EFFECTS OF A FAT-FREE DIET ON GROWING MALE RATS WITH SPECIAL REFERENCE TO THE ENDOCRINE SYSTEM [1]

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FIVE FIGURES

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In a previous publication, the effects of a fat-free diet on growing female rats were described, with particular observations on morphology and function of the endocrine glands and on ratios of organ weight to body weight (Panos and Finerty, '53). A diversity of significant changes was demonstrated, in addition to such characteristic signs of fat deficiency as retardation of growth, dermatitis, loss of hair and increased oxygen consumption. The thyroid and adrenal glands were smaller whereas the liver, heart and kidneys were larger in fat-deficient animals than in controls. Ovarian weight was not affected and although the follicles and corpora lutea appeared normal microscopically, large numbers of "deficiency" nuclei were present in the interstitial tissue. This finding was correlated with a demonstrated decrease in pituitary acidophiles, and, in turn, with observed irregularity of estrous cycles and increased pituitary basophiles to suggest that estrogen secretion is diminished as a result of decreased production of luteinizing hormone by the anterior pitiutary.

The purpose of the present report is to supplement these observations on female rats with those on growing male rats,

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studied under identical experimental conditions. In addition to determining the effects on growth, skin and oxygen consumption, the gross and microscopic characteristics of the endocrine organs and accessories, the brain, liver, heart and kidney were studied.

MATERIALS AND METHODS

Male rats of the Holtzman strain were obtained at weaning, the average weight at the beginning of the experiment being 67 gm. Thirty-five rats were placed on the fat-free diet and 20 controls were fed laboratory chow.[2] In addition to this major group of animals, two other groups were studied on an identical regimen for the purpose of determining organ-body weight ratios after varying periods on the fat-free diet (2 weeks and 5 weeks). Access to urine and feces was prevented by use of false-bottom cages, each of which contained 5 animals. Distilled water was supplied for drinking. Air-conditioning maintained temperature and humidity within a fairly constant range.

The percentage composition of the fat-free ration was as follows: fat-free, vitamin-free casein [3] 18; sucrose 76; salts [4] 4; and cellulose 2. Vitamins were added in the following amounts in milligrams per kilogram of food: thiamine chloride 5, riboflavin 10, pyridoxine 5, calcium pantothenate 50, nicotinic acid 25, para-aminobenzoic acid 10, biotin 2, inositol 1,000, folic acid 2, B_{12} with mannitol 1.8, menadione 50, crystalline vitamin A acetate 1, crystalline vitamin D_2 [5] 20 and alpha-tocopherol 100. The last three vitamins were combined in a special "mix," which was made up in small amounts at frequent intervals: the vitamin A acetate was dissolved in petroleum ether, the alpha-tocopherol in ethyl ether and these two and the vitamin D_2 were added to sucrose which was then thoroughly stirred until dry. The complete ration was mixed in amounts which would

[2] Purina.

[3] Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[4] Salt mixture U.S.P. XIV, obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[5] Drisdol[R].

last about 7 days. Food containers were improvised from 8-ounce ointment jars, the caps of which had a central punched-out area, 32 mm in diameter, to allow access to food and to minimize spillage. Food was readily accessible, was replenished daily, and an excess of food was always present.

The animals were weighed weekly as the only means employed to evaluate growth. Examination for skin changes was made during the weekly weighing. The criteria employed for evaluating the severity of change were the same as those described previously (Panos and Finerty, '53). The tail and feet each were graded minimum to +++. Changes in the pelt were recorded only as loss of hair, since no dandruff was observed.

Oxygen consumption rates were measured in animals from each group at 2, 8, 10 and 19 weeks after the onset of the experiment. These measurements were made in order to follow the course of changes in oxygen consumption and for comparison with the previously reported increased consumption in fat-deficient females. The method employed was identical to that in the previous study. Following correction to standard barometric pressure and temperature, the final results are expressed as milliliters of O_2 consumed/100 gm body weight/hour.

After 20 weeks, the experiment was terminated. The animals were weighed and placed in an ether jar. After deep narcosis had been achieved, blood for fatty acid studies was obtained from the abdominal aorta. Skin specimens from the ventral thorax and dorsal interscapular area were taken, after removal of hair by fine clippers. The following organs were weighed on a torsion balance immediately upon removal: brain, pituitary, thyroid, thymus, adrenals, testes, ventral prostate, seminal vesicles, preputial glands, liver, heart and kidneys. The pituitary was fixed in Zenker-formol solution and prepared for histological study by the technique of Briseno-Castrejon and Finerty ('49). A portion of the liver and one adrenal were fixed in 10% formalin and stained with Sudan black B. The remaining tissues were fixed in Bouin's solution for routine

hematoxylin-eosin staining. The brain and most of the liver, heart and kidneys were set aside for tissue fatty acid studies. All tissues were sectioned at 6 μ except the hypophysis, which was sectioned at 4 μ.

Differential cell analyses of the anterior pituitary glands were made by counting all the acidophiles and basophiles in a centrally located horizontal section, under oil immersion and using 20 × oculars. Cellular percentages were calculated on counts of approximately 1,000 cells in each gland.

RESULTS

Effect on growth

Impairment of growth was apparent after three weeks and the maximum weight achieved by the fat-deficient animals was 67% that of the controls. A growth plateau was reached after 91 days at a weight of 288 gm. Five of the original 35 fat-free animals died during the course of the experiment, one after 6 weeks and 4 after 16 weeks; autopsies revealed pulmonary infection with multiple abscesses. Two of the 20 chow-fed animals died, one after 6 weeks and the other after 18 weeks; both showed evidence of extensive pneumonia.

Appearance of skin

Characteristic dermatitis began to appear after 9 weeks and was somewhat more extensive than in females. The tail showed involvement first, the hind feet three weeks later and the fore feet 5 weeks later. All of the animals on the fat-free diet developed dermatitis of the tail and feet as well as loss of hair, especially over the back. No dandruff was observed. The maximum severity of dermatitis was achieved after 16 weeks, in contrast to 24 weeks for female rats.

Oxygen consumption

Figure 1 portrays values obtained for oxygen consumption at various intervals during the experiment. Although the number of determinations is not great, it can be stated that there

is a distinct tendency for increased consumption rates in animals on a fat-free diet and it would appear that this difference is manifested as early as two weeks.

Macroscopic autopsy findings

The organ weights of the animals sacrificed after 20 weeks are shown in table 1. When computed on the basis of organ weight per 100 gm of body weight, the thyroid was significantly

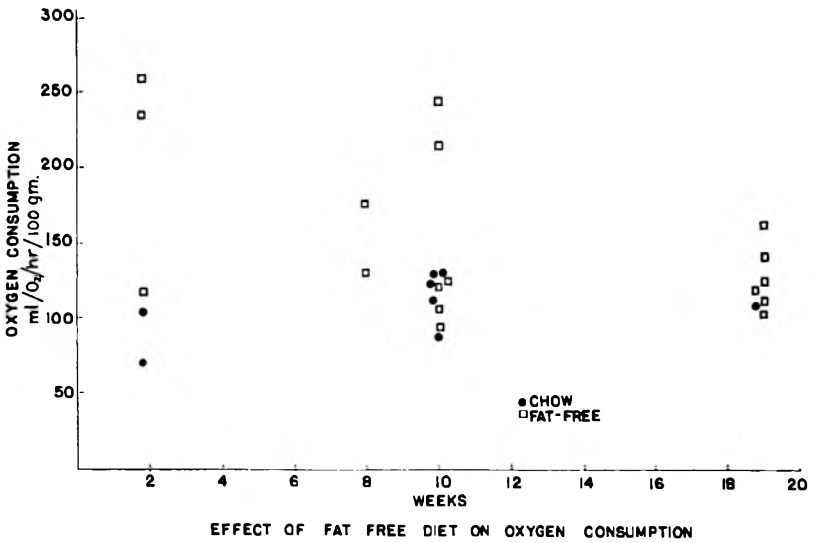

Fig. 1 Graphic representation of oxygen consumption of fat-free and chow-fed male rats.

smaller whereas the brain, liver, heart, kidneys and adrenals were larger in the fat-free group than in the controls. The relative enlargement of the brain, heart and kidneys was particularly pronounced. Except for these weight differences, there were no perceptible changes on gross inspection of the specimens.

Organ weights of animals sacrificed after only two weeks on the fat-free diet are presented in table 2. It may be noted that even after so short a period the brain, liver and heart were

TABLE 1

Effect of a fat-free diet on weight of various organs of the growing male rat

DIET	NO. OF RATS	BODY WT.	BRAIN WT.	PITUITARY WT.	THYROID WT.	THYMUS WT.	ADRENALS WT.	TESTES WT.	VENTRAL PROSTATE WT.	SEMINAL VESICLES WT.	PREPUTIAL GLANDS WT.	LIVER WT	HEART WT.	KIDNEY WT.
		gm	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
Control (20 weeks)	18	431 ± 8	1812 ± 23	11.2 ± 0.2	19.4 ± 0.5	292 ± 17	46.1 ± 2.8	3571 ± 63	557 ± 29	401 ± 15	130.0 ± 14.7	15558 ± 415	1201 ± 25	3004 ± 78
Fat-free (20 weeks)	30	287 ± 5	1794 ± 20	7.5 ± 0.2	11.3 ± 0.3	200 ± 8	38.8 ± 0.8	2534 ± 97	366 ± 18	307 ± 14	97.4 ± 6.5	11665 ± 384	1001 ± 19	2591 ± 70
		gm	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*
Control (20 weeks)	18	431 ± 8	424 ± 11	2.6 ± 0.0	4.5 ± 0.1	68 ± 4	10.7 ± 0.7	834 ± 18	130 ± 7	93 ± 3	29.3 ± 3.0	3642 ± 53	279 ± 3	699 ± 11
Fat-free (20 weeks)	30	287 ± 5	630 ± 13	2.5 ± 0.0	4.0 ± 0.1	70 ± 3	13.6 ± 0.4	882 ± 30	129 ± 6	106 ± 5	35.7 ± 2.3	4052 ± 98	351 ± 7	905 ± 19
t value		24	13	0	3.6	0.4	3.6	1.4	0.1	2.2	1.7	3.7	9	9.4
P		< 0.001	< 0.001	> 0.9	< 0.001	> 0.7	< 0.001	< 0.2	> 0.9	< 0.05	< 0.1	< 0.001	< 0.001	< 0.001

TABLE 2

Effect of a fat-free diet on weight of various organs of the growing male rat

DIET	NO. OF RATS	BODY WT.	BRAIN	THYROID	THYMUS	ADRENALS	TESTES	VENTRAL PROSTATE	LIVER	HEART	KIDNEYS
		gm	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
Control (2 weeks)	10	126 ± 7.3	1567 ± 21	7.6 ± 0.5	361 ± 32	22.4 ± 0.9	1347 ± 42	55.8 ± 4.7	6074 ± 772	437 ± 16	1090 ± 82
Fat-free (2 weeks)	10	106 ± 3.5	1521 ± 23	6.3 ± 0.2	290 ± 19	19.4 ± 0.7	1166 ± 83	49.8 ± 5.8	7423 ± 401	436 ± 13.8	996 ± 42
		gm	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*
Control (2 weeks)	10	126 ± 7.3	1258 ± 35	6.0 ± 0.3	283 ± 17	17.9 ± 1.3	1077 ± 25	44.4 ± 3.1	4838 ± 107	348 ± 6.2	868 ± 9.3
Fat-free (2 weeks)	10	106 ± 3.5	1443 ± 35	6.0 ± 0.2	273 ± 17	18.5 ± 9.9	1095 ± 67	46.3 ± 4.6	7036 ± 383	412 ± 6.7	940 ± 29
P		< 0.02	< 0.001						< 0.001	< 0.001	< 0.05
		gm	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
Control (5 weeks)	5	256 ± 10	....	17.2 ± 1.3	569 ± 63	32.2 ± 1.0	3118 ± 22	224 ± 11	11656 ± 720	829 ± 32	1985 ± 106
Fat-free (5 weeks)	10	209 ± 8	....	11.6 ± 0.7	416 ± 20	30.0 ± 1.3	2666 ± 76	175 ± 10	11595 ± 455	881 ± 41	1730 ± 86
		gm	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*	*mg/ 100 gm*
Control (5 weeks)	5	256 ± 10	....	6.8 ± 0.3	220 ± 18	12.6 ± 0.4	1224 ± 50	87.8 ± 5.1	4531 ± 127	325 ± 15	773 ± 24
Fat-free (5 weeks)	10	209 ± 8	....	5.6 ± 0.3	200 ± 10	14.4 ± 0.8	1313 ± 47	83.6 ± 4.0	5550 ± 128	422 ± 14	824 ± 17
P		< 0.01		< 0.01		< 0.05			< 0.001	< 0.001	< 0.1

relatively larger in the fat-free group. The body weight of the fat-free rats was essentially significantly decreased, while the increased relative weight of the kidneys likewise approximated significance. In the group studied after 5 weeks (table 2), the body weight difference had become highly significant, the relative enlargement of the adrenals and relative decrease of the thyroid became apparent and the liver and heart relationships were unchanged.

Microscopic studies

Microscopic examination of the tissues of the rats of the 20-week groups resulted in a number of significant findings, particularly in the case of the skin, testes, kidneys, pituitary and liver of the fat-deficient animals. There were no significant histologic alterations in the thyroid, adrenals, thymus, prostate or seminal vesicles. The brain was not sectioned.

Skin. Characteristic dermatitis developed in every fat-free animal. The changes found were essentially identical to those described in a previous report (Panos and Finerty, '53).

Kidneys. There were distinct accumulations of blood between tubules in approximately 50% of the fat-deficient animals. This finding is similar to, but less marked than that reported in females. In several specimens, there were relatively large hemorrhagic collections scattered through the cortex usually located near glomeruli. There were no consistent intratubular changes.

Heart. Although it was impossible to determine fiber and capillary size specifically by special injection techniques, careful study of sections from fat-free animals suggested that there was no increase in fiber size but a rather definite increase in capillary vascularity. This was apparent in approximately 50% of the specimens.

Testes (figs. 2 and 3, plate 1). Pronounced degenerative changes were found in the seminiferous tubules of the fat-deficient rats. The tubules were smaller and contained numerous multinucleated giant cells and large vacuoles. In most sections, no spermatids or spermatozoa were observed and

there was a considerable amount of fine cellular debris in the lumen. The number of secondary spermatocytes was markedly reduced and the nuclei of the primary spermatocytes were often pyknotic. The presence of occasional tripolar spindles gave an indication of aberrant mitosis. No consistent changes from normal were observed in the amount or cellular characteristics of the interstitial tissue.

Liver (figs. 4 and 5, plate 1). There was a moderate accumulation of fat in the livers of the fat-deficient animals. When stained with Sudan black B, this fat was found to be in the

TABLE 3

Effect of fat-free diet on cytologic structure of anterior hypophysis of male rats

TREATMENT	NO. OF RATS	PITUITARY CELL ANALYSIS		
		Acidophiles	Basophiles	Chromophobes
Chow-fed	6	39.6 ± 1.8 [1]	4.5 ± 0.8	55.9 ± 2.5
Fat-free	10	27.5 ± 2.1	5.0 ± 0.5	67.5 ± 2.3
t value		4.5	0.6	3.4
P		< 0.001	> 0.9	< 0.01

[1] Standard error.

form of droplets located primarily in cells around the central vein, but also in the region of the portal triad. There was no apparent change in the nuclei of the fat-laden cells.

Pituitary. Reference to table 3 will reveal a highly significant reduction in the number of acidophiles, which finding confirms that previously reported in fat-deficient females. There was, however, no increase in the number of basophiles.

DISCUSSION

In terms of severity of dermatitis, ratio of maximum body weight gain to that of controls and mortality, the manifestations of fat-deficiency were more pronounced in male rats than in females. This is well known and to be expected, since re-

quirements for essential fatty acids are approximately 5 times greater for male than for female rats (Greenberg, Calbert, Savage and Deuel, '50). Male mice have also been shown to be much more severely affected by fat-deficiency than females (White, Foy and Cerecedo, '43; Decker, Fillerup and Mead, '50).

The average weight at plateau (288 gm) was considerably higher than that reported by other workers (Burr and Burr, '29; Deuel et al., '50; and Sinclair, '52). However, the time required to reach plateau (13 weeks) and the ratio of maximum weight achieved by the fat-deficient animals to that of controls (67%) were essentially identical.

Hematuria was not observed in any of the fat-free animals, although microscopic evidence of pathologic changes was present in nearly all. Although there were frequent hemorrhagic collections of blood scattered throughout the cortex in the location of the interlobular arteries, the most consistent change was the accumulation of blood between the tubules. This finding was somewhat less marked in males than in females.

Oxygen consumption studies, though performed in relatively small numbers of animals, not only confirm the finding that consumption is increased in fat-deficiency, as reported in female rats, but suggest that increased oxygen consumption is perhaps the earliest manifestation of fat-deficiency. Loss of weight occurs as a result of an increased expenditure of energy, in spite of the fact that it has been repeatedly demonstrated that fat-free animals eat more food (as much as 40% more, Sinclair, '52) in relation to their body weight (Burr and Burr, '30; Meader, '37). That the increased expenditure of energy is not due to increased activity has been demonstrated by careful prolonged recordings of the activity of fat-deficient rats (Burr and Beber, '37). Greatly increased conversion of carbohydrate to fat, as indicated by respiratory quotients above unity following feeding, has been stated to be the primary factor involved (Krogh and Lindhard, '20; Wesson, '27; Wesson and Burr, '31; Burr and Beber, '37; Brown and coworkers, '38). An additional factor has been suggested by Sinclair ('52)

who determined that fat-deficient animals gained weight when immersed up to the neck in water at 39 to 40°C. for two hours while control rats lost weight and concluded that "water passes with abnormal readiness through the skin in both directions." The possibility that fat-deficiency is associated with either hyperthyroidism or increased tissue responsiveness to thyroid hormone has also received some attention.

Examination of tables 1 and 2 permits evaluation of progressive organ weight changes. The very early relative enlargement of the brain, liver and heart is a conspicuous and significant finding in that it indicates that important metabolic effects of feeding a fat-free diet are evident within a short period and long before the development of skin manifestations of fat deficiency. There is also an early and definite tendency for enlargement of the kidney and by the end of 20 weeks these 4 organs show highly significant relative enlargement. It is important to emphasize that these organs have been shown to be particularly involved in fatty acid metabolism. They normally contain large amounts of unsaturated fatty acids, retain these acids tenaciously during periods of fat-deprivation and show strikingly large re-deposition when fed good sources of unsaturated fatty acids (Rieckehoff, Holman and Burr, '49; Widmer and Holman, '50; Holman and Taylor, '50). As in the case of female fat-deficient rats, the organs which show the greatest fatty acid activity are those which undergo the greatest change in weight and presumably those which have the highest requirement for essential fatty acids.

The relative decrease in weight of the thyroid gland, apparent as early as the 5th week, is in agreement with the finding in female fat-deficient rats and would seem to establish this as a fundamental change.

The relative hypertrophy of the adrenals in fat-deficient males is in contradistinction to the finding in fat-deficient females of relative decrease in size. Such hypertrophy might be expected in view of the pronounced decrease in pituitary acidophiles with resultant presumptive reduced androgen secretion, since there is a known reciprocal relationship between

testicular androgen secretion and adrenal size. However, in these experiments, the Leydig cells appeared normal and there was no change in the weight or histology of the seminal vesicles and prostate.

The degenerative changes noted in the testicular tubules agree closely with those originally described in fat-deficient males by Evans, Lepkovsky and Murphy ('34). The latter authors did not study pituitary cytology and made no mention of regressive change in interstitial tissue, but did emphasize the loss of sex interest in their animals, suggesting that Leydig cell function was reduced. On the other hand, Greenberg and Ershoff ('51) reported that in male rats maintained on a fat-free diet for 21 weeks from the time of weaning, there was no histologic difference between the testes of fat-deficient and of control animals. When their results are expressed in terms of milligrams per 100 gm body weight, it is evident that there is no difference in the weights of the testes or prostate. The weight of the seminal vesicles, however, was markedly reduced and was restored following injections of chorionic gonadotrophin. Thus the findings previously described in this presentation are in agreement with the latter report as regards weight of testis and prostate and the absence of change in histology of interstitial tissue. However, it is difficult to reconcile the difference in observations regarding tubular degeneration, which was a striking finding in the present report and in that of Evans and co-workers ('34).

In recent years, much study has been devoted to the problem of infiltration of the liver with fat as a result of faulty nutrition. It is not surprising that, in an organ of such high metabolic activity, the variety of dietary conditions associated with fatty liver is very great (e.g., diets low in protein, high in fat, high in carbohydrate, deficient in specific amino acids). To this expanding list must be added the fat-free diet. It has been noted that, in both male and female rats, prolonged feeding of a fat-free diet resulted in consistent infiltration of minimum to moderate amounts of fat in the liver cells, especially in those situated around the central vein. The amount of fat was

greater in livers from male rats than those from females, which correlates with the higher requirement of the male for unsaturated fatty acid, but in no case was the amount of fat extremely high. The earliest microscopic study of livers of fat-deficient rats were reported by Rice and Jackson ('34) who described deposition of variable amounts of fat in both glandular and Kupffer cells in the form of fine and coarse granules, the latter located particularly in the periphery of the hepatic lobules. Decker, Fillerup and Mead ('50) found that the livers of mice fed a fat-free diet were fatty, although the description of the microscopic sections did not indicate any preferential localization.

SUMMARY

Thirty male Holtzman rats were fed, from the time of weaning and for a period of 20 weeks, a synthetic diet completely devoid of fat. Eighteen animals maintained under identical conditions were fed Purina Laboratory chow to serve as controls. Constant findings in the rats fed fat-free diets were impaired growth, the body weight attained being 66% that of controls; typical skin lesions on tail, feet and back, beginning after 9 weeks on the deficient diet; and increased oxygen consumption demonstrable as early as two weeks. Autopsy after 20 weeks on the deficient diet revealed the brain, adrenals, liver, heart and kidneys to be significantly heavier than in controls on a relative body weight basis, but the thyroid smaller. Microscopic study showed inter-tubular accumulations of blood in the kidneys, the characteristic thickening of the epidermis, as we have previously described in the female rat, and accumulation of fatty droplets around hepatic veins. There was increased capillary vascularity of the myocardium. In spite of insignificant relative weight changes in the testes, prostate or seminal vesicles, the seminiferous tubules of all deficient rats showed signs of degeneration consisting of varying degrees of inter-cellular and intra-cellular vacuolations and reduction in spermatids and mature spermatozoa. Other groups of male rats maintained on the fat-free diet for periods of only two and 5 weeks showed similar differences from controls in body

weight and weight of brain, liver, heart, kidney and thyroid, indicating that definite effects of fat deficiency are present long before the appearance of skin lesions.

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PLATE 1

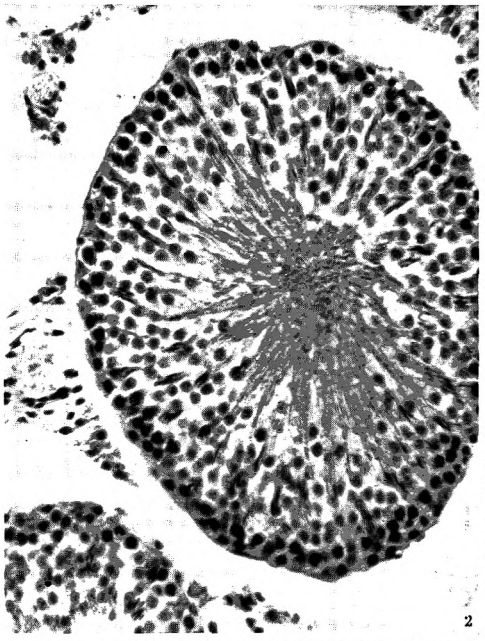
EXPLANATION OF FIGURES

2 Microscopic section of testis of a control rat (H and E; × 166).

3 Microscopic section of testis of a rat fed a fat-free diet from weaning for 20 weeks. Note "shrinkage" of seminiferous tubules, absence of spermatogenesis, multinucleated giant cells and vacuoles (H and E; × 166).

4 Microscopic section of liver of control male rat. Note equal distribution of finely dispersed fat droplets (Sudan black B; × 50).

5 Microscopic section of liver of male rat fed a fat-free diet from weaning for 20 weeks. Heavy deposits of fat may be seen concentrated about the central veins (Sudan black B; × 50).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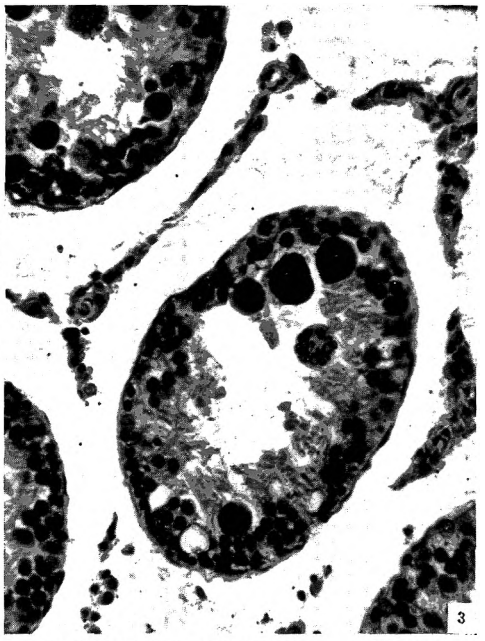
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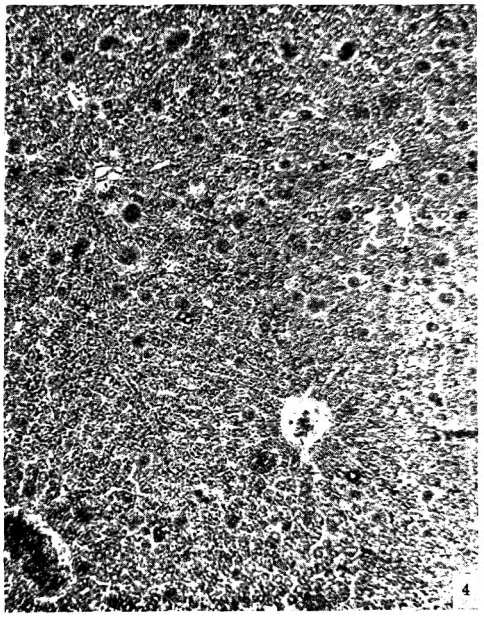
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5

UTILIZATION OF AMINO ACIDS FROM FOODS BY THE RAT[1]

III. METHIONINE

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TWO FIGURES

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In previous studies the quantitative utilization of lysine from foods by the rat, as judged by the weight gain of protein-depleted rats fed graded levels of lysine, was described (Schweigert and Guthneck, '53; Guthneck et al., '53). These studies have now been extended to include methionine, in which the utilization of methionine was measured with the young growing rat and the protein-depleted adult rat. Hydrogen peroxide-treated casein (oxidized casein), with appropriate supplements, was used as the source of amino acids in the methionine-deficient ration. Results are presented for the amounts of methionine utilized for weight gains and the amounts excreted in the feces when foods of animal and plant origin were fed.

EXPERIMENTAL

A basal ration deficient in methionine was designed to include casein treated with hydrogen peroxide in the presence of formic acid (Toennies, '42; Wilkening et al., '47) which destroys all but minute amounts of methionine. The oxidized

[1] We are indebted to the Dow Chemical Company for the DL-tryptophan and to Merck and Company for the vitamin B_{12} used in these studies.
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casein, after being tested microbiologically for methionine content, was incorporated into the rations as the main source of protein. Untreated casein was added to the rations at a 2% level to avoid using large quantities of the oxidized casein and to eliminate the necessity of adding large amounts of purified amino acids in the ration. Cystine, tryptophan and tyrosine, in a dry mix with sucrose, were added to the rations, as shown in table 1. The methionine supplements were added

TABLE 1

Composition of basal ration

INGREDIENT	%	INGREDIENT	MG/100 GM
Oxidized casein	12.0	Thiamine·HCl	0.6
Casein	2.0	Riboflavin	0.6
		Pyridoxine·HCl	0.6
Salts IV [1]	4.0	Ca pantothenate	4.0
Corn oil	4.7	Nicotinic acid	2.0
		Choline chloride	100.0
Fish liver oil (3,000 A, 400 D/gm	0.3	Inositol	100.0
		Pteroylglutamic acid	0.2
Amino acid mix [2]	2.81	Biotin	0.01
Sucrose	to 100	2-methyl-1,4-naphthoquinone	3.0
		p-aminobenzoic acid	30.0
		Vitamin B_{12}	0.002

[1] Hegsted et al. ('41).

