SOME NUTRITIONAL STUDIES ON THE NATURALLY OCCURRING ALPHA-GLYCERYL ETHERS

by

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ABSTRACT

A possible nutritional role for certain of the naturally occurring α-glyceryl ethers has been investigated. Batyl, selachyl and chimyl alcohols have been administered per orum for extended periods to growing dogs and rats at various dosage levels to ascertain if these compounds produce inimical or favourable effects in terms of growth and haematopoietic responses.

Groups of male and female dogs and rats maintained on normal rations were given daily doses of each of the three alcohols at a level of 6 mgs per kilogram of body weight for 180 days. There was no evidence to suggest that these compounds produced harmful effects. Histopathological study of the major tissue system confirmed this conclusion. A favourable response in the form of a slightly increased growth rate was noted in the female rats that received the selachyl alcohol. No evidence of a haematopoietic effect was obtained.

In a second experiment, selachyl alcohol was offered as an addendum to a normal ration, to both dogs and rats at levels that ensured a daily consumption of 600 and 2400 mg per kilogram of body weight of this alcohol for a period of sixty days. Other groups of both species received batyl alcohol at the higher dosage level (2400 mg per kilogram of body weight) daily for the same time period. The results obtained suggest that both compounds when fed at the 2400 mg per kilogram level interfered with the digestibility of the ration offered and, in

so doing, reduced the growth rate of the experimental dogs. A corresponding effect did not occur in the rats. Both alcohols, when administered at the highest dosage level, induced an increase in the reticulocyte count in the blood of the dogs and an increase in the percentage of nucleated red blood cells in the bone marrow smears prepared from the rats.

A glyceryl ether-free synthetic ration supplemented with 0.5, 5 and 50 mg of batyl alcohol per kilogram of body weight was prepared and then offered to groups of young growing rats for a period of five weeks. No growth response was obtained at any level, suggesting that this compound does not have a nutritional function or that the rat is able to synthesize the compound at a rate that is adequate to permit near maximum growth. It is also possible that the animals had sufficient reserves of compounds of this type to permit growth at the measured rate.

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PART I:

LITERATURE REVIEW

INTRODUCTION

The α-glyceryl ethers have been examined as possible therapeutic agents for more than ten years. In 1957, Evans, et al (33), reported on the use of one of these, batyl alcohol, in the treatment of bracken poisoning in the bovine and since then other reports have been published indicating various degrees of success in the use of batyl alcohol for the treatment of certain blood dyscrasias. The α-glyceryl ethers have aroused renewed interest with the finding that they normally exist in all animals in small quantities. This might imply that they possess vitamin-like properties.

An extensive literature review has been carried out covering the occurrence, composition and chemical nature of these ethers. The literature concerning the biological effects of these compounds has been examined and found to be wanting in that much of the experimental work has been carried out under the "ill defined" conditions of the clinic.

The present study was designed to determine if certain of the α-glyceryl ethers can be considered to be essential nutrients. To this end, a series of experiments have been carried out using the dog and the albino rat as experimental subjects. The work has, of necessity, involved screening assays to ascertain if the compounds selected would produce inimical effects in mammalian systems when administered per orum. On the basis of this preliminary work, a feeding response experiment was conducted, using synthetic α-glyceryl free basal diets, to determine if the compounds possess growth promoting properties or desirable effects on the haematopoietic system.

A. THE CHEMICAL NATURE OF THE α-GLYCERYL ETHERS

The three most common, naturally occurring, glyceryl ethers are: α-hexadecylglyceryl ether (chimyl alcohol), α-octadecylglyceryl ether (batyl alcohol), and α-,9-octadecenylglyceryl ether (selachyl alcohol).

TABLE I: MELTING POINT AND STRUCTURE OF THE GLYCERYL ETHERS

Glyceryl Ether	Melting Point	Structure
chimyl alcohol	60° - 61.5°C. (28)	$CH_3(CH_2)_{14}CH_2.O.CH_2.CH(OH).CH_2OH$
batyl alcohol	70° - 71°C. (28)	$CH_3(CH_2)_{16}CH_2.O.CH_2.CH(OH).CH_2OH$
selachyl alcohol	8 - 9 C. (16)	$CH_3(CH_2)_7CH\ CH(CH_2)_8.O.CH_2.CH(OH).CH_2OH$

The ether linkage was first shown to be of the α form by Weidemann (106) in 1926 when he demonstrated that hydroiodic acid acts on batyl alcohol to produce methyl iodide. This established that the third oxygen is present as a methoxy group and, in turn, indicates that an ether linkage exists between the stearyl alcohol and the glycerol moiety. In 1930, Knight (58) reported the surface film activity of the synthetic α form to be identical with that of the natural ethers, but that the activity of the β form was different. The melting point of batyl alcohol is 60° - 61.5°; this is the same as that found for the α form. The β octadecyl ether has a melting point of 62° - 63°C. (26). A mixture of the β form with natural batyl alcohol causes a marked melting point depression, whereas the mixture of α-octadecyl ether with natural batyl produces only a slight depression. This can be explained on the basis of the optical activity of the glyceryl ethers.

Batyl alcohol belongs to the d-series whereas synthetic octadecyl glyceryl ether is a racemic mixture and therefore can be expected to have slightly different properties.

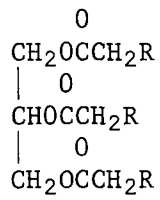
Oxidation of batyl alcohol with lead tetraacetate yields glycol aldehyde octadecyl ether (m.p. 51°C.) and formaldehyde. According to Criege (28) this is a specific reaction for an α,β-glycol. This reaction indicates that the α^1 and β positions of the glycerol moiety must be free and that there is no alternative other than to assume that glyceryl ethers are of the same α type. Finally, the very fact that the glyceryl ethers have optical activity (7) (8) (9) shows that the ether linkage must form an asymetric molecule and hence an α linkage must be involved. It has been observed that batyl alcohol in high concentrations shows a negative rotation, gradually diminishing, to disappear entirely at a concentration of 10 per cent. On further dilution dextrorotation is displayed (Toyama and Isikawa) (16).

Selachyl alcohol, having a double bond in its long sidechain, can exist in either the cis or trans form; the natural compound has the cis configuration, giving it the low melting point of 8° - 9°C. While the trans form, which has been synthesized (8) (9) has a melting point of 48.5° - 49.5°C.

In further investigation of the optical properties, Baer and Fischer (6) (9) synthesized l- and d- forms of batyl, chimyl, and selachyl alcohols. They found the physical properties of the d-forms to be identical with those of the natural compounds, while the l-forms differed slightly.

B. OCCURRENCE OF THE GLYCERYL ETHERS

Glyceryl ethers have been found to be quite wide-spread in their occurrence; they exist almost entirely in an esterfied form and are extracted from tissue with the lipid fraction. Malins and Mangold (48) report finding some of the free form in ratfish liver oil and basking shark liver oil. Their properties are lipid-like in nature since the structure of the glyceryl ethers is identical to the glycerides with the exception that they possess an ether bond instead of an ester bond.



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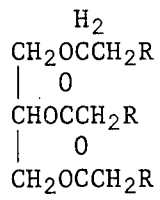


diesterfied glyceryl ether

A glyceryl ether was first described in 1915 by Kossel and Edlacher (10). They assigned the name "astrol" to the alcohol that they isolated (m.p. 71°C.) from the unsaponifiable fraction of the fat of a starfish. Later, Tsujimoto and Toyama (28) reported what they believed to be the first isolation of the glyceryl ethers. In a subsequent communication Toyama established the structure of the component groups. In 1943, Bergman and co-workers (10) showed "astrol" to be identical with batyl alcohol, thus establishing Kossel and Edlacher as the original discoverers of the glyceryl ethers. In 1941, Holmes et al (49) reported the first isolation of batyl alcohol from a source other than the marine organisms; they isolated batyl alcohol from the yellow bone marrow of cattle, thus opening the possibility that this compound might have value as a nutrient. Since 1941,

glyceryl ethers have been isolated from many other tissues. Carter, et al (1958) (23) isolated and identified for the first time a glyceryl ether-containing phospholipid. Since 1948 glyceryl ethers have been found in the phospholipid fractions from many sources. In at least two tissue systems, bovine erythrocytes (44) and red bone marrow (101) glyceryl ether-containing phospholipids account for over 10 mole percent of the phospholipid fraction. Table II provides a summary of the many natural sources from which the glyceryl ethers have been isolated.

TABLE II: OCCURRENCE OF GLYCERYL ETHERS

Tissue	Content	Reference
amniotic fluid (human)	some batyl alcohol	1962 Helmy and Hack (47)
atherosclerotic aortas (human)	285 mg. batyl alcohol from 3,450 gm. fat from 370 aortas (0.008% of oil)	1943 Hardegger, et al (45)
atherosclerotic aortas	0.0782 microMole/100gm. wet tissue 0.0802 microMole/100gm. wet tissue	1964 Miller, et al (75)
beef tallow (P.L. free)	65mg./Kg. tetradecanol	1960 Schogt, et al (91)
bone marrow, yellow (cattle)	batyl alcohol	1941 Holmes, et al (49)
bone marrow (cattle)	0.2 - 0.7% G.E. (free form)	1957 Brohult (17)
bone marrow, red (pig epiphyses)	lecithin fraction has 20% G.E. derivative	1962 Pietruszko (81)
bone marrow (human)	9 - 16% of phospholipids are glyceryl ethers	1965 Wajda (105)
brain (calf) brain (human)	ether-containing phospholipids range from 1 to 2.5% of the total lipids	1960 Svennerholm and Thorin (96)
brain (human) cephalin fraction	inositol phosphate isolated contained 8.4% batyl-chimyl mixture	1961 Klenk and Hendicks (57)
brain (young rat)	G.E. phospholipid is 1.8% of total phospholipids	1963 Ansell and Spanner (3)
brain (adult rat)	G.E. phospholipid is 3.1% of total phospholipids	1963 Ansell and Spanner (3)
Centrina salviani (liver and eggs)	contains glyceryl ethers	1949 Omelik (78)
dog tissues, with the exception of marrow	see Table VIII	1962 Nakagawa, et al (77)
egg yolk	glyceryl ether containing phospholipids	1958 Carter (23)

TABLE II (cont'd)

Tissue	Content	Reference
Elasmobranch fish	see Table V	1962 Hallgren and Larsson (41)
erythrocytes (bovine)	ethanolamine lipids make up 30% of total phosphatides	1961 Hanahan and Watts (44)
erythrocytes (bovine)	75% of phosphatidylethanolamine fraction is glyceryl ethers	1963 Hanahan, et al (43)
Halobacterium cutirubrum	93% of lipidsare phospholipids 73% of phosphatides are glyceryl ethers	1962 Sehgal, et al (94)
heart muscle (human)	mono- and di-ethers occur presumably as their phosphate esters	1965 Popović (83)
heart (ox)(P.L. free)	1000mg. tetradecanol/kg.	1960 Schogt, et al (91)
heart (ox)	glyceryl ether derivative forms 2% of the lecithin fraction	1962 Pietruszko (82)
liver (human)	contains glyceryl ethers	1958 Riley, et al (86)
liver oil of: -soupfin shark (Galeorhemis rond.) -seven-gilled shark (Heptranchias pectorosus) -basking shark (Cetorhinus maximus) -spiny shark (Echinorhinus spinosus)	all contain glyceryl ethers	1948 Karnovsky, et al (51) 1948 Karnovsky, et al (53) 1948 Karnovsky, et al (54) 1948 Karnovsky, et al (54)
liver oil, cow shark (Hexanchus grisen)	16.8% selachyl alcohol 2.38% batyl alcohol some chimyl alcohol	1954 Manforte and Fenech (69)
liver oils (elasmobranch fish)	see Table VI	1956 Hilditch (48)
liver oil (shark)	16 - 17% of oil (expressed as the free form)	1957 Brohult (17)

TABLE II (cont'd)

Tissue	Content	Reference
liver oil (dog-fish)	contains glyceryl ethers	1960 Malins (65)
liver oil (ratfish and basking shark)	free glyceryl ethers as well as glyceryl ether diesters	1960 Mangold and Malins (70)
liver (dogfish)	glyceryl ethers found	1963 Hanahan, et al (43)
mammalian tissue and starfish	see Table IX	1964 Todd and Rizzi (104)
man and cow	see Table IV	1962 Hallgren and Larsson (42)
Milk fat (phospholipid free)	50 mg. tetradecanol/Kg.	1960 Schogt, et al (91)
Phylum Mollusca -chiton (Catherina tunicata) -marine (Thais Lamellosa) -clam (Protothaca staminea) -octopus (Octopus dofleini)	all are rich in glyceryl ethers	1965 Thompson and Lee (102)
reef building coral (Plexaura flexuoso)	batyl alcohol found	1942 Kind and Bergman (56)
sea anemones	batyl alcohol isolated	1956 Bergman, et al (11)
spleen (pig)	contains batyl alcohol	1943 Prelog, et al (84)
starfish fat	astrol isolated	1915 Kossel and Edlbacher (10)
starfish fat	batyl alcohol found	1943 Matsumoto, et al (74) 1954 Matsumoto and Wainai (73)
terrestial slugs (Arian ater) (Ariolimax columbianus)	amounts to more than 25 mole per cent of total phospholipids	1963 Thompson and Hanahan (99)
various	see Table VII	1946 Karnovsky, et al (52)
various	see Table III	1963 Gilbertson and Karnovsky (37)

TABLE III: GLYCERYL ETHERS IN THE UNSAPONIFIABLE FRACTION OF NEUTRAL LIPIDS FROM SEVERAL MAMMALIAN TISSUES
(taken from Gilbertson and Karnovsky)(37))

Tissue	Total lipid as % of fresh wt.	Neutral lipids as % of T.L.	Phosphatide residual % of N.L.	Total G.E. % of N.L.	Vinyl ethers molar % of total G.E.
diverticulum (starfish)	5.7	72.50	0.19	16.1	31.9
brain (beef)	7.4	43.95	0.10	1.9	63.1
bone marrow (beef)	88.9	99.97	0.00	2.4	61.2
heart (calf)	5.1	62.61	0.42	2.4	31.2
adipose tissue (rat)	76.8	98.91	0.01	1.3	82.0
brown fat (rat)	43.1	78.40	0.04	0.4	54.3
leucocytes (polymorphs)(guinea pig)	-	51.92	0.00	2.9	65.8
chylomicrons (human)	-	85.90	-	0.1	78.6
beta-lipoprotein (human)	-	-	-	0.6	29.3

TABLE IV: PERCENTAGE OF GLYCERYL ETHERS IN LIPIDS FROM SOME HUMAN AND ANIMAL SOURCES
(taken from Hallgren and Larsson)(42)

Source	Glyceryl Ethers as % of Lipids
human bone marrow (red)	0.2
human spleen	0.05
human red blood cells	less than 0.01
human milk	0.1
cow bone marrow (yellow)	0.01
cow milk	0.01
egg yolk	none found
liver oil of elasmobranch fish	10 - 30

TABLE V: COMPOSITION OF THE NONSAPONIFIABLE MATTER IN FISH LIVER OILS
(taken from Hallgren and Larsson) (41)

Species	% unsap. in oil	Composition of unsap.
Chimaera monstrosa	33	almost exclusively G.E.
Somniosus microcephalus	8	90% G.E. and 8% cholesterol
Squalus acanthias	7	84% G.E. and 13% cholesterol

TABLE VI: LIVER OILS FROM ELASMOBRANCH FISH
(taken from Hilditch) (48)

Liver fat from	% G.E.*	Composition of unsaponifiables
skate	0.3	mainly cholesterol
angel fish	1.5	mainly cholesterol
thresher shark	1.8	mainly cholesterol
spotted dogfish	2.0	mainly cholesterol
grey dogfish	10.0	mainly selachyl, some chimyl and batyl
ratfish	37.0	almost wholly selachyl, only some chimyl and batyl
shark species	50 - 80	large amounts of squalene, some selachyl, chimyl and batyl

*Glyceryl Ethers

TABLE VII: A SUMMARY OF THE EXTENSIVE TABLE GIVEN BY KARNOVSKY AND RAPSON (51)

Species	Glyceryl ether content of oil calculated as selachyl dioleate
25 species listed under Elasmobranchii	amount varies from nil in the liver of the man eating shark to 77.5% in the liver of the six-gilled shark
21 species listed under Teleostomi	amount varies from nil in the blue hottentot to 7.3% in the liver of the stone bass
6 species listed under Mollusca	quantities vary from 0.7% in the Limpet to 8.0% in the viscera of the rock octopus
1 Arthropoda (crayfish)	quantities vary from 0.9% in the intestine to 6.0% in the flesh
1 Amphibia (cape clawed toad)	quantities vary from nil in the liver to .4% in the body oil
1 Reptillia (mole snake)	0.3% in the total viscera to 1.2% in the body
1 Aves (domestic fowl)	0.1% in the liver to 0.3% in the viscera
6 species of Mammalia; 3 whales, 1 rat, 1 cow and 1 man	quantities range from nil in the liver of a man to 2.5% in the faeces of the rat
4 vegetable oils investigated	only tung nut oil showed any glyceryl ethers (less than 0.1%)

TABLE VIII: α-GLYCERYL ETHER CONTENT OF DOG TISSUES
(taken from Nakagawa, et al) (77)

Tissue and animal no.	Molar ratio to lipid P	microMoles/gm. dry lipid free tissue
Lung 1	.0027	.41
Lung 2	.0043	.70
Kidney 1	.0015	.29
Kidney 2	.0086	.80
Liver 1	.0026	.31
Liver 2	.0077	1.16
Liver 2	.0071	1.07
Spleen 1	.0048	.67
Intestine 1	.0102	1.35
Heart 1	.0069	.59
Aorta 1	.0039	.20
Skeletal muscle 1	.0157	1.02
Skeletal muscle 1	.0159	1.03
Bone marrow (rabbit) 1	.1570	8.73
Bone marrow (rabbit) 2	.3600	14.10

TABLE IX: α-GLYCERYL ETHERS OF MAMMALIAN YELLOW BONE MARROW
(taken from Todd and Rizzi) (104)

Source	NS* as % wt. of marrow	G.E.** as % wt. of NS*	G.E. composition as % of total glyceryl ethers		
			18:0	16:0	18:1
rabbit	0.2	13	0	100	0
cow	0.2 - 0.4	12 - 25	50	50	0
sheep	0.36	11	-	-	-
pig	0.5 - 0.6	4 - 8	-	-	-
rat	-	0	-	-	-
man	-	11.3 - 16.6	25	50	25

*nonsaponifiables
**glyceryl ethers

C. THE COMPOSITION OF THE GLYCERYL ETHERS

For a good many years only the glyceryl ethers batyl, selachyl, and chimyl were mentioned in the literature. Recently there have been several reports of glyceryl ethers having the chain length of the fatty alcohol moiety varying from 12 to 24 carbons, including some odd chain lengths and some chains having more than one double bond (38, 40, 41, 42, 65). The first of these reports was published in 1959 by Hallgren and Larsson (40). The following is a table of their published results.

TABLE X: THE PERCENT COMPOSITION (WT) OF THE ALKOXYGLYCEROLS FROM LIVER OILS*

Alkoxyglycerol	Grey dogfish *Squalus acanthias*	Greenland shark *Somniosus microcephalus*	Ratfish *Chimaera monstrosa*
12:0	trace	trace	trace
14:0	5.7	2.0	1.7
15:0	1.9	0.7	1.1
16:0 (chimyl)	13.2	9.1	10.4
16:1	10.6	10.8	9.1
17:0	3.0	3.6	4.7
18:0 (batyl)	3.4	2.8	6.7
18:1 (selachyl)	47.8	59.4	53.6
18:2	2.4	1.6	2.5
18:3	trace	?	?
19:1	1.2	1.5	2.4
20:1	8.0	6.2	6.4
21:0	trace	?	?
22:1	2.7	2.2	1.0

*The alkoxyglycerols are represented by the long chain part of the molecule.

Hallgren and Larsson published two further papers in 1962, one (42) of which describes the glyceryl ethers occurring in man and in the bovine. Table XI summarizes their composition data on the glyceryl ethers.

TABLE XI: PERCENTAGE COMPOSITION (WT/WT) OF GLYCERYL ETHERS FROM HUMAN BONE MARROW, HUMAN SPLEEN, AND HUMAN MILK (Hallgren and Larsson)(42)

Long-chain component in glyceryl ethers	Human bone marrow	Human spleen	Human milk
unidentified components	3.8	-	2.0
16:0	29.4	33.0	23.9
16:1			trace
17*	7.6	1.0	3.6
18:0	24.6	25.8	22.8
18:1	16.7	27.6	33.8
18:2			1.4
19*	6.1	?	2.4
20:0	2.9	)	1.6
20:1	3.2	) 7.3	2.3
22:0	0.7	)	0.7
22:1	5.1	) 5.2	3.4
24:0			2.1

*Normal and branched

In 1962, Guyer (38), using gas chromatographic techniques on the glyceryl ethers isolated from dogfish liver oil, showed that the components had chain lengths varying from 12 to 20 carbon atoms.

Although glyceryl ether-containing phospholipids are present in bone marrow they are virtually absent from the erythrocytes, in fact human erythrocytes have no detectable glyceryl ethers, but they do contain

plasmalogens. Young bovine erythrocytes which still possess a full complement of nuclei, mitochondria, and microsomes have glyceryl ethers in the mitochondrian fraction; whereas in the older erythrocytes, where all intracellular organelles have atrophied in the cell, the glyceryl ether containing phospholipids are localized in the cell membrane (101).

Studies of the occurrence of glyceryl ethers in various tissues show that bone marrow is the richest site, with the other tissues having lesser amounts. Todd and Rizzi (1964)(104), on the other hand, working with rabbit tissue, found intraperitoneal fat to be the richest source and blood the lowest. In the same paper, Todd and Rizzi report a comparison of mammalian yellow bone marrow from the rabbit, the cow, the sheep, the pig, the rat, and man. (Details are given in Table IX.) It may be noted from this data that the rabbit has only chimyl alcohol, and that the rat appears to have no glyceryl ethers in its bone marrow. Analysis of the glyceryl ethers from starfish show the batyl and chimyl alcohols to be present and the selachyl to be absent (104); this is in contrast to the fish oils which have a high proportion of selachyl as a glyceryl ether.

It is of interest that two species of terrestial slug, Arian ater and Ariolimax columbians, contain α-glyceryl ether phospholipids accounting for more than 25 mole percent of the total phospholipid mixture. There is an absence of unsaturation with the major glyceryl ether being chimyl alcohol, a sharp contrast to the findings with other organisms.

