## PHYSIOLOGY OF THE EXOCRINE PANCREAS

IN RELATION TO PROTEIN UTILIZATION BY THE CHINCHILLA

BY

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Date $\qquad$ July 25, 1973

## ABS TRACT


#### Abstract

PROTEIN UTILIZATION WAS INVESTIGATED IN THE CHINCHILLA FROM BIRTH TO POST-WEANING BY STUDYING THREE IMPORTANT STAGES OF GROWTH AND NUTRITIONAL ADAPTATION, OCCURING AT BIRTH, WEANING, AND IN ADULT LIFE.


THE FIRST AREA OF STUDY WAS CENTERED ON THE PRE-COLOSTRAL CHINCHILLA, A PERIOD WHICH IN MANY SPECIES IS ACCOMPANIED BY ABSORPTION OF INTACT PROTEINS AND IMMUNOGLOBULINS.IMMUNOGLOBULINS OF THE BLOOD SERA FROM BIRTH TO POST-WEANING WERE QUANTITATED BY POLYACRYLAMIDE DISC GEL ELECTROPHORESIS. THE LEVELS OF IMMUNOGLOBULINS DID NOT CORRELATE WITH PANCREATIC ENZYME OR INHIBITORY LEVELS IN ANY WAY SO AS TO SHOW A RELATIONSHIP BETWEEN INHIBITED ENZYME LEVELS AND THE AMOUNT OF IMMUNOGLOBULIN ABSORBED INTO THE BLOOD STREAM. TRYPSIN AND CHYMOTRYPSIN INHIBITORS, ASSAYED SPECTROPHOTOMETRICALLY, WERE NOT APPRECIABLE IN THE PANCREATIC homogenate from the young chinchilla tested.<br>THE STUDY OF ADAPTATION CAN BE BASED ON THE ASSUMPTION THAT AT DIFFERENT STAGES OF GROWTH, THE CHINCHILLA UTILIZES THE PROTEINS OF ITS DIET TO DIFFERENT EXTENTS. THIS IS IN ACCORDANCE WITH THE CHANGE IN LEVEL OF PROTEIN BETWEEN ITS FIRST DIET OF MATERNAL MILK AND THEN ITS SECONDARY DIET OF ROUGHAGE. IN ORDER TO VERIFY THIS ASSUMPTION, PANCREATIC PROTEASE ACTIVITY, OF TRYPSIN AND GHYMOTRYPSIN, WAS STUDIED IN SEVENTEEN CHINCHILLA FROM BIRTH TO POST-WEANING.

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HOMOGENATES OF THE PANCREAS, ASSAYEO` SPECTROPHOTOMETRICALLY, WERE
USED TO MONITOR THE ENZYMATIC ACTIVITY. THOUGH NOT SIGNIFICANTLY
DIFFERENT FOR THE RESPECTIVE AGE GROUPS, TRYPSIN ANO. CHYMOTRYPSIN
LEVELS TENDED TO BE HIGHEST AT BIRTH, DROPPING AT ABOUT DAY THREE
TO DAY EIGHT AND THEN RISING SLIGHTLY AT ABOUT SIX WEEKS OF AGE
WHERE THE vAluES LEVElled off.
    THE THIRD AREA OF STUDY WAS THE JUVENILE AND ADULT ADJUSTMENT
TO VARIOUS RATIONS. IN THIS CASE, FIVE RATIONS VARIED IN PROTEIN
LEVELS, RANGING FROM A LOW PROTEIN LEVEL (11.2%) TO A RELATIVELY
HIGH PROTEIN LEVEL (24.5%). PANCREATIC TRYPSIN AND CHYMOTRYPSIN
LEVELS WERE INVESTIGATED TO DETERMINE IF PANCREATIC PROTEASE WOULD
RESPOND TO THE LEVEL OF PROTEIN IN THE DIET. AGAIN, ENZYME LEVELS
WERE NOT SIGNIFICANTLY DIFFERENT BETWEEN DIETS, BUT THERE WAS A
TENDENCY FOR THE HIGHER PROTEIN DIETS TO RESULT IN HIGHER ENZYME
VALUES.
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## table of contents

## Page

ABS TRACT ..... 1
LIST OF TABLES ..... vi
LIST OF FIGURES ..... VII
LIST OF APPENDICES ..... v:l!
ACKNOWLEDGEMENTS ..... IX
INTRODUCTION ..... 1
LITERATURE REVIEW ..... 3
A. Structure and Development of the Exocrine Pancreas ..... 3
B. The Enzymes of the Exocrine Pancreas ..... 4

1. Trypsin ..... 4
2. Chymotrypsin ..... 6
C. Ultimate Controls of Enzyme Synthesis, Secretion, inactivation and Inhibition ..... 7
3. The Effect of age on Pancreatic Protease Activity ..... 7
4. The Effect of feeding Regimen on Pancreatic Activity ..... 7
5. The Effect of Amino Acids on Pancreatic Activity ..... 9
6. The Effect of Natural Inhibitors on Pancreatic Activity ..... 10
D. Proximate Controls of Enzyme Secretion (reflex, hormonal, humoral) ..... 10
MATERIALS AND METHODS ..... 14
A. Experiment 1. Protease Activity and Absorption of Immunoglobulins by Newborn Chinchilla ..... 14
7. ExpERIMENTAL ANIMALS ..... 14
8. Serum immunoglobulins ..... 14
9. TRYPSIN INHIBITORS ..... 15
B. Experiment 2. Development of Enzyme Profile from Birth to maturity. ..... 16
10. Experimental Animals ..... 16
11. Preparation of Pancreatic Tissue for Enzyme Assay ..... 16
12. Statistical Treatment ..... 17
C. Experiment 3. Role of Protein level of diet on Enzyme Profile ..... 17
13. Experimental Animals ..... 17
14. Preparation of Pancreatic Tissue for Enzyme Assay ..... 17
15. Experimental Design and Statistical Treatments ..... 18
D. BIochemical Analyses for Experiment 2 and experiment 3 ..... 18
16. Homogenization of the Pancreas ..... 18
17. Activation by Enterokinase ..... 19
18. Determination of protein ..... 19
19. ACtivation of Enzymes ..... 20
20. Enzyme Assays ..... 20
A. Total Protease ..... 20
B. Trypsin and Chymotrypsin ..... 21
RESULTS AND DISCUSSION ..... 23
A. Protease Activity and Absorption of Immunoglobulins by Newborn Chinchilla ..... 23
21. Immunoglobulin Absorption ..... 23
22. Trypsin inhibitors ..... 28
B. Development of Enzyme Profile from Brith to
Maturity ..... 29
23. Results ..... 29
24. Discussion ..... 29

## Page

C. Role of Protein level of Diet on Enzyme Profile ..... 42

1. Results ..... 42
2. Discussion ..... 42
SUMMARY AND CONCLUSIONS ..... 55
BIBLIOGRAPHY ..... 57
APPENDICES ..... 62

## LIST OF TABLES

Table Page

1. Routes of Transmission of Maternal Antibody in Various Species . . . . . . . . . . . . . . . . . . ..... 23
2. Placental Types ..... 27

## LIST OF FIGURES

Figure Page

1. Factors Affecting Pancreatic Protease Secretion ..... 12
2. Range of Changes in Level of $\quad$-Globulins in Serum of Chinchillas from Birth to Twelve Weeks of Age ..... 24
3. Relative Changes in Trypsin and Chymotrypsin with Respect to Booy Weight in the Chinchilla from Birth to Twelve Weeks ..... 30
4. Relative Changes in Trypsin and Chymotrypsin from the Pancreatic Homogenate of the Chinchilla from Birth to Twelve Weeks. ..... 32
5. Relative Changes in the Ratio of Chymotrypsin and Trypsin with Respect to Body Weight in the Chinchilla from Birth to Twelve Weeks ..... 34
6. Relative Changes in total Protease activity Per mg. Protein in Pancreatic Homogenate in the Chinchilla from Birth to Twelve Weeks ..... 36
7. Range of Values for Trypsin Units from Chinchilla fed
Rations with Varying levels of Protein ..... 43
8. Range of Values for Chymotrypsin Units from Chinchilla fed rations with Varying Levels of protein ..... 45
9. Ratio Chymotrypsin:Trypsin. Range and average values from Chinchilla fed Rations with Varying Levels of Protein ..... 47
10. Range of Values for total protease Activity from Chinchilla feo Rations with Varying levels of Protein ..... 49

## LIST OF APPENDICES

APPENDIX Page

1. Serum Protein Levels in the Chinchilla from Birth to Twelve Weeks. ..... 63
2. Relative Changes in Trypsin and Chymotrypsin with Respect to Body Weight in the Chinchilla from Birth to Twelve Weeks ..... 64
3. Total Protease activity and Chymotrypsin: Trypsin With Respect to Body Weight in the Chinchilla from Birth to Twelve Weeks ..... 654. TRypsin and Chymotrypsin Units in relation toBody Weight in Chinchilla Fed Rations withVarying Levels of Protein . . . . . . . . . . . . 66
4. TOTAL Protease and Chymotrypsin:TrypsinRelation to booy Weight of ChinchillaFed Rations with Varying Levels ofPROTEIN. . . . . . . . . . . . . . . . . . . . . . 67
5. Protease Activity of Different Species with Respect to \% Immunoglobulins Acquired After Birth, Protein Content of the Milk, and Physiological Age at Birth ..... 68
6. Example Graph to Illustrate Linearity of Regression Line for Analysis of data FOR EXPERIMENT 3 ..... 70

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## INTRODUCTI ON

THE POTENTIAL OF AN ANIMAL FOR GROWTH CANNOT BE FULLY REALIZED UNLESS THE DIET IT IS CONSUMING PROVIDES ALL ITS PROTEIN AND ENERGY NEEDS. IN ORDER TO UNDERSTAND NUTRIENT REQUIREMENTS FOR A SPECIFIC SPECIES OF ANIMAL, ONE SHOULD BE AWARE OF THE PHYSIOLOGY OF ITS DIGESTIVE PROCESSES. THIS STUDY ATTEMPTS TO INVESTIGATE THE PHYSIOLOGY OF THE CHINCHILLA EXOCRINE PANCREAS IN RELATION TO PROTEIN UTILIZATION WITH EMPHASIS ON ELUCIDATING WHAT MAJOR FACTORS ARE INVOLVED IN THE STIMULATION OF THE PROTEASE SYNTHESIS AND SECREATION. GROWTH OF THE YOUNG ANIMAL RESULTS IN CORRESPONDING CHANGES IN THE PHYSIOLOGY OF ITS DIGESTIVE TRACT. IN ORDER TO ADAPT TO NATURAL CHANGES IN THE DIET, ENZYME SYSTEMS OF THE ANIMAL, TO A CERTAIN EXTENT, MUST BE RESPONSIVE TO THESE CHANGES. THE FIRST PERIOD OF MARKED CHANGE IN PROTEIN NUTRITION OCCURS AT BIRTH. THE YOUNG ANIMAL DEPENDS UPON ABSORPTION OF MATERNAL IMMUNOGLOBULINS FROM THE COLOSTRUM. THE DIGESTIVE TRACT IS VERY PERMEABLE TO THESE LARGE PROTEINS FOR A SHORT PERIOD OF TIME AFTER BIRTH. TRYPSIN AND CHYMOTRYPSIN WOULD DEGRADE THESE PROTEINS IF PRESENT IN LARGE QUANTITIES WHEN THE GUT IS MOST RECEPTIVE TO THEM. SIMILARLY, TRYPSIN AND CHYMOTRYPSIN INHIBITORS PRESENT IN COLOSTRUM OR IN THE DIGESTIVE TRACT OF THE CHINCHILLA WOULD PREVENT THESE ENZYMES FROM DIGESTING THE IMMUNOGLOBULINS BY COMBINING WITH THE ENZYMES THEMSELVES. YOUNG CHINCHILLA START LIFE ON A MATERNAL MILK DIET, WHICH IS

