0
	Pantothenic acid	219.2	161 to 173	5.9	3.4 to 3.7
W5 (70 min.)	Riboflavin	376.4	199	1.0	0.5
	Pantothenic acid	219.2	133 to 145	0.4	0.3
	Nicotinic acid	123.1	107 to 156	5.1	3.3 to 4.8

TABLE 36 - continued

Experiment[a]	B-vitamin	Molecular weight[b]	Estimated initial electro-chemical potential difference (millivolts)	Concentration decrease[c]	
				Absolute (microgram-moles per 100 milliliters)	Relative (per cent)
W6 (70 min.)	Riboflavin	376.4	218	1.7	0.8
	Pantothenic acid	219.2	144 to 155	3.0	1.9 to 2.1
	Nicotinic acid	123.1	135 to 184	3.4	1.9 to 2.5
W6 (150 min.)	Riboflavin	376.4	218	5.1	2.3
	Nicotinic acid	123.1	135 to 184	9.9	5.4 to 7.3

[a]The data in parentheses designate the time intervals during which the recorded concentration decreases occurred.

[b]These data, rounded off to one decimal place, are from the Handbook of Chemistry and Physics (167).

[c]These data are taken from Table 35 on page 196.

demonstration of a net movement of a substance against an electrochemical

gradient would be considered as strong evidence for the existence of

one or more other forces being concerned in the movement of the substance.

Unfortunately, the B vitamin concentrations that were used in the present

experiments were so high that in no case was there any possibility of

testing for movement of the vitamins against an electrochemical gradient.

Table 35 records the lowest-found concentrations of the three B vitamins

in the ruminal solution of each experiment, along with the blood and

plasma concentrations reported in the literature for man and sheep. In

every case, the ruminal solution concentration was so high relative to

the plasma concentration that, along with the fact that the electrical

gradient also was orientated from ruminal solution to plasma, the absorp-

tion of the B vitamins always was with the electrochemical gradient. The

present data, therefore, yield no evidence as to whether or not the

lining epithelial cells of the ruminal wall are able to move the B vita-

mins against an electrochemical gradient.

Comment.- The purpose of the previous analyses was to determine if

any evidence existed in the data of the ruminal fistula experiments as

to the manner of absorption of the B vitamins from the rumen. Un-

fortunately, these analyses yielded little information, other than to

suggest that the process of simple diffusion is involved. The nature

of the data was not such as to yield evidence for or against the

involvement of metabolic energy--such as might be utilized by phosphory-

lating enzymes--in the absorption of the B vitamins.[100] As for the amino acids, further experimentation is required to elucidate the manner of absorption of the B vitamins from the rumen.

<div align="center">

B Vitamin Absorption from the Rumen Under Normal

Feeding Conditions

</div>

Introduction

The same question can be asked for the B vitamins that was asked for the amino acids: is there any evidence to support the concept that the absorption of B vitamins occurs from the rumen under normal feeding conditions. To be able to answer this question, there is required a knowledge of the fulfillment or non-fulfillment of the following conditions:

1) the demonstration of the presence of the B vitamins in the rumen;

2) the demonstration of the relation of the concentration of the individual B vitamins in the ruminal liquor to their concentration in the blood plasma: a rumen to plasma, downward electrochemical gradient would permit the possibility of B vitamin absorption without requiring direct participation of metabolic energy, but would not rule out the possibility of metabolic energy also participating--as, for example, in a phosphorylation of the vitamins--; a plasma to rumen, downward electrochemical

---

[100]It is pertinent to note here that Phillipson and Reid (302) found nearly all the extracellular thiamine present in ruminal contents to be already in the phosphorylated form: thiamine pyrophosphate. Now, as Kon and Porter (personal communication cited in 302) have demonstrated that thiamine is absorbed from the rumen, it is to be wondered whether the thiamine is absorbed as such, as the pyrophosphate, or in both forms. Of interest to this question is the suggestion of Snell (357) that the "cell wall" of some microorganisms is impermeable to thiamine pyrophosphate. On the other hand, Rosenberg (327) states that thiamine can be absorbed from the small intestine in the free or in the phosphorylated form.

gradient would limit any B vitamin absorption to that accomplished directly or indirectly by the force of metabolic energy;

3) the demonstration of the absorption of the individual B vitamins from an ingesta-filled rumen, the concentration of each the individual B vitamins at which this absorption occurs being of the same order of magnitude as that found in the fluid of the ruminal ingesta ordinarily. Each of these points will be discussed in turn.

Condition 1: Demonstration of B Vitamins in the Rumen

The B-complex of vitamins is present in the ruminal ingesta in considerable quantities. For many of the B vitamins, columns 2 and 4 of Table 37 record the concentrations of these vitamins that have been found in the ruminal ingesta of the cow and sheep. The concentrations are recorded as micrograms per gram of ruminal ingesta (dried weight). Columns 3 and 5 of Table 37 record again the data of columns 2 and 4, but this time the data are recalculated so as to express the concentrations in terms of the original ruminal ingesta (wet weight). In making this recalculation, the dry matter content of the original ruminal ingesta was assumed to be 15 per cent (404, 405) and a specific gravity of 1 also was assumed. The recalculated data are expressed as micrograms per 100 milliliters of solution. It is not known what proportion of these wet weight concentrations are present as free vitamins in the ruminal liquor. Other than for thiamine and riboflavin, the present author was unable to find any data recorded in the literature for the concentrations of B vitamins in the ruminal liquor. Phillipson and Cuthbertson (299) do suggest, however, that "in all probability much of the B vitamin content of the rumen is in true solution . . . ." This

TABLE 37. - Concentrations of the B-vitamins in the ruminal ingesta and plasma of the sheep and cow (the plasma concentrations for man are included, since in most cases a knowledge of the plasma concentrations of the B-vitamins in the ruminants is lacking)

B-vitamin	Ruminal ingesta[a]				Plasma[b]		
	Sheep		Cow				
	Original data (micrograms per gram of dry matter)	Calculated data (micrograms per 100 milliliters of wet matter)	Original data (micrograms per gram of dry matter)	Calculated data (micrograms per 100 milliliters of wet matter)	Sheep	Cow	Man
					(micrograms per 100 milliliters)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Riboflavin	33	493	4 to 32	60 to 478[c,c']	...	...	2.6 to 3.7[d]
Nicotinic acid	...	...	15 to 92	224 to 1373[e,e']	...	...	25 to 150[f,g]
Pantothenic acid	70	1045	3 to 56	45 to 836	20 to 30	...	6 to 35
Thiamine	7	104[h]	0.12 to 5	1.8 to 75	3.8[i]	...	1 to 9[i]
Pyridoxine	10	149	2 to 10	30 to 149	...	...	1 to 18[j]

TABLE 37 - continued

B vitamin	Ruminal ingesta[a]				Plasma[b]		
	Sheep		Cow		Sheep	Cow	Man
	Original data (micrograms per gram of dry matter)	Calculated data (micrograms per 100 milliliters of wet matter)	Original data (micrograms per gram of dry matter)	Calculated data (micrograms per 100 milliliters of wet matter)	(micrograms per 100 milliliters)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Biotin	⟩2.6	⟩39	0.08 to 0.31	1.2 to 4.6	. . .	. . .	1.0 to 1.7
Folic acid	. . .	. . .	0.24 to 2.3	3.6 to 34	. . .	0.05[k]	0.05[k]

[a]The "original data" recorded here are selected from data that were found by the author in a search of the literature (6, 177, 179, 192, 193, 194, 202, 208, 243, 244, 245, 246, 247, 404, 405). Only those data that had been determined by a biological method--that is, microbiological, chick, or rat assay--were utilized. The data for the cow represent the lowest and the highest concentrations that were found to be recorded.

[b]These data, from Albritton's Standard Values in Blood (8), are the lower and upper limits of the 95 per cent range of the concentrations that have been reported in the literature. Except for the data of riboflavin, biotin, and perhaps pyridoxine, all the data that are recorded were determined by the microbiological method. Riboflavin and biotin were determined chemically. The method of determination of pyridoxine is not stated.

[c]In a preliminary assay, for which the microbiological procedure of Barton-Wright (30) was utilized, the present author found the concentrations of riboflavin in three samples of ruminal liquor from a goat to be 41, 45, and 53 micrograms per 100 milliliters.

[c']Just recently, the author came across the data of Conrad (76) for the concentration of riboflavin in ruminal liquor: for six samples, the concentration ranged between 62.5 and 165.8 with an average of 112.8 micrograms per 100 milliliters. Although the data of Conrad are too few to draw any valid conclusions, in some of his samples a considerable portion of the riboflavin apparently was in the free state in the ruminal liquor (see also footnote h).

[d]In a microbiological assay (procedure of Barton-Wright (30)) of three plasma samples from one goat and of 4 plasma samples from another goat, the present author found the concentrations of riboflavin to range, respectively, between 2 to 5 and between 1 to 4 micrograms per 100 milliliters.

[e]In a preliminary assay, for which the microbiological procedure of Barton-Wright (30) was utilized, the present author found the concentrations of nicotinic acid in three samples of ruminal liquor from a goat to be 350, 430, and 470 micrograms per 100 milliliters.

[e']Conrad's data (76) for the analyses of two composite samples of ruminal liquor are 395.0 and 563.0 micrograms per 100 milliliters. His data do not indicate what proportion of these concentrations were present as free vitamin in the ruminal liquor.

[f]Total nicotinic acid activity.

[g]In a microbiological assay (procedure of Barton-Wright (30)) of three plasma samples from one goat and of nine plasma samples from another goat (see Table 20, p. 110), the present author found the concentrations of nicotinic acid to range, respectively, between 34 to 48 and between 34 to 72 micrograms per 100 milliliters.

[h]Recently, Phillipson and Reid (302) have recorded the thiamine content in 266 samples removed from a total of 49 sheep that were fed different rations. The concentrations of thiamine ranged between 23 and 189 micrograms per 100 grams of rumen sample. Although the authors found appreciable concentrations of thiamine in the small food particles and microorganisms of the ruminal samples, most of the thiamine was extracellular and existed as thiamine pyrophosphate in solution in the ruminal liquor. Contrary to these findings, Conrad and Hibbs (76, 78) state that the B-complex vitamins in ruminal liquor largely are bound in the microbial cells. The data in Table 35 of Conrad's thesis (76) do not appear to provide the necessary basis for such a statement: only thiamine and riboflavin were measured and even for these two

vitamins the data are too few and too variable. In fact, in one sample (#968) the contents of thiamine and riboflavin in the microorganism-free filtrate were greater than the contents of the vitamins in the ruminal bacteria, the content of thiamine in the filtrate being almost double the content in the bacteria. The author makes no comment on this.

