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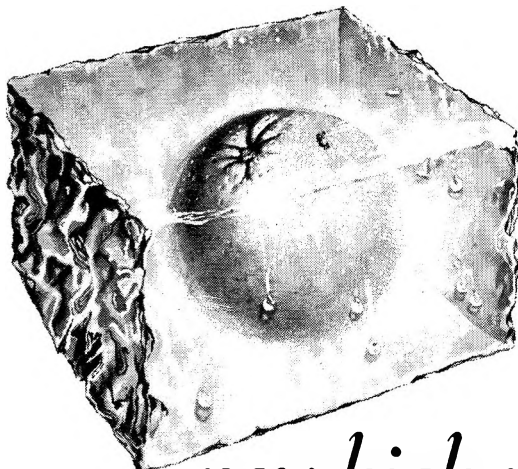
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Meat...

and the Need for Adequate
Protein in Therapeutic Nutrition

Liberal protein intake is considered to be of therapeutic value in a wide variety of pathologic conditions.[1] Advances in the understanding of protein metabolism indicate that dietary protein should provide amino acids in proportions paralleling physiologic needs.[2, 3] In experimental studies with animals, low protein diets supplying amino acids disproportionate to needs have been shown to effect physiologic harm by depressing growth, by inducing amino acid and B-vitamin deficiencies, and by causing deposition of fat in the liver.[4]

Hence not only the *amount* of protein but also its *quality* (in terms of its amino acid proportions) is important. It has been suggested[1] that for therapeutic purposes about two-thirds of the ingested protein come from foods of animal source, whose protein resembles human body protein in amino acid interrelationships. Depending on the needs of the patient, the therapeutic diet may supply 1.0 or more grams of protein per kilogram of body weight. Adequate caloric intake is required to protect the dietary protein from dissipation for energy purposes.

Meat, with its high content of top-quality protein, holds a prominent place among foods which supply this essential for establishing satisfactory levels of amino acids in physiologic proportions. It also contributes valuable amounts of B vitamins and essential minerals— nutrients which play a basic role in intermediate metabolism.

1. Proudfit, P. T., and Robinson, C. H.: Nutrition and Diet Therapy, ed. 11, New York, The Macmillan Company, 1955, pp. 314-320.
2. Harper, A. E.: Amino Acid Imbalance, Toxicities and Antagonisms, Nutrition Rev. *14*:225 (Aug.) 1956.
3. Amino Acid Requirements of Adult Man, Nutrition Rev. *14*:232 (Aug.) 1956.
4. Amino Acid Imbalance and Supplementation, Editorial, J.A.M.A. *161*:884 (June 30) 1956. Council on Foods and Nutrition, American Medical Association: Importance of Amino Acid Balance in Nutrition, J.A.M.A. *158*:655 (June 25) 1955.

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Breakfast Cereals
are Low in Fat

At leading professional meetings, in professional journals, and in health columns and articles in the lay press during the year, there has been a noticeable increase in the tendency to discuss the fat content of the daily food intake.

In dietary regimens recommended by nutrition and medical authorities for the purpose of reducing fat in the diet the importance of the morning meal is given full recognition.

*In the low-fat diet, breakfast cereals deserve con-*sideration because they are low in fat as shown in the following table. Whole grain, enriched and restored cereals, hot and ready to eat, considered as a group can be counted on to supply vitamins of the entire B-complex, important minerals including iron, appreciable quantities of protein in addition to the carbohydrates needed for energy. Thus, breakfast cereals merit inclusion in dietary regimens planned for the purpose of reducing the fat intake in the daily diet.

Nutritive Value of Breakfast Cereals
(Based on composite average)

	Cereal,* 1 oz. Dry Weight Basis
Calories	104
Protein	3.1 gm.
Fat	0.6 gm.
Carbohydrate	22 gm.
Iron	1.4 mg.
Thiamine	0.12 mg.
Riboflavin	0.04 mg.
Niacin	1.3 mg.
Cholesterol	0

*Cereal Institute, Inc.: The Nutritional Contribution of Breakfast Cereals. Chicago: Cereal Institute, Inc., 1956.

In almost Every Diet...

in health and disease

Enriched Bread

remains basic

Regardless of the dietary
adjustment indicated,
Enriched Bread helps supply
needed nutrients...protein,
energy, vitamins, minerals...
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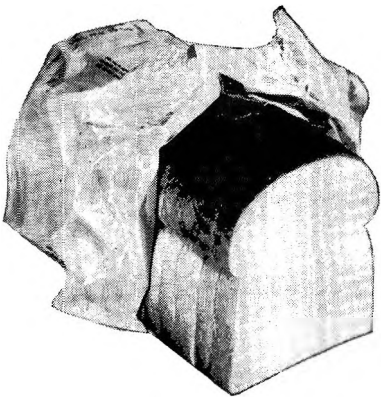
AMERICAN BAKERS ASSOCIATION

20 North Wacker Drive   •   Chicago 6, Illinois

THE VITAL STORY OF

Breakfast Cereals

with essential vitamins and minerals restored

by Science Writer

New Edition

AMERICA LIKES BREAKFAST FOODS

Let no one doubt the popularity of breakfast cereals among Americans. The chart below traces the consumption of these fine foods between 1950 and 1955. During that period annual consumption rose by *76,000 tons*. In just one year, 1955, Americans ate 2½ lbs. of hot and 4.8 lbs. of cold cereals per person!
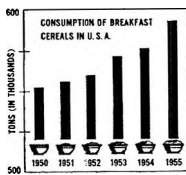
Why are breakfast cereals so well-liked? They are tasty; they are easily served; they appeal to busy homemakers, as well as institutional dietitians, because they are readily available in a variety of flavors at a modest cost. They add interest and value to an important but sometimes neglected meal—breakfast. Their use is extending to between-meal and party snacks, too.

Many grains are processed to make breakfast cereals: wheat, corn, oats, rice. Eaten with fruit and milk or light cream, they contribute an excellent combination of basic, flavorful, nutritious foods to the diet.

Better Foods for Better Health
Through Restoration

The science of nutrition has advanced rapidly. In the manufacturing process of some cereals, some of the essential "B" vitamins and minerals are subject to some loss, just as with other foods. These losses are inescapable when such grains are prepared for human use. When this became known, manufacturers acted to overcome the losses. They adopted restoration.

Restoration simply means that certain important vitamins and minerals are restored to the cereal food during processing, so that the vitamin and mineral values in the finished product are generally equal to the whole grain values of those elements. Wheat, corn and rice products are customarily so treated. Vitamins B_1 (thiamine), B_2 (riboflavin), niacin (another "B" vitamin), and the mineral, iron, are those most widely restored. Vitamins C and D are also sometimes added.

Pre-sweetened cold cereals emphasize the nutritional importance of added vitamins. Increased calories require more "B" vitamins for best utilization of the food.