HIGHER IN PROTEIN THAN THEIR WEANLING DIET OF.ROUGHAGE. SINCE IT WAS FOUND THAT THE ACTIVITIES OF CERTAIN CARBOHYDRASES INCREASE WITH AGE TO MEET WITH AN INCREASING DEMAND TO DIGEST CARBOHYDRATES (45), A HYPOTHESIS WAS TESTED TO SEE IF AGE AND/OR FEED AND FEEDING REGIMEN WERE MAJOR FACTORS IN THE STIMULATION OF PANCREATIC DEVELOPMENT. AS WELL AS MONITORING PANCREATIC PROTEASE DEVELOPMENT IN THE GROWING CHINCHILLA, AN ATTEMPT WAS MADE TO TEST THE PROPOSAL THAT THE PANCREAS OF THE WEANED CHINCHILLA ADAPTS TO CHANGES IN PROTEIN LEVEL of the diet. Weaned chinchilla were fed varying levels of proteln IN THEIR RATION RANGING FROM ABOUT $11 \%$ TO $24 \%$ PROTEIN AND TRYPSIN AND CHYMOTRYPSIN ACTIVITIES WERE QUANTITATED.

The objectives of this study were as follows: (a) to I NVESTIGATE WHETHER IMMUNOGLOBULIN ABSORPTION WAS AFFECTED BY PROTEASE LEVELS; (B) TO DETERMINE WHETHER PANCREATIC ENZYME INHIBITORS WERE RELATED TO THE ENZYME LEVELS IN THE DIFFERENT AGE GROUPS; (C) TO MEASURE THE CHANGES IN PANCREATIC PROTEASE LEVELS WITH GROWTH OF THE CHINCHILLA, AND (D) TO DETERMINE WHETHER OR NOT THE FPAANCREEASS WOULD REACT TO A CHANGE OF PROTEIN LEVEL IN THE DIET.

## LITERATURE REVIEW

## A. Structure and Development of the Exocrine pancreas



Histologically, the acinar cells are characterized by the PRESENCE OF ABUNDANT ROUGH-SURFACED ENDOPLASMIC RETICULUM AND LARGE ZYMOGEN GRANULES IN THEIR CYTOPLASM; THEIR APICAL SURFACES POSSESS microvilli. The lumen of the acini is continuous with that of small DUCTS LINED BY CENTROACINAR CELLS. THE DISTAL PORTION OF THE SMALL dUCTS EXtENDS ItSElf Into the intralobular or intercalated ducts. The latter are lined by a columnar epithelium in which occasional goblet cells are present and have a moderately thick wall of dense connective tissue that contains some muscle fibres and nerve elements. THE MAIN FUNCTION OF THE LARGE DUCTS IS THE TRANSPORT OF THE PANCREATIC SECRETION FORMED BY ACINAR AND POSSIBLY CENTROACINAR AND ductal cells to the duodenum. The mucous layer that lines the lumen of these ducts seems to act as a protective barrier between the ENZYME RICH DUCTAL CONTECTS AND THE UNDERLYING PARENCHYMA (4).

## B. The Enzymes of the Exocrine pancreas

Pancreatic juice contains several enzymes of importance to the digestive process. These include trypsinogen, chymotrypsinogen, PROCARBOXYPEPTIDASES, A LIPASE, AN $\alpha$-AMYLASE, MALTASE AND ribonuclease (56). The proteases with which this study is concerned, TRYPSIN AND CHYMOTRYPSIN, WILL BE DISCUSSED.

1. TRYPSIN.

The proteolytic enzyme trypsin and its inactive precursor, TRYPSINOGEN, WERE FIRST OBTAINED IN CRYSTALLINE FORM FROM BOVINE pancreatic tissue by Northrop and Kunitz (37). Trypsinogen
(M.W. $=24,500$ ) IS TRANSFORMED INTO TRYPSIN AS THE RESULT OF THE

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cleavage of a single peptide bond (Lys}\mp@subsup{\sigma}{6}{-ILE}7\mathrm{ ) near the N-terminus of
the zymogen, and the appearance of activity is accompanied by
conformational changes. The activation process is catalysed by a
variety of enzymes including enterokinase, mold proteases, and trypsin
itself. The latter autocatalytic process is accelerated by calcium
ions which bind to the N-terminal region of the zymogen and promote
the specific bond cleavage. A striking feature of the enzyme is the
NARROW SPECIFICITY OF ITS ACTION, WHICH IS ALMOST EXCLUSIVELY
dIrected toward L-lysl and L-argininyl bonos of polypeptides.
Biologically, trypsin serves as the activator of all the other zymogen
of pancreatic tissue. Thus, the control of the activation of
trypSINOGEN has broad consequences in terms of the formation of the
ENDOPEPTIDASE AND EXOPEPTIDASE COMPONENTS OF PANCREATIC JUICE.
    The substrates of trypsin can be described by the general
formula, r-CO-X, Where the Narrow specificity of trypsin is determined
by the acyl moity (r-co-). The type of bond cleaved (i.e. peptide,
amide or ester) is defined by the nature of X.
    The most widely used assays for trypsin follow the esterase
ACtivity toward benzoyl-L-ARginine ethylester (bAEE) or p-toluene
sulfonyl-L-ARGinine methylester (tame) by potentiometric or
SPECTROPHOTOMETRIC PROCEDURES. TAME hAS GREATER SENSItIVIty ANO
greater selectivity (chymotrypsin does not hydrolyze tame): than
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BAEE.

Trypsin is stable at ph 3 and low temperatures where it can be stored for weeks without loss of activity. the addition of calcium IONS to trypsin solutions retards autolysis. This stabilizing effect

IS ACCOMPANIED BY A CONFORMATIONAL CHANGE IN THE TRYPSIN MOLECULE, APPARENTLY INDUCED BY CALCIUM AND RESULTING IN A MORE COMPACT STRUCTURE.

AN IMPORTANT FEATURE OF TRYPSIN IS ITS NARROW SPECIFICITY OF ACTION WHICH RENDERS IT SUITABLE FOR SPECIFIC CLEAVAGE AT LYSYL OR ARGINYL RESIDUES IN AMINO ACID SEQUENCE ANALYSIS. THE SPECIFICITY OF TRYPSIN IS DIRECTED TOWARD LYSL AND ARGINYL RESIDUES IN NATURAL AND SYNTHETIC SUBSTRATES, THUS EXHIBITING THE MOST RESTRICTED SPECIFICITY OF ACTION OF THE KNOWN ENDOPEPTIDASES (54).

## 2. CHYMOTRYPSIN.

Chymotrypsinogen A (M.W. $=25,000$ ) is converted to active ENZYME AT PH 7.6 BY TRYPTIC CLEAVAGE OF THE BOND BETWEEN ARG 15 AND ILE $16^{\circ}$ IT AND CHYMOTRYPSINOGEN B, REPRESENT $32 \%$ OF THE PROTEIN CONTENT OF PANCREATIC EXTRACTS. DEPENDENT UPON THE CONDITIONS, CHYMOTRYPSINOGEN A MAY BE ACTIVATED TO $\alpha-, \beta-, \gamma-, \delta-, O R \quad \pi-$ CHYMOTRYPSIN (59). CRUDE CHYMOTRYPSINOGEN SOLUTIONS (AT PH 7.O-8.0) ARE RAPIDLY ACTIVATED BY AN EXTRACT OF THE SMALL INTESTINE (ENTEROKINASE), BUT CANNOT BE ACTIVATED BY•SMALL AMOUNTS OF TRYPSIN. RECRYSTALLIZED CHYMOTRYPSIN, HOWEVER, CAN ONLY BE ACTIVATED BY TRYPSIN. CHYMOTRYPSIN BINDS ONE CALCIUM ION (CA ${ }^{++}$) AND THEREBY BECOMES STABHLIZED AGAINST DENATURATION. THE PRESENCE OF THE CA ++ HAS NO EFFECT ON THE RATE OF ACTIVATION OF THE ZYMOGEN BY TRYPSIN (57). CHYMOTRYPSIN PREFERENTIALLY CATALYSES THE HYDROLYSIS OF PEPTIDE BONDS INVOLVING THE L-ISOMERS OF TMROSINE, PHENYLALANINE AND TRYPTOPHANE. THE ENZYME READILY ACTS ON AMIDES AND ESTERS OF SUSCEPTIBLE AMINO ACIDS (59).
C. ULTIMATE CONTROLS OF ENZYME SYNTHESIS, SECRETION, INACTIVATION AND INHIBITION

1. The Effect of Age on Pancreatic Protease Activity.

Young mammals of most species may change the composition of the feedstuffs they consume during early life. A milk diet, gradually REplaced by a vegetable or meat diet, would require changes in the CONCENTRATION OF ENZYMES IN THE DIGESTIVE TRACT RESPONSIBLE FOR BREAKING DOWN THESE NUTRIENTS.

RESULTS VARY FROM AUTHOR TO AUTHOR AND ALSO FROM SPECIES TO SPECIES, AND THEREFORE, CAN BE CONFLICTING. It has been demonstrated that the pancreatic secretion in the baby pig is highly irregular and UNPREDICTABLE WITH RESPECT TO THE PROTEOLYTIC ENZYMES (23), HOWEVER, Carbohydrases have been quantitated (1, 25). Pancreatic proteases of the chinchilla as yet have not been studied.
2. The effect of feeding regimen on pancreatic activity.