[i]Total thiamine. Rosenberg (327) states that there seems to be a constant level of free thiamine-- i.e., non-phosphorylated thiamine--of about 1 microgram per 100 milliliters of plasma.

[j]These concentrations are for the plasma of monkey.

[k]Free folic acid.

statement appears to be valid for thiamine: in footnote h of Table 37 there is indicated that most of the content of thiamine in the ruminal ingesta is extracellular and present as thiamine pyrophosphate in the ruminal liquor. For riboflavin and nicotinic acid, in footnotes c and e of Table 37 there are recorded the present author's own preliminary data for the concentrations of these two B vitamins in ruminal liquor. Unfortunately, as the ruminal liquor in the author's experiments was a filtrate that resulted from filtering the ruminal ingesta through four layers of cheesecloth, the liquor still contained solids such as small fragments of food and microorganisms. This means that, as some of the measured riboflavin or nicotinic acid content of the ruminal liquor could have been molecular constituents of these solids, the data do not yield a knowledge of the free concentration of riboflavin and nicotinic acid in the ruminal liquor. The data of Conrad (see footnote c' in Table 37) do indicate, however, that possibly a considerable proportion of the riboflavin may be in the free form. More work is required, however, on this point.

Condition 2: Demonstration of an Electrochemical Gradient

For many of the B vitamins, columns 6, 7, and 8 of Table 37 record the concentrations of these vitamins found in the plasma. Where available, the data for ruminants are given, otherwise the data for man are utilized. A comparison in Table 37 between the concentrations of the B vitamins in the ruminal ingesta (wet weight) and in the plasma reveals that the vitamin concentrations in the ingesta are usually much greater than the concentrations in the plasma. If any considerable proportion of these vitamin concentrations that are recorded for the ruminal ingesta

are present in the ruminal liquor in the free state, then these data could be evidence for the existence of downward concentration gradients for the B vitamins in the direction rumen to plasma. Such may be the case for thiamine, as the information contained in footnote h of Table 37 indicates that most of the thiamine in the ruminal ingesta is extra-cellular and present in the free state as thiamine pyrophosphate. For riboflavin and nicotine acid there exist the goat data in footnotes c, d, e and g of Table 37. Comparison of the found-concentrations of ribo-flavin in the ruminal liquor (41 to 53 micrograms per 100 milliliters) with the found-concentrations in the plasma (1 to 5 micrograms per 100 milliliters) reveals that a strong, downward concentration gradient from rumen to plasma probably existed for riboflavin. The same type of comparison for the found-concentrations of nicotinic acid in the ruminal liquor (350 to 470 micrograms per 100 milliliters) with the found-concen-trations in the plasma (34 to 72 micrograms per 100 milliliters) reveals that a strong, downward concentration gradient from rumen to plasma probably existed for nicotinic acid as well. Unfortunately, as it already has been noted previously, it is not known how much of the measured concentrations of riboflavin and nicotinic acid in the ruminal liquor were in the free state. Except for thiamine it is not possible, there-fore, to make any statement as to the direction of the electrochemical gradient across the ruminal wall for any of the B vitamins until more data becomes available on the free concentrations of the B vitamins in the ruminal liquor.

Condition 3:   Demonstration of Absorption from an Ingesta-Filled Rumen

The only data that are available to examine on this point are the

results presented in Table 20 (p. 110) for nicotinic acid. These results failed to demonstrate the absorption of nicotinic acid from an ingesta-filled rumen. It is to be noted, however, that the method of detection of nicotinic acid absorption was by the making of a comparison of the concentrations of nicotinic acid in the portal and peripheral vessel plasmas. The lack of sensitivity of this procedure for the detection of substances absorbed continuously and in small amounts has been comment-ed upon previously (see p. 116). By contrast, when there was utilized the more sensitive procedure of sampling at periodic intervals a solu-tion placed in an empty rumen--that is, as in the ruminal fistula experiments--, absorption of the B vitamins was demonstrated. Although the data that are assembled in Table 38 reveal that the vitamin concen-trations in these ruminal fistula experiments were usually higher than the concentrations reported in the literature for the ruminal ingesta (wet weight), the data also reveal that in many of the experiments the concentrations in the ruminal fistula experiments were of the same order of magnitude as those reported for the ruminal ingesta.

Comment

    The previous discussion indicates that many of the B vitamins are present in the rumen in appreciable amounts. These amounts may be large enough that rumen to plasma, downward electrochemical gradients can exist for many of the B vitamins. Further measurements must be made, however, to determine if such gradients really do exist. If they are found to exist, then these gradients should permit the B vitamins to be absorbed from the rumen, even if the ruminal epithelium does not possess the ability to absorb B vitamins against an electrochemical gradient. As well, there

TABLE 38. - Ruminal fistula experiments Wl, W2, W4, W5, and W6: a comparison, for each the three B-vitamins whose concentrations were measured, between the lowest-found concentrations in the ruminal solutions and the concentrations in the ruminal ingesta (all concentrations are expressed as microgram-moles per 100 milliliters of solution)

Vitamin	Ruminal solution in experiment[a]					Ruminal ingesta[b]	
	Wl	W2	W4	W5	W6	Sheep	Cow
Riboflavin	2.7	2.3	14.9	4.5	10.7	1.3	0.1 to 1.3
Nicotinic acid	5.7	...	...	24.0	58.1	...	1.8 to 11.2
Pantothenic acid	3.8	...	13.6	7.0	8.4	4.8	0.2 to 3.8

[a]See footnote a of Table 35 on page 243.

[b]These data are from Table 37. Their unit of measurement has been changed, however, from micrograms to microgram-moles per 100 milliliters of ruminal ingesta.

still remains the possibility that all or some of the B vitamins can be absorbed from the rumen, even against an unfavourable electrochemical gradient. Finally, although the previous discussion failed to present evidence of B vitamin absorption from the normal ruminal contents, evidence is presented which indicates that ruminal absorption of the vitamins did occur from ingesta-free rumens at concentrations which possibly were of the same order of magnitude as the concentrations that have been reported for ruminal ingesta. This finding suggests that, with suitable methods of experimentation, it should be possible to demonstrate the absorption of the B vitamins from the ruminal contents. One such suitable method of experimentation might be to use radioactive-labelled vitamins.

<div align="center">

The Effect of Ruminal Absorption on the Fate

of B Vitamins Present in the Rumen

</div>

Introduction

Figure 23, modelled after the figure previously presented to summar-ize the principal reactions of nitrogen compounds in the rumen (Fig. 22, p. 214), is proposed by the present author to summarize the principal reactions of the B vitamins in the rumen. Figure 23 suggests that the fate of the B vitamins that enter or are formed in the ruminal ingesta is manifold:

1. the B vitamins are utilized in the synthesis of microbial enzyme systems, or they are degraded to simpler molecules;

2. the B vitamins pass with the rest of the constituents of the ruminal ingesta into the more distal parts of the alimentary tract;

3. the B vitamins are absorbed from the ruminal ingesta by passage
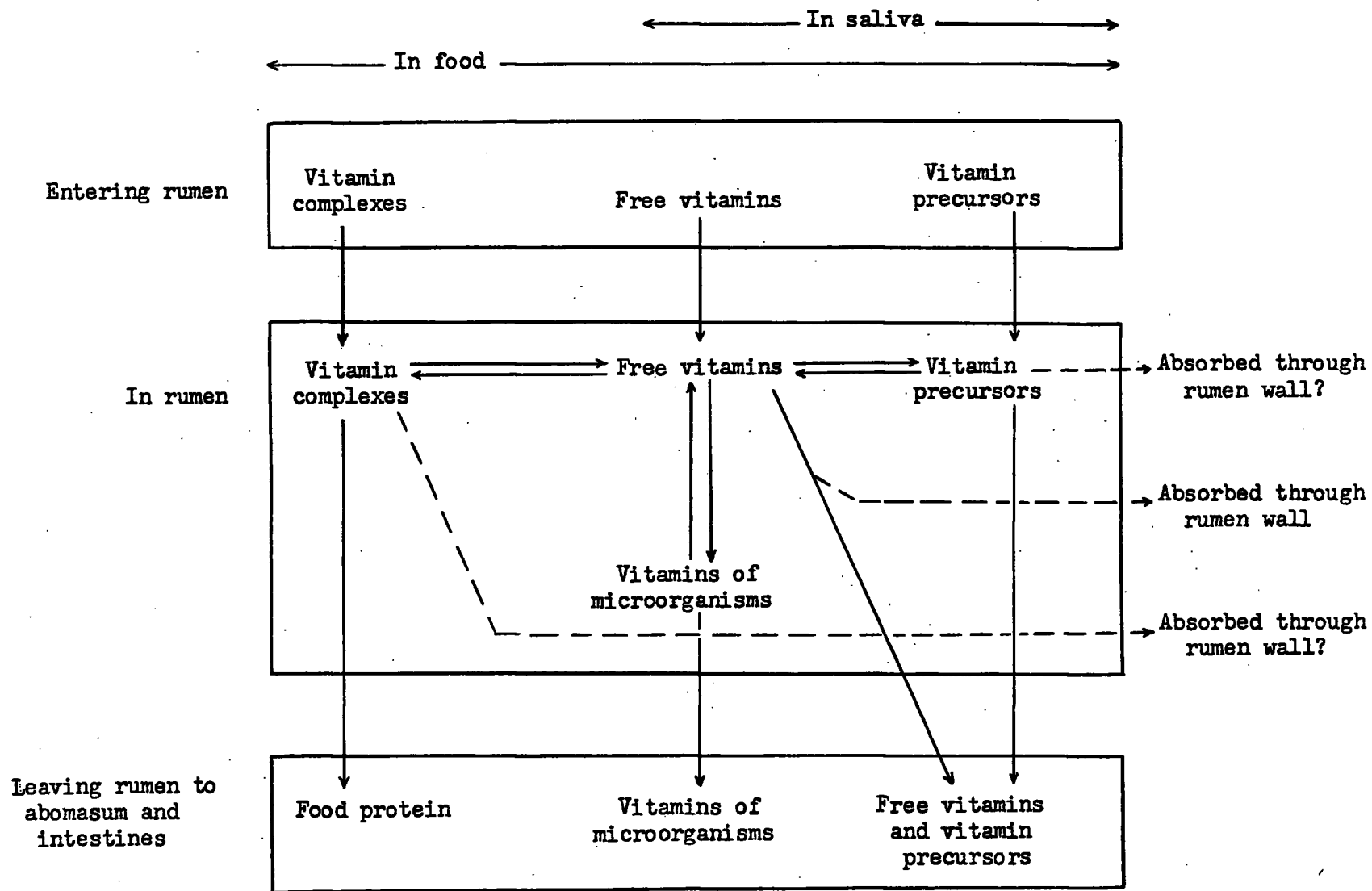
Fig. 23.