Why the Vitamins are Important

Physicians and diet experts have proved that vitamins are essential to prevent certain deficiency diseases and to contribute to robust good health.

Vitamin B_1 (thiamine) helps build and maintain physical and mental health. It is essential for normal appetite, intestinal activity, and sound nerves. A lack of this vitamin leads to beriberi, a rarity in the U. S. A., but still a very serious health problem in other parts of the world.

Vitamin B_2 (riboflavin) is essential for growth. It helps to keep body tissues healthy and to maintain proper function of the eyes.

Niacin is needed for healthy body tissues. Its use in the American diet has been largely responsible for the virtual disappearance of pellagra, a serious disease.

Vitamin D helps children develop normal teeth and bones. It prevents the development of certain abnormal bone conditions in adults.
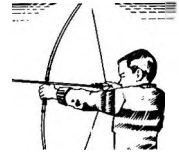
Iron is essential for making good red blood and for the prevention of nutritional anemia.

Where Do the Vitamins Come From?

At about the same time that processing losses in breakfast cereals became known, other developments in the scientific world made available ample supplies of vitamins at economical prices. Thus, the nutritional contribution of some breakfast cereals could be, and was, greatly improved through restoration.

Since the early days of breakfast food restoration and of white flour and white bread enrichment, the world-famous firm of Hoffmann-La Roche has supplied top quality vitamins *by the tons*. Pioneering work in its laboratories and by its collaborators resulted in the "duplication" of some of nature's extremely complex substances. First, the chemical composition of the vitamin was learned. Second, the pure substance was isolated. Third, the "duplicate" was made by synthesis. And fourth, the laboratory techniques were extended to large scale commercial operations.

The manufactured "duplicate" is identical chemically and in biological activity with nature's own product. A vitamin is still a vitamin regardless of whether nature or man made it. So efficient is large-scale manufacturing, that vitamins are sold at a lower cost than if they were extracted from natural sources.

This article is one of a series devoted to the story of vitamin enriched or restored cereal products: white flour, white bread and rolls, corn meal and grits, macaroni products, white rice, breakfast cereals, farina. Reprints of this article, of any other in the series, or of all are available without charge. Please send your request to the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada, Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

APPLICATION OF THE PROTEIN DEPLETION-REPLETION TECHNIQUE IN BABY PIG FEEDING EXPERIMENTS

I. A COMPARISON OF LEVELS AND SOURCES OF PROTEIN FOR BABY PIGS [1,2]

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C. H. LIU AND D. V. CATRON

*Department of Animal Husbandry, Iowa Agricultural
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The most widely used method for testing the adequacy of protein in baby pig diets has been to compare the responses of the pigs fed different kinds and amounts of protein in terms of efficiency of feed utilization, and the magnitude of weight gain during periods of uninterrupted growth. However, this method has been criticized on the grounds of sensitivity. It appeared, therefore, that there is need for a more sensitive method that would allow the detection of smaller differences in responses both between sources of protein and between levels of protein.

There are several methods that have been used for this purpose. These methods include the protein efficiency method

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proposed by Osborne, Mendel and Ferry ('19), Mitchell's
('24) nitrogen balance method, the measurement of liver
enzyme activity as proposed by Lightbody and Kleinman
('39), Potter and Klug ('47), Miller ('48), Seifter et al. ('48),
and Williams and Elvehjem ('49) and the rat repletion method
of Cannon and associates ('44).

The rat repletion method employs the production of a
biological deficit in the animal in order to measure the replace-
ment value of a test material. It provides a rapid assay since
the feeding period is relatively short. Cabell and Earle ('54)
made an extensive comparison of the rat repletion method with
other methods of assaying the nutritive value of proteins in
cottonseed meal. They reported the method to have marked
advantages such as the elimination of effects of palatability
and of unequal protein intake by quantitatively measuring or
restricting protein intake.

This paper reports the results of a study of levels and
sources of proteins in baby pig nutrition using the protein
depletion-repletion technique.

EXPERIMENTAL

The results to be reported here were obtained from Iowa
State College Swine Nutrition Experiments 692 and 699.

Animals. Experiment 692 called for a total of 12 pigs, two
per pen (two replications of three ration treatments). The
pigs were three-way crossbreds taken from the sows at an
average weight of 8.6 lbs. and at an average age of 11.4 days.
Initially, 60 pigs were started on experiment 699 but after
a one week pre-experimental period during which all pigs
were fed a common ration, the pig whose weight was most
distant from the mean pig weight of each pen was removed,
this left 4 pigs per pen for the experimental period. They
also were three-way crossbreds and were weaned at an average
weight of 7.4 lbs. at an average age of 15.0 days.

The pigs were confined in concrete floored pens equipped
with self-feeders and float operated water founts. Wood

shavings were used for bedding and the pens were cleaned daily. Thermostats were set to hold the temperature of the rooms at 18 and 21°C. for experiments 692 and 699, respectively.

Rations. In both experiments a 24% protein prestarter ration (I.S.C. Pre-starter "75," Speer et al., '54) was fed

TABLE 1

Composition of basal rations

INGREDIENT	PROTEIN-FREE DEPLETION RATION	20%-PROTEIN REPLETION BASAL RATION	12%-PROTEIN REPLETION BASAL RATION
	lbs.	lbs.	lbs.
Dried skimmilk (low-heat, spray-dried)	....	58.80	35.30
Sucrose	10.00	10.00	10.00
Lactose	78.02	20.17	42.29
Lard (stabilized)	2.50	4.70	4.82
Dicalcium phosphate	4.70	1.50	2.76
Calcium carbonate	0.30	0.35	0.35
Trace mineral mixture [1]	1.63	1.63	1.63
Woodflock [2]	2.00	2.00	2.00
Iodized salt	0.50	0.50	0.50
Vitamin-antibiotic premix [3]	0.35	0.35	0.35

[1] Contributed the following minerals in p.p.m.: Fe, 362.5; Cu, 7.6; Co, 3.1; Zn, 32.4; Mn, 101.5; K, 3979.0; I, 0.4.

[2] Manufactured by Brown Company, 110 South Dearborn, Chicago, Illinois.

[3] Each pound of complete ration contained at least the following amounts of vitamins and antibiotics: vit. A, 5000 I.U.; vit. D_2, 1000 I.U.; riboflavin, 5.0 mg; pantothenic acid, 10 mg; niacin, 30 mg; choline chloride, 450 mg; ascorbic acid, 300 mg; α-tocopheryl acetate, 10 mg; biotin, 20.0 μg; folic acid, 9 μg; inositol, 250 mg; Menadione, 3.0 mg; PABA, 8.0 mg; pyridoxine HCl, 1.2 mg; thiamine·HCl, 5.0 mg; vit. B_{12}, 20.0 μg; chlortetracycline, 15.0 mg; oxytetracycline, 15.0 mg; penicillin, 10.0 mg; bacitracin, 10.0 mg.

to the pigs for a pre-experimental period of one week. All rations were fed in the meal form. Feed consumption was determined weekly and the feeders were meticulously cleaned between the pre-experimental, the protein-depletion and the protein-repletion periods.