[2] A dry mix with sucrose which provided 225 mg of tryptophan, 800 mg of tyrosine, and 400 mg of cystine per 100 gm of ration.

from a 20% mix of methionine with sucrose. The amounts of the other amino acids contributed by 14% casein (12% oxidized casein and 2% untreated casein) were shown by calculation to exceed the estimated requirements for the protein-depleted rat (Wissler et al., '48). All additions to the rations were made at the expense of sucrose.

The preparation and description of the test products have been presented previously (Guthneck et al., '53). Due to the limited quantities of certain of the test products available, it was not possible to include in the present tests all of those used previously. The methionine content was determined

microbiologically, and these data and the protein content are presented in table 2.

Weanling male rats (Holtzmann strain) were randomized into groups of 7 rats each. Food and water were provided ad libitum and the rate of gain for each animal and the food efficiency were determined over a 17-day period. A composite fecal collection for all groups was made from the 11th to the 14th days, and the methionine excreted in the feces was determined. The feces were collected each day from all animals in each group for the three-day period. The fecal matter was collected in 50 ml of 3N HCl, and was autoclaved at 15 pounds pressure for 16 hours in preparation for methionine determinations. In this way, the total methionine excreted for each group for the three-day period was determined.

The 2% untreated casein incorporated into all the rations contributed 57 mg of methionine per 100 gm of ration. Six groups of animals received supplements of 0, 60, 120, 180, 240, and 360 mg of L-methionine per 100 gm of ration. One group received a supplement of 120 mg of DL-methionine per 100 gm of ration to provide information on the activity of the D-isomer. The test products were added to the rations in amounts to approximate levels of 80 or 160 mg of methionine per 100 gm of ration.

The rates of gain for the animals receiving the graded methionine supplements are presented in figure 1. From these data and the rates of gain observed when the test products were fed, the percentage of methionine utilized from the test product was calculated. These results with the weanling rats and the percentage that was excreted in the feces are presented in table 2.

Subsequent to these experiments, a second experiment was conducted to test the reproducibility and specificity of the dietary response when young adult protein-depleted rats were used. All animals from the first experiment, except those receiving the basal ration or basal plus soybean flakes, were fed a stock diet [2] for 6 weeks. At this time they were fed a protein-

[2] Rockland rat pellets.

deficient diet (Schweigert and Guthneck, '53) for two weeks. After depletion, the rats (94 days of age) were randomized into groups of 7 rats each, and the rates of gain and food efficiencies were determined over a 14-day period. Fecal

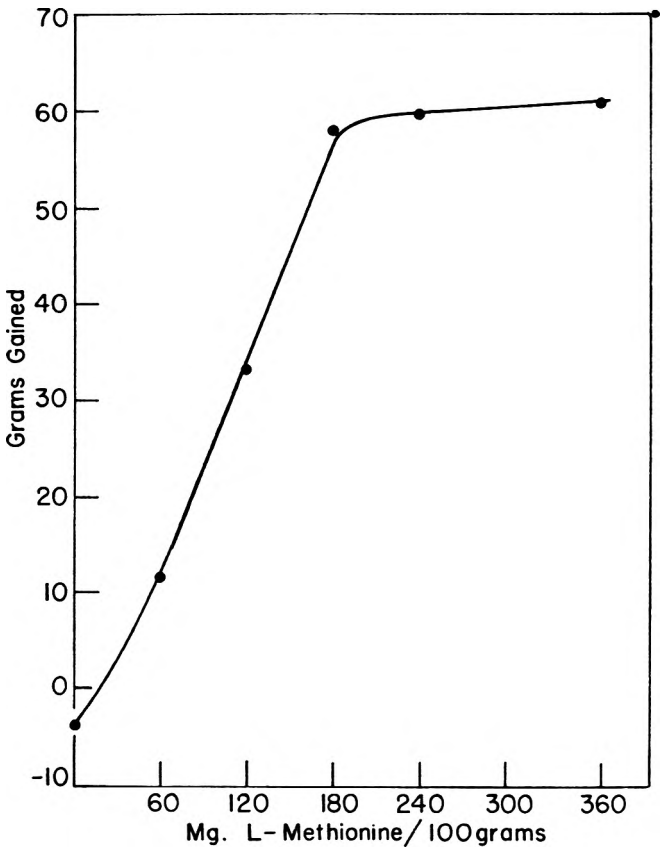

Fig. 1 Rate of gain of weanling rats fed graded levels of L-methionine for a 17-day period (7 rats per group).

collections were made from the 5th through the 8th day. Supplements of 0, 40, 80, 120, 160, and 360 mg of L-methionine per 100 gm of ration (table 1) were added for the 6 groups used in order to establish the standard curve. A supplement of 100 mg of DL-methionine per 100 gm of ration was fed to one group. The test products were added in amounts to ap-

TABLE 2

Per cent methionine utilized for gain and the amount excreted in the feces when various products were fed to weanling and protein-depleted rats

PRODUCTS [1]	PROTEIN CONTENT	METHIONINE IN DRIED SAMPLE	LEVEL OF METHIONINE PROVIDED	PRODUCTS FED TO: Weaning rats Methionine		PRODUCTS FED TO: Protein-depleted rats Methionine	
				Utilized	Excreted [2]	Utilized	Excreted [2]
	%	%	*mg/100 gm ration*	%	%	%	%
DL-Methionine			120	95 ± 2.4 [3]	2.4		
DL-Methionine			100			96 ± 4.9 [3]	3.1
Lactalbumin	73.8	1.79	76	54 ± 4.1	4.1	50 ± 3.9	4.5
Wheat germ	34.6	0.65	80	66 ± 4.5	10.4		
Skim milk	33.1	1.07	80	60 ± 4.1	5.8	59 ± 7.1	4.5
Casein			80	70 ± 2.4	3.5	80 ± 6.9	2.3
Casein	90.2	2.86	120			87 ± 2.8	3.2
Casein			160	92 ± 2.9	4.6		
Split peas	22.9	0.24	57	65 ± 4.7	17.1		
Rolled oats	16.8	0.32	80	70 ± 4.5	19.4		
Soybean oil meal	51.5	0.73	80	80 ± 3.6	10.4	61 ± 6.3	7.3
Soybean grits			80	78 ± 3.1	8.3	55 ± 5.9	10.0
Soybean grits	52.0	0.76	120			75 ± 3.0	6.6
Soybean grits			160	86 ± 5.4	8.6		
Soybean flakes (unheated)	48.9	0.75	80	48 ± 4.2	15.2	40 ± 6.6	9.2
Sesame meal	46.0	1.26	80	76 ± 5.8	8.8	59 ± 4.3	6.2
Pork and beef (canned)	66.3	1.60	80	74 ± 3.5	3.7		
Pork luncheon meat	55.7	1.55	80	66 ± 4.4	4.0	69 ± 9.2	5.8
Pork ham (fresh)	89.3	2.45	80	76 ± 4.1	3.5	73 ± 9.0	1.6
Pork ham (fresh)			160	89 ± 2.9	4.6		
Pork ham (cooked)	92.2	2.52	80	76 ± 2.5	4.7	65 ± 6.4	1.2
Pork ham (cooked)			160	83 ± 5.0	4.8		
Beef round	88.1	2.32	76	79 ± 2.2	2.2	68 ± 6.3	3.3
Beef round			109			89 ± 10.8	5.0
Beef rib	90.0	2.35	80	66 ± 3.3	1.5		
Beef rib (cooked)	90.1	2.42	80	59 ± 1.7	4.5	81 ± 6.5	1.5

[1] The meat samples were lyophilized and extracted with ether.

[2] The amount of methionine ingested was calculated from the food consumption data. After subtracting the amount of methionine excreted in the feces of the basal group from that excreted by the groups receiving the test products, the per cent of the ingested methionine that was excreted was calculated. (See text.)

[3] Mean and standard error.

proximate levels of 80 or 120 mg of methionine per 100 gm of ration. All the products used had been included in the tests conducted with the weanling rats.

The rate of gain for the 14-day test period for groups receiving the graded levels of methionine are presented in figure 2. The percentage of methionine utilized from the test products was calculated as described above and is presented in table 2. The data for the amount of methionine excreted in the feces by each group is also presented in table 2.

The results on all products for both experiments are summarized in table 3.

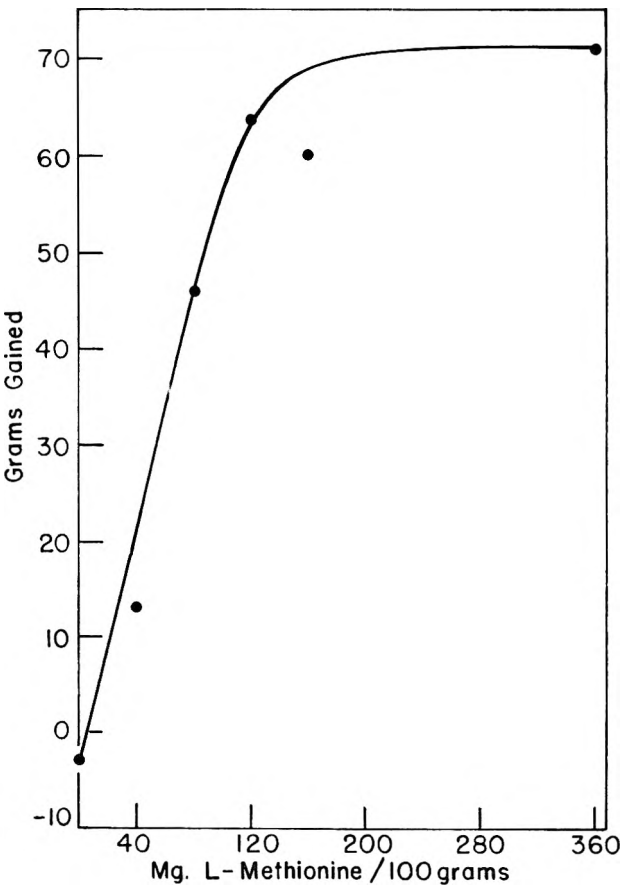

Fig. 2 Rate of gain of protein-depleted rats fed graded levels of L-methionine for a 14-day period (7 rats per group).

TABLE 3

Summary of methionine utilization from various foods by the rat

TEST PRODUCT	% UTILIZED FOR GAIN			% EXCRETED			% TOTAL ACCOUNTED FOR		
	Weanling	Adult	Av.	Weanling	Adult	Av.	Weanling	Adult	Av.
DL-Methionine	95	96	96	2.4	3.1	2.8	97	99	98
Casein	81	84	83	4.1	2.8	3.5	85	87	86
Lactalbumin	54	50	52	4.1	1.9	3.0	58	52	55
Skim milk (dried)	60	59	60	5.8	4.5	5.2	66	64	65
Wheat germ	66		66	10.4		10.4	76		76
Rolled oats	70		70	19.4		19.4	89		89
Split peas	65		65	17.1		17.1	82		82
Soybean oil meal	80	61	71	10.4	7.3	8.9	90	67	79
Soybean grits	82	65	74	8.5	8.3	8.4	91	73	82
Soybean flakes (unheated)	48	40	44	15.2	9.2	12.2	63	49	56
Sesame meal	76	59	68	8.8	6.2	7.5	85	65	75
Pork and beef (canned)	74		74	3.7		3.7	78		78
Pork luncheon meat	66	69	68	4.0	5.8	4.9	70	75	73
Pork ham (fresh)	83	73	78	4.6	1.6	3.1	88	75	82
Pork ham (cooked)	80	65	73	4.8	1.2	3.0	85	66	76
Beef round	79	79	79	2.2	5.0	3.6	81	84	83
Beef rib	66		66	1.5		1.5	68		68
Beef rib (cooked)	59	81	70	4.5	1.5	3.0	64	83	74

RESULTS AND DISCUSSION

The basal ration and levels of methionine supplementation chosen for these studies were satisfactory as judged by the rates of gain observed with methionine supplements (figs. 1 and 2) and by other criteria. On the basis of the rate of gain and food efficiency data, 217 mg of methionine per 100 gm of ration (a supplement of 160 mg plus 57 mg contributed by the basal ration) met the requirement for the weanling rat. It should be pointed out here that the basal ration was designed to be adequate in cystine, in methyl donors (choline, serine, glycine, etc.), and in B-vitamins involved in one carbon metabolism, and that the only obligatory role of dietary methionine was for tissue (protein) synthesis. The methionine requirement per unit weight of diet for the protein-depleted rat was somewhat less and approximated 177 mg of L-methionine per 100 gm of ration (120 mg supplement plus 57 mg contributed by the basal ration).

The amount of food supplements added was designed to provide intermediate levels of methionine (80 mg supplement of methionine). In some cases two levels of a food product were added to ascertain the effect of the level of methionine fed in the form of food products (and thereby the level of protein also) on the amount utilized for gain and excreted in the feces (table 2). On the basis of these tests, as in the case of lysine reported previously, increasing the protein (methionine) level did not decrease the percentage of methionine utilized within the limits investigated (table 2) and appeared to increase the utilization for the protein-depleted rat. It should be stressed here that the amounts of food products added were chosen to provide limiting levels of methionine in all cases, and that the amount of a specific food product used was designed to provide the same level of methionine in all cases (approximately 80 mg) so that the relative values at least for utilization and excretion would be valid.

In accord with earlier reports, the D-isomer of DL-methionine was completely utilized by both weanling rat and protein-depleted adult rat.

In comparing the results obtained with the weanling rat and the adult rats, for 8 of the 13 food products the percentage utilization of methionine did not differ by more than 10% (0 to 10%) and for 5 of the 13 the percentage utilization differed by more than 10% (15 to 22%). Four of the 5 which varied by more than 10% showed lower utilization for the tests conducted with the protein-depleted rat. The amounts of methionine excreted in the feces were the highest when the cereal and legume products were fed. Limitations to these measurements have been commented on in previous papers on lysine utilization. It is of interest to note that less variation among individual animals within each group was observed with the weanling rat than with the adult rat as indicated by the smaller standard errors of the mean for weanling rats (table 2).

The percentage of methionine utilized for weight gain varied for the different foods from 48 (unheated soybean flakes) to 83 (fresh pork) with the weanling rat and from 40 (unheated soybean flakes) to 81 (cooked beef rib) with the protein-depleted rat. The methionine utilization from casein was high in both tests (81 and 84%) and lower for lactalbumin (54 and 50%) and dried skim milk (60 and 59%). It is of interest that among the products tested the lowest utilization of lysine (49%) was also observed with the unheated soybean flakes. Lysine and methionine utilization was also low (60 and 52%, respectively) from the lactalbumin preparation.

The percentage of the methionine ingested that was accounted for (percentage utilized plus the percentage excreted in the feces) ranged from 56 (unheated soybean flakes) to 86 (casein) as shown in table 3 as the average for both experiments. Considerably less methionine than lysine was accounted for by these calculations for the same test products, and the results suggest that significant amounts of methionine

were metabolized and not utilized for tissue synthesis, or excreted in certain cases. This aspect would be particularly important to follow up with isotopically labeled methionine and the techniques used here. No consistent effects of the standard cooking procedures used or processing methods for meat on methionine utilization were noted.

Many other techniques for evaluating protein quality are based on rates of gain or nitrogen utilization (irrespective of the nature of the nitrogen utilized or excreted). In the present series of studies with lysine and methionine, we have attempted to refine such techniques in terms of specific amino acids on a quantitative basis by combining measurements of rates of gain and of the digestibility of the amino acid when the amino acid being studied is supplied at a limiting level of various foods. It would appear that further work with these techniques in which the utilization of a specific amino acid from foods is measured when the foods are added to rations containing protein preparations selectively deficient in the test amino acid would be very worthwhile.

SUMMARY

The percentage of methionine utilized by the weanling rat and protein-depleted rat from various protein preparations, cereal, legume and animal protein foods, supplementing a diet limited only in methionine, has been studied. The ingested methionine that was utilized differed by 10% or less for 8 of the 13 products when tested with the weanling rat as compared to the protein-depleted rat, and by 15 to 22% for the other 5 products tested by the two methods.

The percentage of methionine utilized from various foods ranged from 44 for unheated soybean flakes to 79 for beef round, determined as the average of the two methods. The amount of methionine excreted in the feces (digestibility) was also determined and the percentage of the ingested methionine that was excreted ranged from 1.5 for beef rib to 19.4 for rolled oats. The significance of these studies for measurement of protein (amino acid) quality of foods is discussed.

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THE PHYSICAL AND CHEMICAL CHARACTERIZATION OF RAT'S MILK [1]

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TWO FIGURES

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The physical and chemical properties of the milk of laboratory mammals have not been studied in enough detail to give records comparable to those available for human, cow or goat milk (see compilations of Macy et al., '50, and Kon and Mawson, '50). However the gross composition and some mineral and vitamin analyses are recorded for most laboratory mammals and data are particularly abundant for the dog. Most data reported for rat milk were obtained from pooled samples which allows no evaluation of individual variation. Cox and Mueller ('37) give some analyses at different stages of lactation starting after the first week.

A more complete characterization of rat milk is of importance to those concerned with the pre-experimental diet of the weanling rat, the nutrition of suckling rodents, or comparative physiology and nutrition. These analyses were performed to provide knowledge useful in compounding diets fed to Cesarean-born rats reared from birth through weaning without contact with the dam as described by Reyniers et al. ('46). The data obtained are recorded below with the hope that they will prove equally valuable to others.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy and the University of Notre Dame, NR131-067, and by grants from Eli Lilly and Co.

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METHODS

Lactating dams in the Lobund colony (predominately of the Wistar strain) were prepared for milking by taking them from the nest at 5 P.M. and keeping them in a small cage with water and commercial food [3] until the following morning. To collect colostrum the dam was separated from the litter for from one to three hours. The rat was then given 12.5 mg of Nembutal [4] subcutaneously, allowed to rest for 15 to 40 minutes, shaved with an electric shaver over the abdomen and chest, washed with soap and warm water, rinsed thoroughly with distilled water and dried. The milking operation was done by massaging the mammary tissue and stripping the teat in one motion with gentle pressure applied by the thumb and forefinger. Clean surgical gloves were worn. The droplets of expressed milk were collected in a pipette of suitable size (0.1 to 2.0 ml capacity). The milking time was about 30 minutes per rat irrespective of the volume obtained. Milking machines of the type described by Cox and Mueller ('37) could be used if the dam had large quantities of milk, but were not of value with a "hard milker" or to obtain colostrum, so they were not used. The rats were never milked twice since hand milking might change the character of subsequent milk production. When the dams recovered from the anesthesia they reared their litters with no apparent difficulty. Milking without anesthesia was possible with some rats but was generally considered to be too much trouble. The use of oxytocin did not materially aid the flow of milk in anesthetized rats.

The specific gravity was taken directly from the pipettes at milking. Osmotic pressure determinations were accomplished with the method of Hill and Baldes as modified by Lifson and Lorber ('45), using temperature measurements of a hanging droplet in a chamber having standard vapor density in terms of a standard NaCl solution. Relative viscosity was determined at 25°C. with a small Ostwald viscometer. Surface ten-

[3] Rockland rat checkers.

[4] Twenty-five hundredths milliliter of Nembutal sodium, Abbott Laboratories.

sion was determined with a bubble pressure tensiometer as described by Weissberger ('45). The pH was measured with both glass electrode [5] and paper.[6] A special container was made for the pH meter by connecting two wax wells about 1 mm larger than the electrodes. The pH of samples as small as 0.3 ml could be readily determined with this cup.

Dry weight was determined gravimetrically after drying under vacuum at 50°C. Gross fat content was estimated by the Rose-Gottlieb method after a 1:1 dilution. A micromethod was also used in which the fat was liberated with sulphuric acid in capillary tubes, extracted with toluene using an eccentric centrifuge according to Schmidt-Nilsen ('49), and determined gravimetrically after evaporation of the solvent using 0.002 to 0.02 gm samples weighed on the Kuhlmann microbalance. Standard AOAC methods were used for the Wijs' iodine number and the saponification number. Microdetermination of volatile fatty acids was made by chromatographic separation with subsequent titration according to the method of Nijkamp ('51).

The gross protein was calculated as $N \times 6.38$ using the Hengar method of Henwood and Garey ('36) for nitrogen. Casein was determined by Nesslerization after rennin precipitation. Other protein fractions were determined after fractional precipitation with methanol according to Pillemer and Hutchinson ('45). Amino acids were analyzed microbiologically by the methods of Henderson and Snell ('48) and its adaptation by Agren ('48).

Ash was determined gravimetrically after heating at 550°C. for 24 hours with the addition of HNO_3 as needed. Calcium was determined by the Fiske-Subbarrow method, phosphorus by the method of King ('32), and potassium by microbiological assay as suggested by Luckey et al. ('44). The flame photometer was used to determine sodium as well as to check the values obtained for potassium. Carbohydrate was determined by the method of Folin-Wu.

[5] Beckman Model G pH meter.

[6] Accutint Indicator Paper no. 110 from Anachemia Ltd., Montreal, Canada.

Carotene and vitamin E were determined with the micro-method of Quaife et al. ('49) and the Carr-Price reaction as described by Dan and Evelyn ('38) was used for estimation of vitamin A. Vitamin C was determined on the protein free filtrate with the method of Roe and Kuether ('43) modified by using a 16-hour reaction time at 37°C. Hydrolysis for the B-vitamins was done with clarase and papain following the method of Cheldelin et al. ('42). Pyridoxine determinations were made with *S. carlsbergensis* according to the method of Atkin et al. ('43). The other B vitamins were determined by modification of the method of Luckey et al. ('44) using *L. casei* for riboflavin, biotin, and folic acid and *L. arabinosis* for niacin and pantothenic acid.

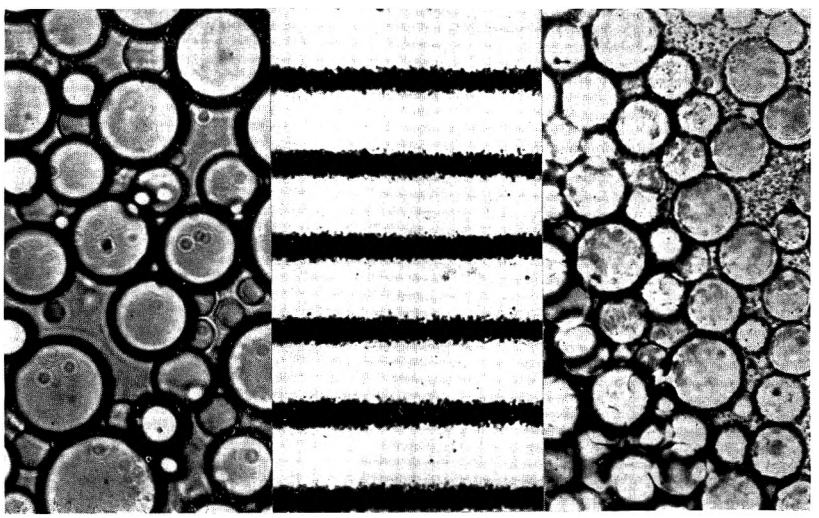
In the majority of cases, results from duplicate samples from one rat were averaged to obtain each value reported.

RESULTS

The gross appearance of rat colostrum and milk are very similar; each is an opaque, white fluid. Small volumes appear to be chalky white; larger volumes, usually obtained after 6 days, have a blue cast. Cream separates readily on standing and redisperses readily. The protein coagulates on standing, or when heated, into soft, small white curds. The milk has little, if any, odor, whereas diets containing cream from cow's milk smell highly of butyric acid when digesting in the stomach of the rat. The taste is quite salty.

The photomicrograph (fig. 1) illustrates the large amount of fat. The size of the fat globules in the expressed colostrum appears to be somewhat larger than was seen in the sample of colostrum taken from a rat's stomach. The average size of the fat globules in the rat colostrum is much greater than that found in later milk. Small, large and average size globules, estimated in microns, were respectively 2.0, 40.0 and 11.0 for rat's colostrum from stomach contents, 2.4, 74.0, and 15.0 for expressed rat's colostrum, and 1.0, 11.9, and 4.0 for expressed rat's milk.

The volume of milk produced per rat per day has been estimated by different methods, none of which are accurate. In our experience with hand milking small volumes of milk were obtained during the first few days (0.02 to 1.0 ml); later as much as 6 ml could be obtained in one milking. Some rats appeared to have relatively large quantities of milk at 21 to 23 days; others had very little. Apparently the latter had almost



(a) (b) (c)

Fig. 1 Photomicrograph of (a) rat's colostrum, (b) stage micrometer (10 μ rulings) and (c) rat's milk showing the relatively large fat globules of hand-expressed colostrum when compared with hand-expressed late milk.

weaned their young at three weeks. It is probable, as stated by Espe ('46), that even with the most complete milking little more than 80% of the total milk is obtained. Another method to estimate milk volume is to feed the dam in a separate cage for two to 4 hours, then weigh her before and after nursing. Despite decreased milk production from the handling and any material picked up in washing the young with her tongue, one rat suckling 5 young was found to produce 16.4 gm of milk at the 8th day of lactation. A more reliable method consists sim-

ply of weighing the litter at the same time each day. The daily weight increments are an index of the minimum quantity of milk produced by the dam until the young begin to nibble at solid material (about 12 days). The daily weight increments of the litters on different days of lactation were: 8.9 gm (first day), 11.7 gm (third day), 12.8 gm (8th day), and 14.5 gm (14th day). The data of Brody and Nisbet ('38) indicate that these values may be doubled to give the quantity of milk produced by the dam.

The physical analyses of rat's milk (table 1) indicate that both the specific gravity and the osmotic pressure of later milk

TABLE 1

Physical characteristics of rat's milk

DAY OF LACTATION	SPECIFIC GRAVITY [1]		OSMOTIC PRESSURE [2]		pH		VISCOSITY [3]		SURFACE TENSION [4]	
	No. of samples	Average and range	No. of samples	Average and range	No. of samples	Average and range	No. of samples	Average	No. of samples	Average
0			1	0.100	5	6.3 (6.1–6.4)				
1	1	0.9977								
4–6	1	0.9944	2	0.170 (0.165–0.175)	2	6.6 (6.6–6.6)	1	5.4	1	49.1
8–10	4	0.9915 (0.9523–1.0085)	2	0.150 (0.120–0.180)	3	6.5 (6.4–6.6)				
14–17	2	1.0422 (0.9930–1.0913)	2	0.170 (0.165–0.175)	5	6.5 (6.4–6.6)	1	7.3	1	50.0
21	6	1.0140 (0.9886–1.058)	2	0.160 (0.145–0.175)						
22	5	1.0950 (1.046–1.126)			15	7.0 (6.6–7.4)				
24	1	1.0891								

[1] Expressed as grams per milliliter.

[2] Expressed as molarity of NaCl.

[3] Centipoises.

[4] Dynes per centimeter.

is somewhat greater than that of early milk. This may well be a reflection of the high fat and low ash content of colostrum and transition milk. The osmotic pressure of rat's milk roughly parallels that of young rat's serum; at 0 days post-partum rat's milk and newborn rat's serum are comparable to 0.1100 and 0.113 molar NaCl respectively; at two weeks the values are

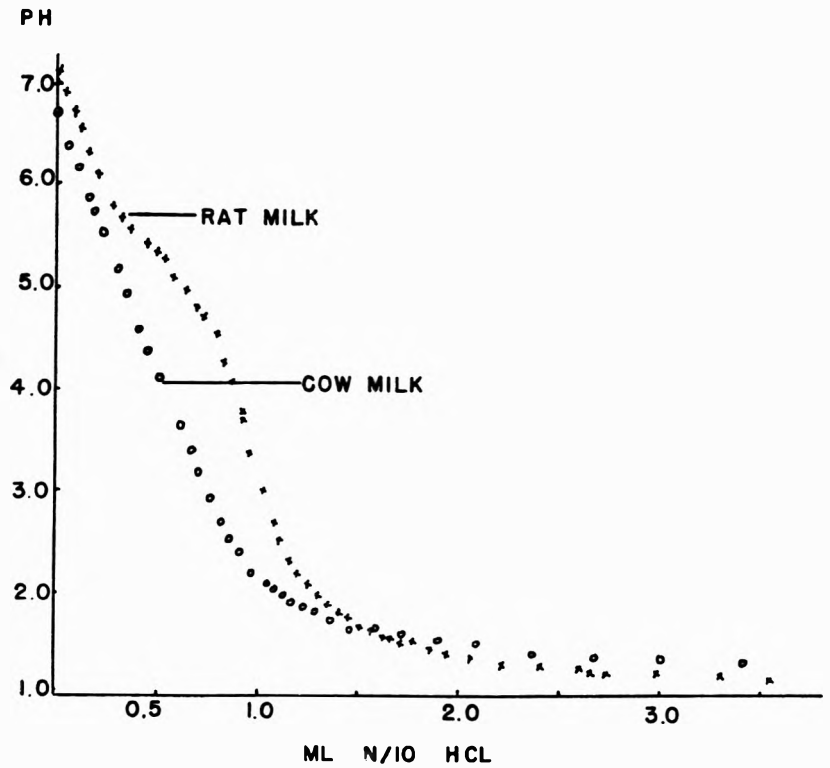


Fig. 2 Titration of 1 ml of rat's milk compared to that of cow's skim milk.

