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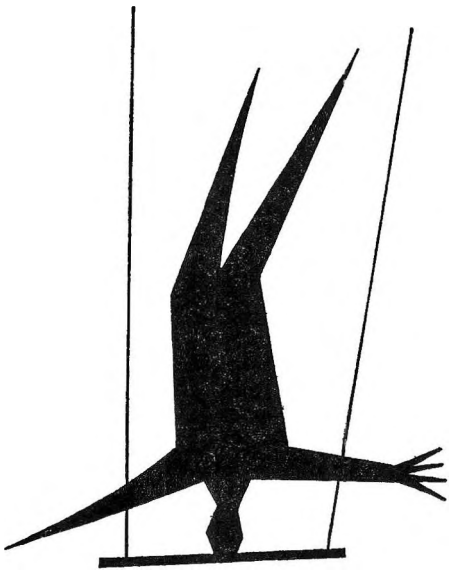
moderate low-fat and well-balanced food values in cereal and milk serving



Among breakfast main dishes, the cereal and milk serving merits consideration when a reduction of dietary fat is indicated because *it ranks low in fat and provides well-balanced food values.* Few foods at such small cost can better its moderate low-fat, well-balanced nutritional contribution as shown in the table below. Served with nonfat milk, the fat content is very low.*

nutritive composition of average cereal serving

	Cereal, 1 oz. Whole Milk, 4 oz. Sugar, 1 teaspoon	Cereal** 1 oz.	Whole Milk 4 oz.	Sugar 1 teaspoon
CALORIES	203	104	83	16
PROTEIN	7.3 gm.	3.1 gm.	4.2 gm.	
FAT	5.3 gm.	0.6 gm.	4.7 gm.*	
CARBOHYDRATE	32.2 gm.	22 gm.	6.0 gm.	4.2 gm.
CALCIUM	0.169 gm.	0.025 gm.	0.144 gm.	
IRON	1.5 mg.	1.4 mg.	0.1 mg.	
VITAMIN A	195 I. U.	—	195 I. U.	
THIAMINE	0.16 mg.	0.12 mg.	0.04 mg.	
RIBOFLAVIN	0.25 mg.	0.04 mg.	0.21 mg.	
NIACIN	1.4 mg.	1.3 mg.	0.1 mg.	
ASCORBIC ACID	1.5 mg.	—	1.5 mg.	
CHOLESTEROL	16.4 mg.	0	16.4 mg.*	

*Nonfat (skim) milk, 4 oz., reduces the Fat value to 0.1 gm. and the Cholesterol value to 0.35 mg.
**Based on composite average of breakfast cereals on dry weight basis.

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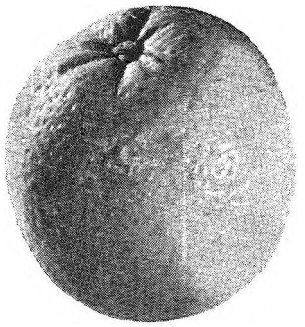

in the season of

acute infections, extra

CITRUS

provides the increased

VITAMIN C

and fluid needed during

FEVER

to prevent deficiency and

help maintain resistance*

*Tisdall and Jolliffe note the systemic relation in animals between vitamin C and resistance to infection, with increased needs evident in upper respiratory streptococcal infections.

– In: Clinical Nutrition ed. by Norman Jolliffe et al. New York, Paul B. Hoeber, Inc., 1950, pp. 590-91, 637-38.

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The metric system should be used in preference to others for measurements, weights, etc. Temperatures should always be expressed in Centigrade scale. Metric abbreviations listed below are shown in the approved lower-case style, *without periods:*

	Weight		*Length*
kg	kilogram	km	kilometer
gm	gram	m	meter
mg	milligram	cm	centimeter
μg	microgram	mm	millimeter
mμg	millimicrogram	μ	micron
μμg	micromicrogram	mμ	millimicron
		μμ	micromicron
	Volume		
m^3	cubic meter		
cm^3	cubic centimeter		*Area*
mm^3	cubic millimeter	m^2	square meter
l	liter	cm^2	square centimeter
ml	milliliter	mm^2	square millimeter

Symbols. When preceded by a figure, the following symbols are used:

A	Angstrom units	°	degree
%	per cent	ppm	parts per million

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Dietary Modification of Serum Cholesterol in the Chick[1]

B. E. MARCH AND JACOB BIELY with the *technical assistance of* J. TOTHILL AND S. A. HAQQ

Poultry Nutrition Laboratory,[2] The University of British Columbia, Vancouver, Canada

In mature roosters, dietary protein is more closely related to serum cholesterol levels than is dietary fat (Kokatnur et al., '58). Likewise in the growing chick low protein diets result in elevated plasma cholesterol (Nishida, Takenaka and Kummerow, '58; Johnson, Leveille and Fisher, '58).

Dietary fat may either increase or decrease blood cholesterol depending upon the level of fat fed, the degree of saturation of the fat, its fatty acid composition and the cholesterol content of the diet. Many reports of this have been reviewed by Felch et al. ('58).

Increasing the level of protein fed increases fat digestibility. This has been shown to be the case with the rat (Barnes, Primrose and Burr, '44), with dogs and rats (Magee, Kim and Ivy, '53), and with the chick (Biely and March, '57). Biely and March ('57) showed also that utilization of a more saturated fat (tallow) is affected more by dietary protein level than utilization of a less saturated one (corn oil). Since the effect of fat on blood cholesterol level differs among various fats and since protein level is a factor in the efficiency of utilization of fat, the following experiment was designed to compare the effect on serum cholesterol of different fats when fed in basal diets containing 20 and 26% of protein.

March and Biely ('57) observed that high levels of dietary fat reduced thyroid size in chicks. The reduction in thyroid size probably indicates a reduction in thyroid activity (Schultze and Turner, '45). Ershoff ('49) reviewed the work of several investigators who found that high-fat diets lowered metabolic rate, and reported additional experiments in support of a beneficial effect of fat for hyperthyroid animals. It is also recognized that there is some connection between thyroid activity and the level of circulating cholesterol (Oliver and Boyd, '58). In the following experiment the thyroid weights of the chicks were determined in order to ascertain if there were a three-way interrelationship among diet, thyroid activity, and serum cholesterol level.

EXPERIMENTAL

Two basal diets formulated to contain 20 and 26% of protein respectively were employed. The composition of the diets is shown in table 1. In the diets supplemented with 8% of various fats the fat was substituted for the cornstarch in the basal diets. The fats used with their iodine values (Hanus) were Crisco[3] (74), lard (62), chicken fat (78), unsalted butter (32), corn oil (127), and light-pressed herring oil (137). Crisco and the lard were labelled as being stabilized with antioxidants.

Each diet was fed to 22 Single-Comb White Leghorn male chicks kept under the customary conditions of management with ad libitum feeding. The chicks were weighed individually at weekly intervals until they were 5 weeks old. Serum cholesterol was determined by the method of Zlatkis, Zak and Boyle, ('53) when the chicks were 3 to 5 weeks old. A total of 35 determinations was made on chicks selected at random from each lot.

After the final weighing when the chicks were 5 weeks old, 10 chicks were taken at

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[1] This investigation was supported by a grant from the National Research Council (Canada).

[2] Contribution no. 114.

[3] Hydrogenated vegetable oil, Proctor and Gamble Co. of Canada, Ltd.

TABLE 1
Composition of basal diets

Ingredient	20% protein diet	26% protein diet
	lb. per 100 lb.	*lb. per 100 lb.*
Ground wheat	28.0	20.0
Ground yellow corn	31.75	23.0
Soybean oil meal (44% protein)	18.0	28.0
Cornstarch	8.0	8.0
Herring meal (70% protein)	5.0	12.0
Dehydrated cereal grass	2.0	2.0
Limestone	1.0	1.0
Bonemeal	1.75	1.5
Iodized salt	0.5	0.5
Choline chloride (25%)	0.4	0.4
Nicarbazine (25%)	0.05	0.05
	gm	*gm*
Manganese sulphate	6.0	6.0
Folacin	0.0375	0.0375
Riboflavin	0.2	0.2
Calcium pantothenate	0.6	0.6
Niacin	1.8	1.8
Menadione	0.027	0.027
Vitamin A	2000 I.U./lb.	2000 I.U./lb.
Vitamin D_3	120 I.C.U./lb.	120 I.C.U./lb.

TABLE 2
Body weights, thyroid weights and serum cholesterol values of chicks fed different fats

Supplementary fat	Av. wt. at 5 wks.	Thyroid wt.[1]	Serum cholesterol[2]	Serum cholesterol after feeding 1% cholesterol[1]
	gm	*mg/100 gm body wt.*	*mg %*	*mg %*
20% protein diet				
None	388	8.65	167	211
Herring oil	393	9.32	166	315
Corn oil	393	8.88	176	297
Chicken fat	414	7.87	183	319
Crisco	401	8.73	181	296
Lard	408	7.78	187	275
Butter	414	8.11	179	247
26% protein diet				
None	424	10.35	157	207
Herring oil	453	10.18	152	257
Corn oil	467	9.97	157	271
Chicken fat	449	8.98	166	273
Crisco	497	8.90	162	241
Lard	469	9.63	168	307
Butter	471	9.02	169	336

[1] Ten determinations. [2] Thirty-five determinations.

TABLE 3
Grouping of different fats according to their effect on serum cholesterol level[1]

20% protein fat	herring oil	control	corn oil	butter	Crisco	chicken fat	lard
26% protein diet	herring oil	control	corn oil	Crisco	chicken fat	lard	butter
20% protein diet, 1% cholesterol	control	butter	lard	Crisco	corn oil	herring oil	chicken fat
26% protein diet, 1% cholesterol	control	Crisco	herring oil	corn oil	chicken fat	lard	butter

[1] Average serum cholesterol levels with any two fats not underscored by the same line are significantly different ($P < 0.05$).

random from each lot and transferred to another set of brooder compartments. The transferred chicks were continued on the respective diets which they had been receiving with the difference that 1% of cholesterol was added to the diets. The birds remaining in the original brooder compartments were continued on the diets unsupplemented with cholesterol. After 48 hours, blood was taken for cholesterol determinations from chicks in all 28 lots.

After the blood for the final cholesterol determinations was taken, 10 of the birds fed the original diets throughout the experiment were killed. Individual body weights were recorded and the thyroid glands from each bird were removed and weighed. Thyroid size relative to body weight was determined separately for each bird.

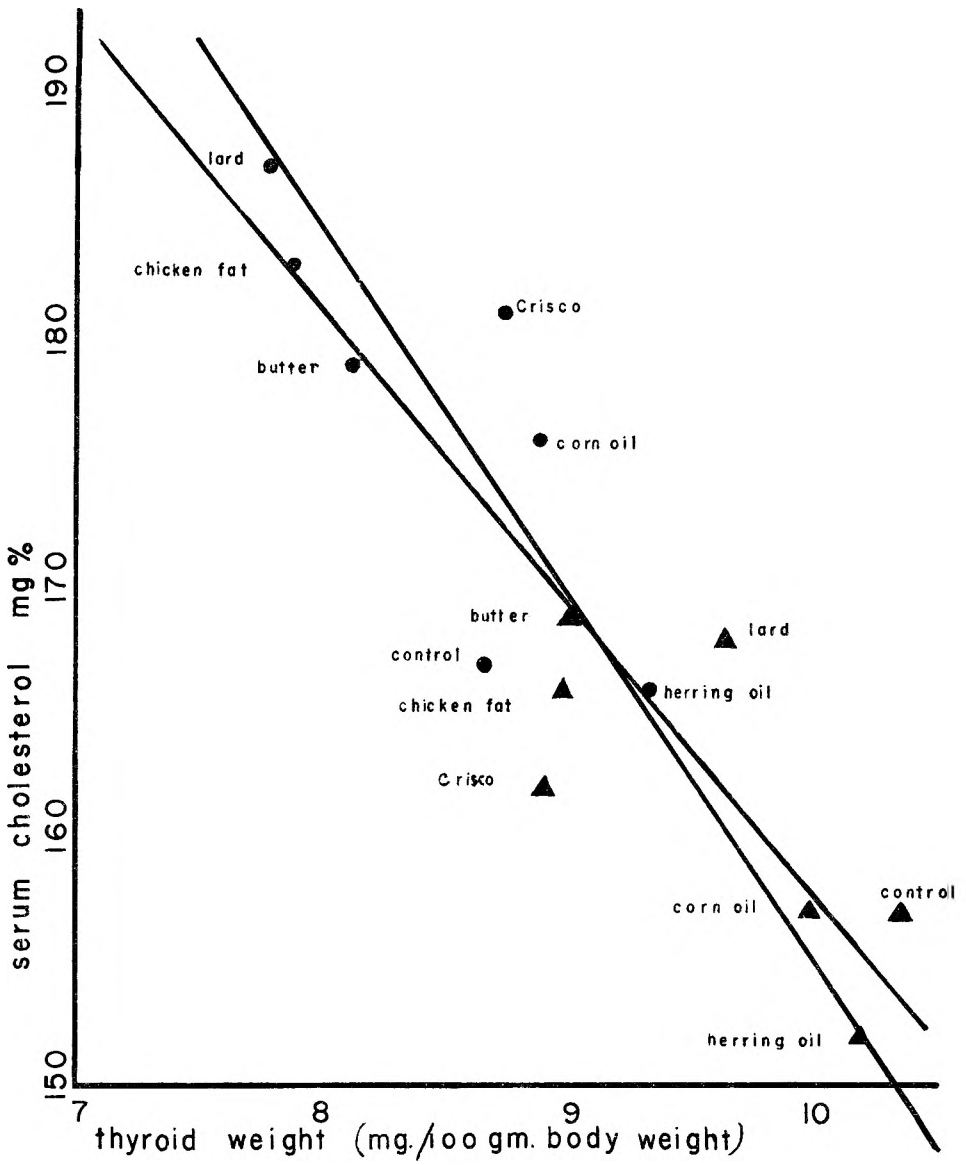

Fig. 1 Scatter diagram of the relationship between thyroid size and serum cholesterol level, r = −0.89. ● 20% protein diet; ▲ = 26% protein diet.

RESULTS

The 26% protein diet promoted a faster rate of growth than did the 20% protein diet (table 2). The difference between growth rate with the diets of different protein content was evident when the diets contained fat in place of cornstarch.

Serum cholesterol levels in the chicks fed the 20% protein diets were consistently higher than in the chicks fed the 26% protein diets. The values obtained are shown in table 3. The differences among them were analyzed statistically and grouped in table 3 according to the multiple range test of Duncan ('55). The effect of protein level on serum cholesterol level was apparent both in the chicks fed the basal diets and those fed the diets containing 8% of added fat. With the 20% protein diet, the inclusion of any of the fats tested, with the exception of herring oil, increased the level of serum cholesterol. When the basal diet contained 26% protein, Crisco, chicken fat, lard or butter increased serum cholesterol significantly.

One per cent of cholesterol fed to the chicks in the various groups for a 48-hour period elevated serum cholesterol in all instances. With the low-fat diets serum cholesterol level was similar whether the diet fed contained 20 or 26% of protein. When the diets contained supplementary fat there were marked differences in serum cholesterol level between the chicks receiving the respective levels of protein. As was the case with the diets without added cholesterol, chicks fed the lower protein diet showed a higher level of serum cholesterol than those fed the higher protein diet when the supplementary fat was Crisco, corn oil, herring oil or chicken fat. When the fat added to the diets was lard or butter, the chicks fed the higher protein level showed the higher levels of serum cholesterol.

The thyroid glands of the chicks fed the 26% protein diets were heavier than those of the chicks fed the 20% protein diets (table 2). Within each protein level there were further differences in thyroid size depending upon the particular fat used to raise the fat level of the diet. In most instances thyroid weight relative to body weight was decreased when the fat content of the diet was raised. However, herring oil did not affect thyroid size when fed in either the 20 or the 26% protein diet. The values for thyroid size and for serum cholesterol level showed a highly significant negative correlation (fig. 1).

DISCUSSION

In every instance increasing the protein level of the diet from 20 to 26% reduced the serum cholesterol level in the chicks fed various fats without additional cholesterol. This is in accord with the findings of other investigators mentioned earlier.

Herring oil did not elevate serum cholesterol in either the 20 or the 26% protein diet and, of the other fats tested, corn oil produced the least increase in serum cholesterol. The more saturated fats, Crisco, butter, chicken fat and lard all increased the cholesterol level when fed to the chicks not receiving a supplement of cholesterol in the diet.

When the chicks were fed 1% of cholesterol for 48 hours the influence of the type of fat contained in the diet differed from that observed in the chicks not receiving the cholesterol supplement. Stamler and Katz ('51), and Peterson et al. ('53), showed that fat increases cholesterol absorption in chicks. This has been shown to be the case with other species also.

Absorption of fats is more efficient in high- than in low-protein diets and the difference in absorption is more marked when the fat is relatively saturated (Biely and March, '57). In the present experiment, therefore, although absorption of corn oil and herring oil might be expected to be similar with both the 20 and 26% protein diets, the absorption of the other fats was probably greater at the higher protein level. It is accordingly probable that butter and lard, added to the 20% protein diet containing supplementary cholesterol, increased serum cholesterol to a lesser extent than did the other fats because butter and lard were not so well absorbed. Lin, Karvinen and Ivy ('55) showed that the percentage of dietary cholesterol absorbed generally varied with the absorption of the fat fed with the cholesterol. Because of poorer absorption from the intestine the absorption of cholesterol

would consequently be relatively poor in the diets containing these fats. When, on the other hand, lard or butter was added to the 26% protein diet with added cholesterol, serum cholesterol was elevated to a greater extent than with the other fats. If absorption was similar in the chicks fed the 26% protein diets, the various fats would then affect serum cholesterol in the same way as they did in the chicks fed the diets without supplementary cholesterol. That is, the predominating effect became that of the characteristic of the fats responsible for reducing the level of circulating cholesterol or that responsible for suppressing the removal of cholesterol from circulation, or both.

It thus appears from the above that two factors may be involved in the effect of dietary fat on serum cholesterol. When the diets contain a normal amount of cholesterol so that endogenous cholesterol is responsible for the major part of circulating cholesterol, the effect of fat is predominantly with respect to disposal of the cholesterol. When on the other hand, cholesterol is added to the diet, the amount of cholesterol absorbed from the intestine then plays the major role in determining the level of serum cholesterol. Accordingly the more readily utilized fats, although accelerating the withdrawal of cholesterol from the circulation may, when the diet contains high levels of cholesterol, increase absorption of cholesterol from the intestine to a greater extent than less readily utilized fats. This latter effect then predominates over the former so that an unsaturated fat may produce a higher level of serum cholesterol than a more saturated one.

When the protein level of the diet is high, the absorption of the more saturated fats is increased with the result that cholesterol absorption is similar with the saturated and unsaturated fats. The net result is that, as with diets not supplemented with cholesterol, the effect of fat on serum cholesterol is principally due to the effect on removal of cholesterol from the circulation.

The negative correlation between thyroid size and serum cholesterol level might be an indication that the fat and protein content of the diet had a direct effect on thyroid activity, or that, by modifying the cholesterol content of the serum there was an indirect effect on thyroid activity. There are reports in the literature that serum cholesterol and thyroid hormone have counteracting effects. Weiss and Marx ('55) have shown in animal experiments that thyroid hormone stimulates the degradation of cholesterol to acid derivatives. On the other hand, Winebrenner and Marx ('49) found that the addition of cholesterol to the diet of rats receiving desiccated thyroid partially reduced the metabolic rate but had no effect on the metabolic rate of rats not receiving thyroid.

A comparison of post-mortem findings in persons dying of coronary sclerosis and those dying from other causes showed a higher incidence of enlarged thyroid gland in the coronary sclerotic group and led to the suggestion "that hypothyroidism may be a cause and not an effect of arteriosclerosis" (Uotila, Raekallio and Ehrnrooth, '58). It has also been reported that dietary fat is beneficial to the hyperthyroid animal. Papers in this regard have been reviewed by Ershoff ('49), including one in which it is reported that protective effects of fats against the rise in metabolic rate following thyroid feeding are correlated with the degree of saturation of the various fats (Guerra, '47). Thyroid size in the chicks fed the high-protein diets was greater than in the chicks fed the low-protin diets. If the energy content of the diet is low relative to the protein content, protein is utilized to meet energy requirements. The data show that the ratio between protein and fat levels in the diet is more important than the absolute levels with respect to the effect on the thyroid gland. It appears possible that some of the dietary effects upon serum cholesterol levels may be mediated through the thyroid gland.

SUMMARY

Chicks were fed herring oil, corn oil, Crisco, lard, butter and chicken fat in diets containing 20 and 26% of protein. The chicks fed the higher protein level showed lower serum cholesterol levels than those fed the lower protein level whether or not the diet was supplemented with fat and regardless of the type of fat added to

the diet. With both protein levels Crisco, butter, lard and chicken fat increased serum cholesterol. Herring oil did not increase serum cholesterol with either diet and corn oil did so to a significant degree only in the case of the chicks fed the 20% protein diet.

When the chicks were 5 weeks old, 1% of cholesterol was fed in the respective diets for 48 hours. Serum cholesterol was markedly increased by the treatment in every instance with the chicks receiving supplementary fat showing higher cholesterol values than the chicks fed the diets without added fat. Butter and lard resulted in the least increase over the control diet with the chicks on the 20% protein diet, but the greatest increase with the chicks on the 26% protein diet. The differences in the effects of the various fats, depending upon the protein content of the diet are considered to result from increased absorption of the fats and cholesterol from the intestine in the chicks fed the higher level of protein.

The weights of the thyroid glands of chicks fed the various diets (without added cholesterol) were determined. There was a negative correlation between thyroid size and serum cholesterol level. Relative to body weight the thyroid glands were lighter in the chicks fed the lower level of protein. The data suggest that the effect of diet upon serum cholesterol level may be mediated to some extent through the thyroid gland.

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Effects of Excess Thiamine and Pyridoxine on Growth and Reproduction in Rats

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INTRODUCTION

Although studies on vitamin interrelationships have shown little or no effect of excess thiamine or pyridoxine in growing rats (Scott and Griffith, '58; Morrison and Sarett, '59), the study of Richards ('45) and the report of Hunt et al. ('54) suggest that excess amounts of either of these vitamins may have deleterious effects in pregnancy. Richards ('45) found that administration of excess thiamine to female rats fed a white flour-casein diet, simulating a low-quality human diet, had no effect on growth, but adversely affected reproduction, as evidenced by high mortality and poor growth of the young. Convulsions similar to those found in pyridoxine deficiency were observed in some of the offspring, and could be prevented by giving the dams additional pyridoxine during lactation. However, signs of other deficiencies were also observed in the young rats. Hunt et al. ('54) reported that an infant, born to a mother who had received excess pyridoxine during pregnancy, exhibited convulsions which responded to pyridoxine. They postulated that excess pyridoxine intake during pregnancy may increase the pyridoxine requirement of the infant.

The present experiment was conducted to determine whether excess amounts of thiamine or pyridoxine or both affected growth, reproductive performance and vitamin stores in female rats fed an adequate diet, and to what extent these high levels of vitamins in the mothers' diet affected weight gain and vitamin stores in the young when raised on a pyridoxine-deficient diet.

MATERIAL AND METHODS

The basal 18% casein diet used in the experiment was similar to that of Sarett and Snipper ('54) except that sucrose was used as the carbohydrate, and ascorbic acid was omitted from the vitamin mixture. In addition, the levels of thiamine·HCl and pyridoxine·HCl were changed to 150 μg per 100 gm of diet. These levels are slightly greater than those listed by Brown and Sturtevant ('49) as the levels required by the growing rat. Four comparable groups of 12 female weanling rats each (McCollum-Wisconsin strain) were carefully selected from 19- to 21-day-old animals weighing approximately 50 gm. The animals were individually housed in screen-bottom cages and given the basal diet (diet 1), or this diet supplemented with excess thiamine (diet 2), excess pyridoxine (diet 3), or excess thiamine and pyridoxine (diet 4). Excess thiamine and pyridoxine were added at 50 times the levels used in the basal diet.

Records were kept of the amounts of food and water consumed by each rat, and the animals were weighed individually at weekly intervals. After the animals had been on experiment for 12 weeks, they were mated with stock males of proven fertility. As soon as possible after each litter was born, the number and weight of the young were recorded. When necessary, the number of young per litter was reduced to 8 at 5 days of age.

When the young had reached 21 days of age, the dams were sacrificed (without fasting) by intraperitoneal injection of Nembutal[1] solution and the livers, kidneys and adrenals were removed and weighed. The livers of the dams fed each diet were pooled and analyzed for solids, total lipid, thiamine, riboflavin, pyridoxine, pantothenate and vitamin B_{12}. The levels of solids and total lipid were determined by

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[1] Abbott.

the method of Sarett and Jandorf ('47). Thiamine was determined by the thiochrome method (Pharmacopeia, '55), while riboflavin was assayed fluorometrically (Pharmacopeia, '55). Pyridoxine was extracted by autoclaving the samples at 121°C for 5 hours in 0.055 N HCl, according to the method of Rabinowitz and Snell ('47), and assayed microbiologically with *S. carlsbergensis*. Pantothenate was determined microbiologically with *L. plantarum* by the method of Toepfer et al. ('54), following release of bound forms of the vitamin by the double enzyme system of Novelli et al. ('49). Vitamin B_{12} was extracted with a buffered cyanide solution according to the procedure described by Gregory ('54), and assayed using *L. leichmannii* (Pharmacopeia, '55).

At weaning (19 to 21 days of age) representative young were killed and the livers of the animals from each group were pooled (sexes kept separate) and analyzed for solids, total lipid, thiamine and pyridoxine, by the methods outlined above. The remaining animals were individually housed in screen-bottom cages and were given a diet similar to diet 1, except that pyridoxine was omitted, and the level of thiamine was increased to 200 μg per 100 gm of diet. The weight gains of these animals were followed. After two and 5 weeks, one-third of the surviving males and females from each group were sacrificed. The livers were pooled as before and analyzed for solids, total lipid, thiamine and pyridoxine. The data on liver solids and total lipid levels in these animals showed no effect of maternal diet and are not reported herein. Most of the remaining rats died before the planned 8-week terminal analyses could be performed.

RESULTS AND DISCUSSION

Data on food and water intakes, weight gains and food efficiency values obtained before the animals were mated are summarized in table 1. In 12 weeks, the animals fed the control diet (diet 1) gained 145 gm, with an average food efficiency of 17.9 gm gain per 100 gm food intake. Addition of excess thiamine (diet 2) or excess pyridoxine (diet 3) had no significant effect on weight gain or food efficiency. However, supplementation with high levels of both thiamine and pyridoxine (diet 4) resulted in slightly greater weight gain and somewhat increased efficiency of food utilization. Although these differences were not significant, the results suggest that the thiamine and pyridoxine levels in diet 1 may have been borderline for maximum growth.

The data on reproductive performance (table 2) show that the average birth

TABLE 1

Data on weight gains, food and water intakes and food efficiencies of female rats fed diets containing various levels of thiamine and pyridoxine for 12 weeks

	Diet 1 Adequate thiamine, pyridoxine	Diet 2 Excess thiamine	Diet 3 Excess pyridoxine	Diet 4 Excess thiamine, pyridoxine
No. of survivors[1]	11	12	12	11
Initial weight, *gm*	49	49	49	49
6 weeks				
Weight gain, *gm*	106 ± 11[2]	109 ± 12	112 ± 9	116 ± 16
Food intake, *gm*	372	380	387	393
Water intake, *ml*	557	658	664	583
Food efficiency[3]	28.5 ± 2.1	28.8 ± 1.8	29.0 ± 2.6	29.5 ± 2.8
12 weeks				
Weight gain, *gm*	145 ± 19	146 ± 15	153 ± 14	158 ± 19
Food intake, *gm*	811	816	827	827
Water intake, *ml*	1354	1702	1620	1443
Food efficiency[3]	17.9 ± 1.4	18.0 ± 1.1	18.6 ± 1.8	19.2 ± 1.8

[1] Each group contained 12 animals initially.
[2] Standard deviation.
[3] Average grams gain per 100 gm food intake.

TABLE 2

Data on reproductive performance of female rats fed diets containing various levels of thiamine and pyridoxine

No. and description of diet	No. of animals	No. of litters	No. of young	Av. birth weight of young	Survival data				Av. weight of young, 21 days
					5 days		21 days		
				gm	*no.*	*%*	*no.*	*%*	*gm*
1. Adequate thiamine, pyridoxine	10	6	44	6.5	43	98	32	73	37
2. Excess thiamine	12	11	75	6.3	72	96	57	76	43
3. Excess pyridoxine	11	9	75	6.1	67	89	56	75	41
4. Excess thiamine, pyridoxine	10	9	87	6.2	78	90	56	64	39

weight, survival of the young to weaning, and average weight of the young at weaning were not significantly influenced by addition of excess thiamine or pyridoxine or both to the basal diet. However, the animals which received excess thiamine and pyridoxine (diet 4) had more young per litter than those fed the other diets. The lack of any deleterious effect of excess thiamine on reproduction observed in the present study is in contrast to the findings of Richards ('45). The discrepancy in the results may be due, in part, to the relative inadequacy of the diet used by Richards, since Morrison and Sarett ('59) have found that excess amounts of a single B vitamin may retard weight gain of animals fed diets low in several B vitamins, but usually have no effect in animals fed adequate diets.

The data on organ weights of the post-parturient females showed some hypertrophy due to pregnancy, but little effect of vitamin excess. The liver weights averaged 4.6 to 5.1% of the body weight, and the adrenal glands averaged 21.3 mg to 23.5 mg per 100 gm body weight. The kidney weights of the animals on diets 2, 3 and 4 averaged 0.73 to 0.78% of body weight, whereas those of the rats on diet 1 were slightly heavier, namely 0.82% of body weight.

The levels of solids and total lipid in the livers of the post-parturient females (table 3) were not significantly influenced by addition of excess amounts of thiamine or pyridoxine or both to the basal diet. The levels of riboflavin, pantothenate and vitamin B_{12} were also not significantly influenced by the diet fed. The addition of excess thiamine to the basal diet increased liver thiamine levels from 2.6 to 8.7 μg per gm. In contrast to the findings with thiamine, the addition of excess pyridoxine to the diet had no significant effect on liver pyridoxine levels. Other workers have also found that increasing the level of thiamine in the diet above that needed for maximum growth, increases tissue concentrations of the vitamins (Ochoa and Peters, '38; Byerrum and Flokstra, '51), whereas the concentration of pyridoxine in the liver of growing rats is not influenced by addition of pyridoxine to an adequate diet (Sheppard and McHenry, '46). Excess pyridoxine apparently had no effect on liver thiamine levels, nor did excess thiamine influence the level of pyridoxine in the liver.

The data on weight gains of the young on the pyridoxine-deficient diet are summarized in table 4. The young of mothers which received excess pyridoxine (diets 3 and 4) gained significantly more weight than did those of mothers which received the basal diet. These findings do not support the hypothesis of Hunt et al. ('54) that administration of excess pyridoxine to the mother during pregnancy increases the pyridoxine dependency of the young. The presence of excess thiamine in the maternal diet (diet 2) appeared to slightly increase weight gain in the young fed a pyridoxine-deficient diet.

The data on thiamine and pyridoxine levels in the livers of the young at weaning, and after two and 5 weeks on the pyridoxine-deficient diet, are summarized in table 5. Liver thiamine levels in the young at weaning reflected maternal thiamine intakes, in a manner similar to that found in the livers of the mothers. The pyridoxine levels of the livers of the young at weaning also reflected maternal pyridoxine intake, although those in the mothers showed no effect of excess pyridoxine (table 3). At weaning, the young of ani-

TABLE 3

Data on liver composition of post-parturient female rats fed diets containing various levels of thiamine and pyridoxine

	Diet 1 Adequate thiamine, pyridoxine	Diet 2 Excess thiamine	Diet 3 Excess pyridoxine	Diet 4 Excess thiamine, pyridoxine
Number of animals per group	6	11	8	9
Solids, %	30.5	29.4	29.3	29.4
Total lipid (ether extract), %	2.7	2.7	2.4	3.3
Thiamine, $\mu g/gm$	2.6	8.7	2.3	8.4
Riboflavin, $\mu g/gm$	33.3	31.5	28.8	29.1
Pyridoxine, $\mu g/gm$	11.2	10.2	10.4	11.0
Pantothenate, $\mu g/gm$	61.2	56.4	56.4	56.1
Vitamin B_{12}, $m\mu g/gm$	133.5	133.8	131.7	131.7

TABLE 4

Influence of maternal diet on weight gain of male and female weanling rats fed a pyridoxine-deficient diet for 5 weeks

	Maternal diet			
	Diet 1 Adequate thiamine, pyridoxine	Diet 2 Excess thiamine	Diet 3 Excess pyridoxine	Diet 4 Excess thiamine, pyridoxine
Males				
Two weeks				
No. of animals	12	12	12	12
Weight gain, *gm*	11	16	31	32
Five weeks				
No. of animals	5	8	8	8
Weight gain, *gm*	12 ± 7[1]	24 ± 8	56 ± 6	55 ± 7
Females				
Two weeks				
No. of animals	12	12	12	12
Weight gain, *gm*	11	14	33	30
Five weeks				
No. of animals	6	7	8	8
Weight gain, *gm*	14 ± 9	18 ± 7	50 ± 7	47 ± 3

[1] Standard deviation.

mals fed excess thiamine (diets 2 and 4) had higher liver thiamine levels than did their mothers. However, in the animals which received excess pyridoxine (diets 3 and 4), the levels of pyridoxine in the liver were lower in the young at weaning than in the mothers, suggesting limited placental transfer of this vitamin. In man, placental transfer of vitamin B_6 also occurs, as shown by the work of Wachstein et al. ('57), who found that administration of pyridoxine to pregnant women resulted in elevated pyridoxal phosphate levels in the umbilical cord blood at birth. During the 5-week period on the pyridoxine-deficient diet, the greater liver vitamin stores in the young of mothers fed excess thiamine or pyridoxine were decreased to the levels found in the young of mothers fed the basal diet.

SUMMARY

An experiment was conducted to determine whether excess thiamine or pyridoxine or both affect growth and reproduction in rats receiving an otherwise adequate diet. Groups of female weanling rats were fed a basal diet containing 150 μg of thiamine and of pyridoxine per 100 gm of diet, alone or supplemented with 50 times this level of thiamine or pyridoxine or both. After a 12-week growth period, the

TABLE 5

Influence of maternal diet on levels of thiamine and pyridoxine in the livers of male and female weanling rats fed a pyridoxine-deficient diet for 5 weeks

	Maternal diet			
	Diet 1 Adequate thiamine, pyridoxine	Diet 2 Excess thiamine	Diet 3 Excess pyridoxine	Diet 4 Excess thiamine, pyridoxine
Males				
Thiamine, μg/gm				
Initial	1.9(6)[1]	14.7(17)	1.8(9)	12.0(16)
Two weeks	2.5(4)	5.7(4)	2.6(4)	6.0(4)
Five weeks	1.6(2)	1.8(4)	2.1(4)	2.5(4)
Pyridoxine, μg/gm				
Initial	4.8(6)	5.8(17)	8.8(9)	8.7(16)
Two weeks	3.6(4)	4.0(4)	5.1(4)	5.0(4)
Five weeks	5.6(2)	4.0(4)	5.3(4)	4.8(4)
Females				
Thiamine, μg/gm				
Initial	2.0(9)	14.4(16)	1.7(15)	12.6(8)
Two weeks	2.6(4)	6.0(4)	2.7(4)	6.0(4)
Five weeks	1.6(3)	2.0(3)	2.0(4)	1.8(4)
Pyridoxine, μg/gm				
Initial	5.2(9)	5.5(16)	9.1(15)	9.7(8)
Two weeks	3.9(4)	4.0(4)	4.6(4)	5.1(4)
Five weeks	2.9(3)	3.1(3)	5.2(4)	4.2(4)

[1] Figures within parentheses indicate number of animals sacrificed to obtain pooled samples of liver.

animals were mated to stock males for study of reproductive performance. The mothers were sacrificed after the young were weaned, and liver vitamin levels were determined. The young were given a pyridoxine-deficient diet for 5 weeks, and growth and liver thiamine and pyridoxine levels were measured at intervals.