In experiment 692 a single cycle of a one-week depletion and a one-week repletion was employed; in experiment 699 the pigs were carried through three such cycles in succession.

The depletion ration and two repletion rations (12 and 20% protein) are presented in table 1.

Sucrose and lactose were the main sources of carbohydrate. The proportion of sucrose was standardized for all rations. A constant level of 5% fat was maintained in all repletion rations.

The protein in the three different rations in experiment 692 was supplied at the 20% level by dried skimmilk, or by solvent-processed soybean oil meal (50% protein) unsupplemented or supplemented with 0.1% of DL-methionine. In experiment 699 dried skimmilk supplied all of the protein but it was incorporated in the ration in proportions to give 6 levels of protein, namely, 12, 14, 16, 18, 20, and 22%.

Analysis of the data. Both experiments were conducted using a randomized block design. The pigs were alloted to the experimental rations at random within a replication in each experiment with the restriction that no littermates appear in the same pen. In experiment 692 the pen of two pigs was used as the experimental unit while in experiment 699 the pen of 4 pigs constituted the experimental unit. The average per pig response for live weight, repletion gains and feed per pound of repletion gain were analyzed statistically according to the analysis of variance plans below:

Analysis of Variance Plan

Experiment 692		*Experiment 699*	
Source of variation	*D.F.*	*Source of variation*	*D.F.*
Replication	1	Replication	1
Ration treatments	2	Ration treatments	5
DSM vs both SBOM	1	Linear regression	1
SBOM rations	1	Quadratic regression	1
		Cubic regression	1
		Remainder	2
Exp. error	2	Exp. error	5
Total	5	Total	11

Unless otherwise specified all statements concerning statistical significance of ration treatment effects are at $P = 0.05$ or less.

RESULTS AND DISCUSSION

Experiment 692

A summary of the effect of protein depletion and protein repletion on gains and feed efficiencies of baby pigs is presented in table 2.

Gain. During the one-week protein-depletion period, the pigs lost approximately 0.3 to 0.5 lb. of body weight per pig. Depletion starting weight did not appear to influence the amount of weight lost during the depletion period. During

TABLE 2

Exp. 692. Summary of the effect of protein depletion and repletion on gains and feed efficiences of baby pigs

ITEMS	RATION TREATMENT		
	Dried skimmilk	50% Soybean oil meal	50% Soybean oil meal + 0.1% DL-methionine
	lbs./pig	lbs./pig	lbs./pig
Depletion starting weight	9.8 [1]	9.9	9.4
Depletion final weight	9.3	9.5	9.1
Depletion loss	0.50	0.37	0.31
Repletion final weight	14.2	12.9	12.4
Repletion gain [2]	4.9	3.3	3.2
Repletion feed/gain	1.51	1.84	1.69

[1] Values are averages of 4 pigs per treatment.

[2] The experimental error mean squares for repletion gain and feed/pound gain were 0.7615 and 0.1887, respectively.

the one-week repletion period, those pigs repleted with the dried skimmilk diet gained approximately 1.6 lbs. more per pig than those fed the diets in which soybean oil meal was used as the source of protein. Supplementation of the soybean oil meal diets with 0.1% of DL-methionine did not improve gains. Statistical analysis of the gain data did not reveal any significant differences with the replications and degrees of freedom available in this experiment.

Feed per pound of gain. As expected from the gain data, it required less feed to produce a pound of repletion gain with the dried skimmilk than with soybean oil meal ration. Supple-

mentation of soybean oil meal with 0.1% of DL-methionine appeared to improve feed efficiency over unsupplemented soybean oil meal. The magnitude of the apparent differences in these feed efficiency data were not great enough to attain statistical significance.

Experiment 699

Gain. A statistical analysis of the gain data showed the coefficients of variation were decreased from 4.81 for the first repletion period gains to 2.94 for the summed gains of the three repletion periods and the treatment F (treatment mean square/experimental mean square) values were increased from 33.6 to 48.3 for these two periods, respectively. Both of these F values are beyond the 14.94 required for significance at the probability level of 0.5%. In view of this reduced variability by successive depletion-repletion cycles the repletion gain and feed efficiency data used for evaluating the ration effects were those obtained by summing gains and feed intakes over the three repletion periods.

Weight losses during the depletion period did not appear to be affected by previous treatment as evidenced by the close parallelism which exists in the growth curves of the pigs for the depletion periods (fig. 1). Figure 1 shows that the spread between the average live weights of the pigs on the different levels of protein increased with each depletion-repletion cycle. After the first cycle the live weights for the different levels of protein remained in the same order and increased regularly with each increment of protein with the exception of the 14 and 16% levels. This retainment of relative position might be expected in view of the parallelism of the depletion losses. That is, if the pigs that gained the most during the repletion period lost no more weight than the pigs that gained the least during the repletion period, then the initial advantage would still be maintained by the pigs that had previously made the greater gains.

The most rapid gains were made by the pigs repleted with 22% protein. As expected, the least gains were made by the

pigs fed 12% protein. However, the pigs on 16% protein failed to gain as rapidly as those on 14% protein. In view of the superiority of 20% protein over 18% protein for repleting protein-depleted pigs, one would expect 16% protein to replete the pigs as well or better than the 14% protein ration. Kjeldahl nitrogen determinations on the rations left no doubt in the authors' opinion that the pigs did receive their proper rations.

Examination of the growth curves (fig. 1) and the gain data in table 3 reveal that the average initial weight of the
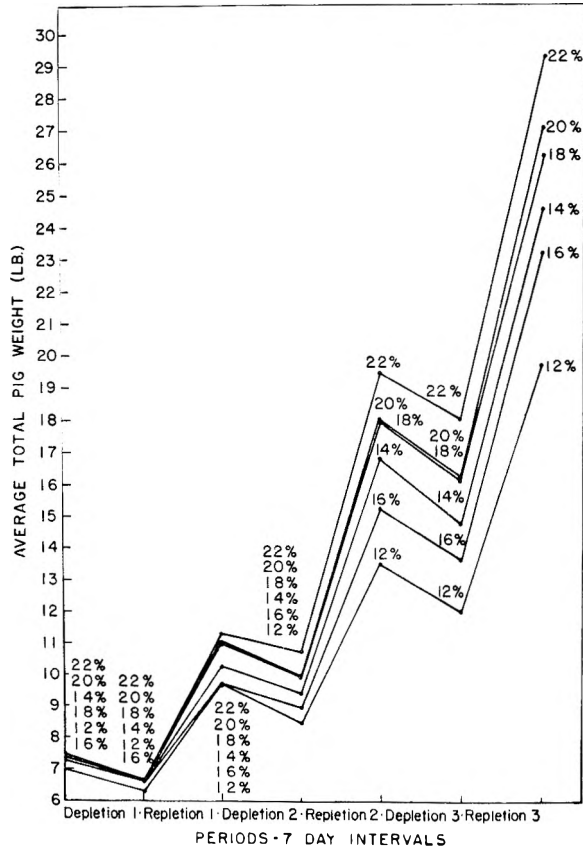
Fig. 1 Exp. 699. Growth curves of protein-depleted and protein-repleted baby pigs.