Fig. 23. - The principal reactions of the B vitamins in the rumen (based on information contained in references 203, 267, 357, 388).

through the ruminal wall into the portal circulation.

As with the amino acids, it is to be expected that the distribution of each B vitamin among the various possible pathways just described will be influenced by the particular set of environmental conditions prevailing in the rumen, by the known influence of the diet on the composition of the population of microorganisms in the rumen, by the physical and chemical nature of the dietary constituents, by the relative stability of the B vitamins in the ruminal ingesta, and by the physiological state of the animal. Each of these influencing factors previously was discussed in the section dealing with the amino acids (see pp. 216 to 231). Much of that discussion can find application here; hence, it is felt that it is not necessary to present here what largely would be a repetition of the discussion that was presented for the amino acids. The reviews of or reports of Lannen and Tanner (388), Lardinois et al (208), Snell (357), Najjar and Barrett (267), Kon and Porter (203), Hunt et al (177, 180) and Agrawala et al (6), Conrad and Hibbs (78), Phillipson and Reid (302), and Hollis et al (169) provide many examples of how variations in different environmental conditions can influence the activities of the population of ruminal microorganisms in relation to B vitamin synthesis and utilization. Only the influence of the diet on the composition of the microorganism population will be discussed again, as there do exist several interesting examples of how this influence will help to determine the fate of the B vitamins in the rumen.

Influence of the Diet on the Composition of the Population of Micro-
organisms in the Rumen and, therefore, on the Fate of B Vitamins in the
Rumen

Snell (357) has described the effects produced on the vitamin re-

quirements of individual microorganisms when certain constituents of the cultural medium are varied. As these results could have application and be of value in gaining an understanding of the conditions in the rumen, some details are presented here of the description given by Snell for the "sparing action" produced by certain compounds on the vitamin requirements of microorganisms. For example, the requirement of _Acetobacter suboxydans_ for para-aminobenzoic acid in a cultural medium free of purine bases is about ten times as high as in a medium which contains purine bases. Again, the amount of pyridoxine required for the growth of many lactic acid bacteria depends upon the number and type of amino acids present in the cultural medium: _Lactobacillus arabinosus_, for example, can grow in the absence of pyrodoxine, if the medium contains hydrolyzed casein or a matching mixture of synthetic amino acids; if, however, anyone of a number of amino acids—such as cystine, histidine, phenylalanine, tyrosine, or arginine—is not present, then the microorganism cannot grow in the absence of pyridoxine. The production by other molecular compounds of the same type of "sparing action" has been demonstrated for folic acid, biotin, and nicotinic acid. In most these cases, the "sparing action" is suggested as indicating that an individual vitamin is concerned in the microbial synthesis of the particular compounds whose presence "spares" the vitamin. Apparently, when the "sparing" compound is present preformed in the cultural medium, the compound is assimilated directly. Under these circumstances, an otherwise essential function of the spared vitamin is made non-essential and the requirement of the microorganism for the vitamin is reduced or eliminated. The implication of these results with respect to the action of the ruminal population of microorganisms on the B vitamins is clear: variation in the quantity

and quality of the molecular constituents of the diet should influence the requirements of the microorganisms for the B vitamins, thus producing variation in the distribution of the B vitamins among the three possible pathways that previously were suggested for the B vitamins that enter or are synthesized in the ruminal ingesta.

Of more immediate application to the question of the effect of diet on the fate of B vitamins in the rumen are experiments that have been concerned with the effect of diet on the population of ruminal micro-organisms as it exists in the ruminant. The population of ruminal micro-organisms is known to be capable of synthesizing all the members of the B vitamin complex (see p. 9). The extent of this synthesis, under different dietary conditions, is not, however, clear. This situation more readily is appreciated when the method that often was utilized to demonstrate B vitamin synthesis by the ruminal population of micro-organisms is considered. The method of demonstration often was to feed to the experimental animals diets that were deficient or almost devoid in their content of the B vitamins. With such diets, it was possible to demonstrate larger concentrations of B vitamins in the ruminal ingesta as compared to the concentrations in the feed. Several early groups of workers, utilizing this method of demonstration, were able to demonstrate increased microbial synthesis of many of the B vitamins in response to an increased content in the diet of carbohydrate (76, 177, 178, 179, 180, 208), silage (372), urea (76, 208), or thiamine (201, 404, 405). When, however, diets are fed that already are rich in B vitamins, it is difficult to demonstrate larger concentrations of the B vitamins in the ruminal ingesta as compared to the concentrations in the feed (6, 202, 203). In fact, two groups of workers (6, 203) found that the concentra-

tions of the B vitamins were as high in the ruminal ingesta of animals
that were fed a ration which was poor in its content of the B vitamins
as in the ruminal ingesta of animals that were fed a ration which was
rich in its content of the B vitamins. The microbial synthesis of the
B vitamins in the animals that were fed the ration which was poor in its
content of these vitamins apparently was sufficient to increase their
concentrations to the higher concentrations that were present in the
ration which was rich in its content of the B vitamins. From these
results, it appears that when a B vitamin-rich ration is fed, the amount
of microbial synthesis of the B vitamins is not extraordinary. It is
only when a B vitamin-poor ration is fed, that the amount of B vitamin
synthesis becomes extraordinary—and easily demonstrable. Coates et al
(72) comment on these results as follows:

> These findings indicate a balance between the extent of
> synthesis and absorption of vitamins by the microflora and
> ... the concentration [of the vitamins] in the rumen.

A possible explanation of these results can be deduced from the known
effect that a change of diet can have on the proportions of the different
species of microorganisms present in the rumen (see p. 220). A ruminant
subsisting on a B vitamin-rich diet would be able to support a popula-
tion of microorganisms which either absorbs its B vitamin requirements
from the environment or which synthesizes its own required B vitamins.
Many of the microorganisms which synthesize their required B vitamins
probably would decrease or even eliminate, however, the synthesis of
these vitamins under the condition of high concentrations of the B vita-
mins in the diet. Now, changing the B vitamin-rich ration of a ruminant
to a B vitamin-poor ration would produce a new nutritional environment
in the rumen. This new environment would favour the growth and multipli-

cation of those microorganisms that can synthesize their own B vitamin requirements, as opposed to those microorganisms that obligatorily require preformed B vitamins in order to grow and multiply. This dependence of the character of the population of ruminal microorganisms on the character of the diet of their host means that, when a B vitamin-poor ration is fed, the greater proportion of the total B vitamin content of the ruminal ingesta will be that which has been formed by microbial syntheses; on the other hand, when a B vitamin-rich ration is fed, the greater proportion of the total B vitamin content of the ruminal ingesta will be that which has been ingested with the ration. This variation in the source of the B vitamins in the ruminal ingesta, with the attendant variation in the metabolic activities of the ruminal microorganisms, most certainly will influence the distribution of the B vitamins among the three possible pathways previously described (see p. 259).

Comment

Keeping in mind the discussion previously presented for the amino acids (see pp. 216 to 231), it becomes apparent from the discussion just presented for the B vitamins that their distribution in the rumen among the various possible pathways will depend upon the influences of many factors. The proportions of the individual B vitamins that are absorbed from the rumen will be greater when the interactions of the many factors are such as to produce higher concentrations of the B vitamins in the ruminal ingesta. Such higher concentrations of B vitamins, occurring together with a metabolically-active ruminal wall and a high rate of flow of blood and lymph through the ruminal wall, should lead to an

increased amount of B vitamin absorption. This increased absorption of

the B vitamins would be of great nutritional significance to the ruminant

(see below).

Part C: The Possible Nutritional Implications of the Absorption of

Amino Acids and B Vitamins from the Rumen

The standard thinking of nutrition is so often applied to
the ruminant in a way that suggests "if we could only
stop a ruminant being a ruminant what a much better animal
it would be". . . . The ruminant is not likely to be
badly adjusted to its digestive apparatus and as animal
husbandry experts we would be well advised to approach the
ruminant with some humility bearing this in mind. To suc-
cumb to the temptation of forcing the ruminant into the
standard mold of nutritional thought which is based largely
on the rat is to display a lack of proper appreciation of
what is known about ruminant digestion.
A. T. Phillipson and D. P. Cuthbertson (299).

Introduction

If it be assumed that at least some amino acids and B vitamins can

be absorbed from the rumen, then the question of the nutritional implica-

tions of this absorption can be raised. It is probable that the implica-

tions are many. Most of the present discussion will be limited, however,

to the making of two suggestions as to the possible nutritional signifi-

cance of the absorption of amino acids and B vitamins from the rumen.

The remainder of the discussion will be devoted to an application of the

concept of ruminal absorption to explain certain results obtained in

nitrogen retention trials with ruminants.

The Time Factor

Several workers (107, 130, 131, 366; see 105 for citations of

earlier work) have demonstrated the importance of the time factor in the

utilization by an animal of its ingested nutrients. It has been found,

for example, that the administration to an animal of an amino acid mix-
ture which otherwise would maintain the nitrogen balance of the animal
will not do so if one or more of the component amino acids is omitted
and administered several hours later (107). The explanation of this
result apparently is that the capacity of the liver to store amino acids
for any length of time is limited. This limited capacity becomes mani-
fested when there arrives at the liver a mixture of amino acids that is
lacking in one or more of the amino acids that constitute the proteins
synthesized in the liver: the liver deaminates the incoming amino acids
and the resulting ammonia rapidly is excreted. Only when the mixture
of amino acids is not lacking in any one of the required amino acids
does the liver utilize the mixture of amino acids in the synthesis of
protein (257, 389, 390). This behaviour of the liver towards amino
acids is epitomised by Geiger's (130) statement that "protein synthesis
occurs only when all the essential building stones are available simul-
taneously." A second example of the importance of the time factor in
the utilization by an animal of its ingested nutrients is that noted by
Ellman (107). He and others have found that a reciprocal relation
exists between amino acids and carbohydrates in that their simultaneous
administration to an animal results in better utilization of both amino
acids and carbohydrates--as compared with the degree of utilization of
these two classes of nutrients when each is administered separately.
Ellman suggests that the explanation of this reciprocal effect is that
"the simultaneous entrance of carbohydrate and amino acids into the
metabolic pool enhances the storage or oxidation of carbohydrates or
both." To be more specific, it is suggested here that the simultaneous
presence of amino acids during the utilization of carbohydrates may

increase the efficiency of this utilization because of the fact that amino

acids are necessary components of the enzymes concerned in the metabolic

oxidation of carbohydrates. The presence of these amino acids at the

time of carbohydrate utilization, along with an adequate concentration

of the necessary B vitamin components of the enzymes, would help to

ensure an optimal concentration of the enzymes concerned in this utiliza-

tion. Further, it is suggested here that the simultaneous presence of

carbohydrate during the utilization of amino acids may increase the

efficiency of this utilization because of the fact that the presence

of carbohydrate would reduce or eliminate the need of the animal to

utilize amino acids as an energy source. The elimination of this need

would permit the animal to utilize the amino acids for protein synthe-

sis--that is, the well-known "sparing action" of carbohydrates on protein

or amino acid utilization.[101] Other examples of the importance of the

time factor in nutrition could be presented, but the two examples just

given should be sufficient to illustrate the point.