The results showed that excess thiamine or pyridoxine or both had no effect on weight gain, reproductive performance or the levels of solids, total lipid, riboflavin, pyridoxine, pantothenate and vitamin B_{12} in the livers of the animals after parturition and lactation. Liver thiamine levels in these animals, and in their young at weaning, were markedly increased by excess thiamine. Liver pyridoxine levels in the mothers were not increased by excess dietary pyridoxine, although pyridoxine stores in the livers of the young at weaning were greatly increased by excess pyridoxine intake of the mothers.

During 5 weeks on a pyridoxine-deficient diet, the young of mothers which had received excess pyridoxine gained significantly more weight than did those of mothers which had received the basal diet.

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Protein Depletion and Amino Acid Requirement in the Growing Chicken[1]

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Previous studies with the adult rooster (Leveille and Fisher, '58) have shown quantitative differences in the requirement for maintenance of nitrogen balance between protein-depleted and non-depleted animals. Allison ('51), working with dogs, and Scrimshaw et al. ('58), in their studies of growing infants and children, recognized the possibility that the optimum pattern of amino acid requirement may vary with the "physiological state" of the individual. Indirect evidence for such a possibility was presented by Bressani et al. ('58) who observed that children recovering from kwashiorkor went into positive nitrogen balance on a corn masa diet when either of the two most limiting amino acids (tryptophan or lysine) was added to the diet. In normal animals the addition of only one of two equally limiting amino acids will not improve, but will, in fact, further decrease the value of the protein mixture.

The present experiments were undertaken in an effort to elucidate the effect of "physiological state" (protein depletion) on the methionine and lysine requirement of the rapidly growing chicken. Although protein depletion in the growing chicken will be shown to increase the requirement for both amino acids, data are presented which resolve these findings on the basis of a greater total protein requirement and a relatively constant amino acid-protein proportionality.

EXPERIMENTAL AND RESULTS

In all three trials, day-old male chicks were fed a standard 22% protein starting ration for two weeks.[2] At the end of this period, half of each group, selected at random, was placed for one week on a nitrogen-free purified ration (for composition see table 1) while the remaining half was continued on the 22% protein starting ration. After the third week both the protein-depleted and non-depleted chicks were assigned, on a weight basis, to their respective lots. In both trials 1 and 2, 10 lots of 10 birds each were fed 5 graded levels of the critical amino acid (methionine or lysine) for each of the two protein-depletion states. The basal diets as shown in table 1 were formulated to contain 20% protein.

Table 2 shows the growth response to the different amino acid levels for the protein-depleted and the non-depleted birds. While the depleted birds responded with increased growth to each additional increment of the amino acid under study, the growth response of the non-depleted birds appears to plateau at the second level of amino acid supplied.

Trial 3 was designed to test the following hypothesis as a possible explanation for these findings: The non-depleted birds might require less than the 20% protein that had been provided in the experimental diets for trials 1 and 2, and therefore utilize lysine or methionine only to the extent of their optimum protein requirement. The depleted birds, on the

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[2] Composition in pounds per ton: ground yellow corn, 1080; soybean oil meal (50%), 680; alfalfa meal, dehydrated, 60; dried distillers' solubles, 80; butyl fermentation product, 20; dicalcium phosphate, 44; limestone, 16; salt, 10; mineral concentrate, 4; vitamin A and D concentrate (1,000,000 I.U. vitamin A and 250,000 I.C.U. vitamin D/lb.), 4; antibiotic supplement, 1; vitamin B_{12} concentrate (20 mg vitamin B_{12}/lb.), 1; 2110 gm choline chloride; 10 gm niacin.

other hand, representing "physiologically younger" animals, would utilize and require all the protein offered (20%) and would thus require proportionately more of the limiting amino acid under study.

As shown in table 3, groups of non-depleted birds were fed 5 levels of protein varying from 14 to 22% in two series. One series was supplemented with lysine in such a way that this amino acid constituted 1% of the diet at each protein level (the amount previously found optimal). In the second series lysine was adjusted at 5% of the protein level (on the basis of 1% lysine on the previously fed 20% protein diet). Similarly, for protein-depleted chickens two series of protein levels were fed varying from 16 to 24%, one series supplemented at a constant 1.2% of the diet and the second at a constant

TABLE 1

Composition of experimental rations

Ingredient	Nitrogen-free ration	Ration for trial 1[1]	Ration for trials 2 and 3[2]
	%	%	%
Fiber	3.00	3.00	
Corn oil	3.00	3.00	3.00
Mineral mix[3]	5.34	5.34	5.34
B vitamins[3]	0.15	0.15	0.15
Vitamins A, D and E[3]	0.10	0.10	0.10
Choline Cl	0.20	0.20	0.20
Soybean protein[4]		23.40	
Glycine		0.50	
L-Cystine		0.10	
Dextrin	44.10		
Sesame meal			41.90
Alfalfa meal			3.00
DL-Methionine			0.10
L-Lysine·HCl (95%)			0.34
Glucose (cerelose)			

[1] Calculated methionine content is 0.20% and cystine content 0.22%.
[2] Calculated lysine content is 0.80%.
[3] For composition see Fisher and Johnson ('56).
[4] Assay protein C-1.

TABLE 2

Final body weights of protein-depleted and non-depleted chicks fed graded levels of methionine or lysine

Amino acid level	Non-depleted[1] chicks	Depleted[1] chicks
%	*gm*	*gm*
	Methionine	
0.20	627 ± 26[2]	308 ± 24[2]
0.25	680 ± 17	377 ± 22
0.30	645 ± 18	395 ± 13
0.35	683 ± 20	420 ± 25
0.40	699 ± 20	445 ± 26
	Lysine	
0.8	545 ± 18[3]	297 ± 17[3]
0.9	617 ± 13	365 ± 18
1.0	635 ± 17	380 ± 17
1.1	620 ± 13	418 ± 13
1.2	626 ± 13	446 ± 12

[1] Averages for lots of 10 chicks ± standard error of the mean.
[2] Average weights of Vantress cockerels at 5 weeks of age. Average lot weights at start of experiment (three weeks of age): non-depleted 351 gm, depleted 159 gm.
[3] Average weight of crossbred (New Hampshire ♂ × Columbian ♀) cockerels at 34 days of age. Average lot weights at start of experiment (three weeks of age): non-depleted 283 gm, depleted 136 gm.

TABLE 3

Response of protein-depleted and non-depleted chicks to different protein and lysine levels

Protein	Lysine	Weights[1]	Lysine[2]	Weights[1]
%	%	*gm*	%	*gm*
		Non-depleted birds		
14	1.0	618 ± 12	0.7	582 ± 16
16	1.0	691 ± 10	0.8	651 ± 16
18	1.0	666 ± 20	0.9	667 ± 15
20	1.0	679 ± 15[3]	1.0	679 ± 15[3]
22	1.0	677 ± 14	1.1	701 ± 16
		Protein-depleted birds		
16	1.2	416 ± 12	0.96	399 ± 10
18	1.2	440 ± 19	1.08	434 ± 16
20	1.2	469 ± 13[3]	1.2	469 ± 13[3]
22	1.2	437 ± 14	1.32	478 ± 15
24	1.2	476 ± 10	1.44	508 ± 12

[1] Duplicate groups of 6 chicks each; when the birds were placed on the above diets (after the three-week preliminary period) they weighed 348 and 160 gm for the non-depleted and depleted chicks respectively. The above weights were recorded after 13 days on the experimental diets.

[2] Lysine at a constant percentage of the protein: 5% and 6% respectively, for the non-depleted and depleted groups.

[3] For the non-depleted series the 20% protein, 1% lysine level and for the depleted series the 20% protein, 1.2% lysine level were included only once in the experimental design.

6% of the protein. If the above hypothesis were correct the non-depleted birds should perform equally well at a protein level lower than 20% but containing the same absolute amount of lysine (1%) as was previously shown necessary on the 20% protein diet. They should not perform as well at lower protein levels containing lysine at 5% of the protein since the lysine requirement for both depleted and non-depleted birds is expected to be alike when expressed as a percentage of the protein. By the same token, the depleted birds when fed levels of protein lower than 20% should not perform as well on lower levels of protein with lysine held constant at 1.2% of the diet. The depleted birds might actually perform better at a protein level above 20% with the lysine content at 6% of the protein (the same ratio which had previously resulted in optimum growth, namely 1.2% lysine and 20% protein).

The data in table 3 support the hypothesis advanced. The non-depleted chicken required no more than 16% protein in combination with 1% dietary lysine (or 6.2% when expressed on a protein basis). The depleted chicken required approximately 20% protein when the diet contained 1.2% lysine (or 6% on a protein basis). These chickens also responded to higher protein levels *provided the lysine: protein ratio remained constant.*

DISCUSSION

The results of these studies indicate that protein-depleted chickens require higher absolute levels of methionine and lysine than non-depleted birds of the same age. However, this greater need for amino acids appears to be only a reflection of a greater protein requirement. Thus, the lysine requirement of the depleted chicken, when expressed as a percentage of the protein level required, remained essentially unchanged. It appears reasonable that for purposes of growth the protein-depleted chick has an increased nitrogen requirement since this requirement is inversely related to body size. The effect of protein depletion on the maitenance requirements in the adult rooster (Leveille and Fisher, '58) was to decrease the protein needs for positive nitrogen balance. It is possible that the reduced amount of body protein in the mature and depleted animal is responsible for this observation.

Children, compared with growing animals, exhibit an unusually prolonged period of slow growth; it might therefore be expected that the amino acid requirements

of kwashiorkor cases would follow a less predictable course than that observed in the depleted chick. During early stages of recovery, the requirements of protein-depleted children might well depend to a larger extent upon their maintenance than upon their growth requirements. It has yet to be determined to what extent protein depletion as defined for the experimental animal[3] correlates with the depletion state of children suffering from kwashiorkor.

SUMMARY

The methionine and lysine requirement of normal and protein-depleted chicks was determined on diets containing 20% of protein. For both amino acids a higher absolute requirement could be demonstrated for the depleted birds. Data are presented which explain these findings on the basis of a greater protein requirement for the depleted chicks. Thus, for lysine, the requirement for depleted and non-depleted chicks remained essentially constant when expressed on the basis of the dietary protein level required for maximum growth.

ACKNOWLEDGMENTS

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[3] In the case of the mature rooster (Leveille and Fisher, '58) the animals were depleted of their protein reserves until a constant and minimal nitrogen excretion was reached. The growing chicks averaged a 24-gm loss in weight during the one-week depletion period.

An Experimental Study of Nail Growth

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This paper is concerned mainly with the linear growth rate of the nail of the rat. A simple method of measuring this parameter is given, together with results obtained when the animals are subjected to different experimental treatments. The object of the work was to extend certain observations on nail growth that have been made in the past, almost exclusively on the human subject, and in particular to confirm some of these observations in the experimental animal, where conditions can be controlled more rigorously. In the discussion at the end of the paper an attempt is made to evaluate the physiological importance of nail growth in relation to current problems in research, and to suggest further avenues for investigation, some of which are already giving interesting results.

METHOD

Measurement of nail growth in the human is relatively simple; Babcock ('55) deals with a variety of available techniques. To obtain growth rates for the nail of the rat did not prove to be a simple matter; because of this, and because the early results given in this paper were obtained by a method which was subsequently revised, a brief account of the development of the method in current use will be given.

Examination of the claw of the anaesthetized rat suggested that the major problem was the establishment of a reference point in the dermis or epidermis. Hamilton, Hollander and Andervont ('58), studying nail growth in the mouse, made a tattoo on the dorsal surface of one phalanx of the paw, and used this as a reference point to measure the peripheral migration of a scratch on the dorsal surface of the nail. This is the only other paper known in which nail growth has been measured in an experimental animal. In the rat another method suggested itself since the lateral aspect of the claw, when viewed under the dissecting microscope, is relatively flat, a cross section of the nail is shown diagrammatically in figure 1.

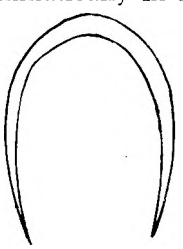

Fig. 1 Cross section through rat's nail.

The animal was anaesthetized lightly with ether, or wrapped tightly in a cloth with just its left rear limb free, then laid on its side beneath the dissecting microscope. The digit under inspection was gently pressed into a small piece of modelling clay.[2] This method of holding the digit has been used throughout and has been found most satisfactory.

The first type of marking was simply a small nick in the nail where the lower edge meets the digital pad, see figure 2 at a. The distance this mark moved away from the front of the pad was measured after a number of days. Bleeding, and cracking of the nail sometimes occurs, however, so this method was given up, since these changes might interrupt normal growth of the nail.

The second method adopted was to make three marks in the digital pad, and a cross in the nail substance, see b_1, b_2, b_3 and c, in figure 2.

The growth of the nail was measured by observing the distance that the cross c

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[2] Plasticine.

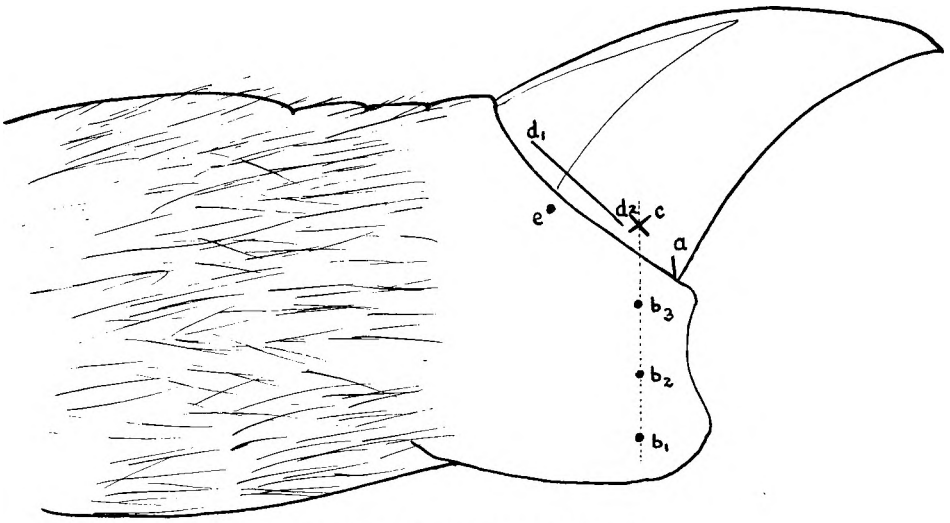

Fig. 2 Line drawing of rat's nail.

moved away after a few days from the vertical line b_1, b_2 and b_3. This method, although accurate, was tedious.

Finally it was found that a line made by a shallow cut with the scalpel at d_1–d_2 in figure 2, adjacent to the line of junction between nail and epidermis remained parallel during its migration for the next few days. The skin just behind the nail is firm, on this lateral aspect, so a puncture was made at e, using a fine sewing needle held in a pair of "Mosquito" forceps. The puncture was just deep enough to penetrate the epidermis so that a reasonably permanent mark could be made in the dermis with a concentrated solution of Evans Blue solution applied to the skin with a camel's-hair brush. By the second day the marks had become clearly defined and could be measured accurately; readings were obtained with a Filar ocular micrometer. Two or three days later the distance between the marks was again measured and the daily rate of growth calculated. Three separate readings were made each time of the distance between marks, and an average value calculated.

Figure 3 is a photograph showing the marks in place.

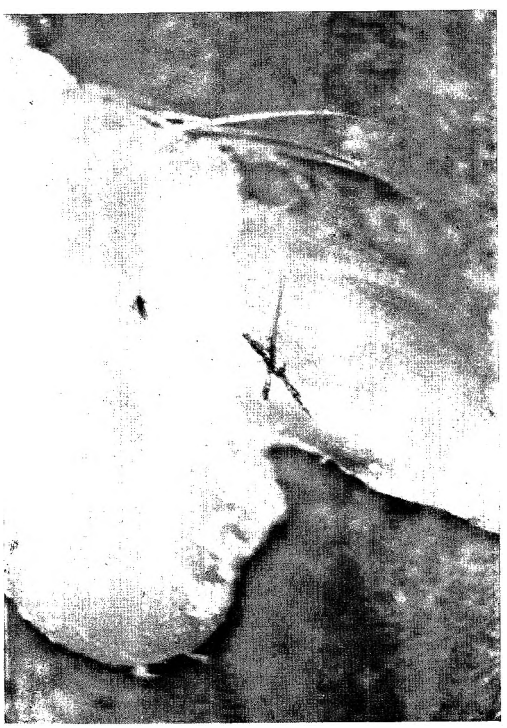

Fig. 3 Photograph showing marks for measuring nail growth.

RESULTS

The linear rate of growth of the nail of the rat was measured in animals under the following conditions: when fed a stock diet; given diets of different biological value, for 10 days; maintained on "low 8% protein diet"[3], with and without vita-

[3] Supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio.

min A; maintained on "vitamin A test diet,"[3] those of a second group were fed the same diet but were given adequate vitamin A; kept in a hot or cold environment; given cortisone or ethionine daily; restrained for 24 hours; kept in cages where they were exercised. Comparisons were made in all cases between animals of the same litters, litters being equally distributed between the control and experimental diets; sexes were evenly distributed wherever possible.

Table 1 gives results obtained using diets of different Net Dietary-Protein Value (Platt and Miller, '58); the first method of measurement was used. There is a correlation ($+ = 0.62$, $P > 0.02$) between Net Dietary-Protein Value and the linear rate of nail growth. Diets containing uncooked haricot bean (*Phaseolus vulgaris*) caused marked reduction, or even cessation, of nail growth; however, it is known that toxic factors are present in these beans.

TABLE 1

Influence of dietary protein on linear rate of nail growth in the rat

No.	Diet[1]	N.D.-p.V.[2]	Nail growth
			mm/24 hrs.
1	Non-protein	0	0.045
2	Non-protein	0	0.044
3	Cassava	0.5	0.031
4	Cassava and *Vigna sincusis*	1.3	0.045
5	10% *V. sincusis*	1.6	0.078
6	Yam and *V. sincusis*	1.7	0.048
7	Cassava and coconut	1.9	0.044
8	Cassava and fish	3.1	0.057
9	5% fish	3.7	0.089
10	Yam and fish	3.9	0.062
11	10% peanut	4.5	0.044
12	Maize and coconut	4.7	0.069
13	10% casein	7.6	0.072
14	Cassava and milk	8.1	0.059
15	10% egg	8.7	0.091
16	10% *Phaseolus vulgaris*	—[3]	0.000
17	5% *P. vulgaris*	—[3]	0.000
18	2% *P. vulgaris*	—[3]	0.044

[1] Diets 5, 9, 11, 13, 15–18 were as diet 1 but with starch replaced by the materials indicated at such a level that they contained the percentage of protein shown.

Diets 3, 4, 6–8, 10, 12 and 14 were human dietaries; the main constituents (staple + chief protein source) are indicated.

[2] N.D.-p.V. = Biological Value × digestibility × N × 6.25. It is a measure of the utilizable protein (Platt and Miller, '58).

[3] Diets 16, 17 and 18 contained decreasing amounts of raw haricot bean; a toxic factor is present.

TABLE 2

Effect of vitamin A deficiency and protein shortage on nail growth in the rat

Dietary group[1]	No. of rats	Mean growth	S.D.	P value grid			
				1	2	3	4
		mm/24 hrs.					
1. Control[2]	9	0.1060	±0.027		0.01	0.01	0.01
2. Vitamin A test[3] + vitamin A	8	0.0729	±0.016	0.01		0.2	
3. Vitamin A test[3]	10	0.0580	±0.027	0.01	0.2		
4. Low 8% protein[4]	7	0.065	±0.023	0.01			

[1] Animals from same stock, weighing between 30 and 35 gm when placed on diets; nail growth was measured for three days after two weeks on diet.

[2] Approximately 18% protein.

[3] As supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio.

[4] As mentioned in the text, a slightly reduced nail growth was found in animals fed this same diet with vitamin A omitted; the difference was not significant (mean difference 0.0115 mm/24 hrs., $P = 0.2$).

In table 2 are shown later results; the method of measurement described last was used. It will be seen that reduced nail growth is associated with a low protein diet; an additional suppression brought about by omission of vitamin A from this diet was not significant (the mean difference of 0.0115, P = 0.2). With diets containing adequate protein, omission of vitamin A causes a reduction in nail growth (see also table 3). It will be noticed in table 2, however, that the same diet, with vitamin A added, does not give as good nail growth as the stock diet. This may be because the test diet is rather unpalatable. Figure 4 shows growth curves for 4 groups of 4 animals placed on different diets. Nail growth was measured in the 16 animals at the three separate periods shown. On the stock diet the rate of nail growth remained constant throughout; the animals increased considerably in weight. The vitamin A test diet, supplemented with vitamin A, caused a slower weight increase but did not affect nail growth to the same extent. At first the low 8% protein diet caused a great reduction in weight increase but no change in nail growth; by

TABLE 3

A further second and third series of animals maintained on vitamin A test diet

Dietary group[1]	No. of rats	Mean growth	S.D.	P
		mm/24 hrs.		
1. Vitamin A test + vitamin A	10	0.0659	±0.029	0.03
Vitamin A test	8	0.0370	±0.020	
2. Vitamin A test + vitamin A	12	0.0796	±0.014	> 0.001
Vitamin A test[2]	11	0.0465	±0.022	

[1] Food intakes measured at this time showed that the animals of the vitamin A test group were eating more than those of the vitamin A supplemented group. On two successive days the intakes were 7.5 and 5.3 gm per animal and 6.5 and 3.1 gm respectively.

[2] Although all animals were on the diet for 4 weeks, deficiency symptoms developed more rapidly in this group.

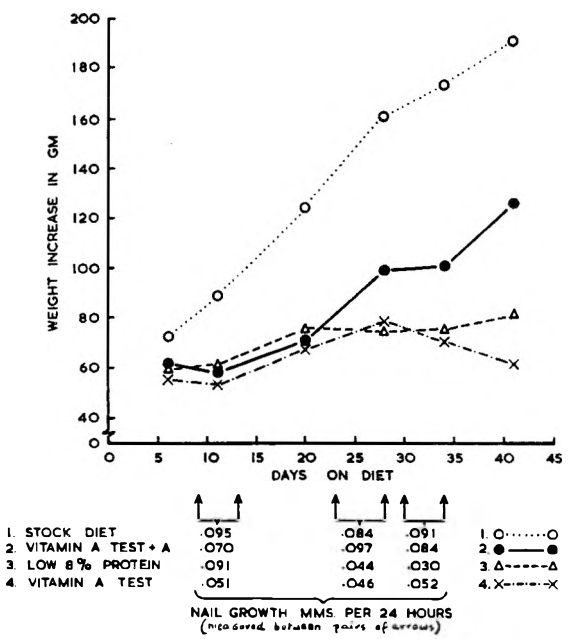

Fig. 4 Weight curves and nail growth measurements in relation to diet.

TABLE 4

The effect of a cold environment on nail growth in the rat

Group treatment	No. of rats	Mean growth	S.D.	P
		mm/24 hrs.		
No acclimatization	6	0.0098	±0.011	
				> 0.001
Control	6	0.0975	±0.024	
				> 0.001
Acclimatized	7	0.0045	±0.007	

TABLE 5

Nail growth in rats before and during cortisone administration

Treatment	No. of rats	Mean growth	S.D.	P
		mm/24 hrs.		
Before cortisone	11	0.090	±0.022	
During cortisone[1] administration (duration was three days)	8[2]	0.050	±0.019	> 0.001

[1] Each animal received 5 mg of sterile cortisone acetate suspension in 0.2 ml of saline, daily, by intramuscular injection.

[2] Three animals died under anesthetic; a fresh bottle was substituted.

the 20th day there was a reduction in the rate of nail growth. The vitamin A test diet caused an early reduction in weight increase and nail growth. This diagram suggests that nail growth is not related simply to weight increase.

There is (table 4) almost complete cessation of nail growth at 6°C, whether the animals are put into the cold room immediately or allowed to acclimatize. There is, therefore, no initial stimulus to nail growth which might have been thought to accompany an initial rise in metabolic rate; Herrington ('51) has shown that at 12°C there is a 100% increase in heat production over that at 28°C. There was no increase in nail growth in a series of animals on the low 8% protein diet, when placed in the hot room, nor was there a rise in a group of animals on the stock diet. Animals receiving ethionine daily (50 mg each of a solution in saline buffered to neutrality with sodium carbonate) showed no alteration in nail growth. However, 5 mg of cortisone acetate given daily caused a reduction in nail growth (see table 5).

Two animals were restrained for 24 hrs. in an attempt to measure the diurnal variation in nail growth. However, it proved impossible as there was complete cessation of growth over the entire period.

Again, two animals were placed in cages where they could exercise on a revolving drum. Although they both exercised a considerable amount, no increase was found in the rate of nail growth.

DISCUSSION

Human nail growth has been followed by a few investigators. Mitchell, in 1871, found a slow rate of nail growth on the hand of a 56-year-old woman, following a stroke. He then studied nail growth in two men who suffered strokes and noticed that it ceased in the early stages, and resumed at 12 and 21 days respectively, about a week, in each case, before some return of motor power occurred.

Head and Sherren ('05) confirmed Mitchell's observations, finding that nail growth was retarded on the fingers of the paralyzed hand. Similar retardation occurred when a splint or cast was worn. It is of interest that the same thing was found when two animals were restrained, in this study. Ivanovsky ('23) noticed retardation of growth of the nails in famine victims.

LeGros Clark and Buxton ('38), working with adults and children, found several facts about nail growth including a pronounced seasonal variation. The in-

fluence of cold in the experimental animal is very striking, and a similar phenomenon has recently been demonstrated by Geoghegan, Roberts and Sampford ('58) in reduced nail growth among men in the Arctic. They argue, from their own work, from that of Sunderland and Ray ('52) who showed reduced nail growth following arterial ligation, and the known facts about blood circulation of the extremities, that the reduction in nail growth in a cold environment may be due to normal physiological adjustment of peripheral circulation. Mitchell had concluded, on the basis of skin temperature and peripheral nerve injury that a central trophic influence was being exerted in his patients, rather than a local ischemic effect. In the animal experiments peripheral circulation is by no means down to zero, although in a number of the animals there was no nail growth at all. A factor that may operate in the cold condition, both in the results of Geoghegan et al., and the animal results reported here, may be the local temperature of the dividing cells of the nail matrix.

Gilchrist and Buxton ('39) showed that nail growth varied with nutritional status in school children. The measurements made on the rat support these results. Vitamin A may or may not have a specific effect on nail growth; the experiments cited do not enable us to decide, as the diet itself, even when vitamin A is supplied, does not give such good nail growth as the stock diet. The further reduction due to vitamin A deficiency may be associated with a further reduction in food intake. However, table 3 shows that the difference was becoming evident when the animals of the vitamin A test group were eating more than those being supplemented with vitamin A. Pair feeding studies will have to be done to decide this point. The suggested difference between the two groups on low 8% protein, with and without vitamin A, there being no difference in the weight of the animals, and the amount of food eaten, rather points to a specific effect due to vitamin A deficiency.

Cortisone is known to affect the rate of many processes, e.g., wound healing and collagen formation; the significance of its effect on nail growth is difficult to assess. It may reflect a general decrease in metabolic rate, a relative increase in catabolism and decrease in anabolism. When cortisone is given to rats daily, weight is found to decrease but food intake to increase (Godwin, '56).

Ethionine, which might have altered the uptake of a protein rich in sulfur-containing amino acids, being an antimetabolite to methionine, exerted no apparent effect on nail growth.

There are at least two ways of viewing nail growth; as a phenomenon of protein replacement throughout the lifespan (Hamilton, Terada and Mestler, '55), and as an example of hard keratinization. From the former viewpoint the present paper offers some evidence that factors which affect the rate of metabolism such as food intake, quality of food, temperature, and perhaps cortisone, do influence the growth of the nail; whether the converse is true, that observations on nail growth may, under certain conditions, give a useful indication of the state of metabolism seems to be worthy of further investigation. The intake of food is obviously an important factor, which must be considered in the interpretation of any data.

The second viewpoint serves to introduce some work which is to be the subject of a further publication on nail growth. It is taught at present that nail growth commences at the nail matrix and dead material is pushed over the nail bed by the developing cells (LeGros Clark, '55). Using cystine, labelled with S^{35} (the cystine content of keratin is 15% of the amino acid present, according to Rothman, '54) and making autoradiographs of the rat's claw, it was found that within an hour or two a considerable quantity of the cystine appears in the keratinized cells throughout the nail substance, and in the keratogenous zone of the hair follicles (Godwin, '58). This is being investigated further.

It has been shown by Rosenberg and Oster ('55) that gelatin has remarkable effects on the nail; it can influence the condition of the whole nail in a very much shorter time than it takes for the nail to regrow. Reference is made, in this last paper, to studies with Atabrine; it has been noticed that the whole nail becomes fluorescent after a short time; however, Atabrine did not diffuse through hair in the same

way. Evidence would seem to be accumulating to suggest that the nail is not a dead structure; these results may have a bearing on the mechanism of hard keratinization.

SUMMARY

A technique is described for the measurement of the linear growth rate of the nail of the rat. Accurate assessment of the rate can be made in two to three days. The growth rate of the nail has been measured in animals kept under a variety of experimental conditions, including the effects of poor diets, heat, cold, restraint, cortisone and ethionine. Conditions which affect the metabolic status of the animal are accompanied by alterations in the growth rate of the nails. Data, previously published, relating to nail growth in the human subject, are supported by these experiments with the rat.

It is suggested that further work seems to be worth while in the experimental animal, to decide the physiological significance of nail growth; particularly its use as an indicator of metabolic status.

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Influence of Body Composition of Weanling Pigs on Survival under Stress

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The stresses of work load and specialized dietary intake make effective tools for use in comparative studies in both man and animals. Ryer et al. ('54) used cold exposure and exercise as a means of determining the need for and effectiveness of extra vitamin supplementation of the diet of the American soldier. An alteration in physiologic responses of the human to a standardized cold stress, as a result of modification of physical fitness of the individual has been demonstrated by Adams and Heberling ('58). Holt and Kajdi ('44) measured the effects of feeding all fat, all carbohydrate or all protein on the survival of rats. Samuels, Gilmore and Reinecke ('48) have shown that there is a difference in activity of rats in the starvation state, after being fed different levels of fat, carbohydrate and protein.

In this experiment, an attempt was made to correlate the body composition of 8-week-old pigs with their response to a variety of dietary stress tests. Two groups of animals were fed to produce two different body compositions. Animals of each group were then subjected to conditions of complete fasting and thirsting, fasting only, or thirsting only.

MATERIALS AND METHODS

The test animals in this investigation were three-day-old farm piglets weighing from 1.33 to 1.93 kg. They were procured from local farms, and were predominantly Hampshire, a lean, or "meat type" hog. The animals were housed individually in screen-bottom cages equipped with heat lamps, in an air-conditioned room held at 72 to 75°F. Diet 1 was a commercial liquid infant food,[2] and diet 2 a dry commercial animal milk substitute.[3] The latter was moistened to a gruel-like consistency for a few days to encourage the young pigs to eat, but the amount of added water was gradually reduced so that at the end of three to 5 days these animals were adapted to the dry diet. A mineral supplement containing copper, iron and manganese salts was added to both diets.[4] Food and water were supplied ad libitum; however, solid food consumption was measured. The animals were weighed weekly. Distribution of animals by sex was approximately equal on the two diets.

At the end of 8 weeks of feeding, some of the animals on each diet were sacrificed by exsanguination, the hair clipped, and the carcasses completely eviscerated. Heart, liver, kidneys and spleen were weighed. These organs and the carcasses were preserved for analysis by freezing.

Animals fed each of the diets were subjected to the following stress conditions, until death:

A. Complete deprivation of calories, complete deprivation of water.

B. Calories available,[5] complete deprivation of water.

C. Complete deprivation of calories, water available.

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[1] Deceased April 8, 1959.

[2] Similac Liquid: cow's milk protein 3.45%, lactose 13.1%, fat 6.8% (mixture of corn, cocoanut, olive, milk fat and cocoa butter), minerals 0.75% including milk ash plus potassium citrate, fish liver oil concentrate, ascorbic acid, niacin, thiamine, and pyridoxine.

[3] Purina Nursing Chow: Crude protein not less than 25.0%, crude fat not less than 2.5%, crude fiber not more than 1.5%, N. F. E. not less than 46.0%, ash not more than 9.0%, moisture not more than 12.0%.

[4] The supplement for the liquid diet, added at the rate of 2.2 ml/kg contained $FeSO_4 \cdot 7H_2O$, 43.9 gm; $MnSO_4 \cdot 4H_2O$, 4.5 gm; $CuSO_4 \cdot 5H_2O$, 0.9 gm; in water to make 600 ml. The mixture for the dry diet, added at the rate of 2.2 gm/kg, contained in grams: $FeSO_4 \cdot 7H_2O$, 26.8; $MnSO_4 \cdot 4H_2O$, 2.7; $CuSO_4 \cdot 5H_2O$, 0.55; starch 69.9.

[5] A commercial spray-dried whole milk powder.

Some of the animals were housed in metabolism cages, allowing collection of urine. Blood for analysis was drawn from all animals at frequent intervals during the stress period. At death, necropsy was performed on all animals, and the carcasses and organs were frozen.

For chemical analysis, the pig carcass was split in half longitudinally, and the half to be analyzed was cut into thin slices and dried in an air oven at 100°C for two days. The free fat was poured off, and the residue returned to the oven overnight. After removal from the oven, the major portion of the remaining fat was removed by extraction and decantation with acetone. The largely defatted residue was then ground with acetone in a Waring Blendor, the acetone filtered off, and the remainder of the solvent removed by heating in the oven. After thoroughly blending the powdery residue, the final extraction of lipid material was made from an aliquot, using hot ethyl alcohol-ethyl ether in a Goldfisch extractor. Water was calculated by difference, protein in the fat-free tissue was determined by a micro-Kjeldahl procedure, and ash was measured by burning the material in a muffle furnace at 500° to 550°C. Moisture, fat, protein and ash in liver were determined by similar procedures. Sodium and potassium contents of the tissue samples were determined by use of the Perkin-Elmer flame photometer, using the method of Wallace et al. ('51). The procedure was modified slightly, in that calcium chloride and phosphoric acid were added to the standard solutions to correct for quenching effects. Chloride was determined by the method of Lowry and Hastings ('42). Standard procedures were employed for the determination of total serum protein, nonprotein nitrogen, and blood urea nitrogen. Urine osmolar concentration was measured in a Fiske Osmometer.

RESULTS AND DISCUSSION

After 8 weeks of feeding, the 16 pigs fed diet 1 averaged 16.5 ± 3.4 kg in weight; the 19 fed diet 2 averaged 18.4 ± 1.8 kg. The difference in mean weight was not statistically significant. To provide control data, against which to compare similar data from stressed animals, pigs in both groups were sacrificed. The carcass compositions are shown in table 1.