Figures in chart indicate percentage of protein in the ration. The protein was supplied by dried skimmilk.

pigs fed 16% protein was on the average 0.4 lb. per pig less than that of the pigs fed 14% protein. In fact, the pigs fed 16% protein had the smallest average initial weight in the experiment. The pigs repleted with 16% protein overcame this handicap in comparison with those repleted with 12% protein during the first repletion period, but did not do so

TABLE 3

Exp. 699. Effect of protein depletion and repletion on gains and feed efficiencies of baby pigs[1]

ITEM	PROTEIN LEVEL. %[2]					
	12	14	16	18	20	22
	lbs./pig	*lbs./pig*	*lbs./pig*	*lbs./pig*	*lbs./pig*	*lbs./pig*
Initial weight	7.3	7.4	7.0	7.3	7.5	7.5
First depletion loss	0.65	0.65	0.65	0.60	0.80	0.80
First repletion gain	2.61	3.66	3.44	4.21	4.38	4.69
Second depletion loss	0.84	1.03	0.90	1.03	1.19	0.75
Second repletion gain	5.16	7.25	6.66	8.03	8.12	8.81
Third depletion loss	1.44	1.78	1.78	1.75	1.62	1.37
Third repletion gain	7.69	9.78	9.62	10.06	10.75	10.00
Depletion loss	2.93	3.46	3.43	3.38	3.61	2.92
Summed repletion gain[3]	15.5	20.7	19.7	22.3	23.2	23.4
Feed consumed[4]	26.4	26.6	24.9	27.5	27.4	28.5
Feed/pound gain[3]	1.71	1.29	1.26	1.24	1.18	1.21

[1] Analyses of variance performed on the summed gains and average feed consumed for the three repletion periods using the average of 4 pigs as the experimental observation.

[2] Protein supplied by dried skimmilk.

[3] Quadratic effect of protein levels significant at $P = 0.05$ or less.

[4] Linear effect of protein levels significant at $P = 0.05$ or less.

The experimental error mean squares for repletion gain, feed consumed and feed per pound of gain were 0.5009, 1.0949 and 0.00134, respectively.

compared to the pigs fed the other levels of protein. Even with this reversal of the 16 and 14% protein-repletion response, statistical analysis of the data revealed that the linear regression component for level of protein was statistically significant.

Feed per pound of gain. The averages for total feed consumed and feed required per pound of gain are presented in table 3. The feed required per pound of gain was remarkably

low for all levels of protein. The pigs repleted with 12% protein required the most feed, 1.71 lbs. per pound of gain. The value of 1.18 lbs. for the pigs fed 20% protein was the least amount of feed required per pound of gain. Statistical analysis of the data revealed that the linear and quadratic regression components of the protein level effects were statistically significant. This parabolic curvature in the response to protein level is largely the result of the high value of 1.71 lbs. of feed required per pound of gain for the 12% protein ration, since the values for the other levels were relatively similar.

It is realized that a direct comparison of the depletion-repletion technique with uninterrupted feeding is needed to evaluate the reliability or validity of the results obtained in this manner. However, the low variability of response obtained in these experiments as compared to similar uninterrupted feeding trials indicates that this type of experiment may prove useful in future work of this nature.

SUMMARY

Sixty baby pigs were used in two experiments where the protein depletion-repletion technique was employed to measure the effectiveness of three different sources and 6 different levels of protein in promoting growth.

In the first experiment, the pigs that were protein repleted with dried skimmilk diets showed greater repletion gains on less feed per pound of repletion gain than those repleted with soybean oil meal diets with or without 0.1% of DL-methionine. There was little difference in the repletion gains or in feed utilization by the pigs fed the two soybean oil meal diets. Less of the methionine supplemented ration was required to produce a pound of gain, however this difference was not statistically significant.

When 6 levels of protein were tested using dried skimmilk as the source of protein, the greatest gains were made by the pigs repleted with 22% protein whereas the least gains were made by the pigs repleted with 12% protein. Statistical

analyses of the data revealed that the quadratic regression component of ration treatment effects on gain was significant.

The feed required per pound of repletion gain decreased as the levels of protein were increased from 12 to 20%. The decrease was large from the 12 to 14% protein level and relatively small per interval of protein increment thereafter. The quadratic regression component of ration treatment effect on feed efficiency was statistically significant.

LITERATURE CITED

CABELL, C. A., AND I. P. EARLE 1954 Comparison of the rat repletion method with other methods of assaying the nutritive value of proteins in cottonseed meals. J. Agr. Food Chem., 2: 787–790.

CANNON, P. R., E. M. HUMPHREYS, R. W. WISSLER AND L. E. FRAZIER 1944 Chemical, clinical and immunological studies on the products of human plasma fractionation. XXIII. The effects of feeding possible blood substitutes on serum protein regeneration and weight recovery in the hypoproteinemic rat. J. Clin. Invest., 23: 601–606.

LIGHTBODY, H. D., AND A. KLEINMAN 1939 Variations produced by food differences in the concentration of arginase in the livers of white rats. J. Biol. Chem., 129: 71–78.

MILLER, L. L. 1948 Changes in rat liver enzyme activity with acute inanition. Ibid., 172: 113–121.

MITCHELL, H. H. 1924 A mehtod of determining the biological value of protein. Ibid., 58: 873–904.

OSBORNE, T. B., L. B. MENDEL AND E. L. FERRY 1919 A method of expressing numerically the growth-promoting value of proteins. Ibid., 37: 223–229.

POTTER, V. R., AND H. L. KLUG 1947 Dietary alteration of enzyme activity in rat liver. Arch. Biochem., 12: 241–248.

SEIFTER, S., D. M. HARKNESS, L. RUBIN AND E. MUNTWYLER 1948 The nicotinic acid, riboflavin, D-amino acid oxidase, and arginase levels of the livers of rats on a protein-free diet. J. Biol. Chem., 176: 1371–1381.

SPEER, V. C., G. C. ASHTON, F. DIAZ AND D. V. CATRON 1954 I.S.C. Pre-Starter "75". Iowa Farm Sci., 8: 3–6.

WILLIAMS, J. N., JR., AND C. A. ELVEHJEM 1949 The relation of amino acid availability in dietary protein to liver enzyme activity. J. Biol. Chem., 181: 559–564.