SEcretion of pancreatic juice is under both neural and hormonal CONTROL. THE PRESENCE OF LARGE POLYPEPTIDES OR ACID IN THE UPPER duodenum results in liberation of a hormone, secretin, into the CIRCULATION, RESULTING IN AUGMENTED FLOW OF PANCREATIC JUICE AND, TO A lesser extent, of bile and intestinal juices. A second intestinal HORMONE, PANCREOZYMIN, UNLIKE SECRETIN, STIMULATES SECRETION OF enzymes by the pancreas (56).

By Lett.ing animals go without food but administering water it is possible to almost halt secretion by the pancreas. The flow of PANCREATIC JUICE UNDER CONDITIONS OF STARVATION VAARIEES IN DIFFERENT

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species. IN thE mOUSE AND thE GUINEA PIG, thE ENZYME CONTENT OF THE
PANCREAS IS UNAFFECTED AFTER TWENTY-FOUR HOURS STARVATION (16).
DIfferences obSERVED betwEEN THE REST|NG aND ACTIVE cells of RABBIT
PANCREAS WERE NOT CORRELATED WITH PHASES OF DIGESTION SINCE IN THIS
ANIMAL DIGESTION IS ALMOST A CONTINUOUS PROCESS (51). IN CONTRAST TO
THE RABBIT AND RODENTS, THE DOG AND THE CAT SECRETE VERY LITTLE
DURING STARVATION, AND RESPOND WELL TO STIMULUS BY SECRETIN OR
PANCREOZYMIN (16). THE VOLUME AND PROTEIN CONTENT OF THE PANCREATIC
JUICE VARIES CONSIDERABLY WITH THE TYPE OF FOOD EATEN. FOR EXAMPLE,
A MEAL RICH IN FAT CAN RESULT IN THE JUICE BEING VERY RICH IN
PROTEIN (2).
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PANCREATIC AND INTESTINAL PROTEASES ARE RESPONSIBLE FOR
CARRYING OUT THE DIGESTION OF BOTH EXOGENOUS AND ENDOGENOUS PROTEIN.
THEREFORE, PROTEOLYTIC ENZYME SECRETION MUST CONTINUE EVEN WHEN DIETS
DEVOID OF PROTEIN ARE FED. LIKEWISE, PROTEOLYTIC ENZYME SECRETION
MUST BE INCREASED WHEN PROTEIN IS FED TO COMPENSATE FOR THE INCREASE
IN THE LOAD OF DIGESTION.

There is ample evidence in the literature to support the CONCLUSION THAT THE EXOCRINE ENZYMES OF THE PANCREAS OF MOST SPECIES ADAPT TO THE COMPOSITION OF THE DIET. THE EFFECT OF FEEDING REGIMEN ON THE DEVELOPMENT OF ENZYME ACTIVITY IN THE WEANED, GROWING CHINCHILLA HAS NOT BEEN STUDIED EXTENSIVELY. SMITH (45) SHOWED THAT PANCREATIC AMYLASE INCREASED STEADILY IN THE CHINCHILLA FROM BIRTH TO MATURITY, HOWEVER, THE EFFECTS OF VARIOUS DIETS WERE NOT STUDIED. AOAPTION OF THE EXOCRINE PANCREAS TO CHANGES IN THE AMOUNT OF PROTEIN IN THE DIET HAS NOT BEEN STUDIED IN THE CHINCHILLA TO DATE.
3. The Effect of Amino Acids on Pancreatic Activity.
LYMAN AND WILCOX (32) OBSERVED THAT ESSENTIAL AMINO ACID deficiencies lowered the enzyme content of the rat pancreas with CERTAIN ENZYMES BEING AFFECTED MORE BY ONE DEFICIENCY THAN OTHERS. VEGHELYI ET AL. (52) SHOWED THAT THE PANCREATIC GLANDS OF RATS FED METHIONINE-DEFICIENT DIETS FOR FOUR WEEKS CONTAINED NORMAL AMOUNTS OF AMYLASE, REDUCED AMOUNTS OF CHYMOTRYPSIN AND NO TRYPSIN. MAGEE AND ANDERSON (33) CONCLUDED THAT PANCREATIC SECRETION IS MORE DEPENDENT ON DUODENAL STIMULATION THAN IT IS UPON THE COMPLETENESS OF THE PROTEIN.EATEN WITHIN LIMITS SHORT, PRESUMABLY, OF EXTREME INANITION. Magee and hong (34) demonstrated that compared with controls, ISOLEUCINE, METHIONINE, PHENYLALANINE, LYSINE OR TRYPTOPHANE supplements did not increase amylase. Methionine increased lipase AND protease activity and phenylalanine and isoleucine increased PROTEASE ACTIVITY ALONE.
Rats fed a low-protein diet containing additional valine had HIGHER PANCREATIC AND PROTEOLYTIC ACTIVITY IN THE RESTING PANCREAS than controls fed the same low-protein diet (3З). Thus, it was felt THAT VALINE ACTS AS A STIMULUS FOR THE RELEASE OF PANCREATIC HORMONES in the duodenum. Even on a diet so deficient in protein that the animals lost weight, the pancreas increased its manufacture of LIPASE AND PROTEOLYTIC ENZYMES APPARENTLY BECAUSE THE DUODENUM WAS adequately stimulated. The valine-fed animals in these experiments gained less weight than the controls. Animals fed diets high in zein, GELATIN OR CARBOHYORATE HAD LESS LIPASE AND PROTEOLYTIC ENZYMES IN the resting pancreas than the casein-fed controls. The duodenal

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STIMULUS FROM THESE DIETS MAY HAVE BEEN INADEQUATE.DUE TO INSUFFICIENT
PROTEIN, POORLY DIGESTED PROTEIN OR PROTEIN DIFICIENCY IN THE
PANCREOZYMIN STIMULATING AMINO ACIDS. EBISUZAZI ET AL. (9) SUGGESTED
THAT THREONINE INHIBITS PANCREOZYMIN RELEASE.
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4. The effect of Natural Inhibitors on Pancreatic activity. Further proof that the pancreas adapts to the amount of PROTEIN IN THE DIET COULD BE OBTAINED bY FEEDING A PROTEASE INHIBITOR. Lyman and Lepkovsky. (31) observed enlargment of the pancreas CONTAINING EXCESSIVE AMOUNTS OF PROTEOLYTIC ENZYMES IN CHICKS AND rats fed a raw soybean diet. The increased pancreatic secretion COULD BE A MEANS OF OVERCOMING AN INHIBITION OF PROTEOLYTIC ENZMYES IN THE INTESTINE.

DIETARY INHIBITORS ASSUME GREATEST IMPORTANCE AT THE TIME OF BIRTH WHEN THE ANIMAL FIRST CONSUMES COLOSTRUM. COLOSTRAL TRYPSIN INHIBITORS PLAY AN IMPORTANT ROLE IN THE MECHANISM OF ABSORPTION OF Immunoglobulins by a newborn animal (26). They are supposedly IMPORTANT AS PROTECTIVE AGENTS FOR THE IMMUNOGLOBULINS IN THE COLOSTRUM AGAINST TRYPTIC DEGRADATION IN THE INTESTINAL TRACT OF the newborn animals (10).

## D. Proximate Controls of Enzyme Secretion (reflex, HORMONAL, HUMORAL)

A consideration of the possible mechanisms of enzymatic ADAPTATION TO THE MAJOR CONSTITUENT OF THE DIET SUGGESTS THREE possibilities: (1) a REFLEX SECRETORY MECHANISM IN. WHICH INCREASED PROTEIN IN THE INTESTINE WOULD REFLEXLY STIMULATE THE PRODUCTION OF



## MATERIALS AND METHODS

A. EXPERIMENT 1. PROTEASE ACTIVITY AND ABSORPTION OF IMmunoglobulin by Newborn Chinchilla

1. Experimental Animals.

Serum from seventeen chinchilla, species Chinchilla lanigera, housed at the University of British Columbia chinchilla unit was ANALYSED FOR CHANGES IN GAMMA-GLOBULINS (IMMUNOGLOBULINS) AND albumin. Two animals were sacrificed at each time interval of SAMPLING: AT BIRTH, THREE DAYS, EIGHT DAYS, TWO WEEKS, SIX WEEKS, eight weeks, ten weeks, twelve weeks, and adult. the animals were EUTHANIZED BY AN INTRAPERITONIAL INJECTION OF ONE TO TWO MILLILITRES OF SUCCINYL CHOLINE CHLORIDE, DEPENDING ON THE SIZE OF THE CHINCHILLA. After euthanization, blood samples were collected by severing the AORTA IN THE THORACIC CAVITY AND TAKING UP THE BLOOD WITH A PASTEUR pipette. Samples were collected in non-heparinized test tubes, centrifuged at 10,000 rpm for ten minutes, serum collected and then FROZEN AT $-20^{\circ} \mathrm{C}$ UNTIL ANALYSED.
2. Serum Immunoglobulins.

POLYACRILAMIDE DISC GEL ELECTROPHORESIS (38) WAS USED IN ORDER TO MONITOR THE CHANGES IN GAMMA-GLOBULINS AND ALBUMIN OF THE SERA. UPON ANALYSIS, 5 NL OF THE bLOOD SERUM WERE PIPETTED DIRECTLY ONTO the stacking gel on top of the $7 \%$ separating gel, and run at 2.5 mamp

PER COLUMN FOR 10 MINUTES THEN SWITCHED TO 4.75 MAMP PER COLUMN UNTIL the tracer dye went off the end of the column. gels were stained WIth amido black for about 30 minutes, destained with $7 \%$ acetic ACID AND STORED IN $7 \%$ ACETIC ACID AT $4^{\circ} \mathrm{C}$. READINGS WERE TAKEN ON A Photovolt densitometer (Model 52C). Proteins were quantitated by measuring peak height oirectly.
3. TRypsin Inhibitors.

Blood serum samples and pancreatic homogenates from the five yOUNG EHINCHILLA (1-DAY TO 3-DAYS OF AGE) FROM THE ABOVE EXPERIMENT and Experiment 2 respectively were investigated for the presence of trypsin inhibitor. The method of fritz, hartwich and Werle (11) was FOLLOWED EXACTLy USING THE Synthetic substrate $\alpha$ - N-benzoyl-DL-ARGININE-P-NITROANILIDE (BAPNA). THE SUBSTRATE SOLUTION WAS PREPARED By dissolving 1 mg bapna per ml distilled water at $100^{\circ} \mathrm{C}$. The ENZYME SOLUTIONS WERE PREPARED BY DISSOLVING 1 MG OF TRYPSIN PER ML of 0.001 M HCl. Dilutions to 0.5 and 0.1 mg per ml were made. The enzyme standard solutions were then kept at $4^{\circ} \mathrm{C}$ until used; O.2 M TRIS-HCL BUFFER, PH $7.8\left(22^{\circ} \mathrm{C}\right)$, WAS USED FOR THE DETERMINATIONS: Measurements were performed at $30^{\circ} \mathrm{C}$ by first mixing 0.025 to 0.100 ml of the sample with buffer in a glass cuvette and then ADDING 0.050 to 0.100 mL OF the enzyme solution (0.5 or 0.1 mg PER ml). The volume was adjusted to a final volume of 2.0 ml with buffer solution and incubated at $30^{\circ} \mathrm{C}$ for 3 minutes. The enzyme reaction WAS STARTED By adoing 1.0 ml OF SUBStrate $\left(30^{\circ} \mathrm{C}\right)$. The release of p-Nitroaniline was then registered at 405 nm for 2 minutes.