0.170 and 0.153 respectively. The average relative viscosity is 6.4 and surface tension is 49.6 dynes/cm for rat's milk. The pH gradually rises during the lactation from pH 6.4 of colostrum to 7.0 in rat's milk at 22 days. The buffer capacity of rat's milk at 22 days is indicated in figure 2. A comparison with that of cow's milk shows no great difference although rat's milk had slightly more buffer action between pH 4.5 and 5.6.

TABLE 2

Composition of rat's milk (% wet basis)[1]

DAY OF LACTATION	0–1		3–6		8–11		13–17		20–23	
	No. of samples	Average and range	No. of samples	Average and range	No. of samples	Average and range	No. of samples	Average and range	No. of samples	Average and range
Dry weight	2	32.4 (32.0–32.7)	1	26.3					2	27.6 (26.4–28.8)
Fat	11	22.2 (21.7–24.9)	3	7.49 (4.80–9.20)	10	8.42 (4.20–13.7)	8	9.19 (7.10–14.9)	8	10.5 (8.23–14.3)
Protein [2]	8	9.0 (8.2–9.7)	6	9.5 (8.7–10.8)	3	8.3 (8.1–8.4)	6	8.8 (7.9–10.4)	9	8.8 (8.1–9.9)
Carbohydrate			2	2.89 (2.70–3.08)			3	3.77 (3.56–4.00)	4	3.60 (3.31–3.82)
Ash	3	0.45 (0.40–0.56)	1	0.86	1	1.23			4	1.49 (1.35–1.56)
P	3	0.140 (0.125–0.171)	3	0.200 (0.160–0.220)			1	0.260	4	0.251 (0.195–0.293)
Ca	2	0.099 (0.075–0.102)	2	0.225 (0.210–0.240)			2	0.275 (0.250–0.300)	9	0.237 (0.151–0.293)
K	1	0.0925			2	0.0854 (0.0815–0.0893)			4	0.119 (0.107–0.146)
Casein [2]	1 [3]	7.25	1	9.20	1	6.50	2	7.35 (6.99–7.70)	2	7.53 (7.20–7.85)
Globulin [2]	1 [3]	1.61	1	1.12	1	0.72	1	1.08	2	0.87 (0.80–0.84)
Albumin + NPN [2]	1 [3]	0.57	1	0.67	1	0.62	2	0.52 (0.34–0.70)	2	0.55 (0.54–0.56)
NPN	1	0.092			1	0.081			1	0.075
Riboflavin	1	2.21			2	2.54 (2.46–2.61)			2	2.74 (2.52–2.96)
Niacin	1	20.0			2	18.4 (17.5–19.3)			2	16.6 (14.3–18.8)
Pantothenate	1	2.9			2	4.6 (4.5–4.7)			2	6.4 (5.0–7.8)
B_6					1	0.43			1	1.08
Biotin	1	0.092			2	0.079 (0.058–0.100)			1	0.085
Folic acid	1	0.30			2	0.28 (0.23–0.32)			2	0.37 (0.33–0.41)

[1] Vitamin values are expressed as μg/gm, wet basis.

[2] N × 6.38.

[3] Pooled sample.

Chemical analyses of rat's milk, summarized in table 2, show the remarkably high fat content (which is reflected in and verified by the dry weight values) of rat's colostrum. The fat content of the transition milk drops rapidly and later milk contains about 9% fat. The fat content of late milk was found to rise as the termination of lactation was approached. On one occasion the fat content of the 1st, second and third consecutive samples from a single teat was found to be 9.8, 7.0 and 4.6% respectively. The colostrum of the milk of mice does not appear to be similar to that of rats: mouse's colostrum contains 12.6% fat (7.4 to 17.6% range for 5 samples) while later mouse's milk contains 10.2% of fat (one sample). The iodine number of fat from a pooled sample of rat's milk is 43.54. The saponification number is 219.5 from one sample. These values agree with the data of Cox and Mueller ('37). No butyric or caproic acids were found in the volatile fatty acid fraction of the fat. Two samples of pooled late milk indicate the following quantities of volatile acids (expressed as per cent of the fat): caprylic 3.81 (3.50, 4.11); capric 6.34 (6.25, 6.42); and lauric, 4.12 (3.96, 4.28) (the values for lauric acid seem to include 2 to 15% higher acids).

The data for both fat and total protein show considerable individual variation between 6 and 18 days while duplicate samples give agreement within 2%. The protein content of rat's colostrum is not materially greater than that of later milk. Thus, on a dry weight basis, the protein is lower in colostrum than in milk. An electropherogram, according to the method of Cremer and Tiselius ('50), on the whey from rat milk shows two peaks corresponding to the fractionation findings. Analysis of the protein for its component parts using the Pillemer-Hutchinson fractionation indicates that the globulin fraction and the non-protein nitrogen compounds are somewhat higher in colostrum than in later milk. The amino acid content of the protein (dialyzed, acetone precipitated) of pooled rat's milk (14 to 20 days lactation) is given as percentage of dry protein: glycine, 1.65; valine, 5.02; leucine, 5.54; isoleucine, 4.67; proline, 7.84; tyrosine, 3.85; phenylalanine,

3.75; tryptophan, 4.57; aspartic acid, 5.78; glutamic acid, 20.20; arginine, 3.42; histidine, 2.50; lysine, 5.28; threonine, 4.49; cystine, 3.99; and methionine, 1.87. Concurrent analyses of casein from cow's milk give results which agree well with data from the literature.

The mineral content of rat's milk increases from 0.4% in colostrum to 1.5% in milk. On a dry weight basis this increase would be considerably greater (as the fat decreases). This is another marked difference from the pattern found with cow's or human's milk. Analyses for individual minerals show considerable variation in rat's milk even when milk of the same lactation day is analyzed. Data obtained with the flame photometer compare well with the results of microbiological methods for the determination of potassium. One pooled sample of rat's milk taken at about 20 days contained 0.14% sodium.

The carbohydrate in rat's colostrum and milk appears to be one of the most stable components when measured on the wet weight basis. Thus the ratio of carbohydrate to protein is constant while the ratio of carbohydrate to fat changes almost daily. Malyoth et al. ('53) have indicated that part of the carbohydrate in rat's milk is glucose and galactose.

We found no carotene in pooled rat milk; Houston and Kon ('37) report a similar finding. The vitamin A content of rat's milk appears to be low since no values could be obtained using the Carr-Price test with 1 ml of milk; although reports from Houston and Kon ('37) and Piccioni and Piccioni ('47) indicate that vitamin A is present. The vitamin E content of two samples of pooled milk was 2.7 (2.4 to 2.9) μg/gm.

Analyses for the B vitamins indicated that riboflavin, biotin, and folic acid are relatively constant throughout lactation while niacin tends to decrease in concentration, and the concentration of pantothenic acid increases as lactation proceeds. This represents a considerable increase in daily output by the rat when the volume of milk is correlated to the pantothenate content. The apparent ascorbic acid content as measured by osazone

formation is 1.6 (1.0 to 1.8) μg/gm. This value could not be confirmed by titration with dichlorophenolindophenol.

Rat's colostrum has no essential factor for newborn rats which is not present in rat's milk (or in formulae prepared from cow's milk). This was shown by repeated weaning of normal-born, non-suckled or Cesarean-born rats placed with a foster dam which had been lactating for 9 to 10 days. This view is supported by the fact that scores of rats have been reared at Lobund on synthetic-type formulae in the complete absence of the dam, after a Cesarean operation. However, rat's colostrum appears to contain more of the bifidus factor of György ('53) than does rat's milk.

DISCUSSION

While considering rat's milk from the viewpoint of the nutrition of the suckling rat, it is of interest to consider the gross constituents on the dry weight basis. On this basis rat's colostrum is quite different from later milk. The ratio of protein to fat is much different and the ash is lower. The rat thus begins life with a food extraordinarily high in calories. Such information proved to have immediate application in the formulation of milk formulae for the artificial feeding of Cesarean-born rats reared in the germ-free system. In the two years preceding the appreciation of these data over 70% of these rats died during the first 5 days of life. Mortality during this period was virtually eliminated when high fat formulae were used.

The analytical data from over 200 rats are summarized in table 3 and compared to data from other laboratories. The variations in the fat content at different stages of lactation make it difficult to compare data from pooled milkings with those from any one stage of lactation. The discrepancies in protein values are disconcerting. Cox and Mueller ('37) speak of "seasonal variation"; again Mueller and Cox ('37) indicate that the protein content of rat's milk may vary with dietary changes. The vacuum of milking machines may concentrate the milk to give high dry-weight data.

The vitamin B_{12} content of rat's stomach curd (11.6 to 22.4 mμg/gm) found by Daniel et al. ('53) is similar to the vitamin B_{12} content of rat's milk (11.0 to 95 mμg/ml) reported by Collins et al. ('51). The folic acid values reported by Collins et al. (2.0 to 3.5 mμg/ml) are much lower than the values reported herein.

TABLE 3

Summary of data from the literature

COMPONENT		LUCKEY ET AL.[1]	COX-MUELLER	MAYER [2]	SPRAY	HOUSTON AND KON	HATAI [2]
Dry wt.	%	27.6	31.7	22.2		27.9	45.9
CHO	%	3.67	2.83	3.39			2.42
Fat	%	9.30	14.79	12.4	14.2	13.8	31.6
Protein	%	8.72	11.0	6.89	10.0		10.1
Casein	%	7.25	8.60				
Globulin	%	0.89	0.53				
Albumin	%	0.052	0.85				
NPN	%	0.078	0.21	0.438			
Ash	%	1.44	1.50				1.61
Ca	%	0.268	0.349		0.384		
P	%	0.253	0.272		0.226		
K	%	0.108	0.170				
Na	%	0.140	0.076				
Fe	%		0.0007				
Mg	%		0.031		0.024		
Cu	%		0.0007		0.00072		
Cl	%		0.117				
Zn	%				0.0055		
Vit. E	mg %	0.27					
Vit. A	μg/gm					9.2	
Vit. C	mg %	1.2				0.35	
Thiamin	μg/gm					1.44	
Riboflavin	μg/gm	2.64				6.07	
Niacin	μg/gm	17.5					
Pantothenate	μg/gm	5.5					
Pyridoxine	μg/gm	0.76					
Biotin	μg/gm	0.082					
Folic	μg/gm	0.33					
pH		6.8	6.7	6.6			
Sp. G.		1.037	1.047				

[1] Exclusive of the first 6 days lactation.

[2] Stomach contents.

SUMMARY

The physical and chemical characterization of rat's milk on a wet weight basis is presented. The gross appearance of rat's colostrum and milk is very similar. Rat's colostrum contains 22% fat while rat's milk appears to vary considerably with 9.3% as an average value. The quality of this fat differs from that of cow's milk in having no butyric or caproic acids. The 8.7% protein in rat's milk shows no characteristic differences from that of other milks. Rat's colostrum and milk contain the same amount of protein. The amino acid components are quantitatively estimated. The 1.4% ash in the milk is about three times greater than is found in colostrum. The carbohydrate content is the most consistent of the major constituents (3.7% on wet basis). No carotene or vitamin A was found. Other vitamins analyzed are present in quantities similar to those of cow's milk.

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FURTHER GROSS OBSERVATIONS ON THE B_{12}-DEFICIENT CHICK EMBRYO

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TWO FIGURES

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Congenital anomalies in the chick embryo, obtained from hens fed a diet low in vitamin B_{12}, were described by Olcese et al. ('50). The characteristic gross symptoms reported by these workers consisted of myoatrophy of the leg muscle, slender legs that were frequently hemorrhagic, and the malposition of the embryo described as the "head between the thighs." Perosis, general hemorrhages of the embryo and hemorrhages in the allantois were also observed. The peak of embryonic mortality was found to be around the 17th day of the incubation period. The administration of "animal protein factor" concentrates as a source of vitamin B_{12} decreased the incidence of the anomalies to zero. Further studies on the effect of vitamin B_{12} on embryonic development have been carried out over a period of 18 months, involving 422 embryos. This paper presents additional gross observations of the B_{12}-deficient embryo.

EXPERIMENTAL

Single Comb White Leghorn hens were placed in individual laying cages with raised screen floors and fed a diet low in vitamin B_{12}. The hens were artificially inseminated with mixed semen from New Hampshire cockerels. Food and water were supplied ad libitum. The composition of the diet was

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as follows: 68% sucrose, 24% soybean protein (Drackett 220), 3% soybean oil and 5% salts IV. The following amounts of vitamins were added in milligrams per kilogram: thiamine hydrochloride 4, riboflavin 6, calcium pantothenate 15, niacin 100, pyridoxine hydrochloride 4, alpha-tocopherol 6, folic acid 2, menadione 0.5, biotin 0.2, choline chloride 2000, para-aminobenzoic acid 20, and inositol 1000. Procaine penicillin was added in the amount of 33 mg per kg and methionine and glycine were added at the levels of 7.5 gm and 4.0 gm per kg respectively. Vitamin A was included at 10,000 U.S.P. Units per kg and vitamin D_3 at 2250 I.C.U. per kg.

After the hens had been on the diet for a period of 4 weeks, to allow time for B_{12} depletion, the eggs were collected daily and marked with the hen number and date. Eggs thus obtained were set and the embryos removed at the end of 17 days incubation. New hens were used to replace those that died or were sacrificed during the experimental period. Some eggs from the hens fed the diet low in vitamin B_{12} were injected with water (0.1 ml) and others with vitamin B_{12} (2 μg or 10 μg in 0.1 ml), using the method described by Olcese and Couch ('50). After the depletion period, some of the hens maintained on the low B_{12} diet were injected parenterally with vitamin B_{12} at varying levels (10 μg to 100 μg B_{12} in 1.0 ml). The vitamin B_{12} content of the egg yolk was determined at intervals by the method of Skeggs et al. ('50). It should be pointed out that values obtained by this method are not absolute and are interpreted to be a measure of *Lactobacillus leichmannii* activity. Embryos removed from the eggs on the 17th day of incubation were examined for gross external characteristics, and the body cavity was opened to observe gross internal changes. Partial or complete dissection of the embryos was made.

OBSERVATIONS AND DISCUSSION

A record of observations made on embryos removed from the eggs on the 17th day of incubation is shown in table 1. A total of 291 embryos from hens fed the low B_{12} diet were removed from eggs on the 17th day. Some of the eggs had

been injected with water while others had not. Of this number, 23% were classed as B_{12}-deficient embryos according to the previously described external characteristics (Olcese et al., '50). In addition to these characteristics, edema occurred in varying degress in almost all of the deficient embryos. Poor feathering was present in the typical B_{12}-deficient embryo,

TABLE 1

Incidence of abnormal heart and liver of embryos removed from eggs on the 17th day of incubation

SOURCE OF EGGS	NUMBER OF EMBRYOS [1]	ABNORMAL HEART OR LIVER [2] OR BOTH	ABNORMAL HEART [3]	ABNORMAL LIVER [4]
		%	%	%
Hens on B_{12}-deficient diet (Egg + H_2O or no injection)	291	55	46	41
Hens on B_{12}-deficient diet Eggs injected with B_{12} or, Hens injected with B_{12}	131	1.5	0	1.5

[1] Include embryos from 15th and 16th day.

[2] Fatty liver with hemorrhages; pale, dilated, irregular-shaped heart with hemorrhagic areas.

[3] Heart as described in 2.

[4] Liver as described in 2.

and profuse bleeding often occurred when feathers were removed from their follicles. The percentage of B_{12}-deficient embryos includes some where the time of death was estimated at 15 or 16 days. In contrast to the above, 131 embryos receiving vitamin B_{12}, either by injection into the egg or into the hen, did not exhibit the gross symptoms of the B_{12}-deficient embryo previously described.

Gross examination of the organs within the body cavity revealed a fatty liver with hemorrhagic areas and a pale, en-

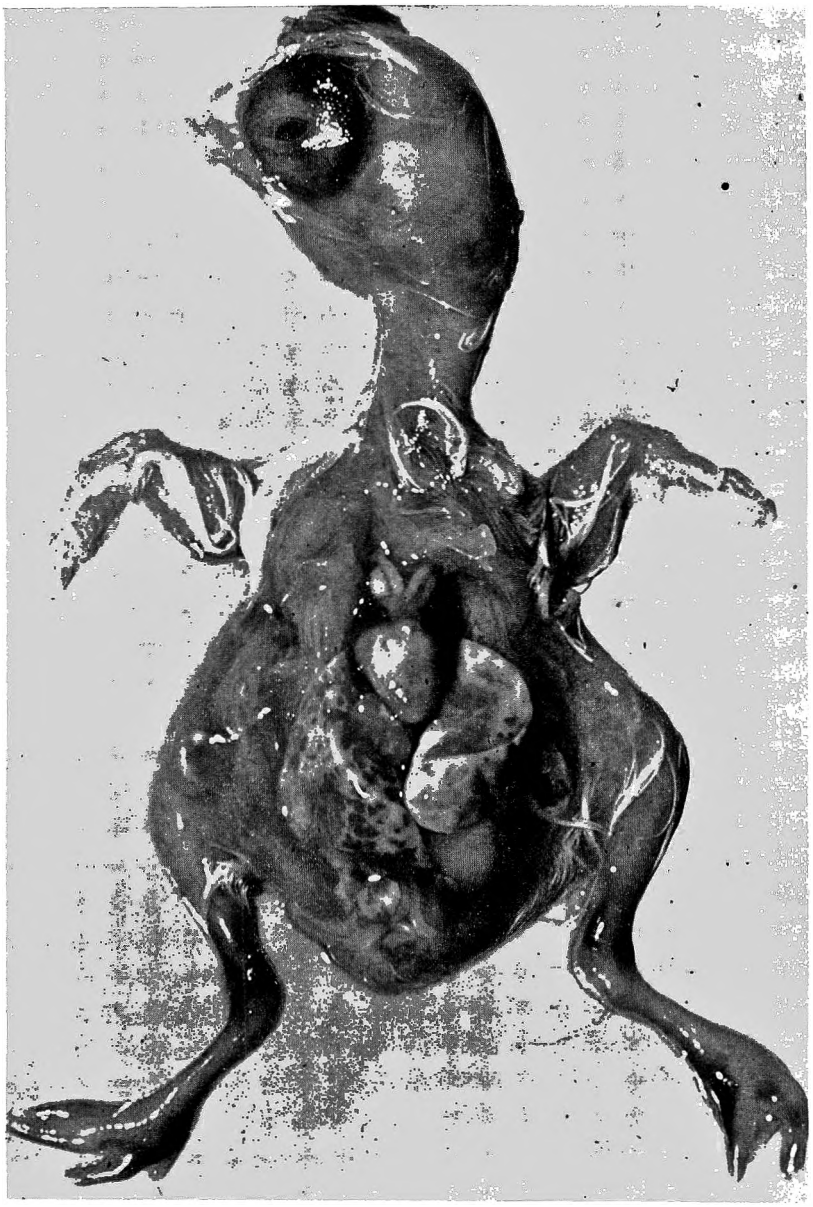

Fig. 1 Seventeen-day embryo from a hen maintained on a low-B_{12} diet. Embryo was extremely red due to excessive hemorrhaging. Note light areas of liver indicating excessive fat, and dark areas indicating hemorrhagic spots. Abnormalities of the heart are not too discernible from this photo; however, the light areas indicate fat and the dark areas are the hemorrhagic portions of the ventricles.

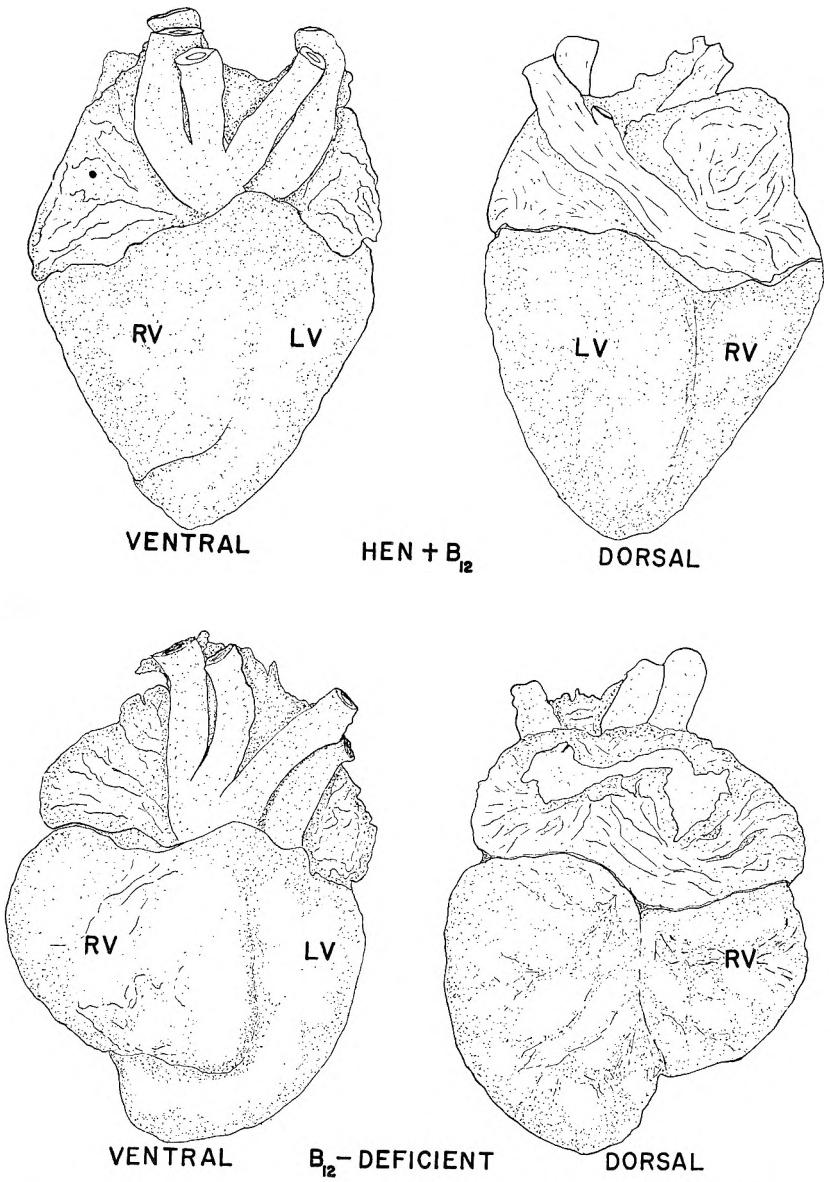

Fig. 2 Camera lucida drawings, dorsal and ventral views, of the heart from 17-day chick embryos, from a hen maintained on a diet low in vitamin B_{12} and from a similar one injected with vitamin B_{12}. Note the dilated ventricles and irregular shape of the deficient heart.

larged, irregular-shaped heart in most of the B_{12}-deficient embryos (figs. 1 and 2). Some of the embryos from B_{12}-deficient hens that were apparently normal externally showed the above listed characteristics internally. The fatty liver varied from a small cream-colored area on the tips of the borders of the liver lobes to an extremely fatty liver with creamy areas interspersed by hemorrhagic areas.

Figure 1 shows a 17-day B_{12}-deficient embryo that exhibits an extreme condition of a fatty and hemorrhagic liver. The light areas are liver fat, and the dark areas are from hemorrhages. This embryo was almost entirely red in color due to a general hemorrhaged condition and the light spots are due to the reflection of light from the edematous tissues. The heart is pale in this embryo with some hemorrhagic areas, but does not show the extreme deviations in color or shape which often occur with the fatty liver.

The heart was often enlarged and irregular in shape, and the auricles usually engorged with blood. Camera lucida drawings (fig. 2) show the irregular shape of the heart in the deficient embryo and the effectiveness of injections of B_{12} into the hen maintained on the low B_{12} diet in preventing this embryonic condition. The drawing was not made from the embryo in figure 1. The ventricles were dilated, pale, almost white in color in many instances, and hemorrhages were frequently observed (fig. 1). Histological sections showed an extremely large deposit of fat in the cardiac muscle. Of the 291 embryos examined, 41% were found to have a fatty liver as determined by gross examination, and 46% showed the pale, dilated, irregular-shaped heart. A total of 55% of these embryos showed the liver or the heart or both with the above mentioned disorders. Results of histological studies of the liver and the heart will be published later.

In 131 embryos examined from hens fed the low-B_{12} diet, but obtained from eggs or hens that had been injected with vitamin B_{12}, only two embryos had a slight amount of fat on the border of the liver, and none of the hearts were judged abnormal.

The kidneys of the B_{12}-deficient embryos were often pale, almost yellow in some instances, and apparently very hemorrhagic in others. Fat has been observed in the kidney by histological techniques, and further experiments are in progress. In embryos observed thus far, the administration of vitamin B_{12} either to the deficient hen or by injection into the deficient egg relieved the fatty condition. A detailed histological report on the kidneys will be made at a later date.

The digestive tract of the 17-day B_{12}-deficient embryo often appeared to be exceedingly thin and in many instances the ileum was enlarged and filled with a greenish watery fluid. Hemorrhages were observed in the crop, gizzard, duodenum, ileum and peritoneal region. The yolk mass was large and hemorrhages in the yolk sac were frequent. The pale yellow yolk varied from a thin, watery to a dark green viscous condition. The amount of remaining albumen was larger in the deficient embryos than in embryos which received a source of vitamin B_{12}. An enlarged gall bladder was not uncommon.

An enlarged thyroid gland was often found in B_{12}-deficient embryos. This prompted a gross and histological investigation into earlier and later stages of development of the thyroid gland which will be described elsewhere. Grossly, the thyroid is usually larger than normal in the deficient embryo on the 17th day, and the size increases considerably from the 17th day to the end of the incubation period.

Measurements were made of the two largest diameters of the left thyroid gland of 17-day embryos. The thyroids of the deficient embryos were usually more rounded than in those provided a source of vitamin B_{12}. In 41 embryos from hens on the low-B_{12} diet, 49% of the thyroids had both diameters 2.0 mm or larger. In 45 embryos receiving vitamin B_{12} by way of parenteral injections to the hen or by injections of B_{12} into the deficient egg, only 30% were found with both diameters 2.0 mm or greater.

The origin, action and insertion of the following muscles of the B_{12}-deficient embryo were checked, according to de-

scriptions in Kaupp ('18) and Potter ('41): biceps, deltoid, pectoralis major, pectoralis secundus, pectoralis tertius, thigh muscles and the tibial group muscles. These muscles were found to be present in the embryos examined, and were often very small and seemed to be tendinous. Hemorrhages were observed in the thigh, shank, synsacral and coccygeal muscles. The muscles of the synsacral region were very thin with the origins apparently low on the synsacrum. Hemorrhages were found in the tibiotarsal-tarsometarsal region of the bones, and these bones would bend easily. Most of the deficient embryos had thin legs and toes. Some appeared short and chubby, probably due to the edematous condition of the embryo. Curled toes were found occasionally.

The brain was exposed in an occasional embryo, and a rare condition found was the absence of one or both eyes. These may or may not be due to the B_{12} deficiency, but may be genetic in origin as reported in a review by Landauer ('51). Subdural hemorrhages were apparent in a few embryos.