APPLICATION OF THE PROTEIN DEPLETION-REPLETION TECHNIQUE IN BABY PIG FEEDING EXPERIMENTS

II. EFFECT OF LEVELS OF PROTEIN ON REPLETION GAINS AND BLOOD SERUM COMPONENTS OF BABY PIGS [1,2]

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In the first paper of this series, it was reported by Peo et al. ('57) that the greatest repletion gains were made by pigs fed 22% protein, the highest level fed. Therefore, it would appear that this level, or perhaps even a higher level of protein, is necessary to obtain maximum repletion gains. It was the purpose of this experiment, therefore, to establish the minimum level of protein (employing dried skimmilk as the source of protein) for maximum response from protein-depleted baby pigs.

Although the repletion gain was accepted as a good criterion of response, it was considered that other criteria might be more sensitive. Thus, it was also the purpose of this experiment to determine the effect of protein depletion and repletion on the albumin/globulin ratio and on other blood components of the blood serum of baby pigs.

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Animals. Thirty-six crossbred pigs weaned at an average age of 15.5 days and at an average weight of 7.8 lbs., were used in this experiment. The pigs were allotted to 6 ration treatments on the basis of initial weight within litters (in most instances pigs from the same litter constituted a replication), and were confined to individual pens for the entire experimental (one week preliminary, 5-week experimental) period. A group of pigs was also maintained under approximately the same experimental conditions as the individually-fed pigs until the end of the first depletion period. This provided a source of replacement for any pigs that died or were unintentionally lost during heart puncture for blood samples during this first part of the experiment. After a one-week preliminary period, the pigs selected for the experiment were depleted one week, repleted one week, depleted two weeks and repleted one week. The pigs were weighed at weekly intervals and blood samples were obtained at the end of the preliminary, the depletion, and the repletion periods.

The pens were equipped with self-feeders and constant-flow type water cups. The floors of the pens were heated by a thermostatically controlled radiant heat system. Initially the floor temperature was held at 28°C., and was then reduced 5°C. each week to 18°C. and held at this level for the remainder of the experiment. Room temperature was thermostatically held at 24°C. for the first three weeks and at 20°C. for the last three weeks of the experiment.

Rations. During the one-week preliminary period, all pigs were fed a 24% protein diet (Speer et al., '54). Protein levels of 12, 15, 18, 21, 24 and 27% were fed during the repletion periods. Composition of the protein-free diet and 12% protein-repletion basal ration was the same as that previously described by Peo et al. ('57). For this experiment, a 3% protein interval was selected in order to expand the range of the 6 levels of protein used by Peo et al. ('57) to higher levels and yet still allow the use of a 12% protein-repletion basal ration. Twenty-seven per cent protein was the maximum level

of protein that could be obtained with dried skimmilk as the sole source of protein and with the other ration components (and levels of each) used.

Blood. The pigs were bled by the heart puncture technique at the end of the preliminary, the depletion, and the repletion periods. A 5-ml syringe equipped with a number 19 needle was used to obtain a blood sample from each pig. The needles were autoclaved and the syringes were scrupulously cleaned after each bleeding period. Immediately after the blood was drawn from the pig, a portion was allocated (in a citrated tube) for hemoglobin, hematocrit and red and white blood cell determinations. The remainder of the blood was allowed to clot and then centrifuged at 1500 revolutions per minute for 15 minutes. After centrifugation, the serum was removed by pipette, transferred to a test tube and taken to the laboratory for serum protein determinations. All determinations were made within 24 hours after the blood samples were taken. The serum proteins were determined according to the method of Kingsley, described by Hawk et al. ('51). Globulin concentration was taken as the difference between the determined total serum protein and the determined albumin concentrations. Hemoglobin determinations were made according to the acid hematin method as outlined by Klett-Summerson ('47).

Analysis of the data. All data (gain, feed and blood) were analyzed by pooling the data for the two depletion and two repletion periods and testing according to the following analysis of variance plan.

Analysis of Variance Plan

Source of variation	Degrees of freedom	
Replication (litters)		5
Levels of protein		5
Linear regression component	1	
Quadratic regression component	1	
Cubic regression component	1	
Remainder	2	
Remainder (Experimental error)		25
Total		35

All statements concerning statistical significance are made at a probability level of 5% or less.

RESULTS AND DISCUSSION

The results of the treatment effects on repletion gains and feed efficiencies are shown in figure 1.

Gains. For the first protein-repletion period, as the protein levels were increased, pig gains also increased and reached a maximum at the 21% level of protein. Gains made by the pigs repleted with 24 and 27% protein were, on the average, 0.6
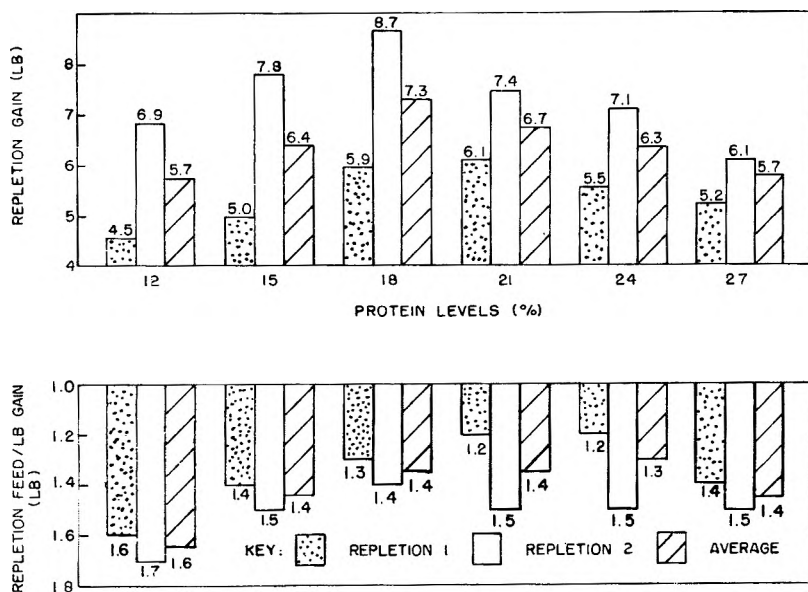
Fig. 1 Effect of protein levels on repletion gains and feed required per pound gain in baby pigs.

and 0.9 lb., respectively, per pig less than those made by the pigs repleted with 21% protein. During the second repletion period, the greatest gains were made by the pigs fed 18% protein. With the exception of 18% protein, the gains made by the pigs fed 15% protein were greater than those made by the pigs repleted with the other levels of protein. When the gains for the two repletion periods were averaged, the greatest gains were made by the pigs fed 18% protein. Statistical analyses of the pooled repletion gain data revealed

that the quadratic regression component of the effects of protein levels was significant.