With the previous information as background, the first suggestion

can be made as to the nutritional significance of the absorption of

amino acids and B vitamins by the ruminal wall. As most the volatile

fatty acids that are formed in the rumen by cellulose fermentation are

known to pass through the ruminal wall into the portal blood, the

simultaneous passage of amino acids and B vitamins through the ruminal

wall into the portal blood would permit all these compounds to arrive at

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[101]The article of Geiger (131) should be referred to for a more
detailed consideration of the reasons as to why the simultaneous presence
of carbohydrate during the metabolism of amino acids promotes their
utilization for protein synthesis.

the liver at approximately the same time. Following from the discussion

on the importance of the time factor in the utilization by the animal

of its nutrients, the simultaneous arrival at the liver of the volatile

fatty acids, amino acids, B vitamins, and inorganic ions should promote

more efficient utilization of each these nutrients (131). Such an ad-

vantageous timing of the arrival of all these components at the liver

(or elsewhere in the body) would not occur if the absorption of the

amino acids and B vitamins occurred only from the small intestine.

The Competitive Factor

Introduction. - The second suggestion to be made for the nutritional

significance of amino acid and B vitamin absorption from the rumen con-

cerns the relation of this absorption to a competition that probably

exists between the ruminant host and its contained microbial population.

The competition referred to is that which probably exists for the amino

acids and B vitamins that are ingested as a part of the host's ration (202,

302, 368, 369, 373) or that are liberated[102] into the ruminal liquor by

some members of the ruminal population. Before suggesting the character

of the effect of ruminal absorption of amino acids and B vitamins on this

competition, the probable roles played by the host and microbial popu-

lation in the competition first will be delineated briefly.

Competitive Actions of the Microbial Population for Amino Acids and

B vitamins.- The competitive actions of the microbial population would

be the result of some of its members either absorbing or inactivating

---

[102] Some of this liberation of amino acids and B vitamins would be
by autolysis of microorganisms. In addition, however, there is evidence
that microorganisms also liberate amino acids and B vitamins while still
living (54, 104, 132, 375).

the amino acids and B vitamins. It is well-known that many microorganisms utilize amino acids for protein and nucleic acid synthesis or enzymatically degrade amino acids through decarboxylation or deamination. Likewise, Van Lannen and Tanner (388) cite experiments which demonstrate microbial inactivation of the B vitamins thiamine, nicotinic acid, and biotin. Finally, considerable evidence exists that certain microorganisms absorb B vitamins in relatively large amounts (197, 357, 388). Of special interest in this latter respect is the behaviour of some microorganisms towards thiamine and vitamin B_6: "luxury consumption"--the accumulation within the microorganisms of these vitamins in amounts considerably greater than the amounts sufficient for normal growth (357).

Competitive Actions of the Host for Amino Acids and B Vitamins.-
The competitive actions of the host ruminant for the available amino acids and B vitamins is exerted primarily through the digestive and absorptive capacities of its alimentary canal. The digestive action of the alimentary canal, limited to the abomasum and small intestine, not only liberates amino acids and B vitamins from those feed constituents that have escaped the fermentative action of the microbial population in the ruminant stomach, but also liberates amino acids and B vitamins from the cells of the microbial population as they pass into the digestive parts of the alimentary canal. This digestive action would permit the absorptive action of the alimentary canal to function in the abomasum, small intestine, and large intestine. In addition, the possibility also exists that the absorptive action of the alimentary canal occurs in the first three compartments of the ruminant stomach. In this case, the absorptive action would be limited to those compounds that are ingested in the absorbable state or to those soluble compounds that are formed by

the fermentative action of the microbial population. The combined effect of these digestive and absorptive actions provides to the ruminant host a means of obtaining its requirement of amino acids and B vitamins.

The Beneficial Effect of Ruminal Absorption to the Host in its Competitive Actions for Amino Acids and B Vitamins.- The absorption of amino acids and B vitamins by the ruminal wall is of benefit to the ruminant host in that this absorption conserves for the host amino acids and B vitamins that otherwise would be inactivated by the microbial population. This conservation should be of considerable nutritional significance when feeds are given that are relatively rich in their content of free amino acids and B vitamins. There remains, however, a possibility that ruminal absorption may provide an additional benefit of conservation to the host of many of the amino acids and B vitamins that otherwise might be contained within the protoplasm of ruminal microorganisms. Now, it is accepted quite generally that the amino acids and B vitamins, contained in one form or another in the microbial protoplasm, later become available to the host after the microorganisms pass with the ingesta into the digestive parts of the alimentary canal (18, 21, 23, 154, 200, 228). Indeed, several authors (21, 22, 23, 228, 304), by microscopic examination of the alimentary tract ingesta, have provided indirect evidence that the digestive enzymes of the abomasum and small intestine can digest ruminal microorganisms. The extent of this digestion remains in doubt, however, because as yet there exists no data to indicate what proportion of the ruminal microorganisms are digested (33, 92). One author (198), on the basis of comparative direct bacterial counts between the ruminal and intestinal ingesta, even has suggested that little of the protein of the ruminal microorganisms is digested.

Cuthbertson (86) has commented as follows (see also the discussion by

McDonald (237)):

> The question of disintegration of bacteria in the ali-
> mentary tract is important, for the liberation of their intra-
> cellular products, i.e. polysaccharides, as well as the proto-
> plasmic nitrogen, presumably depends on this. The hypothesis
> that rumen bacteria are digested as they pass through the
> abomasum and small intestine rests on circumstantial evidence.
>
> It is . . . not likely that all bacteria are killed by the
> change in pH [that occurs when the ingesta passes from the
> omasum to the abomasum with its low pH] , by the action of
> pepsin, or by the subsequent action of the duodenal and
> intestinal juices.

Again, Pounden et al (304), for example, have found that not all types

of ruminal microorganisms can be digested in the abomasum or small

intestine. Their observations demonstrate that, although some of the

ruminal microorganisms undergo rapid disintegration in the abomasum or

small intestine, "others manage to hold their form until they reach the

more posterior parts of the digestive tract, while still others can

withstand all the post-ruminal digestive activities of the animals."

This evidence, that some of the ruminal microorganisms are passed out

of the animal in the feces, indicates that amino acids and B vitamins

are being lost by the host. Even that microbial protein which does

become exposed to the digestive enzymes of the small intestine probably

does not yield all its constituent amino acids to the host. This is so

because the ruminal microbial protein is not digested completely

when it is fed to rats: true digestibilities have been demonstrated

of about 74 for bacteria[103] and of about 90 for protozoa. If the

---

[103]Several authors (21, 186, 249) have suggested that the ruminal
protozoa ingest ruminal bacteria and, by converting the bacterial protein
to protozoan protein, thereby increase the digestibility of the microbial
protein. As yet, however, no evidence has been found that the ruminal
protozoa actually do ingest bacteria (86).

digestibilities of microbial protein are the same in the small intestine

of the ruminant as in the small intestine of the rat, then this lack of

complete digestibility of the microbial protein is another cause for the

loss of amino acids to the host. Further, as it has been pointed out by

McDonald (237), because bacteria have a considerable proportion of their

nitrogen in the form of nucleic acids[104] and because most of these nucleic

acids probably are not utilized by the host, then the amino acids that

have been converted in the rumen into these nucleic acids also are being

lost to the host. The host, however, by removing amino acids and B vita-

mins from the ruminal liquor before they can be absorbed or utilized by

the ruminal microorganisms, conserves many of these nutrients. Further,

because of this continual removal of the amino acids and B vitamins from

the ruminal liquor, it is probable that, under conditions of medium or

low content of amino acids and B vitamins in the feed, the ruminal

population of microorganisms would contain an increased proportion of

those types of microorganisms that can synthesize their own amino acids

and B vitamins. This, in turn, should lead to an increased liberation

of these nutrients into the ruminal liquor from the microorganisms--either

by excretion or by autolysis of the cells. In summary, the ruminal

absorption of amino acids and B vitamins not only conserves for the

ruminant host many of those amino acids and B vitamins that otherwise

would be inactivated or be passed out of the animal in the feces, but

also, under certain feeding conditions, probably promotes an increased

microbial synthesis and, hence, an increased liberation of these nutrients

into the ruminal liquor. The total effect of this ruminal absorption is

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[104]Not less than ten per cent of the total nitrogen in ruminal
microorganisms occurs as nucleic acids (239).

to help to assure for the ruminant host an optimal supply of the required

amino acids and B vitamins.

Special Comment: The Application of the Concept of Ruminal Absorption of

Amino Acids to Explain Certain Results of Nitrogen Retention Trials in

Ruminants

One of the ways in which the ruminant differs from the non-ruminant

is that part of the ruminant's dietary protein and amino acids is exposed

to the fermentative action of ruminal microorganisms. As a result of

this fermentative action, a significant proportion of the dietary protein

and amino acids may be utilized for the synthesis of microbial protein

(103, 140, 143, 170, 188, 237, 241, 248, 260, 287, 352, 369). Among a

large number of different types of microorganisms--including ruminal

microorganisms--, the qualitative and quantitative amino acid constitution

of this microbial protein has proved to vary within relatively narrow

limits (40, 58, 104, 121, 170, 184, 364).[105] This means that the conver-

sion of dietary protein and amino acids to microbial protein, if consider-

able, would tend to equate the digestibilities and biological values[106]

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[105] The degree that changes in the environment of microorganisms
can influence their amino acid constitution is something that needs
further investigation (104, 121, 184, 318, 364), especially relative to
an understanding of protein metabolism in the rumen.