In some instances, embryos exhibiting the B_{12}-deficient characteristics from the hens on the low-B_{12} diet were obtained after the hens had been on the diet for only 4 weeks, while at other times approximately two months elapsed before the deficient embryos appeared. In 4 weeks time the vitamin B_{12} content of the egg yolk was found to be less than 2 mμg/gm in eggs from some hens, while in others a longer time was required to reach this level. This was interpreted as a factor of the amount of vitamin B_{12} stored in the body. Five hens on the low-B_{12} diet that had an average B_{12} egg yolk assay of 0.78 mμg/gm were injected parenterally with 100 μg of vitamin B_{12} each day for 5 days. The B_{12} content rose to an average of 243 mμg/gm within one week. This is in agreement with the report of Halick et al. ('53). Following this, 4 weekly injections of 200, 100, 100, and 100 μg respectively were given these birds. Eggs from two of these hens had an average of 7.0 mμg/gm of vitamin B_{12} three months later. The B_{12} content of eggs from hens that remained

on the low B_{12} diet during the experimental period fluctuated from low values of approximately 0.2 mμg/gm to 4.0 mμg/gm. This variation may be due to the hen obtaining vitamin B_{12} from unknown sources, or it may have been related to egg production of the hen. The degree of abnormal characteristics exhibited in embryos from the hens on the low-B_{12} diet may be due to varying degrees of storage of the vitamin in the hen.

SUMMARY

The characteristics of the B_{12}-deficient embryo as described by Olcese et al. ('50) have been confirmed. In addition, embryos produced from hens fed a diet low in vitamin B_{12} were found to have an enlarged thyroid, hemorrhages in the yolk sac, a thin-walled digestive tract, and to be edematous. The muscles examined were small and tendinous. Of 291 embryos examined on the 17th day of incubation, 23% exhibited the typical B_{12}-deficiency symptoms as described by Olcese et al. ('50). A fatty liver or a pale, dilated, irregular-shaped heart or both occurred in 55% of the cases and fatty kidneys were also observed. Parenteral injections of vitamin B_{12} into hens maintained on the low-B_{12} diet, or injections of vitamin B_{12} into the eggs from these hens prior to incubation prevented the anomalies described.

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NUTRITIVE VALUE OF THE DIETS OF IOWA SCHOOL CHILDREN[1, 2]

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ONE FIGURE

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Little is known about the progressive changes in nutrient needs of school children as they grow older. The limited information from studies of diets, nutritional status, and metabolism has been pooled to form a basis for the Recommened Allowances of the National Research Council, proposed for children of specific age-sex groupings. In contrast with the lack of information concerning changes in nutrient needs during childhood, many data are available to show the yearly changes in physical growth. This information has been obtained largely from cross-sectional studies of samples of various types. It seemed possible, therefore, that cross-sectional studies of well-chosen samples of healthy children in times of prosperity and ample food supply might similarly indicate their changing nutrient needs from year to year, especially when combined with physical, biochemical, and clinical observations.

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[2] Supported in part by a grant and other assistance from General Mills, Inc., as a part of its nutrition education program.

This paper reports the average daily nutrient intakes of a sample of Iowa school children, classified by sex and by age in years, consecutively from 6 to 18. Subsequent papers will report physical and biochemical data concurrently obtained. This study has been conducted in cooperation with the Agricultural Research Administration of the United States Department of Agriculture. A part of this study was conducted cooperatively with the Ohio and Kansas Agricultural Experiment Stations.

THE SAMPLE

In selecting the public schools to be sampled, three sub-populations of Iowa were considered: (1) rural communities with schools having grades 1–12; (2) towns of less than 50,000 with separate elementary and high schools; (3) cities of 50,000 or more. In the last group elementary schools only were sampled. The one-room rural schools of the state were not included since they are being reorganized. Within these sub-populations, 61 schools were selected at random to represent both those participating and those not participating in the Federal type A school-lunch program. Within each school, children were sub-sampled by age, sex, and lunch participation. A total of 1,188 children were studied. Further details of the sampling and a table showing the sample have been presented elsewhere (Patton et al., '53; Sidwell and Eppright, '53). Analysis of the dietary data obtained for the 9-, 10-, and 11-year-old children of the state revealed only a few differences in mean daily nutrient intake among the schools of the strata studied in Iowa. Therefore, the data from all the schools were pooled in determining the statistical means of nutrient intake for boys and girls at each age from 6 to 18.

METHODS USED

All of the dietary data were collected under the supervision of one dietitian using essentially uniform methods. The dietitian visited the homes of the elementary children except those

in the rural communities, explained to the mother the purpose of the study and the record form, and obtained some information about the family, the home and the child. Seven-day dietary records with the amounts of foods indicated in common household measures or servings were obtained. The information was collected throughout the school years of 1948–52. Mothers kept the records for the children in grades 1–4; older children cooperated with their mothers in keeping their records, or kept the records themselves. When possible a second visit was made to the home. The record was discussed with the children at school once during the week, when body measurements were made and samples of blood taken for determination of concentrations of hemoglobin, serum alkaline phosphatase, ascorbic acid, carotenoids, and vitamin A. Facilities were available for making the last 4 analyses only for the children in groups 2 and 3. Methods have been discussed in other papers (Patton et al., '53; Eppright et al., '52).

Food items listed on the dietary records were coded and punched on I.B.M. cards. Nutritive values of all items listed were calculated mainly from the values given in Handbook 8, Composition of Foods, Raw, Processed, and Prepared (Watt and Merrill, '50). These figures were punched on cards, and the mean nutritive values of the daily diets, with subtotals of individual meals and snacks, were computed by machine.

Means, standard deviations, and standard error of the mean for food energy and 10 nutrients are given in tables 1 and 2. Some of the data are presented graphically (see fig. 1). As a basis for comparison the graphs also show the Recommended Allowances of the National Research Council (1953 revision, unpublished). Figures for each year not given in the table of Allowances were obtained by interpolation. Because of the unequal distribution of calcium, vitamin A and ascorbic acid in foods, skewed distributions of the mean daily intakes of these nutrients may be expected. Therefore data for these nutrients were considered in percentiles as well as means and standard deviations. The 25th

TABLE 1

Nutritive values of the daily diets of Iowa school children — girls [1]

AGE		GIRLS	FOOD ENERGY VALUE	PROTEIN	FAT	CARBOHYDRATE	CALCIUM	IRON	VIT. A VALUE	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
yrs.		*no.*	*Cal.*	*gm*	*gm*	*gm*	*mg*	*mg*	*I.U.*	*mg*	*mg*	*mg*	*mg*
6	Mean	50	1961	60	94	226	918	9	6175	1.0	1.7	10	66
	S.D.[2]		314	11	18	38	261	1.4	3779	0.17	0.45	1.8	29
	St.Er.[3]		44	2	3	5	37	0.2	534	0.02	0.06	0.3	4
7	Mean	48	1987	61	99	220	877	9	5691	1.0	1.7	11	77
	S.D.		355	12	20	46	269	1.7	3607	0.21	0.48	2.1	35
	St.Er.		51	2	3	7	39	0.2	521	0.03	0.07	0.3	5
8	Mean	43	2025	63	96	240	1009	9	6208	1.0	1.8	10	76
	S.D.		345	12	18	49	246	1.8	4232	0.17	0.48	2.2	32
	St.Er.		53	2	3	8	38	0.3	646	0.03	0.07	0.3	5
9	Mean	62	2282	70	111	267	956	11	6977	1.1	1.8	13	82
	S.D.		322	10	17	44	246	1.9	3851	0.19	0.46	2.2	29
	St.Er.		41	1	2	6	31	0.2	489	0.02	0.06	0.3	4
10	Mean	62	2280	68	108	278	936	11	8010	1.1	1.8	12	86
	S.D.		359	14	20	52	298	2.0	6408	0.21	0.58	2.4	39
	St.Er.		46	2	2	7	37	0.2	814	0.03	0.07	0.3	5
11	Mean	58	2262	69	109	264	1004	10	6458	1.1	1.9	12	80

	St.Er.		55	2	3	7	34	0.3	544	0.09	0.06	0.3	4
13	Mean	44	2471	74	116	296	994	11	6773	1.2	1.8	13	76
	S.D.		576	20	28	74	416	3.0	3645	0.31	0.59	3.6	33
	St.Er.		87	3	4	11	63	0.4	550	0.05	0.09	0.5	5
14	Mean	37	2487	75	120	291	987	12	7219	1.2	1.9	13	82
	S.D.		501	17	26	62	325	2.3	4324	0.28	0.68	2.7	29
	St.Er.		82	3	4	10	53	0.4	710	0.04	0.11	0.4	4
15	Mean	39	2594	75	126	303	899	12	6943	1.2	1.8	14	91
	S.D.		395	14	21	53	302	2.4	4707	0.28	0.47	3.2	43
	St.Er.		63	2	3	9	48	0.4	754	0.04	0.07	0.5	3
16	Mean	36	2312	69	112	271	811	11	5771	1.2	1.6	12	90
	S.D.		437	14	22	62	296	2.6	3620	0.29	0.47	2.7	42
	St.Er.		73	2	4	10	49	0.4	603	0.05	0.07	0.4	4
17	Mean	26	2374	73	114	276	838	11	7140	1.1	1.7	13	87
	S.D.		633	22	36	66	316	3.1	3913	0.31	0.56	3.9	36
	St.Er.		124	4	7	13	62	0.6	767	0.06	0.11	0.8	7
18	Mean	13	2420	73	118	283	809	11	6596	1.2	1.6	13	92
and	S.D.		469	14	24	58	262	2.4	4211	0.28	0.39	3.7	34
older	St.Er.		130	4	7	16	73	0.7	1168	0.08	0.11	11.0	9

[1] Fignres are group means of the average daily intakes obtained from 7-day dietary records.
[2] Standard deviations.
[3] Standard error of the mean.

TABLE 2

Nutritive values of the daily diets of Iowa school children — boys [1]

AGE		BOYS	FOOD ENERGY VALUE	PRO-TEIN	FAT	CARBO-HYDRATE	CAL-CIUM	IRON	VIT. A VALUE	THIA-MINE	RIBO-FLAVIN	NIACIN	ASCORBIC ACID
yrs.		*no.*	*Cal.*	*gm*	*gm*	*gm*	*mg*	*mg*	*I.U.*	*mg*	*mg*	*mg*	*mg*
6	Mean	37	2201	67	104	260	1061	9.6	5671	1.1	1.9	11	81
	S.D.[2]		334	12	19	54	303	1.5	3078	0.19	0.48	2.0	30
	St.Er.[3]		57	2	3	9	50	0.2	506	0.03	0.08	0.3	5
7	Mean	56	2166	65	104	253	1026	9.6	5835	1.0	1.8	11	72
	S.D.		327	11	19	42	266	1.7	2455	0.20	0.44	2.5	29
	St.Er.		44	2	2	6	36	0.2	328	0.03	0.06	0.3	4
8	Mean	54	2270	71	110	260	1124	10.2	6865	1.1	2.0	12	78
	S.D.		367	12	21	48	293	2.0	4430	0.21	0.47	2.3	32
	St.Er.		50	2	3	6	40	0.3	603	0.03	0.06	0.3	4
9	Mean	53	2433	74	117	289	1093	11.3	8427	1.1	2.0	12	84
	S.D.		469	17	27	53	307	2.8	5489	0.25	0.63	3.0	30
	St.Er.		64	2	4	7	42	0.4	753	0.03	0.08	0.4	4
10	Mean	60	2417	74	117	283	1041	11.2	7541	1.1	2.0	13	77
	S.D.		398	13	24	50	286	2.0	4262	0.21	0.52	3.1	29
	St.Er.		51	2	3	6	37	0.2	550	0.03	0.07	0.4	4
11	Mean	50	2615	79	125	310	1128	11.6	7637	1.2	2.1	13	85

	St.Er.		76	2	4	10	42	0.4	651	0.04	0.08	0.4	4
13	Mean	45	2877	86	138	336	1139	13.6	8303	1.4	2.2	15	97
	S.D.		595	19	31	77	442	3.3	6217	0.30	0.75	3.6	51
	St.Er.		89	3	5	12	66	0.5	927	0.04	0.11	0.5	8
14	Mean	39	3088	91	150	361	1113	14.2	9037	1.5	2.2	16	91
	S.D.		543	17	30	76	354	2.7	5665	0.30	0.58	3.5	41
	St.Er.		87	3	5	12	57	0.4	907	0.05	0.09	0.6	7
15	Mean	32	3252	93	159	378	1176	15.4	9800	1.5	2.5	17	97
	S.D.		623	17	32	84	366	4.5	7210	0.36	0.80	4.7	48
	St.Er.		110	3	6	15	65	0.8	1274	0.06	0.14	0.8	8
16	Mean	31	3421	99	167	396	1314	15.6	9180	1.6	2.5	16.8	111
	S.D.		624	21	39	68	370	3.4	4456	0.34	0.60	3.8	39
	St.Er.		112	4	7	12	66	0.6	800	0.06	0.11	0.7	7
17	Mean	21	3399	105	171	381	1441	16.0	8796	1.6	2.7	17	102
	S.D.		681	17	34	100	498	3.4	4300	0.34	0.69	2.8	41
	St.Er.		149	4	8	22	109	0.7	938	0.07	0.13	0.6	9
18	Mean	17	3439	102	175	377	1181	15.3	8862	1.7	2.5	18	86
and	S.D.		468	22	27	66	344	2.8	5206	0.44	0.81	4.1	46
older	St.Er.		114	5	6	16	84	0.7	1268	0.11	0.20	1.0	11

[1] Figures are group means of the average daily intakes obtained from 7-day dietary records.
[2] Standard deviations.
[3] Standard error of the mean.

and 50th percentiles for these three nutrients are given in table 3. The high variability in the vitamin A value of diets noted in other dietary studies (Young et al., '52) was evident

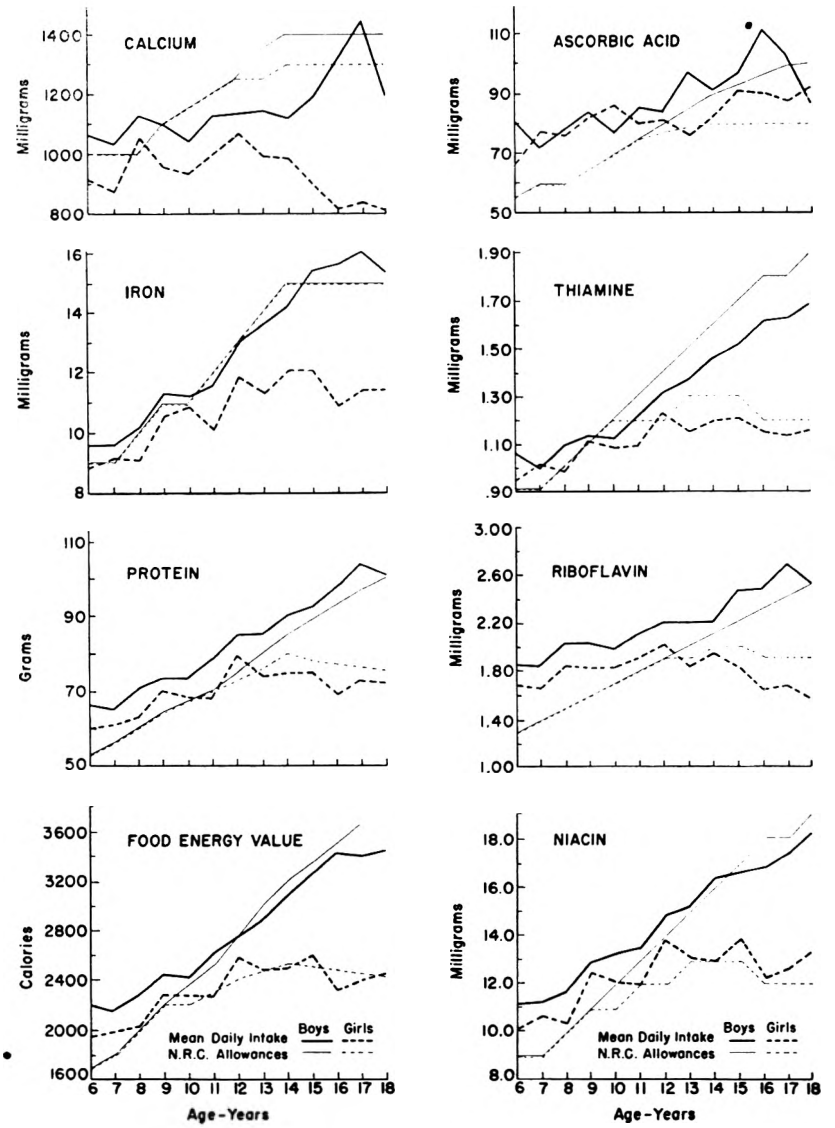

Fig. 1 Mean daily nutritive value of the diets of Iowa school children.

TABLE 3

Calcium and ascorbic acid content and the vitamin A value of the mean daily diets of Iowa school children arranged in 25th and 50th percentiles

AGE IN YEARS	CALCIUM				ASCORBIC ACID				VITAMIN A VALUE			
	Girls		Boys		Girls		Boys		Girls		Boys	
	Percentiles				Percentiles				Percentiles			
	25th	50th	25th	50th	25th	50th	25th	50th	25th	50th	25th	50th
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*International Units × 100*			
6	716	922	810	1051	41	63	58	72	39	51	41	50
7	640	913	821	1047	57	71	51	71	34	47	38	54
8	882	1001	959	1122	48	74	55	76	36	49	36	56
9	758	943	918	1082	59	80	63	79	41	57	49	63
10	759	977	868	1026	56	77	56	72	38	54	40	61
11	788	974	948	1157	60	74	58	85	41	52	41	65
12	884	1070	841	1138	57	75	53	77	47	67	44	60
13	698	950	816	1040	46	73	60	85	39	55	42	69
14	731	949	857	1076	66	82	63	82	46	61	45	77
15	650	872	1015	1126	53	93	65	91	34	55	53	76
16	601	833	1029	1258	50	92	86	104	31	49	57	85
17	718	798	1028	1362	56	80	73	86	39	60	59	68
18 +	610	742	925	1187	62	88	53	75	39	50	50	75

in this investigation. The data, however, seemed to indicate certain trends and are therefore included.

The data herein presented represent the calculated nutrients in the mean daily diets. No allowance for cooking losses has been made beyond those included in certain food items in the tables of food composition. More than 8,000 daily records have been studied. The number of daily records obtained for boys at each age ranged from 119 for boys of 18 and above to 637 for boys of 12; correspondingly for girls, the number of daily records ranged from 91 to 588. For most age-sex groups at least 280 daily records were collected. The analysis was not expected to reveal the actual nutrient intakes of individuals. However, because of the uniformity of methods used throughout the study, the adequacy of the sampling, and the reproducibility of the results as shown by the standard errors, the means are believed to reveal trends of nutrient intake of a population of children who were well enough to be attending school regularly.

The reliability of the data in terms of relative standard error was highest for mean food-energy values and for the protein content of the diets, lowest for the mean vitamin A value, and next lowest for ascorbic acid. It was higher for the younger children than for those in the teens. For the children of 12 years and younger the standard errors of the means for calories were approximately 50 at each year. Throughout the age-range the standard errors of protein means were between 2 and 5 gm.

NUTRITIVE VALUE OF THE DIETS OF THE GIRLS

Between the ages of 6 and 12, the daily food-energy value of the diet increased from approximately 2,000 to 2,600 Cal. From 12 through 15, the mean values were between 2,500 and 2,600 Cal., whereas in the later teens the values were slightly lower, 2,300 to 2,400 Cal. The mean daily food-energy value of the diets of the girls conformed at most ages to the N.R.C. Recommended Allowances within about 100 Cal. The

largest negative deviation from the Allowances was noted at age 16.

The mean daily protein values varied from 60 gm at 6 years to 30 at 12, with values of approximately 70 gm at 9, 10, and 11. At each year beyond 12, mean protein intakes tended to remain at about 75 gm daily. These mean values conformed to the Allowances within about 5 gm, though they tended to be slightly higher before 12 and lower after 12. The peak of protein intake for girls came at 12 rather than between 13 and 15 as indicated by the Allowances. Negative deviation of mean daily protein intake from the Allowances was most marked at 16.

The mean daily calcium intake of the girls varied from 900 to 1,000 mg daily between the ages of 6 and 12. After 12 it declined until, for girls of 16 and above, the diets provided approximately 800 mg daily. At all ages except 8 the mean daily values were less than the Allowances; beyond 12 the means were only about two-thirds of the Allowances. The analysis of the calcium intakes by percentiles showed the 50th percentile and the means to be practically the same at all ages (see table 3). Of the girls of 12 years or younger 25% had mean daily intakes of less than 800 mg, whereas of the older girls 25% had mean daily intakes less than 600 to 700 mg. The minimum 7-day average intakes of calcium were about 400 mg at most ages.

The mean daily intake of iron by girls varied from 9 mg at 6, 7, and 8 years to 12 mg at 12 through 15 years, but after 15 years it declined to an average of 11 mg daily. The iron intake of the girls under 12 approximated the Allowances usually within about 1 mg, but for girls over 12 it was far less. For girls 14 and older the mean daily intake of this nutrient was only about three-fourths of the amount given in the Allowances.

The mean daily values of thiamine and niacin followed trends similar to those of the mean food-energy, protein, and iron values. The riboflavin values of the diets of girls increased from 1.7 mg for the 6- and 7-year-olds to 2.0 for the

12-year-olds. After that age the values decreased to 1.6 or 1.7 mg for the three highest-age groups. As with protein, the trend was toward values higher than the Allowances for groups from 6 to 12 years but lower for groups above 12.

The mean vitamin A values of the diets of the Iowa girls of the sample varied from approximately 6,000 to 8,000 I.U., and were about 1,000 to 2,000 I.U. higher than the 50th percentiles. At 15 and 16 years 25% of the girls had diets with only about 3,000 I.U. of vitamin A. Maximum values fluctuated widely, whereas minimum values varied over a narrow range around 1,000 I.U., which perhaps represents the amount of vitamin A in diets containing only small amounts of whole milk and few green leafy vegetables or yellow vegetables and fruits.

The mean daily ascorbic acid intakes of girls increased irregularly from approximately 70 to 90 mg through the school years, and were usually larger than the Allowances and somewhat larger than the 50th percentiles. Approximately one-fourth of the girls had diets which on the basis of the weekly record averaged 60 mg of ascorbic acid or less. The minimum values ranged around 30. This figure probably represents the ascorbic acid potentiality of a diet consisting mainly of meat, gravy, cereal foods, and potatoes with few other vegetables and fruits. In view of the small allowance for cooking loss the ascorbic acid consumption of a large number of girls must be less than recommended. Contrary to the situation with most nutrients, girls increased their intake of ascorbic acid during the teen ages.

Ages at which the diets of girls appeared to have considerably lower intakes of several nutrients than in the preceding years were 13 and 16. At 16 the diets of the girls tended to deviate most in the negative direction from the Allowances. Further study should be made of the actual intake and needs of girls in consecutive years, especially during the teen-age. Activity records and metabolic studies are needed to show whether or not the total energy needs of teen-age girls actu-

ally decrease. The long-time effects of temporary periods of low-nutrient intakes in girlhood should be investigated.

NUTRITIVE VALUES OF THE DIETS OF THE BOYS

There was little difference between the nutritive values of the diets of boys between the ages of 6 and 7. From 7 to 16 years, there was a fairly continuous increase in the calculated amounts of food energy, protein, iron, thiamine, niacin, riboflavin afforded by the mean daily diet. Intakes of these substances approximated or exceeded the Allowances at most ages.

The mean daily calcium content of the diets of boys followed a distinctly different trend. From 6 through 14, the consumption of this nutrient varied between 1,000 and 1,100 mg daily; at 16 and 17 it was considerably higher, but at 18 it decreased somewhat. At 6, 7, 8, 9 and 17 years the mean daily intake equalled or exceeded the Allowances, but at other ages it was less. The 50th percentiles coincided with the means at most ages (see tables 2 and 3).

In round numbers the mean vitamin A values of the diets of boys varied from 6,000 I.U. at 6 and 7 years to 9,000 from 14 through 18. The means were greater than the 50th percentiles by 1,000 to 2,000 I.U. At each age 25% of the boys had mean daily intakes of less than 4,000 to 5,000 I.U.

The mean daily ascorbic acid intakes of boys increased somewhat irregularly from 81 to 72 mg at 6 and 7, respectively, to 111 mg at 16 years. Means were slightly larger than the 50th percentiles at all ages. Twenty-five per cent of the boys from 6 through 15 had mean daily ascorbic acid intakes of 65 mg or less. After adjustments for cooking losses are made, these intakes might be somewhat low. Although the mean daily intakes both of vitamin A and of ascorbic acid compared favorably with the Allowances, about 25% of the boys had diets relatively low on these nutrients.

COMPARISON OF DIETS OF GIRLS AND BOYS

The diets of the girls and boys differed considerably at all ages. Between 6 and 12 the variation in means for girls roughly paralleled that for boys in food-energy value and all nutrients but vitamin A and ascorbic acid. Intakes of girls, however, were approximately 10% less than those of boys throughout these years. Beyond 12 the differences widened; boys tended to increase their intakes until 16 or 17, whereas the girls either maintained or decreased their nutrient intakes as observed at 12, except for ascorbic acid.

Yearly increments of 5 to 8% occurred somewhat regularly in the mean daily food-energy value of the diets of the boys from 6 through 16 years. In contrast, for girls the increments occurred in spurts, of which the two largest, 12.7 and 13.5%, were noted from 8 to 9 and from 11 to 12 respectively. For boys below 16 years, the period of least change in nutrient intake was between 9 and 10. A similar plateau was noted for girls of this age, but it extended to 11 years.

At all ages boys obtained more protein than girls, both per person and per kilogram of body weight. The mean daily intake of protein per kilogram of body weight varied from 2.9 to 2.0 gm between the ages of 6 and 12 years, and from 2.0 to 1.5 from 12 to 18 years. Corresponding figures for the two age groups of girls were 2.7 to 1.7, and 1.7 to 1.5.

The differences noted between the nutrient intakes of these Iowa school boys and girls suggest that the needs of two sexes may not be identical at any of the ages from 6 to 18. The steady regular increments noted for boys indicate that recommendations for them might best be presented at yearly intervals from 6 or 7 until 16 or 17. The intakes of girls appear to fall in slightly different groupings from those used in the Allowances; namely, 6 to 8, 9 to 11, and 12 to 15 years rather than 7 to 9, 10 to 12, and 13 to 15 years.

INTERRELATIONSHIPS AMONG THE NUTRIENTS

For boys and girls of each age group approximately 12, 42, and 46% of the calories in the average daily intake were

obtained from protein, fat, and carbohydrate respectively. This distribution is similar to that reported by Phipard ('52) in the average American food supply.

Trends in mean food-energy value of the diet and mean intakes of protein, iron, thiamine, niacin, and riboflavin were strikingly similar. It seems possible that the intakes of several of the nutrients for groups of children with food

TABLE 4

Ratios of mean daily intakes of thiamine, riboflavin and niacin to mean daily intakes of protein

AGE IN YEARS	THIAMINE-PROTEIN RATIO		RIBOFLAVIN-PROTEIN RATIO		NIACIN-PROTEIN RATIO	
	Girls	Boys	Girls	Boys	Girls	Boys
	$\times 10^{-5}$		$\times 10^{-5}$		$\times 10^{-4}$	
6	1.7	1.6	2.8	2.8	1.7	1.6
7	1.6	1.6	2.7	2.7	1.8	1.7
8	1.6	1.6	2.8	2.8	1.6	1.7
9	1.6	1.5	2.6	2.7	1.9	1.6
10	1.6	1.5	2.6	2.7	1.7	1.8
11	1.6	1.5	2.7	2.7	1.7	1.6
12	1.5	1.5	2.5	2.5	1.7	1.6
13	1.6	1.6	2.4	2.5	1.8	1.7
14	1.6	1.6	2.5	2.4	1.7	1.8
15	1.6	1.6	2.4	2.6	1.9	1.8
16	1.7	1.6	2.3	2.5	1.7	1.7
17	1.5	1.5	2.3	2.5	1.8	1.6
18	1.6	1.7	2.2	2.4	1.8	1.7

habits similar to those of the Iowa children might be estimated from the calorie or protein intakes. The figures given in table 4 represent the fractional relationships of the mean daily intakes of thiamine, riboflavin, and niacin to the mean daily intake of protein.