Feed per pound of gain. For the first repletion period, the feed required per pound of gain (fig. 1) decreased as protein levels were increased up to 21% protein. Except perhaps for 12% protein, the feed efficiencies for all levels of protein were exceptionally good. At the end of the second repletion period,
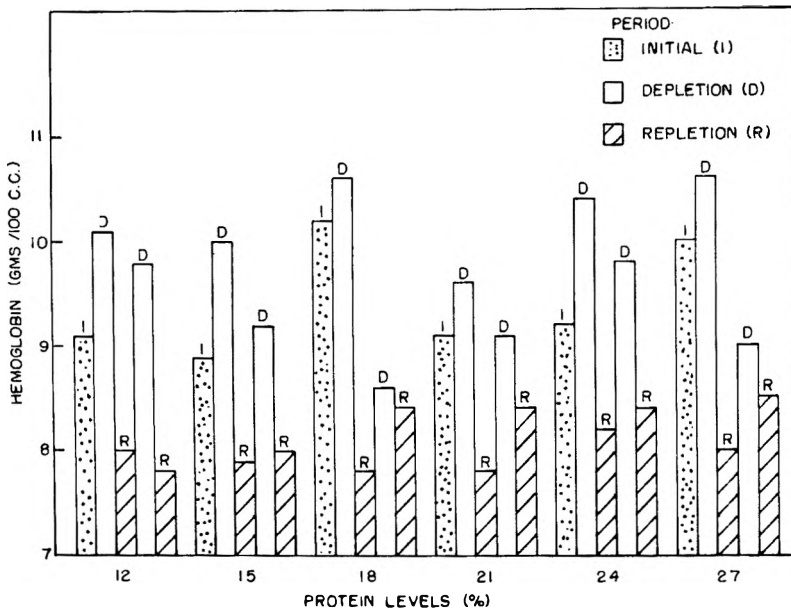
Fig. 2 Effect of protein depletion and repletion on hemoglobin levels of baby pigs.

less feed was required to produce a pound of repletion gain with 18% protein than with any of the other levels of protein. The feed data for the two repletion periods were pooled and analyzed according to the previously described analysis of variance plan. Both the linear and quadratic regression components of the treatment effect of protein levels were statistically significant with the largest portion of the mean squares for protein levels being accounted for by the quadratic component.

Blood data. With the exception of the hematocrits, the blood data were considered in two ways; namely, (1) as averages of the values at the end of the two depletion periods and similarly at the end of the two repletion periods, and (2) as differences between the averages of the values observed at the end of these two regimes (average change over the repletion period).

Hemoglobin. Hemoglobin levels of the protein-depleted and protein-repleted pigs are shown graphically in figure 2. During the depletion period, hemoglobin values averaged over all protein levels showed an apparent increase followed by a severe decrease after protein repletion. This was contrary to what was expected. One would expect hemoglobin levels to decrease or at least remain relatively constant during protein depletion and to either increase or remain relatively constant during protein repletion. Two possible explanations of the results obtained are (1) the changes observed in hemoglobin levels are real changes or (2) the changes in hemoglobin observed are a reflection of alterations in blood volume. This latter explanation appears to be more plausible in view of the work of Allison et al. ('46) and Hegsted and associates ('53) who observed that plasma volume is decreased during protein depletion and extracellular fluid outside the blood system is incerased. This, then, could account for the measured increase in hemoglobin during protein depletion and its decrease during protein repletion. If the plasma volume did fluctuate during protein depletion and repletion as evidenced by the hemoglobin levels, then the results observed with the other blood components are also affected by this change in plasma volume. However, the work of Allison et al. ('46) with dogs showed that plasma proteins decreased on depletion and returned to normal on repletion. They also observed changes in blood volume on depletion and repletion but these did not mask the changes in plasma proteins.

The highest hemoglobin level during the first repletion was for the pigs fed 24% protein. There appeared to be no differences in hemoglobin levels of the pigs fed protein levels

above 18% protein for the second repletion period. On the average, the lowest level of hemoglobin was observed in the pigs fed 12 and 15% protein during the repletion period. However, statistical analyses of the pooled data failed to reveal any significant difference in hemoglobin levels attributable to the protein levels fed during the repletion periods. This was also the case for the depletion periods and for the average differences between the two periods.
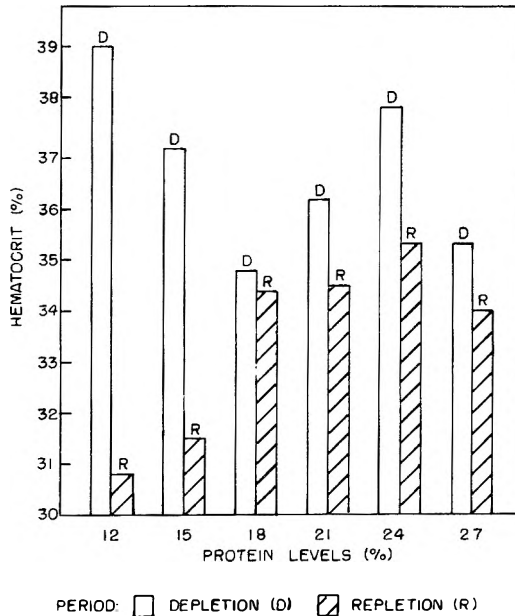
Fig. 3   Effect of protein depletion and repletion on the hematocrit of baby pigs.

Hematocrits. During the second protein depletion and repletion periods, hematocrit determinations were made to help clarify plasma volume changes. As shown in figure 3, the hematocrit was definitely lowered from the depletion level after the pigs were protein repleted. This indicates (although not conclusively) that plasma volume decreases during protein depletion and increases with protein repletion. Hematocrit changes were greatest on 12 and 15% protein. This indicates,

perhaps, that either plasma volumes fluctuated to a greater extent on these levels of protein (12 and 15%) or that these levels were not adequate for maintaining or repleting cell volume to the same extent as were the other levels of protein.

Red blood cells. The red blood cell counts of protein depleted and protein repleted pigs are presented graphically in figure 4. The trends observed with red blod cell counts
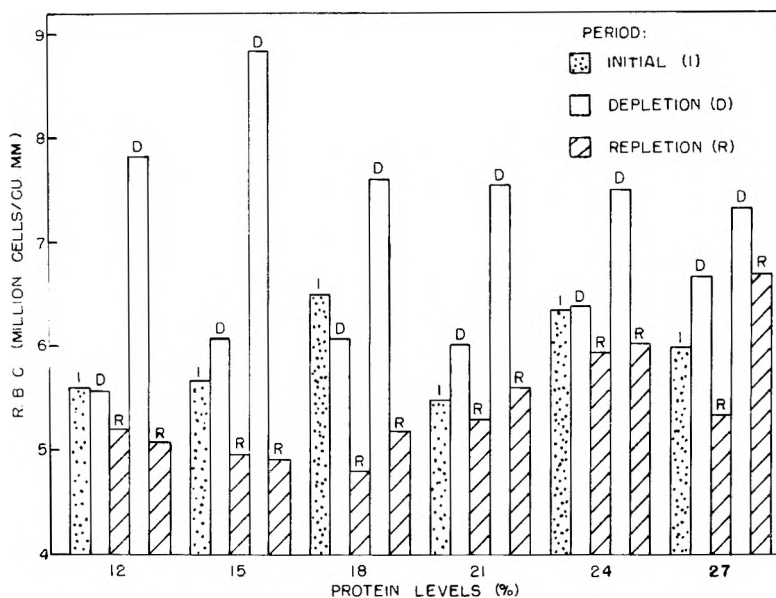
Fig. 4   Effect of protein depletion and repletion on the red blood cell count of baby pigs.