[106] The biological value of a protein is dependent on the qualitative
and quantitative amino acid constitution of the protein. The closer the
amino acid constitution of a protein is to an animal's balanced require-
ment of amino acids, the more efficiently is the protein utilized by the
animal (see 41). Holmes et al (170) have compared the amino acid
composition of ruminal bacterial protein with that of egg protein--a
protein that is regarded as possessing an almost ideal balance of
essential amino acids for producing optimal growth in the rat. By this
comparison they found isoleucine and methionine each to be quite low in
concentration and, therefore, to be the "limiting" amino acids in the
biological value of ruminal bacterial protein. It is, perhaps, of interest

of the dietary proteins and amino acids, irrespective of their digestibilities

and biological values when fed to the non-ruminant (185, 186, 250). Yet

several workers (216, 411; see also 61 and 62 for general commentaries),

feeding cattle and sheep different readily-soluble proteins at concentra-

tions (12 per cent or less) in the diet that should have ensured that at

least a major portion of each protein was hydrolyzed by the ruminal

microorganisms,[107] found that the type of protein which was fed still

determined the nitrogen retention[108] of the animal. At least a partial

explanation for this variation in nitrogen retentions is to be found in

the present experimental results which demonstrate the ability of the

ruminal wall to absorb amino acids. The ruminal absorption of amino

acids that are present in the feed, or that are formed during the micro-

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to note here that several workers (249, 251, 318; also see the reviews in
109, 250, 282) have attempted to determine the biological value of ruminal
microorganisms for the rat. For example, values of 78-80 (318) and 81 or
88 (249, 251) have been found.

[107]The situation would be different, of course, if the protein that
was fed were relatively insoluble or were fed in larger amounts than the
ruminal population of microorganisms could handle (see 412). In this
case, some of the protein would pass into the abomasum and small intestine
and, presumably, this part of the protein would have about the same
biological value as when it is fed to a non-ruminant. Consequently,
when insoluble or very large amounts of protein are fed, absorption from
the small intestine of dietary amino acids would be an additional factor
to consider in explaining differences among animals in their nitrogen
retention when they are fed different types of proteins (see also 143,
240, 241, 250, 282, 411).

[108]The nitrogen retention of an animal is one index that is used to
compare the digestibilities and biological values of proteins. Those
proteins whose digestibilities and amino acid constitutions are such as
to undergo maximal utilization yield maximal nitrogen retention--that is
there is maximal absorption of the amino acids from the alimentary canal
and minimal deamination of the amino acids in the liver, with a consequent
minimization of the nitrogen excretion in the feces and urine, respectively.

bial fermentation of the dietary protein, provides to the ruminant a different array of amino acids than would be the case if all the amino acids provided to the animal were consequent upon the digestion of microbial protein that had passed into the abomasum and small intestine. This absorptive behaviour of the ruminal wall should favour increased or decreased nitrogen retention, depending on whether the dietary proteins and amino acids are of greater or lesser biological value than the microbial protein. It is not unexpected, therefore, that the type of protein fed to a ruminant produces variation in its nitrogen retention. This variation of nitrogen retention implies, in turn, that the digestibilities and biological values of proteins are not necessarily equated when fed to a ruminant. The following quotation from Reid (319; see also 216) serves to summarize the previous discussion:

> In view of the wide range in the biological value of nitrogen for ruminants, it seems possible that, under certain conditions and particularly those in which certain nitrogen sources are fed, a portion of the nitrogen utilized may come directly from the dietary protein and the remainder may be provided as bacterial protein. Of course, this partition would depend upon the amount of required amino acids absorbed directly from the rumen or other parts of the digestive tract but, nevertheless, escaping bacterial action. The relatively high biological value of the proteins of various feeds fed to ruminants may be partially explained on this basis.

CONCLUDING COMMENT

The stated objective of the present investigation was to determine whether ruminal absorption of amino acids and B vitamins does or does not occur. The results of the investigation appear to support the conclusion that ruminal absorption of amino acids and B vitamins does occur. In the General Discussion, an attempt has been made to determine the manner of this absorption. Although the attempt was only partly successful, enough information was gained to make the following statement of Phillipson (297) an especially appropriate comment at this point:

> The concept has slowly developed . . . that the epithelium lining the alimentary tract has a greater metabolic importance than is usually appreciated and that its metabolism besides contributing to the total metabolism of the animal regulates not only the absorption of molecules and ions but also prevents loss of molecules and ions from the body into the gut. Transport through the cell is presumably intimately related to the metabolism of the cell and a study of one is incomplete without a study of the other.

The possible nutritional implications of the ruminal absorption of amino acids and B vitamins also were discussed. It may well be that the present investigator has placed too much stress upon the possible importance of the ruminal absorption of amino acids and B vitamins. It is quite true that too often one or a few aspects of a problem become overstressed with a concomittant neglect of other aspects of the problem. It is, however, the hope of the author that, overstressed or not, the present investigation will have raised the question of the quantitative nutritional significance of the ruminal absorption of amino acids and B vitamins and, therefore, will stimulate future research.

APPENDIX

Ruminal Fistula Operation

The purpose of a ruminal fistula operation is to provide the experimentalist with easy access to the interior of the rumen and its contents. Stoddard et al (363) and Phillipson and Innes (300) cite the early workers who first developed the technique of making a ruminal fistula. In recent times many groups of workers have modified the early technique (see, for example, 14, 272, 300, 312, 331, 336). The present author, in adapting the operation to the goat, likewise has modified the general procedure. The modified procedure, developed according to a suggestion of Dr. P. A. Klavano[109] that the operation be divided into two parts, is described below. It since has been discouvered that Rankin (315), in 1940, devised a similar modification for making ruminal fistulae in sheep.

The site of the operation is located in the left paralumbar fossa (see Fig. 24). This area was clipped and shaved the day before the operation. On the day of the operation, the skin in the area was well-lathered and washed. Two applications of tincture of iodine were made to the operative area. Although occasionally general anesthesia[110] was used, more frequently the operation was performed under local anesthesia. A 2 to 4 per cent solution of procaine was infiltrated first cranially

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[109]Associate Professor and Chairman of the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Washington State University.

[110]General anesthesia was induced by intravenous administration of sodium pentobarbital and maintained by inhalation administration of ethyl ether.

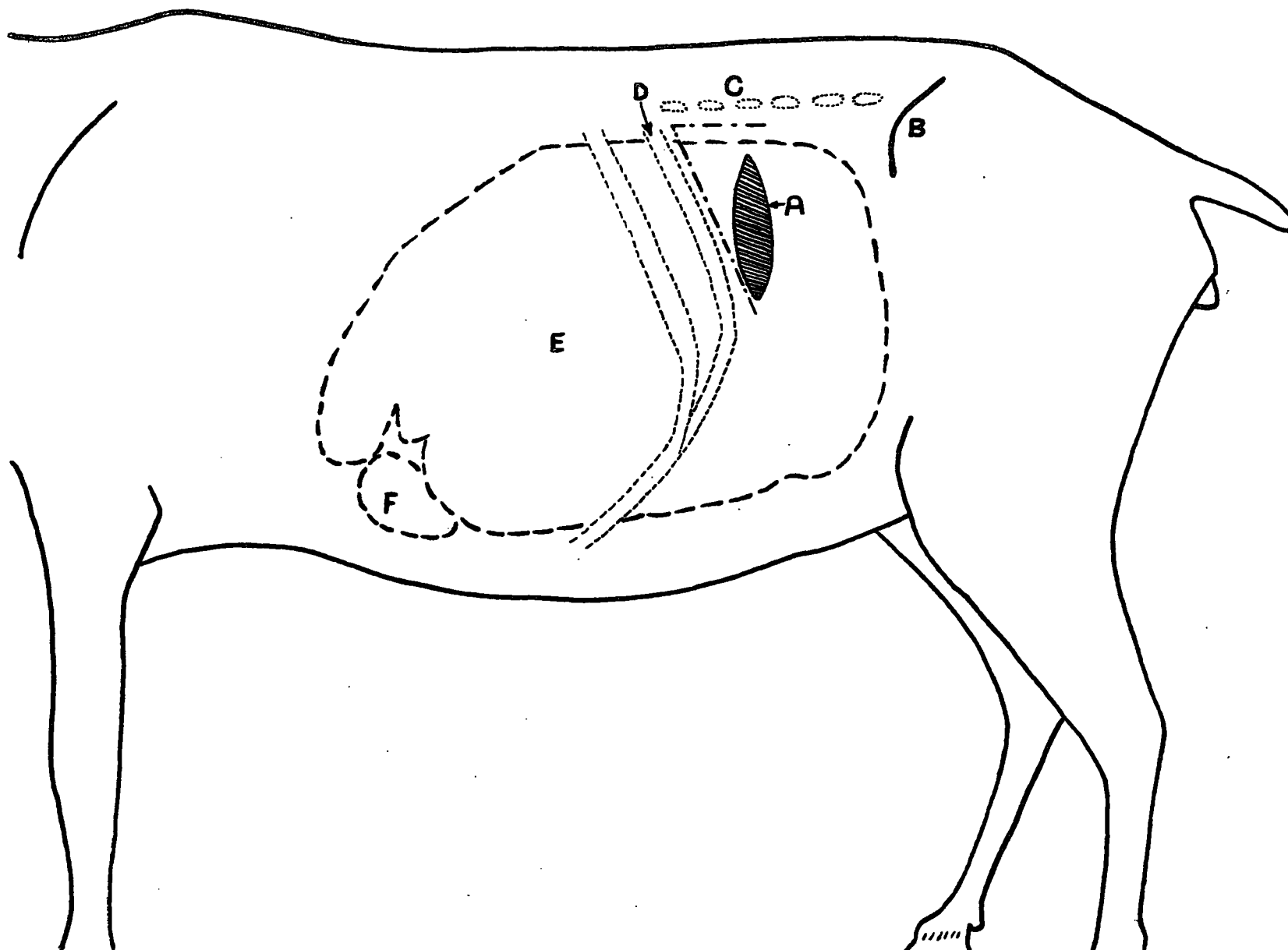Fig. 24.

Fig. 24. - A drawing to show the position of the ruminal fistula

A, ruminal fistula in the left paralumbar fossa; B, tuber coxae; C, transverse processes of lumbar vertebrae; D, thirteenth rib; E, rumen; F, abomasum; the inverted L (—.—.—.—) designates the lines of infiltration that were used for producing local anesthesia of the left lumbar flank during the ruminal fistula operation.

and then dorsally to the site of the incision (Fig. 24). The procaine
solution was injected intradermally, subcutaneously, and intamuscularly
in the different muscle layers. Such a procedure blocks the medial
and lateral branches of the ventral divisions and the lateral branches
of the dorsal divisions of the last thoracic and first two or three
lumbar nerves. If carried out properly, this procedure provides complete
desensitization of the skin, fascia, muscles, and parietal peritoneum in
the operative area.