Bodies with different levels of water, fat and protein are produced by the two diets. On a fat-free basis, the difference in protein content is less pronounced, although still significant ($t = 5.81$). The protein values correspond to those reported by Spray and Widdowson ('50) for 56-day-old pigs.

Among the factors leading to the differing carcass nitrogen content may be the low (for the pig) protein content of diet 1. A level of 13.75% protein has been shown to be suboptimal for the growth of the young pig (Jensen et al., '57; Peo et al., '57). Furthermore, the two diets differ markedly in their fat content, a factor that regulates the fat content of the pig carcass.[6]

The mean time of survival of the pigs subjected to the three stresses is shown in table 2. It was assumed that the animals, at the time of being subjected to stress, had body composition similar to that of controls of like age, on the same diet. The length of survival in response to the various conditions of starvation and water deprivation was spectacularly varied. With stresses A and C, there was no overlap in range of survival time between the animals reared on the different diets. All animals under stress B died within a day of each other. Although the number of pigs in these two categories is low, it is believed

[6] Unpublished data, Filer et al.

TABLE 1

Percentage body composition of control pigs at eight weeks

Diet	No. animals	Water	Fat	Protein	Ash	Protein, fat-free basis
1	6	49.9 ± 1.15[1]	35.2 ± 0.69	11.2 ± 0.29	2.7 ± 0.23	17.2 ± 0.42
2	8	62.8 ± 3.46	16.7 ± 4.03	16.0 ± 1.14	3.8 ± 0.84	19.2 ± 0.84

[1] The ± values represent standard deviation.

TABLE 2

Relationship between previous diet and average survival time of pigs under stress

(days survived under stress)

Diet	Stress A			Stress B			Stress C		
	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range
1	5	21	19–22	2	15.5	15–16	3	89	84–93
2	5	28	25–31	2	16	16	4	36	34–40

TABLE 3

Average percentage weight loss and percentage carcass composition after stress

	Wt. loss	Water	Fat	Protein	Ash	Fat-free basis	
						Water	Protein
	%	%	%	%	%	%	%
Stress A							
Diet 1	34.8	44.8	37.5	13.3	3.7	71.8	21.3
Diet 2	47.4	63.5	11.1	19.4	5.8	70.7	21.6
Stress B							
Diet 1	32.2	46.4	34.8	14.5	3.4	71.3	22.3
Diet 2	38.7	60.0	14.7	20.4	4.8	70.5	24.1
Stress C							
Diet 1	60.7	76.7	2.1	15.6	5.6	78.3	15.9
Diet 2	40.9	77.3	0.9	16.5	5.2	78.0	16.7

that reliance may be placed on the figures in view of the clear-cut data obtained with stresses A and C.

During the stress periods, the animals catabolized much of their own energy stores. Although the loss of weight of the animals varied from one stress to another, the average body composition at death for pigs on stresses A and B was not much different from that of the control animals, as shown in table 3.

However, when water was available, body composition after stress was very much altered. The pigs had used almost all of their fat stores and much of their body protein, before succumbing to the stress of starvation. On a fat-free basis, body water remained quite constant for stresses A and B, but as fat stores decreased under stress C, body water content increased. Starvation also led to a change in the water/dry matter ratio in the carcasses of pigs under stress C. The mechanism of this altered relationship between water and solids was recently described by McConkey ('59). At necropsy, the heart, spleen, kidneys and liver were removed from each animal and weighed separately. Mean weights of the organs per kilogram of body weight for the control and the stressed animals are shown in table 4. It is quite evident that organ weight in relation to body weight is the same for the control animals on both diets. Under stress, the ratios of heart to body and kidney to body generally tended to increase. Spleen definitely decreased in weight more rapidly than body tissue under stress A, but this was not so with stress B or C. Liver size was unaltered except with stress C. Here, there appeared to be more rapid wastage of liver than of body tissue. This would tend to indicate that liver protein stores are more labile than body tissue protein under conditions of starvation without thirsting.

Livers of control and stressed animals were analyzed for water, fat, protein and ash content, and the results are shown in table 5. Deviations from controls were noted in the livers of two diet 1 animals subjected to stress A. In these two pigs the livers showed grossly some evidence of fatty infiltration. Histopathologic examination[7] confirmed fatty infiltration in the peripheral lobes of the liver of one animal only; sections on the remaining animals

[7] Kindly conducted for us by Dr. William A. Newton, Jr. of Children's Hospital, Columbus, Ohio.

were normal. Changes in the livers of animals under stress C tended to follow those of the carcass, inasmuch as the water content was somewhat elevated, with lowered tissue fat and protein levels.

Sodium, potassium and chlorides were determined on the dry, fat-free carcasses of control animals and those subjected to stress. These data are shown in table 6. The alterations in carcass sodium and chloride of animals deprived of both food and water are in line with the observations reported by Winkler et al. ('44). In those studies, dogs deprived of food and water lost water out of proportion to the loss of salt. Under conditions of starvation with water available, the concentrations of carcass sodium and chloride increased remarkably. Potassium was reduced in amount. In this situation, potassium shifts from the cells and interstitial fluid into the plasma, to be eliminated in the urine, whereas sodium is retained in an effort to maintain homeostasis.

Because of limited availability of metabolism cages, only a few studies were made on the urine of the stressed pigs. Animals deprived of water under stresses A and B very soon voided a more concentrated urine, the specific gravity changing from a normal value between 1.010 and 1.018 to a high of 1.035 to 1.060 within two days.

TABLE 4

Weight of heart, spleen, kidney and liver in grams per kilogram of body weight

	Heart	Spleen	Kidney	Liver
Control				
Diet 1	5.7 ± 0.72[1]	2.1 ± 0.48	6.5 ± 0.82	34.5 ± 5.10
Diet 2	5.7 ± 0.91	2.5 ± 0.23	7.9 ± 0.94	36.1 ± 3.40
Stress A				
Diet 1	7.3 ± 0.94	0.9 ± 0.08	8.8 ± 0.91	33.0 ± 2.70
Diet 2	7.2 ± 0.93	1.3 ± 0.07	8.3 ± 0.83	35.1 ± 3.72
Stress B				
Diet 1	7.8 ± 0.57	1.9 ± 0.56	8.4 ± 0.25	34.4 ± 1.65
Diet 2	6.6 ± 0.64	2.3 ± 0.11	8.5 ± 0.96	37.3 ± 5.09
Stress C				
Diet 1	10.6 ± 0.73	1.8 ± 0.22	9.8 ± 0.76	29.4 ± 1.70
Diet 2	8.4 ± 0.41	2.4 ± 0.51	8.1 ± 0.48	25.0 ± 1.58

[1] In view of the small numbers of samples (see table 2), the ± values are given in terms of average deviations rather than standard deviations.

TABLE 5

Percentage composition of liver from control pigs and those under stress

	Water	Fat	Protein	Ash
Control				
Diet 1	71.4 ± 0.71	4.6 ± 0.30	18.5 ± 1.80	1.4 ± 0.10
Diet 2	73.1 ± 1.40	3.9 ± 0.35	18.8 ± 0.52	1.2 ± 0.10
Stress A				
Diet 1	68.5 ± 3.93	11.8 ± 8.35	18.5 ± 2.50	1.4 ± 0.10
Diet 2	75.5 ± 0.96	4.7 ± 0.98	17.4 ± 1.30	1.4 ± 0.06
Stress B				
Diet 1	74.1[1]	4.2	19.9	1.4
Diet 2	73.8 ± 0.10	4.2 ± 0.15	20.2 ± 0.10	1.3 ± 0.0
Stress C				
Diet 1	79.5 ± 0.05	3.2 ± 0.25	15.6 ± 1.3	1.3
Diet 2	80.6 ± 1.33	2.2 ± 0.07	15.0 ± 2.28	1.2 ± 0.25

[1] Where average deviations are not given only one liver is involved.

TABLE 6

Sodium, potassium and chloride concentrations of pig carcasses, control and stressed animals

(mEq/100 gm dry, fat-free tissue)

	Sodium	Potassium	Chloride
Controls			
Diet 1	25.1 ± 1.84[1]	29.8 ± 0.64	14.4 ± 2.20
Diet 2	23.6 ± 1.44	28.5 ± 1.46	12.7 ± 0.69
Stress A			
Diet 1	30.9 ± 1.92	21.8 ± 0.90	17.9 ± 0.86
Diet 2	31.5 ± 1.78	20.8 ± 1.26	17.7 ± 1.30
Stress B			
Diet 1	28.3 ± 0.0	25.2 ± 0.5	15.8 ± 1.34
Diet 2	28.4 ± 0.15	24.5 ± 0.70	15.5 ± 0.85
Stress C			
Diet 1	43.6 ± 2.57	16.1 ± 1.23	25.1 ± 2.52
Diet 2	37.7 ± 1.68	17.3 ± 0.45	20.8 ± 0.33

[1] Average deviation.

Fiske Osmometer readings rose from 360 milliosmols/liter as a normal value to as high as 2324. The pigs retained their ability to concentrate urine up to the time of death.

Two diet 1 animals on stress C voided an average of 4.0 l of urine per week. The output was only 1.0 to 2.0 l during early fasting and increased to 4.0 to 6.0 l during the latter stages. The specific gravity fell from an average of 1.032 during the first week to levels of 1.002 to 1.004 during the 10th to 12th week of starvation. Two diet 2 pigs under stress C, on the other hand, produced urine at an almost constant rate of 13.0 to 14.0 l per week, with a specific gravity that ranged from 1.002 to 1.005.

The pigs subjected to stress B, permitted ad libitum feeding of powdered whole milk, consumed some food up to the 10th day of water deprivation, with those originally on diet 1 eating an average of 2.3 pounds and those on diet 2, 3.5 pounds. It was surprising that these animals continued to eat as long as they did, since each additional increment of ingested food would tend to further jeopardize their precarious water reserves. As expected, the pigs with the greater body water content (diet 2) ate more than those with more fat and less water (diet 1).

It would appear that under conditions of fasting and thirsting (stress A) the animal with the greater amount of body water can withstand the rigors of this stress longer than the animal with smaller reserves of water. While catabolism of body fat produces additional water, this is a self-defeating process, as pointed out by Grande et al. ('57), since the water so created falls far short of the needs for excretion of waste products. Animals with high body fat would compromise their water reserves more rapidly, and, unable to meet obligatory water requirements for removal of catabolic end products, would become toxic and die.

The general subject of body protein stores, and their availability during stress situations, has been ably discussed by Allison ('55). It is commonly believed that the animal with greater stores can withstand caloric restriction or starvation to a greater degree, and that accelerated metabolism of depot fat occurs first, with the protein being utilized only after the fat has been partially or completely depleted. This does not seem to be the case for the pigs subjected to the three stress conditions. Table 7 shows that the daily utilization of fat remained reasonably constant for all animals, regardless of stress or previous diet. However, such was not the case for protein utilization. In every instance the average daily loss of protein by those animals having greatest protein stores was from two to three times as much as that lost by the animals with smaller amounts of body protein. Blood urea ni-

TABLE 7

Daily utilization of body constituents by pigs during stress periods[1]

	Water	Fat	Protein
	gm/day	*gm/day*	*gm/day*
Stress A			
Diet 1	136	73	15
Diet 2	186	68	35
Stress B			
Diet 1	154	100	12
Diet 2	86	86	31
Stress C			
Diet 1	36	68	10
Diet 2	86	82	31

[1] This calculation was made by dividing the difference in body components pre- and poststress by the days of survival.

trogen values remained at a more or less constant level throughout the stress period (except from 4 to 7 days terminally) in all animals fed diet 2. This would indicate that protein catabolism was proceeding at a constant rate, even early in stress. The overall indication is that the body catabolically accustomed to a higher protein intake is unable to gear down its metabolic processes when suddenly faced with a dietary emergency. Protein stores continue to be utilized at the previously established rate.

Studies on the rate of degradation of radioiodinated serum albumin by Iber et al. ('58) in adult humans show that periods of high-protein feeding are associated with an increase in catabolism of this protein. Similar findings for total plasma proteins have been reported by Steinbock and Tarver ('54) in the rat and by Yuile et al. ('59) in the dog. Danowski et al. ('44) related survival time of dogs subjected to food and water deprivation to the amount of protein metabolized daily. The influence of body composition on survival was not apparent, since it is highly likely that their dogs were similar in that respect. Henry and Kon ('53) observed that rats accustomed to a high dietary calcium intake were unable to decelerate calcium turnover when faced with a restricted intake in later life. Similar results were reported in dogs by Gershoff, Legg and Hegsted ('58).

SUMMARY AND CONCLUSIONS

1. By the use of two diets, one high fat, low protein, and the other low fat, high protein, the carcass composition of the 8-week-old pig can be markedly altered.
2. The ability to survive stress in the form of starvation, thirsting or both is influenced by body composition and prior plane of protein nutrition.
3. Survival of 8-week-old pigs, deprived of water and calories, was longest for those animals having the greatest body water content.
4. Water deprivation, with calories available, considerably shortened the period of survival, and body composition seemed without influence in this stress situation.
5. Survival of 8-week-old animals deprived of calories, with water available, was longest for pigs with the greatest fat stores.
6. Under all three types of stress, the rate of protein catabolism was two to three times greater for those animals fed a high-protein diet during the first 8 weeks of life.
7. Changes in liver composition during stress parallel the changes observed in carcass composition.
8. In considering survival in situations of reduced calorie and water supplies, body composition and prior plane of protein nutrition become decisive factors.

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Volatile Fatty Acid Rations for Growing Lambs[1]

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It is generally considered that roughage is a necessary constituent of satisfactory rations for ruminants (Davenport, 1897; Thomas and Okamoto, '54). In addition to possible beneficial effects from the physical characteristics of fiber, roughage also provides a source of energy for the ruminant through fermentation by micro-organisms in the rumen to form short-chain fatty acids (Phillipson and McAnally, '42). These fatty acids have been administered intrarumenally as energy sources; as their sodium salts by Danielli et al. ('45), and as the free acids by Armstrong and Blaxter ('57a). Popov ('36) fed mixtures of lactic, acetic, butyric and formic acids with a basal ration and showed that they increased digestibility of protein and fat, but lowered digestibility of crude fiber and nitrogen-free extract. Sodium acetate and sodium propionate were used as supplemental energy sources for growing-fattening lambs by Bentley et al. ('56), while Matrone et al. ('57) used salts of acetic, propionic and butyric acids as energy sources for lambs that had never received roughage. The experiments reported in this paper were conducted to compare salts of volatile fatty acids with roughages as energy sources for lambs with a functional rumen and to find ratios of salts of volatile fatty acids that would promote optimum gain.

EXPERIMENTAL AND RESULTS

All animals used in these experiments were grade lambs weighing approximately 55 lb. All had previously been fed normal roughage-containing rations. The average daily gains were determined over 49- to 70-day experimental periods; the animals were weighed at 14-day intervals. The lambs were fed individually in wooden pens with hardware-cloth-covered sides and metal grating floors.

Experiment 1. The ration used by Matrone et al. ('57) containing salts of fatty acids was compared with two rations containing roughages (table 1): one, a practical type ration containing corn, soybean oil meal and alfalfa hay, and the other, a synthetic type ration containing dehydrated corn cobs in place of the salts of the volatile fatty acids. Three lambs were used per group.

It was planned to keep these animals on an equal estimated digestible energy intake. It was found that the animals would not satisfactorily consume the fatty acid salts ration (A-1) nor the corn cobs ration (B-1), eventually making it impractical to control feed intake. The actual calorie intakes are given in table 1. This poor consumption resulted in very poor gains or in loss in weight. Lambs on the normal ration did maintain their weight even on this low intake, while the lambs on the ration containing corn cobs lost 0.43 lb. per day for 37 days, at which time they were removed from the experiment.

Experiment 2. Because of the failure of the animals to voluntarily consume the original fatty acid salts diet, this experiment was designed to find a palatable diet containing salts of fatty acids which the animals would readily consume. Four rations were formulated to contain the following ingredients in addition to 31.9% salts of volatile fatty acids, 3% mineral mix, 5% vitaminized starch and 4% corn oil:

Ration A-2. Soybean protein,[2] 30%; cerelose 24.1%; starch 2%.

Ration B-2. Soybean protein,[2] 20%; cerelose 10%; starch 16.1%; casein 10%.

Ration C-2. Soybean protein,[2] 30%; starch 26.1%.

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[2] Drackett Assay Protein C-3.

TABLE 1

Fatty acid salts compared to roughage

Experiment 1

Rations	A-1	B-1	C-1
	%	%	%
Ingredients			
Casein (crude)	30.0	29.0	—
Cerelose	26.1	26.1	—
Corn oil	4.0	4.0	—
Vitamin mix (see table 2 footnotes)	5.0	5.0	—
Mineral mix (see table 2 footnotes)	3.0	3.0	—
Volatile fatty acid salts (see table 2 footnotes)	31.9	—	—
Dehydrated corn cobs	—	32.9	—
Alfalfa hay (chopped)	—	—	51.0
Corn (cracked)	—	—	37.0
Soybean oil meal	—	—	9.0
NaCl	—	—	1.0
Steamed bone meal	—	—	2.0
Results			
Av. daily calorie intake (gross energy)	1532	968	2775
Av. initial weight (lb.)	60.6	62.0	60.3
Av. final weight (lb.)	54.0	43.6	61.0
Days on experiment	49	37	49

Ration D-2. Soybean protein,[2] 30%; sucrose 10%; starch 16.1%.

The compositions of the mixtures of vitamins, minerals, and salts of fatty acid are shown in the footnotes to table 2. Three lambs were assigned at random to each of these rations. Daily consumption was determined for a two-week period. Lambs fed ration D-2 showed the best consumption at the end of the two-week period, with a daily intake of 850 gm, and this ration was used as a basic ration for the following experiment.

Experiment 3. Using the soybean protein basal ration, this experiment was designed to test the contribution of the volatile fatty acids to the energy value of this diet for gain and to compare this with the value of corn cobs and of starch. Three lambs were assigned at random to each ration and fed ad libitum. The composition of the rations and the average daily gain per animal are shown in table 2.

Lambs on the starch ration were removed at the end of 28 days because of excessive loss of weight due to low intake of the ration, again illustrating that the ruminant does not perform satisfactorily on energy sources that are satisfactory for the non-ruminant. Lambs fed ration A-3 had the best average daily gain of 0.31 lb. for the 56-day period. Lambs on this ration appeared entirely normal. The pH of the urine was 8.7. During the first week of the experiment there was an attempt to feed 100 gm of rations A-3, C-3, and D-3 for each 68.1 gm of ration B-3; however, due to lower consumption of ration B-3 (the negative control) and ration D-3 (the starch ration), an ad libitum feeding regime was adopted for the remainder of the experiment. At the end of the experiment, calculation of feed consumption showed that for each 100 gm of ration A-3, 51.7 gm of ration B-3 were consumed. When the basic ingredients were compared, 68.1 gm of ration A-3 had been consumed for each 51.7 gm of ration B-3.

Ration A-3, containing salts of volatile fatty acids, was significantly better than rations B-3, C-3, D-3. The difference in average daily gain of animals fed rations B-3 and A-3 was 0.36 lb. Lambs on diet B-3 showed an average loss of 3 lb. each (average daily loss 0.05 lb.) for the 56-day period. They ate wool on body areas that could be reached. The comparison between A-3 and C-3 showed that this mixture of salts of volatile fatty acids gave better results than did dehydrated corn cobs.

Experiment 4. This experiment was designed to determine the ratios of volatile fatty acid salts and free fatty acids which would result in the best average daily gain

[2] See footnote 2, page 136.

TABLE 2

Ration composition and results (56 days)

Experiment 3

Diets	A-3	B-3	C-3	D-3
Ingredients	%	%	%	%
Soybean protein[1]	30.0	30.0	30.0	30.0
Sucrose	10.0	9.5	9.5	9.5
Starch (granular)	16.1	16.1	16.1	48.0
Corn oil	4.0	4.0	4.0	4.0
Mineral mix[2]	3.0	3.0	3.0	3.0
Vitamin mix[3]	5.0	5.0	5.0	5.0
Limestone	—	0.5	0.5	0.5
Dehydrated corn cobs	—	—	31.9	—
Volatile fatty acid salts[4]	31.9	—	—	—
Results				
Av. daily gain (lb.)	0.31	− 0.05	0.17	− 0.26 (4 wk.)
Av. daily calorie intake	3322	1983	3107	1332 (4 wk.)
Av. daily calorie intake without the fatty acids	2283	1983		

[1] Drackett Assay Protein C-3.

[2] Mineral mix per 100 lb. diet: NaCl 405.9 gm; K_2HPO_4 678.5 gm; $MgCO_3$ 204.4 gm; $FeC_6H_5O_7 \cdot 6H_2O$ 63.4 gm; $MnCl \cdot 4H_2O$ 2.9 gm; KI 1.8 gm; $CuSO_4 \cdot 5H_2O$ 1.2 gm; NaF 1.0 gm; $ZnCl_2$ 0.6 gm; $CoCl_2 \cdot 2H_2O$ 0.6 gm; MoO_3 0.5 gm.

[3] Vitamin mix per 100 lb. diet: Thiamine·HCl 200 mg; riboflavin 400 mg; nicotinic acid 1 gm; calcium pantothenate 1 gm; pyridoxine·HCl 240 mg; choline chloride (25% dry mix) 320 gm; vitamin B_{12} (with mannitol, 1 mg B_{12}/gm) 500 mg; biotin 30 mg; folic acid 100 mg; vitamin A (250,000 I.U. per gm) 0.8 gm; vitamin D (15,000 I.C.U. per gm) 2 gm; 2-methyl-1-4-naphthoquinone 100 mg; starch 1947.32 gm.

[4] Salts of volatile fatty acids: When the mix constituted 31.9% of diet A-3, the following salts were furnished in the indicated percentages of the total diet: calcium propionate 4.8%; sodium propionate 3.0%; sodium acetate 9.0%; potassium acetate 8.5%; magnesium acetate 3.0%; sodium butyrate 3.6%.

for growing-fattening lambs. Twelve grade lambs averaging 55 lb. were assigned at random to 4 rations containing different percentages of acetic, propionic and butyric acids fed partly as salts and partly as free acids (table 3). The same basic ration, consisting of soybean protein, starch, sucrose, corn oil, vitamins and minerals, was used in all rations. The sheep were fed ad libitum and the results compared on the basis of weight gain, carcass grade, and dressing percentage. Samples of rumen contents were taken at time of slaughter, 14 hours after feeding, to determine the ratios of volatile fatty acids present. Rumen contents were microscopically examined for protozoa.

The results of this experiment indicate a very marked superiority of the ration (A-4) which contained all the volatile fatty acids as salts. The ratio of volatile fatty acids expressed as a percentage of total volatile fatty acids was 62.5% acetic acid, 25% propionic acid, and 12.5% butyric acid. The average gain on this ration (A-4) was excellent, 0.5 lb. gain per day, which compares favorably with gains on a good practical ration. The poorer results on rations B-4, C-4, and D-4 were due to the low feed intake of the rations containing the free volatile fatty acids.

Carcass grades and dressing percentages showed only the differences that would be expected due to differences in gain. Microscopic examination of the rumen contents showed practically no protozoa, possibly indicating little activity of micro-organisms in the rumen.

The results of volatile fatty acid determinations of the rumen contents are shown in table 4. The animals fed the largest proportion of acetic acid in their rations had the highest proportion of acetic acid in their rumen contents. When the

TABLE 3

Ration composition and results (56 days)

Experiment 4

Ration	A-4	B-4	C-4	D-4
	%	%	%	%
Ingredients				
Basal	49.0	49.0	49.0	49.0
Starch	16.5	18.9	19.8	19.8
Mineral mix (no. 2)[1]	3.0	3.0	3.0	3.0
Volatile fatty acids mix	31.5	29.1	28.2	28.2
Ca propionate	4.7	4.7	4.7	4.7
Na propionate	2.9	2.9	2.9	2.9
Propionic acid	—	6.0	6.0	6.0
Na acetate	8.8	—	—	—
Ca acetate	8.4	8.7	4.9	3.0
Mg acetate	2.9	3.0	2.9	1.0
Na butyrate	3.8	3.8	3.8	7.6
Butyric acid	—	—	3.0	3.0
Amount of volatile fatty acids expressed as free acids				
Total, %	24	24	24	24
Acetic acid, %	15(62.5)	9(37.5)	6(25.0)	3(12.5)
Propionic acid, %	6(25.0)	12(50.0)	12(50.0)	12(50.0)
Butyric acid, %	3(12.5)	3(12.5)	6(25.0)	9(37.5)
Results				
Av. daily gain (lb.)	0.50	0.29	0.10	0.26
Av. daily calorie intake	3636	2844	2155	2828
Feed/lb. gain	3.9	5.1	10.6	5.4
Carcass grade[2]	6.0	4.0	2.8	4.5
Dressing percentage	52.8	53.9	50.4	53.0

[1] Replaced $MgCO_3$ with starch, change from K_2HPO_4 to KH_2PO_4.

[2] High choice, 6; Av. choice, 5; Low choice, 4; High good, 3; Av. good, 2; Low good, 1.

TABLE 4

Volatile fatty acids (VFA) in rumen contents 14 hours after feeding

Rations	Acetic		Propionic		Butyric	
	Molar % in rumen	% total VFA fed	Molar % in rumen	% total VFA fed	Molar % in rumen	% total VFA fed
A-4	60.55	62.5	21.52	25	17.94	12.5
B-4	51.09	37.5	31.08	50	17.83	12.5
C-4	57.08	25	24.45	50	18.48	25
D-4	49.63	12.5	32.08	50	18.28	37.5
All hay[1]	72.62		18.43		8.96	

[1] Sheppard et al. ('58).

propionic acid percentage of the ration was increased, the propionic acid content of the rumen content was increased. However, an increase in butyric acid percentage of the diet did not result in an increase in the butyric acid content of the rumen.

Experiment 5. Due to the poor consumption of the rations containing free volatile fatty acids in experiment 4, experiment 5 was planned to test the effects of variations in the relative amounts of each of the three volatile fatty acids, all fed as their salts. The rest of the diet and the ratios of volatile fatty acids fed in this experiment were identical to those in experiment 4. Table 6 shows the composition of the mixtures of volatile fatty acid salts and the amount of starch used in each ration. Twelve crossbred lambs were assigned at random to the 4 rations which were fed ad libitum. This experiment was conducted for 70 days, and weight gain, feed efficiency, carcass grade, and dressing percentage were determined.

TABLE 5

Analysis of variance of weight gains for experiment 4

Source	Df	ms
Total	11	—
Treatment	3	249.13[1]
A-4 vs. B-4, C-4, D-4	1	552.25[1]
B-4 vs. C-4, D-4	1	78.13[1]
C-4 vs. D-4	1	117.04[1]
Error	8	6.48

[1] $P < 0.01$.

In this experiment there was no significant difference in average daily gain. Upon plotting the average gain per animal as shown in figure 1, it was found that ration A-5 had the advantage of a more rapid adjustment to diet during the first 4 weeks; from then on, rates of gain of sheep on all 4 diets were essentially the same. Carcass analysis and dressing percentage showed no significant differences.

DISCUSSION

The results of experiment 1 showed that the first important problem was to develop a volatile fatty acid-containing diet which the animals would consume reasonably readily. Such a diet was found in ration D-2 of the ration acceptability experiment, experiment 2. The basic problem with the rations used appeared to be related to the presence of casein and cerelose, and as a result of the change to soybean protein and granular starch, very greatly improved feed intake was obtained.

This ration was used as the basic ration in experiment 3 in which dehydrated corn

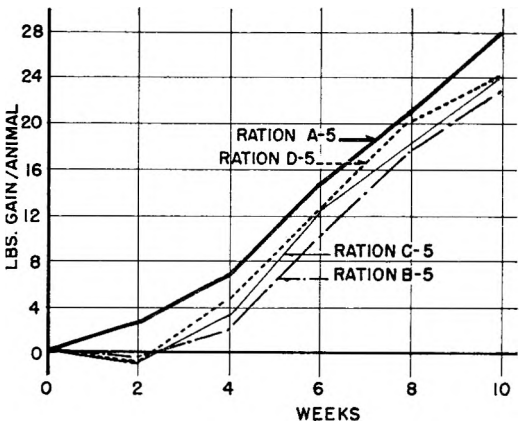

Fig. 1 Experiment 5. Average gain per animal for each ration.

TABLE 6

Ration composition and results (70 days)

Experiment 5

Ration	A-5	B-5	C-5	D-5
Ingredients	%	%	%	%
Basal (table 2)	52.0	52.0	52.0	52.0
Starch (granular)	16.5	17.3	17.4	17.4
Volatile fatty acid salt mix	31.5	30.7	30.6	30.6
Ca propionate	4.7	9.4	9.4	9.4
Na propionate	2.9	5.8	5.8	5.8
Na acetate	8.8	—	—	—
Ca acetate	8.4	8.7	4.9	3.0
Mg acetate	2.9	3.0	2.9	1.0
Na butyrate	3.8	3.8	7.6	11.4
Amount of volatile fatty acids expressed as free acids				
Total, %	24	24	24	24
Acetic acid, %	15.0	9.0	6.0	3.0
Propionic acid, %	6.0	12.0	12.0	12.0
Butyric acid, %	3.0	3.0	6.0	9.0
Results				
Av. daily calorie intake	3908.3	3311.9	3601.8	3565.3
Av. daily gain (lb.)	0.39	0.33	0.34	0.34
Feed/lb. gain	5.5	5.5	5.6	5.3
Carcass grade	4.7	4.0	4.3	5.3
Dressing percentage	54.4	53.8	54.7	54.4

cobs and starch were compared with volatile fatty acids. When the energy intakes of rations A-3 and B-3 were compared (omitting from the calculation the salts of volatile fatty acids of ration A-3), the total caloric intake of ration A-3 was only 300 Cal. more than that of ration B-3. Thus the superior results obtained from ration A-3 indicate that the volatile fatty acids of this ration were indeed being used as an energy source by the lambs, and the additional energy supplied in ration A-3, as salts of volatile fatty acids, must have been responsible for the additional gain. It was shown by Barcroft et al. ('44) that volatile fatty acids are absorbed from the alimentary tract. Armstrong and Blaxter ('57b) showed that fat is synthesized from short-chain volatile fatty acids and that these acids have no effect on fecal energy loss or methane production.

Lambs on ration A-4, experiment 4 (table 3), performed at a rate that is comparable to, or better than, that expected from an average fattening ration. Table 5 shows that ration A-4 was significantly better than rations B-4, C-4 and D-4, and that ration C-4 was significantly poorer than the other diets directly compared to it. The difference in gain obtained with the various rations used in this experiment can be attributed to the caloric intake, which was highly correlated (0.997) with gain. The lack of acceptability of rations B-4, C-4, and D-4 was presumably due to the free volatile fatty acids in these rations. Volatile fatty acid analysis of rumen contents 14 hours after feeding indicated that acetic and propionic acids tend to remain at a ratio similar to that fed to the animal. However, the rumen contents contained approximately the same amount of butyric acid regardless of amount of butyric acid fed. This is similar to the findings of Brown et al. ('58), who found that the butyric acid concentration of rumen contents remained constant on diets containing hay and concentrates in ratios of 4:1, 3:2, 1:3 and 1:4. Using acetate-1-C^{14} and butyrate-1-C^{14}, they also found that there was cycling of acetate to butyrate and butyrate to acetate. The constancy of the butyric acid in the rumen contents indicates that there was an equilibrium in the concentration of butyric acid, maintained either by bacterial fermentation or by preferential absorption through the rumen epithelium.

In experiment 5, where all volatile fatty acids were fed as salts, average daily gain, carcass grade, dressing percentage and feed per pound of gain were not significantly different, indicating that apparently equal gains can be obtained from various ratios of volatile fatty acids. Armstrong et al. ('57) found that when various ratios of acetic, propionic and butyric acids were supplied by means of intra-rumenal drip, there was little change in heat increments.

SUMMARY

Rations containing casein and cerelose in combination with salts of volatile fatty acids were not satisfactory for 55-lb. lambs which had been receiving roughage. A new ration was evolved, containing soy protein, starch, sucrose and volatile fatty acid salts, which gave adequate feed intakes and normal gains. Such rations with salts of volatile fatty acids as a major energy source produced average daily gains that were equal to, or better than, those with dehydrated corn cobs. Rations containing free volatile fatty acids were not consumed as readily as those containing salts of volatile fatty acids. Various ratios of acetic, propionic and butyric acids fed as their salts produced equal gains.

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Galactose Ingestion and Urinary Excretion of Calcium and Magnesium[1]

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INTRODUCTION

Calcariuria has been reported in rats fed diets containing large amounts of galactose or lactose. Handler ('47) reported that rats fed an 80% galactose or lactose diet excreted significantly higher amounts of urinary calcium than rats ingesting diets containing comparable amounts of sucrose. McCay et al. ('52) observed that in rats fed a diet of cows milk 11% of the excreted calcium was in the urine; while in rats fed sucrose diets renal excretion amounted to only 2% of the total. Outhouse et al. ('38) reported that rats fed a 25% lactose diet excreted more urinary magnesium than pair-fed animals ingesting glucose. Lactose is known to enhance the intestinal absorption of calcium and it has been suggested that the elevated urinary excretion of these ions follows increased absorption from the intestinal tract. In this study the relationship between the galactose-induced renal excretion of magnesium and calcium and intestinal absorption was studied by paired-feeding balance techniques.

METHODS

Albino rats of the Rochester colony weighing 125 to 150 gm were housed in individual metabolism cages. Water was provided ad libitum. Animals were fed a diet consisting of 60% carbohydrate (galactose or glucose) 21% casein, 14% fat[2] and 4% salt (Wesson, '32) supplemented with a complete vitamin mixture.[3] In balance studies on low calcium intakes the salt mixture was prepared without the calcium salts.

Paired-feeding balance studies were carried out over 7-day periods but calcium balances and renal excretion were calculated per 24-hour period. Absorbed calcium was calculated as the difference between intake and fecal excretion. The urine was acidified, filtered and analyzed for sodium, potassium and calcium, using Coleman Model 21 flame photometer. Magnesium determinations were done by a modification of Titian yellow method (Orange and Rhein, '51). Acid-washed charcoal was used as fecal marker. Aliquots of the homogenized stool collection were ashed with nitric acid and cation analyses were similarly carried out. Blood pH was determined on samples collected under mineral oil.

RESULTS

Condition of animals. Animals fed the galactose diet gained weight at approximately half the rate of those fed glucose but they did not show the ruffed fur, paw edema and diarrhea that accompany very high galactose intakes. Cataracts developed in three or more weeks. Galactosuria and polyuria developed when the animals were started on the galactose diet and persisted throughout the duration of galactose feeding. Water intake of animals fed a 60% galactose diet averaged 60 ml per day as compared to 15 ml for animals pair-fed the glucose diet.

Calcium excretion in urine. Animals fed a 60% galactose diet excreted significantly greater amounts of calcium than pair-fed animals ingesting glucose (table 1). The difference was approximately 7 mg per week. The calcariuria developed immedi-

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[1] Supported by a research grant (RG-4996) from the National Institutes of Health.
[2] Crisco.
[3] Each kilogram of diet contained: menadione, 50 mg; thiamine, 10 mg; pyridoxine, 40 mg; calcium pantothenate, 25 mg; nicotinamide hydrochloride, 50 mg; *p*-aminobenzoic acid, 50 mg; folic acid, 0.6 mg; biotin, 0.6 mg; riboflavin, 20 mg; inositol, 100 mg; vitamin B_{12}, 0.010 mg; choline chloride, 1 gm; and 19 gm of U.S.P. cod liver oil.

TABLE 1

Calcium balance and urinary excretion

(Expressed as mEq/24 hrs.)