## B. EXPERIMENT 2. DEVELOPMENT OF ENZYME PROFILE FROM BIRTH TO MATURITY

1. Experimental Animals.

Data were obtained from the same seventeen chinchilla, species CHinchilla lanigera, used in experiment 1. The animals were fed a COMMERCIAL CHINCHILLA RATION THROUGHOUT THE DURATION OF THE EXPERIMENT AND itihe young were naturally weaned.
2. Preparation of Pancreatic Tissue for Enzyme Assay.

After weighing the carcass, the pancreas was quickly dissected OUT AND PLACED in A WEIGHING DISH ON CRUSHED ICE. THE PANCREAS WAS RINSED WITH ICE-COLD, O. 15 M NACL AND DISSECTED FREE OF ADHERRING FAT. (Pancreatic tissue can be distinguished from fat tissue since, when COOLED ON IICE, tHE fAT TISSUE TAKES ON A CHARACTERISTICALLY LIGHTER color than the more pink color of the pancreatic tissue.) The pancreas was then placed in a vial, sealed and frozen at - $20^{\circ} \mathrm{C}$ until ANALYSED. THE LUNGS, HEART, SPLEEN, ADRENALS, LIVER AND KIDNEYS WERE ALSO DISSECTED OUT AND WEIGHED FOR A GROWTH CHART.
(USING INTESTINAL CONTENTS FOR ENZYME ASSAYS FOR ABSOLUTE TRYPSIN AND CHYMOTRYPSIN VALUES WOULD NOT BE AS VALID AS USING pancreatic tissue extracts. PElot and Grossman (40) found that CHYMOTRYPSIN IS INACTIVATED MORE RAPIDLY THAN TRYPSIN IN THE RAT Intestine, therefore, enzymatic ratios would not be reliable. Using TISSUE EXTRACTS ALLOWS ONE TO MEASURE MAXIMUM TRYPSIN OR CHYMOTRYPSIN ACTIVITY IN VITRO BEFORE INACTIVATION, OR PROTEOLYSIS, BEGINS.)
3. Statistical Treatment.

Protease levels for the different age groups were analysed by the least squares method (19). Independent variables used were age, SEX, AND BODY WEIGHT. DEPENDENT VARIABLES USED WERE MG PROTEIN/ML homogenate, mg nucleic acio/ml homogenate, trypsin units, chymoTRYPSIN UNITS, TRYPSIN UNITS/BODY WEIGHT, CHYMOTRYPSIN UNITS/BODY WEIGHT, TOTAL PROTEASE ACTIVITY, TOTAL PROTEASE ACTIVITY/BODY WEIGHT, CHYMOTRYPSIN:TRYPSIN AND CHYMOTRYPSIN:TRYPSIN/BODY WEIGHT.
C. EXPERIMENT 3. ROLE OF PROTEIN LEVEL OF DIET ON ENZYME PROFILE

1. Experimental Animals.

Data were collected from sixteen young adult chinchilla, SPECIES CHINCHILLA LANIGERA, WHICH WERE COLLECTED FROM VARIOUS FARMS in the vicinity and then housed at the University of British Columbia CHINCHILLA UNIT.

The animals were divided into five groups and fed diets with VARYING LEVELS of protein. THE different protein levels were $11.2 \%$, $12.6 \%, 18.4 \%, 21.0 \%$ and $24.5 \%$. THE ANIMALS WERE ETHERIZED, WEIGHED AND then killed by cervical dislocation.
2. Preparation of Pancreatic Tissue for Enzyme Assay.

The pancreas was immediately oissected out, rinsed in ice-cold 0. 15 m NaCl, placed on ice and dissected free of fat. After placing in a vial, the pancreas was frozen in $\mathrm{CO}_{2}$-Me OH and stored at - $20^{\circ} \mathrm{C}$ UNTIL ANALYSED.


#### Abstract

3. Experimental Design and Statistical Treatments.

THE LEAST SQUARES METHOD OF ANALYSIS OF DATA WAS CARRIED OUT FOR THE THIRD EXPERIMENT FOR DIFFERENT LEVELS OF PROTEIN IN THE DIET. DEPENDENT VARIABLES WERE THE SAME AS FOR EXPERIMENT 2 EXCEPT IN ONE CASE WHERE THERE WERE NO COVARIABLES USED AND ONLY DIET WAS USED AS THE MAIN EFFECT. IN THE OTHER CASE, BODY WEIGHT, SEX, FEED INTAKE, AND INITIAL WEIGHT WERE USED AS COVARIABLES.

A SIMPLE LINEAR REGRESSION WAS ALSO CONDUCTED ON THE THIRD EXPERIMENT USING THE ABOVE DEPENDENT VARIABLES PLUS BIOLOGICAL VALUE, NITROGEN DIGESTION, NET PROTEIN UTILIZATION, NITROGEN RETENTION, GAIN AND FINAL BODY WEIGHT.


## D. BIOCHEMICAL ANALYSES FOR EXPERIMENT 2 AND EXPERIMENT 3

1. HOMOGENIZATION OF THE PANCREAS.

The pancreas was homogenized with a mortar and pestle with ABOUT ONE GRAM OF WASHED, IGNITED SEA SAND AND ABOUT 5 ML OF 0.15 M NaCl. The mortar and pestle and NaCl solution were cooled on ice BEFORE USE. (TRITON X- 100 WAS NOT USED WITH THE NACL SOLUTION SINCE IT SHOWED A PEAK ON THE SPECTROPHOTOMETER 800 WHICH INTERFERED WITH THE DETERMINATION OF THE AMOUNT OF PROTEIN SHOWN PER SAMPLE. THOUGH IT IS PRESUMED TO LYSE THE ZYMOGEN GRANULES AND SHOW APPROXIMATELY $30 \%$ I NCREASED ACTIVITY IN THE HOMOGENATE (12), ITS ADDITION WAS NOT NECESSARY SINCE THIS STUDY WAS COMPARING ONLY RELATIVE AND NOT absolute enzyme values.) The homogenate was then centrifuged at 7,OOO RPM. FOR 15 MINUTES TO OBTAIN A RELATIVELY CLEAR SUPERNATANT. THE SUPERNATANT WAS THEN USED OR REFROZEN UNTIL REQUIRED FOR ANALYSIS.

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Refreezing had no effect on enzyme aCtivity. In some cases Increased
ACTIVITY FOLLOWED PRESUMABLY DUE TO LYSING OF THE ZYMOGEN GRANULES (12).
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## 2. Activation by Enterokinase.

The enterokinase solution was prepared mainly by the method of Gorrill and Thomas (12). Technical grade enterokinase was prepared IN O.1 M TRIS-HCL BUFFER, PH 7.2, CONTAINING O.1 MCACL2. (MAXIMUM ACTIVITY AND STABILITY OF BOTH TRYPSIN AND CHYMOTRYPSIN ARE enhanced by CaCl $_{2}$. It also prevents trypsin from being converted IInto the non-active form (12). It was pre-incubated at $37^{\circ} \mathrm{C}$ In a WATER BATH FOR 30 MINUTES AND THEN CENTRIFUGED AT 5, OOO RPM. FOR 15 MINUTES TO REMOVE SOLID MATERIAL. THIS STOCK SOLUTION COULD THEN BE STORED FOR ABOUT ONE WEEK AT $4{ }^{\circ} \mathrm{C}$.

## 3. Determination of Proteins.

The amount of protein in the pancreatic sample was determined SPECTROPHOTOMETRICALLY BY THE METHOD OF WARbURG AND ChRISTIAN (55). The presence of tirrosine and tryptophan inpproteins results in a Strong absorption in the region of 280 mN . Nucleic acids also ABSORB LIGHT IN THIS REGION BUT HAVE A STRONGER ABSORPTION IN THE region of 260 mu . The enzyme sample was diluted with sufficient 0.15 M NaCl to obtain optical density readings on the Spec. 800. Readings were then taken at 280 mN and $260 \mathrm{mN} . \mathrm{The}$ Ratio 280mn/260mN Is the Warburg correction factor used in the equation: $280 \mathrm{MN} / 260 \mathrm{MN} \times 280 \mathrm{MN} \times$ DILUTION FACTOR $=$ MG. PROTEIN/ML. to calculate the milligrams of protein present per millilitre of SUPERNATANT.
4. ACTIVATION OF ENZYMES.

ACtivation of the proteolytic enzymes of the pancreas was CARRIED OUT BY MIXING EQUAL VOLUMES OF PANCREAS SUPERNATANT AND THE $1 \%$ enterokinase stock solution. Depending upon the amount of protein present in the sample, the mixture was incubated for about 5 to 6 HOURS FOR TRYPSIN AND 3 TO 4 HOURS FOR CHYMOTRYPSIN (OR UNTIL MAXIMUM activation) at $37^{\circ} \mathrm{C}$ in a water bath. The trypsin sample contained FROM AbOUT 1 to 4 mg protein/ml of InCubation mixture. The chymoTRYPSIN SAMPLE CONTAINED LESS PROTEIN THAN THE TRYPSIN MIXTURE, ABOUT 0.5 TO $1.5 \mathrm{mg} / \mathrm{ML}$ incubation mixture.
5. Enzyme Assays.
(a) Total protease.-- Total proteolytic activity of the Pancreatic homogenates was measured by the release of trichloroacetic (TCA) SOLUBLE PRODUCTS DURING THE HYDROLYSIS OF CASEIN BY TRYPSIN (42). THE CASEIN WAS aCid-precipitated, semi-purified. The TYROSINE AND TRYPTOPHAN CONTENT OF THESE END PRODUCTS WAS DETERMINED by the measurement of the optical density at 280 m .

THE BLANK WAS PREPARED BY ADDING 3 MLS. OF 5\% TCA TO 1 ML of the substrate solution (casein dissolved in O. 1 M phosphate buffered saline (PBS) to 1 mg per Ml, pH 7.6). After shaking on a VORTEX-JR. MIXER, 1 ML OF ENZyME SOLUTION (25 NG TRYPSIN PER ML O. 001 N HCL ) WAS added and allowed to stand for 30 minutes. For the samples, the substrate solution was pre-warmed at $35^{\circ} \mathrm{C}$ in a water bath. The enzyme (different concentrations of supernatant made up to 1 ml With 0.1 M PBS) was then added and mixed on a Vortex mixer.