D. THE MESENCHYMAL ORIGIN OF THE α-GLYCERYL ETHERS

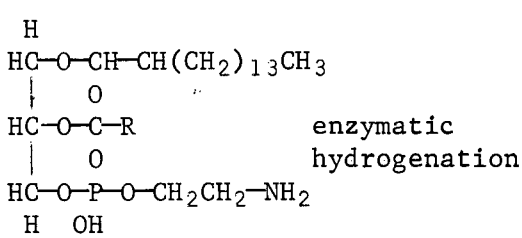
Bodman and Maison (16) report, perhaps with insufficient supporting evidence, that the α-glyceryl ethers are found present in those tissues containing cells of mesenchymal origin, from which tissue cells are continually shed into a body cavity. They support this by referring to the association of α-glyceryl ethers with saturated fatty acids in the unsaponifiable fraction and the association between squalene and α-glyceryl ethers. Squalene rich oil has been found in the stomach of the shark and in the faeces of land animals; if we assume glyceryl ethers to be present along with the squalene in these cases, then it is reasonable to assume that the gut mucosa is concerned with α-glyceryl ether synthesis.

E. BIOSYNTHESIS OF THE GLYCERYL ETHERS

In 1955, Karnovsky and Brumm (50), using the starfish, reported the incorporation of radiocarbon into α-glyceryl ether moities at rates comparable to those observed in the more commonly studies lipid fractions. This may indicate that the starfish does not simply accumulate these compounds from its food.

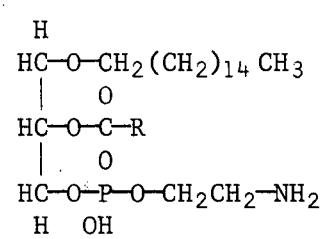
Data presented by Thompson and Hanahan (1962) (100), obtained from experiments using C^{14} labelled glucose, indicate that the glyceryl moiety of the glyceryl ethers can be formed from this sugar in bovine marrow. The distribution of C^{14} would strongly suggest that the glucose metabolite directly involved is l-α-glycerophosphate. This is similar to the pathway proposed in the biosynthesis of phosphatidic acid, or some other

metabolically analogous compound. In a more recent paper (1963) the same authors describe in vitro bone marrow incubation experiments (101). The radioactivity from glucose-6-C^{14}, sodium palmitate-1-C^{14} and tritiated water were incorporated into glyceryl ether phospholipids by bone marrow extracts. At the end of the incubation times used, radioactivity of the glyceryl-ether phospholipids was less than that of the nonglyceryl-ether phospholipids. Almost all of the radioactivity of the synthesized glyceryl ethers was located on the alpha carbon of the glyceryl moiety, again suggesting that α-glycerophosphate may be a direct glucose-derived precursor. Both ethanolamine and choline phosphatides contain the ethers. There appears to be a metabolic relationship between glyceryl ethers and plasmalogens. One possible route by which glyceryl ethers may be synthesized lies in the formation of a plasmalogen type molecule followed by enzymatic hydrogenation of the vinyl ether double bond.



a plasmalogen

enzymatic hydrogenation



α-glyceryl ether containing phospholipid

F. DIGESTION AND METABOLISM OF α-GLYCERYL ETHERS

Much as dietary glycerides are digested by the action of the pancreatic lipase present in the intestine, to glycerol, fatty acids, and partially split products such as monoglycerides and diglycerides; the esterfied glyceryl ethers are broken down from glyceryl ether diesters to glyceryl ether monoesters, free glyceryl ethers and fatty acids. Then the fatty acids, with the aid of the bile salts, carry the glyceryl ether mono- and diesters and the free glyceryl ethers into the mucosal cells of the small intestine. Once within the mucosal cells, the ether linkage can be broken to give free glycerol and free fatty acids.

Several papers have been published which give data about digestion and to some extent, the metabolism of glyceryl ethers. (Very little is known about the role of glyceryl ethers in cell metabolism.) Bergstrom and Blomstrand (1956)(12), Blomstrand (1959((15) and Blomstrand and Ahrens (1959)(14) report data on the absorption of chimyl alcohol in rats; Blomstrand and Ahrens (1959)(14) report data on absorption in man and Malins, et al (1964)(66) report data on absorption in the rainbow trout. In man and the rat, well over 90% of fed glyceryl ethers were absorbed. That which passed into the faeces retained its ether linkage intact, the only thing that happened while in the intestine was the hydrolysis and resynthesis of the ester linkages on the glyceryl ether molecule. On the other hand, the glyceryl ethers that were absorbed into the mucosal cells and traced in the chyle had their ether linkages broken. The following table is taken from Blomstrand's paper (1959)(15):

TABLE XII: RECOVERY AND DISTRIBUTION OF RADIOACTIVITY IN LYMPH LIPIDS AFTER FEEDING C^{14}-LABELLED CHIMYL DIOLEATE*

% of administered activity absorbed	% of absorbed activity recovered in lymph lipids	% of activity in lymph lipids recovered as:			
		chimyl alcohol		triglyceride fatty acids	phospholipid fatty acids
		free	esterfied		
90	60	6	18	74	2
85	45	5	20	73	2

All this work done by Blomstrand and his co-workers with the rat and the human is substantiated by Malins, et al (66) in his work with the rainbow trout (Salmo gairdneri). The trout were fed batyl alcohol and C^{14}-glyceryl ethers. At the end of a feeding period the lipids of the intestinal contents were analysed; although there was simultaneous esterfication and hydrolysis in the lumen of the intestine, there was no cleavage of the ether bond. No appreciable amounts of glyceryl ethers were found deposited in the flesh of the fish which suggests metabolic breakdown of the glyceryl ethers in the fish as well as in rats and in man.

Swell, et al (1965)(98) report on the comparative absorption of α- and β-octadecyl glyceryl ethers. Lymph fistulated rats were fed test rations containing 35 mg. of α- or β-octadecyl glyceryl-1-C^{14} ether. They found that β-glyceryl ether was absorbed into the lymph to a greater extent (96%) than the α-glyceryl ether (76%). Fractionation of the lymph lipids indicated that 37 to 48 percent of the C^{14} activity was in the form of C^{14}-labelled fatty acids, and from 55 to 63 percent as free and esterfied glyceryl C^{14}-ethers. The α-glyceryl ether was split in the intestine to a

*The chimyl dioleate was dissolved in olive oil and fed to 2 rats using a thoracic duct fistula.

greater extent than the β-glyceryl ether. Most of the C^{14} activity of the lymph lipids was in the alkoxydiglyceride fraction. The β-glyceryl ether was more efficiently converted to the alkoxydiglyceride than the α-glyceryl ether. More alkoxymonoglyceride appeared in the lymph when the α-glyceryl ether was fed than when the β-glyceryl ether was administered.

G. α-GLYCERYL ETHERS AND HAEMOPOIESIS

As early as 1949 Sandler (90) suggested that glyceryl ethers, in particular batyl alcohol, had the ability to stimulate reticulocytosis in man. Then in 1951 Arturson and Lindback (4) reported that batyl alcohol given at a dosage level of 34 and 70 mg/Kg of body weight to male white mice caused a significant increase in reticulocytes while red cell counts were little affected by the treatment. A slight contradiction to these positive effects is given by Evenstein, et al (34) who dosed rats at levels of 2 to 20 mg/Kg of body weight, without significant effect on the red blood cell count, reticulocyte number, hematocrit or hemoglobin values. In 1958 Linman, et al (61) reported the subcutaneous injection of female rats with 150 and 300 mg of batyl alcohol per Kg of body weight, and obtaining a significant response in erythropoiesis, thrombopoiesis and granulopoiesis. The response appeared to be one of accelerated cellular division. In 1959 Linman, et al (62) published another paper reporting the administration, by stomach tube, of approximately 30 mg/Kg of batyl alcohol to rats; the results were: erythrocytic response, production of microcytes, reticulocytosis, thrombocytosis and leucocytosis. Although Linman (62) found batyl alcohol effective orally and parenterally, independent of optical activity, he

found selachyl alcohol (natural and synthetic) devoid of activity. The selachyl was administered over a ten day period by gastric intubation to normal rats at a dosage rate of 50 mg/rat/day (312 - 356 mg/Kg.) The inactivity of selachyl alcohol was confirmed by Osmond, et al (79) in their experiments using guinea pigs. Both selachyl and batyl alcohols were given at a dosage level of approximately 10 mg/Kg of body weight. The results suggest that the batyl alcohol may be an erythropoietic stimulant in the guinea pig whereas selachyl alcohol is not. Negative results were also obtained by Penny, et al (80) working with sheep, cattle, and mice. Sheep were dosed intravenously at a rate of 2 and 4 mg of batyl alcohol per Kg of body weight for 21 days with no response. In one experiment, sheep were dosed intravenously with 10 mg of batyl alcohol per Kg of body weight for 4 days which resulted in a rise in polymorphonuclear leucocytes, but no increase in white blood cell count. Two heifers given 1 gm of d-batyl alcohol (3 mg/Kg of body weight) intravenously for four days yielded no haematopoietic response. Mice were given approximately 110 mg/Kg of body weight subcutaneously yet they showed no stimulation, in fact, there was a slight decrease in the red cell, reticulocyte and marrow erythroblast counts.

Under specific conditions of disease and stress to the body, glyceryl ethers in general and batyl alcohol in particular stimulate the formation of blood cells and appear to alleviate some of the toxic side effects. The first reported use of bone marrow was in 1930 by Giffin and Watkins (36) in the treatment of secondary anemia. They used normal bone marrow in the hope that some factor in it would stimulate or alleviate the diseased bone marrow in secondary anemia. In general, the bone marrow

proved to be unsatisfactory in the treatment of secondary anemia, except in one case of agranulocytosis. Over the years 1930 to 1938, Giffin and Watkins treated 24 cases of agranulocytosis with bone marrow, and obtained a favourable response in all but three cases. In the original work, Giffin and Watkins administered about 0.4 grams of marrow (60 mg/Kg of body weight) to their patients. In 1936, Marberg and Wiles reported the use of a concentrate of the unsaponifiable portion of bone marrow dissolved in a bland oil (equivalent to 2 grams of marrow per drop) administered to patients suffering from agranulocytosis. As a result, there was in most cases a rise in the number of granulocytes which began with 24 to 36 hours and values returned to normal if the causative infection persisted during the period of treatment.

One of the promising uses of glyceryl ethers is said to be for the treatment of leucopenia caused by irradiation. In 1954, a paper by Brohult and Holmberg (22) describes the successful use of the non-saponifiable portion of bone marrow fat and concentrates of alkoxyglycerols and their esters (the concentrates had a higher potency than the bone marrow preparations). Of 36 human cases suffering from irradiation leucopenia, 25 cases responded to the treatment by an immediate increase in the white cell count. In 9 cases there was no further decrease while two showed a continued decrease in leucocyte counts. In 1957, Brohult published another paper (17) with similar results, when she used alkoxy-glycerol esters administered to 100 patients suffering from irradiation leucopenia. She reports an optimum dosage of 1.2 grams of oil per day (equivalent to 2.74 mg of free glyceryl ethers per Kg of body weight) and an actual decrease in leucocyte numbers

at dosage levels above 2.5 grams of oil per day (equivalent to 5.70 mg of free glyceryl ethers per Kg of body weight). Brohult (1958)(18) used alkoxyglycerol esters for the prophylactic treatment of rats that were subsequently exposed to total body X-irradiation. A significant higher count of both megakaryocytes and nucleated cells was found in the marrow of the prophylactically treated animals. Table XIII gives a summary of the results obtained by Brohult.

Mizuno, et al (76) treated calves, exposed to whole body irradiation, with autologous bone marrow. Nine out of 15 recovered after a severe hematologic crisis.

TABLE XIII: ALKOXYGLYCEROL ESTERS FED TO RATS RECEIVING TOTAL BODY X-IRRADIATION (taken from Brohult) (18)

Experimental group	Number of rats	(mg/day)*	(M%)**
Normal	70		100
Control	87		19
Prophylactic groups			
Shark liver oil	18	0.25	26
Shark liver oil	9	0.40	33
Shark liver oil	23	0.50	27
Pure esters	3	0.25	29
Batyl alcohol	11	0.14	32
Selachyl alcohol	8	0.12	61
Selachyl alcohol	6	0.14	49
Selachyl alcohol	12	0.18	27

*0.15 mg is equivalent to 25 mg of free glyceryl ethers per Kg of body weight.

**Megakaryocyte count (M) in % of the megakaryocytes for normal rats.

There have been other reports of the therapeutic use of glyceryl ethers in the treatment of irradiation sickness. Rusanov, et al (87) treated X-irradiated rats with batyl alcohol and obtained an activation of blood production as well as a faster recovery from the radiation sickness. Prokhonchukov and Panikarovskii (85) used both selachyl and batyl alcohols and found batyl to give a satisfactory therapeutic response, while selachyl gave an insignificant response. Sviridov, et al (97) studied the therapeutic effect of selachyl alcohol administered to dogs, rabbits and rats that had been exposed to X-irradiation. The alcohol alleviated the gravity of the radiation sickness, increased survival rate, normalized tissue respiration in the brain and myocardium, and somewhat stimulated the haemopoietic organs (the follicular appendages of the lymph nodes and the spleen). Another report of the use of batyl alcohol is given by Dudin (29), where primagravid rats on the 15th day of pregnancy were irradiated and injected intravenously with cystamine (65 mg/Kg) and batyl alcohol (0.5 mg/Kg) daily for 10 days after exposure to X-rays. There was no influence on the gravity of the X-ray sickness. In controls, 50 percent of the young were born dead; with the combined treatment of batyl and cystamine only 22 percent of the foeti were born dead and, of those born alive, the development of anemia and leucopenia was completely prevented.

Batyl alcohol has been effective in stimulating haemopoiesis and alleviating certain conditions of toxicity. One of these is in benzene poisoning. Sandler (1949) (90), examined the possibility that yellow bone marrow extracts might have an antitoxic effect rather than containing a specific leucopoietic principle. He injected, subcutaneously, benzene and

preparations made from nonsaponifiable yellow bone marrow extracts to one group of benzene depleted rats. Another group of depleted rats were injected with benzene and batyl alcohol. A number of animals were given benzene solely and served as controls. The bone marrow and the batyl alcohol therapy increased the number of erythrocytes, decreased the tendency to benzene necrosis at the site of injection, and lowered mortality. De Gaetani and Baiotti (1959)(27) report that batyl alcohol given to rats with acute benzene poisoning, relieves intoxication, prolongs life, increases osmotic resistance of red cells, favours reticulocytosis, and restores the activity of bone marrow. Hasegawa, et al (1961) (46), report that batyl alcohol had effective haemopoietic activity in rabbits suffering from experimental anemia caused by Nitromin injection or solvent intoxication. However, in the case of induced aplastic anemia in calves caused by feeding trichloroethylene-extracted soybean oil meal, batyl alcohol failed to prevent development of anemia (92). Batyl alcohol treatment was tried because aplastic anemia so produced is very similar to bracken poisoning in cattle. Batyl alcohol has been used with varying degrees of success in the treatment of bracken poisoning. Evans, et al (1957) (33), report the successful use of batyl alcohol in the treatment of bracken poisoning and in 1958, Evans, et al again reported the use of batyl alcohol; 23 out of 31 poisoned cattle recovered. The validity of these reports is questioned by Dalton (25) who claims that normal cattle may vary their leucocyte count as much as 1000 to 4000 cells per cmm during the day and from day to day. The increase in leucocyte count reported by Evans was 1000 to 2000 cells per cmm. Dalton also treated cattle affected with bracken poisoning, but found no obvious effect on the haematology.

H. SUMMARY

These natural occurring compounds are of interest because they appear to be useful and perhaps essential to all animals including man. They may be useful as a stimulant, a drug, or as a nutrient. They are normally found in the animal body, they are absorbed from the intestine and metabolized within the body. These compounds are not toxic, although there has been some report of toxicity in early work done by Agduhr (1934) (21), Berger (1948) (21), and Emmerie, et al (1952) (31). It is possible that the toxicity reported resulted from impurities in the alkoxyglycerols that were used.

TABLE XIV: BIOLOGICAL EXPERIMENTS INVOLVING THE USE OF GLYCERYL ETHERS

Glyceryl Ether	Animal	Condition	Dosage	Response	Reference
batyl and selachyl	rats	transplanted malignant tumors	1 ml of preparation in sunseed oil/day for a month	these injections caused the tumors to develop slower in the treated rats; selachyl gave the greatest inhibition	Abaturova and Shubina (1964)
batyl (purity unknown)	mice	normal	.01 to .048 mg/Kg/day 160 - 210 days	lesions of heart, kidneys and liver	Agduhr (1934) (cited in Brohult)
alkoxyglycerols	mice	radiated	590 mg/Kg in food	both glyceryl ether esters and essential fatty acids promote longer life	Alexander, et al (1959)
batyl	mice	normal	34 mg/Kg and 70 mg/Kg (intraperitoneal)	both levels gave a significant increase in reticulocytes; red blood cell counts remained normal	Arturson and Lindback (1951)
various	mice	normal	large (subcutaneous)	LD_{50} for batyl alcohol was determined to be 3 gm/Kg	Berger (1948) (cited in Brohult)
C^{14}-chimyl	rats	normal	5 mg by stomach tube	absorption and breakdown studied	Bergström and Blomstrand (1959)

TABLE XIV (cont'd)

Glyceryl Ether	Animal	Condition	Dosage	Response	Reference
C^{14}-chimyl	rats	normal	.5 ml of a 2% solution by stomach tube	absorption and break-down studied	Blomstrand (1956)
C^{14}-chimyl	man		25 mg by mouth	absorption and break-down studied	Blomstrand and Ahrens (1959)
alkoxy exters	man		various	2.74 mg gave the optimum 5.7 mg and higher gave results lower than the controls	Brohult (1958)
alkoxyglycerols including selachyl	man		25 mg/Kg with meals	of some value where leuko- or thrombo-cyto-penia has already occurred	Brohult (1958)
alkoxyglycerols	rats	radiated		the growth response was as follows: normal, 1.7 gm/day; irradiated and treated, 1.3 gm/day; irradiated without treatment, 1.1 gm/day	Brohult (1960)
alkoxyglycerols	bacteria		20 mcgm/ml of media	marked growth response	Brohult (1960)
			2 mcgm/ml of media	observable growth response	
alkoxyglycerol esters	300 cancer of the cervix patients	radiated		white cell and thrombocyte counts were higher for the treated patients	Brohult (1962)

TABLE XIV (cont'd)

Glyceryl Ether	Animal	Condition	Dosage	Response	Reference
batyl	cattle	normal	.5 mg/Kg on alternate days	no haematological response	Dalton (1964)
batyl	pregnant rats	radiated	.5 mg/Kg with and without cystamine	less stillborn, anemia and leucopenia prevented	Dudin (1961)
batyl	mice	radiated	10 mg every other day	lethality is lower in the treated group	Edlund (1954)
unsaponifiables	bacteria		1 mcgm/ml	inhibited growth, marked	Emmerie, et al (1952)
batyl	cattle	bracken poisoning	.5 to 1 mg	promoted recovery from	Evans, et al (1958)
batyl	cattle	bracken poisoning	1 mg and 2 mg	gave a leucocyte response	Evans, et al (1957)
batyl	rats	normal	0.5 mg for 11 days; 1 mg for 20 days, followed by 5 mg for 10 days (subcutaneous)	there was no response in the blood picture	Evenstein (1958)
batyl	rats	benzene poisoning		relieves toxicity	de Gaetani (1959)
Kaby 700	man	radiated		response in only 4 out of 15 cases	Ghys (1960)
batyl	rabbit	anemia by Nitromin		a haemopoietic response	Hasegawa (1961)

TABLE XIV (cont'd)

Glyceryl Ether	Animal	Condition	Dosage	Response	Reference
batyl	rats	normal	12.5 and 25 mg	response in erythrocytes, thrombopoiesis, and granulopoiesis	Linman (1958)
batyl	rats	normal	50 mg	a haemopoietic response	Linman (1959)
batyl	rats	normal	165 - 330 mg	a haemopoietic response	Linman (1960)
selachyl	rats	normal	312 - 356	no activity	Linman (1960)
glyceryl ethers	man			effective in treating wounds caused by X-ray burns	Maisen, et al (1959)
glyceryl ethers	rainbow trout	normal		digestion and metabolism data obtained	Malins, et al (1964)
unsaponifiables of bone marrow	man	granulo-cytopenia		favourable results	Marberg (1936)
alkoxy esters	mice	normal	193 mg/Kg	four died with catarrhal enteritis, survivors showed no path. changes	Melander (1954) (cited in Brohult)
alkoxy esters (mainly selachyl)	mice	normal	167 mg/Kg	no deaths; weight gain normal	
batyl	mice	normal	73 mg/Kg	no deaths, weight gain normal	

TABLE XIV (cont'd)

Glyceryl Ether	Animal	Condition	Dosage	Response	Reference
alkoxy esters (mainly selachyl)	mice	normal	533 mg/Kg 1467 mg/Kg	no deaths; weight gain normal	
batyl and selachyl	guinea pig	normal	10 mg/Kg	batyl alcohol gave significant changes in both bone marrow and blood; selachyl showed no changes	Osmond (1963)
batyl	cattle	normal	3.1 mg/Kg intravenous	no response	Penny, et al (1964)
batyl	sheep	normal	2 to 10 mg/Kg intravenous	no response	
batyl	mice	normal	114 mg/Kg subcutaneous	no response	
selachyl	rats	radiated		ineffective	Prokhonchukov and Panikarovskii (1963)
batyl	rats	radiated		satisfactory therapeutic response	Prokhonchukov and Panikarovskii (1963)
batyl	rats	radiated		activation of blood production	Rusanov, et al (1962)
chimyl	blood cells			inhibited lysolecithin hemolysis	Safanda and Holecek (1965)

TABLE XIV (cont'd)

Glyceryl Ether	Animal	Condition	Dosage	Response	Reference
batyl	rats	benzene depleted		rise in erythrocytes and a decrease in necrosis	Sandler (1949)
batyl	man	normal	0.3 to 0.65 mg/Kg	reticulocytosis	Sandler (1949)
batyl	calves	trichloro-ethylene extracted soybean meal		no response	Schultze, et al (1958)
selachyl	pea stems			stimulates auxin and giberellin action	Stove (1960)
selachyl	dogs, rabbits, rats	radiated		alleviated sickness	Sviridov and Abaturova (1964)
C^{14} α and β batyl	rat	normal	35 mg fed	digestion and metabolism studied	Swell, et al (1965)
bone marrow	man	granulo-cytopenia		favourable results	Watkins (1933) (cited in Marberg)

PART II:

EXPERIMENTAL

A. PRELIMINARY EVALUATION - TRIAL I

1. Preamble

The experimental work carried out as a part of this study can conveniently be divided into two sections; preliminary trials and nutritional investigations. Prior to any nutritional investigations, it was necessary to ascertain if the α-glyceryl ethers would produce any deleterious effects on animal growth and development. In the absence of quantitative information on the dose-response relationship of the α-glyceryl ethers for mammalian systems it was necessary to establish a more or less arbitrary dosage level with which to begin the preliminary trials. The amounts of these compounds in various tissue systems is in the range normally expected for certain of the vitamins. Hallgren and Larsson (42) report bovine milk to have 0.01 percent of its lipid fraction as glyceryl ethers. A calculation for milk containing four percent fat suggests a figure of 400 mcg of glyceryl ethers per 100 grams of milk. This level is of the same order of magnitude as that normally recorded for a number of the vitamins present in milk (see Table XV).