If the mean daily protein intake of a group of Iowa school children is known the mean daily thiamine content of the diets may be estimated by using the factor 1.6×10^{-5}; the riboflavin, by using 2.7×10^{-5} for children below 12 and 2.4 or 2.5×10^{-5} respectively for girls and boys above 12; the

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VARIABILITY IN THE MEASURE OF TOTAL ASCORBIC ACID UTILIZATION BY THE HUMAN [1,2,3]

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ONE FIGURE

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The utilization of ascorbic acid by the human has been repeatedly measured using analyses of blood plasma levels and urinary excretion of the vitamin as criteria. Variability in these measures is encountered when subjects are on identical intakes. One factor which accounts for a part of the variation is indicated by the work of Dodds et al. ('50) who demonstrated a carry-over effect of known prior intake for as long as 12 days. Foods low in total ascorbic acid have been used in this laboratory as the source of a test dose in human utilization studies (Fisher, '53). It was necessary to divide these large amounts of the low vitamin test foods between two meals in order to feed the required amount of the vitamin. This spacing of the ascorbic acid intake may introduce yet another source of variation in the blood plasma and urine values. The variability, whatever the cause, poses the question of the sensitivity of these measures, blood plasma and excretion of ascorbic acid as a means to differentiate small changes in ascorbic acid intake.

[1] Paper 1871, Journal Series, Pennsylvania Agricultural Experiment Station.

[2] Research Contribution no. 123, College of Home Economics.

[3] Portions of these data were obtained from research supported in part by the United States Department of Agriculture through a contract sponsored by the Human Nutrition Branch of the Agricultural Research Service.

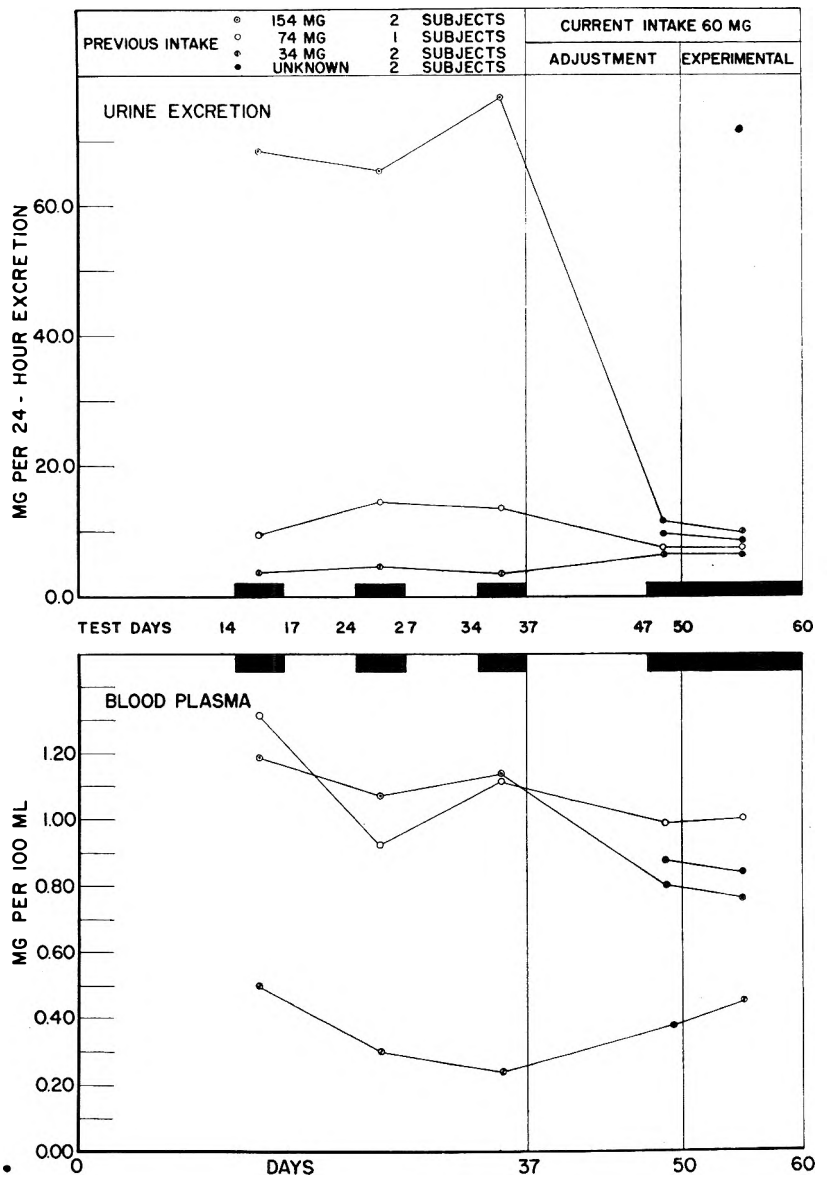

Fig. 1 Carry-over effect of previous intake of ascorbic acid on subsequent data.

second might affect kidney threshold and alter urinary excretion of the vitamin. The 37-day study was divided into a 17-day adjustment period and two 10-day test periods. Each subject served as her own control and received the same amount of supplement for 10 days as a single intake and for 10 days as a divided intake. The divided supplement was given in equal amounts at the noon and evening meals and the single supplement was given at the noon meal. Blood plasma and urine analyses for total ascorbic acid were done on the last 4 or 5 days of each 10-day test period.

The blood and urine data showed no significant difference when the total ascorbic acid was ingested at a single time or divided by an interval of time for these two levels, 75 and 150 mg (table 1).

Sensitivity of blood plasma levels and excretion of total ascorbic acid as criteria

The magnitude of the difference in total ascorbic acid intake which will result in significant changes of blood plasma and urine total ascorbic acid values has been a persistent question. Data which were obtained on 43 young women were collected to judge the sensitivity of these measures to a change in intake of 15 mg total ascorbic acid. The two levels of intake studied were 60 mg (35 subjects) and 75 mg (8 subjects). In one group, 6 subjects were studied simultaneously, three subjects receiving each intake. It was of special interest to see if the criteria for so few subjects would differentiate between these two intakes of total ascorbic acid. This range of 60 to 75 mg is of special interest since it is that of recommended adequacy. The plan of the experiment was the same for all subjects. An adjustment period from 10 to 17 days preceded a 10-day test period. Daily analyses of the total ascorbic acid-containing foods of the basal diet were made throughout. Blood plasma and urine analyses were made each day of the 10-day test period.

2. There are no significant differences in blood plasma or urinary total ascorbic acid values when the same amount of the vitamin, either 75 or 150 mg, is ingested as a single or divided intake.

3. Blood plasma levels and 24-hour urine returns of total ascorbic acid are demonstrated to differentiate between intakes of 60 and 75 mg for 8 subjects at each level (significant 1% level). Differentiation is suggested by as few as three subjects at each level (significant 5% level).

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INCISOR ASH VERSUS FEMUR ASH IN SWEET PEA LATHYRISM (ODORATISM)

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The occurrence of drastic skeletal changes has been emphasized repeatedly as one of the major manifestations of the experimental lathyrism or odoratism which develops in the rat as the result of the ingestion of the seed of the sweet pea, *Lathyrus odoratus* (Geiger, Steenbock and Parsons, '33; Lewis, Fajans, Esterer, Shen and Oliphant, '48; Vivanco and Díaz, '51; Ponseti and Baird, '52). Attention recently has been called also to a peculiar notching of the incisors which occurs in growing rats with advanced odoratism (Dasler, '54a). The suggestion was made that this notching is mechanical in origin in that the overgrowth of bone in the proximal portions of the mandible probably limits the range of movement of the jaw and thus interferes with the normal attrition of the incisors. The possibility remains, however, that some other factor, such as an altered mineralization of the incisor, might be at least partially responsible. It was therefore thought to be of interest to obtain, by means of ashing, a measure of the degree of mineralization in the lower incisors of rats with advanced odoratism and to compare this to that of the femurs in the same animals. Quite unexpectedly, it was found that in odoratism the percentage of ash in the whole incisors actually showed a significant increase, while the level of ash in the femurs decreased.

approach the severity of that found in rickets. The mineralization of the whole incisor is, in fact, increased. These results, together with the fact that serum alkaline phosphatase levels are apparently unaffected (Chu, Christman and Lewis, '48; Dasler, '54b), support the view that odoratism is not primarily a disturbance in calcium and phosporus metabolism. The decreased mineralization must be entirely unrelated to the bone deformities since no such deformities are seen in rickets.

It was also found in these and in other experiments that both the proximal and the distal ends of the femurs pull away very easily from the shaft at the epiphyseal lines. This occurs much more easily than in the case of rachitic femurs having a much lower ash content. There therefore seems to be in the bone a deficiency in some kind of cementing substance which can vary independently of the degree of mineralization. It was also noted that the incisors of rats in which odoratism had been produced were much more easily extracted than those of the control rats. A change in the periodontal membrane is therefore indicated.

Since it appears to be collagenous or connective tissue which is primarily affected, it is felt that odoratism involves a disturbance in the metabolism of connective tissue or of some connective tissue component (Dasler, '54a, b, c). All of the observations reported herein are consistent with this hypothesis. Ponseti and Shepard ('54) have also concluded that it is the mesodermal tissues which are primarily implicated in odoratism and have surmised that the disturbance may specifically involve the chondroitin sulfate of the ground substance.

The increase in the ash content of the incisors of rats receiving sweet pea diets is of especial interest. The ash content of the incisor of the rat usually decreases less than that of the tibia or the femur when diets affecting the mineralization of the bones are fed (Karshan and Rosebury, '33; Templin and Steenbock, '33). On certain diets (high-calcium rachitogenic diet) the ash content of the incisor may even re-

main unchanged while the tibia ash shows a marked decrease (Karshan, '33). The author is unaware of any other studies, however, in which incisor ash increased while bone ash decreased. No explanation for this effect of sweet pea diets is offered.

SUMMARY

Rats which were fed diets containing 50% sweet peas *(Lathyrus odoratus)* for three weeks showed decreases in femur ash and increases in incisor ash when compared to rats fed control diets ad libitum or by the paired-feeding technique.

It is concluded that a disturbance in calcium and phosphorus metabolism is not involved in odoratism (sweet pea lathyrism), but that the effects on the mineralization of the skeleton are secondary to a disturbance in the metabolism of connective tissue or of a connective tissue component.

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ADRENAL FUNCTION IN THE PANTOTHENIC ACID-DEFICIENT RAT [1,2]

LIVER GLYCOGEN, BLOOD GLUCOSE, ADRENAL CHOLESTEROL AND ADRENAL ASCORBIC ACID LEVELS

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INTRODUCTION

Pantothenic acid has been known to be essential for the maintenance of normal adrenal structure since 1939 (for review see Morgan, '51). More recently, however, several studies concerning the biochemical role of pantothenic acid in adrenocortical function have been reported. Hurley and Morgan ('52) observed an adrenal insufficiency in pantothenic acid-deficient rats as measured by their inability to produce an increase in liver glycogen in response to anoxic anoxia. The metabolic defect was not an inability of the peripheral tissues to respond to adrenal steroids, since the administration of adrenal cortical extract to the deficient rats enabled them to produce the normal carbohydrate response. Winters and his associates (Winters et al., '52a, '52b, '52c and Schultz et al., '52), using other techniques, have also demonstrated a depressed adrenal function in pantothenic acid-deficient rats. Eosinophile and lymphocyte counts, insulin sensitivity, excretion of glucose and nitrogen and fasting liver glycogen levels were used by these workers as indices of adrenal activity. Additional evidence

[1] This study was supported (in part) by funds provided under contract AF18 (600) 558 with the USAF School of Aviation Medicine, Randolph Field, Texas.

[2] A preliminary report of this work was presented before the American Physiological Society (Hurley, '54).

was presented by Ershoff ('53), who found a drastically reduced survival rate in pantothenic acid-deficient rats exposed to cold stress as compared with their adequately-fed controls.

The investigations cited, in addition to earlier work, would appear to provide ample evidence for the hypothesis that pantothenic acid deficiency produces an adrenal insufficiency; but Perry et al. ('53) and Ershoff et al. ('53) conclude, on the basis of other criteria including those of adrenal cholesterol and ascorbic acid depletion, that this is not necessarily the case.

In view of these divergent opinions, it was thought desirable to examine the adrenal cholesterol response of pantothenic acid-deficient rats to stress, simultaneously with several other indices of adrenal function. For this purpose the following experiments were designed, using the previous conditions (Hurley and Morgan, '52) of reduced oxygen tension as a stress. Measurements were made of the adrenal cholesterol, adrenal ascorbic acid, liver glycogen and blood sugar levels of normal and pantothenic acid-deficient rats before and after exposure to lowered oxygen tension.

MATERIALS AND METHODS

Animals and diets. All animals in this study were albino rats, both male and female, of the University of Denver strain,[3] born in this laboratory. Mother rats were given the pantothenic acid-deficient diet when their young were 16 days of age. At 21 days of age, the young were placed in individual screen-bottom cages, grouped according to sex, weight and litter, and given purified diets either complete or deficient in pantothenic acid. Food and tap water were supplied ad libitum.

The diets[4] had the following composition in per cent: vitamin-free casein 18, sucrose 68, cottonseed oil 10 and salts 4.[5] The diets were supplemented with vitamins in the following amounts, in milligrams, for each kilogram of diet: calcium

[3] This strain has been inbred for 60 generations during the past 20 years at the University of Denver.

[4] Obtained from Nutritional Biochemicals, Inc., Cleveland, Ohio.

[5] U.S.P. Salt Mixture no. 2.

pantothenate 44, vitamin A concentrate (200 units per milligram) 99, vitamin D concentrate (400 units per milligram) 66, alpha-tocopherol 225.5, inositol 301.4, menadione 4.4, biotin 0.7, niacin 595.5, para-aminobenzoic acid, riboflavin, pyridoxine hydrochloride, and thiamine hydrochloride, each 22, choline chloride 6.01 gm and folic acid 0.2 μg. The deficient animals received the vitamins listed with the exception of pantothenic acid.

The experimental animals were maintained on the respective diets for 6 weeks and were then divided into three groups: (1) untreated, (2) fasted for 24 hours at atmospheric pressure,[6] and (3) fasted for 24 hours under reduced oxygen tension, 349 mm of mercury, corresponding to 20,000 feet of elevation.

The anoxia apparatus consisted of a series of Mason jars connected to a vacuum pump by rubber tubing and copper tubes sealed in the lids, as described by Wickson and Morgan ('46). At the beginning of the test period each rat was placed in a glass jar, those taking the test period at atmospheric pressure being under identical conditions except that there was no reduction in pressure. During this time the animals received no water.

After the test period, the rats were anesthetized with sodium nembutal and blood was taken from the tail. The adrenals were then removed, cleaned of extraneous tissue and each weighed rapidly on a Roller-Smith balance. One gland was placed in a solution of fresh 2.5% metaphosphoric acid for the determination of ascorbic acid and the other was frozen with dry ice for subsequent determination of cholesterol. The liver was then removed, weighed rapidly on a trip balance and plunged whole into hot 30% KOH for glycogen analysis.

Chemical methods. Blood sugar was determined by the Nelson method ('44). Adrenal ascorbic acid was determined by a method based on the Lowry et al. modification ('45) of the Roe and Kuether procedure ('43), in which the gland was extracted with a 2.5% metaphosphoric acid and the ascorbic acid

[6] Normal barometric pressure in Denver, elevation 5280 feet, is 627.6 mm of mercury.

TABLE 1

The effect of pantothenic acid deficiency on response of rats to anoxic anoxia

TIME		GROUP	NO. RATS	BODY WEIGHT	LIVER GLYCOGEN	BLOOD GLUCOSE	ADRENAL ASCORBIC ACID	ADRENAL WEIGHT	ADRENAL CHOLESTEROL
				gm	*mg/100 gm*	*mg %*	*mg/100 gm*	*mg/100 gm*	*%*
				Summary of data					
	I	Normal untreated	30	145 ± 5 [1]	5390 ± 488	140 ± 4	274 ± 9	21.2 ± 1.1	3.5 ± 0.30
	II	Normal fasted [2]	30	146 ± 5	95 ± 15	99 ± 2	321 ± 12	26.7 ± 1.6	3.1 ± 0.26
	III	Normal fasted anoxia	19	145 ± 7	1545 ± 310	144 ± 11	286 ± 15	26.4 ± 2.1	1.3 ± 0.10
	IV	Deficient untreated	19	53 ± 2	3043 ± 388	112 ± 5	323 ± 12	32.1 ± 2.0	2.2 ± 0.17
	V	Deficient fasted	19	54 ± 3	65 ± 10	76 ± 4	355 ± 15	32.0 ± 1.7	2.8 ± 0.35
	VI	Deficient fasted anoxia	19	50 ± 2	99 ± 24	75 ± 6	328 ± 21	31.1 ± 1.6	1.3 ± 0.16
				Data obtained at different times					
Feb. and March	I	Normal untreated	6	142 ± 4	6600 ± 95	114 ± 8	221 ± 9	21.7 ± 3.7	5.9 ± 0.66
	II	Normal fasted	7	137 ± 11	74 ± 17	105 ± 4	286 ± 41	23.1 ± 4.5	3.3 ± 0.55
	III	Normal fasted anoxia	6	156 ± 15	1859 ± 790	177 ± 24	278 ± 19	22.6 ± 4.5	1.5 ± 0.14
	IV	Deficient untreated	6	56 ± 5	3490 ± 450	120 ± 7	347 ± 18	26.3 ± 2.2	2.8 ± 0.33
	V	Deficient fasted	5	67 ± 6	58 ± 7	77 ± 6	314 ± 37	27.0 ± 1.5	4.5 ± 0.15
	VI	Deficient fasted anoxia	6	54 ± 3	45 ± 10	63 ± 15	319 ± 24	27.4 ± 3.0	1.8 ± 0.29

TABLE 1 *(continued)*

The effect of pantothenic acid deficiency on response of rats to anoxic anoxia

TIME	GROUP	NO. RATS	BODY WEIGHT	LIVER GLYCOGEN	BLOOD GLUCOSE	ADRENAL ASCORBIC ACID	ADRENAL WEIGHT	ADRENAL CHOLESTEROL
			gm	*mg/100 gm*	*mg %*	*mg/100 gm*	*mg/100 gm*	*%*
			Data obtained at different times					
July	I Normal untreated	7	136 ± 10	4562 ± 840	148 ± 6	231 ± 21	22.2 ± 2.2	3.0 ± 0.36
	II Normal fasted	6	161 ± 10	46 ± 7	81 ± 3	297 ± 23	23.6 ± 3.2	2.1 ± 0.66
	III Normal fasted anoxia	5	141 ± 11	792 ± 288	163 ± 21	212 ± 27	21.3 ± 1.3	0.8 ± 0.23
	IV Deficient untreated	4	53 ± 4	2248 ± 621	92 ± 11	294 ± 18	30.8 ± 2.2	2.2 ± 0.24
	V Deficient fasted	5	54 ± 4	48 ± 19	81 ± 3	359 ± 14	29.5 ± 1.9	3.3 ± 0.62
	VI Deficient fasted anoxia	5	51 ± 2	33 ± 13	91 ± 13	254 ± 27	29.5 ± 2.7	1.3 ± 0.47
Sept. 15–Oct. 15	I Normal untreated	12	145 ± 8	5098 ± 910	150 ± 7	305 ± 11	18.2 ± 1.6	2.9 ± 0.25
	II Normal fasted	12	149 ± 5	67 ± 12	99 ± 3	328 ± 15	22.5 ± 2.1	3.5 ± 0.14
Dec. and Jan.	I Normal untreated	9	152 ± 10	5797 ± 936	131 ± 4	285 ± 16	24.4 ± 2.1	2.7 ± 0.34
	II Normal fasted	8	132 ± 5	179 ± 43	95 ± 4	350 ± 23	31.4 ± 3.5	2.7 ± 0.39
	III Normal fasted anoxia	9	139 ± 10	1754 ± 42	115 ± 6	329 ± 15	27.6 ± 3.1	1.5 ± 0.17
	IV Deficient untreated	9	56 ± 3	3086 ± 718	117 ± 5	320 ± 20	37.1 ± 3.0	1.7 ± 0.13
	V Deficient fasted	9	47 ± 5	80 ± 19	72 ± 7	376 ± 22	36.1 ± 3.3	1.6 ± 0.29
	VI Deficient fasted anoxia	9	47 ± 2	165 ± 42	72 ± 7	368 ± 35	40.0 ± 3.4	1.0 ± 0.12

[1] Mean ± standard error.

[2] All fasted animals in these experiments received no water during the test period.

estimated by the use of 2,4-dinitrophenylhydrazine. Total adrenal cholesterol was determined by the method of Sperry and Webb ('50). Liver glycogen was determined by the anthrone method (Seifter et al., '50) after precipitation with 60% ethyl alcohol.

RESULTS

Table 1 shows a summary of the results of these experiments. In this table data from all of the animals are combined in the respective groups and are also shown broken down according to the various times at which experiments were made. The time referred to in the table is the time during which the animals were sacrificed, the period of deficiency being the previous 6 weeks. Table 2 shows the statistical significance of the data.

The results confirm the previous work of Hurley and Morgan ('52) and of Guehring, Hurley and Morgan ('52) with respect to the liver glycogen and blood sugar values. After subjection to anoxic anoxia with fasting, normal animals showed a 15-fold increase in liver glycogen and a significant rise in blood sugar as compared with normal animals subjected to fasting alone. Pantothenic acid-deficient rats, however, exhibited no significant increase in either of these constituents. In addition, the present experiments show that pantothenic acid-deficient untreated rats have lower liver glycogen and blood sugar levels than do normal untreated rats.

With respect to adrenal ascorbic acid, however, the present data are at variance with the previous work (Hurley and Morgan, '52; Guehring et al., '52). Although the analytical method was the same, the experiments presented here show no significant differences in adrenal ascorbic acid levels between comparable normal and deficient groups. In the previous work the deficient animals were consistently lower in their ascorbic acid content than the normal rats. No explanation for this difference between the sets of data is at hand, but it may be related to the different conditions of the experiments, i.e., different strains of rats, different diets, different conditions of altitude, humidity and temperature range, etc. These considerations also apply, of course, to the untreated rats, both normal and

deficient, although no untreated animals were included in the previous studies. Here we find a significant difference, with the deficient animals having a higher level of ascorbic acid than the normal ones. It should also be noted that the adrenal ascorbic acid values of the normal rats tend to be lower than those usually quoted in the literature, although they fall within the accepted normal range.

An examination of the adrenal weights shows that fasting produced an increase in adrenal weight of the normal but not of the deficient animals. Fasting with anoxia had no significant effect on either group as compared with its fasting control. The deficiency itself, however, stimulated a significant increase in adrenal weight.

The adrenal cholesterol levels of the deficient untreated rats were lower than those of their normal controls. This finding confirms the work of others (Winters et al., '52c; Morgan and Lewis, '53; Perry et al., '53; Dumm et al., '53; Ershoff et al., '53). Fasting had no significant effect on the adrenal cholesterol level of normal rats. This has earlier been demonstrated by Sterling and Longwell ('53) and many other workers. In the deficient rats, fasting produced a probably insignificant rise in the adrenal cholesterol. Exposure to anoxic anoxia with fasting had the same effect in both normal and deficient animals, namely, a depression of adrenal cholesterol.

The results recorded in the second part of table 1 provide striking evidence of the extreme variability observed in adrenal cholesterol levels in both normal and deficient rats. Even in the normal, untreated animals, the adrenal cholesterol varied from 2.71 to 5.87% and in the deficient rats, fasting produced a large rise in this constituent in two experiments, but not in the third. Two findings concerning cholesterol appear to be consistent (table 1) and are statistically significant (table 2): (1) a lowered adrenal cholesterol level in deficient, untreated rats as compared with their normal controls and (2) a fall in adrenal cholesterol of both normal and deficient animals on exposure to anoxia.

The adrenal ascorbic acid data presented in the second part of table 1 also show great variability. Here again, two findings are fairly consistent: (1) the increase in ascorbic acid in both normal and deficient rats after fasting and (2) the higher (but probably insignificant, according to table 2) levels of this compound in the deficient groups.

Extreme precautions were taken to keep all conditions constant. The rats were housed in individual cages; they were

TABLE 2

The statistical significance between various groups

GROUPS COMPARED	LIVER GLYCOGEN	BLOOD GLUCOSE	ADRENAL ASCORBIC ACID	ADRENAL WEIGHT	ADRENAL CHOLESTEROL
	P[1]	P	P	P	P
I– II	< 0.01	< 0.01	< 0.01	< 0.01	> 0.3
II–III	< 0.01	< 0.01	> 0.1	> 0.9	< 0.01
I– IV	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
IV– V	< 0.01	< 0.01	> 0.05	> 0.9	> 0.05
II– V			> 0.3	> 0.02	
V– VI	> 0.1	> 0.8	> 0.2	> 0.6	< 0.01
III– VI			> 0.3	> 0.05	

[1] P values were obtained from "t" table in Fisher, Statistical Methods for Research Workers.

handled periodically so that handling itself at the time of sacrifice would not constitute a stress; the test and sacrifice were always made at the same time of day; the methods of sacrifice, removal of blood and organs were always the same; the chemical methods were carefully checked.

That such extreme variability should occur in these adrenal components seems even more striking in view of the relative constancy of pattern exhibited by the blood sugar and, to a lesser degree, by the liver glycogen.

DISCUSSION

These experiments demonstrate the extreme variability of both adrenal cholesterol and ascorbic acid levels in spite of

particular care to keep conditions constant. This would suggest that the practice of using one of these components as the sole index of adrenal function in an experiment could lead to erroneous conclusions. In view of cholesterol variability, in addition to the observation that cholesterol decreases concomitantly with lack of gluconeogenesis, the necessity of using more than one measure of adrenal function under any given set of conditions seems imperative. At least in pantothenic acid-deficient or otherwise abnormal animals, changes in adrenal cholesterol levels alone cannot be regarded as an infallible index of increased hormonal output.

One of the most vexing practical problems connected with the study of pantothenic acid deficiency has been that of finding a reliable criterion of the state of deficiency of the experimental animal. Cessation of growth and outward appearance are not adequate measures of the metabolic state. As was found previously (Hurley and Morgan, '52), and again in the present investigation, deficient animals of the same strain maintained under exactly the same conditions often give highly variable responses. A possible explanation of such variability may be found in the recent work of Klein and Lipmann ('53). These workers measured the incorporation of labeled acetate into fatty acids and cholesterol by liver slices from normal and pantothenic acid-deficient rats. It was found that the synthesis of these lipids paralleled the coenzyme A content of the livers. With liver coenzyme A above a certain concentration, there was no depression of lipid synthesis, in spite of clinical symptoms of severe deficiency. Perhaps coenzyme A determinations would provide a better biochemical criterion of pantothenic acid deficiency than has hitherto been available. If the degree of deficiency were expressed in terms of coenzyme A content of the appropriate tissues, it might be possible to systematize investigation and knowledge in this field more readily.

The present investigation demonstrates a severe adrenal insufficiency in pantothenic acid-deficient rats as measured by carbohydrate metabolism during stress. Simultaneous with this, there occurred a depletion of adrenal cholesterol. Ac-

cording to current concepts, such a depletion is indicative of increased adrenal activation (Sayers, '50). There is now some evidence that cholesterol synthesis is impaired in pantothenic acid deficiency (Guehring et al., '52; Morgan and Lewis, '53; Dumm and Ralli, '53; Klein and Lipmann, '53). A depressed rate of cholesterol synthesis could be one factor explaining the apparent paradox of adrenal cholesterol decrease in the presence of an adrenal insufficiency. However, the fact that in the present work cholesterol dropped sharply after the stress period would seem to necessitate the postulation of an additional mechanism. Exposure to stress must have effected either (or both) an increased rate of utilization or a decreased rate of synthesis in normal as well as in deficient animals. Unless we assume that stress causes a decrease in the rate of cholesterol synthesis (for which there is no evidence whatever and which would be at variance with all current thought), the question of the fate of the original cholesterol present in the adrenal gland still remains. According to present theory, this cholesterol is utilized in the synthesis of the adrenal cortical hormone or hormones. In the case of pantothenic acid-deficient animals, under the conditions of the present experiment, such synthesis seems to be impaired. The following hypothesis is therefore postulated: in the normal animal subjected to stress, cholesterol is used in the synthesis of adrenal steroids, with coenzyme A as a necessary component of the system. In the pantothenic acid-deficient animal, the synthesis of cortical steroids is begun, but in the absence of adequate amounts of coenzyme A the process cannot be carried to completion and the synthesis stops at some intermediate step. The compound or compounds elaborated are therefore not effective for gluconeogenesis. Such an hypothesis would fit all the facts available at present and would be amenable to investigation, such as analysis of the steroids produced by deficient adrenals. It would explain the fate of the cholesterol which had disappeared from the adrenal glands of pantothenic acid-deficient animals in spite of the absence of increased gluconeogenesis. It would resolve the apparent contradiction between the work

of those investigators who concluded that an adrenal hypofunction exists in pantothenic acid deficiency and those workers who came to the opposite conclusion. Although the state of adrenal function with respect to salt and water metabolism is almost completely unkown in pantothenic acid deficiency, it is possible, according to this hypothesis, that the steroid or steroids elaborated by the adrenal cortices of deficient animals are capable of performing this function, although completely ineffective in gluconeogenesis.