are similar to those observed with hemoglobin levels. Within each level of protein, red blod cell counts were higher during protein depletion than during protein repletion. For the first repletion period, maximum red blood cell counts were observed in the pigs repleted with 24% protein. For the second repletion period, maximum red blood cells were observed in the pigs fed 27% protein. On the average for both repletion periods, minimum red blood cell counts were observed in the pigs fed 15% protein. Statistical analyses of the pooled

repletion red blood cell counts revealed that the linear regression component of the effect of protein levels was statistically significant. While no regression component was significant for the depletion period, the linear component was for the difference between the two periods. As the level of protein was increased the difference in the red blood cell count became less (fig. 4).
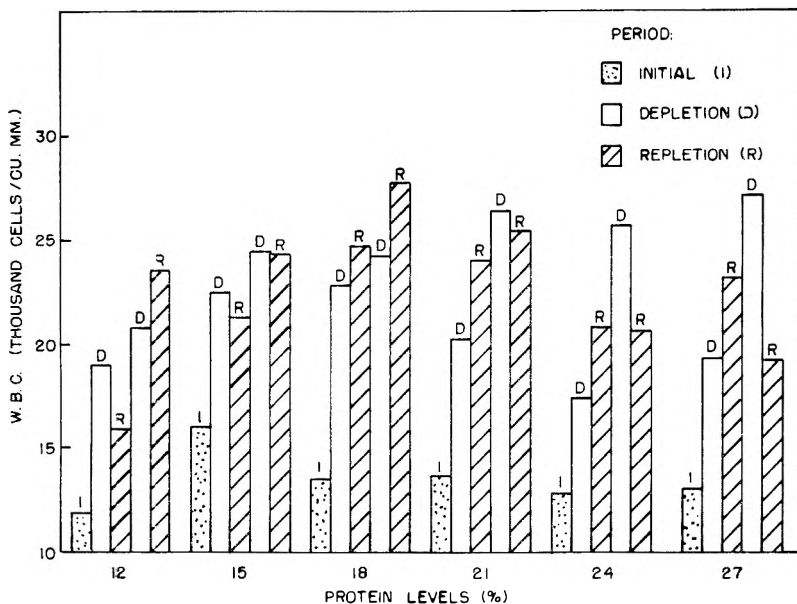
Fig. 5 Effect of protein depletion and repletion on the white blood cell count of baby pigs.

White blood cells. The white blood cell counts are presented in figure 5. Since white blood cells fluctuate in accordance with disease level, the observed white blood cell count does not in all instances follow the same trends as the hemoglobin level or the red blood cell count. The pigs were bled by heart puncture once each week at the experiment station farm and extreme aseptic techniques such as might be practiced in an animal clinic were not feasible. This may account for the relatively high white blood cell count observed in these pigs (Scarborough, '31, reported that on an average,

the normal white blood cell count of pigs was 15,280 cells per cubic millimeter). Except for the two highest levels of protein (24 and 27%), the white blood cell count did not fluctuate a great deal between the depletion and repletion periods. White blood cell count of the pigs fed 12, 15 and 18% protein increased from the initial to the end of the second repletion periods. From the second depletion to the second repletion, the white blood cell count of the pigs repleted with 21, 24 and 27% protein decreased. This may indicate that factors responsible for leucocytosis were decreased or eliminated with these levels of protein, since the management of the pigs on all treatments was essentially the same. Statistical analyses of the white blood cell repletion data gave a quadratic regression mean square for protein levels just short of the 5% probability level of significance.

Total serum. The results of protein depletion and protein repletion on total serum protein of baby pigs are summarized in figure 6. There was little difference in the total serum protein of the pigs fed any of the levels of protein during the first repletion period. For the second repletion period, the pigs repleted with 15% protein maintained a somewhat higher level of total serum protein than those repleted with the other levels of protein. Here again, the actual effect of protein repletion on total serum protein is probably masked by changes in plasma volume between the protein depletion and repletion periods. Consequently, any positive interpretation of the results may not be valid. However, it would appear from the observed data that there was little effect of protein levels on total serum protein.

Albumin and globulin. The levels of the albumin and globulin fractions of the blood serum of protein-depleted and protein-repleted baby pigs are presented in figure 7. The albumin levels of the pigs repleted with 15 and 18% protein were higher after protein repletion than they were after protein depletion for both of the depletion and the repletion periods. The albumin levels of the pigs fed 12% protein showed a consistent decline from the initial level to the final

level observed at the end of the second protein-repletion period. Such a consistent decline in serum albumin was not quite as evident in the pigs repleted with higher levels of protein. The globulin fraction of the serum protein (fig. 7) did not appear to change as much as the albumin fraction. This is in agreement with the results of Zeldis et al. ('45) who showed that restriction of dietary protein results in a
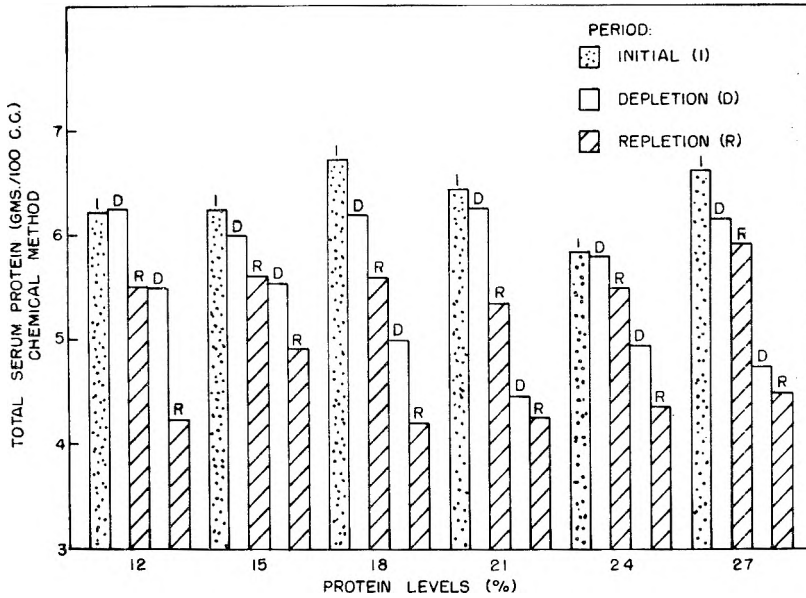
Fig. 6 Effects of protein depletion and repletion on total serum protein of baby pigs (chemical method).