Dietary carbohydrate	Pairs of animals	Average intake	Absorbed	Diff.	S.E.[1]	Urinary Excretion	Diff.	S.E.[1]
60% galactose	12	2.04	1.33	0.12	0.16	0.079	0.054	0.008
60% glucose	12	2.04	1.21			0.025		
30% galactose + 30% glucose	7	2.09	1.37	0.15	0.09	0.023	0.008	0.004
60% glucose	7	2.09	1.52			0.015		
60% galactose, low Ca	7	0.014	− 0.20	− 0.17	0.006	0.052	0.031	0.004
60% glucose, low Ca	7	0.014	− 0.03			0.021		
60% glucose − chloramphenicol	7	1.00	0.69	0.39	0.04	0.019	0.006	0.004
60% glucose	7	1.00	0.30			0.013		
60% galactose + chloramphenicol	7	0.53	0.35	0.16	0.05	0.042	0.020	0.050
60% galactose	7	0.53	0.18			0.062		

[1] S.E. of mean difference.

ately and persisted for the longest period of observation (21 days). Differences in renal calcium excretion were much less when the diet contained only 30% galactose.

The differences in excretion were not related to enhanced intestinal absorption during galactose feeding (table 1). Calcium absorption was the same in rats fed diets containing 60% galactose or glucose. In rats fed diets sufficiently low in calcium to produce a negative balance, galactose-fed animals continued to excrete significantly more urinary calcium than pair-fed control animals. The addition of antibiotics to the diet has been shown to enhance the absorption of calcium and magnesium from diets containing 60% galactose or glucose (Heggeness, '59). Urinary excretion in animals fed a glucose diet containing chloramphenicol is the same as in pair-fed control animals ingesting glucose but not the antimicrobial agent. In rats fed a galactose diet urinary excretion is likewise not modified by the addition of chloramphenicol to the diet. Seven rats allowed only 10% galactose solution to drink excreted 0.22 ± 0.07 mEq of calcium per day as compared to only 0.02 ± 0.01 mEq per day by rats fed a 10% glucose solution.

Urinary sodium and potassium. Urinary sodium and potassium were elevated in animals fed the galactose diet (table 2) but the elevated renal excretion of these cations did not follow the same time course as that of calcium. The elevated calcium excretion developed immediately and continued unchanged but differences in renal excretion of sodium and potassium usually occurred only after several days of galactose feeding. Table 2 shows urinary calcium, sodium and potassium excretion during two successive three-day periods of feeding a diet containing 60% of galactose or glucose. Animals fed a 30% galactose diet showing only a small elevation in urinary calcium excretion have significantly elevated urinary sodium and potassium losses compared to glucose fed control animals (table 2).

Bone ash values. Bone ash and calcium content were determined in weanling rats fed ad libitum a 60% galactose or glucose, low-calcium diet for 28 days. The 6 galactose-fed animals after 28 days weighed only 74 gm, while 5 animals fed the 60% glucose diet weighed an average of 149 gm. The marrow-free shaft of the femurs of the galactose-fed animals contained 46.7 ± 1.1% ash with 6.29 ± 0.13 mEq of calcium per gram of fresh bone. Bone of the animals fed a glucose diet contained 37.2 ± 1.7% ash with 5.34 mEq of calcium per gm.

TABLE 2

Urinary electrolyte excretion

(Expressed as mEq/day)

Diet	Pairs of animals	Days	Ca^{++}	Diff.	S.E.[1]	Na^{+}	Diff.	S.E.[1]	K^{+}	Diff.	S.E.[1]
60% galactose			0.072			0.63			0.90		
	7	1–3		0.056	±0.010		0.06	±0.09		0.03	±0.02
60% glucose			0.028			0.57			0.93		
60% galactose			0.096			0.90			1.87		
	7	4–6		0.075	±0.010		0.30	±0.06		0.52	±0.11
60% glucose			0.021			0.60			1.32		
30% galactose + 30% glucose			0.021			0.80			1.10		
	7	1–3		0.011	±0.004		0.45	±0.05		0.48	±0.07
30% glucose			0.010			0.35			0.62		

[1] Standard error of mean difference.

TABLE 3
Urinary magnesium excretion

Diet	No. of animals	Urinary magnesium
		mEq/24 hrs
60% glucose	27	0.104 ± 0.008[1]
30% glucose + 30% galactose	7	0.105 ± 0.019
60% galactose	8	0.198 ± 0.011

[1] Mean ± S.E.

Magnesium excretion. Urinary magnesium excretion was also found to be greater in animals ingesting galactose than in those fed glucose. The data for magnesium excretion were collected from several feeding experiments and, although the animals were not pair-fed, daily intakes approximated in all cases 10 gm of diet containing 0.6 mEq of magnesium. Again the effect was evident with intakes of 60 but not 30% of galactose. Table 3 summarizes the data.

Serum calcium and magnesium and blood pH. The serum calcium concentration of 9 animals fed galactose was 7.50 ± 0.20 mEq per liter as compared to 6.12 ± 0.20 mEq per liter in 12 animals ingesting glucose. In 11 animals fed a 60% galactose low-calcium diet the serum calcium level was 5.90 ± 0.12 as compared to 5.18 ± 0.15 mEq per liter in 7 animals fed the glucose diet.

The serum magnesium levels were not different in galactose and glucose-fed animals. In 9 control animals serum magnesium was 1.45 ± 0.19 mEq per liter as compared to 1.24 ± 0.16 in 5 animals fed the galactose diet. In 6 animals fed a 60% galactose diet the blood pH was 7.39 ± 0.12 as compared to 7.48 ± 0.03 in 5 animals fed a diet of dog checkers.

DISCUSSION

Bonnamour and Escallon ('13) reported that the daily intravenous administration of lactose to a rabbit for three months resulted in marked depletion of bone ash. In studies reported here the depletion by galactose feeding of bone calcium in rats on low-calcium intakes was less than that in a comparable group of animals fed glucose. The glucose-fed animals, however, trebled their weight while the galactose-fed animals failed to double theirs. Assuming the total body calcium initially present to be the same in both groups, the dilution of the original calcium by growth in the glucose-fed animals resulted in a lower bone ash than that produced by the calcariuria in rats fed galactose. Further experiments with diets isocalorically utilized will be necessary to determine the extent of decalcification of the body that galactose feeding will produce.

Galactose-induced renal excretion of calcium is not related to over-all balance. In these studies the degree of galactose-induced calcariuria was unchanged during negative and positive calcium balance. In rats fed a 60% glucose diet renal calcium excretion did not change in response to changes in absorptive rates.

The polyuria of galactose-fed animals is not a factor in the elevated renal excretion of calcium and magnesium. Elevated renal excretion of potassium and sodium usually develops only after several days of galactose feeding, while calcariuria develops immediately. Rats fed a diet containing 30% of galactose had fluid intakes of 30 ml per day (twice that of controls) with natriuria and kaliuria but without calcariuria.

Increased renal excretion of calcium and magnesium is not related to the development of an acidosis with galactose feeding. In these studies blood pH was not affected by galactose feeding. Handler ('47) found that the serum CO_2 combining power of rats fed an 80% galactose diet was 57% by volume as compared to 56% in rats fed sucrose diets and not showing calcariuria.

The total serum calcium concentration is higher in the galactose-fed animals as compared to those on the glucose diet. Handler ('47) reported that animals fed an 80% galactose diet had a serum calcium concentration of 5.9 mEq per liter as compared to 5.0 mEq per liter for glucose-fed animals. Serum magnesium values, on the other hand, are the same in animals fed glucose or galactose diets. Whether this involves more efficient renal handling of magnesium, or a different mechanism to account for its excretion, is not known.

Human infants with congenital galactosemia develop proteinuria (Butler and Flynn, '58) and amino-aciduria (Bickel and Hickmans, '52; Holzel et al., '52) during galactose feeding. Rats fed diets containing galactose do not show amino-aciduria (Human et al., '58). The amino-aciduria of congenital galactosemia is explained by development of renal tubular dysfunction (Cusworth, Dent and Flynn, '55). Further studies are needed to determine whether the elevated renal excretion of calcium and magnesium are due to a direct action of galactose on the kidney.

SUMMARY

1. Rats ingesting a 60% galactose diet excrete more calcium and magnesium in the urine than pair-fed animals ingesting glucose.

2. The increased excretion of these ions during galactose feeding is independent of the rate of intestinal absorption.

3. Serum calcium but not magnesium levels are elevated in galactose-fed animals.

4. Animals fed a 60% galactose diet do not develop an acidosis to account for the urinary excretion of calcium and magnesium.

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The Importance of Arginine and Methionine for the Growth and Fur Development of Mink Fed Purified Diets[1]

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Evidence available from studies with mink fed purified diets has indicated that the mink require three unidentified factors, in addition to the known crystalline vitamins, for growth and survival (Schaefer, Tove, Whitehair and Elvehjem, '48; Tove, Lalor and Elvehjem, '50a; Leoschke, Lalor and Elvehjem, '53). Two of the unknown factors are found in liver and the third unidentified factor is present in hog intestinal mucosa. One of the unknown factors present in liver has been designated the residue factor as it is present in the insoluble residue from a 60% methanol extract of liver. Studies by Tove, Lalor and Elvehjem ('50b) have indicated a relationship between the residue factor required by the mink and the fur quality of the mink. Mink placed on the purified diet developed depigmented, poor quality underfur while animals receiving the purified diet supplemented with the residue fraction of liver were observed to have dense, fine quality underfur equal to that of mink receiving liver.

The results of the present studies indicate that arginine and methionine may replace the residue factor required by the mink for growth and quality fur production.

EXPERIMENTAL AND RESULTS

Weanling male mink kits weighing 400 to 500 gm were used in these studies and were given a purified diet similar to that employed in the earlier studies. This purified diet consisted of sucrose 66, casein[3] 19, cottonseed oil 11, and salts IV (Phillips and Hart, '35) 4%. Each 100 gm of ration were supplemented with 0.2 mg thiamine·HCl, 0.2 mg pyridoxine·HCl, 0.4 mg riboflavin, 1.5 mg Ca pantothenate, 4.0 mg nicotinic acid, 100 mg choline, 25 mg i-inositol, 50 mg *p*-aminobenzoic acid, 0.5 mg 2-methyl, 1-4 naphthoquinone, 0.1 mg pteroylglutamic acid, 0.025 mg biotin and 0.004 mg of vitamin B_{12}. Haliver oil fortified with α-tocopherol acetate and vitamin D_3 was added so that 100 gm of ration contained 1,200 I.U. vitamin A, 120 I.U. vitamin D and 4 mg α-tocopherol acetate. Feed consumption of the mink on the purified diet varied from 75 to 150 gm per animal per day.

The kits were fed the purified diet starting early in July. By September or October they exhibited the characteristic signs of the residue factor deficiency. It should be pointed out that the time of occurrence of the residue factor deficiency in the mink is concurrent with the fur production phases of the animal's life. A few mink were able to survive through the fall and winter months on the unsupplemented purified diet. They exhibited only a slight growth depression during the fall fur production period. However, with the initiation of the spring fur production phase, April and May, these animals developed the residue factor deficiency. With the onset of the residue factor deficiency in the fall or spring fur production periods the mink were given different supplements mixed daily with the purified diet. Supplementa-

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[2] Present address: Chemistry Department, Valparaiso University, Valparaiso, Indiana.

[3] General Biochemicals, Inc.

TABLE 1

Weight responses of mink with the residue factor deficiency to different dietary supplements

Dietary supplement	Gain 6 weeks[1,2]
	gm
4% liver residue	280 ± 34 (7)
200 ml milk	257 ± 12 (9)
10% skim milk powder	234 ± 37 (10)
10% cottage cheese	278 ± 17 (10)
0.50% L-arginine 0.25% DL-methionine 0.30% DL-threonine 0.30% DL-tryptophan	236 ± 11 (7)

[1] Mean and standard error of the mean.
[2] The number of mink per group is indicated by the number within parentheses.

tion of each mink's diet was initiated when the animal manifested significant weight loss (150 gm or more) and severe anorexia (feed consumption of less than 20 gm per day). With the addition of active supplements an immediate recovery of appetite was noted with subsequent weight gains. Mink receiving inactive preparations or no dietary supplements continued to refuse to eat, lost weight and died. Upon autopsy these animals exhibited severe fatty degeneration of the liver.

The weight responses of mink with the residue factor deficiency to different dietary supplements are presented in table 1. Excellent weight gains were obtained with the liver residue, whole milk, skim milk powder and cottage cheese. The good weight responses of the animals with the nutritional deficiency to the addition of cottage cheese to the diet were interesting. These data indicated that the residue factor deficiency might be related to the quality of protein or level of protein in the mink's diet, i.e., the residue factor deficiency might be a deficiency of a specific amino acid or combination of amino acids. Thus a number of different amino acid mixtures were added to the diet of animals exhibiting symptoms of the residue factor deficiency. Good responses in weight gains were obtained with an amino acid mixture containing arginine, methionine, threonine and tryptophan.

The weight gain of a mink with the residue factor deficiency in response to the addition of the 4 amino acids to the purified diet is shown in figure 1. It is interesting to note that although excellent growth is obtained on the purified diet containing 19% of casein during July and August, an amino acid deficiency is precipitated in September at the beginning

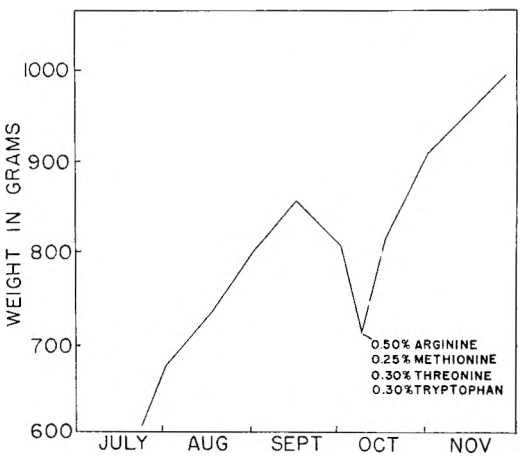

Fig. 1 Responses of mink with the residue factor deficiency to amino acid supplementation.

TABLE 2

Weight responses of mink with the residue factor deficiency to amino acid supplementation

Protein and amino acid composition of diet	Amino acid supplement	Gain 6 weeks[1,2]
		gm
19% casein 0.25% DL-methionine 0.30% DL-threonine 0.30% DL-tryptophan	0.50% L-arginine	224 ± 20 (7)
19% casein 0.50% L-arginine	0.25% DL-methionine	250 ± 46 (6)
19% casein	0.50% L-arginine 0.25% DL-methionine	346 ± 52 (5)

[1] Mean and standard error of the mean.
[2] The number of mink per group is indicated by the number within parentheses.

of the fur production period. Data on the weight responses of mink with the residue factor deficiency to the addition of specific amino acids to the purified diet are shown in table 2. These data indicate that the residue factor deficiency of mink on purified diets is a critical deficiency of two amino acids, arginine and methionine. Mink on diets supplemented with only one of the required amino acids developed distinct nutritional deficiencies showing anorexia and loss of weight. With the addition of the required amino acid, arginine or methionine, to the diet, an immediate recovery of appetite was noted with the subsequent weight response shown in the table. The large weight responses of the mink in the third group to the addition of both arginine and methionine to the diet was primarily due to a greater loss of weight in these animals prior to the development of severe anorexia and subsequent dietary supplementation.

Figure 2 shows the growth curves of mink on the purified diet containing arginine and methionine and on a practical ranch diet. The ranch diet contained 40% of horsemeat, 30% of fish, 10% of liver and 20% of cereal mix. With the grading of the mink in November it was noted that the fur quality of the animals on the purified diet was comparable to that of those on the practical ranch diet. Although the mink on the purified diet grew slowly in the early part of the growth period, at pelting in December these animals were equal in size and fur quality to those on the ranch diet.

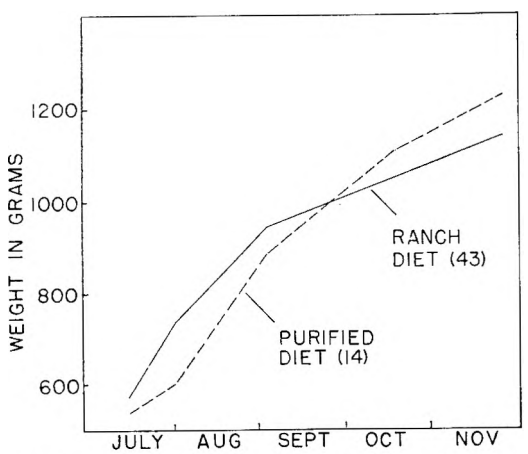

Fig. 2 Growth of mink fed a purified diet containing arginine and methionine or a practical ranch diet. The number of mink per group is indicated by the number within parentheses.

DISCUSSION

From the data presented it is evident that the residue factor deficiency of the mink fed purified diets is a deficiency of two amino acids, arginine and methionine. The weight responses of deficient mink to supplements of arginine and methionine were equal to the weight responses to the liver residue obtained by Tove and coworkers ('50b). With reference to Block and Bolling's ('45) data on the amino acid composition of casein, the purified diet employed in these studies contained approximately 0.6% methionine and 0.8% arginine. The addition of 0.25% DL-methionine and 0.50% L-arginine to this diet brought about the immediate recovery of mink exhibiting symptoms of the residue factor deficiency. Inasmuch as only a limited number of animals were available for the experimental studies, no attempt was made to ascertain the minimum level of arginine and methionine supplementation required by the mink for growth and fur production.

There is a striking correlation between the time of onset of the residue factor deficiency in the mink and the initiation of the fur production phases of the mink's life. It is apparent that the mink are able to show good growth on the purified diet containing 19% casein until the beginning of fur production in the fall. Then, with a critical demand for amino acids for the synthesis of new fur, an amino acid deficiency is precipitated. Recent studies on the amino acid composition of mink fur by Moustgaard and Riis ('57) offer further evidence that arginine and the sulfur amino acids are important for the fur production of the mink. Their data show that mink fur contains 7.6% arginine, 1.0% methionine and 15.1% cystine. The analyses of the mink fur indicated that, with the exception of cystine, arginine was present in highest concentration.

The importance of arginine for the nutrition of the chick has been known for many years (Arnold, Kline, Elvehjem and Hart, '36). The critical value of arginine

and methionine for the nutrition of the guinea pig has been shown recently by Heinicke, Harper and Elvehjem ('55).

The mink's requirement for arginine when fed on purified diets containing casein as the sole source of protein may be accentuated by an incomplete availability to the mink of the arginine present in the casein. Arnold et al. ('36) found that the arginine in casein was only partially available to the chick.

As seen from figure 2, mink can be raised from weanling to maturity on a purified diet. However, the knowledge of the nutrition of the mink on purified diets is not complete. The exact nature of the other two unidentified factors required by the mink is still unknown. These two factors are the one present in whole liver and the hog mucosa factor. Nutritional deficiencies requiring these two unknown factors do not develop until the mink have been fed the purified diet for at least one year. Preliminary data indicate that the unknown factor present in whole liver may also be found in lard. Thus the liver factor may be an animal fat factor, an unknown factor present in such animal products as liver, spleen and lard but lacking in a vegetable fat such as cottonseed oil.

SUMMARY

1. The liver residue factor deficiency of the mink fed purified diets has been shown to be a deficiency of two amino acids, arginine and methionine.

2. Arginine and methionine have been shown to be of critical importance for the fur production of the mink fed purified diets containing casein as the sole source of protein.

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Serial Determinations of Serum Protein-bound Carbohydrates and Proteins During Protein Depletion and Repletion in the Adult Rat[1]

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The systemic effects of nutritional states as reflected by changes in the concentration and distribution of the protein-bound carbohydrates and proteins in the serum of the adult rat have been the subject of several recent communications from this laboratory (Weimer and Nishihara, '57, '59a, '59b). Inanition, acute and chronic protein depletion, and the level of dietary protein were found to affect the concentrations of the serum glycoproteins and proteins. The present study was initiated to investigate the effects of the degree of protein depletion and repletion on the serum components.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were employed. The animals weighed between 350 and 380 gm at the time of receipt and were approximately 4 months old at the initiation of the study. They were housed singly in suspended type wire cages with wire bottoms and were maintained on an antibiotic-free commercial chow[2] and tap water, both supplied ad libitum. The use of larger animals was dictated by the difficulties in obtaining adequate blood samples from smaller rats, especially when depleted and by their satisfactory response in earlier studies (Weimer and Nishihara, '57, '59a, '59b). The animals were sacrificed by exsanguination from the heart while under ether anesthesia.

Diets. Due to limitations in cage space, some rats in groups A1, B2, C3, C4 and groups A2, B1, B3, C1 and C2 were fed simultaneously. Additional animals in groups A1, B2, C3 and C4 were fed in another trial with results in good agreement with those obtained previously.

Normal. Two normal groups were employed. Group A1 served as a base line control and A2 as an age and weight control. They were maintained under the conditions described above.

Depletion. The animals were fed a protein-free diet[3] previously described (Weimer and Nishihara, '57) until the desired weight loss was achieved. Groups of rats were sacrificed after losing 10, 25, and 40% of their original weight.

Repletion. A commercial chow[2] with a protein content of approximately 25% was fed ad libitum to animals which had sustained a 25% weight loss on the protein-free diet. The choice of this ration for repletion was based on earlier studies in which (1) the diet was found to be adequate for weight and serum protein repletion (Weimer and Nishihara, '59b) (2) the observation that there were no significant differences in serum glycoprotein and protein concentrations between rats which were repleted on the commercial chow and those repleted on a purified diet of comparable protein content containing casein (cf. Weimer and Nishihara, '59a, '59b). Groups of rats were sacrificed after regaining 35, 70, and 100% of their lost weight. In addition, one group was allowed to survive 4 weeks following weight repletion.

Chemical and hematologic determinations. Serum chemistry and hematologic

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[1] Supported in part by grants from the National Cancer Institute (C-2368) and the California Institute for Cancer Research.
[2] Purina Laboratory Chow.
[3] Nutritional Biochemicals Corporation, Cleveland, Ohio.

TABLE 1

General and hematologic data

Group	No. rats	Initial wt.[1]	Depletion wt.[1]	Depletion time	Final wt.[1]	Repletion time	Hemoglobin[1]	Hematocrit[1]
		gm	*gm*	*days*	*gm*	*days*	*gm %*	*%*
A1, normal (400 gm)	40	404 ± 3.0					14.3 ± 0.19	45 ± 0.3
A2, normal (460 gm)	14	457 ± 2.9*					13.8 ± 0.20**	46 ± 0.5
B1, 10% depleted	13	400 ± 4.3	357 ± 3.9*	7.0			14.7 ± 0.15	49 ± 0.3*
B2, 25% depleted	26	399 ± 3.2	301 ± 2.9*	26.2			13.5 ± 0.28*	43 ± 0.4*
B3, 40% depleted	10	402 ± 5.4	239 ± 3.4*	62.0			11.8 ± 0.33*	42 ± 1.7*
C1, 35% repleted	12	397 ± 5.5	299 ± 4.0*	27.2	333 ± 4.2*	2.5	11.8 ± 0.24*	38 ± 0.6*
C2, 70% repleted	13	399 ± 6.4	297 ± 5.1*	27.2	368 ± 5.9*	5.0	11.8 ± 0.30*	37 ± 0.5*
C3, 100% repleted	28	403 ± 3.8	303 ± 2.7*	21.5	402 ± 4.0	8.6	12.4 ± 0.17*	40 ± 0.5*
C4, 4 wks. post-repletion	26	401 ± 3.1	300 ± 2.5*	24.2	459 ± 4.0*	33.8	14.0 ± 0.20	45 ± 0.5

[1] Including the standard error of the mean.

Statistically significant differences from normal values (Group A1) are indicated: * $P = < 0.01$; ** $P = < 0.05 > 0.01$.

analyses were carried out by methods previously reported in detail (Weimer and Nishihara, '59a). The mean, standard error of the mean, *t*, and probability values were determined by standard statistical procedures (Snedecor, '56).

RESULTS AND DISCUSSION

General and hematologic data are summarized in table 1. Changes in plasma volume as indicated by hematocrit values were in general agreement with the earlier studies reviewed by Keys et al. ('50) and by Allison ('55). In the early stage of protein depletion (group B1) there was a marked hemoconcentration which was followed by hemodilution as depletion progressed. Pronounced hemodilution occurred during repletion. Four weeks following repletion, hematocrit levels were in the normal range. Hemoglobin was restored more slowly than the serum glycoproteins and proteins (figs. 1, 2).

The results of the chemical determinations are presented for whole serum and the globulin fraction in figure 1 and for the albumin and seromucoid fraction in figure 2. During depletion the rate of decrease of the serum components was greatest during the first week (B1) with the exception of the bound carbohydrate of the albumin fraction. After a weight loss of 25% (B2), all serum constituents were significantly decreased. In the 36-day time interval between the sacrifice of the 25% and 40% (B3) depleted groups, no significant alterations were observed in the total serum values. Pronounced changes, however, had occurred in the subfractions of serum. Globulin protein declined significantly, while marked increases were found in the seromucoid and albumin fractions. The elevations of the protein components were somewhat greater than those of the polysaccharide moieties. Apparently in severe depletion there occurred either in-

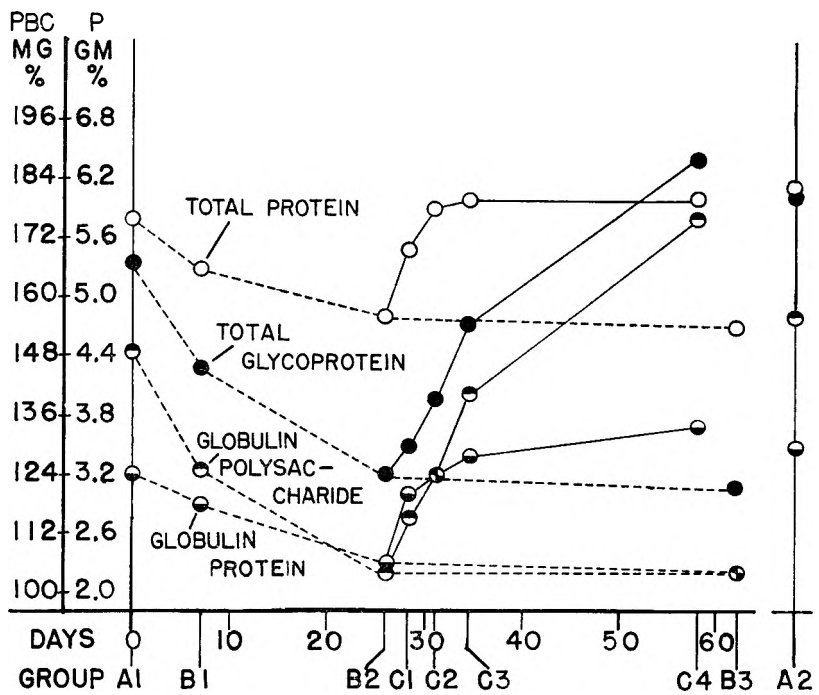

Fig. 1 Effects of protein depletion and repletion on protein-bound carbohydrates and proteins of whole serum and the globulin fraction. Dashed and solid lines indicate when protein-free and stock diets, respectively, were fed. ●, Total serum glycoprotein; ○, Total serum protein; ◓, Globulin polysaccharide; ◒, Globulin protein. Protein-bound carbohydrate and protein values are expressed in milligrams per cent and grams per cent respectively. Plotted values are not corrected for experimental hematocrit. Statistical data are available upon request from the authors.

creased synthesis, a slower rate of turnover, or the release of tissue proteins with the solubility properties of seromucoid and of albumin.

Weight repletion was characterized by rapid increases in total serum and globulin fraction values and considerable fluctuation in the concentrations of the components of the albumin and seromucoid fractions. Due to the marked hemodilution which was still present at the end of the repletion period (C3), serum values were corrected for the reduced hematocrit by the formula:

$$\frac{(100-H2)\ (\text{observed concentration})}{(100-H\ 1)}$$

in which H1 = normal hematocrit and H2 = experimental hematocrit (Boas and Peterman, '53). The adjusted values with the notable exception of albumin polysaccharide were either in the normal range (seromucoid protein, total glycoprotein, globulin polysaccharide, seromucoid polysaccharide) or significantly increased (total, albumin, and globulin proteins).

Four weeks after repletion (C4), total serum glycoprotein and globulin polysaccharide levels were significantly increased over both normal groups (A1, A2). A comparison of the data for the normal groups indicated an increase in these serum constituents with age. The observation was in accord with that of Shetlar et al. ('48) for man and was presumably a major factor in the noted increases for group C4. The fact that certain significant differences existed between the experimental and control groups suggested that stimulation of glycoprotein synthesis caused by protein depletion may also have been involved. The protein components of the seromucoid and globulin fractions continued to increase following repletion (C4) concomitant with a significant decline in albumin concentrations. Serum total and seromucoid protein values for the group were in the normal range for rats of this weight but considerable var-

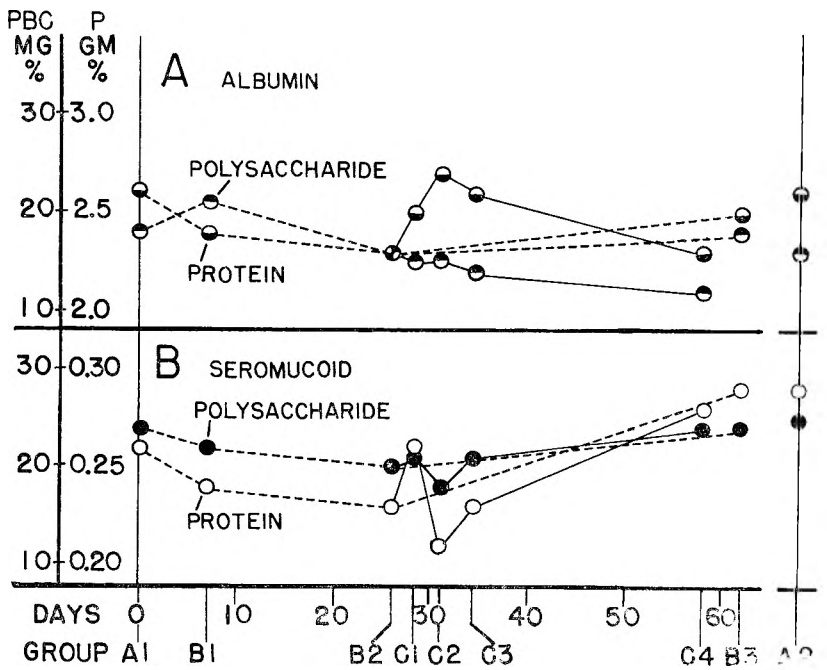

Fig. 2 Effects of protein depletion and repletion on protein-bound carbohydrates and proteins of albumin (A) and seromucoid (B) fractions. Dashed and solid lines indicate when protein-free and stock diets, respectively, were fed. ◓, Albumin polysaccharide; ◒, Albumin protein; ●, Seromucoid polysaccharide; ○, Seromucoid protein. Protein-bound carbohydrate and protein values are expressed in milligrams per cent and grams per cent respectively. Plotted values are not corrected for experimental hematocrit. Statistical data are available upon request from the authors.

TABLE 2

Protein-bound carbohydrate/protein[1] and A/G ratios during protein depletion and repletion

Group	Protein-bound carbohydrate/protein				A/G	
	Total serum	Seromucoid fraction	Albumin fraction	Globulin fraction	Protein-bound carbohydrate	Protein
	%	%	%	%		
A1, normal (400 gm)	2.9 ± 0.03[2]	9.2 ± 0.19	0.7 ± 0.03	4.7 ± 0.08	0.12 ± 0.005	0.81 ± 0.018
A2, normal (460 gm)	3.0 ± 0.04	8.6 ± 0.29	0.6 ± 0.05	4.7 ± 0.08	0.10 ± 0.008**	0.74 ± 0.012**
B1, 10% depleted	2.8 ± 0.04	9.2 ± 0.15	0.9 ± 0.05*	4.3 ± 0.04*	0.17 ± 0.010*	0.83 ± 0.033
B2, 25% depleted	2.6 ± 0.04*	8.7 ± 0.10**	0.7 ± 0.02	4.3 ± 0.11*	0.15 ± 0.007*	0.92 ± 0.033*
B3, 40% depleted	2.6 ± 0.12*	8.3 ± 0.24**	0.7 ± 0.05	4.7 ± 0.12	0.17 ± 0.017*	1.14 ± 0.053*
C1, 35% repleted	2.4 ± 0.04*	8.1 ± 0.51**	0.6 ± 0.05	3.8 ± 0.07*	0.13 ± 0.012	0.84 ± 0.022
C2, 70% repleted	2.4 ± 0.04*	8.6 ± 0.18	0.6 ± 0.06	3.9 ± 0.08*	0.12 ± 0.011	0.84 ± 0.021
C3, 100% repleted	2.6 ± 0.02*	9.1 ± 0.24	0.5 ± 0.04*	4.1 ± 0.04*	0.10 ± 0.007**	0.76 ± 0.018
C4, 4 wks. post-repletion	3.1 ± 0.03	8.6 ± 0.11	0.5 ± 0.05	4.8 ± 0.03	0.07 ± 0.005*	0.62 ± 0.023*

[1] Protein-bound carbohydrate ÷ protein × 100.

[2] Findings include standard error of the mean.

Statistically significant differences from normal values (A2, B1, 2, 3, C1, 2, 3 were compared with A1; C4 with A2) are indicated: * P = <0.01; ** P = <0.05 >0.01.

iance was observed for the albumin and globulin levels which resulted in significantly decreased A/G ratios (table 2). A significant decrease in the albumin fraction following repletion has been reported for man (Keys et al., '50) but the cause of the phenomenon has never been established.

To compensate for the changes in plasma volume and further to define their differential response, a study was made of the relationships of the protein-bound polysaccharides and proteins of serum and of the A/G ratios (table 2). The most pronounced early change during depletion was the significant decrease of the ratio in the globulin fraction. In severe depletion, however (B3), the ratio for the fraction was restored to normal, notwithstanding a significant decrease in the ratio for total serum determinations.

A general decrease in the ratios occurred in the early stage of repletion (C1). At repletion (C3), only the ratio for the seromucoid fraction was in the normal range. Ratios for the post-depletion group, C4, were in the normal range when compared with their weight control group, A2. It was apparent, however, that in the 4-week period following repletion, the protein-bound carbohydrates of the serum globulins had increased to a greater extent than the protein components.

The results of the A/G ratio computations emphasized the relatively greater stability of the albumin fraction of the adult, male rat to protein depletion. When the stock diet was introduced (C1), the ratios rapidly returned to normal. Following repletion (C4) and with increasing age and weight (A2) there were inversions of the ratios which were more marked in the experimental group.

The present study has demonstrated the diversity of the responses of the protein-bound carbohydrates and proteins of serum during the course of protein depletion and repletion. Recent investigations on the development and application of the techniques of immunoelectrophoresis (Williams and Grabar, '55), starch gel electrophoresis (Poulik and Smithies, '58) and anion-exchange cellulose chromatography (Fahey et al., '58) have provided adequate evidence for the heterogeneity of the major fractions of the serum proteins. It seems probable that the concentration changes observed in the current study reflected alterations in the metabolism of the carbohydrate-rich and carbohydrate-poor proteins of serum in adjusting the tissue requirements of the organism to the available diet. Significantly decreased polysaccharide-protein ratios for total serum and the globulin fraction suggested that carbohydrate-rich proteins were utilized to a greater extent than carbohydrate-poor during moderate depletion (B2) and in repletion (C1, 2, 3).