SUMMARY

The adrenal function of normal and pantothenic acid-deficient rats was investigated by subjection of the animals to anoxic anoxia equivalent to 20,000 feet of altitude for 24 hours without food or water. Measurements of adrenal cholesterol and ascorbic acid, liver glycogen and blood sugar were made before and after the stress period and after fasting alone.

Extreme variability was observed in the levels of adrenal cholesterol and ascorbic acid in both normal and deficient rats examined at different seasons. In contrast, liver glycogen and blood sugar levels were fairly constant both as to absolute values and as to pattern among the various experimental groups.

The deficient, untreated animals were found to have lower adrenal cholesterol, liver glycogen and blood sugar levels and higher adrenal weights and ascorbic acid levels than comparable normal animals.

After exposure to the stress period, the normal rats showed a 15-fold increase in liver glycogen and a significant rise in blood sugar as compared with their fasted controls. Pantothenic acid-deficient animals showed no change in these constituents. A significant decrease in adrenal cholesterol levels following anoxia was observed in both normal and deficient rats.

The apparent paradox of an adrenal insufficiency, as indicated by lack of carbohydrate accumulation, concomitant with

a decrease in adrenal cholesterol is discussed and a possible hypothesis is postulated.

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AN UNIDENTIFIED CHICK-GROWTH FACTOR FOUND IN LITTER[1]

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Prior to the isolation of vitamin B_{12}, chick growth responses were obtained with dried whey (Berry et al., '43; Hill et al., '44); with distillers dried solubles (Synold et al., '43; Novak et al., '47); and with dried brewer's yeast (Schumacher et al., '40; Hill et al., '44). After the isolation of vitamin B_{12} by Rickes and co-workers ('48), attempts were made to explain the growth-promoting activity of some of these supplements on the basis of the vitamin B_{12} content (Lindstrom et al., '49; Lillie et al., '48; Nichol et al., '49). However, there were other growth-promoting factors as evidenced by chick growth responses obtained from these substances in the presence of an adequate amount of vitamin B_{12}. This growth response obtained in the presence of vitamin B_{12} has been demonstrated with dried whey by Menge et al. ('49), Reed et al. ('51), Menge et al. ('52), and Combs et al. ('54); with fish meal and dried brewer's yeast by Carlson et al. ('49); with liver by Savage et al. ('50,), Sunde et al. ('50), and Combs et al. ('54); with fish solubles by Fuller et al. ('52),

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Heuser and Norris ('51), Sunde et al., ('52), and Combs et al. ('54); and with distillers dried solubles by Couch et al. ('51, '52, '54) and Norris ('54). Jacobs and associates ('53) reported that dehydrated alfalfa leaf meal also contained an unidentified factor necessary for maximum hatchability and fertility in the mature fowl.

Jacobs et al. ('53) reported that antibiotics (directly or indirectly) stimulated growth by reducing the total number of *Clostridia* in the intestinal tract of the chick and that antibiotics might be ineffective in stimulating growth in a clean environment where the *Clostridia* population was low. Elam et al. ('54) obtained a decrease in growth in a clean environment by feeding *Clostridia* isolated from the feces of chicks maintained in old quarters. This decrease was overcome by the addition of penicillin to the diet.

Drug-fastness (antibiotic resistance) in the intestinal flora has been reported by Elam et al. ('51a, '51b), Starr and Reynolds ('51) and Johansson et al. ('53), and may be noteworthy because of the altered nutritional status of bacterial cultures when resistance to penicillin (Gale, '48) or streptomycin (Sevag and Rosanoff, '52) is induced.

The object of this study was to investigate the presence of an unidentified growth factor in litter which would increase the growth rate of birds fed a diet complete with respect to the known nutrients. This factor could conceivably be produced by the microbial inhabitants of litter.

EXPERIMENTAL

Data from two experiments are reported in this study. The New Hampshire chicks used in these studies were obtained from hens that had been fed the diet described earlier by Elam et al. ('51b). The birds were maintained in batteries with raised screen floors. Feed and water were supplied ad libitum and the chicks were weighed at weekly intervals during an experimental period of 10 weeks.

The basal diet used in this study consisted of 60.75% ground yellow corn, 35% soybean oil meal, 2% bone meal,

1.5% oyster shell flour and 0.5% NaCl. In addition, the diet was supplemented with 2 mg riboflavin, 5 mg calcium pantothenate, 12.5 mg niacin, 200 mg choline chloride, 100 mg manganese sulfate, 4,000 I.U. vitamin A (stabilized), 600 I.C.U. vitamin D_3 and 13.6 μg vitamin B_{12} per pound of feed.

Penicillin was fed at a level of 2 mg per pound of feed in the first experiment. An antibiotic combination consisting of penicillin 0.5, aureomycin 1.25, bacitracin 1.25, and terramycin 1.25 mg per pound of diet was fed in the second experiment. Fish solubles was fed at a level of 3% and the protein content of this diet was adjusted so that it did not exceed that of the control group. The litter and autoclaved litter extracts were fed at a level of 8 ml (which contained 0.350 gm of litter per milliliter of water) per pound of feed.

Number of birds. For the first experiment 200 chicks were randomized into 5 groups of 40 chicks each, and in the second experiment 800 sexed chicks were randomized into 8 groups of 100 chicks each (50 males and 50 females).

Preparation of the litter extracts. Samples of litter were obtained from poultry quarters where the litter had not been changed for at least three months prior to their collection. The litter samples were made into a water suspension by suspending 0.350 gm of litter per milliliter of water. The resulting suspension was then filtered through several layers of cheesecloth and the residue was discarded. Portions of this extract were then autoclaved for 15 minutes at 15 pounds of pressure at 121 to 125°C.

Bacteriological techniques. Fecal samples were collected once a week and were prepared as described earlier by Elam, Gee and Couch ('51a). The total number of *Clostridia* per gram of feces was determined by the method described by Jacobs et al. ('53). Antibiotic-resistant bacteria were determined by plating in nutrient agar and Difco eosin methylene blue agar (coliform count) which contained 25 μg per milliliter of each of the 4 antibiotics mentioned above.

Statistical methods. An analysis of variance for counts and weights was made, and the F value (ratio of appro-

priate mean square values from an analysis of variance table) was determined. Least significant differences (LSD) were calculated where the F value was significant at the 1 or 5% level of probability.

TABLE 1

Microorganisms found in chick litter

MEDIA AND TYPE OF COUNT	NUMBER OF MICROORGANISMS
	per gram of litter
Thioglycollate (Total dilution count)	511,800,000 [1]
Lactobacillus-selection (Lactics)	12,395,500
"SF" broth (Enterococci)	15,800,000
Eosin methylene blue (Coliform)	7,924,000
Potato-dextrose (Yeast)	1,350
Potato-dextrose (Mold)	3,970
Sulfite-glucose-iron *(Clostridia)*	23,780

[1] These figures represent an average of 5 different determinations.

RESULTS AND DISCUSSION

First experiment

In the first experiment the autoclaved litter extract produced an increase in the growth rate of birds when added to an all-vegetable protein diet adequately supplied with the known B-vitamins (table 2). The addition of the litter extract (not autoclaved) failed to stimulate growth. This may be explained by the high *Clostridia* population of the raw litter extract (table 1), since *Clostridia* has been shown to depress growth when incorporated into the diet of the chick (Elam et al., '54). Another possible explanation is that the growth factor is activated by the autoclaving process.

The addition of penicillin to the diet increased the growth rate of birds and decreased the *Clostridia* content of the feces. Fish solubles likewise increased the growth rate.

The autoclaved litter extract was assayed for antibiotic activity using known sensitive strains of *Bacillus subtilis, Bacillus cereus, Micrococcus flavis* and *Micrococcus pyogenes* var. *aureus* and found to contain no detectable antibiotic

TABLE 2

The effect of feeding penicillin, fish solubles, litter extract and autoclaved litter extract upon the weights of chicks and total number of fecal Clostridia

GROUP	AVERAGE WEIGHT AT 10 WEEKS	TOTAL NUMBER OF CLOSTRIDIUM ISOLATES
	gm	*per gram of feces*
Control	1134	12,450
Penicillin	1262 [1]	640 [1]
Litter extract	1170	16,710
Litter + penicillin	1310 [1]	740 [1]
Autoclaved litter extract	1407 [1]	11,150
Fish solubles	1316 [1]	14,750
Fish solubles + penicillin	1334 [1]	790 [1]

[1] Significant at the 1% level of probability.

activity against these sensitive organisms. This finding was not surprising since it is not likely that an antibiotic would retain full activity during autoclaving. Furthermore the autoclaved litter extract was found to have no effect in reducing the fecal *Clostridia* count.

Microbiological assays of the autoclaved litter extract showed a very small amount of vitamin B_{12} and pantothenic acid activity. It is quite unlikely that the increase in growth was due to any of the known B-vitamins which might have survived autoclaving since they were all fed in excess of the basal diet. It is believed, however, that the unidentified factor is synthesized by one of the microbial inhabitants (or group) of the litter (table 1).

Second experiment

The results obtained in the second experiment were similar to those observed in the first experiment (tables 2 and 3). The growth rate of birds was increased when a combination of antibiotics, autoclaved litter extract or fish solubles was added to the basal diet (table 3). When autoclaved litter extract was fed in combination with fish solubles or a combination of antibiotics, the weight of the birds was further increased. This indicates that the growth factor in autoclaved litter extract differs from the unidentified factor found in fish solubles since the effect of these two supplements is additive. The mode of action of the unidentified factor in autoclaved litter extract appears to be different from that of antibiotics since the autoclaved litter extract was ineffective in reducing the *Clostridia* count or increasing the antibiotic-resistant microorganisms (table 4). The autoclaved litter extract when fed with a combination of antibiotics was effective in producing an additional growth response over that by either of the supplements fed alone.

When fish solubles were fed with a combination of antibiotics there was an increase in growth rate over that when the fish solubles were fed alone. When fish solubles, autoclaved litter extract and the antibiotic combination were added to the diet, the growth response was no greater than when two of the supplements were fed, with the exception of the males. The males showed an additive growth response to a combination of the three supplements (table 3).

The addition of a combination of antibiotics was effective in reducing the total number of *Clostridia* per gram of feces in all cases (table 4). When the combination of antibiotics was added to the diet the number of antibiotic resistant microorganisms was increased (table 4). At the present time the significance of this increase in antibiotic-resistant microorganisms is not known, but is definitely interesting since the organisms are resistant to 4 antibiotics. Transfer of the resistant organisms were made to a medium containing the same antibiotic combination to confirm the resistance. These

TABLE 3

The effect of a combination of antibiotics, fish solubles, and an unidentified factor found in litter extract upon the ten-weeks' weight of chicks

GROUP	MALES	GAIN OVER CONTROL	FEMALES	GAIN OVER CONTROL	AVERAGE	GAIN OVER CONTROL
	gm	*gm*	*gm*	*gm*	*gm*	*gm*
Control	1198		990		1094	
Combination of antibiotics	1314	116	1164	174	1239	145
Autoclaved litter extract	1306	108	1109	119	1208	114
Autoclaved litter extract + combination of antibiotics	1378	180	1177	187	1278	184
Fish solubles	1289	91	1098	108	1194	100
Fish solubles + combination of antibiotics	1395	197	1246	256	1321	227
Fish solubles + autoclaved litter extract	1414	216	1169	169	1288	194
Fish solubles + autoclaved litter extract + combination of antibiotics	1457	259	1171	181	1314	220
LSD [1]						
1% level		106		78		65
5% level		81		60		49

[1] Least significant difference.

TABLE 4

The effect of the different dietary supplements upon the total number of Clostridia and the antibiotic resistant organisms

GROUP	TOTAL NUMBER OF CLOSTRIDIA	ANTIBIOTIC-RESISTANT ORGANISMS*	
		Coliforms	Nutrient agar
	per gram of feces		
Control	9,645	10	85
Combination of antibiotics	5 [1]	1,790 [1]	7,365 [1]
Autoclaved litter	8,185	5	195
Combination of antibiotics + autoclaved litter	55 [1]	1,735 [1]	64,725 [1]
Fish solubles	7,985	20	495
Fish solubles + penicillin	680 [1]	3,465 [1]	40,065 [1]
Fish solubles + autoclaved litter	6,675	65	295
Fish solubles + autoclaved litter + combination of antibiotics	35 [1]	4,005 [1]	20,200 [1]

[1] Significant at 1% level of probability.

resistant organisms are now in the process of being characterized.

SUMMARY

A hitherto unrecognized chick-growth factor present in litter is reported. The factor is thermostable and probably produced by microbial fermentation. Data from two separate experiments are reported. The factor gave a significant growth response when added to an all-vegetable protein diet adequate with respect to the known vitamins. The factor gave a growth response comparable to that produced by fish solubles or a combination of 4 antibiotics and produced an additive effect when fed with either of the latter supplements.

It is concluded that the litter factor is not an antibiotic and differs from the growth factor present in fish solubles.

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EFFECT OF METHIONINE, VITAMIN B_{12} AND α-TOCOPHEROL ON THE GROWTH-PROMOTING AND HEPATIC-NECROGENIC ACTIVITY OF PUERTO RICAN TORULA YEAST

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Previous investigations indicate that the protein of Puerto Rican *Torula* yeast has a lower biological value than that of brewers' yeast (Goyco and Asenjo, '47a; '47b; '48; '49; Seeley and Crafa, '51). Also it has been observed (Goyco and Asenjo, '46; Goyco, '51) that rats receiving, as the sole source of protein, Puerto Rican *Torula* yeast grown on sugar cane molasses, tend to develop liver lesions similar to those described by Glynn and Himsworth ('44) using British yeast and by Schwarz ('51b) using American *Torula* yeast grown in a simple medium of sulphite liquor.

The present work deals with the effect of methionine, vitamins E and B_{12}, alone or in combination, on the growth promoting value, protein efficiency and hepatic necrogenic activity of Puerto Rican *Torula* yeast, using albino rats as experimental animals.

EXPERIMENTAL

Twenty-eight-day-old male rats (Wistar-School of Tropical Medicine strain), raised on the laboratory stock ration [1] and

[1] Rockland complete rat diet, ad libitum, and ground beef and lettuce once a week.

weighing about 45 gm, were fed a basal diet in which the protein was supplied by *Torula* yeast.[2]

The basal diet had the following percentage composition: dry torula yeast 37.9, cornstarch 35.1, salt mixture (U.S.P. no. 2) 3.0, sucrose 10.0, sodium chloride 1.0, vegetable oil (Argo)[3] 9.0, cod liver oil 1.0 and cellu flour 3.0. The protein content of the *Torula* yeast used was 47.5% (N × 6.25). The crude protein content of this diet was 18%; the methionine content, 0.24%.

The rats were ear-marked and kept in cages with raised bottoms, in groups of two or three per cage. The growth experiments lasted for 4 weeks.

Autopsies were performed on all animals at the time they died or at the end of the 4-week-experimental period.

RESULTS AND DISCUSSION

Effect on growth and protein efficiency

Methionine. Table 1 records the growth and protein efficiency at different levels of DL-methionine supplementation.

Even at the lowest levels of DL-methionine supplementation, a slight improvement in growth and protein efficiency was observed. However, it was not until a level of 0.05% had been reached that this improvement became statistically significant. Maximum growth and protein efficiency were attained when 1.0% of DL-methionine was added to the diet (1.24% total methionine in the diet).

The presence of 2.24% total methionine in the ration retarded growth and lowered protein intake and efficiency. This confirmed the observations made by others (Wretlind, '50; Van Pilsum and Berg, '50; Wretlind and Rose, '50) as to the toxicity of this amino acid when furnished at levels of 2%, or more, in the diet.

[2] The *Torula* yeast used was supplied by the laboratories of the Puerto Rico Economic Development Co., at Hato Rey, P. R.

[3] Refined Milo (sorgo) oil, manufactured by Corn Products Refining Company, New York.

TABLE 1

Effect of DL-*methionine, vitamin* B_{12} *and* α-*tocopherol on the growth and protein efficiency of rats on an 18% Torula yeast protein diet*

(Four-week-experimental period)

SUPPLEMENT	ESTIMATED TOTAL METHIONINE IN THE DIET	NUMBER OF RATS	AVERAGE GAIN IN WEIGHT	AVERAGE PROTEIN INTAKE	PROTEIN EFFICIENCY [1,2]	RATS THAT DIED BEFORE THE END OF THE 4-WEEK-EXPERIMENTAL PERIOD
	%		*gm*	*gm*		
None	0.24	16	34.0	27.8	1.2	3
0.01% DL-methionine	0.25	12	43.6	33.5	1.3	4
0.03% DL-methionine	0.27	12	43.3	31.8	1.4	2
0.05% DL-methionine	0.29	12	59.5	36.1	1.6	2
0.07% DL-methionine	0.31	12	59.9	35.7	1.7	2
0.50% DL-methionine	0.74	12	77.5	36.3	2.1	none
1.00% DL-methionine	1.24	12	106.8	40.6	2.7	none
2.00% DL-methionine	2.24	12	84.7	34.2	2.5	none
2 mg α-tocopherol per week	0.24	15	31.3	26.7	1.2	none
200 μg vitamin B_{12} per kg of diet	0.24	16	46.7	35.5	1.3	8
2 mg α-tocopherol per week + 200 μg vitamin B_{12} per kg of diet	0.24	10	39.0	25.0	1.6	none
0.5% DL-methionine + 200 μg vitamin B_{12} per kg of diet	0.74	10	135.1	53.2	2.5	none
0.5% DL-methionine + 2 mg α-tocopherol per week	0.74	10	104.8	36.4	2.7	none
0.5% DL-methionine + 200 μg vitamin B_{12} per kg of diet + 2 mg α-tocopherol per week	0.74	10	163.0	58.9	2.8	none

[1] Protein efficiency = $\frac{\text{Av. gain in weight}}{\text{Av. dietary protein consumed}}$.

[2] In the case of those groups in which some of the animals died before the end of the 4-week-experimental period the protein efficiency was computed on the basis of the animals that survived.

Vitamin B_{12}. The minimum requirement of vitamin B_{12} for optimum growth of the rat is unknown so far as we know, but Hartman et al. ('49) determined that the male rat, which shows a greater demand for this vitamin than the female, does not require more than 1 or 2 μg per day.

The vitamin B_{12}[4] was incorporated into the basal *Torula*-yeast diet at a level of 200 μg per kilogram of ration. Diets thus supplemented supplied well over 1 μg per day of vitamin B_{12} even to those animals that consumed the least amount of basal diet during the 4-week-experimental period.

As can be seen in table 1, supplementation with vitamin B_{12} increased the protein intake as well as the gain in weight, thus maintaining the protein efficiency at practically the same value as when the basal diet was taken alone. The protein efficiency of the particular batch of *Torula* yeast used in these experiments was 1.2; this value was a little higher than that reported previously by the writers (0.9) (Goyco and Asenjo, '49). Seeley and Crafa ('51) have reported a value of 1.0 for another batch of Puerto Rican *Torula* yeast assayed by them.

Alpha-tocopherol. Supplementation with α-tocopherol improved neither growth nor protein efficiency; on the contrary, the animals thus supplemented grew more slowly than the controls on the basal diet, although the difference was not statistically significant. The protein efficiency remained at the same level as in the control group due to the fact that the food intake was proportionally lower.

These results seemed to confirm those of Hove and Harris ('47), who observed that α-tocopherol does not benefit the growth or the protein efficiency of rats receiving a basal ration containing 15% yeast protein.

Effect of combined supplementation. It has been pointed out that a significant increase in protein intake was observed when the basal *Torula* yeast diet was supplemented with either DL-methionine or vitamin B_{12}. The increase in protein intake above that of the basal diet only was of about the same order

[4] Cobione was kindly supplied by Merck and Co., Rahway, New Jersey, through the courtesy of Dr. R. A. Peterman.

when either one of these two factors was given (about 8 gm increase in the 28-day-experimental period). However, when both were given together, the protein intake was almost doubled; that is, it was raised from 27.8 gm on the basal diet to 53.2 gm on the DL-methionine-vitamin B_{12}-supplemented diet. As the weight gain was quadrupled, the protein efficiency was a little more than doubled, increasing from 1.2 to 2.5.

Combined supplementation with DL-methionine and α-tocopherol significantly improved growth, and even more so, protein efficiency. This was due to the fact that the protein intake remained practically at the same level as when the basal ration was supplemented with methionine only. The effect produced by the α-tocopherol in this case was that of improving the efficiency of protein utilization. A similar type of improvement in protein utilization was reported by Hove and Harris ('47) when they added α-tocopherol to a ration containing 10% casein. They observed a significant increase in growth and protein efficiency although the food intake remained practically the same as in the group without α-tocopherol.

Addition to the basal *Torula*-yeast diet of vitamin B_{12} and α-tocopherol produced what might be considered anomalous results. Although both the gain in weight and food intake were lower than in the group receiving vitamin B_{12} only as a supplement, the protein efficiency was higher. This difference, however, was of doubtful statistical significance. According to the results, it seems that supplementation with vitamin B_{12} or α-tocopherol or both does not produce significant changes in the protein efficiency exhibited by rats fed a diet in which the protein is supplied by *Torula* yeast alone.

Supplementation of the basal diet with the three factors simultaneously resulted in a protein efficiency slightly higher than that obtained when supplementing with DL-methionine and α-tocopherol. However, this protein efficiency was attained at a much higher level of protein intake and gain in weight. The group of animals consumed a little over twice the amount of protein and gained nearly 5 times as much weight as those that received the basal diet only.

Gross hepatic necrosis

Table 2 records the gross post mortem observations made on the livers of rats fed the basal ration alone or together with the various supplements indicated.

TABLE 2

Effect of DL-*methionine, vitamin* B_{12}*, and* α*-tocopherol on the gross hepatic necrosis of rats on an 18% Torula yeast protein diet*

SUPPLEMENT	NUMBER OF RATS	NUMBER OF RATS THAT DIED BEFORE THE END OF THE 4-WEEK-EXPERIMENTAL PERIOD	NUMBER OF RATS WITH GROSS HEPATIC NECROSIS
None	16	3	3
None (new batch of yeast)	12	0	0
0.01% DL-methionine	12	4	4
0.03% DL-methionine	12	2	2
0.05% DL-methionine	12	2	2
0.07% DL-methionine	12	2	2
0.50% DL-methionine	12	none	none
1.00% DL-methionine	12	none	none
2.00% DL-methionine	12	none	none
2 mg α-tocopherol per week	15	none	none
200 μg vitamin B_{12} per kg of diet	16	7	8
200 μg vitamin B_{12} per kg of diet (new batch of yeast)	12	none	none
2 mg α-tocopherol per week + 200 μg vitamin B_{12} per kg of diet	10	none	none
0.5% DL-methionine + 200 μg vitamin B_{12} per kg of diet	10	none	none
0.5% DL-methionine + 2 mg α-tocopherol per week	10	none	none
0.5% DL-methionine + 200 μg vitamin B_{12} per kg of diet + 2 mg α-tocopherol per week	10	none	none

Gross hepatic necrosis [5] developed in 13 (20%) out of 64 rats that received the basal *Torula* yeast diet only, or this diet supplemented with minimum amounts of DL-methionine (0.07% or less). On the other hand, the incidence of the lesion in the

[5] The criteria were: presence in the liver lobes of bright red hemorrhagic spots averaging from 3 to 4 mm in diameter, or wide congestive areas along the border, or both. Some of these liver lesions were checked by microscopic examination.

group supplemented with vitamin B_{12} was much higher, or 50% (8 out of 16). It is interesting to note that György and Rose ('50) reported a 100% incidence of liver necrosis in rats receiving a basal diet containing as the sole source of protein a "necrogenic" British yeast with and without vitamin B_{12} supplement. Their diet contained about half the amount of yeast and, therefore, of protein, of our diet.

As this finding was somewhat unexpected, considering the high protein content of the diet, these trials were repeated, using this time a new batch of *Torula* yeast, as the previous one had been exhausted. Twelve rats were used in each group (basal yeast diet and this same diet supplemented with 200 μg of vitamin B_{12} per kilogram). Although the growth and protein efficiency obtained compared closely with the values previously reported, none of these animals exhibited gross liver lesions when the autopsy was performed.

Any speculations on the frequency of liver lesions in the various groups of animals studied must take into consideration the fact that the experimental rats received, during the pre-experimental period, a standard stock colony ration and therefore could have had variable initial stores of vitamin E. Goettsch ('51) has pointed out that rats vary greatly in their ability to store vitamin E and that the divergent results in the production of massive hepatic necrosis among different laboratories presumably have been associated with the amount of vitamin E supplied during the pre-experimental period. Apparently this lesion results not from a simple deficiency of sulphur amino acids, but from a simultaneous deficiency of these amino acids, vitamin E, and, possibly also of a third not yet well-defined substance called Factor 3 (Schwarz, '44; '51a; '52).

SUMMARY

1. DL-methionine induced maximum food intake, growth and protein efficiency when about 1% was added to a *Torula*-yeast diet.

2. Neither vitamin B_{12} (200 μg per kilogram of diet) nor α-tocopherol (2 mg per week) improved the protein efficiency of

the rats receiving the *Torula*-yeast diet. Vitamin B_{12} increased growth and protein intake; α-tocopherol did not. Combined supplementation improved the protein efficiency slightly.

3. Addition of vitamin B_{12} (200 μg per kilogram of diet) increased protein intake, growth and protein efficiency when a *Torula*-yeast diet supplemented with 0.5% DL-methionine was fed to the rats.

4. Addition of α-tocopherol (2 mg per week) increased growth and protein efficiency but not protein intake, when a *Torula*-yeast diet supplemented with 0.5% of DL-methionine was fed to the rats.

5. Maximum protein intake, growth and protein efficiency were obtained on a combined supplementation of the *Torula*-yeast diet with 0.5% of DL-methionine, 200 μg of vitamin B_{12} per kilogram of diet and 2 mg of α-tocopherol per week.

6. A level of 0.5%, or more, of DL-methionine in the diet or 2 mg per week of α-tocopherol or both imparted full protection against gross hepatic necrosis to rats maintained on the *Torula*-yeast diet.

7. Vitamin B_{12} did not protect against the gross hepatic necrosis observed in rats maintained on the *Torula*-yeast diet.

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THE EFFECTS OF INORGANIC SALTS ON FLUORINE STORAGE IN THE RAT[1]

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The importance of fluorine in human nutrition is perhaps best exemplified by the fact that approximately 20 million Americans are presently ingesting water containing 1.0 p.p.m. or more of fluorine (Anonymous, '54). These persons may be ingesting a total of 1.5 to 2.0 mg of fluorine per day from the combined food and water sources (McClure, '53). However, in warm, humid regions, the amount may be considerably larger. If the fluorine were completely available for absorption, a high percentage might be stored in the body, preferentially in the skeleton. Thus, it seems of practical interest to determine the amount of fluorine stored in the skeleton as well as in the entire carcass of the rat after the administration both in the presence and absence of common inorganic ions of a quantity of fluorine similar to that ingested by people living in a community in which the water supply is fluorinated. The inorganic salts chosen for study were those of calcium, magnesium and aluminum. Calcium and magnesium are of particular interest since it is advocated by some that fluorine could logically be placed in milk instead of in the community water supply and thus provide the element only to that portion of the population interested in fluorine therapy. In addition, a study was conducted in order to determine the storage rate of identical amounts of fluorine administered in the dry diet

[1] This work was supported in part by a grant from the Indiana State Board of Health, Indianapolis, Indiana.

and in the water. Previous workers have considered these problems and have arrived at several different conclusions, but no one has measured the amount of fluorine storage in the total animal nor used techniques similar to those described in this paper.

EXPERIMENTAL

The experiments were divided into two series: series I, in which the fluoride was placed in aqueous solutions together with the salts of the various inorganic elements, and series II in which identical amounts of fluoride were given to the rat in the dry diet or in the drinking water. In series I, approximately 100 weanling rats of the Sprague-Dawley strain were divided into 6 groups. Each animal in group I received 2.0 ml of an aqueous fluoride (sodium fluoride) solution containing exactly 1000 μg F/ml, administered daily by stomach tube for 14 days. For the animals of group 2 sufficient calcium chloride solution was added to the stock fluoride solution to give final concentrations of 1.0, 0.1 and 0.01% calcium and to make the fluorine concentration identical to that received by the animals of group 1. Similarly, the animals of group 3 received calcium pyrophosphate at a calcium concentration of 0.1 and 0.01%, those of group 4, magnesium chloride at a level of 1.0, 0.1 and 0.01% of magnesium; group 5, aluminum chloride at 1.0, 0.1 and 0.01% of aluminum; and group 6, aluminum oxide at 0.1 and 0.01% levels of aluminum. All of the salts were soluble at the concentrations used except the calcium pyrophosphate and the aluminum oxide.