To locate the position of the initial incision, the last rib and
the ends of the transverse processes of the lumbar vertebrae were
palpated. The initial incision was made 1 to 2 inches caudal to the
last rib (Figs. 24 and 25). The dorsal limit or commissure of the
incision was about 1 inch below a frontal plane through the ends of the
transverse processes. From its dorsal limit, the incision was extended
ventrad about 4 inches. This size of incision permitted the formation
of a fistula that was large enough to allow the insertion of the experi-
mentalist's hand into the rumen. Fascia, abdominal muscles, and parietal
peritoneum were incised to expose the dorsal sac of the rumen (Fig. 26).
Pushing the fascia, abdominal muscles and parietal peritoneum aside, the
wall of the dorsal sac of the rumen was sutured to the deep surface of
the free margin of the skin around the incision. Care was taken in the
suturing not to penetrate to the interior of the rumen. Using monofila-
ment nylon[111] of size 0 or 1, continuous sutures were made along each
side of the incision. Interrupted sutures were placed at the dorsal and
ventral commissures. This attachment of the ruminal wall was done in

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[111]Ethicon, Ethicon Suture Laboratories, New Brunswick, New Jersey.

Fig. 25 - The initial skin incision.
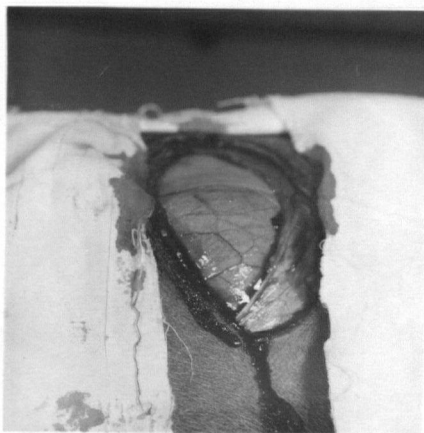
Fig. 26. - The fascia, muscles, and parietal peritoneum
have been incised to expose the ruminal wall.

such a manner as to expose an oval shaped portion of the ruminal wall
(Fig. 27). At the finish of the suturing, the operative area was dusted
lightly with an antiseptic powder. Seven to ten days later, the sutures
were removed. This completed the first stage of the operation.

Two or more days after the removal of the sutures, the second stage
of the ruminal fistula operation was performed. An oval-shaped section
of the ruminal wall was excised, the incision being made about 1/4 inch
inside the newly-formed junction between the skin and the ruminal wall
(Fig. 28). After the excision of the section of ruminal wall was
completed, the edge of the new incision was dusted with a powder having
antiseptic and astringent properties. A fistula plug (see description
below) was then inserted to close the new opening into the rumen (Fig.
29). About 1/2 hour was required to complete this second stage of the
fistula operation.

Once a ruminal fistula is formed, it usually is necessary to keep
it closed by means of a fistula plug. Such a plug prevents leakage of
ruminal contents and probably also helps to maintain anaerobic conditions
in the rumen. Different types of plugs have been devised by different
workers (25, 74, 187, 315, 331, 403; see also 312 and 363). Some of
these plugs have been simple, others have been complex. For the fistu-
lated goats that were used in the present experiments, a simple plug of
soft neolite was designed. It consisted of circular inner and outer
flanges, with an intermediate pad of sponge rubber between them. The
three parts were held together by two ordinary screws and nuts. The
intermediate pad was of such size and shape as to fit snugly inside the
fistula opening. Although the plug was not effective completely in
preventing leakage of the ruminal ingesta, the plug was inexpensive,
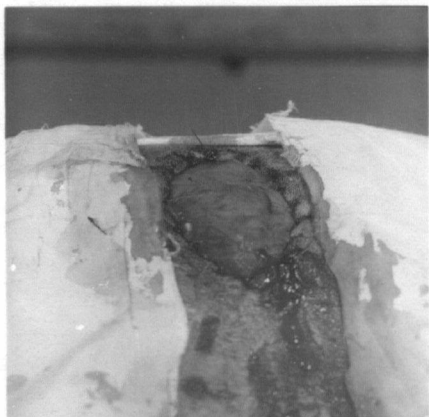
Fig. 27. - The ruminal wall has been
sutured to the deep surface of the free
margin of the skin.
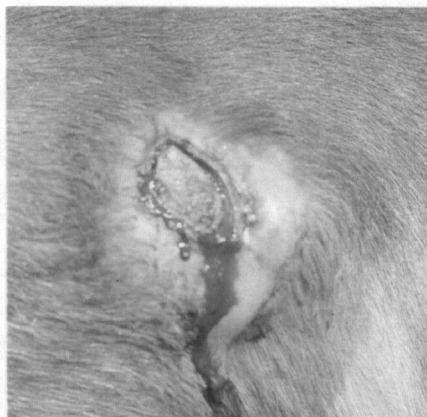
Fig. 28. - This photograph shows
the animal several weeks later. The line
of excision just has been made, prior to
the removal of the exposed ruminal wall.
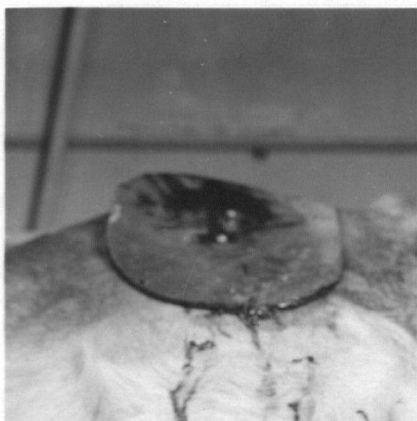
Fig. 29. - After removal of the exposed ruminal
wall, the fistula plug is inserted. Only the external
flange of the plug is visible.

easy to make, and simple to remove and reinsert. In addition, the soft texture of the sponge rubber center pad minimized irritation at the edge of the fistula.

Carotid Loop Operation

The term "carotid loop operation" refers to the permanent exteriorization of a section of the common carotid artery. Graham et al (137), using the goat, were the first to develop this type of exteriorization. McClymont (236) has adapted the operation to the cow and Schambye (332) has modified the operation for use in sheep and calves. The value of such an exteriorization is in the provision of a blood vessel from which frequent arterial blood samples can be obtained with ease and with little disturbance to the animal. Other approaches that have been proposed for obtaining arterial blood are sampling from the radial artery (36), the heart, the internal iliac artery by rectal puncture (136), the internal pudendal artery by vaginal puncture, and the middle coccygeal artery of the tail (329). Except for the method of sampling the middle coccygeal artery, all these other methods have the disadvantage of either exciting the animal or allowing only infrequent sampling. Further, although applicable to the cow, not all the above methods are applicable to the goat because of its smaller size. The carotid loop operation was chosen as the method for obtaining arterial blood in the present investigation because of its applicability to the goat as a method that permits frequent sampling, with small or no disturbance of the animal.

General anesthesia always was employed for the carotid loop operation. Either one of two methods of anesthesia was employed. One method

was an adaptation of a procedure developed in England by Longley (217, 218) for general anesthesia of the horse. Longley rapidly administered sodium thiopental intravenously to induce anesthesia and utilized ether by intravenous drip to maintain anesthesia. Conner (75) has utilized a similar procedure to produce general anesthesia in the bovine. In the other method, the present author administered sodium pentobarbital to induce anesthesia and utilized ether by inhalation to maintain anesthesia. Providing that care was taken to maintain the goat in light anesthesia, both methods of anesthesia proved to be applicable to the goat. It is to be noted that care was taken to pillow the neck of the goat in such a manner that the head was tilted toward the floor. This position of the head permitted regurgitated ruminal ingesta or saliva to drain out of the mouth, thereby reducing the possibility of ingesta being aspirated into the trachea and lungs—an event that could cause suffocation of the animal immediately or foreign body pneumonia later.

The site of the operation was located on either the left or right side of the neck, and about midway between the base and apex of the neck. The preparation of the operative area followed the same pattern that already has been described for the ruminal fistula operation (see p. 279). The initial incision, 3 inches in length, was made just lateral to the trachea. By dissecting deeply between the sternomandibularis muscle and the trachea, the common carotid artery was located (Fig. 30). The artery and its associated vagosympathetic nerve trunk were dissected free from the fascia of the carotid sheath and brought to the surface (Fig. 31). The common carotid artery then carefully was dissected free from the nerve trunk (Fig. 32), the dissection extending slightly beyond the cranial and caudal limits of the initial skin incision. It sometimes
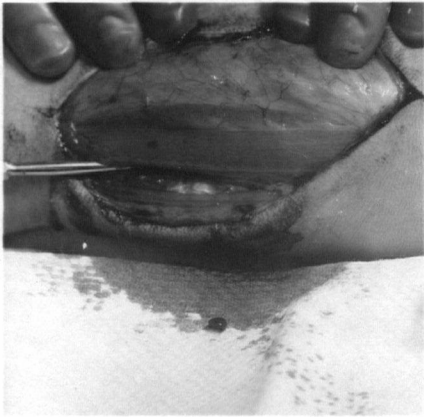
Fig. 30
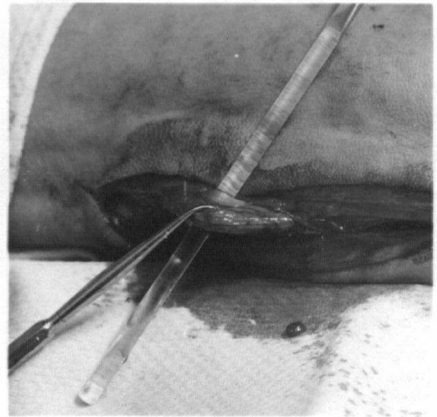
Fig. 31
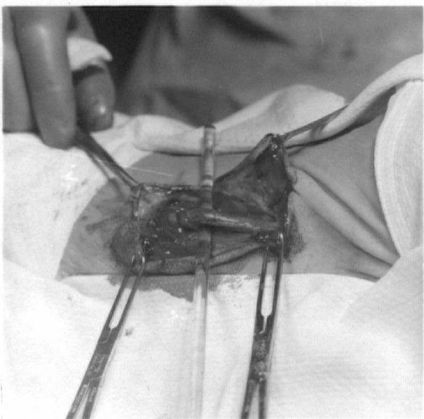
Fig. 32
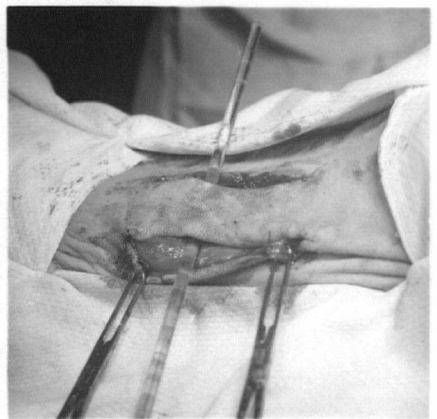
Fig. 33

Fig. 30 - A scalpel is inserted between the sternomandib-
ularis muscle and the trachea to show the approach for finding
the common carotid artery.

Fig. 31 - The common carotid artery and the vagosympathetic
nerve are shown before being dissected free from one another. They
are held in view by a glass rod, with the point of a probe lying
between the nerve above and the artery below.