On the basis of the above, a dosage level of 6 mg per kilogram of body weight per day was arbitrarily selected for preliminary toxicity trials. Table XVI affords a comparison of this dosage level with recommended levels for several of the vitamins. Both sets of values are expressed as mcg per kilocalorie of digestible energy in the diet to permit direct comparison. These values are all computed for rats; similar estimates can be made for the dog. In these calculations it was assumed that the digestible energy of the feed was 3800 kilocalories per kilo. The

TABLE XV: REPRESENTATIVE VALUES OF IMPORTANT VITAMINS IN MILK
(from Rusoff) (88)

Vitamins	mcg per 100 gm of milk
Fat soluble vitamins	
vitamin A activity	42 - 63
vitamin D	0.045 - 0.058
vitamin E	60
Water-soluble	
thiamine	40 - 45
riboflavin	150 - 200
nicotinic acid	80 - 100
pantothenic acid	350 - 400
pyridoxine	25 - 48
folic acid	0.1 - 0.23
biotin	2.0 - 3.5
cobalamin	0.3 - 0.5
vitamin C	2000
choline	13000
inositol (total)	13000

TABLE XVI: AMOUNT OF GLYCERYL ETHER ADMINISTERED COMPARED WITH THE VITAMIN REQUIREMENTS OF THE ALBINO LABORATORY RAT

	mcg per kilocalorie of digestible energy
dosage of glyceryl ether per kilogram of body weight	
6 mgs	11
Vitamin	
A	1.58
cobalamin	0.0013
riboflavin	0.76
choline chloride	197
niacin	4
pyridoxine HCl	0.32
thiamin HCl	0.66

apparent digestible energy intake (A.D.E.I.) in kilocalories per day for the rat may be estimated from the expression (Cheeke) (24)

$$A.D.E.I. = 6.82\ W^{0.451}$$

where W represents body weight in grams. On the basis of this equation, a 100 gram rat would ingest 54 kilocalories of digestible feed energy per day. In order that this rat may receive 6 mg of a glyceryl ether per kilogram of body weight per day, its ration would have to provide 11 mcg of the ether per kilocalorie of ration digestible energy (54 times 11 equals 594 mcg per 100 gram rat which is approximately 6 mg per kilogram of rat.)

2. Experimental Design

Eighty male and female rats were randomly allotted into four groups with equal numbers of each sex in each group. Four groups each containing five dogs were used. Each of the four groups of rats and dogs were allocated to a treatment as follows: one control group and one group for each of the three important naturally occurring glyceryl ethers. These animals were fed normally and presented with their respective doses daily for a period of 6 months. The rats were weighed three times weekly (Monday, Wednesday, and Friday); the dogs were weighed once each week. Daily feed intake data were recorded for the rats, but not for the dogs. Blood samples were obtained from both the dogs and the rats according to the schedule given in Table XVII. Erythrocytes and leucocytes were counted using standard methods (see Appendix XVI). After 180 days all the rats and one dog from each group were killed. Gross pathology was noted and tissues were recovered in methanal acetate. Sections from the heart, lungs, liver, kidneys, adrenals, intestine and spleen were prepared for histological study.

TABLE XVII: SCHEDULE OF BLOOD COUNTS

	Time of blood sampling*
Rats	
erythrocytes	180
leucocyte counts	90 and 180
Dogs	
erythrocytes	110
leucocyte count	180

* Days from the beginning of the trial

3. Animals

Albino rats of the UBC Wistar strain and Labrador dogs, both reared at the university animal units, were used in this experiment. The rats were placed on experiment at a body weight of approximately 90 grams and the dogs at 10 kilograms. The details of the weight changes in both groups of experimental animals are given in Appendix II.

4. Ration

The rats were fed UBC stock rat ration in ground form to facilitate the measurement of feed intake. A correction was not applied for feed spillage. The dogs were fed UBC stock dog crumbles. Both the rats and the dogs were fed ad libitum. The constituent composition of these rations is given in Appendix I.

5. Housing

The rats were housed individually in wire cages in a room with controlled ambient temperature and humidity. The dogs were housed in individual concrete block pens using wood shavings for litter. They were exercised outdoors from 10:00 a.m. to 4:00 p.m. each day, weather permitting.

6. Administration of glyceryl ethers

The rats were dosed with each of the three glyceryl ethers suspended in a safflower oil/safflower ethyl esters/oleic acid (55/40/5) diluent. The control rats received the diluent only. The dogs received the batyl and chimyl alcohols in tablet form, the selachyl alcohol was suspended in the above diluent. As was the case with the rats, the control dogs received the diluent only. The glyceryl ethers, in diluent suspension, were administered per orum with a syringe. Those dogs receiving their glyceryl ethers in tablet form, were forced to swallow their daily dose.

7. Results

All of the pertinent data concerning feed intake, body weight changes and blood counts are given in Appendices II, III and IV. After considering all the data collected during the 180 day trial and the data collected at the time of slaughter, there appeared to be no evidence of deleterious effects from the treatments. Table XVIII, XIX and XX summarize the data given in the Appendices II and III. They reveal that the weight gains and feed efficiencies of the treated dogs and rats were essentially the same as the controls. A "t" test indicates that the rate of gain of

TABLE XVIII: SUMMARY OF MEAN BODY WEIGHT GAIN AND MEAN FEED-INTAKE DATA FOR MALE RATS

		Selachyl	Batyl	Chimyl	Control
final weight	(gm)	421.5	413.9	412.8	418.9
initial weight	(gm)	95.1	94.4	94.0	88.7
total weight gain	(gm)	326.4	319.5	318.8	330.2
gain/day	(gm)	1.79	1.76	1.75	1.81
total feed consumed	(gm)	3,925	3,736	3,666	3,829
feed intake/day	(gm)	21.6	20.5	20.1	21.0
gm of feed/gm of gain		12.0	11.6	11.5	11.6

TABLE XIX: SUMMARY OF MEAN BODY WEIGHT GAIN AND MEAN FEED-INTAKE DATA FOR FEMALE RATS

		Selachyl	Batyl	Chimyl	Control
final weight	(gm)	267.3	255.4	253.3	256.4
initial weight	(gm)	85.9	86.4	88.1	85.7
total weight gain	(gm)	181.4	169.0	165.2	170.7
gain/day	(gm)	1.0	0.93	0.91	0.94
total feed consumed	(gm)	3,263	3,041	3,132	2,930
feed intake/day	(gm)	17.9	16.7	17.2	16.1
gm of feed/gm of gain		18.0	18.0	19.0	17.2

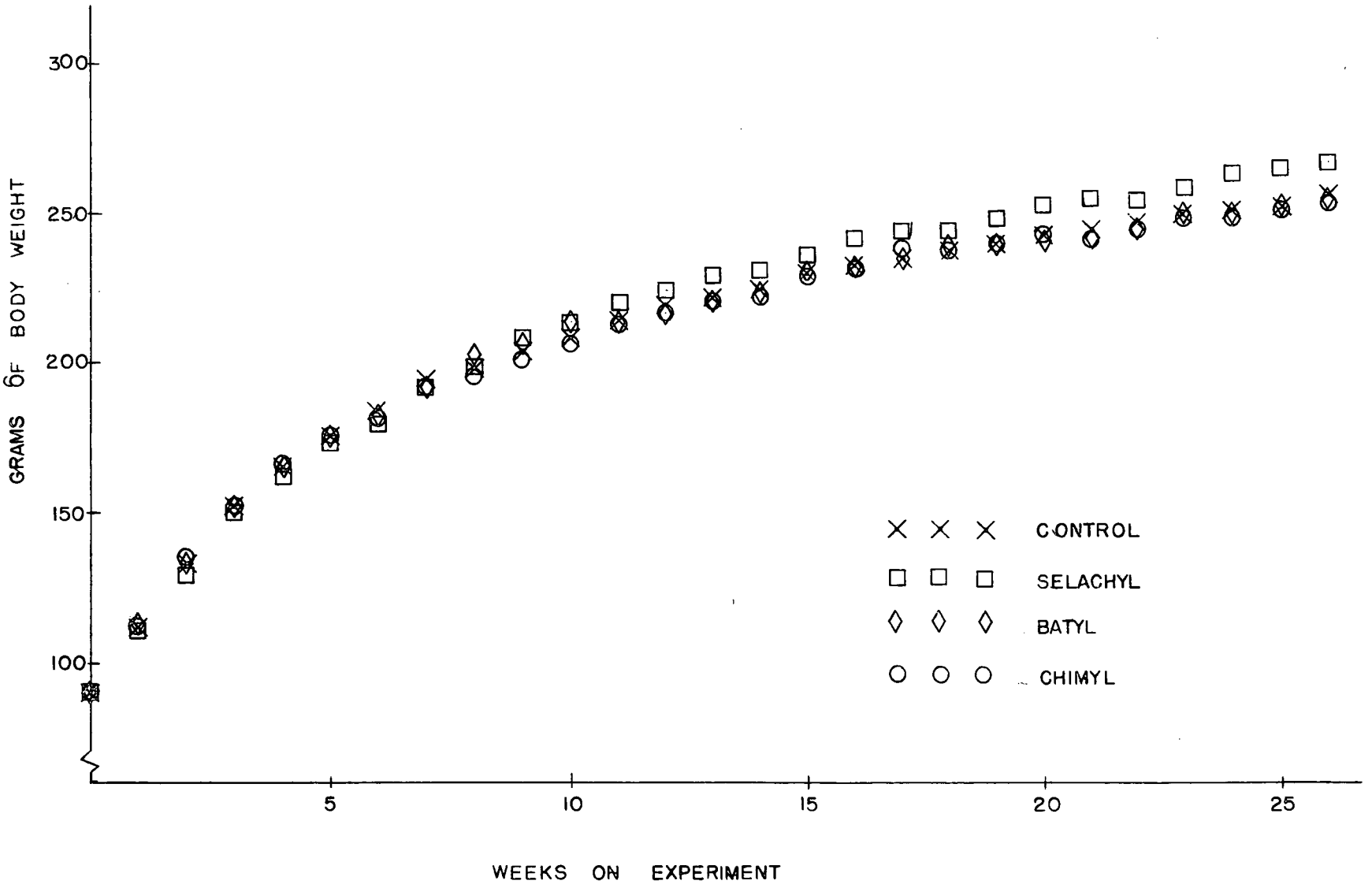
TABLE XX: SUMMARY OF BODY WEIGHT GAIN FOR DOGS*

		Selachyl	Batyl	Chimyl	Control
final weight	(Kg)	109.7	103.7	115.0	104.0
initial weight	(Kg)	51.6	53.6	58.1	47.6
total weight gain	(Kg)	58.1	50.1	56.9	56.4
weight gain/week	(Kg)	2.23	1.93	2.19	2.17
weight gain/dog/week	(Kg)	0.45	0.39	0.44	0.43

*There were five dogs in each of these groups

FIGURE I:

GROWTH CURVES FOR FEMALE RATS


GRAMS OF BODY WEIGHT
300
250
200
150
100
5
10
15
20
25
WEEKS ON EXPERIMENT
CONTROL
SELACHYL
BATYL
CHIMYL

the female rats in the selachyl group is significantly higher than that of the control female rats. However, the significance of this growth response is questioned because it did not occur in the male rats on the selachyl treatment. The batyl and chimyl treated groups of female rats have identical growth curves to the control group. The growth curve (Figure I) for the female rats that received the selachyl alcohol was identical with that of the other groups to a mean body weight of about 200 grams. Above this body weight this group gained weight at a slightly faster rate and hence reached a slightly heavier weight at the end of the experiment. This increase in body weight above the other experimental groups was significant (at the .05 level). This increase did not occur until after puberty. It did not appear in the male rats receiving these alcohols.

The growth curves of the male rats and of the dogs are not presented here because the different experimental groups displayed identical growth responses.

Table XXI gives a summary of the blood counts which were taken during this trial and which are given in detail in Appendix IV. There was a large variation in leucocyte counts between animals in any one group (especially in the case of the rats), but very small differences in average counts between the control and treated groups. This variability in leucocyte count probably arose because the rats were suffering from a low level intercurrent lung infection. Using the "t" test on the leucocyte count data, at 90 days, revealed that female rats given the selachyl treatment had significantly (.05) higher counts. At variance with this result, the

TABLE XXI: SUMMARY* OF BLOOD COUNTS TAKEN IN TRIAL I

	Selachyl	Batyl	Chyml	Control
Rats				
leucocytes after 90 days				
female	18,340	15,440	15,690	14,600
male	18,550	17,665	22,110	19,800
leucocytes after 180 days				
female	15,560	16,010	14,510	14,320
male	18,200	19,340	19,540	17,560
erythrocytes after 180 days				
male	9.11	8.82	9.58	8.87
Dogs				
erythrocytes after 110 days	0.28	0.39	0.52	0.46
leucocytes after 180 days	17,200	17,200	16,900	17,500

*For rats, these are means of 10 animals and for dogs these are means of 5 animals. Leucocytes are given as number of cells per cmm of blood and erytrocytes in millions of cells per cmm of blood.

leucocyte counts for the male rats at 90 days showed that both the selachyl and batyl groups had significantly (.05) lower counts than the control animals. At 180 days the "t" test did not reveal any significant differences, positive or negative, between the control and treated male and female rats. The leucocyte counts taken of the dogs' blood after 180 days did not show any significant (.05) differences. Despite the fact that statistically significant differences have been noted in a few comparisons in relation to time of examination and treatment, the overall pattern of the results does not permit the derivation of any final conclusions.

Erythrocyte counts showed much less variation between individuals than did the leucocyte counts, but the differences between groups was still quite small. Statistical analysis of erythrocyte counts from both dogs and rats revealed a significant difference in only one case, in which the chimyl group of male rats showed higher counts than the controls.

The histopathological preparations from the various tissue systems of the rats and the dogs revealed no apparent differences between the treatment and the control groups.

8. Conclusion

Glyceryl ethers administered at a level of 6 mg per kilogram of body weight over prolonged periods of time are not deleterious to the growth of rats or of dogs. With the methods used here, it was not possible to demonstrate any effect of the α-glyceryl ethers on the haematopoietic systems of either species.

B. PRELIMINARY EVALUATION - TRIAL II

1. Preamble

Having established that the α-glyceryl ethers, when fed at a level comparable to demonstrated vitamin requirements, produced no deleterious effects on the rat or dog, it was considered necessary to ascertain if these natural compounds had such effects at much higher dosage levels. For this purpose an upper level of 2400 mg per kilogram of body weight per day was selected (this is 400 times the dosage level used in Trial I). Because of the considerable expense associated with these compounds and with trials with dogs, only batyl and selachyl alcohols were examined in this phase of the work. Chimyl alcohol was eliminated since its level in tissue relative to the others is comparatively low. Batyl alcohol is a suitable representative of the group of saturated glyceryl ethers and selachyl of the unsaturated ethers.

One group of rats and one group of dogs were offered a ration containing selachyl alcohol at a level which provided a daily intake of 600 mg per kilogram. This was done to provide data on the effects of an intermediate dosage level.

2. Design

Forty rats were randomized into four groups of ten animals containing five males and five females. Sixteen dogs were divided randomly into four groups of four, with two males and two females in each group. The treatment of each of the four groups from each species was as follows: a control group, two groups fed selachyl alcohol and a group fed batyl

alcohol. The details of animal grouping and dosage schedule are given in Table XXII. The glyceryl ethers were incorporated into the feed to facilitate administration. Feed intake was restricted to ensure complete consumption of all the feed offered each day. The rats were offered feed at a level calculated to be 80 percent of maximum intake to a body weight of 160 grams, then 90 percent of maximum intake to a body weight of 190 grams and, finally, 100 percent of the calculated maximum feed intake to the end of the 60 day trial period. The dogs were fed 80 percent of maximum feed intake for the full two month period. The rats were weighed three times per week (Monday, Wednesday, and Friday); the dogs were weighed once per week. At the end of one month and again at the end of two months, blood samples were taken from all of the animals; red blood cell counts, white blood cell counts, and differential counts of the white blood cells were made. Feed and faeces samples were taken every two weeks during the two month experimental period. After two months all of the rats and one-half of the dogs (two from each group) were killed, posted, gross pathology noted, and femur bone marrow smears made. The remaining eight dogs were treated to alleviate an intestinal parasite infestation and were then placed on the control ration for one month. Subsequently they were killed, posted, gross pathology noted, and femur bone marrow smears made. In addition, tissue sections were taken from each animal; heart, liver, spleen, kidney, adrenal and intestine.

TABLE XXII: GROUPING AND DOSAGE LEVELS USED IN TRIAL II

Group*	Daily Dose	Rats		Dogs	
		male	female	male	female
S^{400}	2.4 gm selachyl/Kg	5	5	2	2
B^{400}	2.4 gm batyl/Kg	5	5	2	2
S^{100}	0.6 gm selachyl/Kg	5	5	2	2
C^{400}	2.4 gm safflower oil/Kg	5	5	2	2

* The superscript ^{400} and the superscript ^{100} refer to 400 times and 100 times the 6 mg dosage level used in Trial I.

3. Animals

Albino rats of the UBC Wistar strain and Labrador dogs, both reared in the university animal units, were used in this experiment. The rats were placed on experiment at a weight of approximately 80 grams and the dogs at approximately 12 kilograms. The actual initial weights are given in Appendix V.

4. Housing

The rats were housed individually, in wire cages, in a room with controlled ambient temperature and humidity. The dogs were housed in individual concrete block pens using wood shavings for litter. The dogs were exercised from 10:00 a.m. to 4:00 p.m. every day in an outside run, weather permitting.

5. Ration and administration of glyceryl ethers

Both the dogs and the rats were fed the stock UBC dog crumbles (UBC ration No. 14 - 63 given in Appendix I). This differs from the ration arrangements used in the first trial in which the rats received UBC ration

No. 10 - 63. The appropriate amounts of glyceryl ethers, and for the control group, safflower oil were incorporated into the dog crumbles and fed as a dry mash. A regression equation relating maximum feed intake to body weight was calculated from feed intake values recorded for the rats used in Trial I. A corresponding regression equation for dogs was calculated from data collected during a six day preliminary feeding trial when dry dog crumbles were fed to 16 dogs having a body weight range of 18 to 31 pounds (8.2 to 14.1 kilograms). The ration used to establish feed intake was of lower fat content than that used in this experiment, hence the standard selected was reduced to 80 percent, by weight, of predicted full feed-intake. The addition of 5.8 percent glyceryl ether and, in the case of the controls, safflower oil, to the experimental rations increased the energy content by approximately 7 percent so the dogs being fed 80 percent of their maximum calculated feed intake were actually receiving 87 percent of their maximum energy intake. The regression equations so obtained are given below:

rats - grams of feed intake = $6.68W_{gm}^{0.167}$

dogs - grams of feed intake = $57.79W_{Kg}^{0.956}$

The exponent 0.956 for the dogs was not significantly different from unity hence the feed intake standard for this species was taken to be linear over the weight range used. The animals were fed less than their maximum calculated feed intake to ensure that all feed offered was eaten; this made it possible to add sufficient glyceryl ethers to the ration to ensure that the S^{400}, B^{400} and C^{400} groups received 2.4 gm/Kg and S^{100}, 0.6 gm/Kg of

body weight, daily. The blending of the glyceryl ethers into the ration was adjusted after each weighing of the rats to keep the level of administration at the prescribed point. For the dogs, the glyceryl ethers formed 5.7 percent of their ration and for the rats it varied from 1.7 percent at the beginning of the experiment to 3.5 percent of their ration at the end. This was necessary in the case of the rats since feed intake per unit of body weight decreases markedly as body weight increases. By increasing the relative level of glyceryl ethers in the ration as body weight increased, it was possible to ensure that the daily intake of the ethers per unit weight of animal remained at the prescribed level.

The regression equation predicting maximum feed intake for the rats was found to be in error, as the initial restricted level of 80 percent had to be raised to 90 percent at the end of 30 days and to 100 percent at the end of 45 days, in order to maintain a reasonable rate of growth (approximately 2.3 grams/day). With the dogs, feeding at 80 percent of the predicted maximum level was sufficient throughout the 60 day feeding period to cause the control animals to grow at a rate of 0.78 kilograms per week.

6. Digestibility procedures

(a) Digestibility of the experimental rations

(i) Rats

The feed intake was recorded for the rats each day. Total 48 hour fecal collections were made at two week intervals. The digestibility values calculated from this data are presented in Table XXVIII.

(ii) Dogs

Chromic oxide (0.1%) was added to the dog ration and fecal samples were collected from the individual dogs at two week intervals. In the determinations, the C^{400} and S^{100} groups were treated as a whole, while the B^{400} and S^{400} groups were treated according to whether they ate the same amount as the controls or not. The digestibility of the dog rations was determined by the increase in the concentration of chromic oxide in faeces over that in the feed, following the method proposed by Schürch, et al (93). The digestibility values so obtained are used in Table XXVII.

(b) Isolation and determination of glyceryl ethers*

(i) Extraction of lipids from feed and faeces**

Firstly, samples of the material to be extracted were oven dried at 110 degrees centigrade for two hours. These samples were subsequently used for chromate digestibility determinations. Another representative sample (5 gm on a dry weight basis) of the material was weighed into a 16 ounce jar. A calculated amount of water was added to increase the percentage of water to 80 percent. This total sample size was taken to be unity. An equal amount of chloroform and twice this amount of methanol was added. This mixture was then blended

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* These are the methods approved and employed by Western Chemical Industries Limited of Vancouver.