SEVERAL Dilutions of the sample, from 0.05 mg to about 0.7 mg protein per ml, were analysed. After exactly 20 minutes, 3.0 ml of $5 \%$ TCA Was added to stop the reaction. After standing at room temperature for at least 30 minutes the samples were filtered through Whatman \#z filter paper and read on a spec. 800. The optical density of the EXPERIMENTAL TUBE AFTER SUBTRACTION OF THE BLANK WAS USED TO calculate the enzyme activity. Activity was expressed as change in AbSORBANCE PER MG OF PANCREATIC PROTEIN. AN AVERAGE OF AT LEAST three values was used.
(b) Trypsin and Chymotrypsin.--Activities of trypsin and CHYMOTRYPSIN WERE ASSAYED SPECTROPHOTOMETRICALLY USING TOLUENE ARGININE METHYL ESTER (TAME) AND BENZOYL TYROSINE ETHYL ESTER (BTEE), RESPECTIVELY, AS SUBStRATES. THE PROCEDURES OUTLINED by the Worthington Biochemical Corporation (59) were essentially the ones used. Trypsin preferentially catalyses the hydrolysis of PEPTIDE BONDS BETWEEN THE CARBOXY GROUP OF ARGININE OR TYROSIINENAND THE AMINO GROUP OF ANOTHER AMINO ACID. THE RATE OF HYDROLYSIS OF TAME is measured by the increase in absorbancy at 247 mN , based on the method of hummel (24). Substrate ( 0.3 ml of 0.01 M tame) and BUFFER (2.6 MLS TRIS, 0.046 M , PH 8.1 , CONTAINING $0.0115 \mathrm{M} \mathrm{CACL}{ }_{2}$ ) WERE PIPETTED INTO A GLASS VIAL AND THEN TAKEN UP INTO A 3 ML syringe. At zero time, O. 1 Ml of the activated enzyme solution was PIPETTED INTO A ONE CM DIAMETER SILICA CUVETTE AND THE SUBSTRATE AND buffer added. (The force of the syringe mixed the sample adequately.) The blank was prepared the same way except 0.1 ml of 0.001 N HCl was


#### Abstract

substituted for the O. 1 ml of enzyme. Absorption was taken for about 10 MINUTES ON A SPEC. 800. IF A STRAIGHT LINE WAS NOT OBTAINED FOR at least 3 minutes the activated enzyme solution was diluted with 0.15 N NACl. ACtivity WAS measured as change in absorbancy/minute/ MG. PROTEIN, USING THE EQUATION:


UNITS/MG. PROTEIN $=\frac{A_{2} 47 \text { PERMIN. } \times 1000 \times 3 \mathrm{ML} .}{* 540 \times \text { MG. PROTEIN IN CUVETTE }}$
CHyMOTRYPSIN PREFERENTIALLY CATALYSES THE HYDROLYSIS OF PEPTIDE BONDS INVOLVING THE L-ISOMERS OF TYROSINE, PHENYLALANINE AND tryptophan. The rate of hydrolysis of bTEE is determined from the Change in absorbancy at 256 m $\mu$ according to Hummel (24). Substrate (1.4 ML OF $0.00107 \mathrm{M} \mathrm{BTEE} \mathrm{IN} 50 \% \mathrm{METHANOL}$ ) AND BUFFER (1.5 ML OF 0.08 M TRIS, $0.1 \mathrm{MCACL}_{2}$, PH 7.8) WERE PREPARED AS FOR TRYPSIN. AT zero time, O. 1 ml activated enzyme solution was pipetted into the cuvette and the same procedure was followed as for trypsin. ACtivity was measured as change in absorbancy per minute per mg PROTEIN USING THE EQUATION:

$$
\text { UNITS/MG PROTEIN }=\frac{A 256 / \mathrm{MIN} . \times 1000 \times 3 \mathrm{ML} .}{* * 964 \times \mathrm{MG} \cdot \text { PROTEIN } 1 \mathrm{~N} \text { CUVETTE }}
$$

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## RESULTS AND DISCUSSION

## A. Protease activity and Absorption of Immunoglobulin by NEWBORN CHINCHILLA

1. Immunoglobulin Absorption.

The electrophoretic profile of the blood sera samples was RELATIVELY CONSTANT OVER THE TIME PERIOD STUDIED (FIG. 2 AND Appendix I). ALbumin levels were uniformly high and Immunoglobulin LEVELS WERE CONSTANT EXCEPT FOR A SLIGHT DROP AT ONE WEEK OF AGE AND AGAIN AT EIGHT WEEKS OF AGE. THESE CONSTANT LEVELS OF IMMUNOGLOBULINS COULD BE EXPLAINED BY THE FACT THAT THE COLOSTRUM OF THE RODENT IS NOT THE MAJOR PATHWAY OF ANTIBODY TRANSFER FROM the mother to the young (10)(Table 1).
table ${ }^{*}$
Routes of Transmission of Maternal Antibody in Various Species

## Species

Route of Transmission

| HORSE, COW, SHEEP, PIG | COLOSTRAL-INTESTINAL |
| :--- | :--- |
| DOG | PLACENTAL AND COLOSTRAL-INTESTINAL |
| RAT, MOUSE | YOLK SAC AND COLOSTRAL-INTESTINAL |
| RABBIT, GUINEA PIG, BIRDS | YOLK SAC |
| MAN, MONKEY | PLACENTAL |

*Soloman (49).
figure 2. Range of Changes in levels of $\gamma$-globulins
in SERUM OF CHINCHILLAS from Birth to
Twelve Weeks of Age.



TRANSFERENCE MOST LIKELY WOULD DEPEND UPON YOLK-SAC TRANSFER AS IN THE RABBIT AND GUINEA PIG SINCE THE GESTATION PERIOD OF THE

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CHINCHILLA IS MORE THEIR LENGTH THAN THAT OF THE RAT OR MOUSE.
SOLOMAN (49) NOTES THAT IT IS SIGNIFICANT THAT ANIMALS SUCH AS THE
MOUSE AND RAT BORN AFTER SHORT PERIODS OF GESTATION HAVE A PROLONGED
POST-NATAL PERIOD OF INTESTINAL ABSORPTION OF MATERNAL ANTIBODIES
FROM THE COLOSTRUM WHEREAS INTESTINAL ABSORPTION IN ARTIODACTYLA IS
LIMITED TO A DAY OR SO AFIER BIRTH AND IN PRIMATES DOES NOT OCCUR
TO ANY SIGNIFICANT EXTENT.
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    TABLE 1l*
    Placental Types
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| SPECIES | MATERNAL TISSUES | FETAL TISSUES |
| :--- | :---: | :---: |
|  |  |  |
| HORSE, SWINE | 3 | 3 |
| CATTLE, SHEEP | 2 | 3 |
| RABBIT, RAT, GUINEAPIG | 0 | 1 OR 2 |

*Windle (58).

DO CONDITIONS WITHIN THE STOMACH AND INTESTINE PERMIT ORALLY INGESTED GAMMA-GLOBULINS TO REMAIN INTACT IN THE CHINCHILLA IF THIS IS THE ROUTE OF TRANSFER ON WHICH IT RELYS? HILL (2O) NOTED THAT IN SPECIES SUCH AS THE RAT, THERE IS A DEFICIENCY OF PEPSINOGEN IN THE PEPTIC CELLS UNTIL FIFTEEN DAYS AFTER BIRTH MAKING THE LACK OF ENZYMES AND HIGH PH UNRELIABLE FOR DIGESTION OF THE MATERNAL ANTIBODIES. LECCE ET AL. (29) THOUGHT IT POSSIBLE THAT A HORMONE IN THE MILK MAY STIMULATE IMPERMEABILITY OF THE INTESTINAL WALL AFTER 24-36 HOURS SINCE THEY FOUND THAT STARVED NEW-BORN PIGLETS CAN ABSORB ANTIBODIES

FOR A LONGER PERIOD OF TIME THAN THE UNSTARVED.
2. Trypsin inhibitors.

Trypsin inhibitors from the pancreatic homogenates of the YOUNG CHINCHILLA WERE NOT LARGE ENOUGH TO BE SIGNIFICANTLY DETECTED. THIS COULD AGAIN BE EXPLAINED BY THE NATURE OF ANTIBODY TRANSFER; THE YOLK SAC ALLOWS FREE TRANSFER OF MATERNAL ANTIBODIES TO THE PRENATAL ANIMAL THEREBY REDUCING THE IMPORTANCE OF THE COLOSTRAL IMMUNOGLOBULIN TRANSFER SYSTEM. IN OTHER MAMMALS, SUCH AS CATTLE AND PIGS, THE TRYPSIN INHIBITOR PRESENT IN COLOSTRUM FURTHER INCREASES THE EFFECTIVENESS OF THIS SYSTEM BY REDUCING DIGESTION OF PROTEINS In the gut lumen (35, 36). In human colostrum the content of the TRYPSIN INHIBITOR IS MUCH LOWER THAN IN THE CASE OF OTHER MAMMALS. THIS CAN BE EXPLAINED BY DIFFERENT PATHWAYS OF ANTIBODY TRANSFER; PREDOMINANTLY VIA THE PLACENTA IN MAN AND VIA COLOSTRUM IN OTHER MAMMALS (44). THE COLOSTRUM TRYPSIN INHIBITOR IS MOREOVER PROBABLY ABSORBED VIA THE INTESTINAL EPITHELIUM INTO THE BLOOD, AND IN THIS WAY REDUCES THE CATABOLISM OF THE PROTEINS IN THE SERUM. SLIGHT INHIBITION WAS DETECTED IN ONE OF THE THREE-DAY CHINCHILLA BLOOD SERUM SAMPLES. THE INCREASING TRYPSIN INHIBITING CAPACITY OF SERUM DURING THE FIRST WEEK AFTER BIRTH MUST BE OF GREAT IMPORTANCE especially for the Immune system, since the passively transferred COLOSTRAL IMMUNOGLOBULINS ARE PROTECTED FROM CATABOLISM. THIS MECHANISM MAY THUS SERVE TO OVERRIDE THE LAG PERIOD EXISTING BEFORE THE SYNTHESIS OF IMMUNOGLOBULINS STARTS DE NOVO IN THE NEWBORN (5).