Fig. 32 - The common carotid artery has been dissected
free from the other structures of the carotid sheath.

Fig. 33 - A second skin incision has been made 1 1/2
inches lateral to the first skin incision to form the band of
skin passing over the glass rod.

was necessary, depending upon the location of the initial skin incision, to ligate and cut a small branch of the common carotid artery. A second skin incision now was made parallel and about 1 1/2 inches lateral to the first skin incision (Fig. 33). The band of skin so formed was dissected free from the muscles and fascia lying deep to it. Next the common carotid artery was placed in the band of skin (Fig. 34). The edges of the skin band were sutured together with either size 0 monofilament nylon or stainless steel. This procedure formed a tube of skin with the common carotid artery passing through the tube (Fig. 35). The two free edges of skin left on the neck now were sutured together (Figs. 35 and 36). Figure 37 demonstrates the relative position of the completed carotid loop on the neck of the goat. The operation was completed in 1 to 1 1/2 hours. Although for the first few animals extensive bandaging was applied to protect the newly-formed carotid loop, it later was learned that this was unnecessary. The sutures were removed about 2 weeks after the operation was completed.

Cannulation of Ruminal Veins

Several workers have reported on the cannulation of blood vessels in man and animals. Ralston et al (314) have reviewed much of the literature on this technique and, in addition, have reported on the cannulation of the external jugular veins in the dairy cow. In 19 experiments, they found the length of time that the cannulation tubes remained patent was 2.1 hours to 14.5 days. Cresson and Glen (83) reported on 24 portal vein cannulations in dogs. These workers concluded that when large veins are cannulated with polyethylene tubes of small caliber, blood samples may be obtained over an average period of approximately 3 weeks.
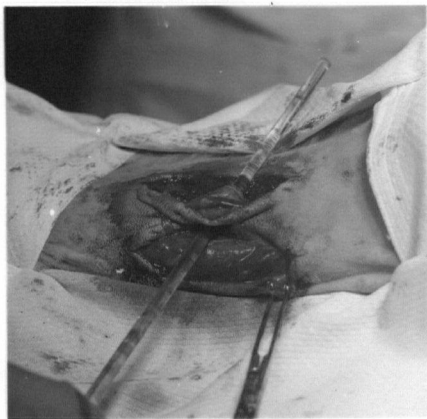
Fig. 34

Fig. 35
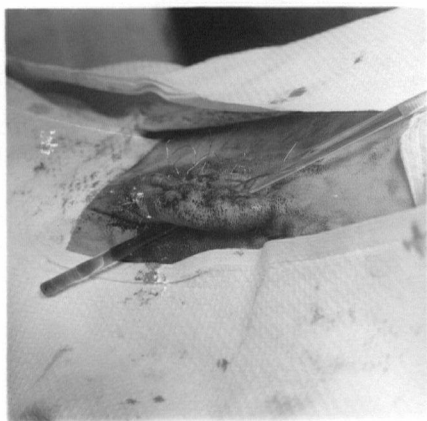
Fig. 36

Fig. 37

Fig. 34. - The common carotid artery has been placed in the band of skin. The band was twisted only for the purpose of taking the photograph.

Fig. 35. - The edges of the skin band have been sutured together to form a skin tube containing the common carotid artery. The first suture has been made to join the free edges of the skin that are left on the neck after the formation of the skin tube.

Fig. 36. - The suturing has been completed. A glass rod is inserted between the skin tube and the neck.

Fig. 37. - The photograph shows the position of the skin tube or "carotid loop" on the neck of the goat.

Annison et al (17) and Schambye (335) have reported on techniques for
cannulating the portal vein in sheep. In the present cannulation experi-
ments of the ruminal veins, the longest time that the cannulas were
maintained patent was 7 days. The procedure for the cannulation of the
ruminal veins is outlined below.

The site of the operation was located in the left paralumbar fossa.
The preparation of the operative field was the same as for the ruminal
fistula operation (see p. 279). General anesthesia was employed with
sodium pentobarbital as the induction anesthetic and inhalation ether
as the maintenance anesthetic. The method of entering the peritoneal
cavity was as for the ruminal fistula operations, except that the incision
usually was placed midway between the last rib and a transverse plane
through the level of the tuber coxae. This surgical approach exposed
the left face of the rumen and, in addition, made accessible the left
ruminal vein and the terminal part of the right ruminal vein (see Fig.
14, p. 103). Further, by applying traction to the dorsal sac of the
rumen, it was quite easy to expose the right face of the dorsal sac of
the rumen and, thereby, make accessible the main part of the right
ruminal vein and its radicles.

The cannulation of the ruminal veins was accomplished as follows.
A large radicle of the vein to be cannulated was selected. As loose
areolar tissue that contained a variable amount of fat and a thin layer
of smooth muscle always covered the veins, it first was necessary to
dissect a small section of the vein free from these tissues. The part
of the radicle vein that was dissected was the last 1 to 2 inches before
it jointed its parent vessel. During the dissection, two loops of catgut,
size OO, were placed around the vein. A number 12 or 14 gauge needle,

cut to a 3/4 inch length and containing the end of a polyethylene cannula[112], was thrust through the wall of the radicle vein. After the needle had pierced the wall of the vein, the cannula was pushed 1 to 2 inches into the lumen of the parent vein. The needle was withdrawn and the two ligatures were tied around the vein to hold the cannula in place. After checking on the patency of the cannula by aspirating a small quantity of blood, the cannula was rinsed with physiological saline and then filled with a solution of heparine (1:1000). In order to hold the heparin solution in the cannula, the free end of the cannula was sealed by heating its tip with an alcohol flame and pinching the tip with forceps. The free end of the cannula then was brought outside the animal through a stab wound that was made cranial to the original skin incision. That part of the cannula that was external to the skin was coiled and then covered by a pad of cotton, the pad being held in place by adhesive tape. The suturing of the original incision completed the operation. Each day subsequent to the day of surgery, the cannula was flushed with physiological saline, aspirated for blood to see if still patent, flushed again with physiological saline, refilled with heparin solution, and heat-sealed as before.

Operative Procedure for Experiments B3 to B9

A typical protocol is presented here to illustrate the procedure followed in Experiments B3 to B9.

1. Clip goat on right flank and neck.

[112]The tubing used for the cannula was Intramedic polyethylene tubing, PE 90 (I.D. 0.034" x O.D. 0.050"). Intramedic is the trade name for the polyethylene tubing of Clay-Adams Co., Inc., New York. The

2. Anesthetize the goat using pentobarbital (lightly) and inhalation ether. Record the time that the goat is placed on the table.

3. Expose the common carotid artery and the external jugular vein, cannulate them, and take two 5 cc. blood samples. Record the time.

4. Make an inverted-L incision on the right flank.

5. Isolate the portal vein and the distal parts of the gastro-splenic and mesenteric veins. Take two 5 cc. samples from each these two veins, also two 5 cc. samples from each the common carotid artery and the external jugular vein. Record the sampling times.

6. Open the rumen, remove the ingesta from it and the reticulum, and rinse both of these compartments with warm water.

7. Make a longitudinal incision on the greater curvature of the omasum to facilitate the insertion of a cotton plug in the reticulo-omasal orifice. After the plug is in position, ligate the connection between the reticulum and omasum.

8. Aspirate any fluid that still remains in the rumen and reticulum. Suture the ruminal incision, leaving a small opening through which the experimental solution can be poured later. Suture also the omasal incision.

9. While step 8 is being carried out, take two 5 cc. samples from each blood vessel sampled in step 5. Record the sampling times.

10. Complete the isolation of the branches of the portal system. Cannulate the gastrosplenic and mesenteric veins. Bring the free ends of the cannula tubes outside the animal by passing them through the openings of stab wounds.

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company states that their tubing "has been animal tested and found free of tissue reaction."

11. Take two 5 cc. samples from each the four blood vessels. Record the sampling times. Place 1 liter of the experimental solution, warmed to 35° C., into the rumen. Record the time: zero time.

12. While the sampling of step 11 is being carried out, close with temporary sutures the incision previously made on the right flank of the goat. Check temperature of goat.

13. Approximately 5 minutes after zero time, remove via the cannulas two 5 cc. samples from each the four blood vessels. Record the sampling times.

14. At approximately 10, 15, 30 and 60 minutes after zero time, repeat the procedure of step 13.

15. Euthanize the goat.

16. Record the pH of the experimental solution. Check the ligature around the connection between the reticulum and the omasum and check the position of the cotton plug within the reticulo-omasal orifice. Check the position of the cannulas in the blood vessels.

Additional information now is presented to clarify some of the above steps.

Step 3. Briefly, the method of inserting the cannulas in the blood vessels was as follows. The end of the cannula to be inserted in a blood vessel was placed within a size 14 needle that had been shortened to 3/4 inches. The needle was inserted into the blood vessel, the cannula pushed through the needle into the blood vessel, and the needle withdrawn. After the withdrawal of the needle, the application of direct pressure for a few minutes at the site of entry of the cannula usually was sufficient to arrest any hemorrhage that occurred. The cannula was

filled with a solution of heparin to prevent the formation of blood clots. The free end of each cannula was closed with a forcep in order to prevent the heparin solution from being drawn into the blood vessel.

Step 4. The inverted-L incision consisted of two abdominal incisions made at right angles to one another. The first incision, 4 to 5 inches in length, was made about 1 inch caudal to the last rib. The dorsal commissure of the incision was just below a frontal plane that passed through the ends of the transverse processes of the lumbar vertebrae. The second incision began at the dorsal commissure of the first incision and extended in a frontal plane 4 to 5 inches caudad. These incisions formed a triangular section of the abdominal wall which could be drawn caudoventrad to expose that part of the portal venous system which lies just caudal to the liver.

Step 5. By drawing the caudate lobe of the liver dorsocraniad, the portal vein can be observed to enter the porta of the liver. At this location, the hepatic lymph node lies adjacent to the portal vein and can serve as a guiding landmark. About 1 to 2 inches caudal to the entrance of the portal vein into the liver, the gastrosplenic vein joins with the mesenteric vein to form the portal vein (see Figure 14, p. 103).

Step 7. In placing the ligature about the reticulo-omasal junction, great care had to be taken to pass the ligature so that, when it was tied, it would not compress the large veins in the area.