**This is a modified Bligh and Dyer procedure (13) that was used for lipid extraction of fish.

with a propeller-type stirrer at about 2000 rpm for 5 minutes. Then one unit of chloroform was added and blended for one minute. This was followed by one unit of water blended in for one minute. The whole mixture was then transferred to a stainless steel funnel and the solvent filtered into a 250 ml cylinder using a slight negative pressure. The residue was washed with a further one and one-half units of chloroform to give a mixture of solvents with the final composition of 2/2.5/1.8 (chloroform/methanol/water). This mixture separated into two phases. The lipid-containing chloroform phase was transferred to an evaporating flask. The chloroform was stripped off, and the total lipid residue dried to a constant weight in a vacuum oven at 60 degrees centigrade.

(ii) Determination of neutral lipids

The total lipid fraction was taken up in 100 ml of acetone per gram of lipids. Four ml of ethyl ether was added and the stoppered flask placed in running water at 14 degrees centigrade. The precipitated phospholipids were removed by filtration and the neutral lipids were recovered by stripping off the acetone and drying the residue to a constant weight under vacuum at 60 degrees centigrade.

(iii) Determination of the unsaponifiable lipid fraction

The neutral lipids were saponified according to the following scheme. Thirty ml of ethanol and three ml of 50 percent potassium hydroxide were added to approximately

one gram of neutral lipids and refluxed for one-half hour. Then thirty ml of water were added and the mixture allowed to cool. This mixture was extracted four times with ethyl ether (30 ml each time). This extract was washed alternately with 20 ml of water and 20 ml of 0.5 N potassium hydroxide and finally with water until the washings were clear to phenolphthalein. The ether was then stripped off and the unsaponifiable material dried to a constant weight in a vacuum oven at 60 degrees centigrade.

(iv) Qualitative and quantitative determination of glyceryl ethers

Qualitative pre-eluted 10 by 20 cm thin layer plates (250 micron layer of silica gel) were scored with 6 to 7 lanes. About 100 gamma of glyceryl ether residue was applied to a lane. Identification of the components in the residue was made by applying suitable reference compounds to corresponding lanes. These plates were eluted with 50/50/1 ($CHCl_3/CH_3OH$/acetic acid). Upon the completion of the elution the plates were sprayed with 20 percent sulphuric acid and charred at 150 degrees centigrade for 20 minutes or until the spots were sharply charred. The amount of glyceryl ether in the glyceryl ether residue was determined by comparison of the spot size with the spot size of known amounts of reference glyceryl ethers. The actual weight of glyceryl ethers in the glyceryl ether residue from the preparatory plate was determined by using the correction factor determined from the qualitative thin-layer plate.

7. Discussion of feed-intake, digestibility and weight gain

The consideration of the experimental results from the dogs gives rise to a number of problems. Firstly, there was a large variation in the initial weights of the animals. It was necessary to accept this variation since a limited number of dogs was available from which the experimental groups could be constituted. Sixteen pups were selected from some two dozen weanlings. Selection was carried out by elimination of the smallest and the largest animals. The sixteen so selected varied in weight from 18 to 31 pounds (8.2 to 14.1 kilograms).

The second consideration was one concerning the acceptability of the prepared rations. All the dogs were fed the stock dog ration for a four week period prior to the commencement of the experimental trial. During this first period all of the dogs ate and gained normally. After the dogs were placed on the experimental rations, it became apparent that all rations were not equally acceptable to the animals. The S^{100} and C^{400} groups continued to eat normally, but those offered the B^{400} and S^{400} rations did not eat with relish. In fact, some of the dogs refused to eat all the feed that they were offered. In the case of two dogs in the B^{400} group, the self-imposed feed-intake restriction actually led to a body weight loss. The acceptability of the S^{400} and B^{400} experimental rations was markedly less than that of the S^{100} and C^{400} rations. Glyceryl ethers in contact with water form complexes which adhere to adjacent surfaces. Thus the rations high in glyceryl ethers tended to interfere with the prehension and ingestion of the feed. Furthermore, the high levels of glyceryl ethers in the rations gave them an unusual odor which may have been repellent to the animals.

TABLE XXIII: SUMMARY OF THE WEIGHT GAIN AND FEED INTAKE DATA OF DOGS

S^{400} Individual Animals

		#25	#10	#9	#23	Mean
final weight	(Kg)	12.7	15.9	12.7	12.3	13.4
initial weight	(Kg)	10.9	14.5	12.3	10.0	11.9
total gain	(Kg)	1.8	1.4	0.4	2.3	1.5
gain/Kg of weight	(Kg)	1.5	0.9	0.03	0.21	0.66
gain/week	(Kg)	0.200	0.155	0.044	0.255	0.164
feed offered	(gm)	29,715	38,955	30,380	28,910	31,990
feed refused	(gm)	2,940	10,805	7,095	nil	5,210
feed consumed	(gm)	26,775	28,150	23,285	28,910	26,780
feed consumed/week	(gm)	2,975	3,128	2,587	3,212	2,975
gm feed/gm gain		14.9	20.2	58.8	12.6	26.6
gm feed/gm gain/Kg wt		1.26	1.33	4.70	1.13	2.11
gm feed/gm gain/$W^{0.73}$		2.46	2.77	9.30	2.16	4.17

B^{400} Individual Animals

		#20	#34*	#14	#12	Mean
final weight	(Kg)	9.5	14.1 (15.0)	10.9	12.7	11.8 (12.1)
initial weight	(Kg)	10.5	12.3	8.6	13.2	11.2
total gain	(Kg)	- 1.0	1.8 (2.7)	2.3	- 0.5	0.65 (0.88)
gain/Kg of weight	(Kg)	- 0.1	0.13 (0.20)	0.26	- 0.04	0.06 (0.08)
gain/week	(Kg)	- 0.11	0.20 (0.30)	0.26	- 0.06	0.07 (0.10)
feed offered	(gm)	26,530	35,595	24,780	32,970	29,969
feed refused	(gm)	8,835	nil	nil	9,460	4,574
feed consumed	(gm)	17,695	35,595	24,780	23,510	25,395
feed consumed/week	(gm)	1,966	3,955	2,753	2,612	2,822
gm feed/gm gain			19.8 (13.2)	10.8		
gm feed/gm gain/Kg wt			1.5 (0.97)	1.1		
gm feed/gm gain/$W^{0.73}$			3.0 (2.0)	2.1		

*Dog #34 was sick the last week of the experimental period and, as a result, lost weight. If he had gained normally then we would expect those figures which appear in brackets.

TABLE XXIII (cont'd)

S^{100} Group Individual Animals

		#26*	#33	#21	#13	Mean
final weight	(Kg)	15.9 (16.8)	19.5	15.0	18.7	17.3 (17.5)
initial weight	(Kg)	11.4	13.2	9.5	12.7	11.7
total gain	(Kg)	4.5 (5.4)	6.3	5.5	6.0	5.6 (5.8)
gain/Kg body weight	(Kg)	0.33 (0.38)	0.39	0.45	0.38	0.39 (0.40)
gain/week	(Kg)	0.5 (0.6)	0.7	0.61	0.67	0.62 (0.65)
feed offered	(gm)	34,860	40,915	30,835	39,690	36,575
feed refused	(gm)	nil	nil	nil	nil	nil
feed consumed	(gm)	34,860	40,915	30,835	39,690	36,575
feed consumed/week	(gm)	3,873	4,546	3,426	4,410	4,068
gm feed/gm gain		7.75 (6.46)	6.49	5.62	6.58	6.61 (6.29)
gm feed/gm gain/Kg wt		0.57 (0.40)	0.40	0.46	0.42	0.46 (0.42)
gm feed/gm gain/$W^{0.73}$		1.15 (1.12)	0.84	0.91	0.88	0.95 (0.94)

*Dog #26 was sick during the last week of the experimental period and, as a result, lost weight. If he had gained normally then we would expect those figures which appear in brackets.

C^{400} Individual Animals

		#11	#19	#22	#17	Mean
final weight	(Kg)	24.5	16.4	16.8	18.2	19.0
initial weight	(Kg)	15.5	10.5	10.9	10.9	12.0
weight gain	(Kg)	9.0	5.9	5.9	7.3	7.0
gain/Kg body weight	(Kg)	0.45	0.44	0.43	0.50	0.46
gain/week	(Kg)	1.00	0.66	0.66	0.81	0.78
feed offered	(gm)	50,330	33,110	35,805	36,611	38,964
feed refused	(gm)	nil	65	850	415	333
feed consumed	(gm)	50,330	33,045	34,955	36,196	38,632
feed consumed/week	(gm)	5,592	3,672	3,884	4,022	4,293
gm feed/gm gain		5.59	5.50	5.92	4.96	5.49
gm feed/gm gain/Kg wt		0.28	0.41	0.43	0.34	0.37
gm feed/gm gain/$W^{0.73}$		0.63	0.82	0.87	0.70	0.76

The third consideration concerns the presence of intestinal parasites in the experimental dogs. During the course of the project definite signs of ascariasis appeared, but no corrective measures were taken for fear of confounding the experimental design. Post mortem of those dogs killed after nine weeks revealed a very heavy infestation of ascarids (Toxocara canis) and whipworms (Trichuris vulpis). This intestinal infestation, coupled with the physical difficulties of ingesting feed containing high levels of the ethers, probably accounts for the feed refusals noted.

The experimental results show large variations in the individual response to the treatments. This makes it necessary to consider each of the animals separately. Feed intake and body weight gain relationships have been calculated for each dog to facilitate consideration of the findings (see Table XXIII for a summary of the results).

A comparison of the results from the S^{100} group with those from the control animals shows a significantly slower growth rate and a lower feed efficiency. The difference in the energy level of the two rations may be enough to account for the difference in growth rate between the groups. The C^{400} ration had 5.8 percent safflower oil mixed in the basic dog ration, while the S^{100} ration had only 1.45 percent selachyl alcohol. Looking closer at the individual results from the animals in the S^{100} group it becomes apparent that dog #26 had a significantly slower growth rate than the other three animals in the group. This can be explained by examination of the growth data presented in Appendix V. It may be noted that dog #26 lost one-half a kilogram of body weight during the last week of the experiment

while the other dogs of the group gained from 0.5 to 0.9 kilograms. It is suggested that the parasite infestation in this animal (#26) had reached a level that interfered with feed utilization and hence growth rate.

A close examination of the individual results of the animals in the B^{400} group shows that two of the animals responded in a manner that is quite different from the other pair. Dogs #20 and #12 refused to eat all of the feed that they were offered and as a result lost some weight during the two month trial. The other two dogs (#34 and #14) in this group gained slowly and hence, because they consumed their allotted amount of feed, yield a feed efficiency ratio that is approximately one-half that of the animals from the control group. This suggests that the batyl alcohol when fed at a level of 2400 mgs per kilogram of body weight interfered with the digestion of the feed. Evidence for this interference with digestion will be found in the data on glyceryl ether digestibility (see Tables XXVII and XXVIII). This aspect will be elaborated upon later.

Group S^{400} may be similarly divided; dogs #25 and #23 ate all or nearly all the feed that was offered to them. The feed conversion ratios for these two animals were 14.9 / 1 and 12.6 / 1, or about one-half of that obtained for the control group.

The large variation in feed efficiency between the control animals and certain of the animals in the experimental groups (see Table XXIV) can probably be explained on the basis of the effect of the ethers on the digestibility of the various experimental rations. The extremely low feed conversion efficiencies for the second and third dog in the S^{400} group and the negative values for the first and fourth dogs in the B^{400} group is

TABLE XXIV: SUMMARY OF FEED EFFICIENCIES

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	14.9	$-\infty$	6.46	5.59
2	20.2	13.2	6.49	5.50
3	58.8	10.8	5.62	5.92
4	12.6	$-\infty$	6.58	4.96

inevitable since they refused to eat all the feed that was offered to them. This led to very low or negative weight gains, hence the feed utilized per unit of weight gained becomes infinitely large. Dogs #1 and #4 in the S^{400} group and dogs #2 and #3 in the B^{400} group ate according to the same feeding schedule as the control animals yet their gain fell well below that of the controls. The following model shows how a small variation in digestibility can markedly change feed conversion efficiency.

MODEL

In this model both body weight and feed intake are held constant, while the digestibility of the ration is varied from 48 to 66 percent. This model could be a typical animal from the control group in this experiment. In this model the following assumptions are made:

body weight ———— 15 kilograms
feed-intake/day ———— 620 grams
gain/day ———— 110 grams
energy content of gain ———— 3 kcal/gram
metabolizable energy of ration ——— 3 kcal/gram

If we accept a daily intake of 1860 kcal of metabolizable energy when the digestibility of the feed is 60 percent, it is possible to compute the values given in the following table:

TABLE XXV: THE RELATIONSHIP BETWEEN DIGESTIBILITY AND FEED EFFICIENCIES FOR A MODEL DOG

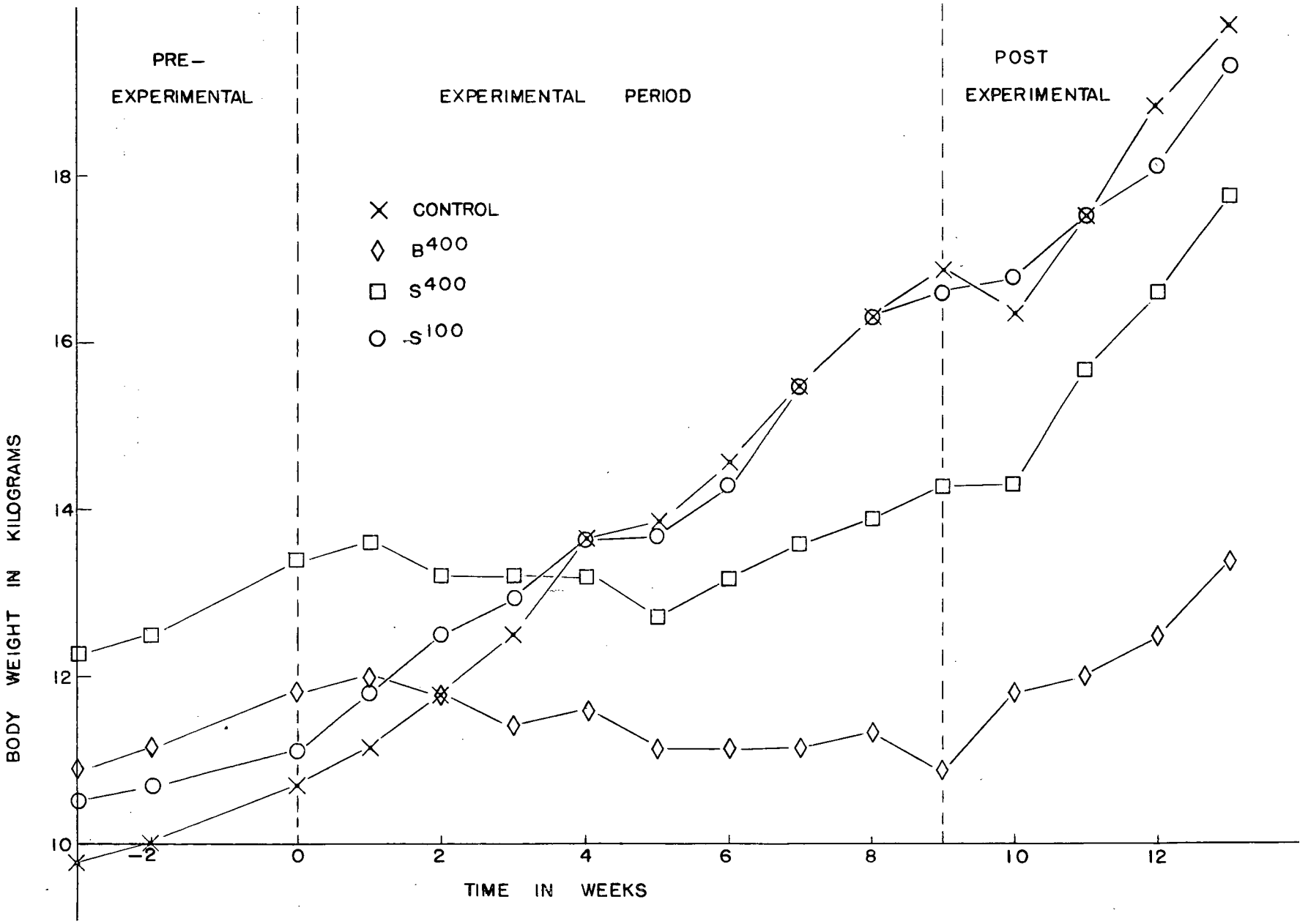
% Digestibility	Metabolizable energy kcal	Energy content of gain kcal	Grams of gain	Grams feed gram gain
66	2,046	516	172	3.6
64	1,984	454	151	4.1
62	1,922	392	131	4.7
60	1,860	330	110	5.6
58	1,798	268	89	7.0
56	1,736	206	69	9.0
54	1,674	144	48	12.9
52	1,612	82	27	22.9
50	1,550	20	6.7	92.5
48	1,488	- 42	negative gain	

These calculations show that if the digestibility of the ration falls from 60 to 54 percent, the feed efficiency ratio changes from 5.6 to 12.9 grams of feed per gram of gain. Thus a small change in the digestibility of the ration results in a relatively large change in the feed efficiency ratio.

At the end of the experimental period two of the four dogs in each group were slaughtered as outlined earlier. The dogs to be held over were selected at random from the C^{400} and S^{100} groups, but in the B^{400} and S^{400} groups, the dogs that had been refusing feed were selected. These eight dogs were all fed the control ration at a rate of 80 percent of their calculated maximum intake. The finding of the heavy infestation of

FIGURE II:

THE MEAN GROWTH RATES OF THE TWO DOGS FROM EACH GROUP MAINTAINED ON THE CONTROL RATION FOLLOWING REMOVAL FROM THEIR EXPERIMENTAL DIETS


PRE-
EXPERIMENTAL
EXPERIMENTAL PERIOD
POST
EXPERIMENTAL
CONTROL
B400
S400
S100
BODY WEIGHT IN KILOGRAMS
TIME IN WEEKS
10
12
14
16
18
-2
0
2
4
6
8
10
12

intestinal parasites in the dogs that were killed dictated that the remaining dogs all be treated for worms. A response to the worm treatment appeared in the control group. The B^{400} and S^{400} dogs gave a response both to the worming and to the control ration. They grew at or near the same rate as the C^{400} and S^{100} dogs. From this response it seems logical to conclude that the experimental treatments had no lasting effects on the treated animals. All of the experimental dogs so treated, including the controls, showed an immediate response by gaining weight at a higher rate than before the treatment. This response suggests (1) that all of the dogs, including the controls, were suffering from the worm infestation to a variable extent that impaired growth rate, and (2) that the treated dogs had apparently suffered no lasting effects from the alcohols since they responded immediately when placed on the control ration. Figure II presents the average growth curve from 4 weeks prior to the experimental period to 4 weeks after for the two dogs held over from each group. The figure shows the response to worming in all the groups as well as the response of the B^{400} and S^{400} dogs to the control ration.

Table XXVI explores the feed intake-gain relationships for the experimental groups of rats. No significant differences are revealed. The fact that the S^{100} ration had a slightly lower feed efficiency than the control can be explained on the basis of the energy level of the ration. The physical nature of the α-glyceryl ethers did not appear to have any effect on the feed consumption of the rats. It should be pointed out, however, that the ration offered to the dogs contained almost twice as much of the ethers per unit weight as that offered to the rats.

TABLE XXVI: FEED INTAKE-GAIN RELATIONSHIPS* FOR THE RATS IN TRIAL II

		S^{400}	B^{400}	S^{100}	C^{400}
final weight	(gm)	216.1	227.8	212.6	218.1
initial weight	(gm)	79.6	79.7	83.6	80.7
total gain	(gm)	136.5	148.1	129.0	137.4
gain/gm of weight	(gm)	.92	.96	.87	.92
gain/day	(gm)	2.24	2.43	2.11	2.25
feed offered	(gm)	825	827	822	824
feed refused	(gm)	30	14	8	19
feed consumed	(gm)	795	813	814	805
feed consumed/day	(gm)	13.0	13.3	13.3	13.2
gm feed/gm gain		5.82	5.58	6.31	5.86
gm feed/gm gain/Kg wt		39.3	36.2	42.6	39.3
gm feed/gm gain/$W^{0.73}$		24.9	22.5	28.2	24.9

*These figures are the mean for 10 animals.

8. Glyceryl Ethers and Digestibility

Faeces samples taken two weeks and six weeks after the beginning of the trial were anlyzed for the presence of glyceryl ethers. Using the average feed intake for the week prior to the sampling and the digestibility values arrived at by the chromic oxide method in the dog and by total collection in the rat, the digestibility of the glyceryl ethers was determined. In these determinations the dogs in the S^{400} and B^{400} groups were separated into those which ate according to the feeding standard and those that voluntarily restricted their feed intake. The results are summarized in Table XXVII. A similar summary for the rats is given in Table XXVIII. The values for the digestibilities of the glyceryl ethers in both the dogs and the rats are presented in the form of a bar graph in Figure III. The rats digested the glyceryl ethers more completely than did the dogs. Batyl

alcohol was less digestible than selachyl in both the dogs and the rats. In the rats selachyl alcohol fed at the level of 2.4 grams per kilogram of body weight was digested equally as well as was selachyl fed at the 0.6 grams per kilo of body weight level, whereas in the dogs there was a marked difference in the digestibility of selachyl alcohol between those two dosage levels. This is quite reasonable since the percent of the glyceryl ethers in the S^{400} dog ration was 5.8 while the S^{400} rat ration was in the range of 1.7 to 3.5 percent.

The dogs eating to the feeding standard digested a smaller portion of the glyceryl ethers ingested than did the dogs which voluntarily restricted their own feed intake. This, then, is a partial explanation why the digestibility of the S^{400} and B^{400} rations for the dogs must be lower than for the $S^{100}$and control. It is reasonable to assume that the glyceryl ethers coating the feed particles prevented enzymatic action on some of the feed in the digestive tract and the digestibility of the whole ration was lowered to a point where the S^{400} and B^{400} dogs ate the same amount of feed as the S^{100} and the control groups, but demonstrated little or no gain. A change in digestibility of 6 to 10 percent from the normal is enough to prevent body weight gain.

One point of note is the finding of glyceryl ethers in small quantities in both the control feed and the faeces of the control animals. The total amount in the faeces was greater than that found in the control feed, thus it seems tenable that synthesis of glyceryl ethers occurred in the intestine or that they were secreted into the intestine from the tissues. The presence of glyceryl ethers in the control ration is not unexpected since the ration contained 10 percent herring meal.