## B. DEVELOPMENT OF ENZYME PROFILE FROM BIRTH TO MATURITY

## 1. Results.

In the second experiment, protease levels were not statistically SIGNIFICANTLY DIfFERENT FOR ANY OF THE AGE GROUPS STUDIED. TRYPSIN UNITS/MG PROTEIN/GM BODY WEIGHT WERE HIGH AT O DAYS OF AGE, DROPPED RAPIDLY At 3 days of age, rose slightly at 8 days and then continued TO DECLINE OVER THE PERIOD TESTED. CHYMOTRYPSIN UNITS/MG PROTEIN/GM BODY WEIGHT FOLLOWED A SIMILAR PATTERN AS TRYPSIN EXCEPT TOTAL amounts were less than for trypsin (Fig. 3 and Appendix 2). Trypsin AND CHYMOTRYPSIN UNITS/MG PROTEIN, CHYMOTRYPSIN:TRYPSIN, CHYMOTRYPSIN:TRYPSIN/GM BODY WEIGHT, AND TOTAL PROTEASE/MG HOMOGENATE SHOWED SImILAR PATtERNS TO The AbOVE EXCEPT A SLIGHT DROP OCCURRED at the eight to ten week period (Figs. 4, 5, 6 and Appendices 2 and $3)$.

There was no sex effect for the protease levels.
RATIOS OF CHYMOTRYPSIN:TRYPSIN AND CHYMOTRYPSIN:TRYPSIN/GM BODY WEIGHT WERE SIGNIFICANT ( $\mathrm{P}<0.05$ ) FOR THE AGE GROUPS STUDIED (Fig. 5). Milligrams of protein/ml homogenate were significant $(P<0.01)$ FOR BODY WEIGHT.
2. Discussion.

VARIOUS AUTHORS FOUND THAT IN THE YOUNG PIG PROTEOLYTIC ENZYMES INCREASE WITH ADVANCING AGE DURING THE POSTPARTUM PERIODS Studied (30, 39). Walker (53), Who primarily found changes in the CARBOHYDRASES OF YOUNG LAMBS AND PIGS, ALSO SHOWED DIFFERENCES IN CONCENTRATIONS OF CERTAIN PROTEOLYTIC ENZYMES.
. Figure 3. Relative Changes in Trypsin and Chymotrypsin With Respect to Body Weight in the Chinchilla from birth to Twelve Weeks. (Trypsin Units/ mg Protein/gm body Weight Chymotrypsin Units/mg Protein/gm Body Weight - - - - -. )
Trypsin and Chymotrypsin

Time (Weeks)

Figure 4. Relative Changes in Trypsin and Chymotrypsin from the Pancreatic homogenate of the Chinchilla from Birth to Twelve Weeks. (TRypsin Units/mg Protein from Pancreatic Homogenate $=\square$ Chymotrypsin Units/mg Protein from Pancreatic Homogenate = - - - - - - - )


Figure 5. Relative Changes in the Ratio of Chymotrypsin and Trypsin With Respect to Body Weight in the Chinchilla from Birth to Twelve Weeks. (Chymotrypsin: TRypsin - . - . - -

Chymotrypsin:Trypsin/kg Body Weight
$\mathrm{CH}: \mathrm{T} /$ B.W.


Figure 6. Relative Changes in Total Protease Activity Per mg Protein in Pancreatic Homogenate in the Chinchilla from Birth to Twelve Weeks. (Total Protease/mg protein in Pancreatic homogenate Total Protease/mg Protein/kg Booy Weight - - - - -..)


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A DROP IN PROTEASE ACTIVITY WAS NOTED AT ABOUT EIGHT TO TEN WEEKS OF AGE IN THE CHINCHILLA (FIGS. 3, 5, 6). SIMILARLY, HARTMAN ET AL. (18) NOTED THAT CHANGE OF DIET (WEANING) IN YOUNG PIGS CAUSED A DEPRESSION IN ENZYME SECRETION WHICH WAS SUBSEQUENTLY REGAINED. PROTEASE ACTIVITY FROM THE INTESTINE INCREASED FROM BIRTH TO A MAXIMUM AT ABOUT SEVEN WEEKS. DECREASED ENZYME LEVELS WERE NOTED IN SOME INSTANCES IN THE EIGHT-WEEK-OLD ANIMALS INDICATING THAT the mature pig does not have higher levels of certain digestive ENZYMES IN THE GASTRO-INTESTINAL TRACT THAN DOES THE ONE-TO-TWO MONTH OLD PIG. ENZYMATIC ASPECTS OF DIGESTION ARE CORRELATED, PARTLY, WITH POORER UTILIZATION OF PLANT PROTEINS THAN MILK PROTEINS by the neonatal animal. However, this does not serve completely to ELUCIDATE THE REASONS FOR BETTER UTILIZATION OF CERTAIN PROTEINS BY THE OLDER ANIMAL THAN BY THE ONE-MONTH OLD ANIMAL.
Pancreatic protease levels were found to be highest at birth IN THE CHINCHILLA. IN CONTRAST, OTHER AUTHORS FOUND ENZYME LEVELS TO BE LOWEST AT BIRTH OR ONE DAY AGE. HUBER ET AL. (22) REPORTED THAT IN CALVES PANCREATIC AMYLASE, LIPASE AND PROTEASE ACTIVITY WERE LOWEST AT ONE DAY, INCREASED DURING THE FIRST WEEK, AND CHANGED little thereafter. They also concluded that oiet apparently exerted NO MAJOR EFFECT ON LEVELS OF ANY OF THE DIGESTIVE ENZYMES.
HOWARD AND YUDKIN (20)), WORKING WITH RATS, FOUND A MUCH GREATER INCREASE WITH AGE IN AMYLASE ACTIVITY THAN THAT OF TRYPSIN, AND RAPID CHANGE IN TRYPSIN ACTIVITY WITH DIETARY CHANGE COMPARED WITH THE MUCH SLOWER CHANGE IN AMYLASE ACTIVITY.
EMBRYONIC EXTRACTS OF DIGESTIVE GLAND TISSUES FROM THE BABY
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PIG SHOWED INCREASED CONCENTRATION OF PROTEOLYTIC ENZYMES. OTHER ENZYMES, PARTICULARLY LACTASE, WERE FOUND TO DECREASE IN CONCENTRATION WITH ADVANCING AGE DURING EARLY POSTPARTUM PERIODS (39). GORRILL ET AL. (13) COMPARED THE PROTEOLYTIC ACTIVITY IN SMALL INTESTINAL CONTENTS FROM RUMINANTS AND NON-RUMINANTS OF DIFFERENT AGES. THEY FOUND THAT TRYPSIN ACTIVITY REMAINED FAIRLY CONSTANT IN RAT DIGESTA FROM ONE TO NINE MONTHS OF AGE, BUT CHYMOTRYPSIN ACTIVITY DECLINED. LESS CHYMOTRYPSIN THAN TRYPSIN ACTIVITY WAS RETAINED DURING INCUBATION OF THE DIGESTA. RATIOS OF CHYMOTRYPSIN-TO-TRYPSIN ACTIVITIES RANGED FROM 0.6 TO 0.8 IN THE BOVINE AND OVINE DIGESTA, AND FROM 0.96 TO 2.46 IN THE RAT DIGESTA. LEVELS OF PROTEASE IN THE PANCREAS OF THE CHINCHILLA AGREE PARTIALLY WITH THE ABOVE WORKERS. TRYPSIN ACTIVITY REMAINED FAIRLY CONSTANT (NOT SIGNIFICANTLY DIFFERENT). IN THIS EXPERIMENT, CHYMOTRYPSIN VALUES WERE ALSO LOWER THAN TRYPSIN VALUES, BUT THEY WERE PARALLEL.
SNOOK ET AL. (47) STUDIED THE CHANGES IN THE PANCREATIC CONTENT OF RNA, DNA, AMYLASE, CHYMOTRYPSINOGEN, TRYPSINOGEN AND LIPASE IN RATS BETWEEN O DAYS AND 28 DAYS OF AGE. THE RATS WERE WEANED AT 21 DAYS OF AGE. THEY FOUND THAT PANCREAS WEIGHT, RNA, AND DNA INCREASED SLOWLY DURING THE FIRST FOURTEEN DAYS AND AT AN ACCELERATED RATE THEREAFTER. CHYMOTRYPSINOGEN AND AMYLASE BUT NOT TRYPSINOGEN AND LIPASE WERE HIGH AT BIRTH BUT FELL DRAMATICALLY AFTER ONE DAY. (CHINCHILLA CHYMOTRYPSIN LEVELS FELL AFTER ONE DAY OF AGE BUT TRYPSIN LEVELS ALSO DECLINED.) THEY FOUND THAT ENZYME LEVELS INCREASED WITH PANCREAS SIZE DURING THE LAST TWO WEEKS OF THE NURSING PERIOD ALTHOUGH
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ENZYME CONCENTRATION ALSO APPEARED TO INCREASE AFTER FOURTEEN DAYS. SINCE RAT MILK HAS A HIGH FAT/LOW CARBOHYDRATE CONTENT, THE AUTHORS OF THIS STUDY LOOKED AT THE EFFECT OF CARBOHYDRATE ON PANCREAS DEVELOPMENT AND ENZYME SYNTHESIS IN THE NEONATAL AND YOUNG ADULT RATS. THEY FOUND THAT WHEN ACCESS TO THE MATERNAL DIET WAS PREVENTED AND THE NURSING PERIOD WAS PROLONGED, AMYLASE CONCENTRATION AND THE OTHER THREE ENZYMES WERE ELEVATED. THE SAME EFFECT WAS NOTED IN ADULT RATS FED ISOCALORIC, ISONITROGENOUS MEALS WITH A TOTAL FAT:CARBOHYDRATE RATIO EXCEEDING 50\%. PRELIMINARY STUDIES INDICATED that glucose supplementation had little effect on pancreas developMENT OR ENZYME SYNTHESIS IN RATS YOUNGER THAN FOURTEEN DAYS. SCHINGOETHE ET AL. (43) MADE A COMPARISON OF SIZE AND PROTEOLYTIC ENZYME ACTIVITY OF THE PANCREAS OF SEVERAL SPECIES OF VERTEBRATE ANIMALS. THEY FOUND THAT PANCREAS SIZE PER KILOGRAM OF LIVE BODY WEIGHT WAS GREATER FOR THE NON-RUMINANT THAN FOR THE RUMINANT. IN CATTLE, CHYMOTRYPSIN ACTIVITY PER UNIT OF PANCREAS DRY MATTER WAS HIGHEST IN THE NEWBORN CALF, DROPPED TO ONE-HALF THE LEVEL WITHIN ONE WEEK AFTER BIRTH, AND THEN INCREASED ONLY SLIGHTLY WITH AGE UP TO ONE YEAR. THE RATIOS OF CHYMOTRYPSIN TO TRYPSIN ACTIVITIES IN THE PANCREAS RANGED FROM O. 39 TO 1.20 IN THE RUMINANTS, EXCEPT FOR THE NEWBORN CALF (1.60), AND WERE GREATER THAN 2.O FOR THE OTHER SPECIES. ALl AGE GROUPS OF CATTLE HAD ABOUT THE SAME PANCREAS SIZE AND ENZYME ACTIVITIES PER KILOGRAM BODY WEIGHT, BUT SOME VARIATIONS WERE OBSERVED. CHYMOTRYPSIN ACTIVITY VARIES MORE THAN EITHER PANCREAS SIZE OR TRYPSIN ACTIVITY. CHYMOTRYPSIN ACTIVITY WAS HIGHER
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Lebas et al. (27) demonstrated that the growth rate of the PANCREAS OF RABBITS TO BE LOWER THAN THAT OF THE ANIMAL UP TO DAY FOURTEEN AND PARALLEL TO IT AFTER THAT AGE. THE VARIOUS ENZYMES
investigated were present from birth. They observed a markedInCREASE IN LIPASE, AMYLASE AND CHYMOTRYPSIN TOTAL ACTIVITIES FROMday 24 On; Whereas trypsin total activity remalned constant through-out the range of the experiment.
C. Role of Protein level of diet on Enzyme Profile