The separation of tissue anabolism and catabolism in the intact animal presents formidable difficulties which as yet have not been resolved. It might be anticipated that the application of the more recent and sensitive techniques of protein fractionation cited above would provide additional information on the influence of nutritional states on the simple and conjugated proteins of serum.

SUMMARY

Serial determinations of the protein-bound carbohydrates and proteins of serum and of the seromucoid, albumin, and globulin fractions as well as the hemoglobin and hematocrit values of blood were made during the course of protein depletion, repletion and 4 weeks following repletion of the adult rat. Weight depletion to 25% was accompanied by significant decreases in all serum values. Severe depletion (40%) elicited variable responses; the seromucoid and albumin fractions were restored to normal while the serum globulins declined further. At repletion, all serum constituents were in the normal range or increased with the exception of albumin polysaccharide. Following repletion, the concentrations of the components of the albumin fraction decreased. Other serum constituents remained in the normal range or increased. Some of the elevation was attributable to increased age and weight. Hemoconcentration (10% depletion) was followed by hemodilution during further depletion and in repletion. Four weeks following repletion, hemoglobin and hematocrit values were normal. Hemoglobin was restored at a slower rate than were the serum glycoproteins or proteins.

ACKNOWLEDGMENT

The technical assistance of Mrs. Dorothy M. Roberts is gratefully acknowledged.

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The Reaction of the Rat to Different Levels of Manganese Following Hepatectomy[1]

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Numerous reports have appeared on the effects of dietary manganese in the rat. Von Oettingen ('35) has shown that manganese concentration normally occurs in the liver, and Wachtel et al. ('43) and Holtkamp and Hill ('50) have stated that a diet of less than 2 or 3 ppm of manganese significantly retards the growth rate of rats. Orent and McCollum ('31), Skinner et al. ('32), Amdur et al. ('45), and Hill et al. ('52) have reported ataxia, reproductive failure and poor bone development as effects of manganese deficiencies. Barnes et al. ('41), Boyer et al. ('42), and Shils and McCollum ('43) have reported that to produce these deficiency effects, the rats must be depleted of their manganese stores before the experimental work is initiated. Chornock et al. ('45), and Penalver ('55) are among the few who have reported on the effects of large amounts of manganese in the diet, and consider that this problem warrants further study.

Since manganese normally becomes concentrated in the liver and, in rats, recovery is rapid after partial hepatectomy, as reported by Ralli et al. ('51), the present investigation was undertaken (1) to determine whether partial hepatectomy combined with a low-manganese diet could produce a detectable manganese deficiency in the rat in one generation; (2) to observe the effects of a high-manganese diet upon the rat; and (3) to see if any histological changes could be correlated with the physiology of the animal.

MATERIALS AND METHODS

Sixty white rats of the Holtzman strain, equally divided as to sex, were used in this study. These were divided into 6 groups of 10 each; two groups were fed a high-manganese diet (50 ppm), two a low-manganese diet (0.8 ppm), and two a control diet (2.0 ppm of manganese). In each category there was one operated and one non-operated group. The experimental diets are given in table 1.[2] The rats were fed the diets starting 24 days after birth, and partial hepatectomy, in which 70% of the liver was removed, was performed on the 52nd day. The animals were fed ad libitum and testing continued until day 107. At various intervals rats were sacrificed and histological sections of the liver, spleen, kidney, gonad, thyroid, adrenal and pituitary prepared for study. Weights of all the organs were recorded

TABLE 1
Low-manganese basal diet and mineral mixture

Ingredient	Amount
	%
Basal diet	
Cerelose	37.86
Dried skim milk	46.00
Casein	5.00
Vitamins	0.27
Fat[2]	4.00
Vit. A, D, E (fish oil)[3]	1.00
Minerals	3.87
Celluflour	2.00
Mineral mixture[1]	
NaCl	4.85
$MgSO_4$ (anhyd.)	7.43
NaH_2PO_4	9.71
K_2HPO_4	26.64
$CaH(PO_4)_2 \cdot H_2O$	15.07
Ca lactate	36.24
$CuSO_4$	0.026
$ZnCO_3$	0.026
KI	0.008

[1] Manganese sulfate (anhyd.) was added to form the control and high-manganese diets.
[2] Partially hydrogenated vegetable oils (Primex).
[3] Alpha tocopherol was added.

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[1] This research was supported by U. S. Public Health Grant no. A-1009.
[2] These diets were devised in the Department of Biochemistry at Purdue University, Lafayette, Indiana.

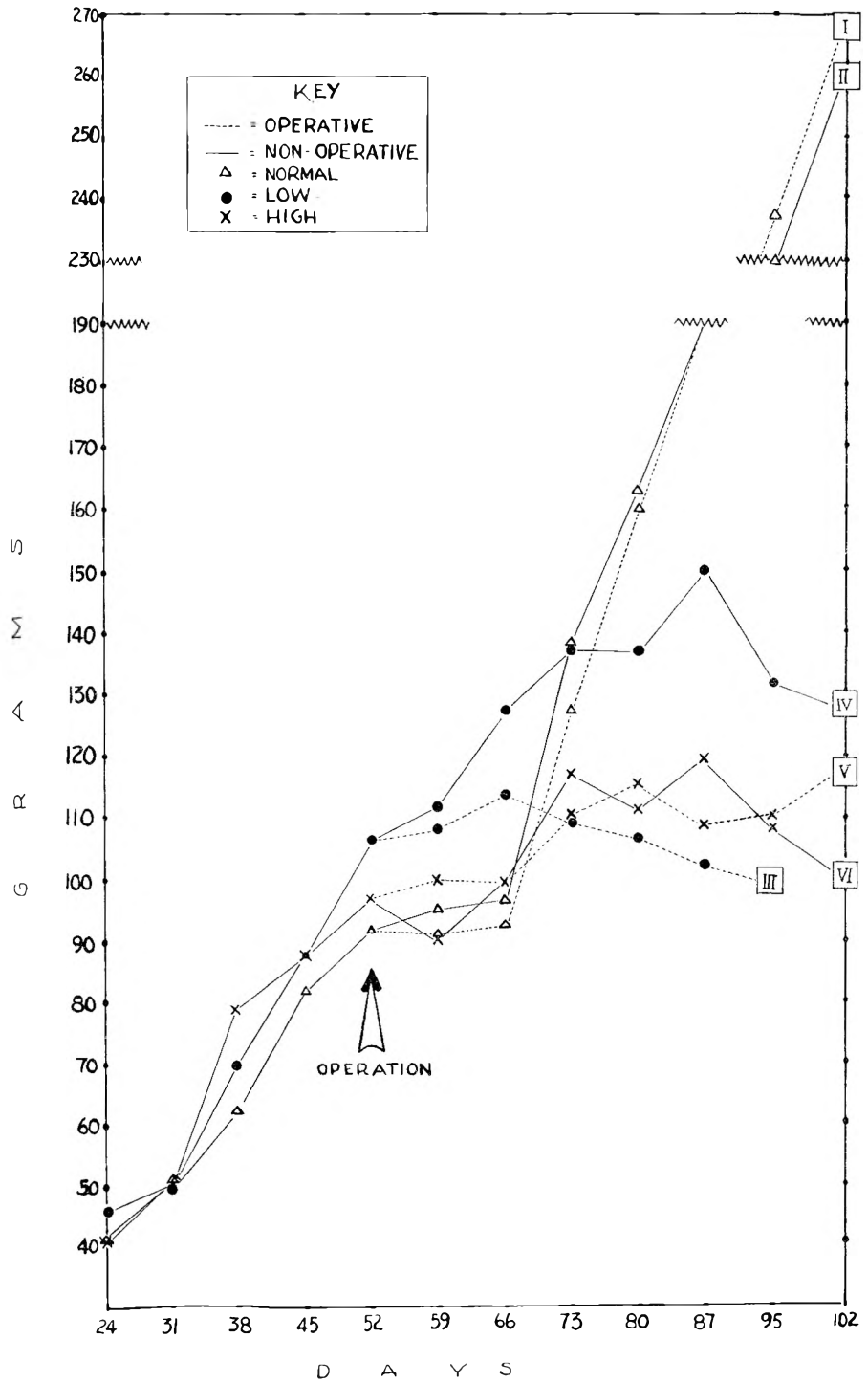

Fig. 1 Effect of partial hepatectomy and level of manganese on the growth rate of rats.

as percentage of body weight. Weekly weights were taken of all groups and the gain per day computed.

Twelve rats were used to measure thyroid activity. Five animals were treated with 5 microcuries of iodine^{131} injected intraperitoneally at day 105 and subjected to a 27- to 30-hour uptake study,[3] using a Scintillation Counter Thallium Activated Sodium Iodide Crystal with the pulse height analyzer set for the iodine energy peak. To obtain the count, 5 one-minute counts were averaged. Studies of resistance to anoxia were done on the other 7 rats, using the technique of Zarrow et al. ('51). A one-liter Atlas special Mason jar was used and the weights of the animals in relation to the time of death were computed. All the anoxia work was carried out at a temperature of 25°C.

RESULTS

It can be seen (fig. 1) that the average weight of the animals in the three dietary groups correlates closely until partial hepatectomy. The control animals gained at a greater rate than those in the other groups (table 2). The growth rates of the animals receiving the manganese-deficient diet began to show a difference after the 59th day. The differences between the operated animals receiving the low-manganese diet and those fed the high-manganese ration were not significant at 87 days. However, the difference between the unoperated animals receiving the manganese-deficient diet and those fed high intakes of manganese was significant at the 0.05 level. The differences between the control animals and those receiving the diets high in manganese or low in manganese were significant at the 0.01 level at the 87th day. On day 100 the differences between the control animals and those fed experimental diets were significant at the 0.01 level. The differences between the animals fed a low-manganese diet and those fed the high-manganese diet were not significant at the 100th day.

The operated animals receiving the diet low in manganese exhibited symptoms of manganese deficiency by day 72, and by the 96th day all were dead. The non-operated group supplemented with a low-manganese diet showed signs of a slight manganese deficiency by the end of the experiment. The rats on the diets augmented by 50 ppm of manganese did not grow well and exhibited signs of nervousness. Their coats were poor and their testes were undersized.

Except in one instance, the weights of the organs exhibited no significant differences when expressed as a percentage of body weight. There was a change in the testis weights from an average of 0.49% in normal males to 0.37% in rats receiving the high-manganese rations. This was significant at the 0.01 level.

The effects of the diets upon the histology of the rat varied. The histology of the control animals was normal for all organs studied. The animals fed the low-manganese ration exhibited excessive bile duct proliferation, though in the livers of these rats, the hepatic cells appeared normal. The epithelial cells of the thyroid were

[3] Compliments of Dr. R. Ogburn, Chief, Radioisotope Laboratory, Veterans Memorial Hospital, Omaha, Nebraska.

TABLE 2

Effect of manganese level of the diet and of partial hepatectomy on the growth of rats

Dietary treatment	Hepatectomy	Group	Gain	
			Day 87	Day 100
			gm/day	*gm/day*
Control	Operated	1	2.33[1]	2.61[1]
	Non-operated	2	2.28	2.59
Low Mn	Operated	3	1.18[2]	None
	Non-operated	4	1.72	1.29
High Mn	Operated	5	1.24[1]	1.00[1]
	Non-operated	6	1.37	1.16

[1] Differences not significant.
[2] Differences significant beyond the 0.02 level.

quite flat and there was an excess of colloid. Since the cell heights of the epithelium indicate the physiological condition of this gland, a hypothyroidal condition was indicated. The kidney tubules, in some cases, showed signs of parenchymatous degeneration in which the cells appeared swollen and cloudy. There was also a slight amount of hyalinization present in these kidneys. This correlated with the observation that some of the operated animals receiving a diet low in manganese had urinary bladders filled with a hard crystalline substance.

The histological changes in the animals fed the high-manganese diet were limited to the thyroid and the testes. The thyroid exhibited a hypothyroidal condition and the germinal cells of the testis never progressed to produce sperm. This effect seen in the testis correlated with the underdevelopment of the testis noted grossly and as a percentage of body weight.

The thyroid activity studies showed that the uptake of iodine^{131} at 27 to 30 hours was 10.2% for the rats on the normal diet, 3.2% for those receiving the high-manganese ration, and an average of 3.05% for those fed the low-manganese diet. Thus the iodine^{131} uptake of the control animals was at least three times that of those fed the other two diets, indicating that the control animals had a more active thyroid gland than the animals of the other groups. The data on resistance to anoxia showed oxygen uptake averages of 0.394, 0.195, and 0.087 minutes per gram of body weight, respectively, for the animals receiving the high, low, and control rations. Though there were too few animals to permit satisfactory statistical analysis, this was taken to indicate that animals receiving a high-manganese diet have an increased resistance to anoxia. These studies indicated that the thyroids of the rats which had received either the deficiency diet or the diet supplemented with high amounts of manganese were in a hypothyroidal state, a conclusion that correlates well with the histological picture reported above.

DISCUSSION

It has been shown that partial hepatectomy causes a manganese deficiency by the 72nd day in rats fed a low-manganese diet. Since manganese accumulates mainly in the liver in animal tissue, as shown by von Oettingen ('35) and Maynard and Cotzias ('54), this operation has the effect of depleting the animals' store of this element. The reason the operated rats which received the low-manganese diet died by the 96th day is still unknown. There is a possibility that this could be due either to an altered Krebs urea cycle in the production of urine or to the lack of protection of the rats against ammonia toxicity. Greenstein et al. ('56) showed that arginine, which is activated by manganese, protects against ammonia toxicity, and Shils and McCollum ('43) have shown that the level of arginine is reduced in rat livers when there is a manganese deficiency. Since arginine is necessary for the formation of urea and manganese is a catalyst in this reaction, in a deficiency state, the urea cycle could be changed and cause ill effects in the rats. Further work is necessary to determine the cause of death in a manganese deficiency of this type and to see if the physiological effects can be correlated with the altered kidney histology. The bile duct proliferation found in these deficiency rats has been reported before in cattle by Bentley and Phillips ('51), but never in rats.

Though partial hepatectomy had no particular effect on the rats receiving the high-manganese diet, these animals showed an obvious response to the manganese in the diet. They were the most nervous animals, their coats lacked luster, and the males exhibited lack of testicular development. In contrast to the report of Chornock et al. ('45), these effects were noted with a diet containing 50 ppm of manganese. These results might be due to a toxic effect of too much manganese or to an antagonism of similar elements in the body.

The hypothyroidal condition in animals fed diets both high and low in manganese was shown histologically and by physiological tests. It had been reported previously in rats fed a low-manganese diet but not in rats on a diet high in manganese (Richards, '30). Whether this is due to a blocking of the thyroid hormone output or to a decrease in the pituitary trophic hormones is not known. Decreased pituitary activity in manganese deficiencies has

been mentioned by Orent and McCollum ('31), but this concept does not correlate with the percentage of cell types observed in the anterior pituitary with altered manganese diets as found by Van Donk et al. ('33), Boyer et al. ('42), Wachtel et al. ('43), and Urban ('54). Further investigations are necessary to study the mechanism causing this condition, though it has been reported by Reinke and Turner ('45) that manganese does activate the formation of thyroxine, and it has been stated by Ray and Deysach ('49) that the element accumulates in the thyroid in guinea pigs.

SUMMARY

A manganese deficiency was produced in rats easily after partial hepatectomy. In addition to the expected deficiency symptoms, a hypothyroidal condition and a degenerative condition in the kidney were noted. The high-manganese (50 ppm) diet caused manganese toxicity, as evidenced by nervousness, lack of coat luster, underdeveloped testis, and poor growth rate. A hypothyroidal condition was also caused by the high-manganese ration. Partial hepatectomy did not alter the effects upon control animals or those on a high-manganese diet.

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Biochemical Observations on Aortas of Chickens

EFFECT OF DIFFERENT FATS AND VARYING LEVELS OF PROTEIN, FAT AND CHOLESTEROL[1]

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Limited information is available on the effect of dietary components on the chemical constituents of the aorta. Studies with human aortas have attempted to differentiate chemically between normal and atherosclerotic tissues (Buck, '51; Buck and Rossiter, '51; Nobel et al., '57; Luddy et al., '58; Buddecke, '58 and others) but necessarily without relating the findings to diet except in a general way.

In the rabbit and also the chicken studies have been reported relating dietary cholesterol primarily to the lipid components of the aorta (Weinhouse and Hirsch, '40; Dauber and Katz, '43; Herrmann, '47; Peterson and Hirst, '51; Dam et al., '56; Hirsch and Nailor, '56; Dury, '57; Warnock et al., '57; Stamler et al., '58a and b and others). In the present investigation, analyses of cholesterol, polyunsaturated fatty acids and hydroxyproline of both thoracic and abdominal segments of the aorta were carried out in chickens receiving various dietary combinations of saturated or unsaturated fat, cholesterol and protein.

Aortic cholesterol concentration has been widely used as an indication of atherosclerosis; aortic hydroxyproline has been recently used to evaluate the fibrotic proliferation also associated with atherosclerosis (Buddecke, '58; Nobel et al., '57); the usefulness of aortic fatty acid analyses in assessing atherogenesis has received scant attention (Luddy et al., '58) but was included in this study in view of the current emphasis regarding fatty acids in the human diet. For similar reasons a highly saturated dietary fat (tallow) was compared to a highly unsaturated fat (linseed oil).

It has been inferred (Katz and Stamler, '53) that the spontaneous type of atherosclerosis in the chicken differs from the cholesterol-induced type. Other studies indicate a quantitative rather than a qualitative difference between the induced and the spontaneous type of avian atherosclerosis (Lindsay et al., '55). It was therefore a further purpose of the present study to compare analytically as well as by visual scoring the aortas from young growing cockerels with cholesterol-induced atherosclerosis to those of two- and three-year-old hens fed essentially cholesterol-free diets of known and experimentally varied composition for a 12-month period.

EXPERIMENTAL

The studies were carried out in two parts: the first was designed to evaluate the effects of fat, cholesterol, and protein level in growing cockerels, and part 2 was carried out with equal numbers of 8- and 20-month-old hens fed diets containing differently saturated fat.

Part 1. Day-old crossbred roosters were fed a practical starting ration for one week and then were assigned to the experimental rations (the composition of basal ration is given in table 1) for the subsequent 10 weeks. The experimental design as given in table 1 was essentially factorial with two levels of protein, corn oil, and cholesterol. One cholesterol level differed for each of the two protein levels and was chosen on the basis of the results shown in table 2. It was found that as little as 0.3%

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of cholesterol in the low protein diet induced a marked hypercholesterolemia which surpassed that induced with 2% cholesterol in a 20% protein diet.

The birds (10 per lot) were individually weighed every other week and feed consumption recorded on a group basis. At the end of the 10-week feeding period blood was drawn from the heart for plasma cholesterol determination before the birds were killed and the aorta removed. The aorta was thoroughly cleaned from the brachiocephalic arteries to the iliac bifurcation and visually scored by one of us (H.S.W.) according to procedures previously discussed (Weiss, '59). This method of scoring gives greater weight to the height and area of lesions protruding into the lumen than it does to the appearance of gross lipid deposition as judged by yellow color. After scoring, the aortas were placed in glass bottles with teflon gasketed metal caps and cold-extracted with chloroform-methanol (2:1) for 24 hours in a shaker. The extract was filtered, made up to volume and aliquots taken for cholesterol analysis by the modified method of Zlatkis (Griminger and Fisher, '58) and for polyunsaturated fatty acid and iodine number determination according to the procedures of Herb and Riemenschneider ('53) and Luddy et al. ('58). All analyses, including the cholesterol determinations, were made on individual aortas while the extracts for the fatty acid determinations were pooled from three or 4 birds resulting in three composite samples for each experimental group. The extracted aortas were dried to constant weight at 100°C and then weighed. They were then hydrolyzed with 6 N HCl at 15 lbs. pressure for 24 hours, neutralized, and hydroxyproline determined according to the method of Neuman and Logan ('50).

Part 2 of the present investigation was designed to study in 8- and in 20-month-old hens the effect of prolonged feeding of fats with varying degrees of saturation. Ten

TABLE 1

Composition of basal ration and experimental design of study with growing roosters

Ingredient	Amount
	%
Soybean meal (50% protein)	16.00
Dicalcium phosphate	2.50
Mico concentrate[1]	1.00
Distillers dried solubles	2.50
NaCl	0.50
B vitamins[2]	0.10
Vitamins A, D, and E[2]	0.10
Choline chloride	0.20
DL-Methionine	0.10
Glucose (cerelose)	to 100

[1] Trace mineral-containing limestone, product of Limestone Corporation of America, Newton, N. J.

[2] For composition see Fisher and Johnson ('56).

Experimental Design

Lot	Dietary variables		
	Protein	Corn oil	Cholesterol
	%	%	%
1	8	2	—
2	8	2	0.3
3	8	10	—
4	8	10	0.3
5	20[1]	2	—
6	20	2	2.0
7	20	10	—
8	20	10	2.0

[1] Additional DL-methionine (0.2%) was added to all high-protein diets; to obtain 20% protein the level of soybean meal in the ration was increased from 16 to 40%.

TABLE 2

Effect of graded levels of dietary cholesterol on plasma cholesterol of three week-old cockerels fed low- and high-protein diets

Dietary cholesterol	8% Protein die		20% Protein diet	
	Body wts.	Plasma cholesterol	Body wts.	Plasma cholesterol
%	*gm*	*mg %*	*gm*	*mg %*
0.1	209 ± 19[1]	299 ± 16	331 ± 16	204 ± 9
0.3	205 ± 16	773 ± 67	348 ± 15	246 ± 14
0.5	197 ± 16	1236 ± 55	344 ± 12	260 ± 13
1.0	197 ± 16	1771 ± 383	356 ± 13	380 ± 28
2.0	197 ± 10	2034 ± 250	345 ± 14	433 ± 46

[1] Mean ± standard error.

hens within each age group composed one treatment group. Treatments together with the composition of the basal ration are listed in table 3. The birds were fed their respective diets for one year. Throughout this period, egg production, feed consumption, mortality, body weight, and plasma cholesterol were recorded at regular intervals for each bird. After the birds had been killed the aortas were removed and analyses carried out essentially as described for part 1 except for the separate chemical treatment of thoracic and abdominal segments of the aorta.

Because of the interesting observations regarding the absence of polyunsaturated fatty acids beyond the tetraenoic acids in aorta fat, plasma polyunsaturated fatty acids were determined on a few birds which were not part of the present studies but had received essentially the same low-fat and linseed-oil-containing diets. The fatty acid analyses of the plasma were carried out according to the procedure of Morris and Riemenschneider ('58).

TABLE 3

Composition of basal ration and experimental design of studies with hens

Ingredient	Amount
	%
Corn meal	to 100
Soybean meal (50% protein)	20.00
Butyl fermentation product	2.00
Corn distillers solubles	2.00
Dried whey	2.00
Alfalfa meal	3.00
Dicalcium phosphate	4.00
Mico concentrate[1]	3.00
Limestone	1.00
NaCl	0.50
Choline chloride (70%)	25 gm/100 lbs.
Vitamin B_{12} supplement	0.50
Methionine hydroxy analogue[2]	0.10
Santoquin[2]	0.10
Vitamins A and D (6600 I.U. of A, 1190 I.C.U. of D_3)	25 gm/100 lbs.
Variables (linseed oil, tallow,[3] corn meal	10

[1] See footnote 1, table 1.
[2] Monsanto Chemical Co., St. Louis, Mo.
[3] For specification on the tallow see footnote 3, table 2 in Leveille and Fisher ('58).

Experimental design

Dietary fat	Age of hens at start	
	8 months	20 months
No supplement	Lot 1	Lot 4
Linseed oil, 10%	2	5
Tallow, 10%	3	6

RESULTS

Part 1. The data collected in the study with growing cockerels are summarized in table 4.[2] The following observations are of particular interest:

1. The low-protein diets, irrespective of dietary fat level, were associated with higher levels of cholesterol in plasma and aorta despite the much reduced cholesterol consumption with these diets compared to the cholesterol intake on the high-protein diets (0.3 vs. 2% dietary cholesterol).

2. Despite the higher blood and aorta cholesterol levels obtained with low-protein diets the subjective score of the abdominal aorta tended to be higher on the high-protein, high-fat 2% cholesterol diet.

3. In all groups the abdominal aorta was more affected than the thoracic aorta.

4. For the high-protein groups the hydroxyproline:cholesterol ratio tended to be higher than for the low-protein groups, suggesting that the lesions were more fibrotic in composition.

5. On the low-protein diets, the amount of aorta fat was significantly higher and the iodine number of the fat lower (more saturated) than on the high-protein diets.

6. No polyunsaturated fatty acids beyond the dienoic acids were found in the aorta fat.[3]

Additional information was revealed by correlation analysis.[4] Those correlation coefficients which were significant and pertinent are listed in table 5. Of particular interest from the viewpoint of developing atherosclerosis was the highly significant negative correlation between aorta cholesterol content and iodine number of aorta fat. Increased aorta cholesterol content therefore appears to be associated with a

[2] Feed consumption and mortality figures are omitted; the former follow the pattern of growth and the latter were negligible since only 5 out of 80 birds died.

[3] Although small readings were obtained at the appropriate wave lengths for trienoic and tetraenoic acids, no peaks were present which justified the inclusion of these readings.

[4] We wish to thank Dr. F. G. Fender of the Rutgers University Computation Center for help with the programming of the data for I.B.M. 650 analysis.

TABLE 4

Aorta and other measurements of growing roosters fed different cholesterol, fat and protein levels

Dietary supplement								
Protein, %	8	8	8	8	20	20	20	20
Corn oil, %	2	2	10	10	2	2	10	10
Cholesterol, %	0	0.3	0	0.3	0	2	0	2
Gain, gm/10 wks.	338 ± 14[1]	521 ± 35	414 ± 51	452 ± 44	1854 ± 43	1822 ± 61	1977 ± 24	1761 ± 67
Plasma cholesterol, mg %	266 ± 17	1568 ± 275	301 ± 13	2591 ± 254	174 ± 9	514 ± 80	177 ± 6	1523 ± 292
Aorta								
Score, thoracic	1.0 ± 0.01	1.1 ± 0.06	1.0 ± 0.02	1.2 ± 0.05	1.1 ± 0.03	1.2 ± 0.03	1.1 ± 0.02	1.2 ± 0.03
Score, abdominal	1.2 ± 0.07	1.3 ± 0.07	1.3 ± 0.09	1.5 ± 0.22	1.4 ± 0.06	1.5 ± 0.12	1.5 ± 0.13	2.0 ± 0.13
Weight, mg/100 gm body wt.	21.2 ± 0.9	19.8 ± 0.70	18.9 ± 0.4	19.8 ± 0.9	9.2 ± 0.3	10.0 ± 0.5	9.4 ± 0.2	10.9 ± 0.63
Cholesterol, mg/gm dry defatted tissue	13.98 ± 0.7	27.40 ± 3.5	18.76 ± 1.6	34.62 ± 3.5	12.39 ± 0.8	19.18 ± 3.1	14.08 ± 0.57	23.65 ± 1.4
Hydroxyproline, % of defatted tissue	3.66 ± 0.07	3.97 ± 0.12	4.00 ± 0.25	3.98 ± 0.48	4.13 ± 0.25	4.08 ± 0.14	4.28 ± 0.03	4.36 ± 0.18
Fat								
% of dry defatted wt.	38	33	36	38	28	25	29	28
Iodine no.	36.2	35.3	36.1	33.0	38.3	37.3	45.0	41.1
Fatty acids								
Saturated, % of fat	63.5	63.5	62.6	66.5	60.1	61.8	53.4	57.5
Oleic, % of fat	32.7	33.8	34.6	30.4	37.3	34.9	43.2	39.4
Linoleic, % of fat	3.79	2.72	2.77	3.19	2.62	3.29	3.41	3.13

[1] Standard error of the mean.

TABLE 5

Correlation coefficients for measurements and treatments in experiment with roosters

Simple correlation coefficients		
Variables		Coefficient[1]
Abdominal score	vs. aorta weight	0.31
Dietary protein level[2]	vs. aorta fat	— 0.92
	vs. aorta weight	0.84
	vs. plasma cholesterol	— 0.33
	vs. aorta cholesterol	— 0.34
Dietary cholesterol level	vs. aorta weight	0.48
Aorta cholesterol	vs. plasma cholesterol	0.69
Iodine no. of aorta fat	vs. aorta cholesterol	— 0.41

Multiple correlation coefficients		
Dietary variables (Independent)	Measurement (Dependent)	Coefficient
Fat and cholesterol level	Plasma cholesterol	0.30
Protein and fat level	Abdominal score	0.38
Protein and cholesterol level	Abdominal score	0.36
Cholesterol and fat level	Abdominal score	0.48
Protein, cholesterol and fat level	Abdominal score	0.47

[1] Significance level (1%) = 0.29.

[2] The authors are aware that differences in body weights, between roosters fed the two protein levels may be reflected in these correlation coefficients, since animals of different body size (physiological age) might have different aortic composition.

more saturated type of fat *despite* the fact that 2 or 10% levels of a highly unsaturated fat (corn oil) were ingested.

Part 2. The results of feeding diets without added fat, 10% tallow or with 10% linseed oil to 8- and to 20-month-old chickens for one year are presented in table 6.[5] The salient features can be summarized thus:

1. The tallow diets were responsible for significantly heavier body weights in both the younger and older hens.

2. Plasma and aorta cholesterol concentrations in the older hens were highest on the tallow diets; in the younger hens plasma cholesterol was not increased by tallow.

3. The subjective (visual) score was greater in both age groups of hens fed the tallow diets.

4. The hydroxyproline and therefore collagen content of the aortas shows a greater increase with age for the abdominal region (14.8% change between the older and younger birds) than for the thoracic region (9.5%).

5. As with the growing chicks, the lesions appeared more severe in the abdominal region; on the basis of an increased hydroxyproline concentration in relation to cholesterol content these lesions are judged to be more fibrotic than lipid in composition.

6. However, in the specific case of the tallow-fed hens which had the highest score but in which the hydroxyproline content remained unchanged whereas the aorta cholesterol was increased, it was judged that the lipid component contributed more to the increased severity of the lesions.

7. The fatty acid analyses indicate that abdominal aorta fat is more saturated (lower iodine numbers), than thoracic aorta fat, irrespective of the degree of unsaturation of the diet fat.

8. For both age groups, no polyenoic acids beyond the tetraenoic acids were found in aorta fat, although the plasma fatty acids for similarly-fed birds contained

[5] Egg production, feed consumption and mortality were omitted for the sake of greater clarity. Dietary treatments did not affect egg production or feed consumption. Mortality was low for all groups so that no significance could be attached to the slightly higher death rate in the tallow-fed groups.

TABLE 6

Aorta and other measurements recorded with hens

	Pullets			Hens		
	No fat	Linseed	Tallow	No fat	Linseed	Tallow
Body wt., lbs.	4.2 ± 0.2	4.0 ± 0.2	4.8 ± 0.2	4.6 ± 0.4	4.6 ± 0.2	5.2 ± 0.3
Plasma cholesterol, mg %	188 ± 11	192 ± 12	176 ± 11	179 ± 12	209 ± 14	241 ± 26
Score						
T[1]	1.1 ± 0.05	1.1 ± 0.05	1.2 ± 0.06	1.2 ± 0.04	1.1 ± 0.05	1.6 ± 0.07
A[2]	1.2 ± 0.09	1.3 ± 0.08	1.5 ± 0.09	2.5 ± 0.6	2.0 ± 0.2	3.3 ± 0.5
Aorta wt., mg/100 gm body wt.						
T	4.1	4.6	3.3	3.8 ± 0.5	3.8 ± 0.3	3.5 ± 0.3
A	3.3	2.7	2.5	2.4 ± 0.3	2.9 ± 0.4	2.5 ± 0.2
Aorta cholesterol, mg/gm dry defatted tissue						
T	—	—	—	23 ± 7	24 ± 4	26 ± 5
A	—	—	—	16 ± 2	24 ± 5	38 ± 11
Aorta OH proline, % of dry defatted tissue						
T	3.9 ± 0.2	4.3 ± 0.2	4.4 ± 0.3	4.8 ± 0.5	4.6 ± 0.1	4.3 ± 0.1
A	5.3 ± 0.5	5.5 ± 0.4	5.3 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	6.2 ± 0.2
Aorta fat, % of dry defatted tissue						
T	20	12	34	30	28	39
A	30	20	43	38	40	39
I_2 no.						
T	77	74	54	—	120	47
A	44	59	48	38	56	50
Aorta fatty acids, % of fat						
Saturated						
T	34.2	31.5	55.0	—	7.2	61.3
A	66.7	45.2	50.9	71.8	67.0	58.1
Oleic						
T	52.7	85.2	32.8	—	65.9	30.0
A	22.2	47.8	46.1	19.9	13.3	32.2
Linoleic						
T	8.1	7.6	8.2	—	17.6	5.6
A	7.4	4.7	2.5	5.7	11.8	6.9
Linolenic						
T	1.6	1.3	0.9	—	4.9	0.8
A	1.6	1.1	0.2	1.5	4.8	0.9
Arachidonic						
T	3.2	1.4	2.1	—	4.9	2.4
A	2.1	1.2	0.3	2.0	3.0	1.8

[1] Thoracic.

[2] Abdominal.

significant amounts of penta- and hexaenoic acids.[6]

DISCUSSION

The present study offered an opportunity of comparing spontaneous aortic lesions (not induced by feeding of cholesterol) in older hens with experimentally cholesterol-induced lesions in growing roosters. The data indicated that in both cases the abdominal section of the aorta is more severely afflicted and relatively more sensitive to treatment manipulation than the thoracic aorta. Furthermore, the two segments differ in proportions of lipid and collagenous material each of which, in turn, may be separately affected by treatment. For example, in the chick experiment comparing the low-protein, high-fat, high-cholesterol group with the high-protein, high-fat, high-cholesterol group no difference is observed in the thoracic score but the abdominal score for the high-protein group is greater. Also the elevated score for the high-protein group appears to reflect the increased hydroxyproline content of the aorta rather than the lower cholesterol concentration (compared to the low-protein group).

It would seem in confirmation of Dauber's theory (Dauber and Katz, '43) that the combination of dietary cholesterol and fat can bring about changes leading to the development of lesions which are not characterized by fat deposits only. However, the study with growing roosters also indicated clearly that high plasma as well as high aortic cholesterol content *per se* is not necessarily the major prerequisite for the development of lesions. Thus, the observed abdominal scores in the low-protein groups were less severe despite significantly higher cholesterol levels than the corresponding scores of the groups receiving the 20% protein level. This raises interesting questions regarding rate of growth and of aging in relationship to the development of atherosclerosis. In contrast to the present observation, Stamler et al. ('58b) observed an increased incidence of lesions with low-protein diets; their data indicate, however, that the protein level considered "low" was adequate for normal growth as judged by a comparison of the final weights of the "low-" and high-protein groups.

In the hen experiments dietary tallow hastened the development of aortic lesions, whereas the unsupplemented and the linseed oil-containing diets behaved similarly to each other giving rise to a degree of aortic involvement previously observed in birds of this age (Weiss, '59). The aortic cholesterol content as well as the aortic weights are of the same order of magnitude reported by Weiss ('59) except that the cholesterol values for the tallow-fed birds are more in line with those of birds at least a year older than their actual age. Aortic weight as expressed per unit of body weight has proved a good criterion of aortic proliferation and plaque development in the hands of several workers (Faber and Lund, '49; Weiss, '59). In the case of the tallow-fed birds, this relationship did not hold, presumably because of the significantly greater body weights which, according to past experience (Weiss and Fisher, '57), are the result of heavy body fat deposits. On a lean body mass basis, the same trend probably would hold for the tallow-fed birds.