TABLE XXVII: RECOVERY OF GLYCERYL ETHERS FROM FAECES AND DETERMINATION OF DIGESTIBILITY OF THE ETHERS FOR DOGS IN TRIAL II

Group	Feed/Day/Dog			% of	Faeces/Dog/Day			G.E.	%	Dog	G.E.
	Dry wt (Grams)	% G.E.	G.E. Wt (Grams)	Feed Digested	Dry Wt** (Grams)	% G.E.*	G.E. Wt (Grams)	Absorbed (Grams)	G.E. Digested	Weight (Kg)	Absorbed mgm/Kg/day
				Samples taken two weeks from the beginning of the trial							
C^{400}	481	0.048	0.231	56.5	209.2	0.13	-	-	-	13.3	-
B^{400} a	413	5.75	23.74	55.5	183.8	6.41	11.78	11.96	50.4	11.4	1.05
B^{400} b	270	5.75	15.52	54.1	123.9	4.75	5.89	9.63	62.1	11.8	0.82
S^{400} c	364	5.80	21.11	55.4	162.3	7.87	12.77	8.34	39.5	10.9	0.77
S^{400} d	286	5.80	16.59	55.4	127.6	3.59	4.58	12.01	72.4	13.2	0.91
S^{100}	465	1.33	6.18	59.2	189.7	0.27	0.51	5.67	91.7	13.0	0.44
				Samples taken six weeks from the beginning of the trial							
C^{400}	598	0.048	0.287	55.1	268.5	0.126	0.34	-	-	16.5	-
B^{400} a	437	5.75	25.13	42.0	253.5	6.05	15.34	9.79	39.0	11.8	0.83
B^{400} b	271	5.75	15.58	56.9	116.8	4.43	5.17	10.41	66.8	11.1	0.94
S^{400} c	423	5.80	24.53	54.4	192.9	6.29	12.13	12.40	50.5	11.4	1.09
S^{400} d	361	5.80	20.94	57.8	152.3	4.52	6.88	14.05	67.1	13.2	1.06
S^{100}	550	1.33	7.32	60.4	217.8	0.39	0.85	6.47	88.4	15.1	0.43

a dogs #14 and #34
b dogs #12 and #20
c dogs #23 and #25
d dogs #9 and #10

Note: The feed per day data is calculated from the feed consumed for the week prior to the faeces sampling. The % G.E. (glyceryl ethers) in the feed for all the groups has 0.05 subtracted from the experimental values and the % G.E. in the faeces has 0.13 subtracted from the experimental values. (This has been done to correct for the glyceryl ethers found in the control ration.)

* As determined in the feed by a modification of the method of Bligh and Dyer (13).

** As computed from ration digestibility using a chromic oxide method (93).

TABLE XXVIII: RECOVERY OF GLYCERYL ETHERS FROM FAECES AND DETERMINATION OF DIGESTIBILITY OF THE ETHERS FOR THE RATS IN TRIAL II

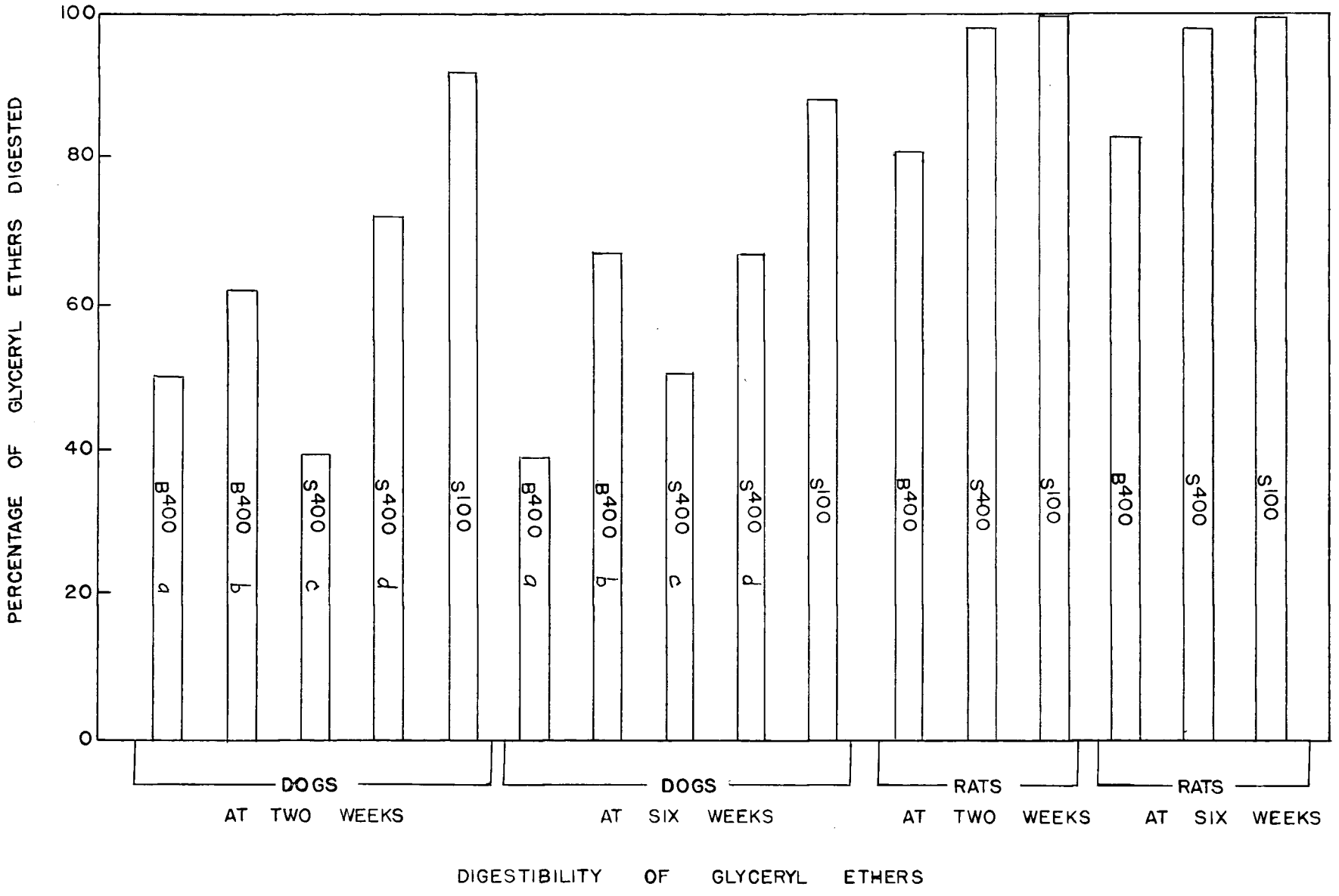
Group	Feed/Day/Group			% of* Feed Digested	Faeces/Day/Group			G.E. Absorbed (Grams)	% G.E. Digested	Group Wt (Kg)	G.E. Absorbed mgm/Kg
	Dry wt (Grams)	% G.E.	G.E. Wt (Grams)		Dry Wt (Grams)	% G.E.	G.E. Wt (Grams)				
	Sample taken two weeks from the beginning of the trial										
C^{400}	105.3	0.048	0.05	71.5	30.1	0.26	0.078	-	-	1.176	-
B^{400}	104.4	2.27	2.37	72.2	29.1	1.55	0.450	1.92	81.0	1.181	1.63
S^{400}	105.3	2.29	2.41	72.1	29.4	0.15	0.044	2.37	98.2	1.196	1.98
S^{100}	105.3	0.50	0.53	72.2	29.3	nil	nil	0.53	100.0	1.189	0.44
	Sample taken six weeks from the beginning of the trial										
C^{400}	128.5	0.048	0.06	72.3	35.6	0.19	0.068	-	-	1.841	-
B^{400}	120.1	2.85	3.42	72.7	32.8	1.78	0.58	2.84	83.0	1.898	1.495
S^{400}	127.6	2.87	3.66	75.4	31.4	0.27	0.085	3.58	97.8	1.856	1.93
S^{100}	128.3	0.64	0.82	70.9	37.3	0.01	0.004	0.82	99.5	1.798	0.454

Note: The feed per day data is calculated from the feed consumed for the week prior to the faeces sampling. The % G.E. (glyceryl ethers) in the feed has 0.05 subtracted from all the experimental values; % G.E. of the faeces taken two weeks from the beginning of the trial has 0.26 subtracted from all the experimental values, and % G.E. of the faeces samples taken six weeks from the beginning of the trial has 0.19 subtracted. (These corrections are made because of the glyceryl ethers found in the control feed and faeces.)

* Digestibility by total collection

FIGURE III:

DIGESTIBILITY OF GLYCERYL ETHERS


PERCENTAGE OF GLYCERYL ETHERS DIGESTED
100
80
60
40
20
0
B400 a
B400 b
S400 c
S400 d
S100
B400 a
B400 b
S400 c
S400 d
S100
B400
S400
S100
B400
S400
S100
DOGS
AT TWO WEEKS
DOGS
AT SIX WEEKS
RATS
AT TWO WEEKS
RATS
AT SIX WEEKS
DIGESTIBILITY OF GLYCERYL ETHERS

9. Results from cell counts

The following table lists the significant differences found in the cell counts derived in this trial. They include erythrocyte, leucocyte and reticulocyte counts of blood, a differential of leucocytes in the blood and a differential of bone marrow cells. All of these counts were made after one month of treatment and again at the end of two months with the exception of the bone marrow differentiation which was done when the animals were killed.

TABLE XXIX: SIGNIFICANT (0.05 LEVEL) DIFFERENCES IN CELL COUNTS

Group	Sex	Species	Blood or Bone Marrow Cells	Higher or Lower Counts
			Counts Taken After One Month	
S^{400}	female	rats	leucocyte counts	lower
S^{400}		dogs	reticulocyte count	higher
			Counts Taken After Two Months	
S^{100}	female	rats	erytrocytes	higher
S^{400}	female	rats	leucocytes	lower
B^{400}	female	rats	reticulocytes	higher
S^{400}		dogs	reticulocytes	higher
B^{400}		dogs	reticulocytes	higher
S^{100}		dogs	reticulocytes	higher
B^{400}	male	rats	polymorphs	lower
S^{100}	male	rats	polymorphs	lower
B^{400}	male	rats	lymphocytes	higher
S^{100}	male	rats	lymphocytes	higher
B^{400}	female	rats	lymphocytes	higher
S^{100}	female	rats	lymphocytes	higher
			Differential of Rat Bone Marrow	
S^{100}	female		promyelocytes	higher
S^{400}	male		myelocytes	lower
S^{100}	male		myelocytes	lower
S^{400}	male		metamyelocytes and 'ring cells'	lower
B^{400}	male		mature granulocytes	lower
S^{400}	female		mature granulocytes	lower

TABLE XXIX (cont'd)

Group	Sex	Species	Blood or Bone Marrow Cells	Higher or Lower Counts
			Differential of Rat Bone Marrow	
B^{400}	female		mature granulocytes	lower
S^{100}	female		mature granulocytes	lower
S^{400}	male		nucleated red blood cells	higher
B^{400}	male		nucleated red blood cells	higher
S^{100}	male		nucleated red blood cells	higher
S^{400}	female		nucleated red blood cells	higher
B^{400}	female		nucleated red blood cells	higher
			Differential of Dog Bone Marrow	
			No significant differences were found in the bone marrow smears of the dogs	

Although some staggered significant differences occur throughout all of the blood counts of the rats, there appears a definite trend only in the case of lymphocytes. Of the three groups of treated rats, male and female, only the S^{100} females show a significantly higher erythrocyte count over the control. Similarly, only the S^{400} females show a significantly different leucocyte count, this count being lower. Only the B^{400} females showed a significantly higher reticulocyte count. Two groups of male rats (B^{400} and S^{400}) showed a significantly lower percentage of polymorphs. In four cases the lymphocyte percentage of the white blood cells was significantly higher; B^{400} and S^{100} groups (male and female). All other categories of leucocytes in the treated animals did not differ from the control.

The dogs showed no significant differences in blood counts or differentials except in the case of reticulocytes where all the treated dogs had significantly higher counts of reticulocytes than had the control animals.

The differential counts of the rat bone marrow shows only two definite trends, the one may be the result of the other. Mature granulocytes are significantly lower in the B^{400} males and the three treated groups of females, while nucleated red blood cells are significantly higher in all the groups of male and female rats except the S^{100} female rats.

No significant differences were found in the differential of cells in the bone marrow smears of the dogs.

The haematopoietic response, then, to the α-glyceryl ethers, administered at extremely high levels, is relatively insignificant, except in the case of the rats, where nucleated red blood cells in the bone marrow are significantly higher and in the case of the dogs, where reticulocytes in the blood are significantly higher. This stimulation of the immature red blood cells is not reflected in an increase in erythrocyte numbers. This suggests that either the life of the immature erythrocyte is extended or the life of the mature red blood cell is shortened.

C. FEEDING RESPONSE EXPERIMENT - TRIAL III

1. Preamble

The analysis of the control feed used in the preliminary trials revealed an amount of glyceryl ethers sufficient to elicit a response if glyceryl ethers possess vitamin-like properties. Hence it is possible that the addition of glyceryl ethers to this ration (Trials I and II) could produce little or no positive response in the experimental animals. The hypothesis that the addition of glyceryl ethers to a glyceryl ether free ration would show a response is tested in this third trial. Batyl alcohol was the glyceryl ether of choice in this feeding response experiment since it was readily available and appears to have been more active than selachyl in alleviating certain conditions of stress (Prokhonchukov and Panikarovskii, 1963). A level of 5 mg per kilogram of body weight was chosen as an appropriate dosage. This was considered an adequate dosage level since it is similar to recommended levels of vitamins as discussed in the preamble to Trial I. Two more dosage levels were chosen: one which was one tenth (0.5 mg) of the 5 mg per kilogram of body weight level and another which was ten times as great (50 mg).

2. Design

Forty female rats were placed at random into four groups of ten; a control group and three groups receiving 0.5, 5, and 50 mg of batyl alcohol per kilogram of body weight per day. Batyl alcohol was incorporated into the feed to facilitate administration. The experimental period was 5 weeks. Cheeke (24) has suggested that the apparent digestible energy

intake of the albino laboratory rat over the body weight range, 50 to 175 grams, be predicted from the following relationship:

$$ADE = 6.82\ W_{gm}^{0.451}$$

This feeding standard was accepted as valid for this experiment and the animals were fed 80 percent of this theoretical maximum intake.

All animals were weighed three times each week. Representative samples of feed and faeces were taken three times during the five week experimental period. During the fifth week blood samples were taken from all the animals; red blood cell counts and white cell counts were made, smears were made for reticulocyte counts and leucocyte differentiation. At the end of the five week period all the animals were sacrificed, organs were inspected, and femur bone marrow smears were made.

3. Animals

Albino Sprague-Dawley (SPF) female rats were used as experimental animals. They were placed on the experiment at a weight of approximately 60 grams. The actual weights are given in Appendix III.

4. Housing

The rats were housed in individual wire cages. Feed and water were supplied in porcelain jars.

5. Ration

A glyceryl ether free ration was made up as follows:

sucrose	56	percent
casein	25	"
safflower oil	10	"
mineral mix	4	"
vitamin mix	2	"
alphacel	3	"

To balance this ration with respect to minerals and amino acids the following additions were made:

11.7 mg $CuSO_4$ per kilogram of basic ration
13.8 mg $ZnCL_2$ per kilogram of basic ration
773 mg cystine per kilogram of basic ration

The ingredients used in this synthetic ration were shown to be free of glyceryl ethers by analysis. The sucrose, casein, mineral mix and alphacel were all free of lipid material and therefore were free of glyceryl ethers. Analysis of the vitamin mix and the safflower oil revealed no trace of glyceryl ethers.

This basic ration was fed to all the rats with the appropriate amounts of batyl alcohol added to the ration for each of the treated groups.

6. Feed intake-gain relationships

Table XXX summarizes the feed intake-gain relationships for the rats used in this trial. No differences were found in the rate of gain or feed efficiencies between the groups. The response of the batyl alcohol treated groups was essentially the same as the control animals on the glyceryl ether free diet.

TABLE XXX: FEED INTAKE-GAIN RELATIONSHIPS* FOR RATS IN TRIAL III

		Control	B - 0.5	B - 5	B - 50
final weight	(gm)	169.3	170.4	169.5	167.8
initial weight	(gm)	57.5	57.3	57.6	56.3
total gain	(gm)	111.8	113.1	111.9	111.4
gain/gm weight**	(gm)	0.99	0.99	0.99	0.99
gain/day	(gm)	3.4	3.4	3.4	3.4
feed offered	(gm)	371.8	370.3	370.9	369.5
feed refused	(gm)	18.3	18.1	20.4	20.3
feed consumed	(gm)	353.5	352.2	350.5	349.2
feed consumed/day		10.7	10.7	10.6	10.6
gram feed/gram gain		3.16	3.11	3.13	3.13

*all figures are the mean of ten rats
**this weight is the mean of the initial and final weights for each group

7. Digestibility

The digestibility values in Table XXXI are based on total faecal collections taken over 72 and 96 hours. They show no significant differences between groups. The extremely small amount of faeces excreted by these animals is a result of the highly digestible nature of the synthetic experimental ration used in this trial.

TABLE XXXI: DIGESTIBILITY OF EXPERIMENTAL RATIONS IN TRIAL III

Group	Control	B - 0.5	B - 5	B - 50
2 weeks (72 hr)	93.4	93.9	94.5	94.9
3 weeks (96 hr)	93.4	93.2	93.5	93.7
4 weeks (72 hr)	93.4	94.2	93.6	93.6

8. Results of blood and bone marrow cell counts

These results are listed in Appendices XIII, XIV and XV and are all summarized in Table XXXII. Analysis of these results failed to show any significant differences between the experimental groups and the control. Large variations existed between individual animals.

Arbitrary categories of bone marrow cells were made to facilitate differentiation. The slides were poorly stained so that the classical cell types were not always recognizable. The categories chosen showed no significant differences between groups.

9. Conclusion

Batyl alcohol added to a glyceryl ether-free synthetic ration and fed for 5 weeks did not appear to affect the rate of body growth or the production of blood cells in normal growing female albino rats.

There was, however, some sign of erythrocyte stimulation in four of the control rats that were fed the B - 50 ration for 4 days after the end of the experiment. Table XXXIII gives the erythrocyte counts. A "t" test indicates a significant increase in the number of erythrocytes. Thus, this limited experimental diversion suggests further nutritional trials where glyceryl ether-deprived rats or other experimental animals are administered glyceryl ethers.

That batyl alcohol stimulates haemopoiesis in normal rats, is suggested in experimental work done by Linman, et al, 1959. The dosage level used was 50 mg/rat/day. This would equal approximately 300 mg per kilogram of body weight or 6 times the highest level used by the author

TABLE XXXII: A SUMMARY* OF THE BLOOD AND BONE MARROW DATA FROM TRIAL III

Group		Control	B - 0.5	B - 5	B - 50
Erythrocytes (millions per cmm)		7.60	7.26	7.44	7.60
Leucocytes (cells per cmm)		11,200	12,800	12,400	12,400
Differential of Leucocytes (percentage of leucocytes)					
Polymorphs		14.4	17.1	12.5	15.2
Eosinophils		1.5	0.6	0.6	1.1
Lymphocytes		81.6	81.0	85.4	81.2
Monocytes		2.2	2.1	2.0	2.2
Differential of bone marrow cell (percentage of cells)					
Category**	1	4.84	5.02	5.48	3.66
	2	5.86	4.39	5.85	5.24
	3	15.32	13.22	14.33	16.47
	4	8.56	11.25	9.12	8.58
	5	19.52	17.15	17.13	14.94
	6	34.30	34.67	34.36	36.16
	7	11.12	12.60	12.46	13.01
	8	0.20	0.63	0.41	0.39

*These are averages of ten animals except in the case of the differential of the bone marrow of the control animals where the numbers are averages of five animals.

**The eight categories of bone marrow cells are explained in Appendix XV.

TABLE XXXIII: ERYTHROCYTE COUNTS (MILLIONS PER CMM) OF FOUR CONTROL RATS FED BATYL ALCOHOL AT THE END OF THE EXPERIMENTAL PERIOD

Rat No.	End of Experiment	Four Days on Feed
7	6.50	8.08
8	7.01	9.80
9	7.93	7.83
10	7.43	8.56
Average	7.60	8.57

in Trial III. Linman does not mention the age of the rats nor the ration that he fed. The batyl alcohol used was a racemic synthetic material and not the optically active natural form used in this study. The form of the batyl alcohol may have some effect on the activity of the compound, since dosage levels as high as 2400 mg per kilogram of body weight were administered to animals in this project, without causing a significant haemopoietic effect. This apparent inconsistency between the experimental results obtained by Linman and those obtained by the author suggest a comparative study of the synthetic form with the natural form.

PART III:

GENERAL DISCUSSION

GENERAL DISCUSSION

The main conclusions to be drawn from these experiments are summarized below:

1. Dogs and rats fed chimyl, selachyl and batyl alcohols at levels of 6, 600 and 2400 mg per kilogram of body weight did not show any signs of intoxication.

2. Except in the case of female rats receiving selachyl alcohol at the 6 mg level for a period of six months, there was no difference in the growth rate between experimental and control animals.

3. Chimyl, selachyl and batyl alcohols administered orally at a level of 6 mg per kilogram of body weight to dogs and rats for six months failed to initiate any definite haematopoietic response.

4. Selachyl alcohol administered at levels of 600 and 2400 mg per kilogram of body weight and batyl alcohol administered at a level of 2400 mg per kilogram of body weight for a period of two months produced relatively insignificant haematopoietic responses in both the dogs and the rats. The only indications of haematopoietic stimulus were a reticulocyte response in the blood of the dogs and a response in the percentage of nucleated red blood cells in the bone marrow of the rats.

5. Batyl alcohol added to a glyceryl ether-free synthetic ration and fed for a five week period did not appear to affect the growth rate or the production of blood cells in normal growing female albino rats.

Throughout this whole series of experiments no sign of toxicity appeared in the experimental animals. Gross pathology, growth rate and histopathology of tissue sections from the heart, liver, lungs, spleen, kidneys, adrenals and intestine showed no indication of an abnormal condition.