1. Results.
In the third experiment, though there was a tendency for the
higher protein diets to give higher protease activities, proteaseactivity was not significantly different for each diet. Results aregiven in figures 7, 8, 9 and 10 and Appendices 4 and 5.
In the analysis by regression, none of the variables were CORRELATED EXGEPT CHYMOTRYPSIN:TRYPSIN AND GAIN WHICH ONLY
approached significance ( $\quad<0.05$ ) with an f-value of 0.0741. (appendix 7).
Using the least squares method of analysis with diet as the MAIN.EFFECT AND BODY WEIGHT, SEX, FEED INTAKE AND INITIAL WEIGHT as covariables (in other words, using each animal as its own constant) did not result in significant f-values for diet. By deleting the covariables and just using diet as the main effect, F-values were higher but still not significant with respect to diet.
2. DISCUSSION.
Evidence from the literature on work done on other rodent species and rabbits shows that. they do adapt to changes in the diet by altering their output ratios of pancreatic enzymes. Work done by Grossman and Greengard (14) demonstrated that in rats maintained

Figure 7. Range of Values for Trypsin UNits FROM CHinchilla fed

Rations with Varying levels
of Protein.


Figure 8. Range of Values for Chymotrypsin Units from Chinchilla Fed Rations with Varying Levels of Protein.

figure 9. Ratio Chymotrypsin:Trypsin. Range and Average Values from Chinchilla fed Rations with Varying Levels of Protein.

figure 10. Range of Values for total protease
Activity from Chinchilla fed
Rations with Varying levels of
protein.


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ON A CONSTANT DIET AN ADAPTATION OF THE CHIEF PANCREATIC ENZYMES TO THE PREDOMINANT CONSTITUENT OF THE DIET OCCURRED. THUS, A HIGH CARBOHYDRATE DIET PRODUCED A PRONOUNCED INCREASE IN THE AMYLASE CONTENT OF PANCREATIC TISSUE, A HIGH PROTEIN DIET RESULTED IN GREATLY INCREASED TRYPSIN CONTENT, WHEREAS A HIGH FAT DIET CAUSED NO IMPORTANT
AlterAtION IN lipASE CONTENT OF THE PANCREATIC TISSUE. It WAS
FURTHER DEMONSTRATED IN THE DOG THAT THE RELATIVE CONCENTRATION OF
THE CHIEF PANCREATIC ENZYMES IN PANCREATIC JUICE PARALLELS THAT
EXISTING IN PANCREATIC TISSUE. BEN ABDILJLIL AND DESNUELLE (3) GAVE
AMPLE PROOF WITH RATS THAT BOTH AMYLOLYTIC AND PROTEOLYTIC ENZYME
ACTIVITY CAN BE INFLUENCED BY THE NATURE OF THE DIET. THEY SHOWED
THAT THE SPECIFIC ACTIVITY OF AMYLASE IS HIGHER IN THE PANCREAS OF
RATS INGESTING A STARCH-RICH DIET THAN IN RATS INGESTING A PROTEIN-
RICH DIET. ON THE OTHER HAND, THE SPECIFIC ACTIVITY OF CHYMOTRYPSINOGEN,
TRYPSINOGEN AND OTHER PROTEOLYTIC PRECURSORS WAS FOUND TO BE HIGHER
IN THE PANCREAS OF RATS EATING THE PROTEIN-RICH DIET. OTHER STUDIES
by HOWARD aND YUDKIN (21) INDICATED THAT AMYLASE AND TRYPSIN levEls
IN TISSUE EXTRACTS VARY INDEPENDENTLY IN THE RAT, AMYLASE BEING
RELATED TO THE LEVEL OF DIETARY CARBOHYDRATE AND TRYPSIN TO DE|TARY
PROTEIN. THIS STUDY IS ONLY CONCERNED WITH THE EFFECT OF DIETARY
PROTEIN ON PANCREATIC ENZYME LEVELS.
    SINCE THE ENZYME CONTENT OF PANCREATIC JUICE PARALLELS THAT
EXISTING IN PANCREATIC TISSUE (14) STUDIES CAN BE UNDERTAKEN USING
FISTULATED ANIMALS TO DETERMINE THE TIME IT TAKES FOR AN ANIMAL TO
ADAPT TO A GIVEN DIET. CORRING ET AL. (8), EXPERIMENTING WITH
FISTULATED PIGS, SHOWED THAT CHYMOTRYPSIN ADAPTS TO THE PROTEIN
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CASEIN RAISEO THE PROTEASE CONTENT OF THE RAT PANCREAS AS mUCH as 100%
WITH CHYMOTRYPSINOGEN BEING AFFECTED MORE THAN TRYPSINOGEN.
    LebAS ET AL. (27) WERE INCliNED to the view that the feed
GREATLY STIMULATES AMYLASE BIOSYNTHESIS, CHYMOTRYPSINOGEN BIO-
SYNTHESIS TO A LESSER EXTENT AND IS INEFFECTUAL ON TRYPSINOGEN IN
the rabbit. The variations of lipase, they demonstrated were rather
PECULIAR AND SEEMED tO bE IRRESPECTIVE OF THE DIET AND ITS LIPID
CONSTITUENTS.
CORRING ET AL. (7) ATTEMPTED TO CONFIRM THE HYPOTHESIS THAT
THE EXOCRINE PANCREAS IN YOUNG SUCKLING RABBITS ADAPTS TO VARIATIONS
IN the diet at weaning. Rabbits WERE fed elther maternal milk to
3O DAYS THEN SUDDENLY WEANED TO A STARCH DIET OR THEY WERE FED
MATERNAL MILK TO 21 DAYS THEN SUDDENLY WEANED TO A STARCH DIET.
TRYPSIN, CHYMOTRYPSIN, LIPASE ANO AMYLASE ACTIVITIES WERE SLIGHTLY
Higher in animals weaned at 21 days of age. This seemed to indicate
THAT THE FEED WAS NOT THE FACTOR STIMULATING PANCREATIC ENZYME
development. However, it coulo possibly modify the amount of
RESPONSE TO THIS STNIMUEATION.
    RESULTS OF THIS EXPERIMENT MAY HAVE BEEN NON-SIGNIFICANT DUE
TO THE FACT THAT RATIONS WERE NOT AS VARIABLE IN CARBOHYDRATE CONTENT
AND PROTEIN CONTENT AS THE RATIONS USED BY OTHER WORKERS STUDYING THE
SAME PROBLEM. AS IN EXPERIMENT 2, RATIOS OF CHYMOTRYPSIN:TRYPSIN
WERE SIGNIFICANTLY DIFFERENT WITH RESPECT TO BODY WEIGHT. THIS
COULD BE DUE TO THE HIGHER VARIABILITY OF CHYMOTRYPSIN THAN TRYPSIN
AS A RESULT OF THE DIET. OR, PERHAPS FEED PALATABILITY AND
COMPENSATORY FEED CONSUMPTION PLAYED AN IMPORTANT ROLE IN INFLUENCING
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THESE RESULTS. ANIMALS CONSUMING THE LOW-PROTEIN RATIONS ATE SLIGHTLY MORE THAN THE ANIMALS CONSUMING THE HIGH-PROTEIN RATIONS. Perhaps this compensated for the differences in protein levels in THE DIFFERENT RATIONS.

THE CHINCHILLA DIFFERS IN VARIOUS WAYS FROM OTHER SPECIES WITH RESPECT TO ITS THREE MAJOR PERIODS OF ADAPTATION IN RELATION TO PROTEIN UTILIZATION. SERUM IMMUNOGLOULIN LEVELS DID NOT INCREASE FROM BIRTH ONWARDS. THIS COULD INDICATE THAT THE YOLK SAC AND/OR PLACENTA IS IMPORTANT IN THE PRE-NATAL CHINCHILLA FOR ANTIBODY TRANSFER FROM THE MOTHER. THIS IS ALSO SEEN IN RABBITS AND SOME RODENTS RATHER THAN COLOSTRAL TRANSFER WHICH IS IMPORTANT IN GROUPS SUCH AS ARTIODACTYLA (APPENDIX 6, FIG. 1).

TRYPSIN AND CHYMOTRYPSIN INHIBITORS WERE NOT FOUND IN APPRECIABLE LEVELS IN THE PANCREATIC HOMOGENATE FROM NEWBORN TO THREE-DAY-OLD CHINCHILLA. THE SUDDEN DROP IN PROTEOLYTIC ENZYME ACTIVITY AT THREE DAYS OF AGE COULD HAVE BEEN EXPLAINED BY HIGH LEVELS OF INHIBITOR IN THE PANCREATIC JUICE AFTER BIRTH. THESE INHIBITORS COULD HAVE COMBINED WITH THE ENZYMES THEREBY PREVENTING COLOSTRAL ANTIBODY DEGRADATION. SUCH INHIBITORS, HOWEVER, WERE NOT DETECTED.