Polyethylene Glycol Assay[113]

Hydén and co-workers (181, 360) introduced polyethylene glycol as a

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[113]The principle of random assignment was followed in assaying the samples (see p. 304).

marker or reference substance in the study of ruminant digestion. They
not only found this substance not to be absorbed from the rumen, but
also not significantly to be broken down or absorbed by ruminal contents
in experiments of less than 10 hours duration. These characteristics of
polyethylene glycol and its relative ease and accuracy of measurement
make this chemical suitable for use as a marker or reference substance.
In the present ruminal fistula experiments, polyethylene glycol
was so used: it was added to the experimental solutions in order to
measure the volume changes of these solutions that might be caused by
the inflow of saliva into the rumen or by water movements across the
ruminal wall. Any such volume changes would be reflected by a change in
the concentration of the polyethylene glycol.

In the present experiments, the gravimetric method described by
Sperber et al (360) was utilized to assay the concentration of polyethylene
glycol. Table 39 presents the results of the assay of 5 samples of a
control solution of polyetheylene glycol. The coefficient of variation
of the data in Table 39 is 6.8 per cent. Sperber et al state that the
accuracy of the method of assay that is used in the present experiments
should be the same as the accuracy of a method that is described by
Oliver and Preston (275). Utilizing data of Oliver and Preston that
contained weights of the polyethylene glycol complex of the same order of
magnitude as the weights in Table 39, the present author has calculated
the coefficient of variation of their data to be 2.0 per cent. As it
was performed by the present author, the accuracy of the gravimetric
method of analysis for polyethylene glycol appears to have been reasonably
good.

TABLE 39. - Weights of polyethylene glycol complex
that were precipitated from five samples of a con-
trol solution of polyethylene glycol

Sample	Milligrams of polyethylene glycol complex
1	58.8
2	59.8
3	50.5
4	59.1
5	58.8

Propionic Acid Assay[114]

The following steam distillation procedure for the assay of propionic acid, essentially an adaptation of the method of Nish (271), was worked out in cooperation with Dr. W. D. Kitts.[115]

1. If protein is present in the sample to be assayed, then the sample should be deproteinized. In the present assay, this step was not necessary.

2. Samples of 10 milliliters were used. The samples were made up to a volume of 25 milliliters by the addition of distilled water.

3. Seventeen grams of $MgSO_4 \cdot 7H_2O$ were added to the sample to raise the boiling point and, thereby, cause the propionic acid to distill over more rapidly.

4. The samples were acidified by the addition of 1 milliliter of 10 N H_2SO_4. This addition was made in order to convert the salts of propionic acid to the undissociated acid.

5. The samples now were steam distilled until the distillate came over acid-free. During the steam distillation, the fluid level in the distilling flask was kept down to the original level by heating the flask with a small bunsen burner. The distillate was collected by running an adaptor tube from the condenser into a receiving flask that contained 100 milliliters of cold (5 - 10° C.) distilled water. Blank determinations, using 25 milliliters of distilled water, also were made.

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[114]The principle of random assignment was followed in assaying the samples .

[115]Assistant Professor of Animal Husbandry, Division of Animal Science, University of British Columbia, Canada.

Table 40 presents the results of the assay of 5 samples of a control solution of propionic acid. In making up the control solution, 3.9 milligrams of propionic acid were dissolved in each milliliter. The mean analytically-determined concentration of 4.14 milligrams per milliliter is, therefore, 106 per cent of the known concentration. The coefficient of variation of the data that are recorded in Table 40 is 3.9 per cent.

Microbiological Assay of Amino Acids and B Vitamins

The use of microorganisms for the quantitative assay of amino acids has been adopted widely. As early as 1945, Stokes et al (365) stated that "microbiological methods for the determination of amino acids, because of their specificity, accuracy, sensitivity, and ability to yield many replicate results within a short time, promise to become of increasing importance ... ." Several reviews (e.g., 29, 339, 365; see also 41 for critical comments) have been published on the theory, methods, and applications of the microbiological method of assay. Recently, Barton-Wright (30) has written a book entitled "The Microbiological Assay of the Vitamin B-Complex and Amino Acids." The following quotation from this book serves as a brief but adequate description of the microbiological assay method.

> The principle upon which all microbiological assays, whether of vitamin or amino acids, is based, is the preparation of a basal medium which shall contain in ample supply all the specific nutrients required by the particular organisms to be used for the assay, except the one to be determined. Graded doses of the particular essential nutrient to be assayed are next added to the basal medium. After the usual microbiological technique of sterilization, inoculation and incubation has been carried out, growth, or lactic-acid production, is measured and will be found over a certain range to be proportional to the concentration of the added essential factor. A parallel series

TABLE 40. - Analytically-determined concentrations
of propionic acid in five samples of a control
solution that contained 3.9 milligrams of propionic
acid per milliliter

Sample	Milligrams of propionic acid per milliliter
1	4.1
2	4.4
3	4.1
4	4.1
5	4.0
	mean = 4.14

of tubes of the test substance at different concentration
levels are set up at the same time and a comparison is
made of the growth in the two series.

In the present experiments, the assay procedures of Barton-Wright
were adhered to carefully. Only for the assays of methionine and tyro-
sine were the procedures of Barton-Wright modified slightly. For these
two amino acids, the procedure of Barton-Wright yielded little or no
growth of the assay microorganism in any of the test tubes. The assay
organism that was used was Leuconostoc mesenteroides P-60, American
Type Culture Collection number 8042. After carrying out a number of
experiments to determine the cause of this lack of growth, it was
found that the addition of 0.1 microgram of biotin per milliliter of
basal medium would permit maximum growth of the assay organism to occur.
The basal medium of Barton-Wright does not contain added biotin, even
although several authors (125, 356, 407) have reported the P-60 and
other strains of Leuconostoc mesenteroides to require biotin for growth.
The reason for the lack of addition of biotin to the basal medium is
not clear. It may be that the reason is one of error of omission. It
also can be suggested that the basal medium as prepared by Barton-
Wright contained biotin as a contaminant. Gaines and Stahly (125)
found that commercial tryptophan often was contaminated with biotin.
As the basal medium of Barton-Wright contains added DL-tryptophan,
perhaps the growth obtained by Barton-Wright in supposedly biotin-
lacking medium was because of contaminating biotin. The lack of growth
response in the present experiments could be, then, because of the
addition to the basal medium of a tryptophan that was free of biotin.
Another possible source of biotin as a contaminant is the Bacto-Peptone
that was used by Barton-Wright. It may be that the peptone that was

added by him to the basal medium contained biotin, whereas the peptone which was used in the present experiments lacked biotin. At present, the complete answer as to why the Barton-Wright basal medium failed to support the growth of <u>Leuconostoc</u> <u>mesenteroides</u> P-60 in this laboratory is not determined.

It should be noted here that, although the assay procedures of Barton-Wright were adhered to carefully, two refinements were made. The first refinement was made in order to allow the analysis of variance to be applied to the assay data. Schweigert and Snell (339) have found that the agreement in data that are obtained from replicate tubes which are prepared and incubated together is considerably better than the agreement in data that are obtained from replicate tubes which are prepared and incubated at random. Now in order to be able to use the analysis of variance method of statistical analysis, it is necessary that a valid estimate be made of the population error term. Such an estimate will not be made unless each of the replicates is a random independent variate (115). In the present experiments, therefore, with only one exception, all the steps of the microbiological assay procedure were applied to the tubes in a random fashion. A table of random numbers was utilized for this purpose. The one exception was the addition of the samples to their replicate tubes. In this case, it was technically quite difficult to use a table of random numbers. It was felt, however, that this was a minor violation of the principle of random assignment. The principle of random assignment also was adhered to in the assays of polyetheylene glycol, propionic acid, and alpha amino nitrogen.

The second refinement that was made was in the method of calculating the concentrations of the assayed amino acids and vitamins. For these

calculations, the coded dose method suggested by Bliss (39) was adopted, but with some modifications. The steps in the calculations are presented below.

1. For the standard solution data, plot the log-log relation of response (acid production in the present experiments) to the dose. Inspect the graph to find the range of doses that yield a straight line relationship. There will be a range of responses that corresponds to this range of dosages. Only those response data of the experimental solution that fall within the straight-line response range of the standard solution are used.

2. For the standard solution data calculate, by the method of least squares, the regression line equation, $Y = a + b(X - \bar{x})$. The Y of this equation represents the estimated response that corresponds to the dose X.

3. Substitute the response data of the experimental solution in the equation as Y and solve for X. The calculated X is multiplied by the appropriate dilution factor to obtain the concentration of the amino acid or vitamin in the experimental solution.

This method of calculation was used for all the amino acids and B vitamins except tryptophan. For tryptophan, the log-log plot did not yield a straight line portion and, therefore, the regression line equation could not be used. An arith-arith plot was made for the data of the tryptophan standard solution and the resulting curve of response on dosage was used for direct reading of the concentrations of tryptophan in the experimental solutions.

Assay of Amino Acids as Alpha Amino Nitrogen[116]

The theory and applications of the Van Slyke manometric apparatus have been presented adequately elsewhere (19, 182, 291). Van Slyke and co-workers (148, 391) have presented a description of the specific use of the manometric apparatus for the ninhydrin method of measuring the concentration of amino acids. The basic reaction of the ninhydrin method is the quantitative release of carbon dioxide from the carboxyl group of alpha amino acids. This release of carbon dioxide from an amino acid is brought about by boiling the amino acid with ninhydrin in a special reaction vessel. The carbon dioxide gas then is transferred from the reaction vessel to the extraction chamber of the manometric apparatus. In the chamber, the pressure produced by the gas at a specified volume is determined. The measured pressure then is multiplied by a conversion factor to yield the weight of alpha amino nitrogen per unit volume of solution. The following statement has been made as to the specificity of the ninhydrin test (391).

> The reaction is specific for free amino acids in that it requires the presence, in the free, unconjugated state, of both the carboxyl and the neighboring NH_2 or $NH-CH_2$ groups.

Table 41 presents the results of control assays for several different amino acids. The percentage recoveries ranged between 96.5 and 100.4 per cent. The average coefficient of variation for the measurements of the amino acids alanine, phenylalanine, and methionine is 1.6 per cent.

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[116] The principle of random assignment was followed in assaying the samples (see p. 304).

TABLE 41. - Analytically-determined concentrations of alpha amino nitrogen in eleven control samples, each sample containing one of four amino acids

Sample	Amino acid	Milligrams of alpha amino nitrogen		Percentage recovery
		By calculation	By analysis	
1	L-alanine	0.786	0.782	99.5
2	L-alanine	0.786	0.759	96.6
3	L-alanine	0.786	0.784	99.8
4	L-aspartic acid	0.526	0.516	98.1
5	DL-phenylalanine	0.424	0.413	97.4
6	DL-phenylalanine	0.424	0.417	98.4
7	DL-phenylalanine	0.424	0.409	96.5
8	L-methionine	0.470	0.472	100.4
9	L-methionine	0.470	0.456	97.0
10	L-alanine	0.786	0.786	100.0
11	L-alanine	0.786	0.788	100.3

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