Any picture of the response to the administration of natural occurring α-glyceryl ethers is obscured by the inconsistencies of the results. There appears to be no growth response due to the administration of glyceryl ethers, except in the case of growing female rats given a low dosage of selachyl alcohol. These rats have a growth curve identical with that of the other groups of female rats to a mean body weight of about 200 grams. Above this body weight, the selachyl females grew at a slightly faster rate to a final weight which was significantly higher than that of the other females. The haematopoietic responses were also inconsistent. For example, in the low dosage group of selachyl-fed rats, at 90 days the females had significantly higher leucocyte counts, while the males had significantly lower leucocyte counts and at 180 days no differences were measured between either the female or male and the control animals.

There appeared to be some stimulation in erythrocyte production when selachyl alcohol was fed at 600 and 2400 mg per kilogram of body weight and batyl alcohol at 2400 mg per kilogram of body weight. The increases were restricted mainly to reticulocytes in the blood of the dogs and to nucleated red blood cells in the bone marrow of the rats. This increase in immature forms of erythrocytes was not accompanied by an overall increase in the red blood cell count. Thus it would appear that

the normal sequence of events in the life of an erythrocyte was altered. This may mean that the immature period is lengthened and that the mature period of a red blood cell is decreased. This suggests that the average red blood cell is more youthful and more capable of fulfilling its role as an erythrocyte, or that the mature erythrocyte is more fragile and is removed from circulation sooner.

The short experimental trial using a glyceryl ether-free synthetic ration showed no change in erythrocyte numbers over a five week period. However, four of the control rats that were fed a glyceryl ether-free ration received batyl alcohol in their feed for four days after the termination of the experiment and they showed an encouraging erythropoietic response. This isolated response is not significant by itself because of the small number of animals involved and because of the lack of control animals, but it does serve as an indication that another experiment could be done in which rats are depleted of glyceryl ethers for varying lengths of time and measure the response to administered glyceryl ethers. If blood cell counts do not decrease when the animals are fed a glyceryl ether-free ration, yet increase over normal values when glyceryl ethers are administered to glyceryl ether-depleted rats, then the problem becomes of interest to the biochemist and the physiologist as well as to the nutritionist.

In any comparison of the experimental results of this project with those reported in the literature, optical isomerism becomes an important factor. The natural occurring α-glyceryl ethers are of the d-configuration. Much of the experimental work reported in the

literature makes use of synthetic racemic mixtures. From our knowledge of enzymes we know that they are most commonly enantiomer specific. This means that we might expect an entirely different physiological response from a racemic mixture than we would from either emantiomer. An experiment specifically designed to compare pure l- and d-forms of α-glyceryl ethers is indicated here.

Since it is now established that the naturally occurring α-glyceryl ethers are not toxic and since the response is variable and inconsistent in the rat and the dog, it may be worth while to use other species as experimental subjects. The dog and the rat were used primarily as models of mammals in general; it may well be that we should use the guinea pig or cat or some other animal to establish the importance of glyceryl ethers. It may even be expedient to experiment with sheep or cattle or even man with respect to the glyceryl ethers as nutritional entities.

This thesis has established the non-toxic nature of the natural occurring α-glyceryl ethers and has indicated the direction in which further research might proceed.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abaturova, E.A., and A.V. Shubina. Ovliyonii batilovogo i selakhovogo spirtov na rost zlokachestvennykh opukholei u krys (Effect of batyl and selachyl alcohols on the growth of malignant tumors in rats). Buyl Eksp Biol. Med. 57(6): 81-83, 1964. Biol. Absts. Feb. 15, 1965, 16020.

2. Alexander, P., D.I. Connell, A. Brohult and S. Brohult. Reduction of radiation induced shortening of life span by diet augmented with alkoxy glyceryl esters and fatty acids. Gerontologia 3:147-152, 1959.

3. Ansell, G.B. and S. Spanner. The probable occurrence of long-chain ether analoques of phosphatidylethanolamine in brain tissue. Biochem. J. 81:36P-37P, 1961.

4. Arturson, G. and M. Lindback. Experiments on the effect of batyl alcohol on the number of erythrocytes and reticulocytes in white mice. Acta Soc. Med. upsalien. 56:19-26, 1951. Nutr. Absts. Rev. 21(2):374, 1951.

5. Ashikawa, J.K. The lipid syndrome in mammalian radiobiology with emphasis on postirradiation protection of animals with lipids. Doctorate Thesis, University of California, 1961.

6. Baer, E. and H.O.L. Fischer. Synthesis of optically active glycerides from d(+)-acetone glyceride. J. Biol. Chem. 128:475-489, 1939.

7. Baer, E. and H.O.L. Fischer. Configuration of the natural batyl, chimyl, and selachyl alcohols. J. Biol. Chem. 140:397-410, 1941.

8. Baer, E. and H.O.L. Fischer. Naturally occurring glyceryl ethers. III. Selachyl alcohol and its geometrical isomer. J. Biol. Chem. 170:337-342, 1947.

9. Baer, E., L.J. Rubin and H.O.L. Fischer. Naturally occurring glyceryl ethers. II. Synthesis of selachyl alcohol. J. Biol. Chem. 155:447, 1944.

10. Bergmann, W. and H.A. Stansbury. Contributing to the study of marine products. XIV. Astrol. J. Org. Chem. 8:283-284, 1943.

11. Bergmann, et al. Marine Products. XL. Waxes and triglycerides of sea anemones. J. Org. Chem. 21:721-728, 1956.

12. Bergström, S. and R. Blomstrand. The intestinal absorption and metabolism of chimyl alcohol in the rat. Acta Physiol. Scand. 38:166, 1956.

13. Bligh, E.G. and W.J. Dyer. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911, 1959.

14. Blomstrand, R. and E.H. Ahrens, Jr. Absorption of chimyl alcohol in man. Soc. Exp. Biol. Med. Proc. 100:802-805, 1959.

15. Blomstrand, R. Digestion, absorption and metabolism of chimyl alcohol fed as free alcohol or as alkoxydiglyceride. Soc. Exp. Biol. Med. Proc. 102:662-665, 1959.

16. Bodman, J. and J.H. Maisin. The alpha-glyceryl ethers. Clin. Chem. Acta 3:253-274, 1958.

17. Brohult, A. Alkoxy-glycerol esters in irradiation treatment. International conference on radiobiology. Proc. 5th conference, Stockholm 1956: 241-247, 1957.

18. Brohult, A. Effects of alkoxyglycerols and especially selachyl alcohol on the bone marrow in connection with irradiation treatment and in leukaemia therapy. Nature 181:1485, 1958.

19. Brohult, A. Alkoxyglycerols as growth-stimulating substances. Nature 188:591-592, 1960.

20. Brohult, A. Alkoxyglycerols in irradiation treatment. Nature 193:1304, 1962.

21. Brohult, A. Alkoxyglycerols and their use in radiation treatment. Acta Radiol. Supp. 223:1-99, Stockholm, 1963.

22. Brohult, A. and J. Holmberg. Alkoxyglycerols in the treatment of leukopaenia caused by irradiation. Nature 174:1102, 1954.

23. Carter, H.E., D.B. Smith and D.N. Jones. A new ethanolamine-containing lipid from egg yolk. J. Biol. Chem. 232:681-694, 1958.

24. Cheeke, P.R. The changes of the serum protein-bound iodine during growth in the Holstein calf and the Wistar albino rat. Masterate Thesis, University of British Columbia, 1965.

25. Dalton, R.G. The effect of batyl alcohol on the hematology of cattle poisoned with bracken. Vet. Rec. 76(15):411-416, 1964.

26. Davies, W.H., I.M. Heilbron and W.E. Jones. The structure of batyl and selachyl alcohols. J. Chem. Soc. 1933: 165-167.

27. de Gaetani, G.F. and G. Baiotti. Hemopoietic activity of batyl alcohol. Boll. soc. ital. biol. sper. 35:1156, 1959. Chem. Absts. 55:16815a, 1961.

28. Deuel, H.J., Jr. The lipids. Interscience Publishers, New York, 1951. Vol. I.

29. Dudin, V.N. Profilaktika i lechenie luchevoi bolezni beremennykh zhivotnykh i ikh plodov tsistaminom i batilovym spirtom. (Prophylaxis and medical treatment of X-ray sickness in pregnant animals and their feti with cystamine and batyl alcohol.) Med. Radiol. 6(11):82-84, 1961. Referat. Zhur. Biol., 1962. Biol. Absts. 42:297, 1963.

30. Edlund, T. Protective effect of d-, l-alpha-octadecylglycerol ether in mice given total body X-irradiation. Nature 174:1102, 1954.

31. Emmerie, A., C. Engel and W. Klip. The tuberculostatic action in vitro of the unsaponifiable fraction of cod-liver oil. J. Sci. Food Agric. 3:264-268, 1952.

32. Evans, I.A., A.J. Thomas, W.C. Evans and C.M. Edwards. Studies on bracken poisoning in cattle. Brit. Vet. J. 114:253-267, 1958.

33. Evans, W.C., I.A. Evans, C.M. Edwards and A.J. Thomas. Bracken poisoning of cattle; therapeutic treatment. Biochem. J. 65:6P, 1957.

34. Evenstein, D., A.S. Gordon and M. Eisler. Lack of influence of batyl alcohol on erythropoiesis in rats. Anat. Rec. 132:435, 1958.

35. Ghys, R. Effects of alkoxyglycerol (Kaby 700) on leucopenia resulting from radiotherapy. Laval Med. 30:331-342, 1962.

36. Giffin, H. and C.H. Watkins. Treatment of secondary anemia. J. Am. Med. Assoc. 95:587-592, 1930.

37. Gilbertson, J.R. and M.L. Karnovsky. Nonphosphatide fatty acyl esters of alkenyl and alyl ethers of glycerol. J. Biol. Chem. 238(3):893-897, 1963.

38. Guyer, K.E. Studies on the isolation and analysis of glyceryl ethers. Doctorate Thesis, Ohio State University, 1962. Dissertation Absts. 24(1):57, 1963.

39. Guyer, K.E. Studies on the composition of glyceryl ethers and their preparation from diacyl glyceryl ethers in liver oils. J. Lipid Res. 4:385-391, 1963.

40. Hallgren, B. and S.O. Larsson. Separation and identification of alkoxyglycerols. Acta Chem. Scand. 13:2147-2148, 1959.

41. Hallgren, B. and S.O. Larsson. The glyceryl ethers in the liver oils of elasmobranch fish. J. Lipid Res. 3:31-38, 1962.

42. Hallgren, B. and S.O. Larsson. The glyceryl ethers in man and cow. J. Lipid Res. 3:39-43, 1962.

43. Hanahan, D.J., J. Ekholm and C.M. Jackson. Studies on the structure of glyceryl ethers and the glyceryl ether phospholipids of bovine erythrocytes. Biochem. 2(4):630-640,1963.

44. Hanahan, D.J. and R. Watts. The isolation of an α-alkoxy-β-acyl α-glycerophosphorylethanolamine from bovine erythorcytes. J. Biol. Chem. 236:PC59-PC60, 1961.

45. Hardegger, E., L. Ruzicka and E. Tagmann. Helv. Chem. Acta 26:2205, 1943. Cited by Hallgren, B. and S.O. Larsson. The glyceryl ethers in man and cow. J. Lipid Res. 3:39-43, 1962.

46. Hasegawa, Y., et al. Batyl alcohol. Nippon Rinsho 19:1793-1800, 1961. Chem. Absts. 56:1968G, 1962.

47. Helmy, F.M. and M.H. Hack. Comparison of the lipids in maternal and cord blood and of human amniotic fluid. Proc. Soc. Exp. Biol. Med. 110:91-94, 1962.

48. Hilditch, T.P. The chemical constitution of natural fats. London, Chapman and Hall, 1956.

49. Holmes, H.N., R.E. Corbet, W.B. Ceiger, N. Kornblum and W. Alexander. The isolation and identification of batyl alcohol and cholesterol from yellow bone marrow. Amer. Chem. Soc. J. 63:2607-2609, 1941.

50. Karnovsky, M.L. and A.F. Brumm. Studies on naturally occurring alpha-glyceryl ethers. J. Biol. Chem. 216:689-701, 1955.

51. Karnovsky, M.L., A.W. Lategan, W.S. Rapson and H.M. Schwartz. South African fish products. Part XXIX. The composition of the liver oil of the soupfin shark. (Galeorhenus, Rond.) J. Soc. Chem. Ind. 67:193-196, 1948.

52. Karnovsky, M.L., W.S. Rapson and M. Black. South African fish products. Part XXIV. The occurrence of alpha-glyceryl ethers in the unsaponifiable fractions of natural fats. J. Soc. Chem. Ind. 65:425, 1946.

53. Karnovsky, M.L., W.S. Rapson and H.M. Schwartz. South African fish products. Part XXVIII. The composition of the liver oil of the seven-gilled shark. (Heptranchias pectorosus) (Garman). J. Soc. Chem. Ind. 67:144-147, 1948.

54. Karnovsky, M.L., W.S. Rapson, H.M. Schwartz, M. Black and N.J. van Rensburg. South African fish products. Part XXVII. The composition of the liver oils of the basking shark (Cetorhinus maximus, Gunner) and the spiny shark (Echinorhinus spinosus, Gmelin). J. Soc. Chem. Ind. 67:104-107, 1948.

55. Kates, M., L.S. Yengoyan and P.S. Sastry. A diether analog of phosphatidyl glycerophosphate in Halobacterium cutirubrum. Biochem. Biophys. Acta 98:252-268, 1965.

56. Kind, C.A. and Bergman. The occurrence of octadecyl alcohol, batyl alcohol, and cetyl palmitate in Gorgonias. J. Org. Chem. 7:424, 1942.

57. Klenk, E. and U.W. Hendricks. An inosital phosphate containing carbohydrate isolated from human brain. Biochem. Biophys. Acta 50:602-603, 1961.

58. Knight, B.C.J.G. Surface films of batyl, chimyl and selachyl alcohols. Biochem. J. 24:257-261, 1930.

59. Linman, J.W. Hemopoietic effects of glyceryl ethers. III. Inactivity of selachyl alcohol. Proc. Soc. Exp. Biol. 104:703-706, 1960.

60. Linman, J.W. and F.H. Bethell. Observations on circulating erythrocytes and bone marrow of rats receiving protein-free extracts of rabbit plasma. Blood 11:310-323, 1956.

61. Linman, et al. The erythropoietic stimulating activity of batyl alcohol. J. Lab. Clin. Med. 52:596-604, 1958.

62. Linman, et al. Studies on the stimulation of hemopoiesis by batyl alcohol. J. Lab. Clin. Med. 54:335-343, 1959.

63. Lovern, J.A. Specific peculiarities in depot fat composition. Biochem. J. 31:755-763, 1937.

64. Maisin, J.H., J. Keusters, H. Guidetto and G. Lambert. The effective use of glyceryl ethers in treating X-ray burn. J. de Radiologie d"Electrologie et Medicine Nucleaire 40:454-460, 1959.

65. Malins, D.C. Fatty acids and glyceryl ethers in alkoxyglycerides of dog-fish liver oil. Chem. Ind. 38D:1359-1360, 1960.

66. Malins, D.C., J.C. Wekell and C.R. Houle. The metabolic fate of ingested glyceryl ethers in rainbow trout (Salmo gairdneri). First World Fat Congress, Hamburg, Germany. Oct., 1964.

67. Malins, D.C., J.C. Wekell and C.R. Houle. Composition of diacyl glyceryl ethers and triglycerides of the flesh and liver of the dogfish (Squalus acanthias). J. Lipid Res. 6(1):100-105, 1965.

68. Malkins, T. Recent work in the phospholipid field. Chem. Ind. 39B:605-611, 1961.

69. Manforte, F. and G. Fenech. Components of the liver oil of Hexanchus griseu. Ann. Chim. 44:804-814, 1954.

70. Mangold, H.K. and D.C. Malins. Fractionation of fats, oils and waxes on thin layers of silicic acid. J. Am. Oil Chem. Soc. 37:383-385, 1960.

71. Maqsood, M. and J.K. Ashikawa. Post-irradiation protection and recovery. I. Effects of lipids on hematopoietic organs of X-irradiated male mice. Internat. J. Rad. Biol. 4:521-531, 1961.

72. Marberg, C.M. and H.O. Wiles. Yellow bone marrow extracts in granulocytopenia. J. Am. Med. Assoc. 109:1965-1966, 1936.

73. Matsumoto, T. and T. Wainai. The saponifiable matter of the Echinoderms. I. Δ^7-Cholesterol in the fat of starfish. J. Chem. Soc. Japan, Pure Chem. Sect. 75:756-758, 1954.

74. Matsumoto, T., et al. Sterols and other unsaponifiable substances in the fats of shell fishes, Crustacea and Echinodermata. J. Chem. Soc. Japan 64:1203-1204, 1943.

75. Miller, B., C.E. Anderson and C. Piantadosi. Plasmalogen and glyceryl ether concentrations in normal and atherosclerotic aortic tissue. J. Gerontol. 19(4):430-434, 1964.

76. Mizuno, N.S., V. Perman, D.D. Joel, F.W. Bates, J.H. Sautter and M.O. Schultze. Survival of calves treated with autologous bone marrow after exposure to lethal dose of whole body irradiation. Proc. Soc. Exp. Biol. Med. 105(2):317-320, 1960.

77. Nakagawa, Sachiko and J.M. McKibbin. Distribution of alpha glyceryl ethers in animal tissues. Pro. Soc. Exp. Biol. Med. 111(3):634-636, 1962.

78. Omelik, S. The adriatic elasmobranch liver oils. IV. Biochemical study of oils from the liver and eggs of Centrina salviani. Juguslavije Acta Adriat. 4:3-23, 1949.

79. Osmond, D.G., P.J. Raylance, A.J. Webb and J.N. Yoffey. The action of batyl alcohol and selachyl on the bone marrow of the guinea pig. Acta Haematol. 29(3):180-186, 1963.

80. Penny, R.H.C., A.J. Wright and J.W. Stoker. Observations on the relationship between batyl alcohol and haemopoiesis in cattle, sheep and mice. Brit. Vet. J. 120:286-293, 1964.

81. Pietruszko, R. Lipids of red bone marrow from pig epiphyses. Biochem. Biophys. Acta 64:562-564, 1962.

82. Pietruszko, R. and G.M. Gray. The products of mild alkaline and mild acid hydrolysis of plasmalogens. Biochem. Biophys. Acta 56:232-239, 1962.

83. Popović, M. Über die glycerinather des menschlichen herzmuskels Z. Physiol. Chem. 340:18-23, 1965.

84. Prelog, V.L., Ruzicka, L., F. Steinmann. The isolation of chimyl alcohol from testes extract and its identification with testriol. Helv. Chem. Acta 27:674, 1944.

85. Prokhonchukov, A.A. and V.V. Panikarovskii. The condition of the maxillodental system during the experimental therapy of radiation sickness. Teoriya i Prakt. Stomatol., Sb. 6:61-63, 1963. Chem. Absts. Biochem. Sections 63(7):8706H, 1965.

86. Riley, R.F., Y. Hokama and P. Kratz. Nonsteroidal components of the unsaponifiable fraction of human liver. Cancer Res. 18:825-832, 1958.

87. Rusanov, A.M., E.N. Mosharova and R.S. Komarova. Therapy with chemical compounds of hematopoiesis inhibition caused by ionizing irradiation. Med. Radiol. 7(2):42-9, 1962. Chem. Absts. Biochem. Sect. 63(6):7325C, 1965.

88. Rusoff, L.L. The role of milk in modern nutrition. Borden's Rev. Nutr. Res. 25:2-3, 1964.

89. Safanda, J. and V. Holecek. Inhibitory effect of chimyl alcohol on lysolecithin hemolysis. Folia Haematol. 83(2):171-175, 1965. Chem. Absts. Biochem. Sect. 63(10):13865G, 1965.

90. Sandler, O.E. Some experimental studies on the erythropoietic effect of yellow bone marrow extracts and batyl alcohol. Acta Med. Scan. Supp. 225:1-99, 1949.

91. Schogt, J.C.M., P. Haverkamp Begemann and J. Koster. Nonphosphatide aldehydrogenic lipids in milk fat, beef tallow and ox heart. J. Lipid Res. 1:446-449, 1960.

92. Schultze, M.O., V. Perman, F.W. Bates and J.H. Saulter. Failure of DL-batyl alcohol to prevent aplastic anemia in calves. Proc. Soc. Exp. Biol. Med. 98(3):470-474, 1958.

93. Schürch, A.F., L.E. Lloyd and E.W. Crampton. The use of chromic oxide as an index for determining the digestibility of a diet. J. Nutr. 41:629-636, 1950.

94. Sehgal, S.N., M. Kates and N.E. Gibbons. Lipids of Halobacterium cutirubrum. Can. J. Biochem. Physiol. 40:69-81, 1962.

95. Stove, B.B. Growth promotion in pea stem segments. I. Stimulation of auxin and gibberellin action by alkyl lipids. Plant Physiol. 35:262-269, 1960.

96. Svennerholm, L. and H. Thorin. Isolation of "Kepalin B" from cerebral lipids. Biochem. Biophys. Acta 41:371-372, 1960.

97. Sviridov, N.K., E.A. Abaturova, A.V. Shubina and G.N. Elpat'evskaya. Therapeutic effect of selachyl alcohol on experimental radiation sickness. Moscow Med. Sb. 1964 254-258. Chem. Absts. Biochem. Sect. 63(10):13676C, 1965.

98. Swell, L., M.D. Law and C.R. Treadwell. Absorption of α- and β-octadecyl glyceryl ethers. Arch. Biochem. Biophys. 110(2):231-236, 1965. Chem. Absts. Biochem. Sect. 63(1):1014D, 1965.

99. Thompson, G.A., Jr. and D.J. Hanahan. Identification of glyceryl ether phospholipids as major lipid constituents in two species of terrestial slug. J. Biol. Chem. 238(8):2628-2631, 1963.

100. Thompson, G.A. and D.J. Hanahan. Origin of the glyceryl component of alpha glyceryl ethers. J. Biochem. Biophys. 96:671-672, 1962.

101. Thompson, G.A., Jr. and D.J. Hanahan. Studies on the nature and formation of alpha-glyceryl ether lipids in bovine bone marrow. Biochem. 2(4):641-646, 1963.

102. Thompson, G.A. and P. Lee. Studies of the alpha-glyceryl ether lipids occurring in Molluscan tissues. Biochem. Biophys. Acta 98:151-159, 1965.

103. Tietz, A., M. Lindberg and E.P. Kennedy. The oxidation of glyceryl ethers: A new enzyme system requiring a pteridine cofactor. Fed. Proc. 22:296, 1963.

104. Todd, D. and G.P. Rizzi. Biochemistry of the alpha-glyceryl ethers. I. Distribution in mammalian tissues and in starfish. Proc. Soc. Exp. Biol. Med. 115:218-222, 1964.