Protease development in the newborn chinchilla may not be as IMPORTANT AS IN RABBITS AND SOME RODENTS SINCE CHINCHILLA ARE BORN IN A MORE ADVANCED CONDITION. ENZYME SYSTEMS COULD INITIALLY BE MORE FUNCTIONAL AND LESS DEPENDENT UPON THE MOTHER'S MILK AS A STIMULUS FOR ENZYME SECRETION. SINCE YOUNG MAMMALS ARE BORN AT WIDELY DIFFERENT STAGES OF PHYSIOLOGICAL MATURITY AND THE MILKS OF

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DIFFERENT SPECIES DIFFER GREATLY IN PROTEIN COMPOSITION, IT IS
LOGICAL TO SUGGEST A RELATIONSHIP BETWEEN MILK COMPOSITION AND
Stage OF mATURITY OF THE NEWBORN. HOWEVER, UPON COMPILATION OF THE
OBSERVATIONS, THIS IS NOT AT ALL APPARENT (APPENDIX 6, FIG. 2).
Protease levels of the newborn of different species are also widely
VARIABLE REGARDLESS OF PHYSIOLOGICAL STAGE OF MATURITY AT BIRTH
(APPENDIX 6, FIG. 3). IN THE YOUNG CHINCHILLA, DIETARY PROTEIN
LEVEL COULD BE CONSTANTLY MAINTAINED SINCE IT QUICKLY SUPPLEMENTS
ITS DIET WITH FOOD OTHER THAN MILK.
    IN STUDIES ON DIET ADAPTION, INVESTIGATORS WORKING WITH RATS
AND MICE USUALLY DEMONSTRATED A PANCREATIC ENZYME LEVEL OR
ENZYMATIC RATIO RESPONSE. THIS MAY HAVE BEEN BECAUSE RATS AND MICE
ARE MORE OMNIVOROUS THAN CHINCHILLA AND CAN BETTER ADAPT TO DIFFERENT
DIETS. CHINCHILLA, IN THE NATURAL STATE, CONSUME A HIGH PROTEIN
LEVEL ALPINE DIET AND MAY NOT BE ABLE TO ADAPT TO A LOWER PROTEIN
LEVEL IN EXPERIMENTAL DIETS.
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APPENDICES

## APPENDIX 1

Serum Protein Levels in the Chinchilla from Birth to Twelve Weeks

| $\begin{aligned} & \text { AGE } \\ & (D A Y S) \end{aligned}$ | Peak height (Relative absorbance) |  |  |
| :---: | :---: | :---: | :---: |
|  | Albumin | Transferrin | Gamma-globulin |
| O day | 9.2 | 3.8 | . 82 |
| O day | 9.8 | 4.1 | 4.9 |
| O day | 9.2 | 3.5 | 1.5 |
| 3 days | 9.8 | 3.5 | 1.48 |
| 3 days | 9.3 | 4.2 | 1.2 |
| 1 wk. | 9.6 | 6.3 | . 96 |
| 1 Wk. | 9.6 | 6.2 | 1.02 |
| 2 wks. | 8.8 | 3.2 | 1.28 |
| 2 wks. | 9.5 | 4.1 | 1.02 |
| 6 wks. | 9.7 | 5.2 | 1.5 |
| 6 wks. | 9.8 | 4.2 | 1.22 |
| 8 wks. | 9.8 | 6.3 | 1.18 |
| 8 wks. | 9.8 | 5.1 | 1.30 |
| 10 wks. | 9.8 | 6.7 | 1.42 |
| 12 wks. | 9.7 | 5.6 | 1.56 |
| 12 wks . | 9.7 | 5.6 | 1.22 |

APPENDIX 2
Relative Changes in Trypsin and Chymotrypsin with
Respect to Body Weight in the Chinchilla
from Birth to Twelve Weeks

| AGE | TRYPSIN UNITS/MG. <br> Pancreatic Protein <br> (TU) | ```CHYMOTRYPSIN UNITS/MG. Pancreatic Protein (CU)``` | $\begin{gathered} \text { TU/KG } \\ \text { BODY WEIGHT } \end{gathered}$ | $\begin{gathered} \text { CU/KG } \\ \text { BODY WEIGHT } \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: |
| O day | 3.26 | 1.73 | 94.4 | 50.1 |
| O DAY | 3.84 | 1.37 | 98.6 | 35.2 |
| O DAY | 2.47 | 1.08 | 61.9 | 26.9 |
| 3 DAYS | 2.19 | . 374 | 43.974 | 7.30 |
| 3 DAYS | 1.85 | . 603 | 32.9 | 10.8 |
| 1 WK. | 3.31 | . 457 | 54.2 | 7.49 |
| 1 WK. | 2.30 | . 096 | 29.5 | 1.20 |
| 2 wks. | 3.28 | . 520 | 52.0 | 8.30 |
| 2 wks. | 1.68 | . 839 | 19.1 | 9.50 |
| 6 wks. | 1.83 | 2.64 | 9.4 | 13.5 |
| 6 wks. | 2.91 | 2.04 | 14.0 | 9.80 |
| 8 wks. | 1.08 | 1.34 | 4.30 | 5.40 |
| 8 wks. | 1.87 | . 631 | 7.20 | 2.40 |
| 10 wks. | 1.25 | 1.27 | 33.67 | 3.74 |
| 10 wks. | . 588 | 1.918 | 3.10 | 10.3 |
| 12 wks. | . 938 | . 128 | 2.70 | . 370 |
| 12 wks. | 2.28 | 1.34 | 7.20 | 4.20 |

## APPENDIX 3

TOtal Protease Activity and Chymotrypsin:Trypsin WIth Respect to Body Weight in the Chinchilla from Birth to Twelve Weeks

| Age | total protease/mg. Pancreatic Protein (Tot. Protease) | Tot. Protease/Kg Body Weight | Chymotrypsin: <br> TRypsin (C:T) | C: t/kg Body WEIGHT |
| :---: | :---: | :---: | :---: | :---: |
| O day | . 247 | 7.17 | . 531 | 15.4 |
| O day | . 273 | 6.99 | . 356 | 9.14 |
| O day | . 256 | 6.40 | . 436 | 10.9 |
| 3 days | . 141 | 2.77 | . 171 | 3.35 |
| 3 days | . 142 | 2.53 | . 327 | 5.83 |
| 1 wk. | . 192 | 3.15 | . 138 | 2.27 |
| 1 wk. | . 051 | . 656 | . 042 | . 535 |
| 2 wks. | . 147 | 2.33 | . 159 | 2.52 |
| 2 wks. | . 103 | 1.17 | . 501 | 5.69 |
| 6 wks. | . 166 | . 853 | 1.44 | 7.39 |
| 6 wks. | . 193 | . 927 | . 701 | 3.37 |
| 8 wks. | . 093 | . 373 | 1.24 | 4.96 |
| 8 wks. | . 066 | . 254 | . 338 | 1.29 |
| 10 wks. | . 109 | . 319 | 1.02 | 2.99 |
| 10 wks. | . 155 | . 827 | 3.26 | 17.5 |
| 12 wks. | . 098 | ¢ 2285 | . 137 | . 396 |
| 12 wks. | . 136 | . 431 | . 588 | 1.86 |

## APPENDIX 4

TRyPSIN AND Chymotrypsin Units in Relation to
Body Weight in Chinchilla fed Rations with Varying levels of Protein

| Diet | TRypsin Units/mg. Pancreatic Protein (TU) | Chymotrypsin UNits/mg. Pancreatic Protein (CU) | TU/KG. BODY WEIGHT | $\mathrm{CU} / \mathrm{Kg}$. BODY WEIGHT |
| :---: | :---: | :---: | :---: | :---: |
| 1 | 2.73 | 1.53 | 7.92 | 4.44 |
| 1 | . 724 | . 529 | 2.59 | 1.90 |
| 2 | 1.13 | . 920 | 2.21 | 1.80 |
| 2 | 1.99 | . 981 | 5.03 | 2.47 |
| 2 | 4.10 | 2.30 | 10.1 | 5.67 |
| 3 | 2.66 | 1.48 | 5.70 | 3.19 |
| 3 | 2.16 | 1.61 | 6.65 | 4.94 |
| 3 | 2.08 | . 925 | 5.23 | 2.32 |
| 4 | 2.02 | 1.87 | 5.35 | 4.97 |
| 4 | 2.31 | 1.13 | 5.32 | 2.61 |
| 4 | 3.64 | 2.24 | 9.42 | 5.81 |
| 4 | 1.74 | 1.08 | 4.11 | 2.56 |
| 5 | 4.41 | 4.94 | 10.4 | 11.6 |
| 5 | 2.93 | 1.53 | 6.01 | 3.14 |
| 5 | 3.13 | 1.92 | 7.52 | 4.60 |
| 5 | 3.66 | 1.48 | 8.78 | 3.56 |

Diet $1=11.2 \%$ Protein
DIET $4=21.0 \%$ PROTEIN
DIET $2=12.6 \%$ PROTEIN
DIET $5=24.5 \%$ PROTEIN
DIET $3=18.4 \%$ PROTEIN

## APPENDIX 5

## Total Protease and Chymotrypsin:Trypsin in Relation

 to Body Weight of Chinchilla fed Rationswith Varying Levels of Protein

| Diet | total Protease/mg. Pancreatic Protein (Tot. Protease) | tot. protease/kg. Body Welght | Chymotrypsin:Trypsin $(C: T)$ | C: $\mathrm{T} / \mathrm{Kg}$. <br> Body Weight |
| :---: | :---: | :---: | :---: | :---: |
| 1 | 2.83 | 8.20 | . 560 | 1.62 |
| 1 | . 949 | 3.40 | . 731 | 2.62 |
| 2 | 1.31 | 2.56 | . 816 | 1.63 |
| 2 | 1.93 | 4.87 | . 491 | 1.24 |
| 2 | 3.52 | 8.68 | . 561 | 1.38 |
| 3 | 2.60 | 5.58 | . 559 | 1.20 |
| 3 | 2.21 | 6.78 | . 744 | 2.29 |
| 3 | 2.35 | 5.89 | . 443 | 1.11 |
| 4 | 2.67 | 7.09 | . 929 | 2.47 |
| 4 | 2.10 | 4.85 | . 491 | 1.13 |
| 4 | 2.99 | 7.72 | . 617 | 1.59 |
| 4 | 2.75 | 6.47 | . 622 | 1.47 |
| 5 | 4.15 | 9.79 | 1.12 | 2.64 |
| 5 | 2.39 | 4.90 | . 522 | 1.07 |
| 5 | 2.63 | 6.32 | . 612 | 1.47 |
| 5 | 3.19 | 7.66 | . 405 | . 970 |


| DIET $1=11.2 \%$ PROTEIN | DIET $4=21.0 \%$ PROTEIN |
| :--- | :--- |
| DIET $2=12.6 \%$ PROTEIN | DIET $5=24.5 \%$ PROTEIN |

DIET $2=12.6 \%$ PROTEIN
DIET $5=24.5 \%$ PROTEIN

Protease Activity of Different Species with respect to \% Immunoglobulins Acquired After Birth, Protein Content of the Milk, and Physiological age at Birth
FIG. 1


Fig. 2


APPENDIX 6 CON'T.

Fig. 3


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## APPENDIX 7

Example Graph to Illustrate Linearity of REGRESSION LINE FOR ANALYSIS OE DATA FOR EXPERIMENT 3



[^0]:    * 540 equals the molar absorbancy index of tame at that WAVELENGTH.
    * 964 EQUALS molar extinction coefficient for bTEE.

[^1]:    R. Jenness and R. E. Sloan. Dalry Science Abstracts. 32:599.