The effect of tallow in increasing the severity of aortic lesions confirms an earlier report from this laboratory (Weiss and Fisher, '57) of a dietary effect of lipids other than cholesterol. The relatively high degree of saturation of the animal tallow permits speculation concerning the relationship between saturated fatty acid intake and atherogenesis as recently reviewed by Bronte-Stewart ('58).

In comparing the younger hens with the older ones it is interesting to note the significantly greater increase in the hydroxyproline content of the abdominal segment of the aorta as compared to the thoracic segment. This faster "aging" appears in line with the greater severity of

[6] Plasma fatty acids as percentage of total fat were as follows: No fat group, dienoic 1.3, trienoic 1.3, tetraenoic 0.9, pentaenoic 0.4 and hexaenoic 0.8; linseed oil group, 4.3, 3.0, 1.4, 1.9 and 3.1. For the aorta fat small readings were obtained at the appropriate wave lengths for the pentaenoic acids and the hexaenoic acids, but no peaks were discernible, thus not justifying the inclusion of these readings; aortic fat from other animals with similar dietary background has shown very slight peaks in the pentaenoic and hexaenoic regions indicating the presence of minute amounts of these polyunsaturated fatty acids.

the abdominal lesions as compared to the thoracic involvement. It also agrees well with the report of Christie and Dahl ('57) which indicates a faster fall in respiration rate for the abdominal as compared to the thoracic segment during aging in the rat. Further evidence for the considerable metabolic differences between thoracic and abdominal segments is evident from the fatty acid analyses for the two segments. The thoracic fat reflected more closely the dietary fat while abdominal fat appeared quite constant, irrespective of dietary fat changes.

Observations on the parallel changes of the major classes of lipids between plasma and aortic tissue led Weinhouse and Hirsch ('40) and Hirsch and Nailor ('56) to postulate that the aortic lipids reflect passive deposition from plasma. The more specific fatty acid examination of the present study shows significant qualitative differences, specifically the absence of polyenoic acids beyond the tetraenoic acids in aortic tissue although present in blood.

SUMMARY AND CONCLUSIONS

Growing cockerels were fed diets varying in level of protein, fat and cholesterol and their aortas examined grossly as well as chemically. Similarly, two age groups of hens were placed for one year on essentially cholesterol-free diets containing fat of different degree of saturation, and their aortas were examined. On the basis of the data collected the following conclusions are in order:

1. The gross appearance of the induced lesion is similar to that of the spontaneous one and occurs predominantly in the abdominal region of the aorta.

2. The abdominal segment of the aorta "ages" more rapidly than the thoracic region as evidenced by the greater increase in hydroxyproline (collagen) content.

3. Cholesterol deposition in the aorta was associated with a more saturated type of aortic fat.

4. Aortic fat differs in fatty acid composition from plasma fat. The abdominal aortic fat is more saturated than the fat of the thoracic region.

5. In the hens, tallow hastened the development of aortic lesions.

6. In the growing rooster the combination of a low-protein level with 0.3% of cholesterol and 10% of corn oil gave rise to higher plasma and aorta cholesterol levels but to less severe atherosclerosis than was induced with high-protein, 2% of cholesterol and 10% of corn oil.

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Beneficial Effects of Alfalfa Meal and other Bulk-containing or Bulk-forming Materials on Symptoms of Tween 60 Toxicity in the Immature Mouse[1]

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Growth retardation, diarrhea and other toxic manifestations have been reported in a number of animal species including the rat (Harris et al., '51a; Chow et al., '53; Poling et al., '56), hamster (Schweigert et al., '50; Harris et al., '51b; Poling et al., '56) and mouse (Brush et al., '57) on diets containing high levels of non-ionic surface-active agents. There is evidence that the extent to which such toxicity is manifest is dependent in considerable degree on the composition of the diet fed. Thus, Chow et al. ('53) observed that whereas a supplement of 5% of polyoxyethylene (20) sorbitan monostearate (Tween 60) resulted in growth retardation and diarrhea when fed to weanling rats in conjunction with a highly purified (casein and sucrose-containing) ration, no deleterious effects were observed following the administration of this surfactant even at a 15% level when fed with a diet containing soybean meal. It was suggested by these workers that the increased toxicity of the Tween 60 when fed with the purified diet was due to the lack of sufficient residues in the ration employed to absorb the surface-active agent, which was irritating to the intestinal tract by virtue of its physical properties and they cite as evidence for this hypothesis their finding that supplementing the purified diet with bulk-forming inert substances such as cellulflour, celite or agar prevented the occurrence of diarrhea (Chow et al., '53). An alternate suggestion, however, for the protective effect of soybean meal, and one also proposed by Chow et al. ('53), was that this material contained a substance other than inert residue *per se* that was effective in counteracting the toxic effects of massive doses of Tween 60 and other surfactant agents. In the present communication data are presented indicating that supplements of alfalfa and other succulent plants were also active in counteracting the toxic effects of massive doses of Tween 60 when the latter was fed with a purified low-fiber diet and that the protective factor (or factors) therein is apparently distinct from any of the known nutrients.

PROCEDURE AND RESULTS

The basal ration employed in the present experiment consisted of cerelose, 64%; casein,[2] 24%; salt mixture,[3] 5%; cottonseed oil, 5%; and cellulose,[4] 2%. To each kilogram of the above diet were added the following vitamins: thiamine·HCl, 10 mg; riboflavin, 10 mg; pyridoxine·HCl, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; *p*-aminobenzoic acid, 400 mg; inositol, 800 mg; vitamin B_{12}, 150 μg; 2-methyl-1,4-naphthoquinone, 5 mg; choline chloride, 2 gm; vitamin A, 5000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and α-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of cerelose. Polyoxyethylene

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[2] Vitamin-free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

[3] Wesson Modification of Osborne-Mendel General Biochemicals, Inc., Chagrin Falls, Ohio.

[4] Solka Floc 200, Brown Co., Boston, Mass.

(20) sorbitan monostearate (Tween 60)[5] and polyoxyethylene (40) monostearate (Myrj 52)[6] as well as the various test supplements were incorporated in the above diet in the amounts listed in tables 1 to 3, replacing equal amounts of cerelose. Male mice of the RAP[7] and Webster strain[8] were selected between 11 and 14 gm in body weight and were fed the various diets indicated in tables 1 to 3. The mice were placed in metal cages with raised screen bottoms (5 animals per cage) and were provided with food and water ad libitum. The animals were fed daily and all food not consumed 24 hours after feeding was discarded. Feeding was continued for either 10 or 14 days in the various experiments or until death, whichever occurred sooner.

Experiment 1. Beneficial effects of alfalfa meal on symptoms of Tween 60 and Myrj 52 toxicity in the immature mouse

In agreement with previous findings (Brush et al., '57) growth was retarded and survival decreased in immature mice fed

[5] Atlas Tween No. 60, Mefford Chemical Co., Los Angeles, Calif.

[6] Atlas Myrj No. 52, Mefford Chemical Co., Los Angeles, Calif.

[7] RAP (Rockland all-purpose) strain, Rockland Farms, New City, New York.

[8] Webster strain, Curd's Caviary and Animal Supply, La Puente, California.

TABLE 1

Beneficial effects of alfalfa meal on the weight increment and incidence of survival of immature mice fed a highly purified diet supplemented with toxic levels of Tween 60 or Myrj 52 (10 animals per group)[1,2]

Supplements fed with basal ration	Average gain in body wt. after following days of feeding		Per cent survival
	7th	14th	
	Experiment 1a		
None	5.0(10)[3]	9.7(10)	100
7.5% Tween 60	3.7(9)	9.0(9)	90
10% Tween 60	1.5(9)	4.6(9)	90
15% Tween 60	0.3(2)	5.5(2)	20
7.5% Tween 60 + 20% alfalfa meal	3.2(10)	7.0(10)	100
10% Tween 60 + 20% alfalfa meal	4.4(10)	9.3(10)	100
15% Tween 60 + 20% alfalfa meal	3.9(9)	9.1(8)	80
7.5% Myrj 52	0.2(8)	2.8(2)	20
10% Myrj 52	—	—	0
15% Myrj 52	—	—	0
7.5% Myrj 52 + 20% alfalfa meal	6.1(10)	9.9(10)	100
10% Myrj 52 + 20% alfalfa meal	4.4(10)	8.8(10)	100
15% Myrj 52 + 20% alfalfa meal	2.1(9)	6.1(9)	90
	Experiment 1b		
None	3.6(10)	7.4(10)	100
7.5% Tween 60	− 0.7(3)	—	0
7.5% Tween 60 + 10% alfalfa meal	4.0(10)	6.4(10)	100
7.5% Tween 60 + 20% alfalfa meal	2.8(10)	5.8(10)	100
10% Tween 60	—	—	0
10% Tween 60 + 10% alfalfa meal	1.9(9)	7.2(5)	50
10% Tween 60 + 20% alfalfa meal	1.7(8)	6.4(5)	50
7.5% Myrj 52	− 1.6(6)	0.1(4)	40
7.5% Myrj 52 + 10% alfalfa meal	3.0(9)	6.3(8)	80
7.5% Myrj 52 + 20% alfalfa meal	2.6(9)	4.2(9)	90
10% Myrj 52	—	—	0
10% Myrj 52 + 10% alfalfa meal	0.9(8)	4.4(7)	70
10% Myrj 52 + 20% alfalfa meal	2.0(8)	4.6(8)	80

[1] Experiment 1a was conducted with male mice of the RAP (Rockland all-purpose) strain; experiment 1b with male mice of the Webster strain.

[2] The average initial body weights of groups in Experiment 1a ranged from 12.5 to 12.8 gm; and in Experiment 1b from 13.2 to 13.6 gm.

[3] The values within parentheses indicate the number of animals which survived and on which averages are based.

a purified low-fiber diet supplemented with high levels of Tween 60 or Myrj 52. A difference in response to Tween 60 administration was noted between mice of the RAP (experiment 1a) and Webster (experiment 1b) strain. In the RAP strain Tween 60 when fed at a 7.5% level in the diet had little if any deleterious effect on either weight increment or survival although growth was retarded at the 10% level and both growth and survival were adversely affected at the 15% level of supplementation. In the Webster strain, Tween 60 when fed at a 7.5% level significantly retarded both growth and survival. The response to Myrj 52 when fed at graded levels in the diet was comparable in the two strains. In both experiment 1a and 1b the retardation in growth, diarrhea, unthrifty appearance and decreased survival which occurred in the mice fed the basal ration supplemented with toxic levels of Tween or Myrj 52 were largely counteracted by the concurrent feeding of alfalfa meal. Results are summarized in table 1.

TABLE 2

Comparative effects of alfalfa meal and other bulk-containing or bulk-forming materials on symptoms of Tween 60 toxicity in the immature mouse[1,2,3]

Supplements fed with basal ration	Average gain in body wt. after 10 days of feeding[4]	Per cent survival
	gm	
None	6.4 ± 0.5(20)[5]	100
7.5% Tween 60	− 1.6(4)	20
7.5% Tween 60 plus the following supplements:		
5% alfalfa meal lot no. 1	4.1 ± 0.6(9)	90
10% alfalfa meal lot no. 1	6.3 ± 0.5(10)	100
10% alfalfa meal lot no. 2	7.2 ± 0.5(10)	100
10% alfalfa meal lot no. 3	5.8 ± 0.8(9)	90
10% alfalfa meal lot no. 4	5.4 ± 0.9(8)	80
10% dehydrated rye grass	6.2 ± 0.6(10)	100
10% dehydrated orchard grass	6.0 ± 0.6(10)	100
10% dehydrated wheat grass	5.5 ± 0.7(10)	100
10% dehydrated fescue grass	5.8 ± 0.8(10)	100
5% Solka Floc BW 200	3.5 ± 0.5(7)	70
10% Solka Floc BW 200	1.4 ± 1.2(9)	90
5% Cellophane Spangles	2.4 ± 0.8(7)	70
10% Cellophane Spangles	0.4 ± 1.2(6)	60
5% carboxymethylcellulose	3.4 ± 0.8(9)	90
5% Celite	1.4 ± 1.1(7)	70
5% calcium silicate	2.4 ± 0.4(9)	90
5% carrageenin	7.3 ± 0.7(10)	100
5% sodium alginate	6.6 ± 0.5(10)	100
5% agar	5.2 ± 0.8(10)	100

[1] Twenty animals per group were employed on the basal ration and basal ration plus 7.5% Tween 60 diets; 10 animals per group on the remaining diets.

[2] The average initial body weights of the various groups ranged from 11.8 to 13.6 gm.

[3] The animals employed in this experiment were male mice of the Webster strain. The alfalfa samples were provided by the Research and Development Division of Nutrilite Products, Inc., Buena Park, California. The dehydrated rye grass, orchard grass, wheat grass and fescue grass were obtained from the National Chlorophyll and Chemical Co., Lamar, Colorado; Cellophane Spangles from the Rayon Processing Company of Pawtucket, R.I.; Solka Floc BW 200 from Brown Co., Boston, Mass.; carrageenin (SeaKem Type 21 Irish Moss Extractive) from Seaplant Corporation, New Bedford, Mass.; Celite Analytical Filter-Aid and calcium silicate (Micro-Cel) from Johns-Manville Products, Los Angeles, California.

[4] Including standard error of the mean calculated as follows:

$$\sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}} \Big/ \sqrt{n}$$

where "x" equals the weight gained and "n" is the number of observations.

[5] The values within parentheses indicate the number of animals which survived and on which averages are based.

Experiment 2. Comparative effects of alfalfa meal and other bulk-containing or bulk-forming materials on symptoms of Tween 60 toxicity in the immature mouse

In agreement with experiment 1b, alfalfa meal when fed at a 10% level in the diet largely counteracted the retardation in growth, diarrhea, unthrifty appearance and decreased survival of immature mice fed the basal diet supplemented with 7.5% Tween 60. Similar effects were obtained with dehydrated rye grass, orchard grass, wheat grass and fescue grass when fed at a 10% level in the diet or with carrageenin, sodium alginate or agar at a 5% level of supplementation. Alfalfa meal when fed at a 5% level in the diet also had a significant protective effect but less

TABLE 3

Comparative effects of alfalfa meal, alfalfa fractions and supplements of the known nutrients on symptoms of Tween 60 toxicity in the immature mouse[1,2,3]

Supplements fed with basal ration	Average gain in body wt. after 10 days of feeding[4]	Per cent survival
	gm	
None	5.8 ± 0.3(20)[5]	100
7.5% Tween 60	− 2.1(3)	15
7.5% Tween 60 plus the following supplements:		
10% alfalfa meal lot no. 1	5.6 ± 0.6(10)	100
7.5% alfalfa residue	5.1 ± 0.8(10)	100
2.5% dried alfalfa juice	—	0
2.5% alfalfa ash	—	0
2.5% salt mixture[6]	1.9(2)	20
Vitamins B, C and K[7]	—	0
Vitamins A, D and E[8]	—	0
10% casein	—	0
10% fish meal	—	0
5% cottonseed oil	1.9(1)	10
10% Solka Floc BW 200	2.2 ± 0.9(8)	80
10% desiccated liver N.F.	—	0
10% penicillin mycelia	0.5(1)	10
10% yeast	1.4 ± 0.5(6)	60
3% fish solubles	—	0
2.5% Vigofac	0.2(1)	10
2% lemon bioflavonoid complex	—	0
Aureomycin·HCl[9]	1.2(2)	20

[1] Twenty animals per group were employed on the basal ration and basal ration plus 7.5% Tween 60 diets; 10 animals per group on the remaining diets.

[2] The average initial body weights of the various groups ranged from 11.4 to 12.8 gm.

[3] The animals employed in this experiment were male mice of the Webster strain. The alfalfa meal and alfalfa fractions were provided by the Research and Development Division of Nutrilite Products, Inc., Buena Park, California. The casein (Vitamin-free Test Casein) was obtained from General Biochemicals, Inc., Chagrin Falls, Ohio; the fish meal (Herring Meal, 70% protein) from the Canadian Fishing Co., Vancouver, B. C.; the desiccated liver N.F. from Armour and Co., Chicago, Ill.; the penicillin mycelia and Vigofac from Chas. Pfizer and Co., Brooklyn, New York; the yeast (Primary Dried Yeast, Strain 200) from Anheuser, Busch, Inc., St. Louis, Missouri; and the fish solubles from Van Camp SeaFood Co., Terminal Island, California.

[4] Including standard error of the mean. See footnote 2, table 2.

[5] The values within parentheses indicate the number of animals which survived and on which averages are based.

[6] Wesson Modification of Osborne-Mendel. See footnote 5 in text.

[7] The following vitamins were added per kilogram of diet: thiamine·HCl, 10 mg; riboflavin, 10 mg; pyridoxine·HCl, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; *p*-aminobenzoic acid, 400 mg; inositol, 800 mg; vitamin B_{12}, 150 μg; 2-methyl-naphthoquinone, 5 mg; and choline chloride, 2 gm.

[8] The following were added per kilogram of diet: 5000 U.S.P. units of vitamin A, 500 U.S.P. units of vitamin D_2 and 100 mg of α-tocopherol acetate.

[9] Aureomycin·HCl, 100 mg per kilogram of diet.

than that when fed at the 10% level. In contrast to the above, cellophane spangles when fed at a 5% or 10% level or Solka Floc, carboxymethylcellulose, celite or calcium silicate at the 5% level of supplementation showed considerable activity in preventing diarrhea and promoting the survival of mice fed the basal ration supplemented with 7.5% of Tween 60, but were less active than the supplements indicated above in counteracting the retardation in growth. Results are summarized in table 2.

Experiment 3. Comparative effects of alfalfa meal, alfalfa fractions and supplements of the known nutrients on Tween 60 toxicity in the immature mouse

In agreement with the findings reported above, alfalfa meal when fed at a 10% level in the diet largely counteracted the retardation in growth, diarrhea, unthrifty appearance and decreased survival of immature mice fed the basal ration supplemented with 7.5% of Tween 60. The protective factor (or factors) was retained in the alfalfa residue fraction (the water-washed pulp remaining after the extraction of the juice). Dried alfalfa juice when fed at a level corresponding to the amount present in 10% alfalfa meal was devoid of activity. Alfalfa ash when fed at a 2.5% level in the diet (corresponding to the ash provided by a supplement of 20% alfalfa meal) was without significant effect. Supplements of the known vitamins, aureomycin·HCl at a level of 100 mg per kg of diet, 2.5% salt mixture, 5% cottonseed oil, 10% casein, 10% desiccated liver, N.F., 10% fish meal, 10% penicillin mycelium, 3% fish solubles, 2.5% of a product derived from fermentation sources[9] or 2% lemon bioflavonoid complex had no protective effect. Yeast and Solka Floc when fed at a 10% level in the diet had some activity in preventing diarrhea and promoting survival but had little if any effect on growth. Results are summarized in table 3.

DISCUSSION

Present findings indicate that alfalfa and other succulent plants contain a factor or factors apparently distinct from any of the known nutrients which largely counteracted the retardation in growth, diarrhea, unthrifty appearance and decreased survival of immature mice fed a highly purified low-fiber diet supplemented with 7.5% of Tween 60. Carrageenin, sodium alginate and agar were also active in this regard. The protective factor (or factors) in alfalfa was retained in the alfalfa residue fraction (the water-washed pulp remaining after the extraction of the juice). This is the same alfalfa fraction that partially counteracted the inhibitory effects of massive doses of estradiol on ovarian development in the immature rat (Ershoff et al., '56), prolonged the survival of immature hamsters fed highly purified diets (Ershoff, '56), promoted growth of immature guinea pigs fed a mineralized dried milk ration (Ershoff, '57a), and counteracted the toxic effects of massive doses of glucoascorbic acid in the rat (Ershoff, '57b). It is also the same fraction that counteracted symptoms of mineral oil toxicity in rats and mice fed a low-fat ration (Ershoff and Hernandez, '58) and prolonged the survival of hyperthyroid rats (Ershoff et al., '59).

No data are available as to the mechanism (or mechanisms) whereby alfalfa and other materials effective in counteracting Tween 60 toxicity exert their protective effect. Chow et al. ('53) have suggested that toxic symptoms resulting from the feeding of Tween 60 with a purified low-fiber diet were due to the lack of sufficient residues in the ration to absorb this surface-active agent, which was irritating to the intestinal tract by virtue of its physical properties. This explanation might account for the beneficial effect of the bulk-containing or bulk-forming materials employed in the present experiment in preventing diarrhea and prolonging survival but fails to account for the variation in response obtained with these materials in respect to growth. Thus cellulose when fed at a 5% level in the diet in the form of Solka Floc or cellophane spangles was just as effective as a supplement of 10% alfalfa meal or 10% dehydrated rye grass, orchard grass, wheat grass or fescue grass

[9] Vigofac, Chas. Pfizer and Co., Brooklyn, New York.

in preventing diarrhea but was less active than the above supplements in promoting growth. If the greater activity of alfalfa and the grass supplements in promoting growth were due to the fact that these were fed at a higher level, then increasing the cellulose content of the diet from 5% to 10% should have a growth-promoting effect. Such, however, was not the case. On the contrary, the weight increment of mice fed Solka Floc or cellophane spangles at a 10% level in the diet was less than that of animals fed these materials at a 5% level. The toxicity of Tween 60 cannot be ascribed solely, however, to its irritating effect in the intestinal tract. Although Wick and Joseph ('56) reported that from 67 to 93% of ingested Tween 60 can be recovered in the feces, sufficient amounts might still be absorbed, particularly if administered for a prolonged period of time, to exert toxic effects in tissues other than the intestinal tract. Thus, Eagle and Poling ('56) observed in rats fed a high level of Tween 60 for 21 weeks and then sacrificed such pathological effects as a 100% incidence of enlarged kidneys, renal calculi, atrophy of the testicular tubules, calcified concretions in the kidney, obstruction of the renal tubules, decreased splenic lymphoid tissue, atrophy of liver parenchymal cells, and decreased lymphoid tissue in the mesenteric nodes, as well as other findings. Similar findings were reported in the hamster. The administration of emulsifiers has also been reported to result in low coliform counts and to decrease the total number of intestinal organisms in the rat, findings correlated with a reduction in growth (Bourke and Fitzhugh, '53). The retarded growth of mice fed the purified diet supplemented with 7.5% of Tween 60 might be due, therefore, to such factors as (1) the toxic effects of Tween 60 *per se* which may be absorbed in small amounts particularly through an intestinal wall that is damaged, (2) the toxic effects of metabolites derived therefrom or produced as a consequence of the interaction between Tween 60 and other chemicals, cells or microorganisms, (3) the inhibitory effect of Tween 60 on the synthesis of a growth-promoting factor (or factors) by the intestinal flora or the animals' own tissues or (4) an increased requirement for such a growth factor(s) in mice fed Tween 60 above the levels of such factor produced by the intestinal flora or the animals' own tissues. Further studies are indicated to determine to what extent the protective (and in particular the growth-promoting) effect of alfalfa and other succulent plants may have been due to the possible effect of these supplements in counteracting toxicity caused by the factors indicated above or in supplying a growth-promoting factor (or factors) whose synthesis by the intestinal flora or the animals' own tissues was impaired in the mice administered Tween 60. The possibility that cellulose as present in alfalfa and other succulent plants may have growth-promoting activity not shared by other forms of cellulose under conditions of the present experiment has not, however, been eliminated.

SUMMARY

Immature mice fed a highly purified low-fiber diet containing 7.5% of polyoxyethylene (20) sorbitan monostearate (Tween 60) exhibited retardation in growth, diarrhea, an unthrifty appearance and decreased survival. These effects were largely counteracted by the concurrent administration of alfalfa meal, or dehydrated rye grass, orchard grass, wheat grass or fescue grass at a 10% level in the diet or carrageenin, sodium alginate or agar at a 5% level of feeding. Cellulose in the form of Solka Floc or cellophane spangles when fed at a 5 or 10% level, yeast at a 10% level and carboxymethylcellulose, celite and calcium silicate at a 5% level of supplementation prevented diarrhea and promoted survival but were not as active as the substances indicated above in counteracting the retardation in growth. The protective factor (or factors) in alfalfa was retained in the alfalfa residue fraction (the water-washed pulp remaining after the extraction of the juice). Supplements of the known vitamins, alfalfa ash, salt mixture, protein in the form of casein or fish meal or fat in the form of cottonseed oil as well as supplements of desiccated liver N.F., penicillin mycelium, fish solubles, a product derived from fermentation solubles, lemon bioflavonoid complex or aureomycin·HCl were without protective effect.

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The Effect of Age and Supplemental Amino Acids on the Utilization of Milk and Soya Protein by the Young Pig[1]

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Work reported by Lewis et al. ('55), Peo ('56) and Hudman ('56) demonstrates that the performance of baby pigs fed all-plant protein rations does not approach that of pigs fed milk protein. Lewis et al. ('55) attributed the poor utilization of soya protein to an inadequately developed digestive enzyme system in the young pig. This theory is supported by enzyme assays of the baby pigs' digestive organs presented by Lewis et al. ('57) and Hudman et al. ('57) and also by digestibility studies reported by Lloyd and co-workers ('57). The latter workers demonstrated that the pigs' ability to digest various nutrients improved as the pig progressed from three to 7 weeks of age.

In the studies reported herein, nitrogen balance techniques were used to study the differences in digestibility of milk and soya protein. These determinations were made at two and again at 5 weeks of age in order to compare the effect of age on utilization of the two protein sources.

A survey of the pigs' amino acid requirements and the quantities contributed by each source (Hays, '57) indicated that methionine was the most limiting amino acid in the soya protein diets and also that if any amino acid were present in excessive quantities, arginine would be one to suspect immediately, as the soya ration contained more than three times the suggested arginine requirement. Also, arginine is the single essential amino acid that differs greatly in its concentration in milk and soya protein. Thus, it was decided to study also the effect of added arginine to the milk and soya diets on the utilization of these proteins by the young pig.

EXPERIMENTAL

Animals. Six litter-mate groups of 6 pigs per group, averaging 10.2 days of age and 6.7 pounds body weight were selected and assigned at random within litters to the 6 ration treatments. The pigs were maintained in individual wire-floor metabolism cages with feed provided ad libitum during the entire 5-week experimental period. Water was provided in pans of half gallon capacity, which were filled a minimum of 4 times daily to provide essentially ad libitum water consumption.

The room temperature was maintained initially at 30°C and reduced three degrees each week. Weight gains and feed consumption data were collected initially, at the start and termination of each collection period and at the end of the experiment.

Rations. The composition of the soybean oil meal and dried skim milk basal diets are presented in table 1. The 6 ration treatments consisted of 4 soybean oil meal-type diets and two dried skim milk-type diets. The 4 soya diets were: (1) basal, (2) basal plus 0.05% DL-methionine, (3) basal plus 0.5% L-arginine and (4) basal plus 0.05% DL-methionine plus 0.5% L-arginine. The two milk diets were: (1) basal and (2) basal plus 0.84% L-arginine. This level of arginine raised the calculated arginine content of the milk diet to that of the soybean oil meal basal diet.

The 20% level of protein in these rations was provided by low heat, spray-dried skim milk (34% protein) or solvent processed soybean oil meal (50% protein) except that contributed by the dried beet pulp which amounted to less than 1% of the total protein fed. Chromium oxide

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TABLE 1

Composition of soybean oil meal and dried skim milk basal rations

Experiment 798

Ingredient	SBOM ration	DSM ration
	%	%
Sucrose	15.00	15.00
Lactose	34.48	17.84
Solvent soybean oil meal (50% protein)	39.68	—
Dried skim milk (34% protein)	—	58.36
Stabilized lard	2.50	2.50
Dried beet pulp	2.00	2.00
Vitamin-antibiotic premix[1]	2.00	2.00
Calcium carbonate	0.04	—
Dicalcium phosphate	2.65	0.65
Iodized salt	0.50	0.50
Trace mineral mix (35-C-41)[2]	0.15	0.15
Chromium oxide[3]	1.00	1.00
Total	100.00	100.00

[1] Calculated analysis per pound of complete ration: vitamin A, 3000 I.U.; vitamin D_2, 500 I.U.; riboflavin, 5 mg; pantothenic acid, 10 mg; niacin, 30 mg; choline, 450 (DSM) 516 (SBOM). Premix contributed the following in milligrams per pound of ration: vitamin B_{12}, 0.02; vitamin E, 10; menadione, 0.5; ascorbic acid, 100; thiamine, 3; pyridoxine, 1.5; folic acid, 0.5; biotin, 3; inositol, 250; chlortetracycline, 50.

[2] Contributed the following in milligrams per pound of ration: Fe, 48; Cu, 3.3; Co, 1.1; Zn, 56; Mn, 39.

[3] Included for other studies being conducted simultaneously.

marker was added to all rations at the rate of 1% as an index substance for other studies being carried out simultaneously on the same animals. All animals were fed the dried skim milk basal ration for the first 4 days of the experiment, after which time they were given their respective test diets.

Collection of excreta. Total urine and fecal collections were made for two periods of 5 days (120 hours) duration beginning with the 8th day and again with the 28th day on experiment. Urine was collected and stored under toluene at 34°F until the end of the 5-day period at which time nitrogen determinations were made. The fecal excreta were collected daily and frozen immediately upon collection.

After each total 5-day collection, the fecal material was pooled for each pig and the total quantity dried at 100°C for 48 hours. Ration samples were taken and dried at 100°C for 24 hours. Standard macro-Kjeldahl procedures were used for the nitrogen determinations.

RESULTS AND DISCUSSION

Growth data. As can be seen from the summary of weight gains and feed required per pound of gain presented in table 2, the animals receiving the milk diets gained an average of 24.8 pounds on 1.58 pounds of feed per pound of gain as compared to an average gain of 14.4 pounds on 2.12 pounds of feed per pound of gain for the pigs receiving the soya diets. These differences in rate of gain and feed conversion were statistically significant.[4]

Two pigs receiving the soya diets died and two others were near death at time of removal from experiment. Post-mortem examinations did not reveal any cause of death that could be associated with the ration treatments. The missing observations for these 4 pigs were calculated and treated accordingly in the statistical analysis. A mild outbreak of scours was observed in the pigs receiving the soya diets during the second week on experiment; however the intensity or persistency of scouring could not be associated with the performance of the pigs.

Supplementation of the diets with arginine had little or no effect on gains or feed conversion. For those pigs receiving milk diets, rate of gain was lower on the

[4] References to statistical significance pertain to a probability level of $P = 0.05$ or less.

TABLE 2

Summary of feed conversion, digestibilities, gains and gains adjusted for differences in digestibility in young pigs fed different diets

Treatment	Feed/gain	Digestibility X	Observed gains Y	Adjusted gains[1] $\hat{Y}$
	lb.	*%*	*lb.*	*lb.*
Soybean oil meal (SBOM)	2.34	79.9	11.0	13.6
SBOM + methionine	1.88	78.9	17.8	20.9
SBOM + arginine	2.45	80.0	11.4	13.9
SBOM + arginine + methionine	1.82	80.4	17.2	19.5
Dried skim milk (DSM)	1.58	95.5	25.8	20.5
DSM + arginine	1.58	95.6	23.7	18.3

[1] $\hat{Y} = Y + b(\overline{X} - X) = Y + 0.506(85 - X)$.

average for those receiving the supplemental arginine, however feed conversion was similar for the two groups. Also if there was a detrimental effect from excess arginine, the pigs receiving the soya diet plus supplemental arginine should have been more severely affected. To the contrary, the pigs receiving soya diets plus arginine showed gains equally as rapid and essentially as efficient as those receiving no supplemental arginine.

Supplemental DL-methionine improved one- to 6-week gains from an average of 11.2 to 17.5 pounds and decreased the pounds of feed required per pound of gain from an average of 2.40 to 1.85. Analysis of the data from the 4 soybean oil meal diets showed that these differences between the methionine-supplemented and non-supplemented diets were statistically significant.

Only the data from the 4 soybean oil meal diets, were used to test the effects of methionine as similar milk diets supplemented with methionine were not fed and also because of the apparent heterogeneity of variances of pigs fed the two types of rations. Bartlett's test for homogeneity of variance (Snedecor, '56, p. 285) demonstrated that this apparent lack of homogeneity was highly significant, therefore only the data obtained with soybean oil meal diets were used to test the effects of methionine supplementation.

Balance data. Summaries of the two- and 5-week digestibility and retention data are presented in table 3.

At two weeks of age the pigs digested 88% of the dry matter of the soya diets as compared to 96% of the dry matter of the milk diets. At 5 weeks of age the digestibility of the dry matter of the soya diets had increased to 92%, whereas the digestibility of the milk rations showed little change with a digestibility coefficient of 97%.

The same pattern of digestibility existed with respect to protein. This would be expected as lactose and sucrose, two readily available carbohydrates, were used as the sources of energy in the rations. The digestibility coefficients for soya protein were 78 and 82% for the two- and 5-week age periods, respectively. The digestibility of milk protein (96%) apparently did not change with age.

The effect of age on digestibility of both the dry matter and protein was statistically significant. The increased digestibility of the soya rations was largely responsible for these effects as indicated by the significant age × treatment interaction on dry matter digestibility; however this interaction was not significant for the protein data.

Averages of the two- and 5-week protein digestibilities were used in the covariance analysis (Snedecor, '56) of gain on digestibility and the adjusted gains arrived at by this analysis are presented in table 2. A correlation coefficient of 0.492 was observed for gains and digestibility with a regression coefficient of 0.506 pounds of gain per percentage unit change in protein digestibility. This adjustment for differences in digestibility resulted in comparable gains for the pigs fed milk diets and soybean oil meal diets supplemented with methionine. Even with the adjustment, the performance on soybean oil meal rations without added methionine failed to approach that on the milk diet. Also, there was evidence that arginine had a depres-

TABLE 3

Summary of apparent digestibility and protein utilization data obtained from young pigs fed different diets

Source of protein	Soybean oil meal					Dried skim milk		
Supplement	O	Methionine	Arginine	Arginine + Methionine	Av.	O	Arginine	Av.
Dry matter digestibility, %								
2 weeks of age	86	89	88	89	88	96	96	96
5 weeks of age	92	91	91	92	92	97	97	97
Nitrogen (protein) digestibility, %								
2 weeks of age	77	76	77	80	78	95	96	96
5 weeks of age	83	81	81	83	82	96	96	96
Apparent nitrogen retention, %								
2 weeks of age	48	51	49	56	51	77	75	76
5 weeks of age	55	52	44	48	50	61	56	58
Nitrogen retained/nitrogen digested, %								
2 weeks of age	62	67	64	70	66	81	79	80
5 weeks of age	66	64	54	58	61	63	59	61

sing effect on gains. An adjustment for differences in digestibility of the arginine-supplemented and unsupplemented rations would be of questionable validity as the addition of 0.5% of arginine to the soybean oil meal diets and 0.84% of arginine to the milk rations should increase the apparent protein digestibility proportionally. However, this was not evident from the observed data.

The percentage nitrogen retention was considerably greater for the milk diets at two weeks of age than for the soya diets (76 versus 51%). At 5 weeks of age, the nitrogen retention had decreased to 58% for the milk diets whereas the retention on the soya diets had remained at 50%. This apparent age × source of protein interaction was significant and was probably a result of the improved digestibility of soya protein with age coupled with the fact that 20% level of milk protein is more than adequate for the pig at 5 weeks of age.

Supplementation of the soya diets with arginine and methionine appeared to increase nitrogen retention at two weeks of age and to decrease nitrogen retention at 5 weeks of age. Analysis of the two-week data showed these differences to be statistically non-significant. These differences did contribute to the significant age and treatment interaction in the combined analysis. The major portion of this interaction was accounted for by the age × source of protein fraction, but the age × supplemental arginine interaction was also significant.