105. Wajda, M. Lipid composition of human bone marrow. Biochem. J. 95:252-255, 1965.

106. Weidemann, G. Studies on the chemical nature of the unsaponifiable fraction of fish oil. Biochem. J. 20:685-691, 1926.

107. Wintrobe, M.M. Clinical Hematology. Philadelphia, Lea and Febiger, 1952.

APPENDICES

APPENDIX I

A. Composition of U.B.C. Ration No. 14-63

Dog Crumbles

Tomato pomace	100
Ground wheat	590
Wheat bran	100
Wheat germ meal	65
Oat groats	150
Fish meal (70%)	200
Soya meal (50%)	200
Linseed oil meal	100
Vitagrass	100
Skim milk powder	100
Brewers yeast	20
Steamed bone meal	20
Iodized salt	5
Stabilized fat	150
Molasses (cane)	100
	2000
Dry vitamin A	1 million i.u.
Dry vitamin D	150,000 i.u.

B. Composition of U.B.C. Ration No. 10-63

Rat Pellets

Ground barley	300
Ground wheat	400
Wheat bran	200
Wheat germ meal	100
Ground oat groats	200
Fishmeal (73%)	250
Soyabean meal (44%)	100
Oilcake meal	50
Vitagrass	100
Skim meal powder (spray)	100
Brewers yeast	24
Irradiated dry yeast	2
Sterilized bone meal	10
Stabilized fat	50
Molasses	100
Iron oxide	4
Iodized salt	10
	2000

¼ lb dry vitamin A

APPENDIX II

Mean Daily Feed Intake In Grams

A. Male Rats

Week	Selachyl	Batyl	Chimyl	Control
1	14.1	14.1	14.7	14.2
2	16.8	15.6	17.3	17.2
3	19.4	18.4	18.5	18.5
4	18.0	18.5	19.0	18.3
5	19.5	18.3	17.7	20.4
6	19.8	18.0	18.8	20.0
7	17.6	15.4	18.0	19.7
8	19.6	18.7	19.6	20.5
9	20.4	19.9	18.8	19.9
10	19.3	18.5	18.3	18.6
11	21.5	20.5	19.4	20.5
12	22.4	21.1	19.9	21.1
13	21.5	20.1	19.5	20.9
14	24.7	22.4	22.9	22.8
15	23.0	22.4	22.3	22.9
16	24.3	22.9	23.8	24.0
17	25.9	23.2	23.5	23.4
18	25.2	23.4	22.6	23.1
19	24.7	24.4	21.5	22.7
20	26.3	23.6	22.8	25.3
21	25.0	23.1	22.1	22.4
22	23.5	23.1	20.5	22.2
23	23.0	24.2	21.1	24.8
24	21.5	22.2	20.0	21.7
25	20.8	19.5	19.4	20.6
26	20.9	20.0	20.8	20.6
27	22.2	20.6	20.1	21.4

APPENDIX II (cont'd)

B. Female Rats

Week	Selachyl	Batyl	Chimyl	Control
1	13.3	13.7	13.8	13.3
2	14.8	16.0	15.8	14.7
3	15.7	15.8	16.3	14.3
4	16.0	16.8	15.8	15.5
5	16.2	16.4	16.5	15.9
6	17.2	16.3	16.7	15.9
7	16.2	14.6	15.8	15.3
8	17.5	16.7	17.4	16.4
9	17.3	15.3	15.5	15.2
10	15.2	14.5	14.6	14.0
11	17.4	16.0	16.4	15.7
12	18.6	16.9	16.8	16.1
13	18.6	15.5	15.9	15.9
14	20.1	18.6	18.8	17.4
15	18.1	18.2	17.9	16.9
16	19.2	18.8	18.9	17.5
17	19.6	16.9	20.0	17.4
18	18.9	17.8	19.0	17.5
19	20.0	18.0	19.5	17.7
20	19.4	18.1	20.8	18.8
21	18.8	17.8	19.4	17.0
22	21.6	18.6	17.7	16.6
23	19.4	17.7	18.3	17.2
24	19.0	16.6	16.6	15.6
25	18.9	15.6	15.3	14.6
26	18.0	15.9	17.4	15.7
27	18.8	17.8	17.1	16.3

APPENDIX III

Mean Weekly Weights

A. Male Rats*

Week	Selachyl	Batyl	Chimyl	Control
1	102.3	99.5	99.0	94.3
2	132.2	119.5	131.7	124.6
3	164.3	149.8	161.5	150.4
4	200.1	184.3	187.0	183.1
5	226.4	201.8	210.4	212.8
6	249.4	228.0	230.6	230.2
7	261.4	237.3	247.2	243.4
8	276.4	254.3	263.9	262.1
9	294.8	274.1	270.0	277.3
10	314.2	288.0	295.0	291.8
11	329.6	307.4	308.7	303.1
12	338.9	319.4	317.7	316.8
13	347.3	329.6	326.1	329.3
14	358.3	338.7	334.2	339.7
15	362.6	347.4	341.8	348.9
16	373.2	356.8	353.6	362.2
17	380.9	364.3	363.1	369.4
18	382.7	368.8	370.2	373.6
19	387.0	374.0	378.1	375.2
20	391.1	379.7	376.4	377.9
21	398.7	390.9	387.4	383.4
22	400.6	391.8	390.1	393.3
23	403.2	402.9	401.7	398.4
24	405.8	407.0	403.1	406.1
25	410.1	410.0	407.3	409.5
26	410.7	410.9	409.3	413.4
27	421.5	413.9	412.8	418.9

* All weights are in grams and are the mean of ten animals.

APPENDIX III (cont'd)

B. Female Rats*

Week	Selachyl	Batyl	Chimyl	Control
1	91.3	90.9	93.3	94.3
2	112.5	113.7	115.8	114.3
3	130.7	133.4	134.9	133.7
4	150.4	151.6	151.8	151.2
5	163.0	164.9	166.1	165.0
6	175.0	175.9	175.6	175.9
7	181.4	183.1	181.8	183.7
8	191.6	192.3	191.1	194.3
9	199.6	202.8	196.3	198.8
10	208.1	206.1	201.4	204.6
11	214.5	211.6	206.5	208.2
12	219.7	213.6	212.7	213.8
13	223.9	216.5	216.2	219.0
14	229.1	220.4	220.2	221.0
15	229.8	224.7	222.7	224.1
16	236.2	229.0	228.8	228.8
17	240.7	232.6	232.7	232.6
18	243.3	234.4	238.0	235.4
19	243.2	238.8	237.5	237.2
20	247.9	238.6	239.2	238.6
21	252.7	241.4	241.9	242.4
22	254.6	241.5	246.9	242.9
23	253.3	245.9	246.0	246.3
24	258.2	248.6	247.9	248.2
25	262.8	250.4	248.3	249.7
26	264.0	251.3	250.0	251.5
27	267.3	255.4	253.3	256.4

* All weights are given in grams and are the mean of ten animals.

APPENDIX III (cont'd)

C. Dogs*

Week	Selachyl	Batyl	Chimyl	Control
1	10.3	10.7	11.6	9.5
2	12.4	12.6	13.1	10.9
3	13.3	13.7	14.2	11.6
4	12.7	14.0	14.7	12.3
5	13.9	14.2	14.9	12.9
6	14.8	15.4	15.6	13.4
7	15.5	16.3	16.6	14.4
8	16.3	16.7	17.2	15.1
9	16.9	17.3	17.3	15.6
10	16.6	17.4	17.7	15.4
11	17.6	17.9	18.3	16.2
12	18.4	18.7	19.1	17.3
13	18.4	18.9	19.3	17.1
14	19.4	19.5	20.0	18.1
15	19.5	19.5	19.7	18.0
16	20.1	20.0	20.5	18.3
17	20.7	20.7	21.1	19.2
18	21.0	20.8	21.2	19.4
19	21.6	21.2	21.8	20.0
20	22.1	21.1	21.5	19.7
21	21.7	21.6	21.9	20.3
22	22.0	20.8	21.7	19.9
23	21.8	21.0	22.0	19.8
24	22.2	20.2	22.3	20.4
25	22.2	20.8	22.6	20.8
26	22.6	20.6	22.7	20.2
27	21.9	20.7	23.0	20.8

* All weights are in kilograms and are the mean of the five animals.

APPENDIX IV

Blood Counts

A. Leucocyte Counts* of Rats Taken Ninety Days From The Beginning of The Trial

Rat	Selachyl	Batyl	Chimyl	Control
female				
1	18,700	11,300	16,300	19,100
2	20,500	11,450	15,900	13,050
3	17,200	21,400	14,800	16,150
4	19,150	10,750	10,550	15,650
5	16,500	20,700	11,150	11,550
6	20,300	18,950	20,950	14,650
7	15,200	17,250	15,800	12,450
8	16,250	6,900	12,400	16,600
9	18,600	15,050	16,200	15,050
10	21,000	20,650	22,850	11,750
Mean	18,340	15,440	15,690	14,600
S.D.**	1,880	4,840	3,720	2,280
male				
1	23,500	19,750	23,700	19,700
2	22,150	19,600	16,400	24,300
3	13,400	20,950	23,700	21,450
4	14,450	17,800	19,500	15,450
5	16,700	18,000	27,700	15,750
6	17,100	20,900	19,650	17,800
7	19,750	13,000	26,300	27,850
8	18,600	14,700	21,200	21,300
9	18,000	16,950	19,650	13,050
10	16,900	15,000	23,300	21,350
Mean	18,055	17,665	22,110	19,800
S.D.**	2,970	2,600	3,290	4,313

* These counts are numbers of leucocytes per cmm of blood.
** Standard Deviation.

APPENDIX IV (cont'd)

B. Leucocyte Counts* of Rats Taken 180 Days From The Beginning of the Trial

Rat	Selachyl	Batyl	Chimyl	Control
female				
1	17,400	11,500	15,000	18,300
2	18,900	12,100	22,100	16,600
3	12,400	20,600	15,000	18,400
4	21,100	17,800	10,000	13,800
5	14,600	18,000	14,800	11,400
6	9,000	18,000	10,300	12,000
7	13,700	19,800	11,300	10,900
8	17,800	12,500	12,000	11,500
9	16,800	15,000	21,800	14,600
10	13,900	14,800	12,800	15,700
Mean	15,560	16,010	14,510	14,320
S.D.**	3,350	3,110	4,110	2,710
male				
1	21,500	26,000	13,000	15,500
2	17,400	26,300	20,500	12,500
3	25,600	27,500	20,400	17,500
4	20,500	26,700	15,400	19,400
5	12,300	16,800	15,000	27,800
6	13,300	15,000	15,900	16,300
7	16,100	18,700	17,500	16,400
8	20,000	13,100	28,300	17,100
9	12,300	11,100	20,500	16,200
10	23,100	12,200	28,900	16,900
Mean	18,200	19,340	19,540	17,560
S.D.**	4,440	6,300	5,150	3,800

* These counts are number of leucocytes per cmm.
** Standard Deviation

APPENDIX IV (cont'd)

C. Erythrocyte Counts* of Male Rats Taken 180 Days From The Beginning of the Trial

Rat	Selachyl	Batyl	Chimyl	Control
1	9.96	10.2	8.3	7.3
2	9.16	9.4	8.7	8.9
3	9.69	9.1	9.5	8.7
4	8.5	8.6	8.9	9.0
5	9.3	7.9	10.2	8.5
6	9.7	7.2	8.2	10.1
7	8.8	8.8	10.0	9.6
8	8.4	8.9	8.9	9.5
9	8.3	9.3	8.7	8.2
10	9.3	8.8	10.4	8.9
Mean	9.11	8.82	9.58	8.87
S.D.	.56	0.78	0.85	0.73

* The erythrocyte counts are recorded in millions of cells per cmm of blood.

APPENDIX IV (cont'd)

D. Erythrocyte Counts* of Dogs Taken 110 Days from The Beginning of the Trial

Selachyl		Batyl	
dog #	count	dog #	count
59	5.22	60	6.08
60a	5.52	69	5.39
74	5.60	66	5.83
72	4.80	70	5.10
65	5.40	64	6.10
Mean	5.31		5.70
S.D.	0.28		0.39

Chimyl		Control	
dog #	count	dog #	count
67	6.29	75	5.08
NT	6.68	76	5.40
58	5.25	68	6.38
71	5.54	77	5.72
63	6.20	62	5.24
Mean	5.99		5.56
S.D.	0.52		0.46

* The erythrocyte counts are recorded in millions of cells per cmm of blood.

APPENDIX IV (cont'd)

E. Leucocyte Counts* of Dogs Taken 180 Days From The Beginning of the Trial

Selachyl		Batyl	
dog #	count	dog #	count
59	15,300	60	16,200
60a	16,100	69	15,500
74	20,300	66	17,300
72	14,900	70	15,100
65	19,300	64	21,100
Mean	17,200		17,200
S.D.	2,200		2,170

Chimyl		Control	
dog #	count	dog #	count
67	15,300	75	17,200
NT	19,900	76	22,200
58	17,000	68	18,100
71	16,900	77	15,700
63	15,200	62	15,200
Mean	16,900		17,500
S.D.	1,700		2,360

* These counts are numbers of leucocytes per cmm of blood.

APPENDIX V

Weight Data for Animals In Trial II

A. Weekly Weights in Kilograms for Dogs

S^{100} Group

Week	#26	#33	#21	#13
Initial	11.4	13.2	9.5	12.7
1	10.9	13.6	10.0	13.6
2	12.3	14.5	10.9	14.1
3	12.7	15.0	11.4	14.5
4	13.6	15.9	11.8	15.5
5	13.6	16.4	12.3	15.4
6	14.5	16.8	12.7	16.4
7	15.5	17.7	13.6	17.3
8	16.4	18.6	14.5	18.2
9	15.9	19.5	15.0	18.7
10			15.0	17.7
11			15.9	19.1
12			16.8	19.4
13			17.7	20.9

C^{400} Group

Week	#11	#19	#22	#17
Initial	15.5	10.5	10.9	10.9
1	16.4	10.9	11.4	11.4
2	17.3	11.4	12.3	12.3
3	18.2	12.3	12.7	12.7
4	19.5	13.2	14.1	14.1
5	20.0	13.2	14.1	15.0
6	21.4	13.6	15.0	15.9
7	22.7	14.5	16.4	16.8
8	23.6	15.5	17.3	17.7
9	24.5	16.4	16.8	18.2
10		16.4	17.3	
11		17.3	17.7	
12		18.2	19.5	
13		19.1	20.5	

APPENDIX V (cont'd)

S^{400} Group

Week	#25	#10	#9	#23
Initial	10.9	14.5	12.3	10.5
1	11.4	15.4	11.8	10.5
2	10.9	15.0	11.4	10.9
3	11.4	15.0	11.4	10.9
4	11.4	15.0	11.4	11.4
5	11.4	14.5	10.9	11.4
6	11.4	14.5	11.8	11.4
7	11.8	15.4	11.8	11.8
8	12.3	15.5	12.3	11.8
9	12.7	15.9	12.7	12.3
10		15.9	12.7	
11		17.7	13.6	
12		19.1	14.1	
13		20.0	15.5	

B^{400} Group

Week	#20	#34	#14	#12
Initial	10.5	12.3	8.6	13.2
1	10.9	13.2	8.6	13.2
2	10.0	13.6	9.1	13.6
3	10.5	13.6	9.1	12.3
4	10.5	14.5	9.5	12.7
5	10.0	14.1	9.5	12.3
6	10.0	13.6	10.0	12.3
7	10.0	14.1	10.5	12.3
8	10.0	14.5	10.9	12.7
9	9.5	14.1	10.9	12.2
10	10.0			13.6
11	10.0			13.6
12	10.9			14.1
13	11.8			15.0

APPENDIX V (cont'd)

B. Mean Weights* in Grams for Rats

Day	S^{400}	B^{400}	S^{100}	C^{400}
1	79.6	79.7	83.6	80.7
3	90.1	92.5	95.0	92.8
5	97.6	101.5	104.6	102.9
8	105.3	108.6	110.0	109.2
10	113.7	112.5	114.9	112.5
12	119.6	118.1	118.9	117.6
15	125.4	124.3	123.8	123.0
17	134.0	131.1	127.9	128.2
19	137.1	134.2	131.3	132.3
22	143.5	140.0	137.3	138.9
24	148.1	145.6	143.0	146.2
26	153.4	149.9	146.8	149.7
29	158.5	156.8	152.0	154.6
31	162.5	160.4	155.9	160.2
33	163.5	160.4	155.1	159.5
36	174.8	172.1	165.8	170.5
38	177.3	176.7	169.6	173.2
40	181.4	185.9	174.1	177.9
43	185.6	189.8	179.8	184.1
45	191.9	195.6	185.2	191.0
47	191.7	196.1	185.7	192.2
50	202.1	210.3	196.2	201.8
52	207.4	215.8	202.9	208.0
54	208.7	220.1	205.7	211.6
57	215.6	222.8	211.9	220.1
59	216.8	225.9	211.2	217.0
61	216.1	227.8	212.6	218.1

* mean of ten rats

APPENDIX VI

Results of Rat Blood Taken One Month From The Beginning of Trial II

A.. Erythrocyte Counts (millions of cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	10.7	10.0	8.5	7.9
2	8.1	7.4	8.9	8.7
3	9.2	8.0	7.8	8.9
4	9.0	8.1	8.3	8.5
5	8.4	8.7	9.0	7.9
average	9.1	8.5	8.5	8.4
Female				
1	7.6	7.8	9.2	8.2
2	8.1	8.1	8.0	7.3
3	7.0	7.3	7.6	8.5
4	7.7	7.7	9.5	7.0
5	7.6	9.1	8.7	7.1
average	7.6	8.0	8.6	7.6

B. Leucocyte Counts (cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	16,100	8,800	8,200	21,500
2	9,700	15,700	17,100	6,800
3	7,500	12,600	5,900	5,900
4	9,300	6,000	11,800	6,600
5	10,000	9,900	8,000	6,300
average	10,500	10,600	10,200	9,400
Female				
1	10,700	19,000	11,000	15,700
2	13,100	9,900	13,900	19,900
3	11,900	15,300	14,000	13,100
4	8,800	13,000	11,700	19,500
5	14,200	7,300	15,300	10,500
average	11,700	12,900	13,200	15,700

APPENDIX VI (cont'd)

C. Reticulocytes (percent of erythrocytes)

	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	4.7	3.0	3.1	4.6
2	4.1	3.8	4.6	4.2
3	3.9	4.6	2.9	2.8
4	6.9	4.2	3.8	4.4
5	3.7	3.2	3.9	3.5
average	4.6	3.8	3.7	3.9
Female				
1	3.0	8.2	4.0	3.7
2	3.6	4.0	3.2	2.8
3	4.4	5.4	4.8	2.2
4	3.8	3.9	4.5	3.6
5	4.8	3.6	6.2	3.9
average	3.9	5.2	4.5	3.2

D. Differential Leucocyte Counts (percent of leucocytes)

Polymorphs	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	16	14	16	31
2	25	21	23	22
3	27	24	22	24
4	18	14	22	22
5	26	17	24	14
average	22.4	18.0	21.4	22.6
Female				
1	17	16	23	26
2	17	19	18	16
3	26	24	19	27
4	16	21	18	23
5	21	24	14	15
average	19.4	20.8	18.4	21.4

APPENDIX VI (cont'd)

Lymphocytes	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	81	76	81	66
2	75	73	75	69
3	70	74	71	74
4	79	82	76	74
5	68	81	75	85
average	74.6	77.2	75.6	73.6
Female				
1	78	82	71	72
2	77	80	75	75
3	74	73	80	69
4	81	74	77	76
5	74	68	80	83
average	76.8	75.4	76.6	75.0

Eosinophils and Monocytes	S^{400}		B^{400}		S^{100}		C^{400}	
Male								
1	2	1	6	4	3	0	1	2
2	0	0	4	2	2	0	5	4
3	1	2	2	0	6	1	1	1
4	1	2	0	4	2	0	3	1
5	6	0	1	1	0	1	1	0
averages	2.0	1.0	2.6	2.2	2.6	0.4	2.2	1.6
Female								
1	3	2	1	1	5	1	1	1
2	2	3	0	1	5	2	8	1
3	0	0	2	1	1	0	2	0
4	1	2	4	1	3	2	1	0
5	5	0	6	2	5	1	1	1
averages	2.2	1.4	2.6	1.2	3.8	1.2	2.6	0.6

APPENDIX VII

Results of Rat Bood Taken Two Months From The Beginning of Trial II

A. Erythrocyte Counts (millions of cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	9.9	10.8	9.2	8.5
2	8.8	8.6	9.1	8.7
3	9.7	9.0	9.2	8.7
4	9.1	9.2	8.6	8.8
5	8.3	9.4	9.0	8.2
average	9.2	9.4	9.0	8.6
Female				
1	8.3	10.9	9.2	8.7
2	9.0	8.6	8.9	7.8
3	8.6	8.6	8.4	8.8
4	9.4	9.4	9.9	7.5
5	8.8	-	9.4	7.7
average	8.8	9.4	9.2	8.1

B. Leucocyte Counts (cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	18,200	10,300	7,800	22,600
2	13,300	14,300	23,000	9,900
3	9,900	10,100	7,900	8,600
4	12,500	5,800	15,200	10,200
5	11,800	9,600	9,200	9,100
average	13,100	10,000	12,600	12,100
Female				
1	13,000	17,900	9,800	15,700
2	14,300	8,900	13,200	20,800
3	10,700	19,000	18,700	15,100
4	11,200	15,000	11,300	23,200
5	13,200	-	19,300	11,600
average	12,500	15,200	14,500	17,300

APPENDIX VII (cont'd)

C. Reticulocytes (percent of erythrocytes)

	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	6.0	5.1	3.7	4.8
2	4.2	5.8	4.9	4.4
3	9.8	5.1	4.2	2.5
4	7.2	5.0	4.6	4.7
5	5.1	4.4	4.1	3.7
average	6.5	5.1	4.3	4.0
Female				
1	3.7	7.9	4.1	3.3
2	4.9	4.9	3.6	2.9
3	5.3	5.8	4.7	2.3
4	4.3	5.2	4.8	3.4
5	5.1	-	6.1	3.3
average	4.7	6.0	4.7	3.0

D. Differential Leucocyte Counts (percent of leucocytes)

Polymorphs	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	18	17	17	31
2	36	16	19	24
3	27	24	23	31
4	26	24	18	28
5	27	16	17	26
average	26.8	19.4	18.8	28
Female				
1	14	14	23	24
2	16	18	18	23
3	22	17	19	27
4	18	17	21	19
5	23	-	19	15
average	18.6	16.5	20.0	21.6