All of the animals received the same stock diet (F = 0.5 μg/gm), and fluorine-low drinking water (F = 0.05 μg/ml), and were housed in raised screen cages in a temperature-controlled room. The composition of the diet has been described previously (Muhler and Day, '50). The fluoride-salt solutions were given by stomach tube once each day for 14 days, after which time the rats were killed by ether, the right femur was removed (Muhler, Nebergall and Day, '54), and the remainder of the animal was skinned and frozen until

ready for total carcass analysis. The reason for choosing the femur for fluorine analysis is because of its convenience, and the fact that it has an adequate fluorine content. The teeth of individual rats, for example, are difficult to analyse accurately when the fluorine concentration in the supplement is low.

The procedure for ashing whole animal carcasses for the determination of fluorine was as follows: the carcass, after removal of the right femur, was skinned in order to prevent fluorine contamination from external sources, placed in a silica dish and charred over a Meeker burner. The ashing was completed by placing the charred carcass in the muffle furnace at 550 to 600°C. for 6 to 8 hours. The ash was cooled, weighed, then pulverized and mixed with the aid of a glass rod. A sample of the ash was then weighed and placed directly in the fluorine stills for analysis. An attempt was made to determine the amount of fluorine lost during charring by drawing the fumes from this procedure through a water cooled condenser and a flask containing a solution of calcium oxide, arranged so that all the condensate passed into the flask. The contents of the flask were then dried, ashed, and analyzed for fluorine. The results indicated a loss of less than 1% of the total fluorine in the animal carcass.

A total of 18 animals were used in the series II experiment. In this series there were three experimental groups, one which received a stock diet to which was added sufficient fluoride (as sodium fluoride) to produce a fluoride concentration of exactly 5.0 μg/gm and group two, which received an identical concentration of fluorine in the drinking water. A third group received no fluoride supplement in either the food or drinking water and served as a control group in order to determine the amount of fluorine storage from the dietary and water sources alone. All of the supplements were analyzed repeatedly by the modified Williams method in order to maintain the correct fluoride levels (Muhler, Nebergall and Day, '54).

In order to be sure that equal amounts of fluoride were ingested by the animals of series II, paired feeding techniques were used. This was accomplished by accurately determining the amount of food eaten by the animals receiving the fluoride in the dry diet and then giving the animals receiving the fluorine in the water the exact amount of fluoridized drinking

TABLE 1

Percentage of the total fluorine ingested which is stored in the femur and whole carcass of the rat when different inorganic salts are administered with the fluoride solution. A total of 24 mg of fluorine was given to all animals by stomach tube.

SALT	CATION CONC.	TOTAL F IN FEMUR	TOTAL F IN WHOLE CARCASS	TOTAL F STORED IN CARCASS IN PERCENTAGE OF TOTAL INGESTED
	%	*mg*	*mg*	
$CaCl_2$	1.0	0.140	3.13	11
	0.1	0.220	5.92	21
	0.01	0.443	10.95	39
$Ca_2P_2O_7$	0.1	0.509	13.91	50
	0.01	0.505	13.59	49
$MgCl_2$	1.0	0.261	5.88	21
	0.1	0.329	7.77	28
	0.01	0.521	11.43	41
$AlCl_3$	1.0	0.206	4.22	15
	0.1	0.422	8.49	30
	0.01	0.509	12.21	44
Al_2O_3	0.1	0.510	13.22	47
	0.01	0.502	12.44	44
None		0.544	11.98	43

water. When the animals had consumed all of the fluoridized drinking water, they were given fluoride-low water and the stock diet ad libitum as were the animals receiving the fluoride in the diet. At the termination of the experimental period of 40 days, it was estimated that both groups had received almost identical amounts of fluorine (1.8 mg). The animals of series II were prepared for analysis as described previously for those of series I.

RESULTS

The results of series I are presented in table I, which indicates the total amount of fluorine found in the femurs of the various groups of animals which received 2.0 mg of fluorine per day by stomach tube in the presence of various inorganic ions, as compared to a control group receiving an identical amount of fluorine in the absence of any of the added inorganic salts. These data indicate that calcium in the form of calcium chloride markedly interferes with fluorine storage at all three levels tested. The greatest decrease in fluorine storage, 74%, occurred with a calcium concentration of 1.0%. However, even at a calcium concentration of 0.01% there was a 19% decrease in fluorine storage. Calcium in the form of calcium pyrophosphate did not affect fluorine storage, due undoubtedly, to the insolubility of this calcium compound. This indicates that the form of the calcium salt is an important factor in the interference produced by this element. This is further strengthened by the observation that aluminum chloride decidedly interferes with fluorine storage while the insoluble aluminum oxide does not. Apparently, aluminum chloride is not as effective in inhibiting fluorine storage as calcium chloride, since at each aluminum concentration studied, more fluorine is stored in the skeleton than at similar levels of calcium as calcium chloride. Magnesium chloride is intermediate in its action between calcium chloride and aluminum chloride. At a magnesium level of 1.0% there is a 52% decrease in fluorine storage, a 40% decrease at 0.1%, while at a 0.01% level the cation does not affect the storage of fluorine in the skeleton. These conclusions differ somewhat from those of McClure ('48) whose work indicates that magnesium does not decrease the anticariogenic activity of fluorine in the rat. Conclusions similar to those reported by McClure were reached by Schuck ('38).

The results of the analysis of the whole carcass are seen in table I and indicate essentially the same relative findings as shown by the femur analysis. However, from these data it

is possible to determine the effect of the various inorganic salts on the total amount of fluorine stored in the whole carcass as well as the amount of fluorine stored in the absence of the added inorganic ions. These data indicate that the calcium chloride decreased the amount of fluorine in the whole animal to the greatest degree. The effect of the calcium interference appears to be almost linearly dependent upon the cation concentration. At a 1.0% level 3.13 mg of fluorine was found in the whole carcass, while 10.95 mg was present when the same amount of fluorine was administered in the presence of 0.01% calcium. The inhibitory effects of magnesium and aluminum are evident at the 1.0 and 0.1% levels,

TABLE 2

A comparison between fluorine storage in the skeleton of the rat when identical amounts of the element are fed in the dry diet or in the drinking water

DIETARY SUPPLEMENT	FEMUR ANALYSIS		WHOLE CARCASS	
	F conc.	Total F	F conc.	Total F
	(μg/gm)	(μg)	(μg/gm)	(μg)
Diet	264	33	182	825
Water	327	45	220	1,029
Controls	34	4	30	135

while the administration of calcium pyrophosphate or aluminum oxide does not significantly decrease the amount of fluorine stored. It is evident that in the absence of any interfering substance, about 50% of the total fluorine administered is retained.

Table 2 shows the data for the animals of series II and indicates that less fluorine is stored by the animals receiving the fluoride diet than by those receiving an identical amount of fluorine in aqueous solution. When the total amount of fluorine stored in the femurs of the animals receiving the fluoride in the dry diet is compared with that in the femurs of the animals receiving the fluoride drinking water, there is a 36% greater storage in the fluoride-water animals. Similar

conclusions can be reached when comparing the results of the analysis of the whole carcass. Furthermore, when the total amount of fluorine ingested throughout the experiment is compared to the total amount found in the whole carcass it is evident that only 46% of the fluorine in the dry diet was stored in the carcass, while 57% was stored when the element was present in aqueous solution. Not only do these data indicate that fluorine storage is affected by the constituents of the diet, but also that even though the element is readily available for absorption, as it undoubtedly is when present in aqueous solution, only about 55% of the total amount ingested is stored in the body. One could predict from these data that it would take a considerably greater amount of fluorine in the presence of dietary components to produce effects similar to those noticed in the teeth when 1.5 to 2.0 mg of fluorine per day are ingested in the communal water supply during the period of tooth development. It would further appear from those data that the addition of fluorine to milk would not be the best method of administration since the amount of calcium present in milk (approximately 0.1%) would decrease fluoride storage markedly. These data appear interesting in light of the report of Massler and Schour ('52) in which it was found that a much lower fluorine concentration in the drinking water will produce marked endemic dental fluorosis when the diet of the children is low in calcium.

SUMMARY

The administration by stomach tube of identical amounts of fluorine (as sodium fluoride), in the presence of various inorganic salts, has shown that not only the form of the inorganic salt but also its concentration and solubility are of importance when considering its effect on the storage of fluorine in the skeleton of the rat. Calcium in the form of calcium chloride decidedly decreased fluoride storage even at a calcium concentration of 0.01%, while calcium pyrophosphate did not alter the fluorine storage, in comparison with

a similar control group which did not receive the added inorganic elements. Magnesium, in the form of magnesium chloride, and aluminum, in the form of aluminum chloride, also decreased fluoride storage at 1.0% cation concentrations, with the effect of aluminum being somewhat more pronounced than that of magnesium.

When the fluoride is added to a dry diet there is less fluorine in the skeleton of the rat than when identical amounts are received by the rat in the drinking water. Of the total amount of fluorine ingested, a maximum of 50% is stored in the entire carcass of young rats when the fluorine is in aqueous solution.

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APPARENT DIGESTIBILITY OF DIETARY PROTEIN AS A FUNCTION OF PROTEIN LEVEL [1]

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TWO FIGURES

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Any estimation of the usefulness of dietary protein involves a consideration of its digestibility.

The figure for apparent digestibility of protein, corrected for urinary loss, is employed in calculating the caloric value of the food protein according to the Atwater (1899) scheme. For a practical purpose such as this, the use of apparent digestibility is preferred to true digestibility, because the former effectively represents that portion of the dietary protein which can be used by the body for general metabolism over and above that required to replace the constant loss as metabolic protein.

It has been generally assumed that there is a relationship between the type of food and the apparent digestibility of its protein. It is interesting to note that Atwater's grouping of foods into animal foods and vegetable foods also divides them into high- and low-protein groups with corresponding high and low figures for apparent digestibility of protein. Mitchell ('42) claims that the relationship between forage protein content and apparent digestibility of protein is of an exponential nature. Homb and Brierem ('52) in experiments with sheep, found that the protein content of the herbage

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada. Journal Series no. 353.

[2] National Research Council Scholar.

had a greater influence on the apparent digestibility of the protein than had "any other factor." Furthermore, Schneider et al. ('51, '52) reported a decrease in error variance if digestion coefficients were adjusted for proximate composition. This evidence indicates that the apparent digestibility of protein may be influenced by the amount of protein in the food.

The experiment herein reported was designed in an attempt to explain such a relationship.

TABLE 1

Composition of basic mixes

INGREDIENTS	SHREDDED WHEAT MIX	EGG OR CHEESE MIX
	%	%
Shredded wheat	97	
Cheese or egg		97
Bonemeal	2	2
Salt	0.5	0.5
Vitamin supplement [1]	0.5	0.5

[1] One thousand grams of vitamin supplement consisted of 0.05 gm thiamine hydrochloride, 0.20 gm riboflavin, 7.50 gm dry vitamin A (10,000 I.U./gm), 87.50 gm dry vitamin D (1,650 I.U./gm), 904.75 gm dextrose.

EXPERIMENTAL

Two series of feeding trials with rats were carried out. Dry cheese in one series and whole egg powder in the other series were fed as 0, 20, 40, 60, 80 and 100% of the ration, the balance being made up of one or another of three ground shredded wheat mixtures. These latter were (1) 100% shredded wheat mix, (2) 80% shredded wheat mix + 20% methocel, (3) 60% shredded wheat mix + 40% methocel. The composition of the two basic mixes is shown in table 1.

Three hundred and six rats averaging 28 days of age were allotted to individual wire-bottom feeding cages and allowed their respective rations ad libitum for the next 28 days. Six rats were assigned to each subgroup of the egg, and 11 to each of the cheese series.

Coefficients of apparent digestibility of protein were determined by the time collection method.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between per cent digestibility and percentage of protein in the diet, and indicates the close relationship between them.

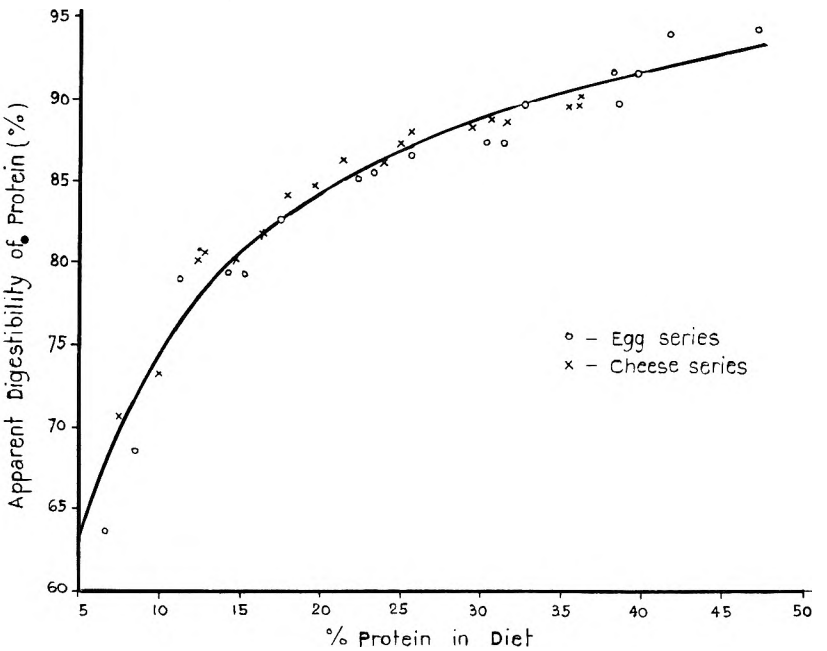

Fig. 1 Relation between the percentage of crude protein in the diet and its apparent digestibility.

Multiple correlation and partial regression were employed to determine the quantitative relationships between the experimentally determined coefficients of protein digestibility (y) as the dependent variable, and the grams of dry matter eaten (x_1), grams of protein eaten (x_2), grams of protein excreted in the feces (x_3), and grams of methocel eaten (x_4) as independent variables.

The first item of significance in the results of the analysis was that 97% of the total variability in the digestion coefficients was accounted for by the 4 variables, as indicated by the value $R^2 = 0.97$.

The beta values or standard regression coefficients show that 73% of this variability is directly traceable to the quantity of protein consumed. Dry matter intake and methocel intake together accounted for only 10% of the variability. The effect of total fecal protein was about one-fifth that of protein intake. Fecal protein, however, is inevitably important in any consideration of protein digestibility and this variable was examined further.

The total fecal protein was adjusted by simple regression to average dry matter intake of the trial (11.6 gm) and these values were plotted against the percentage of protein in the diet as shown in figure 2.

The regression line showing the relationship between fecal protein (Y) and % protein (X) was calculated to be $Y = 0.167 + 0.0074X$.

The extrapolation of this line to the ordinate representing zero protein intake would give a value of 0.167 mg as an estimate of the quantity of fecal protein not arising directly from ingested protein. A part of it presumably arises from previous intake which has been synthesized into digestive enzyme protein. It perhaps includes as well some protein of abraded intestinal mucosa. One might postulate that it would also contain a relatively small amount of protein arising from a greatly reduced microflora able to survive a protein-free diet. In any case, it is generally agreed that the variation in the amount of this metabolic protein is primarily dependent on the amount of dry matter eaten (Schneider, '35).

It is seen in figure 2 that there is a small but steady increase in the total fecal protein with increasing percentage of protein of the ration. Since this rise in fecal protein level was a consequence of additions of egg or cheese, whose apparent protein digestibility was as high as 94%, to wheat, whose apparent protein digestibility was only 80%, then the

increase is not likely to be due to an increase in dietary protein residue.

To explain these observations, it is postulated that this increase over the metabolic excretion of some 7 mg of fecal

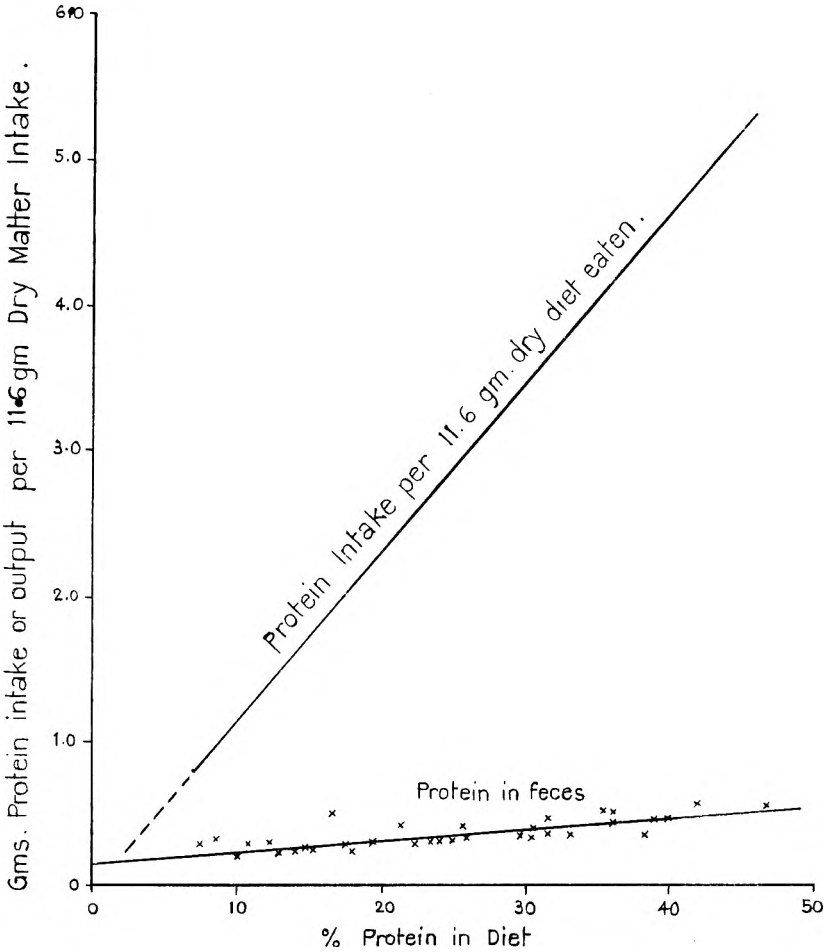

Fig. 2 Daily protein intake and protein in feces at varying levels of dietary protein.

protein for each 1% increase in protein of the diet represents a growth of microflora resulting from increasing protein supplies available to them. This, in turn, leads to the deduction that dietary protein residues were negligible in quantity,

if present at all, and that true digestibility of wheat, cheese, and egg protein is 100%. Confirmation of this hypothesis awaits a method for the specific determination of fecal protein of bacterial origin.

One significant consequence of the relationship between protein level in the diet and the apparent digestibility of the protein is its effect on the calculated "Atwater" physiological fuel value of protein. This is shown in table 2.

TABLE 2

Physiological fuel value of protein according to its level in the food

% PROTEIN IN FOOD (1)	DIGESTION FACTOR (2)	DIGESTIBLE CALORIES PER GRAM PROTEIN (2) × (5.65 Cals)	PHYSIOLOGICAL FUEL VALUES (CALS PER GRAM PROTEIN) (3) — (1.25 Cals)
5	0.63	3.56	2.31
10	0.74	4.18	2.93
15	0.81	4.58	3.33
20	0.84	4.75	3.50
25	0.87	4.92	3.67
30	0.89	5.03	3.78
35	0.90	5.09	3.84
40	0.92	5.20	3.95
45	0.93	5.25	4.00

SUMMARY AND CONCLUSIONS

Coefficients of apparent digestibility of protein for rats were obtained on a wide range of combinations of wheat, methocel, egg and cheese.

Partial regression and multiple correlation analysis of the data revealed that the greatest portion of the variation in the protein digestibility coefficient is accounted for by the percentage of protein in the diet. Therefore, although different types of foods frequently have characteristic protein levels, any relation between type of food and the apparent digestibility of its protein is coincidental.

Adjustment of the total fecal protein to average dry matter intake revealed that there was a small increase in fecal protein with increasing percentage of protein in the diet. It was

postulated that this increase was due to bacterial protein, and that the true digestibility of the protein in all of these diets was 100%.

Thus, differences in apparent digestibility of protein of different types of foods are not primarily due to inherent differences in the true digestibility of their protein, but are mainly a consequence of the effect of a relatively constant metabolic fecal nitrogen on the calculation of the proportion of the protein intake which "disappears" between its ingestion and subsequent fecal output.

ACKNOWLEDGMENT

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WEIGHT INCREMENTS OF SUCKLING RATS AS AFFECTED BY LITTER SIZE AND MATERNAL DIET [1]

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At present the lactation performance of small laboratory animals can be evaluated only by indirect means (Daggs, '35; Brody and Nisbet, '38; Falconer, '47). A criterion of measurement frequently used is the weight increment of the young during the nursing period. With mice and rats it is common practice to restrict the number of suckling young to 6 (Daggs, '35; French et al., '52). The lactation performance is then evaluated by the number or weight of the young which survive to weaning or by the weight increment during specified periods. Under such conditions, however, the mother is not induced to maximum milk production and, hence, her performance and that of the young may not reflect inadequacies of her diet which might become apparent under greater stress of lactation. While the survival and weight increment of individual young are affected by the lactation of the mother, these criteria do not necessarily reflect her lactation potential or the effect of the diet thereon. The total weight increment of large litters during that period when the young consume only mothers' milk might be a better measure of lactation. Cowie and Folley ('47) have recorded the weight increment of litters of rats from the 5th to the 11th day post-partum; French et al. ('52), that of individual rats from the third to

[1] Paper 3167, Scientific Journal Series, Minnesota Agricultural Experiment Station.

TABLE 1

Composition of diets used

YELLOW CORN DIET	%	SUCROSE-CASEIN DIET	%
Ground yellow corn	68.49	Casein, ethanol extracted [1]	24.00
Dried buttermilk	2.25	Hydrogenated vegetable fat (Crisco)	7.00
Tankage, 60%, rendered	9.25	Corn oil + vitamins A, D_3, E [1]	1.00
Soybean oil meal (expeller process)	9.25	Salts 4 [1]	4.00
Alfalfa leaf meal	9.25	Sucrose + B vitamins [1]	2.00
Mineral mixture (Swift and Co.)	1.50	Sucrose	62.00
Irradiated yeast	0.01	Total	100.00
Total	100.00		

ROLLED OATS-CASEIN DIET	%
Rolled oats (ground in hammermill)	84.00
Casein, leached [2]	6.50
Salts 4 [1]	3.00
Wheat germ oil	2.00
Corn oil + vitamins A and D_3 [3]	1.00
Sucrose	1.17
Sucrose + B vitamins [4]	2.00
DL-methionine	0.33
Total	100.00

[1] Schultze, '50. (See table 1.)

[2] Prepared from fresh skim milk by precipitation at pH 4.6 and washing with 40 changes of dilute acetic acid at pH 4.6 during a period of 14 days, followed by dehydration with ethanol and drying.

[3] Contains 1,000,000 I.U. vitamin A acetate and 150,000 I.U. vitamin D_3 per kilogram of the mixture.

[4] See footnote 1 above; 40 μg vitamin B_{12} added per kilogram of ration.

the 14th day and Falconer ('47) the total weight of litters of mice at 12 days of age as indices of lactation.

Incidental to other studies in this laboratory during several years, data have been collected which demonstrate that both the number of suckling young and the diet of the mothers determine the milk output of the rat.

EXPERIMENTAL

Diets. The diets shown in table 1 were fed to rats for other purposes. They supported essentially equal weight increments of young rats after weaning. While these diets were not specifically designed for comparative studies of lactation of the rat or to assess the merits of individual components in this respect, their effect on lactation did reveal nutritional differences which were not apparent by other criteria of performance of the animals.

Animals. The rats used were from the black strain of Line 3 maintained in this laboratory for many years. Each group was fed one of the diets shown in table 1 from weaning and throughout the reproductive and lactation period. In many instances, their ancestors had been fed the same diets for two or three generations. A few days before the end of the first to 4th pregnancies the dams were housed singly in cages bedded with clean wood shavings. The young were weighed shortly after birth and daily thereafter. In no case was the size of the litter changed by removal of young or by the addition of foster young. Only those litters in which no young died were used for calculation of the data presented here. The final weight used for calculation of the weight increment of the young during the nursing period was that attained at the end of the 16th day of life, i.e. before the young consumed any of the maternal diet.

RESULTS AND DISCUSSION

The pertinent data showing the effects of maternal diet and litter size on weight increments of suckling rats are

TABLE 2

Mean weight increments of rats during 16 days after birth

NO. OF ANIMALS PER LITTER	YELLOW CORN DIET			CASEIN-SUCROSE DIET			ROLLED OATS-CASEIN DIET		
	Weight increment		No. of litters	Weight increment		No. of litters	Weight increment		No. of litters
	per rat	per litter		per rat	per litter		per rat	per litter	
	gm	*gm*		*gm*	*gm*		*gm*	*gm*	
1	...	...	..	...	...	..	9.0	9.0	1
2	26.1	52.2 ± 3.1 [1]	4	...	...	..	20.4	40.9 ± 5.3 [1]	10
3	27.9	83.7 ± 1.1	11	23.0	69.0 ± 4.7 [1]	5	24.3	73.0 ± 3.2	13
4	25.1	100.3 ± 3.6	19	26.4	105.5 ± 5.5	10	26.0	104.2 ± 3.4	20
5	24.3	121.4 ± 4.6	16	24.1	120.2 ± 2.8	22	24.7	123.5 ± 2.4	17
6	21.7	130.2 ± 2.5	27	21.7	130.3 ± 4.5	19	22.2	133.3 ± 3.9	16
7	20.4	142.6 ± 4.3	31	19.9	138.5 ± 4.3	19	22.7	159.2 ± 2.9	41
8	19.2	153.5 ± 4.1	29	19.3	154.6 ± 3.3	15	22.7	181.3 ± 1.7	44
9	17.7	158.9 ± 4.8	35	18.7	168.5 ± 2.0	18	21.4	192.7 ± 12.3	40
10	17.1	171.1 ± 5.6	21	16.2	162.4 ± 8.9	7	19.8	198.3 ± 6.9	32
11	17.6	193.2 ± 10.4	13	16.1	177.7 ± 8.6	3	20.1	220.8 ± 13.4	19
12	16.3	196.0	1	18.2	218.5	2	19.8	238.1 ± 12.6	10
13	15.4	199.0	2	...	...	.	18.4	239.7	3
14	...	...	..	...	...	..	18.3	256.0	1
Highest value observed		233.0 (11) [2]			225.0 (12) [2]			285.0 (12) [2]	

[1] Standard error of the mean.

[2] Number of young in litter that had the highest weight increment.

summarized in table 2. As a measure of lactation performance, particular attention should be given to the total weight increment of the litters from birth through the 16th day of life. This parameter is presumably affected by the nutritional quality and quantity of the milk, by the efficiency with which the milk is used to produce weight increments (Brody, '45) and by the inherent growth potential of the young. The latter is not a limiting factor in the weight increment attained by the rats because Cox et al. ('54) reported that through the alternate use of several resting nurses for one litter the weight gains of young rats could be greatly increased over those of rats suckled normally by their own mother. The progressive decline of the mean weight increment of each individual nursling with increasing litter size (when there were more than 3–4 young in a litter) also indicates that the inherent growth potential of the young was not a limiting factor in the large litters. There is no evidence to indicate that either the nutritional qualities of rats' milk with respect to its composition or the efficiency of its utilization by the young decrease with a greater number of young per litter. The data in table 2 demonstrate, therefore, that the greater total weight increment achieved by the litters with increasing numbers of young reflects the production of a larger quantity of milk by the mother. This in turn appears to be the result of the more intense milking by the young. Maximum milk production, with adequate diets, is apparently not attained until the number of young nursed is at least 12 or more per litter. The number of functional mammary glands in this strain of rats is usually 12 and frequently all are functional even when there are only 5 to 6 young in a litter.