The apparent biological values for these rations were no doubt influenced markedly by the overfeeding of protein at 5 weeks of age, especially to those pigs receiving the milk diets. However, a review of these data indicates further that the low digestibility of soya protein is the primary factor responsible for the poorer performance of pigs fed soya protein diets as compared to pigs fed milk protein diets. The apparent biological value of the milk protein was considerably greater than that of the soya protein (80 as compared to 66) at two weeks of age; however the values for each of the proteins had decreased to 61 at 5 weeks of age. This age × source of protein interaction was statistically significant.

The effect of the addition of arginine to the soya diets was to slightly improve the biological value at two weeks of age and to depress it at 5 weeks of age. This age × arginine interaction was statistically significant. Arginine decreased the biological value of milk protein at both two and 5 weeks of age. This coupled with its depressing effect on soya diets at 5 weeks of age, was of such magnitude and consistency that the main negative effect of arginine proved to be statistically significant. This is consistent with the overall average, although not statistically significant, depressing effect of arginine on gains and feed conversion.

Methionine significantly improved gain and feed conversion and one would expect this to be reflected in the nitrogen utilization data. There was an apparent improvement in nitrogen retention and biological value at two weeks of age; however this was not evident at 5 weeks of age.

SUMMARY

Pigs fed skim milk rations gained at a faster rate on less feed per pound of gain than did pigs fed soybean oil meal diets. Supplemental arginine had no significant effect on either gains or feed conversion, even though levels in the diets were three to 4 times the reported requirements. Supplemental DL-methionine significantly improved gains and efficiency of feed conversion in the pigs fed diets containing soybean oil meal.

The apparent digestibility of the dry matter and protein of the milk rations was high at two weeks of age and changed very little as the pigs increased in age to 5 weeks. The digestibility of the dry matter and protein of the soya diets increased as the pigs increased from two to 5 weeks of age. Adjusting the gains to equal digestibilities with the use of covariance analysis resulted in similar gains for the pigs fed methionine supplemented soybean oil meal diets and skim milk diets.

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Interrelation of Cholesterol, Palmitic Acid, and Unsaturated Fatty Acids in the Growing Mouse and Rat[1]

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During the course of a study of the nutritional effects of various fats and fatty acids it was observed that the feeding to weanling mice of a purified type of diet containing 10% of a hydrogenated coconut oil,[2] 20% of palmitic acid, and 1% of cholesterol caused a complete cessation of growth with all animals dying within a period of 5 days. No abnormalities were noted in the mice except for extremely small spleens and lymph nodes and these showed no histological defects.

The literature on atherosclerosis, lipid metabolism and related topics is very extensive and mentions many interesting interrelationships of factors such as saturated and unsaturated fatty acids, cholesterol, pyridoxine and still other substances. None of these interrelationships, to the best of our knowledge, is associated with the phenomenon we observed. This paper is a report of the observations made in the course of experiments designed to throw some light on this peculiar phenomen.

EXPERIMENTAL

Male albino weanling mice[3] weighing 9 to 11 gm were used in these studies. All test groups consisted of 8 mice or multiples thereof. They were housed in individual wire bottom cages. Food and water were supplied ad libitum. All studies were of 12 days duration.

The basal diet employed in these studies consisted of 20% of casein,[4] 4% of salt mixture, (Hegsted et al., '41) 2% of cellulose,[5] 20% of palmitic acid,[6] and 1% of cholesterol. Each diet was supplemented to contain, per 100 gm, 4 mg of α-tocopherol, 0.4 mg of vitamin A palmitate, 0.005 mg of calciferol, 1 mg of 2-methylnaphthoquinone, 0.8 mg of thiamine·HCl, 1.6 mg of riboflavin, 0.8 mg of pyridoxine·HCl, 4.0 mg of niacinamide, 4.4 mg of calcium pantothenate, 4.0 mg of *p*-aminobenzoic acid, 600 mg of choline methionine tartrate, 20 mg of inositol, 0.2 mg of folacin, 0.02 mg of biotin, and 0.03 mg of vitamin B_{12}. The remainder of each diet consisted of glucose,[7] the level of which was varied when supplements were added.

In later experiments, mature male mice, weanling female mice and weanling male rats were used as the experimental animals.

RESULTS

I. Experiments with mice. After our initial observation we found that decreasing the palmitic acid to 10% of the diet greatly reduced the mortality permitting the survivors to grow about 20% as rapidly as those maintained on a good purified diet, i.e., 2 to 3 gm in a 12-day period. Under these less severe conditions it was found that the addition to the diet of 1% of linoleic acid or 2% of oleic acid resulted in near normal growth.

Further preliminary studies showed that the adverse effects were dependent upon the simultaneous feeding of the hydrogenated coconut oil, palmitic acid and cholesterol. The exclusion of any one of these

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[1] A preliminary report of this study was presented before the Federation of American Societies for Experimental Biology, Philadelphia, Pennsylvania, April 18, 1958.

[2] Hydrol, Durkee's Famous Foods, Chicago, Illinois.

[3] Merck Sharp & Dohme strain.

[4] Casein Labco, The Borden Co., New York, N. Y.

[5] Celluflour, Chicago Dietetic Supply Co., Chicago, Illinois.

[6] Prepared by Mr. F. E. Reimers of the Merck Sharp & Dohme Research Laboratories.

[7] Cerelose, Corn Products Refining Co., Argo, Illinois.

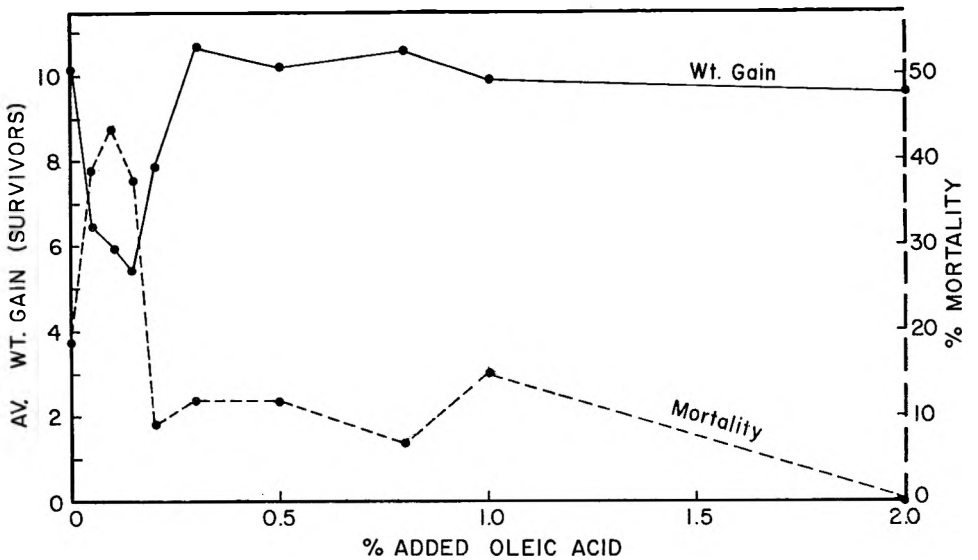

Fig. 1 Average 12-day weight gain and mortality of weanling mice fed a diet containing 20% of palmitic acid, 1% of cholesterol and graded levels of oleic acid.

three materials greatly alleviated or eliminated the adverse growth effect.

Hydrogenated coconut oil, with an iodine number of approximately 6, may contain small quantities of oleic and linoleic acids from the parent coconut oil. Therefore, a study to determine the effects of various combinations of these two unsaturated fatty acids with palmitic acid and cholesterol was undertaken.

The basal diet supplemented with varying levels of oleic acid[6] was fed to groups of 8 mice for 12-day periods. The weight gain and mortality data shown in figure 1 are averages of 4 replicate studies. Thus, at each level of oleic acid, 32 mice were used.

When oleic acid was incorporated into the diet at levels of 0.05 to 0.15% there resulted a marked growth retardation coupled with an increase in mortality. Increasing the dietary level of oleic acid to 0.3% or higher reversed the adverse growth and mortality effects. Although not shown in figure 1, the growth retardation and mortality were not influenced by oleic acid when added to a diet containing 20% of palmitic acid with no cholesterol or to a fat-free diet containing 1% of cholesterol. Under these conditions, 12-day weight gains of 10 to 11 gm were obtained regardless of the level of oleic acid incorporated in the diet.

It was found further that linoleic acid, regardless of the concentration fed, had no deleterious effects when added to the 20% palmitic acid, 1% cholesterol diet. However, the addition of 0.8% of oleic acid to a diet containing 20% of palmitic acid, 1% of cholesterol, and 0.1% of linoleic acid resulted in a marked effect on growth and mortality as shown in figure 2. Each point represents the average results obtained with from two to 4 replicate groups of 8 mice each. Decreasing or increasing the oleic acid to levels below or above 0.8% alleviated or prevented the adverse effects on growth and mortality.

The question of the quality of the oleic acid employed presented itself. However, subsequent studies gave similar results with a triolein prepared in our laboratories[6] and also with a methyl-oleate preparation.[8]

In a further study the effect of the addition of graded levels of olive oil to the 20% palmitic acid, 1% cholesterol-containing diet was determined. Olive oil contains approximately 80% of oleic acid and 7% of linoleic acid. It was found as is shown in figure 3 that the incorporation of 0.05

[6] See footnote 6 on page 185.
[8] Hormel Foundation, Austin, Minnesota.

to 0.1% of olive oil resulted in a growth retardation. These levels would correspond to oleic acid levels of approximately 0.04 to 0.07%. An increase in the amount of olive oil to 0.4% resulted in an improvement in growth. Further increases in the olive oil resulted in a second range of growth depression, the lowest point occurring between 0.8 and 1.0% of olive oil. One per cent of olive oil corresponds to approximately 0.07% of linoleic acid and 0.8% of oleic acid. These values agree closely with the most deleterious combinations shown in figures 1 and 2. Again

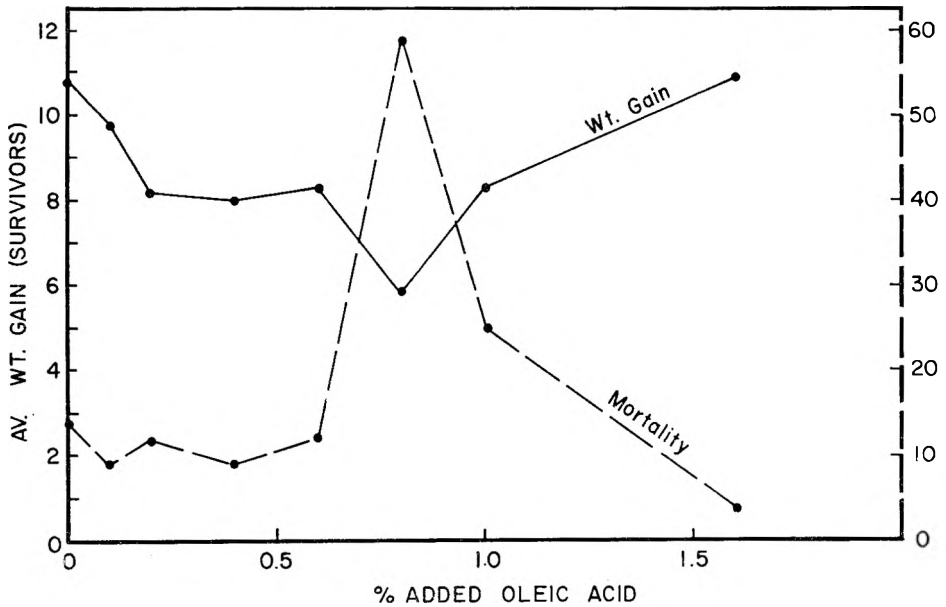

Fig. 2 Average 12-day weight gain and mortality of weanling mice fed a diet containing 20% of palmitic acid, 1% of cholesterol, 0.1% of linoleic acid and graded levels of oleic acid.

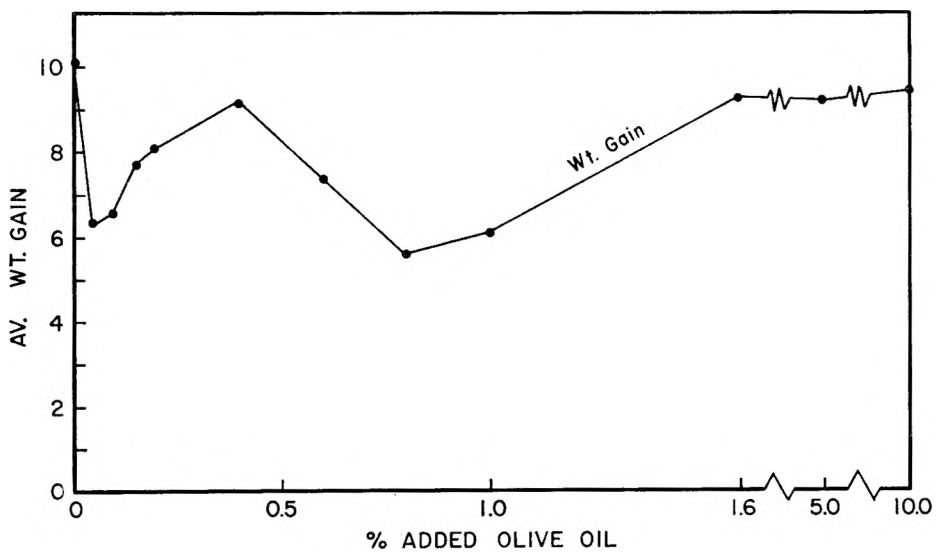

Fig. 3 Average 12-day weight gains of weanling mice fed a diet containing 20% of palmitic acid, 1% of cholesterol and graded levels of olive oil.

growth was improved when the olive oil concentration was increased above 1% of the diet.

In order to determine whether the phenomena observed are peculiar to young growing mice, groups of mature mice, 8 weeks of age, were fed a fat-free basal diet supplemented as shown in table 1. The mice were maintained on the several diets for a period of 12 days. Although no mortality occurred, the greatest weight loss and smallest spleen size were noted in the group receiving a diet containing 20% of palmitic acid, 0.05% of oleic acid and 1% of cholesterol. These conditions were alleviated as the level of oleic acid in the diet was increased. The data are shown in table 1.

In a further extension of the study, female weanling mice were fed diets identical with those given the adult males. To our surprise approximately 50% of these animals were unable to survive on the 20% palmitic acid, 1% cholesterol diet and succumbed even when the cholesterol was removed. As shown in table 2, feeding high levels of oleic acid and other unsaturated fatty acids prevented this high mortality.

Gross and histological examination of morbid animals or of those with depressed body weights revealed no abnormalities

TABLE 1

Effects of palmitic acid, oleic acid and cholesterol on mature male mice

Dietary supplement to fat-free basal diet	Av. 12-day wt. change	Av. spleen wt. after 12 days
	gm	*mg*
9% Crisco, 1% Hydrol 4% corn oil, 1% linseed oil	+ 0.8	197
20% palmitic acid	− 1.0	180
20% palmitic acid + 0.1% oleic acid	− 1.8	156
20% palmitic acid + 1.0% cholesterol	+ 0.5	162
20% palmitic acid + 1% cholesterol + 0.05% oleic acid	− 4.0	112
20% palmitic acid + 1% cholesterol + 0.1% oleic acid	− 2.4	155
20% palmitic acid + 1% cholesterol + 0.15% oleic acid	− 2.2	151
20% palmitic acid + 1% cholesterol + 0.8% oleicacid	− 1.9	172

TABLE 2

Effect of palmitic acid, oleic acid, and cholesterol on growth and mortality of female weanling mice

Dietary supplement to fat-free basal diet	Av. 12-day wt. gain	Mortality
	gm	
10% palmitic acid	9.5	0/8
10% palmitic acid + 1% cholesterol	8.6	0/8
15% palmitic acid	6.2	1/8
15% palmitic acid + 1% cholesterol	6.7	1/8
20% palmitic acid	1.6	4/8
20% palmitic acid + 1% cholesterol	1.3	9/16
20% palmitic acid + 1% cholesterol + 0.1% oleic acid	3.7	7/8
20% palmitic acid + 1% cholesterol + 0.5% oleic acid	4.9	5/8
20% palmitic acid + 1% cholesterol + 5.0% oleic acid	6.5	0/8
20% palmitic acid + 1% cholesterol + 5.0% corn oil	9.4	0/8
20% palmitic acid + 1% cholesterol + 5.0% linseed oil	9.7	0/8
20% palmitic acid + 1% cholesterol + 5.0% cod liver oil	8.9	0/8

TABLE 3

Effect of palmitic acid, oleic acid, and cholesterol on blood glucose and blood urea levels of female weanling mice

Supplement to fat-free diet	Serum glucose	Serum urea
	mg %	*mg %*
9% Crisco, 1% Hydrol, 4% corn oil, 1% linseed oil	155	25.7
20% palmitic acid, 1% cholesterol and 0.1% oleic acid	105	69.6

with the exception of extremely small spleen and lymph nodes. Although the spleens were markedly reduced in size, no histological defects could be found. Pooled blood samples were taken from these same animals by decapitation. Urea and glucose values obtained on these samples are recorded in table 3. It can be seen that urea is elevated and glucose is depressed, but probably not more so than in any terminal state.

Accompanying the growth retardations were marked reductions in food intake resulting in a decrease of approximately 40% in food and protein efficiency ratios. Balance studies suggested that the absorption of fat and protein was not influenced by the low levels of oleic acid employed.

II. Experiments in rats. It seemed desirable to repeat these studies with the laboratory rat not only to obtain data in a second species, but also to gain access to larger quantities of blood and urine for biochemical analyses. Limited supplies of highly purified fatty acids forced a return to the hydrogenated coconut oil-containing diet which was used with mice when the initial observations were made. Four groups of 10 male weanling rats of the Holtzman strain (average weight of 60 gm) were used in this study. They received the cholesterol-containing basal diet used in the mouse studies with the exception that in each case the palmitic acid was replaced as follows:

Group 1—9% Crisco, 1% Hydrol, 4% corn oil, 1% linseed oil and 5% glucose.

Group 2—30% Hydrol (10% glucose eliminated).

Group 3—20% palmitic acid, 10% Hydrol (10% glucose eliminated).

Group 4—30% Hydrol, 1% linoleic acid (11% glucose eliminated).

These animals were maintained on the several diets in individual wire-bottom cages with food and water supplied ad libitum. After 7 days, two rats of each group were sacrificed and spleen weights were obtained. The remaining 8 rats in each group were continued for a total of 91 days. The weight changes, biochemical, and urinalysis obtained at the conclusion of the study are shown in table 4. The urinalysis values were obtained from pooled samples taken the last 4 days of the study. As was found with the mouse, the rats in group 3 showed a marked decrease in growth rate and spleen size. No

TABLE 4

Effect of various fats on rats fed a basal diet containing 1% cholesterol

Fat in diet		Group 1 9% Crisco 1% Hydrol 4% corn oil 1% linseed oil	Group 2 30% Hydrol	Group 3 20% palmitic acid 10% Hydrol	Group 4 30% Hydrol 1% linoleic acid
Av. 7-day wt.,	*gm*	100	96	72	100
Av. 7-day spleen wt.,	*mg*	624	460	216	546
Av. 91-day wt. gain,	*gm*	339	280	130	345
		Plasma values			
Urea N.	*mg%*	11.7	11.3	20.9	11.3
Fibrinogen,	*mg%*	320	275	220	245
Glucose,	*mg%*	110	85	70	105
Total cholesterol,	*mg%*	104	135	104	127
Free cholesterol,	*mg%*	12	14	10	17
Total protein,	*mg%*	6.3	6.3	5.4	6.3
Albumin,	*mg%*	1.50	2.05	2.25	2.05
Globulin,	*mg%*	4.80	4.25	3.15	4.25
Hematocrit		37	40	48	39
		Urine vaues			
Volume,	*ml*	160	155	65	145
Specific gravity		1.011	1.010	1.020	1.009
pH		8.5	8.5	9.0	9.0
Blood		none	none	none	none
Sugar		none	none	none	none
Protein, gm/liter		1.15	1.22	1.55	1.66

pathology was found in any tissue. An explanation for the growth effects of the different diets was not found in the plasma or urine values.

DISCUSSION

The mechanism involved in the mortality and weight losses observed under the conditions described above is not known. One thing seems clear: under the conditions of our experiments oleic acid is synthesized in the rodent body at a very slow rate. Otherwise the extremely low dietary levels used could not be as critical as they were found to be. The inability of the rat to synthesize large quantities of unsaturated fats has been suggested by the observations of Cox and De Eds ('58), and Herting and associates ('58, '59) who found that lipogranuloma, a foreign-body type of reaction in fat cells, develops on a diet containing large quantities of saturated fat and that the lesions can be prevented by increasing the unsaturated fatty acid intake to approximately 50% of the total.

It is of considerable interest that in our experiments conditions have been established under which oleic acid or linoleic acid can be shown to be either lethal or life-saving. These findings point up the very complex interrelationships that exist among all foodstuffs.

SUMMARY

Weanling male mice grow well on a purified diet containing 20% of palmitic acid and 1% of cholesterol, but succumb or fail to grow when 0.1% of oleic acid is added to the diet. Further addition of as little as 0.1% of this acid again affords good growth and survival.

If 0.1% of linoleic acid is incorporated into the diet, the critical lethal level of oleic acid becomes 0.8% and again increasing the oleic acid fed affords survival and a rapid rate of growth.

Mature male mice and weanling male rats survive on these diets but lose weight or grow very poorly.

A high percentage of weanling female mice succumb on a diet containing 20% of palmitic acid with or without cholesterol but survive when unsaturated fatty acids are added to their diets.

ACKNOWLEDGMENT

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Riboflavin in the Blood and Urine of Women on Controlled Diets[1,2]

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The characteristic proportions of various levels of riboflavin intake appearing in the urine allow for a measure of nutritional status in the individual. The limits of riboflavin concentration to be expected in any fraction of the blood have not been established for the well-nourished person nor correlation made with the proportion of the corresponding intake to be found in the 24-hour urine.

This laboratory has reported previously concurrent values for riboflavin in the serum and in the daily urinary excretion for an intake of 1.2 mg of riboflavin per day (Wu et al., '53). In the present study the riboflavin intake has been increased to 1.4 mg per day and the investigations extended to the measurement of the vitamin in whole blood, red cells, white cells plus platelets as well as in serum and urine.

EXPERIMENTAL

Subjects and diet

Seven subjects, three in 1955 and 4 in 1956, from the staff and graduate students of the Department of Foods and Nutrition, received for 30 days the controlled diet (Louhi et al., '52) of ordinary foods. A daily supplement of crystalline riboflavin in hydrochloric acid, the amount of which was determined by previous analysis of the food, brought the intake of riboflavin to 1.4 mg. According to calculation[4] the diet supplied 2000 Cal., 60 gm of protein and satisfactory amounts of the other known nutrients.

The subjects ranged from 23 to 54 years of age. One of them, CAS, took part in the studies in both years. Six of the women remained at their same weights of between 55 and 70 kg but the 7th, FD, started at 110 and lost about 3 kg during the controlled period.

Data were obtained for free and bound riboflavin in the blood fractions of 59 women 19 to 61 years of age, unrestricted as to diet, and of 5 following a test dose of 2 mg of riboflavin.[5]

Preparation and analysis of samples

Analyses for riboflavin were made fluorometrically by the method of Burch and associates ('48). Blood samples obtained before breakfast by finger puncture were prepared as the trichloroacetic acid filtrates of whole blood or its fractions. In 1956 it proved feasible to use the filtrates for parallel assays of oxidized pyridine nucleotides. The 24-hour urinary excretions were preserved with 2% by volume of glacial acetic acid in 1955 and under toluene in 1956 in amber bottles in a refrigerator.

RESULTS

Blood analyses

Average values (table 1) of 8.7, 13.0, 219 and 2.7 μg% were obtained for the concentration of total riboflavin in the whole blood, red cells, white cells and serum respectively. Coefficients of variation were 3% or less as compared with

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[2] This investigation was part of the Western Regional Project on Nutritional Status and was financed in part from funds appropriated under the Research and Marketing Act of 1946.

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[4] Agricultural Handbook No. 8 ('50).

[5] The analyses for 1954 were made by Dr. Mei-ling Wu Chang and Ulla Samvik.

TABLE 1

Average blood and urinary riboflavin values for 7 subjects during 30-day controlled diet periods

Subject	Whole blood total	RBC total	WBC total	Serum total	Serum Free + FMN[1]	Urine Days 17 to 30
1955	*μg %*	*μg %*	*μg %*	*μg %*	*μg %*	*μg per day*
BEH	10.6 ± 0.7[2]	17.2 ± 1.4	231 ± 18[2]	4.3 ± 0.2	2.4 ± 0.1	460 ± 36
MLF	8.5 ± 0.8	14.7 ± 1.8	211 ± 27[3]	2.4 ± 0.1	0.9 ± 0.2	456 ± 53
CAS	8.3 ± 0.8	13.0 ± 1.4	218 ± 17[3]	2.6 ± 0.1	1.4 ± 0.2	376 ± 84
1956						
CAS	8.9 ± 0.6	11.7 ± 1.5	236 ± 14	2.8 ± 0.2		183 ± 33
FD	6.6 ± 0.5	8.7 ± 1.3	209 ± 15	2.0 ± 0.1		373 ± 95
NM	8.6 ± 0.5	11.9 ± 1.9	215 ± 18	2.9 ± 0.2		361 ± 70
AS	9.2 ± 0.6	13.5 ± 1.5	213 ± 14	2.0 ± 0.1		311 ± 51
All subjects 1955 and 1956	8.7	13.0	219	2.7	1.6(3)	360

[1] Flavinmononucleotide.

[2] Standard deviation (SD) calculated from $\pm \sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{N-1}}$.

[3] Days 14 to 30. The values for days 1 to 14 were omitted as unreliable for all subjects.

2% for corresponding control filtrates analyzed simultaneously. The only significant changes observed were slight declines in red cell riboflavin levels for all subjects during the 1956 period and in red cell riboflavin and serum free riboflavin and flavinmononucleotide (FMN) for subject CAS in 1955.

For the random group the ranges for total riboflavin in whole blood, red cells, white cells and serum were 4.3 to 14.6, 7.1 to 25.7, 162 to 292 and 1.8 to 5.5 μg% respectively.

In contrast to 30 to 45 μg% reported by others (Axelrod et al., '41; Strong et al., '41) for total riboflavin in whole blood, our values did not exceed 15 μg% in either the controlled or unrestricted diet groups. Nevertheless they agree with theoretical values derived by calculation from the observed riboflavin contents of the red and white cells and serum. The cell and serum concentrations of riboflavin were similar to those obtained by Bessey and associates ('56) in controlled studies of men for up to 16 months. However only 8 of our random subjects and none of the controlled exceeded the 20 μg% of riboflavin in the red cells suggested by these workers as a mark of adequacy. Even a test dose of 2 mg of riboflavin failed to raise the red cell level to 20 μg% for 4 of 5 subjects. Similar instances of anomalous values for serum riboflavin as demonstrated by Bessey et al. ('56) and Suvarnakich et al. ('52) were found in about 10% of the random subjects with total and free riboflavin as high as 5.5 and 2.6 μg% respectively. BEH, of the controlled group, was probably an "outlier" for serum riboflavin also.

Urine analysis

The average 24-hour urinary excretion of 360 μg of riboflavin during the last 14 of the 30 experimental days agreed with the results of other studies of women following similar periods of adjustment (Brewer et al., '46; Hathaway and Lobb, '46). Since this excretion represented 26% of the intake, 1.4 mg of riboflavin daily would appear to be adequate for all the subjects except CAS who alone showed a steady decrease in urinary riboflavin throughout the 1956 period and an average rate for the latter half of only 13% of the intake.

DISCUSSION

In agreement with many other studies, at least 10 to 14 days were required for the adjustment of the urinary excretion rate to a change in riboflavin intake. No doubt the very heavy losses by the obese subject, FD, of more than 100% of the riboflavin provided during the first 6 days of the study can be attributed also to the instability in nitrogen metabolism indicated by the values obtained for urinary total nitrogen at this time. Similar massive outputs of the vitamin have been reported during starvation (Perlzweig et al., '44) and in suspected negative nitrogen balance (Oldham et al., '47; Lossy et al., '51; Pollack and Bookman, '51). We have observed also an excretion in the urine of 80 to 90% of the riboflavin in the diet by two subjects of normal weight who received daily for three days only one quart of milk as the sole food. In both cases the urinary creatinine was reduced to about half the usual value. In view of the proposal that riboflavin and protein are mutually limiting factors (Bro-Rasmussen, '58), the effect of nitrogen balance on the urinary level of riboflavin ought not to be ignored.

Characteristic levels of riboflavin were maintained in the whole blood and serum not only by the controlled group but also by 7 women with unrestricted diet who were tested at intervals for periods up to three years. However for red cell riboflavin, the ranges of concentration for three of these 7 subjects were almost as widespread as the 7.1 to 25.7 μg% observed for the whole random group of 59 subjects. Increase in serum riboflavin levels, especially in the free riboflavin plus FMN, occurred within half an hour after the test dose in all 5 subjects and was particularly marked for BEH with the original anomalous value. These results suggest that more data are required before the level in any blood fraction may be used to interpret adequacy of riboflavin.

Simultaneous measurement under controlled dietary conditions in 1956 revealed no relationship in the levels of riboflavin and pyridine nucleotides within the group

(Morley and Storvick, '57). The apparent instability in protein metabolism in FD may account for the coincident low riboflavin and high pyridine nucleotide contents in the same samples of whole blood or red cells for this subject.

SUMMARY

Determinations of the riboflavin content of various fractions of fasting blood and of concurrent 24-hour urinary excretions were made for 7 subjects during 30 days of a controlled riboflavin intake of 1.4 mg per day and for bound and free riboflavin in the fasting blood of 59 women with unrestricted intake.

The ability of the individual to maintain a characteristic level of riboflavin in each blood fraction was demonstrated, although individual differences for the white cell fraction were not as distinct as for the other fractions. There appeared to be a gradual trend toward lower levels for red cell riboflavin for all subjects. Adaptation to a change in intake was apparent in the trends for urinary excretion of riboflavin for all subjects during the first 14 days. Very large excretions of riboflavin were observed for an obese subject on the controlled intake.

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Serum Polyunsaturated Fatty Acids in Groups of Africans with Low and High Fat Intake

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Our work on vitamin A deficiency in Ruanda-Urundi (Roels, Debeir and Trout, '58a; Roels, Trout and Dujacquier, '58b) shows that this deficiency in the area may be due partly to a very low fat intake and a resulting poor carotene absorption.

A careful and detailed study by Leurquin ('59) revealed that lipids represent only 6.8% of the total caloric intake in Ruanda-Urundi. We thought it, therefore, of interest to determine the total and the polyunsaturated serum fatty acids of a population group in this area and to compare their serum levels with those of another African tribe with similar caloric intake, but in which the lipids represent 37.8% of the dietary calories.

Although there was no significant difference between the total serum fatty acid content of the various sex and age groups of the two tribes, there were remarkable differences in the polyunsaturated fatty acids in their sera.

GENERAL PROCEDURE AND METHODS

Serum lipids of two African population groups were studied. The first group are Banyaruanda from the Bufundu area (29° 30′ E of Greenwich, 2°40′ S) of Ruanda, where vitamin A deficiency is prevalent. Their staple foods are beans, sweet potatoes and various types of beer made from bananas, sorghum and maize. Their total caloric intake is 2,127 Cal. per inhabitant per day, 11.2% of which are supplied by vegetable proteins, 0.5% by animal proteins (half from meat and half from milk), 81.5% by carbohydrates and 6.8% by lipids (Leurquin, '59). The 6.8% of dietary lipids are not consumed as fat or oil, but are contained mainly in the sweet potatoes, beans and sorghum.

The second group lives in the Djugu area of the Belgian Congo, about 30° E of Greenwich and 2° N. They belong to the Baniari tribe and live nearly all in one village, Sabati. Their staple foods are bananas, green vegetables, palm oil and groundnuts.[2] Their average daily dietary intake is 1,980 Cal. per inhabitant. This figure is slightly underestimated because it does not include their consumption of alcoholic beverages which could not be measured. These Baniari derive 10.9% of their calories from vegetable proteins (mainly from groundnuts), 1.6% from animal proteins (meat and fish), 37.8% from lipids and 49.7% from carbohydrates. The groundnuts furnish 72.5% of their lipid calories, 20.8% are from palm oil and the remainder from the lipids present as minor constituents of bananas, beans, corn and other ingredients of their diet. Figure 1 illustrates the difference between the diets of the two groups.

In each group, fasting (i.e., at least 12 hours after the last meal) blood samples were taken from 25 adult men, 25 adult women, 25 girls and 25 boys. The boys and girls were from 11 to 16 years old. The men and women were generally between 17 and 55 years of age, although it was often difficult to know their exact age. Nothing was added to the blood or serum. The blood was centrifuged after standing for about two hours at room temperature in the dark. The serum was pipetted off and kept at 0°C in the dark until it arrived in the laboratory where it was kept at −25°C in the dark in oxygen-free nitrogen.

For the determination of the total serum fatty acids and the polyethenoid fatty acids, the procedure of Pikaar and Nijhof ('58) was followed.

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[1] Biochemist in the Medical Services of the Belgian Congo Government.

[2] Peanuts.

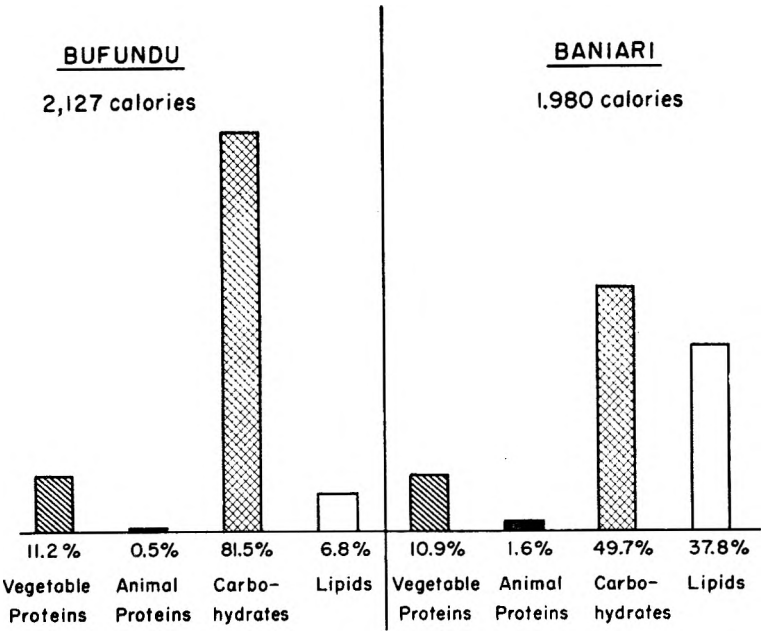

Fig. 1 Polyethenoid acids in African sera. Average daily dietary intake for Bufundu (Ruanda) and for Baniari (Congo). Data taken from work of Leurquin ('59).

RESULTS

The total fatty acids and the polyunsaturated fatty acids of the sera from the various sex and age groups of the different populations examined are summarized in table 1.

There are no significant differences between the two tribes in total serum fatty acids, despite their vastly different dietary fat intake. The differences between the sums of the polyunsaturated fatty acids for both groups are, however, highly significant when this sum is expressed either in milligrams per 100 ml of serum or as a percentage of the total serum fatty acids. Figure 2 illustrates this further and lists the levels of significance for the differences between these mean serum values for both groups.