APPENDIX VII (cont'd)

Lymphocytes	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	80	78	74	67
2	62	78	77	74
3	70	72	74	66
4	70	75	80	69
5	68	80	80	69
average	70.0	76.6	77.0	69.0
Female				
1	81	82	74	72
2	79	81	78	67
3	73	78	77	67
4	76	80	75	72
5	73	-	76	81
average	76.4	80.2	76.0	71.8

Eosinophils and Monocytes	S^{400}		B^{400}		S^{100}		C^{400}	
Male								
1	1	1	3	2	8	1	0	2
2	0	2	2	3	2	2	1	1
3	1	2	2	2	2	1	0	3
4	2	2	1	0	0	2	3	0
5	3	2	1	3	2	1	2	1
average	1.4	1.8	1.8	2.0	2.8	1.4	1.4	1.6
Female								
1	1	3	2	1	2	1	2	2
2	1	4	1	0	1	2	3	7
3	3	2	3	1	2	2	5	1
4	2	4	2	1	3	1	4	4
5	3	1	-	-	4	1	1	1
average	2.0	2.8	2.0	0.8	2.4	1.4	3.0	3.0

APPENDIX VIII

Blood Count Data from Dogs Taken One Month From The Beginning of Trial II

Identification of dogs

The dogs will be identified in this set of data by numbers 1, 2, 3 and 4 as follows:

	S^{400} dog	B^{400} dog	S^{100}dog	C^{400} dog
1	25	20	26	11
2	10	34	33	19
3	9	14	21	22
4	23	12	13	17

A. Erythrocyte Counts (millions of cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	5.6	7.2	6.5	6.2
2	6.0	5.4	5.2	6.1
3	5.3	5.0	6.3	5.5
4	5.7	4.9	5.5	5.6
average	5.7	5.6	5.9	5.9

B. Leucocyte Counts (cell/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	13,300	16,200	14,600	9,400
2	8,600	14,100	11,900	10,700
3	14,600	17,500	17,000	12,600
4	14,800	12,300	13,000	10,000
average	12,800	15,000	14,100	10,800

APPENDIX VIII (cont'd)

C. Reticulocytes (percent of erytrocytes)

	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	1.3	2.2	1.5	1.1
2	1.4	1.1	1.3	1.5
3	1.6	2.6	0.7	0.6
4	1.1	0.8	1.0	0.9
average	1.4	1.7	1.1	1.0

D. Differential Leucocyte Counts (percent of leucocytes)

Polymorphs	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	67	66	50	59
2	57	69	69	65
3	65	72	65	71
4	72	81	59	63
average	65.3	72.8	61.8	64.5

Lymphocytes	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	32	30	39	23
2	36	21	25	32
3	34	27	34	26
4	20	18	37	33
average	30.5	24.0	33.8	28.5

APPENDIX VIII (cont'd)

Eosinophils and Monocytes	S^{400}		B^{400}		S^{100}		C^{400}	
Dog								
1	1	0	0	1	10	1	16	2
2	6	1	10	0	0	2	0	3
3	0	1	0	1	0	0	2	1
4	8	0	0	1	3	1	0	4
average	3.8	0.5	2.5	0.8	3.3	1.0	4.5	2.5

APPENDIX IX

Blood Counts Data Taken From Dogs* Two Months From The Beginning Of Trial II

A. Erythrocyte Counts (millions of cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	6.2	7.9	7.4	6.8
2	6.6	6.6	6.5	6.4
3	6.5	5.7	6.3	5.9
4	7.0	5.3	6.3	5.8
average	6.6	6.4	6.6	6.2

B. Leucocyte Counts (cells/cmm)

	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	15,000	18,900	12,800	15,400
2	10,800	12,400	10,800	11,100
3	13,900	18,100	21,400	11,800
4	18,900	10,700	14,300	12,300
average	14,700	15,000	14,800	12,700

C. Reticulocytes (percent of erythrocytes)

	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	1.8	2.5	1.8	1.4
2	2.0	1.4	1.7	1.4
3	1.9	2.9	1.3	0.7
4	1.7	1.7	1.5	0.9
average	1.9	2.1	1.6	1.1

* The numbering scheme used here identifies the dog as set out in Appendix VIII.

APPENDIX IX (cont'd)

D. Differential Leucocyte Counts (percent of leucocytes)

Polymorphs	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	66	80	57	62
2	73	73	76	68
3	65	69	70	74
4	73	78	67	67
average	69.3	75.0	67.5	67.8

Lymphocytes	S^{400}	B^{400}	S^{100}	C^{400}
Dog				
1	26	20	32	30
2	25	20	23	29
3	33	26	28	20
4	23	21	32	26
average	26.8	21.8	28.8	26.3

Eosinophils and Monocytes	S^{400}		B^{400}		S^{100}		C^{400}	
Dog								
1	8	0	0	0	11	0	7	1
2	1	1	7	0	1	0	0	3
3	1	1	5	0	1	0	6	0
4	4	0	0	1	1	0	5	1
average	3.5	0.5	3.0	0.3	3.5	0	4.5	1.3

APPENDIX X

Differential Counts* of Femoral Bone Marrow Of Rats in Trial II

1. Myeloblasts

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	0.7	0.7	0.3	1.0
2	1.0	1.0	0.7	0.7
3	0.7	-	1.0	0.7
4	0.7	1.0	0.7	0.3
5	1.3	1.3	0.3	-
average	0.88	0.80	0.60	0.54
Female				
1	1.3	1.3	0.7	0.0
2	1.0	1.0	0.3	0.7
3	1.7	1.3	0.3	0.0
4	1.0	0.7	1.0	1.0
5	0.0	0.3	1.3	0.7
average	1.0	0.92	0.72	0.48

2. Promyelocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	2.0	2.7	1.3	3.0
2	2.7	2.7	2.3	3.7
3	2.0	1.0	2.0	4.3
4	1.7	1.7	2.0	1.7
5	3.7	3.0	1.7	1.0
average	2.42	2.22	1.86	2.74
Female				
1	3.0	2.3	2.0	-
2	1.7	2.7	1.7	2.0
3	4.0	2.0	2.0	0.7
4	2.3	1.3	2.3	1.3
5	1.7	1.0	2.3	1.0
average	2.54	1.86	2.06	1.0

* These values are all percentages of 300 differentiated cells.

APPENDIX X (cont'd)

3. Myelocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	2.3	3.0	3.7	4.0
2	2.7	5.0	3.3	5.7
3	2.3	2.3	2.7	5.3
4	2.3	4.3	3.7	4.0
5	4.0	3.7	3.3	2.3
average	2.72	3.66	3.34	4.26
Female				
1	4.3	3.3	3.3	4.3
2	3.0	5.0	3.3	3.0
3	5.0	4.0	4.3	2.3
4	3.0	3.0	4.3	2.0
5	2.7	3.3	2.7	3.0
average	3.60	3.72	3.58	2.92

4. Metamyelocytes and "Ring Cells"

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	9.7	14.3	12.3	12.0
2	10.0	13.3	12.7	16.0
3	11.0	11.7	11.3	13.7
4	9.7	13.0	12.0	9.7
5	11.0	13.3	11.0	10.3
average	10.28	13.12	11.0	12.34
Female				
1	11.3	14.0	13.7	12.3
2	10.7	13.3	12.0	14.3
3	12.3	15.3	11.0	12.3
4	11.7	10.3	14.3	13.0
5	12.7	10.0	11.7	11.0
average	11.74	12.58	12.54	12.58

APPENDIX X (cont'd)

5. Mature Granulocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	29.7	25.3	35.0	30.3
2	30.7	20.7	32.7	33.3
3	23.3	27.3	30.7	28.7
4	24.0	25.0	28.0	35.0
5	22.7	24.7	28.0	37.0
average	26.08	24.60	30.88	32.8
Female				
1	28.0	19.0	28.0	37.3
2	27.7	21.3	32.0	35.3
3	20.3	19.3	28.3	36.0
4	26.0	29.3	28.7	35.0
5	26.7	31.0	30.7	35.7
average	25.74	23.98	29.54	35.14

6. Lymphocyte-Like Cells

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	4.0	7.7	7.7	10.7
2	8.0	8.0	7.0	7.3
3	5.3	9.0	9.7	9.3
4	9.0	7.3	10.7	7.3
5	9.0	9.7	12.0	9.7
average	7.06	8.34	9.42	8.86
Female				
1	10.0	7.3	13.3	8.7
2	8.7	9.0	9.7	7.0
3	9.3	9.0	11.7	10.7
4	7.7	8.7	8.7	7.7
5	7.7	10.7	7.0	8.0
average	8.68	8.94	10.08	8.42

APPENDIX X (cont'd)

7. Nucleated Red Blood Cells

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	49.0	44.3	37.0	36.0
2	41.7	46.0	38.3	31.0
3	50.7	43.0	37.3	35.0
4	48.3	43.3	38.7	37.7
5	43.3	40.7	37.7	35.0
average	46.60	43.46	37.80	34.94
Female				
1	38.3	47.7	34.7	35.0
2	43.7	41.3	36.3	34.0
3	42.7	43.0	37.0	34.0
4	44.0	42.3	37.3	36.7
5	43.0	37.7	39.3	36.0
average	42.34	42.40	36.92	34.14

8. Megakaryocytes, Lymphocytes, Plasma Cells and Monocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Male				
1	2.6	2.0	2.0	3.3
2	3.1	3.3	3.6	2.0
3	3.1	5.6	4.7	3.0
4	4.3	4.3	4.4	4.4
5	3.3	3.7	5.9	5.0
average	3.28	3.78	4.12	3.54
Female				
1	3.7	5.0	4.3	3.0
2	3.3	6.2	4.7	3.6
3	4.6	6.1	5.4	4.1
4	4.4	4.3	3.3	3.3
5	5.7	6.0	5.0	4.6
average	4.34	5.52	4.55	3.72

APPENDIX XI

Differential Counts* of Femoral Bone Marrow Of Dogs in Trial II

1. Myeloblasts

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog.No.				
1	1.7	0.7	1.3	0.7
2	0.7	0.7	1.0	1.0
3	0.7	1.7	0.7	1.3
4	0.7	1.0	1.3	0.7
average	0.95	1.03	1.08	0.93

2. Promyelocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	2.7	1.7	3.3	2.3
2	1.3	2.7	2.0	2.3
3	1.0	2.7	1.7	1.7
4	3.0	1.7	1.7	2.3
average	2.00	2.20	2.18	2.15

3. Myelocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	5.0	2.3	5.6	6.6
2	4.0	4.7	3.7	3.3
3	3.0	4.7	3.7	3.0
4	5.7	2.3	3.0	4.3
average	4.40	3.50	4.00	3.55

* These values are all percentages of 300 differentiated cells. The numbering scheme used is explained in Appendix VIII.

APPENDIX XI (cont'd)

4. Metamyelocytes and Stem Cells

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog. No.				
1	23.6	25.0	22.3	25.0
2	23.7	22.3	24.0	22.6
3	22.3	20.3	23.7	22.6
4	22.0	25.3	23.0	24.0
average	22.90	23.23	23.25	23.55

5. Mature Granulocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	18.0	20.6	19.0	22.3
2	18.6	20.3	19.6	18.0
3	20.7	18.6	19.6	21.4
4	16.4	17.0	19.0	21.0
average	18.43	19.13	19.30	20.68

6. Proerythroblasts

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	3.3	2.3	3.3	2.3
2	2.7	3.7	2.0	2.7
3	2.7	3.7	2.3	2.3
4	3.7	3.3	3.0	2.3
average	3.10	3.25	2.65	2.4

APPENDIX XI (cont'd)

7. Normoblasts

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	37.3	36.3	36.3	34.3
2	36.3	36.3	36.0	36.3
3	36.0	38.3	35.7	35.3
4	36.7	35.7	35.7	34.3
average	36.58	36.65	35.93	35.05

8. Lymphocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	7.7	9.3	7.7	8.3
2	9.7	8.0	9.7	10.7
3	10.3	8.3	10.3	9.3
4	9.0	10.0	9.3	10.0
average	9.18	8.90	9.25	9.58

9. Monocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	0	0.7	0	0.7
2	0.7	1.0	0.7	0.7
3	1.0	0.3	1.0	1.3
4	0.7	1.3	1.3	0.3
average	0.60	0.83	0.75	0.75

APPENDIX XI (cont'd)

10. Megakaryocytes

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	0.7	0.7	0.3	0.3
2	1.3	0.3	0	1.3
3	1.3	0.7	1.0	1.0
4	0.7	1.3	1.3	0.3
average	1.25	0.85	0.58	0.83

11. Plasma Cells

Group	S^{400}	B^{400}	S^{100}	C^{400}
Dog No.				
1	0	0.3	1.0	0
2	1.0	0	1.3	1.0
3	1.0	0.7	1.3	0.7
4	0.3	0.7	0.7	0.3
average	0.58	0.43	1.08	0.50

APPENDIX XII

Mean Weights* In Grams For Trial III Rats

Day	Control	B - 0.5	B - 5	B - 50
Initial	57.5	57.3	57.6	56.3
1	60.5	60.7	61.4	59.5
3	69.5	68.4	69.8	68.3
5	76.6	75.6	78.0	76.6
8	90.5	89.9	91.3	90.1
10	101.1	100.0	102.4	100.5
12	110.1	108.4	111.7	109.5
15	123.0	121.1	122.3	121.4
17	130.4	128.7	129.0	127.7
19	135.8	134.2	134.6	132.9
24	144.2	143.2	143.7	142.4
26	148.7	148.3	148.1	147.4
29	158.3	159.1	158.1	157.4
31	163.9	164.1	163.7	163.1
33	169.3	170.4	169.5	167.8

* mean of ten rats

APPENDIX XIII

Blood Counts Of Rats In Trial III

A. Erythrocyte counts (millions per cmm)

Rat	Control	B - 0.5	B - 5	B - 50
1	8.04	7.72	7.61	7.91
2	9.40	7.00	7.72	8.11
3	8.34	7.70	8.02	8.19
4	6.80	5.80	7.97	7.13
5	7.09	6.64	6.50	10.05
6	7.44	7.31	8.03	6.30
7	6.50	7.03	6.71	7.18
8	7.01	6.74	7.06	8.16
9	7.93	8.76	7.97	6.20
10	7.43	7.91	6.77	6.72
Mean	7.60	7.26	7.44	7.60
S.D.	0.81	0.77	0.59	1.09

B. Leucocytes (cells per cmm)

Rat	Control	B - 0.5	B - 5	B - 50
1	12,000	7,900	13,200	11,700
2	10,400	16,300	12,900	8,900
3	9,700	11,500	9,700	14,100
4	9,500	12,100	12,800	15,700
5	8,700	13,100	10,700	11,100
6	12,000	14,300	11,700	12,300
7	14,600	14,100	13,200	8,400
8	9,600	10,300	15,700	16,400
9	14,300	17,600	14,100	13,000
10	11,300	11,000	9,700	12,700
Mean	11,200	12,800	12,400	12,400
S.D.	1,920	2,670	1,830	2,460

APPENDIX XIV

Percent Differential of Leucocytes in Blood Smears From Group III Rats

1. Control

	Polymorphs	Eosinophils	Lymphocytes	Monocytes
Rat No.				
1	16		83	1
2	11		89	
3	15	1	82	2
4	23	3	73	1
5	22		77	1
6	8		92	
7	7		92	1
8	19		75	6
9	9	11	72	7
10	14		83	3
average	14.4	1.5	81.6	2.2

2. B - 0.5

	Polymorphs	Eosinophils	Lymphocytes	Monocytes
Rat No.				
11	12	1	87	
12	12		88	1
13	24	1	74	3
14	29	3	69	3
15	21	1	77	1
16	21		76	
17	12		84	4
18	4		92	4
19	14		84	2
20	22		75	3
average	17.1	0.6	81.0	2.1

APPENDIX XIV (cont'd)

3. B - 5

	Polymorphs	Eosinophils	Lymphocytes	Monocytes
Rat No.				
21	23	2	75	1
22	9		91	
23	15	3	80	2
24	5	1	94	1
25	24		77	
26	11		83	6
27	7		90	3
28	1		96	3
29	23		75	4
30	7		93	
average	12.5	0.6	85.4	2.0

4. B - 50

	Polymorphs	Eosinophils	Lymphocytes	Monocytes
Rat No.				
31	16		85	1
32	9		90	1
33	16	3	81	
34	21		77	2
35	10		87	1
36	13		86	2
37	23		68	6
38	9	8	78	5
39	8		90	2
40	27		70	2
average	15.2	1.1	81.2	2.2

APPENDIX XV

Percentages of Cells in the Arbitrary Categories* of Cells in the Femur Bone Marrow

Control Group

Category	1	2	3	4	5	6	7	8
Rat No.								
1	4.3	4.9	20.3	5.9	22.0	31.8	9.8	0.33
2	5.3	10.0	13.7	11.0	15.7	39.7	4.7	-
3	4.8	6.9	13.4	10.7	18.3	34.8	10.3	0.34
4	3.5	3.8	17.6	8.2	15.4	37.0	13.5	0.31
5	6.3	3.7	11.6	7.0	26.2	28.2	17.3	-
average	4.84	5.86	15.32	8.56	19.52	34.30	11.12	0.20

B - 0.5

Category	1	2	3	4	5	6	7	8
Rat No.								
11	6.6	3.0	15.0	12.3	16.9	34.9	9.6	0.66
12	5.9	5.3	19.8	6.3	10.2	36.3	14.5	0.33
13	3.7	4.9	10.4	12.3	17.2	35.6	8.9	0.92
14	5.3	2.3	13.0	17.3	19.3	31.9	10.6	0.33
15	3.3	7.7	11.3	7.0	18.3	32.3	19.7	0.70
16	6.5	5.3	8.5	6.3	10.3	43.3	18.7	0.33
17	4.1	4.8	12.4	9.9	18.2	35.0	15.6	-
18	4.1	3.2	11.7	13.6	20.6	35.7	9.2	1.30
19	7.2	6.5	16.6	17.8	18.5	24.0	7.6	1.10
20	3.5	0.9	13.5	9.6	22.0	37.7	11.6	0.63
average	5.02	4.39	13.22	11.25	17.15	34.67	12.60	0.63

*Categories:

1. Eosinophils - 10 - 15 microns, large red granules closely packed in cytoplasm, nucleus; blue, round, kidney or lobed.
2. Doughnut cells - (i) 15 - 20 microns, blue with red hole.
   (ii) 30 microns, large blue ring with light coloured nucleus.
3. Myelocytes - (i) 25 microns, light-coloured large nucleus (takes up most of the cell) and a light blue cytoplasm (ring effect)
   (ii) 15 microns, purple coloured mottled cell

APPENDIX XV (cont'd)

B - 5

Category	1	2	3	4	5	6	7	8
Rat No.								
21	8.7	3.1	16.1	14.6	19.6	29.2	7.8	
22	3.6	5.3	17.2	12.5	16.5	30.4	11.9	1.7
23	8.0	8.7	6.0	0.7	0.7	62.0	14.0	1.7
24	5.6	3.8	17.9	11.9	19.7	28.8	11.0	
25	6.6	7.0	16.2	15.9	23.8	21.5	7.9	0.33
26	4.3	5.0	15.6	10.3	23.9	26.2	10.6	0.33
27	3.0	3.3	13.3	5.3	10.3	38.3	24.7	
28	7.0	5.4	10.2	7.9	19.4	37.8	11.7	
29	4.4	5.9	13.1	8.4	24.4	33.4	10.3	
30	3.6	11.0	17.7	3.7	13.0	36.0	14.7	
average	5.48	5.85	14.33	9.12	17.13	34.36	12.46	0.41

B - 50

Category	1	2	3	4	5	6	7	8
Rat No.								
31	4.3	4.3	14.1	5.3	23.7	40.8	6.9	0.33
32	3.5	5.4	19.6	16.7	14.5	26.8	12.3	0.63
33	1.7	5.0	13.3	7.0	9.3	41.0	12.3	0.33
34	1.6	5.7	14.1	6.1	19.8	35.5	16.9	0.32
35	4.8	2.3	18.9	8.3	16.3	33.3	14.7	
36	3.4	5.6	20.9	8.4	14.3	28.3	15.3	0.31
37	4.5	6.1	13.7	8.9	22.3	32.8	9.6	1.6
38	5.7	5.7	12.3	4.3	10.6	50.3	11.0	0.33
39	4.7	8.7	15.3	9.7	9.3	35.7	17.0	
40	2.4	3.6	22.5	11.1	9.3	37.1	14.1	
average	3.66	5.24	16.47	8.58	14.94	36.16	13.01	0.39

Categories (cont'd)

4. Erythroblasts - 7 - 10 microns, light cytoplasm with dark blue nucleus.
5. Mature granulocytes - 10 - 15 microns, pink granular cytoplasm with nucleus varying from band to kidney.
6. Lymphocyte-like cells - 10 microns, small clear-coloured cells with no nucleus.
7. Nucleated red blood cells - 8 - 12 microns, small clear-coloured cells with a strand in the cytoplasm.
8. Megakaryocytes.

APPENDIX XVI

Standard Method Used for Counting and Differentiating Cells

1. Red blood cell counts

Erythrocytes were counted using Hayem's diluent, Thoma red cell diluting pipettes and an improved Neubauer counting chamber as discussed in Wintrobe (107).

2. White blood cell counts

The procedure as outlined by Wintrobe was followed. The leucocytes were counted using a diluting pipette marked 0.5, 1 and 11 and giving a dilution of 1 in 20, an improved Neubauer counting chamber and the following diluent:

glacial acetic acid	2 ml
distilled water	98 ml
a few drops of methylene blue	

3. Differential of leucocytes

Two blood smears were made from each animal. The slides were stained with Wright stain according to Wintrobe and 100 white blood cells were counted on each slide.

4. Reticulocyte counts

Blood films were made by mixing (50:50) blood with the following solution and then let stand for ten minutes before making smears on glass slides.

100 ml	0.85% saline
0.4 gm	sodium citrate
1 gm	brilliant cresyl blue

The number of reticulocytes were counted per 1000 red blood cells.

APPENDIX XVI (cont'd)

5. Differential of bone marrow cells

Bone marrow was removed from femurs when the animals were killed. An equal quantity of homoserum was mixed with the marrow and slides were made of the mixture. The slides were then stained with Wright stain and a total of 300 cells differentiated.