As shown in table 2, a valid estimate of the maximum lactation potential of the rat cannot be obtained unless 12 or more rats are used as nurslings and the total weight increment of the young serves as a criterion of measurement. Furthermore, it may not be possible to differentiate between the nutritional value of various diets for the support of lactation unless the rat is subjected to a severe stress of

lactation performance produced by large numbers of nurslings. Thus, the total weight increment of those litters containing 6 rats was essentially the same with the three different diets shown in table 1. Only when each litter comprised 7 or more young did the superiority of the rolled oats-casein diet become apparent. The total weight increments produced by the yellow corn diet or the casein-sucrose diet were not consistently different from each other even in the larger litters. However, when maternal diets are very inadequate for the support of lactation, such as in the case of amino acid deficiencies (Schultze, '53, '54) the effect on weight increments of individuals or litters becomes apparent even when only small numbers of young comprise each litter.

The measurement of lactation performance and the evaluation of diets for this purpose through the total weight increment of large litters as suggested above is, of course, applicable only when the mortality of the young is relatively low. Several conditions have been described in which inadequate maternal nutrition caused death of most of the young shortly after birth or in the later stages of the nursing period. In such cases where faulty maternal nutrition leads to intrauterine damage of the young and congenital malformations (Hogan, '53; Glass and Schultze, '54) or to metabolic abnormalities in the newborn such as the syndrome of acute uremia of the young (Schultze, '49) or to the inability of the mother to initiate and maintain a flow of milk, other criteria must be used to evaluate the effect of maternal diets on the postnatal performance of the young. Such conditions may have been operative in those 11 litters cast by mothers fed the rolled oats-casein diet in which only one or two young were born and survived. The performance of these young certainly did not reflect the potential ability of this ration to support lactation.

In many instances rats fed the rolled oats-casein ration maintained or increased their weight while they were nursing 11 or more young successfully and while the young made

weight increments during 16 days about equal to the weight of the dam.

SUMMARY

1. As a criterion of lactation performance, the weight increments from birth through the 16th day of life have been measured in litters of rats containing from one to 14 individuals.

2. With increasing litter size there was a progressive decrease in the mean weight increment of each nursling but a marked increase in the total weight gain of the litter, reflecting greater milk production of the mother.

3. The superiority of certain maternal diets for the support of heavy lactation may become apparent only when the number of young per mother is greater than 6.

ACKNOWLEDGMENTS

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STUDIES OF ANTIBIOTICS IN WEANLING RATS ADMINISTERED SUBOPTIMUM LEVELS OF CERTAIN B VITAMINS ORALLY AND PARENTERALLY

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(Received for publication June 24, 1954)

INTRODUCTION

Reports by Lih and Baumann ('51) and by Sauberlich ('52) have shown that antibiotics stimulate the growth of rats fed diets low in several B vitamins. Investigations at this laboratory have been concerned with the interrelationships of aureomycin and vitamin A (Hartsook et al., '53a), terramycin and methionine (Hartsook and Johnson, '53b), penicillin and thiamine, streptomycin and pantothenic acid as well as the interrelationships of certain surface active agents and of arsanilic acid with thiamine (Johnson et al., '53). It was demonstrated that while certain antibiotics protect rats on low levels of thiamine and pantothenic acid, they had no effect on vitamin A deficiency and, in contrast to the antibiotics, the surfactants and arsanilic acid, had no effect on B-vitamin deficiencies. These latter findings are in agreement with the work of Frost and Spruth ('53). The growth stimulation of terramycin on a diet low in methionine was found to be due to an increased fat deposition.

Recently Waibel et al. ('53) reported that dietary antibiotics failed to increase the growth of chicks given suboptimum amounts of thiamine by subcutaneous injection. Since our previous work indicated that antibiotics increase the synthesis of certain B vitamins by the intestinal microflora

(Johnson et al., '53), we felt that the plan of Waibel et al. would offer us an opportunity to test this theory further. By a comparison of the effects of orally administered antibiotic in rats which received suboptimum amounts of the vitamins, either by mouth or by subcutaneous injection, we hoped to find out whether (a) the antibiotic made available

TABLE 1

Composition of the basal diet

CONSTITUENTS		CONSTITUENTS	
	%		*mg/kg*
Sucrose	71.1	Water soluble vitamins:	
"Vitamin free" casein (Labco)	18.0	Folic acid	4.0
Mineral mixture number 446 [1]	4.0	Biotin	0.6
Corn oil	4.0	Vitamin B_{12}	0.05
Choline dry mix (25% choline)	0.4		
Vitamin premixes	2.5		*per 100 gm*
		Fat soluble vitamins:	
	mg/kg	Vitamin A	2000 I.U.
Water soluble vitamins:		Vitamin D	200 I.U.
Nicotinic acid	100.0	Alpha-tocopherol acetate	12 mg
Riboflavin	16.0	2-Methyl-1,4-naphtho-quinone	0.1 mg
Pyridoxine hydrochloride	6.0		

[1] Composition of mineral mixture number 446:

Constituent	*gm*	*Constituent*	*gm*
NaCl	243.198	$CuSO_4 \cdot 5H_2O$	0.4
$K_3C_6H_5O_7 \cdot H_2O$	533.0	$MnSO_4$	2.8
K_2HPO_4	174.0	$K_2Al_2(SO_4)_4 \cdot 24H_2O$	0.2
$CaHPO_4 \cdot 2H_2O$	800.0	KI	0.1
$CaCO_3$	368.0	$CoCl_2 \cdot 6H_2O$	0.2
$MgCO_3$	92.0	$ZnCO_3$	0.1
$FeC_6H_5O_7 \cdot 3H_2O$	36.0	NaF	0.002
		Total	2250.000

more of the limiting vitamin fed (either by a reduced destruction in the tract or improved absorption), or (b) the antibiotic effect was due to an increased synthesis of the limiting vitamin in the tract.

In the experiments reported here the effects of penicillin and aureomycin were studied in rats receiving suboptimum amounts of thiamine and pantothenic acid respectively by both oral and subcutaneous administration.

EXPERIMENTAL

A total of 128 weanling male albino rats of the Sprague-Dawley strain were used in these experiments. They were housed individually in wire bottom cages in a temperature-controlled laboratory and fed ad libitum. Data on individual food consumption and weight gains were recorded at weekly intervals.

The composition of the basal diet is shown in table 1. In the first study the basal diet was supplemented with pantothenic acid at the levels of two and 40 μg per gm of ration when orally administered and at 20 μg per gm of ration when injected. The effect of this supplementation was studied in the presence and absence of 200 μg of aureomycin per gm of ration.

In the second experiment thiamine was added at levels of 0.5 and 25 μg per gm of ration when administered orally and at 0.25 and 2.5 μg (later 10 μg) per gm of ration when injected. Penicillin was supplied to half of the animals in each treatment at a level of 50 μg per gm of ration. These levels of supplementation were chosen to represent critical amounts as evidenced by earlier work in this laboratory.

Since the basal diet contained neither of the B vitamins in question, an aqueous solution of thiamine was pipetted on to the ration of all animals in the pantothenic acid study at a level of 10 μg per gm of ration. Pantothenic acid was similarly administered to all animals in the second experiment at a level of 40 μg per gm of diet.

The parenterally administered vitamins were injected subcutaneously, at feeding time, in the dorsal thoracic region of the rat. The oral administration was also done at feeding time by pipetting a water solution of the vitamins on to the dry ration in order to prevent destruction of the vitamin.

The animals in the aureomycin-pantothenate study were sacrificed after 7 weeks on experiment and the small intestines excised in groups 1 and 3. The organs were then sectioned longitudinally, pooled into two samples per treatment and

eluted with 250 ml of water. These intestinal contents were assayed for pantothenic acid using *Lactobacillus arabinosus* (Hoag et al., '54). The thiamine-penicillin study was terminated at 4 weeks.

TABLE 2

Response to aureomycin by rats receiving two levels of pantothenic acid

TREATMENT [1]	DAYS ON EXPERIMENT	AVERAGE TOTAL GAIN [2]	AVERAGE DRY MATTER CONSUMED
		gm	*gm*
Oral administration			
2 μg Pantothenic acid/gm ration	49	148	396
2 μg Pantothenic acid/gm ration + aureomycin	49	180	454
40 μg Pantothenic acid/gm ration	42	247	529
40 μg Pantothenic acid/gm ration + aureomycin	42	254	563
Subcutaneous administration			
1 μg Pantothenic acid/gm ration	49	123	361
1 μg Pantothenic acid/gm ration + aureomycin	49	140	399
20 μg Pantothenic acid/gm ration	42	234	516
20 μg Pantothenic acid/gm ration + aureomycin	42	234	514

[1] Aureomycin was supplied in those groups indicated at a level of 200 μg/gm ration.

[2] These figures represent averaged gains of 8 animals per group with the exception of group 3 in which two rats died in the 5th and 6th weeks.

RESULTS

The weights of the rats in the aureomycin study varied initially from 40 to 55 gm and from 35 to 50 gm in the penicillin experiment. The plotting of regression equations of final weights on initial weights revealed this variation to be without significance.

It became apparent by the second week that the subcutaneous administration of 2.5 μg of thiamine per gm of ration was inadequate for maximum growth, under our conditions, when compared with the growth of those animals receiving

25 μg thiamine per gm of ration orally. For this reason the level of thiamine was increased to 10 μg per gm after which the growth stimulus of penicillin observed during the first two weeks disappeared, although its effect is reflected in the 4-week average gain.

The results of the studies are summarized in tables 2 and 3. From the growth data it is evident that the antibiotics, given

TABLE 3

Response to penicillin by rats receiving two levels of thiamine

TREATMENT [1]	DAYS ON EXPERIMENT	AVERAGE TOTAL GAIN [2]	AVERAGE DRY MATTER CONSUMED
		gm	*gm*
Oral administration			
0.5 μg Thiamine/gm ration	28	103	208
0.5 μg Thiamine/gm ration + penicillin	28	161	304
25 μg Thiamine/gm ration	28	181	314
25 μg Thiamine/gm ration + penicillin	28	189	349
Subcutaneous administration			
0.25 μg Thiamine/gm ration	28	66	141
0.25 μg Thiamine/gm ration + penicillin	28	153	268
10 μg Thiamine/gm ration	28	164	314
10 μg Thiamine/gm ration + penicillin	28	187	335

[1] Penicillin was added in those groups indicated at a level of 50 μg/gm ration.
[2] These figures represent average gains of 8 animals per group.

orally, were capable of exerting their B vitamin-"sparing" action whether the limiting vitamin was fed or injected.

The assays of pantothenic acid in the intestinal contents are summarized in table 4. These assays also reveal a greater amount of pantothenic acid in the tract of animals receiving aureomycin and suboptimal amounts of pantothenic acid either orally or subcutaneously than in those which did not receive aureomycin. Similar effects of antibiotics on excretion of a

limiting vitamin have been reported by Guggenheim et al. ('53).

DISCUSSION

An antibiotic growth response to oral vitamin administration with no response to injected vitamin could indicate that the antibiotic either (1) prevented destruction of the dietary vitamin by the flora of the tract or (2) in some way improved the absorption of the vitamin from the tract or both. On the other hand, an antibiotic growth response to both oral

TABLE 4

Effect of aureomycin on the amount of pantothenic acid in the small intestine of weanling rats

TREATMENT	PANTOTHENIC ACID IN INTESTINAL CONTENTS
	μg/gm dry matter
Oral administration	
2 μg Pantothenic acid/gm ration	90.23 [1]
	93.50
2 μg Pantothenic acid/gm ration	112.41
+ aureomycin	99.30
Subcutaneous administration	
1 μg Pantothenic acid/gm ration	73.68
	78.57
1 μg Pantothenic acid/gm ration	83.59
+ aureomycin	99.28

[1] Each figure represents the assay values of pooled intestinal eluates from 4 rats.

and injected low-level vitamin administration could indicate that the antibiotic either (1) increased the intestinal synthesis of the vitamin or (2) prevented destruction by the intestinal flora of vitamin synthesized by other intestinal organisms or (3) improved the absorption of vitamin normally synthesized by the intestinal flora.

Since the antibiotics, when administered by mouth, proved active, whether the vitamin was fed or injected, one or more of the latter hypotheses mentioned above, is indicated; and since more vitamin was found in the tracts of the antibiotic-

fed animals, this would seem to eliminate postulate three and, we believe, favors one. This evidence for intestinal synthesis is in confirmation of the work of Davis and Chow ('51) and of Chow et al. ('53), who found that oral administration of aureomycin increased the amount of radiovitamin B_{12} in feces after feeding Co^{60}. Monson et al. ('54) have also recently demonstrated an increased folic acid synthesis with antibiotic feeding.

The apparent conflict of our results with those of Waibel et al. ('53) probably reflects the wide differences between the rat and the chick in intestinal tract physiology.

It is also conceivable that both the hypotheses which have been suggested are operative in these species but that different ones predominate or are, at least, more apparent in one species than another. Studies conducted here (Scott, '53) reveal that chicks receiving antibiotics have feces of a less sticky consistency which might suggest that antibiotics exert part of their effect, at least, by creating a more "satisfactory" intestinal environment (by way of the gut wall, flora or contents or both) for nutrient absorption. This effect on feces was never observed in the rat.

SUMMARY

Orally administered aureomycin and penicillin markedly increased the growth of 128 weanling rats fed synthetic diets contaning marginal levels of pantothenic acid or thiamine respectively. This effect was observed whether the vitamins were administered orally or subcutaneously.

The antibiotics stimulated growth only slightly or not at all with optimum amounts of thiamine and pantothenic acid present in the ration or administered subcutaneously.

A greater amount of pantothenic acid was observed to be present in the small intestine of those rats receivng aureomycin and suboptimum amounts of pantothenic acid administered either orally or by subcutaneous injection.

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A STUDY OF THE NITROGEN METABOLISM OF LYSINE-DEFICIENT RATS [1]

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Previous studies at this laboratory indicated that when weanling rats were force-fed a synthetic ration devoid of lysine, the nitrogen balance showed a marked tendency to decrease with time, although the animals remained in positive balance until death from the lysine deficiency (Bothwell and Williams, '52). In these studies it was noted that during the first 4-day urine collection period the urinary nitrogen output of the force-fed lysine-deficient group increased and remained at an essentially constant high level for about 10 days. This was in contrast to the comparatively low urinary nitrogen output previously observed when methionine- or histidine-free rations were force-fed (Denton et al., '50; Bothwell and Williams, '51). In all of the experiments, however, the animals remained in positive nitrogen balance.

In an attempt to account for the difficulty of obtaining a negative nitrogen balance in the young animals used in the previous experiments, we have made a detailed study of the nitrogen content and quantitative nitrogen distribution in the blood and urine of young animals force-fed a lysine-deficient diet. The present experiments have been carried

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out using methods as similar as possible to those employed in the nitrogen balance experiments mentioned above.

Although in the lysine-deficiency studies reported earlier, the nitrogen balance was positive throughout the experiment, the positive value of the balance was small and thus might conceivably be accounted for by a retention of the nitrogen in the blood of the animals. An uremic condition might not only help to account for the retained nitrogen but would also indicate a possible cause of death from a lysine deficiency. Therefore, we have studied the changes in total blood nitrogen, blood urea and blood ammonia produced by a lysine deficiency.

In order to identify the component or components that make up the large increase in the output of urinary nitrogen in the force-fed lysine-deficient animals, a study was made of the distribution of nitrogen components in the urine of the force-fed animals. Knowledge of such a distribution might help to clarify the fate of the amino acids of the incomplete protein fed. A significant increase of amino nitrogen excreted would indicate direct excretion of unusable amino acids. Also, since it is known that certain amino acid deficiencies produce losses in tissue enzymes at a faster rate than loss of tissue protein (Miller, '48; Williams et al., '49; Bothwell and Williams, '54), it was considered possible that the enzyme mechanisms involved in urea formation could be adversely affected by the lysine deficiency with a resulting increase of urinary ammonia. Excretion of large amounts of urea would indicate integrity of normal urea-formation and excretion mechanisms, whereas excretion of large amounts of either amino acids or ammonia would indicate a disruption of the normal mechanisms of nitrogen excretion. Therefore, the urinary nitrogen components chosen for study in these experiments were total nitrogen, urea, ammonia and amino acid nitrogen.

EXPERIMENTAL

The nitrogen balance was studied in animals fed a complete and a lysine-free ration by both ad libitum and a force-feeding

procedure. Distribution of urinary nitrogen components was obtained by measuring total, urea, ammonia and amino nitrogen in the urine. Total nitrogen, urea and ammonia of the blood of these groups was also determined. Thirty-six male, weanling rats of the Holtzman strain were separated at random into 4 groups: two groups of 6 rats each (groups I and II), one group of 8 rats (group III) and one group of 15 rats (group IV). Groups I and II were fed ad libitum throughout the experiment while groups III and IV were force-fed by stomach tube. Groups I and III were fed the complete ration and groups II and IV were fed the ration devoid of lysine.

The animals were placed in metal metabolism cages and fed the complete synthetic ration of Ramasarma et al. ('49) either ad libitum or by stomach tube for one week prior to the experiment in order to accustom them to the feeding procedures. This ration consisted of a purified amino acid mix (16%) (Hegsted et al., '41), corn oil (5%), the known vitamins, including vitamin B_{12} (2%), and sucrose to make 100%. All animals were given water ad libitum throughout the experiment.

The animals fed ad libitum (groups I and II) were tongue-fed through wire screen (4 meshes per inch) in order to eliminate as much contamination of the urine and feces as possible. At the end of the one week acclimatization period, the animals had become accustomed to the ration and were gaining weight at a good rate.

Groups III and IV (force-fed control and lysine-deficient groups, respectively) were fed 6 gm of food per day by stomach tube. The ration to be fed was blended with water so that 7 ml of the slurry would be equivalent to 6 gm of dry ration. Feedings were made three times daily, spaced about 6 hours apart.

All urines were collected under toluene acidified with hydrochloric acid. Collections for all groups were made every two days. The urine samples were then diluted to 150 ml for nitrogen determinations. Fecal collections were made at

the same time as urine collections. The feces were covered with 95% alcohol, acidified with one drop of concentrated hydrochloric acid and dried in a steam oven overnight. The dried samples were weighed, ground in a mortar, and portions taken for nitrogen determination.

A semi-micro Kjeldahl procedure was used for all total nitrogen determinations, which were made in duplicate. Urinary ammonia and urea were determined by the urease method (Hawk, Oser and Summerson, '49) using a Nesslerization procedure (Owings and Mandell, '51). Amino nitrogen was determined by the copper method as described by Albanese and Irby ('44).

The urinary nitrogen distribution was not studied for the groups fed ad libitum since the initial observation that led to these studies was concerned with the force-fed animals. The groups I and II fed ad libitum were included to act as nitrogen balance controls to assure consistency with earlier work.

Blood samples, which were obtained from the severed neck vessels of decapitated animals, were oxalated and analyzed for total nitrogen by the semi-micro Kjeldahl procedure. Blood urea and ammonia were determined on tungstic acid filtrate samples of the oxalated blood by the method described by Owings and Mandell ('51).

RESULTS AND DISCUSSION

In tables 1 and 2 are reported results for nitrogen intake, output and balance for the 4 groups of rats over a 10-day period. These values are in agreement with those reported earlier by Bothwell and Williams ('52) for a lysine deficiency. The control group I, fed ad libitum, showed strong positive nitrogen balance throughout the experiment as did control group III, fed the complete ration by stomach tube. As previously noted for the animals receiving the deficient ration ad libitum (group II), the nitrogen intake was markedly decreased while the output was slightly increased so that negative nitrogen balance was achieved within 4 days. This

TABLE 1

Distribution of nitrogen[1] output for animals fed a complete and a lysine-free ration under ad libitum conditions

REGIMEN	PERIOD	NITROGEN INTAKE	NITROGEN OUTPUT			BALANCE
			Urine	Feces	Total	
	days					
Complete ration (Group I)[2]	0	..	72.7	4.9	77.6	....
	1–2	160	81.7	6.2	87.9	+ 72.1
	3–4	162	80.0	5.5	85.5	+ 76.5
	5–6	190	92.2	4.5	96.7	+ 93.3
	7–8	110	73.0	6.3	79.3	+ 30.7
	9–10	153	101	6.3	107.3	+ 45.7
Deficient ration (Group II)[2]	0	..	75.6	5.2	80.8	....
	1–2	123	85.0	7.2	92.2	+ 30.8
	3–4	66.5	90.5	6.1	96.6	— 30.1
	5–6	90.0	92.3	2.1	94.4	— 4.4
	7–8	70.5	85.0	3.7	88.7	— 18.2
	9–10	96.0	104	2.6	106.6	— 10.6

[1] All nitrogen values expressed as milligrams of nitrogen/rat/day.
[2] Group I contained 6 animals; group II contained 6 animals.

TABLE 2

Distribution of nitrogen[1] output for animals fed a complete and a lysine-free ration under force-feeding conditions

REGIMEN	PERIOD	NITROGEN INTAKE	NITROGEN OUTPUT			BALANCE
			Urine	Feces	Total	
	days					
Complete ration (Group III)[2]	0	..	60.5	4.3	64.8	....
	1–2	121	55.7	5.0	60.7	+ 60.3
	3–4	121	61.0	4.8	65.8	+ 55.2
	5–6	121	63.4	4.0	67.4	+ 53.6
	7–8	121	65.3	4.5	69.8	+ 50.2
	9–10	121	89.0	4.8	93.8	+ 27.2
Deficient ration (Group IV)[2]	0	..	61.8	4.5	66.3	....
	1–2	121	111	4.7	115.7	+ 5.3
	3–4	121	108	6.7	114.7	+ 6.3
	5–6	121	109	8.1	117.1	+ 3.9
	7–8	121	107	7.8	114.8	+ 6.2
	9–10	121	113	7.8	120.8	+ 0.2

[1] All nitrogen values expressed as milligrams of nitrogen/rat/day.
[2] Group III contained 8 animals; group IV contained 15 animals at the start and 10 animals at the end of 10 days.

group remained in negative balance throughout the experimental period. Again, the nitrogen balance of group IV, fed the lysine-deficient ration by stomach tube, quickly decreased toward equilibrium by the second day and reached a constant low value. It still remained positive throughout the experi-

TABLE 3

Urinary nitrogen[1] *partition for animals receiving a complete and a lysine-free ration by stomach tube*

REGIMEN	PERIOD	UREA NITROGEN	AMMONIA NITROGEN	AMINO NITROGEN
	days			
Complete ration (Group III)[2]	0	30.6	9.2	11.0
	1–2	30.6	9.6	11.3
	3–4	33.3	10.2	13.4
	5–6	25.7	10.3	12.1
	7–8	42.0	10.6	12.4
	9–10	44.7	10.8	12.4
Deficient ration (Group IV)[2]	0	33.3	9.9	11.3
	1–2	78.4	10.0	15.0
	3–4	70.3	10.6	13.8
	5–6	73.5	10.5	14.4
	7–8	75.0	12.0	13.8
	9–10	68.3	10.3	14.8

[1] All nitrogen values expressed as milligrams of nitrogen/rat/day.

[2] Group III contained 8 animals; group IV contained 15 at start and 10 animals at end of 10 days.

ment, however. The observation noted earlier regarding the immediate increase in urinary nitrogen for group IV was again observed.

In table 3 is reported the distribution of the nitrogen components measured in the urines of the force-fed groups of rats (groups III and IV). It can be seen that there was an essentially constant output of urea nitrogen from the animals receiving the complete ration. Within two days, however, the animals receiving the lysine-deficient ration (group IV) excreted somewhat more than twice the amount

of urea nitrogen as before the introduction of the incomplete ration. This level then remained essentially constant for the remainder of the experiment. Ammonia nitrogen values of the urine remained constant for each group throughout the experiment. The amino nitrogen values were fairly constant for both groups although a slight tendency to increase was noted for the lysine-deficient group after two days. The level of amino nitrogen in both groups was higher than expected. It accounted for about 10 to 20% of the total nitrogen excreted in the urine. These results are in agreement with the recent report by Rose et al. ('54) who indicated that amino acid diets fed adult human males lead to high excretion of alpha-amino nitrogen in the urine. Rose attributed these high values to partial excretion of the D enantiomorphs of valine, isoleucine and threonine. In our rations both D and L forms of these amino acids were used.

If one calculates the numerical difference between the amounts of urea nitrogen excreted by the animals in the force-fed deficient group (group IV) and those in the force-fed complete group (group III) and subtracts this value from the total urinary nitrogen of the deficient group (values in tables 2 and 3), it can be seen that the values obtained are very close to those for the total urinary nitrogen of the control group III. Since there was little difference between the amounts of amino nitrogen excreted by the two groups and no difference between the amounts of ammonia nitrogen, these compounds contributed little to the high level of "extra urinary nitrogen" excreted by the lysine-deficient, force-fed animals. This suggests that almost all of the "extra total nitrogen" present in the urine of the lysine-deficient animals (group IV) was in the form of urea. An inspection of the urinary nitrogen partition for human subjects receiving a lysine-deficient ration as reported by Rose et al. ('54) indicates results very similar to those reported here. The increase in urea nitrogen as reported by Rose accounted for about 97% of the "extra total nitrogen" found in the urine of those receiving the ration with no lysine.

In table 4 is reported the distribution of the blood nitrogen components measured. In these studies measurements were made both for the groups fed ad libitum (groups I and II) and for the force-fed animals (groups III and IV). It can be seen that the total nitrogen concentration of the blood was unaffected by the lysine deficiency when the animals were fed either ad libitum or forcibly. Blood urea nitrogen

TABLE 4

Nitrogen distribution in blood of animals receiving a complete and a lysine-free ration

REGIMEN	TOTAL NITROGEN (mg N/ml blood)	AMMONIA NITROGEN (mg N/100 ml blood)	UREA NITROGEN (mg N/100 ml blood)
Ad libitum complete (Group I)[1]	26.8 ± 2.9[2]	0.2 ± 0.1	10.4 ± 0.6
Ad libitum — lysine (Group II)[1]	27.6 ± 2.2	0.8 ± 0.4	15.4 ± 1.3
Force-fed complete (Group III)[1]	25.4 ± 1.1	2.2 ± 0.7	12.2 ± 0.8
Force-fed — lysine (Group IV)[1]	25.1 ± 1.1	3.1 ± 0.3	17.6 ± 2.8

[1] Group I contained 6 animals; group II, 6 animals; group III, 8 animals; and group IV, 10 animals at the time these analyses were made.

[2] Standard error of the mean.

values all fell within a normal range although there was a tendency for the animals receiving the deficient ration (II and IV) to reflect higher levels than those receiving the complete ration. Ammonia nitrogen levels of the blood appear to be affected much more by the feeding procedure than by the type of ration used. They may reflect results of the absorption of large amounts of amino acids into the bloodstream in a short period because of the force-feeding technique as compared to the small amounts ingested throughout

the day when animals were fed ad libitum. These data indicate that the lysine-deficient animals were not suffering from an uremic condition at a time when they were near death from the amino acid deficiency. The excretory function of the kidney, thus, does not appear to have been impaired by the lysine deficiency. The continued positive nitrogen balance of the lysine-deficient force-fed animals, therefore, cannot be explained by the retention of a large amount of nitrogen in the blood.

That the weanling animals used in these experiments are able to utilize some of the nitrogen of the incomplete protein is indicated by the positive nitrogen balance throughout the experiment. This view is supported by a report of Campbell and Kosterlitz ('48) that when adult rats were fed a protein-free diet and then transferred to a lysine-deficient diet, labile liver cytoplasm was built up in the absence of dietary lysine which must have been obtained by salvage from other tissues of the body. Weanling animals appear to be able to do this to a greater extent than adult animals as reflected by the difficulty of obtaining negative nitrogen balance with young growing animals as compared with the ease that negative balance is induced in adult animals.

SUMMARY

The effects of a lysine deficiency upon nitrogen balance and nitrogen components of the blood and urine have been investigated employing both ad libitum and force-feeding procedures. Under force-feeding conditions a marked increase was noted in urinary nitrogen for animals receiving a lysine-deficient ration. The extra nitrogen was identified as almost entirely urea. As in previous studies, a positive nitrogen balance was obtained throughout the experiment for animals force-fed the lysine-deficient ration. Blood nitrogen could not account for this retained nitrogen. Uremia could not account for the death of the animals from a lysine deficiency as shown by measurement of various nitrogen components as well as the total nitrogen of the blood.

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The formal presentation will be made at the annual meeting of the Institute in the spring of 1955. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1955. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

Chairman, Nominating Committee:

W. D. SALMON
Animal Husbandry and Nutrition
Alabama Polytechnic Institute
Auburn, Alabama

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The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

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Chairman, Nominating Committee:

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