The significantly higher level of polyethenoid fatty acids in the serum of the group with the greater fat intake is due to their considerably higher serum levels of dienoic and tetraenoic fatty acids, although their levels of trienoic, pentaenoic and hexaenoic serum fatty acids are significantly lower than those of the group with the low fat diet. These differences and their levels of significance are illustrated in figure 3.

DISCUSSION

Several workers reported that the levels of dienoic and tetraenoic fatty acids in various tissues of animals were higher for those with a higher fat intake than for the corresponding group on a low fat diet, whereas the opposite was observed for trienoic acid. Thus Wiese, Baughan and Hansen ('55) observed this in dogs when they increased the dietary fat from one to 30% of the calories. Mukherjee et al. ('57), proved this to be the case when they compared blood from rats on a fat-deficient diet and from a control group receiving 6% of their total calories as lipids. Adding 200 mg of linoleate per day to the fat-deficient diet restored the high dienoic and tetraenoic fatty acid levels. Hansen et al. ('58) found that infants on a fat-deficient diet had very low serum levels of dienoic and tetraenoic fatty acids and a high level of trienoic acid. The addition of linoleic acid (2% of the total caloric intake) rapidly reversed this trend, whereas an equivalent supplement of tripalmitin produced no change.

TABLE 1

Serum polyunsaturated fatty acids in two groups of Africans[3] showing values in mg/100 ml, with standard deviation

	No.	Total fatty acids		Dienoic		Trienoic		Tetraenoic		Pentaenoic		Hexaenoic		Total Polyenoic	
Women A[1]	22	292.7	64.67[2]	41.6	11.45	9.6	2.67	16.4	4.61	9.5	2.13	6.4	2.23	83.5	16.42
Women B	26	257.1	39.14	75.6	13.50	4.5	1.27	23.8	4.41	4.1	0.62	4.1	1.54	112.1	17.28
Men A	27	286.6	51.80	41.5	10.87	9.1	2.90	17.6	3.48	8.8	2.42	6.1	1.21	83.0	15.45
Men B	24	277.9	83.23	61.9	16.37	3.7	1.33	22.2	4.84	3.5	0.63	3.7	1.09	94.9	21.36
Girls A	29	249.2	26.61	33.8	7.72	7.5	2.31	15.4	2.91	8.0	2.38	6.7	1.63	71.5	12.66
Girls B	24	265.7	32.30	56.8	13.69	5.5	1.61	21.9	2.46	4.8	0.75	6.8	1.71	95.8	15.15
Boys A	27	253.7	54.43	37.0	12.03	7.7	2.03	15.7	3.08	8.9	1.58	6.9	1.31	76.2	16.40
Boys B	27	257.1	49.18	60.6	15.20	5.2	1.63	21.4	4.99	4.4	1.15	5.6	2.01	97.2	21.38

[1] A = Banyaruanda, low fat intake; B = Baniari, high fat intake.

[2] Standard deviation.

[3] In mg/100 ml.

These observations are in good agreement with our findings concerning the percentages of the dienoic, trienoic and tetraenoic acids in the serum of African subjects with low- and high-fat intakes.

Antonis ('58) found that by substituting 800 Cal. derived from carbohydrates in the diet of Bantus with an isocaloric quantity of sunflower seed oil, the percentage of dienoic and tetraenoic fatty acids in their serum rose, and the trienoic fatty acid decreased. Linoleic acid constitutes about 65% of the total fatty acids in sunflower seed oil.

When comparing the diets of the two groups of Africans in the present study (fig. 1), it is apparent that about 31% of the carbohydrate calories of the Bufundu diet are replaced by groundnut oil, containing about 20% linoleic acid and palm oil, containing about 10% linoleic acid (Hilditch, Meara, Roels, '47) in the Baniari diet. This difference in the diets resulted in changes in the serum fatty acid distribution similar to those reported by Antonis.

Table 2 lists the amount of total serum fatty acids and the percentages of indi-

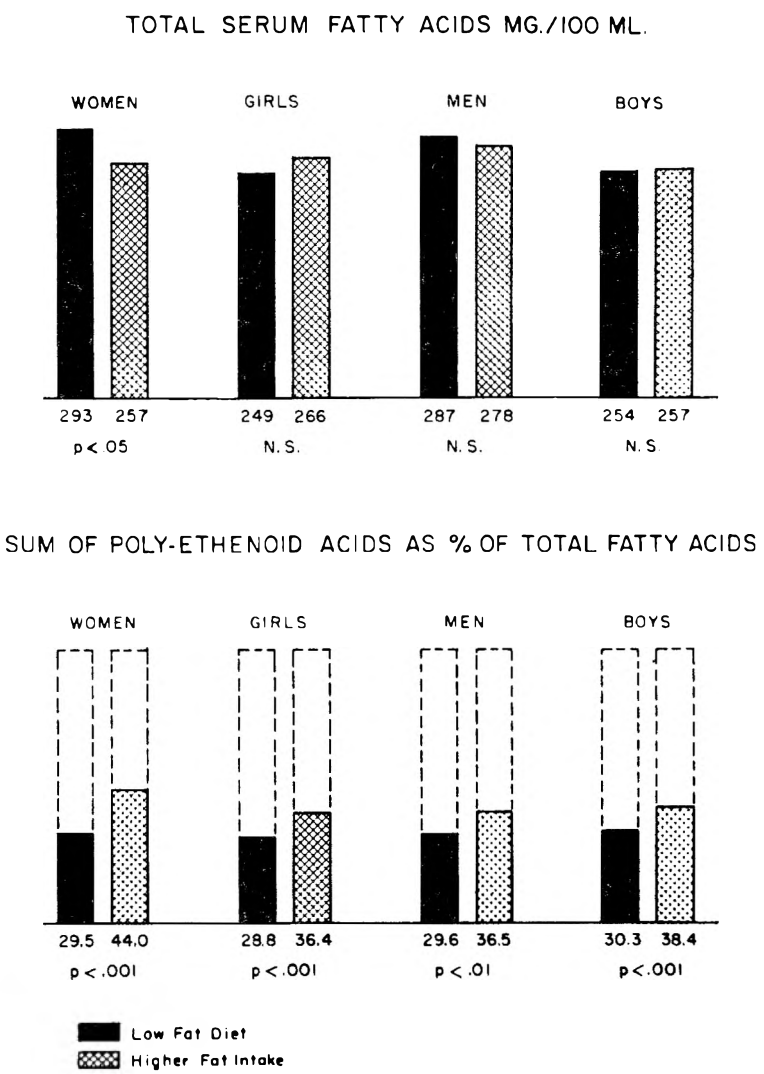

Fig. 2 Polyethenoid acids in African sera.

vidual polyenoic acids reported for different population groups:

(1) by Hammond and Lundberg ('55) for plasma from a blood bank in the United States, for that of Guatemalans and for that of atherosclerotics,

(2) by Antonis ('58) from the Union of South Africa for Bantus, Europeans and atherosclerotics, and

(3) by the authors of the present study for adult men on a high and on a low dietary fat intake.

It is striking that the two groups of atherosclerotics and our group on the low dietary fat intake have the three lowest percentages of total polyenoic acids. It should be noted that there is very little atherosclerosis in the population to which

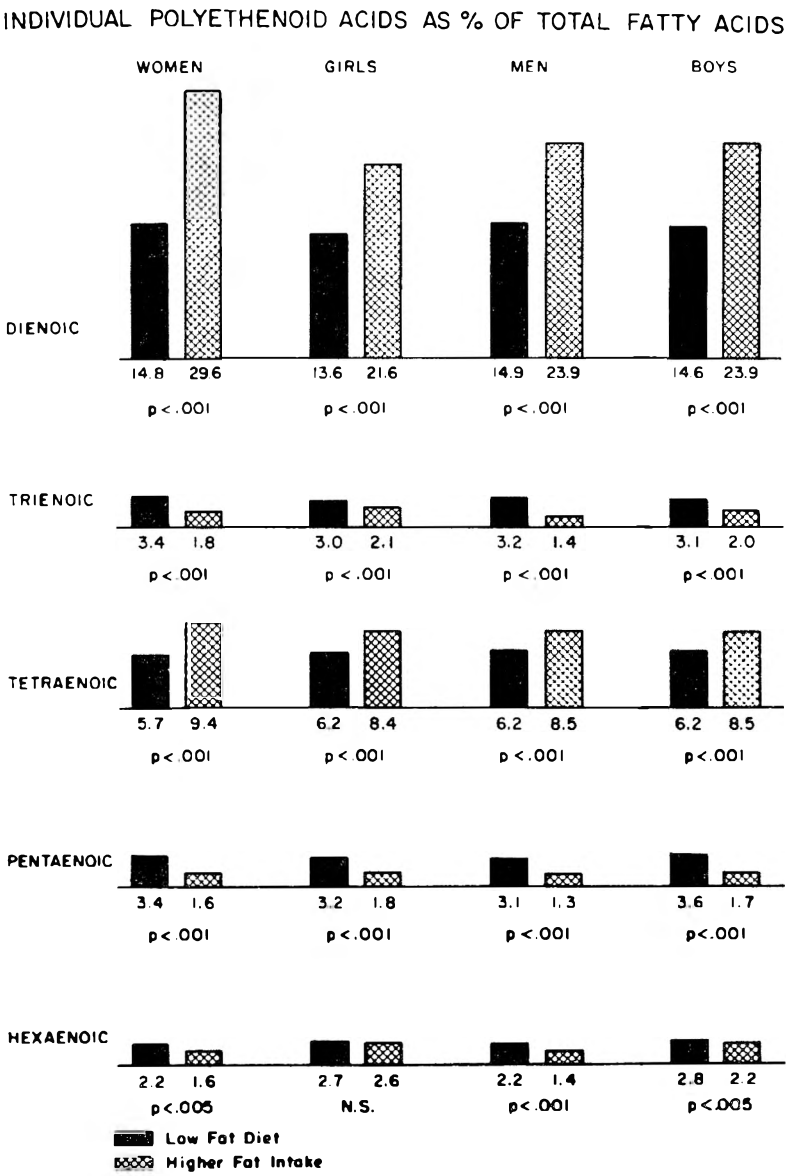

Fig. 3 Polyethenoid acids in African sera. Individual acids as a percentage of total fatty acids.

our low-fat group belongs: in 1955, there were 5,311 deaths in hospitals (where the cause of death is diagnosed fairly accurately) in their country (Ruanda-Urundi) and only one of these deaths was ascribed to atherosclerosis. The total serum fatty acids, however, are very much higher among the two groups of atherosclerotics than among the "low-fat intake" group of the present study.

It should also be noted that the South African Bantu (with a fat intake estimated by Antonis to be 15 to 20% of their total caloric intake) and the "high-fat intake" group of the present study have the highest percentages of dienoic, tetraenoic and total polyenoic fatty acids and the lowest absolute amount of total fatty acids in their serum.

Although it is fully realized that the determination of dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic acids gives only a gross approximation of the great variety of polyunsaturated fatty acids present in human serum, it is suggested as a working hypothesis that when lipogenesis becomes important either as a result of a very low dietary fat intake or of a high caloric intake, the percentage of saturated plus monoenoic fatty acids increases, and under these circumstances more trienoic, pentaenoic and hexaenoic acids appear in the serum. When there are significant amounts of linoleic acid present in the diet, the percentage of the polyunsaturated fatty acids in the serum rises, and this increase is due to greater proportions of dienoic and tetraenoic, with a decrease in the percentage of trienoic acid.

Mead ('58) recently has suggested different pathways for the formation of polyunsaturated fatty acids, a combination of which would readily explain the observations made in the present study. Thus, the pathway from linoleic acid to arachidonic acid seems to be well established, and it would appear that the serum concentrations of both these acids would be dependent to a certain extent upon the dietary supply of linoleic acid. The relatively high serum level of trienoic acid on low-fat diets may perhaps be explained by dehydrogenation of saturated fatty acids (Dauben, Hoerger and Petersen, '53) to monounsaturated acids followed by further dehydrogenation of the so-formed monoenoic acids (e.g., oleic acid) to tri-, penta- and hexaenoic acids (Mead, '58).

In any case, it appears fairly clear that when the linoleic acid intake passes a certain level in the diet, the total serum polyunsaturated fatty acid level rises owing to an increase of the percentages of dienoic and tetraenoic acids. When the level of linoleic acid intake falls below a certain level, another pathway of unsaturated fatty acids seems to become more important and the percentage of trienoic, pentaenoic and hexaenoic acids rises in the serum.

TABLE 2

Comparison of the percentage of polyenoic serum fatty acids obtained in different studies

	No.	Total fatty acids	Di-enoic	Tri-enoic	Tetra-enoic	Penta-enoic	Hexa-enoic	Sum Poly-enoic
		mg/100 ml	%	%	%	%	%	%
Hammond and Lundberg								
Bloodbank U. S.		353	22.0	1.6	6.0	0.8	1.1	31.5
Guatemalans	8	394	20.6	2.2	5.8	0.9	1.5	31.0
Atherosclerotics	8	492	16.2	1.8	5.0	0.8	1.3	25.1
Antonis (S. Africa)								
Bantu	41	234	22.6	2.8	7.8	1.6	3.1	37.9
Europeans	13	464	20.5	2.1	4.8	1.3	3.7	32.4
Atherosclerotics	14	718	13.8	1.9	4.3	1.9	2.8	24.7
Present study (men)								
Low fat	25	287	14.9	3.2	6.2	3.1	2.2	29.6
High fat	25	278	23.9	1.4	8.5	1.3	1.4	36.5

ACKNOWLEDGMENT

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Comparisons of Atherogenesis in Rabbits Fed Liquid Oil, Hydrogenated Oil, Wheat Germ and Sucrose[1]

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INTRODUCTION

The relationship between serum lipids and atherogenesis is not yet firmly established, although investigations with both humans and animals have shown that dietary factors do influence serum lipid levels. We have therefore investigated the relations among diet, blood and liver lipids and atherosclerosis in the rabbit. Van Handel ('55) has suggested that the geographic differences in the incidence of coronary disease might be related to selective hydrogenation of polyunsaturated fatty acids or to degermination of cereals, which removes significant amounts of trace elements and vitamins. In order to test these hypotheses, we supplemented cholesterol-containing rabbit food with liquid or solid fat, with wheat germ or with sucrose. In this manner it was possible to obtain comparisons among two high-fat diets and two low-fat diets, one of which contained an excess of carbohydrate, and one which supplemented the basic diet with large amounts of protein, B vitamins and trace minerals in the form of wheat germ. Most workers studying dietary fats have compared solid fats like butter, hydrogenated coconut oil, safflower oil, or cottonseed oil. To exclude factors not directly related to hydrogenation, we used cottonseed oil and hydrogenated cottonseed oil.

METHODS

Litter mates of three-month-old New Zealand Albino rabbits were selected randomly and distributed among 5 dietary groups of 7 to 9 animals each. Diets were prepared from a commercial chow[3] to which were added fats, wheat germ, or sucrose in equicaloric amounts (table 1). Four diets provided 10% of the fed calories as protein, whereas the wheat germ diet supplied 22.5% of calories in this form. The high-fat diets contained 20% of fat (37% of the fed calories).[4] In order to make the diets calorically comparable, the animals on the high-fat diets received 75 gm of food per day, whereas the animals on the sugar and wheat germ diets received 100 gm. All animals received 200 mg of cholesterol daily, dissolved in fat and mixed with the other ingredients. All diets provided a minimum of 0.5 gm of linoleic acid daily derived from the rabbit chow. Food intake was measured daily and recorded in terms of caloric value of the main constituents (table 2).

The experiment was designed to answer the question how, in an atherogenic diet, equicaloric substitution of hydrogenated oil, liquid oil, or wheat germ for sucrose would affect the aortic lesions. Therefore, the effects of three diets were compared with the sucrose-supplemented diet. A 5th sucrose-supplemented diet without cholesterol was included as a control for spontaneous atherogenesis. The latter group was not used in the statistical evaluations.

Blood samples were taken monthly from the central artery of the ear. Plasma was treated with alcoholic KOH and extracted with petroleum ether according to Abell et al. ('52) and cholesterol was determined with the *p*-toluenesulfonic acid reagent of Pearson et al. ('53). Phospholipids were determined in a chloroform-methanol ex-

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[1] This investigation was supported by research grant H-2181 from the National Heart Institute, U. S. Public Health Service.
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[3] All diets were made from one single batch of oil and hydrogenated fat and from the same shipment of wheat germ and Purina chow.
[4] The calculations are based on the manufacturer's information that the gross energy content of Purina rabbit chow provides 3.74 Cal. per gm, 80% of which is metabolizable.

TABLE 1

Composition of diets

Diet No. of animals	Hydrog. cotton-seed oil + chol. 7	Cottonseed oil + chol. 8	Wheat germ + chol. 8	Sucrose + chol. 9	Sucrose 8
	%	%	%	%	%
Rabbit chow[1]	80	80	62	62	62
Hydrogenated cottonseed oil[2]	20		1	1	1
Cottonseed oil[3]		20			
Wheat germ[4]			37		
Sucrose				37	37
Cholesterol	0.27	0.27	0.20	0.20	

[1] Purina Rabbit Chow: Protein 15%, fat 3% and crude fiber 18%.
[2] Hydrogenated cottonseed oil: Iodine value 63.6, linoleic acid 1.5%, m.p. 37.6.
[3] Cottonseed oil: Iodine value 112, linoleic acid 48%.
[4] Wheat germ (Kretschmer): Protein 33%, wheat germ oil 11.5% and crude fiber 1.7%.

TABLE 2

Daily consumption (in calories) as calculated from food records

Diet No. of animals	Hydrog. cotton-seed oil + chol. 7	Cottonseed oil + chol. 8	Wheat germ + chol. 8	Sucrose + chol. 9	Sucrose 8
Rabbit chow	174 ± 4.3	163 ± 3.6	162 ± 6.0	157 ± 4.2	160 ± 4.0
Hydrogenated cottonseed oil	131 ± 3.2				
Cottonseed oil		123 ± 2.7			
Wheat germ			128 ± 4.7		
Sucrose				123 ± 3.3	125 ± 3.7
Cholesterol (in mg)	195 ± 4.8	184 ± 4.0	174 ± 6.5	166 ± 4.4	

Used for the calculation of the metabolizable portion: Chow, 3 Cal.; Oils, 9 Cal.; Sugar and wheat germ, 4 Cal. per gm.

tract by the method of King ('32). After 160 days, the animals were killed by intracardiac air injection, and the livers, aortas, and hearts immediately removed. Thoracic aortas were graded by 4 independent observers for gross lesions on an arbitrary scale from zero (no lesions) to 5 (90 to 100% of aortas solidly covered with confluent plaques). Samples from each aorta and heart were fixed in 10% formalin, embedded in paraffin, sectioned at 8 microns, and stained with hematoxylin and eosin. Heart valves, endocardium, and the first few millimeters of the coronary arteries were examined grossly and histologically.

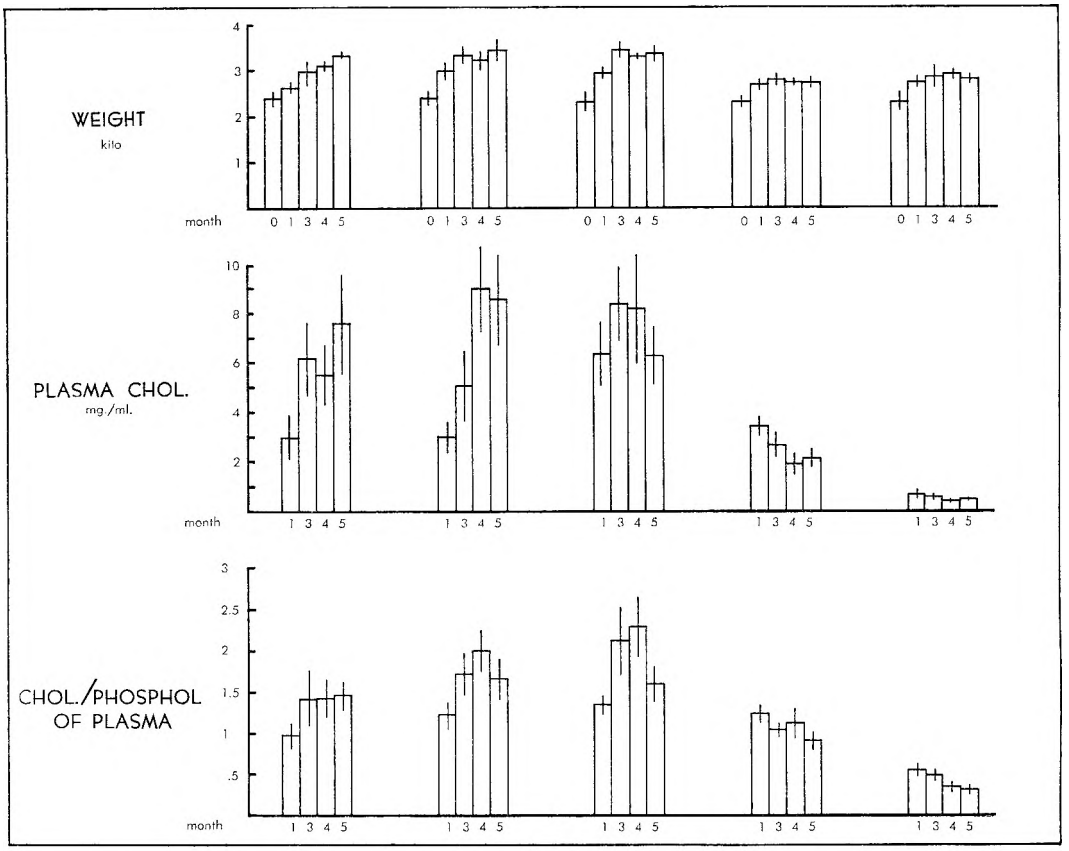
Liver lipids were extracted with chloroform-methanol (2:1) and washed once with an equal volume of water. Aliquots were dried in vacuo, taken up in petroleum ether (bp 60 to 70°C) and placed on a column of 1 cm inner diameter containing 1 gm of non-activated silicic acid-supercel (1:1).[5] Cholesterol esters were eluted with 20 ml of 10% chloroform in petroleum ether, and free cholesterol was eluted with 20 ml of chloroform according to Van Handel ('59). Cholesterol and cholesterol esters were determined with the reagent of Pearson et al. ('53); cholesterol oleate was used as a standard for the cholesterol ester fraction. The values were in agreement with free and total cholesterol determined by the Sperry and Webb ('50) method on the same liver extract.

Fecal lipids were extracted by grinding droppings with methanol and chloroform. They were saponified with alcoholic KOH and the sterols extracted with petroleum ether. Liebermann-Burchard positive digitonin precipitable material was determined according to Sperry and Webb.

RESULTS

All of the animals were in good health throughout the experiment and did not

[5] Silicic acid (Mallinckrodt). 100 mesh A. R. Hyflo Supercel (Johns Manville).



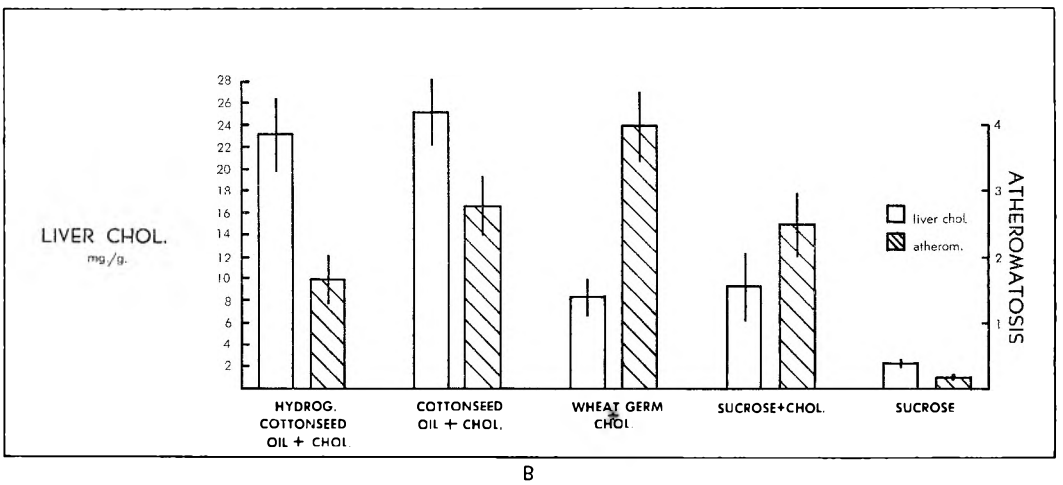


Fig. 1 Monthly weights, plasma cholesterols, cholesterol phospholipid ratios (A) and terminal liver cholesterols and aortic scores (B) of rabbits on various dietary supplements. The vertical line at the top of each bar is two standard errors in length.

TABLE 3

Terminal plasma cholesterol and phospholipids (mg/100 ml), free and esterified liver cholesterol (mg/gm) and aortic score of rabbits on various diets

Litter and sex	Terminal plasma		Liver		Aorta
	Cholesterol	Phospholipid	Free cholesterol	Esterified cholesterol	Score[1]
			Hydrogenated cottonseed oil + cholesterol		
1—M	430	332	7.1	22.9	½
1—M	254	212	4.5	13.7	¼
6—F	1680	770	3.1	26.4	2
7—F	1650	780	2.7	17.3	2½
8—M	220	210	2.5	2.0	1
3—M	940	675	5.1	26.0	3
2—M	320	314	3.5	26.0	2½
Av.	785	470	4.1	19.2	1.7
			Cottonseed oil + cholesterol		
1—M	173	274	4.0	17.5	1
2—M	1250	480	4.0	12.6	2
4—F	510	490	3.2	10.2	2
6—F	1800	785	3.7	26.3	3
7—F	1400	700	6.2	24.8	4½
8—F	760	470	8.1	21.9	2
5—M	785	338	5.2	32.3	3½
6—F	540	760	2.8	14.1	4
Av.	902	537	4.6	20.0	2.7
			Wheat germ + cholesterol		
1—M	83	180	2.2	0.6	¾
2—M	460	520	3.4	4.1	3½
2—M	660	460	4.4	9.4	4
4—M	360	400	1.9	2.3	5
6—F	1030	470	3.7	5.3	5
7—F	650	600	3.2	2.8	5
8—M	560	540	2.5	5.2	4
5—F	300	470	4.3	13.1	5
Av.	513	455	3.2	5.3	4.0
			Sucrose + cholesterol		
1—F	113	200	1.7	0.3	0
2—F	117	167	2.3	11.1	1
4—F	420	270	2.8	6.1	3¼
6—M	250	195	2.3	4.0	3¼
6—M	310	380	1.2	2.8	3
7—M	160	242	2.6	10.4	3¾
3—F	283	322	2.1	1.0	3
4—M	70	131	5.3	25.7	1
7—F	430	334	1.4	1.6	4
Av.	240	249	2.4	7.0	2.5
			Sucrose		
1—M	85	143	1.30	0.62	¼
2—M	30	180	1.50	0.7	¼
4—F	60	184	1.35	0.85	¼
6—F	56	122	1.50	0.30	0
7—F	28	154	1.92	0.30	0
8—M	21	105	1.15	0.30	½
5—M	25	105	1.8	1.2	0
8—M	38	90	1.2	0.80	0
Av.	44	135	1.47	0.63	0.15

[1] Thoracic aortas were graded for gross lesions on an arbitrary scale from zero (no lesions) to 5 (90 to 100% of aortas solidly covered with confluent plaques).

show the jaundice or loss of hair which were observed by Lambert et al. ('58) in rabbits on high cholesterol or high fat diets. According to the records of food intake, the equicaloric substitution of the oils and wheat germ for sucrose was satisfactorily fulfilled (table 2). Total cholesterol and phospholipids of terminal plasma, aortic score and hepatic free and esterified cholesterol of individual rabbits are presented in table 3. Average monthly weight gains, plasma cholesterol, cholesterol/phospholipid ratios, amount of atheromatosis and liver total cholesterol are presented in bar graph form in figure 1.

The least amount of atheromatosis was present in the group consuming hydrogenated cottonseed oil; the most severe atherogenesis occurred in the animals on the wheat germ diet. Hydrogenated fat did not increase atheromatosis when compared with the liquid oil, the wheat germ, or the sucrose diet. This result could not be expected from plasma cholesterol values, since the animals maintained on hydrogenated fat exhibited higher levels than those on the sucrose-supplemented diet. In the group on wheat germ, plasma cholesterol and phospholipids during the first month averaged 650 and 460 mg/100 ml respectively and were significantly elevated above those of the three other groups, which were almost equal and averaged 300 mg/100 ml for cholesterol and 250 mg/100 ml for phospholipids. In subsequent months, however, plasma cholesterol of the animals on the two fat-supplemented and the wheat germ-supplemented diets reached essentially equal levels (750 to 900 mg/100 ml). In the rabbits maintained on the cholesterol-containing, sucrose-supplemented diet, the plasma lipid levels gradually diminished after the first month.

When the aortic scores of the animals on hydrogenated oil, liquid oil, and wheat germ were compared statistically with those on the sucrose-supplemented diet by the procedure of Dunnett ('55), only the wheat germ-supplemented group showed a significant increase ($P < 0.05$). Substitution of hydrogenated fat for cottonseed oil did not aggravate atherosclerosis. One might speculate that this lack of effect of hydrogenated shortening is due to a diminished rate of absorption of dietary cholesterol from a diet containing large amounts of high melting fats. Two arguments speak against this assumption. First, serum cholesterol levels of rabbits on hydrogenated fat were similar to those on the oil diet (fig. 1). Secondly, after 5 months on the cholesterol diet, the amount of Liebermann-Burchard positive, digitonin precipitable material excreted in feces did not differ from one diet to another, and amounted to only 10% of the total cholesterol intake.

Although the addition of large amounts of fat or sugar to commercial rabbit chow might precipitate a relative deficiency of protein, vitamins or minerals, this was probably not the case in our experiment, since the addition of wheat germ to rabbit chow did not increase weight gain above that caused by the high-fat diet. The animals on the sucrose diets, however, gained less weight than those on the fat or wheat germ diets.

Histological examination of the aortas confirmed the information derived from gross examination. Atheromatosis in the heart was exhibited as small, yellowish, raised areas on the valves and in the coronary arteries. In addition, intimal thickening and numerous foam cells, which in some cases occluded the lumen, were observed in the medium and small blood vessels of the heart. The incidence and severity of these valvular, coronary and myocardial blood vessel lesions were closely related to the amount of aortic involvement.

Cholesterol concentrations were elevated in livers of all animals on high-cholesterol diets. The terminal liver cholesterol levels in the rabbits on the two high-fat diets, however, were much higher than in the animals on wheat germ and sucrose (table 3). This impression was confirmed by the multiple comparison test of Scheffé ('53) with $P = 0.01$. There was no correlation between liver cholesterol and plasma cholesterol or aortic score. It is of interest that a large portion of the increase in liver cholesterol occurred in the ester fraction.

DISCUSSION

The effect of different fats on atherogenesis in the cholesterol-fed rabbit has been studied by Kritchevsky et al. ('54)

and by Lambert et al. ('58). Kritchevsky and co-workers used a different strain of rabbits and compared corn oil with hydrogenated shortening, using a 3% cholesterol and a 9% fat level during the two months of their experiment. More atheromatosis was found for the group on hydrogenated fat. Lambert et al. ('58), using a purified diet containing 20% fat and 0.25%, 0.75% and 2% cholesterol, failed to show such an effect when hydrogenated shortening was substituted for safflower oil. The aortic lesions were slightly less in the animals on hydrogenated shortening than in those on safflower oil, a trend comparable to that obtained in our experiments.

In an earlier study, we maintained 5 groups of 5 rabbits each for three months on 500 mg of cholesterol daily and rabbit chow supplemented with different fats or with wheat germ. Here also, the animals on the wheat germ diet showed a significantly greater degree of atheromatous lesions than the animals on rabbit chow plus 20% corn oil, cottonseed oil or hydrogenated cottonseed oil, whereas no significant difference was found between the various fats.

It is difficult to speculate about the mechanism of action of wheat germ, but since wheat germ is rich in protein, the effect of this material should be discussed. Newburgh and Clarkson ('23) found that a diet containing 33% of powdered beef caused more and earlier atheromatosis in the rabbit than a diet with 20% of beef. Loewe et al. ('54) reported a failure of protein to protect underfed rabbits against cholesterol-induced atherogenesis. They found more lesions in the animals on the high-protein diet than in the control animals. Meeker and Kesten ('41) found that in rabbits on diets not supplemented with cholesterol, casein caused some atherosclerosis while soybean protein did not. According to Leveille and Fisher ('58), Nishida et al. ('58) and Stamler et al. ('58) in the cholesterol-fed chicken, an increase in dietary protein diminished or prevented atheromatous lesions. To our knowledge, a similar study on the effect of protein in rabbits has not yet been carried out.

Jones and Huffman ('56) showed that in the chicken on a 1% cholesterol diet, substitution of whole corn germ for corn oil or cottonseed oil depressed atherogenesis. However, this effect has been ascribed by Nash and Wolff ('59) to incomplete absorption of corn germ in the absence of grit. In our experiments, the substitution of wheat germ for liquid or solid cottonseed oil increased the extent of atherogenesis. Therefore, either the chick in its response to protein differs from the rabbit, or else in the rabbit a factor in wheat germ other than protein has a pronounced atherogenic effect.

The observation that in one litter of 6 animals, distributed among all diets, lesions were almost completely absent (litter 1, table 3), suggests that genetic factors play a role in the development of atherosclerosis. Plasma cholesterol and phospholipid levels after the first month of cholesterol feeding were closely related with atheromatosis at autopsy. Plasma cholesterol levels at three, 4 and 5 months did not correlate with degree of atherosclerosis. This obseravtion suggests that early hypercholesteremia triggers vascular damage which develops progressively, relatively unaffected by subsequent levels of plasma lipids. Although the lipid metabolism of the rabbit differs from that of man, the present findings may caution against conclusions about human atherosclerosis based on serum cholesterol data.

SUMMARY

With rabbits fed cholesterol-supplemented chow, equicaloric amounts of the following were compared for their effect on atherogenesis: cottonseed oil, hydrogenated cottonseed oil, wheat germ, and sucrose. Severity of atherosclerosis after 5 months was greatest on the wheat germ-supplemented diet, whereas there were no differences among the other three groups. There was no correlation between the severity of atherosclerosis and either terminal plasma or liver cholesterol concentrations. The animals on the sucrose diet exhibited the lowest serum cholesterol levels. One litter, distributed among all dietary groups, developed practically no lesions. Animals on the high-fat diets exhibited liver cholesterol concentrations about three times as high as those on the

low-fat intake. Most of this increase occurred in the cholesterol ester fraction.

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INVITATIONS FOR NOMINATIONS FOR 1960 AMERICAN INSTITUTE OF NUTRITION AWARDS AND FELLOWS

Nominations are now being invited for the 1960 A. I. N. awards and fellowships.

Nominations for the *1960 Borden Award in Nutrition* must be submitted by December 1, 1959, to Dr. C. A. Baumann, Department of Biochemistry, University of Wisconsin, Madison.

Nominations for the *1960 Osborne and Mendel Award* are due also by December 1, 1959, and should be sent to Dr. D. M. Hegsted, Harvard School of Public Health, One Shattuck Street, Boston, Massachusetts.

The deadline for receipt of nominations for *A. I. N. Fellows* is January 1, 1960. These should be sent to Dr. Cosmo G. Mackenzie, University of Colorado School of Medicine, Denver, Colorado.

Full details of the rules for these awards and lists of former recipients are given in the August 1959 issue of *The Journal of Nutrition.*

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