decreased albumin level while globulin concentrations remain essentially normal. None of the mean squares for the regressions of either albumin or globulin approached statistical significance at the 5% probability level. The albumin/globulin ratio (fig. 7) decreased with protein depletion and increased with protein repletion. This response is in agreement with results reported by Allison ('48). If the globulin fraction remains fairly constant during protein depletion as reported by Zeldis et al. ('45), then the albumin fraction is changing

with protein depletion and repletion. The pigs repleted with
12% protein showed the smallest albumin/globulin ratio. The
average depletion albumin/globulin ratio increased with in-
creasing protein levels in a significantly linear manner. Al-
though a consistent change in the albnmin/globulin ratio was
noted with each protein depletion and repletion period, during
the repletion periods the greatest albumin/globulin ratio
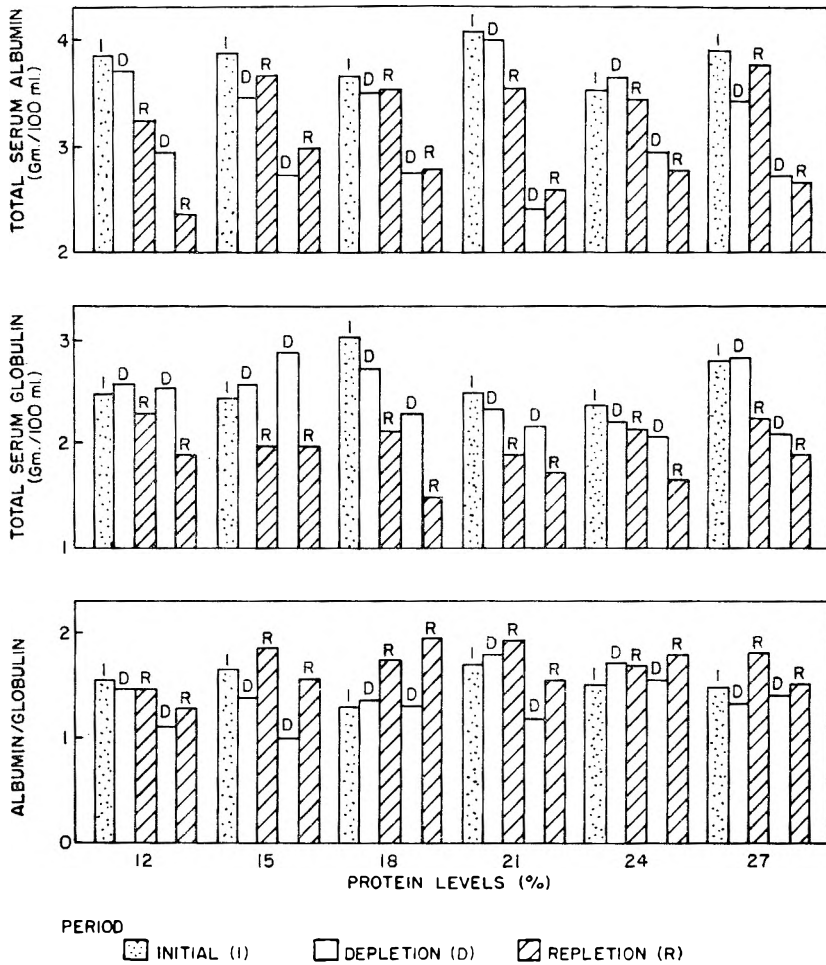
Fig. 7   Effect of protein depletion and repletion on total serum albumin,
globulin and A/G ratio of baby pigs.

was observed with 21 and 18% protein which were also the levels that supported maximum repletion gains for the first and second repletion periods, respectively. Although the albumin/globulin ratio was greater when pigs were repleted with levels of protein higher than 12%, statistical analyses of protein level effects on the albumin/globulin ratio did not reveal this to be a significant difference.

SUMMARY

Thirty-six individually-fed baby pigs were used to determine the effect of 6 levels of protein (using dried skimmilk as the source of protein) on the repletion gains and certain blood constituents of protein-depleted baby pigs.

Maximum repletion gains and feed utilization occurred in the pigs fed 21 and 18% protein during the first and second repletion periods, respectively. Statistical analysis showed the quadratic responses to be significant at $P = 0.05$ or less.

The effects of protein levels on the blood components studied were probably masked by changes in plasma volume. If plasma volumes had been determined, then it is possible that the protein source and levels might have had a significant effect on blood components. Of the blood constituents studied, the albumin/globulin ratio appears to be the most promising criterion of the effects of protein depletion and repletion and warrants further investigation.

ACKNOWLEDGMENTS

LITERATURE CITED

ALLISON, J. B., R. D. SEELEY, J. H. BROWN AND F. P. FERGUSON 1946 Some effects of depletion and repletion in proteins on body fluids in adult dogs. Proc. Soc. Exp. Biol. Med., *63:* 214–217.

ALLISON, J. B. 1948 Utilization of protein hydrolysates by normal and protein-depleted animals. Am. J. Med., *5:* 419–431.

HAWK, PHILIP B., B. L. OSER AND W. H. SUMMERSON 1951 Practical physiological chemistry. Phila., The Blakiston Company, 550–552.

HEGSTED, D. M., J. P. MILNER, D. WILSON AND P. GINNS 1953 Changes in muscle water and composition induced by protein depletion in rats at two environmental temperatures. Am. J. Physiol., *172:* 14–22.

KLETT-SUMMERSON 1947 Photoelectric colorimeter clinical manual. N. Y., Klett Manufacturing Company.

PEO, E. R., JR., V. W. HAYS, G. C. ASHTON, V. C. SPEER, C. H. LIU AND D. V. CATRON 1957 Application of the protein depletion-repletion technique in baby pig feeding experiments. I. A comparison of levels and sources of protein for baby pigs. J. Nutrition, *62:* 1–10.

SPEER, V. C., G. C. ASHTON, F. DIAZ AND D. V. CATRON 1954 I.S.C. Pre-Starter "75". Iowa Farm Sci., *8:* 3–6.

SCARBOROUGH, R. A. 1931 The blood picture of normal laboratory animals. Yale Jour. Biol. and Med., *3:* 547–548.

ZELDIS, L. J., E. L. ALLING, A. B. McCOORD AND J. P. KULKA 1945 Plasma protein metabolism-electrophoretic studies. Chronic depletion of circulating proteins during low protein feeding. J. Exp. Med., *82:* 157–179.

THE CALCIUM, PHOSPHORUS AND MAGNESIUM BALANCES OF YOUNG COLLEGE WOMEN CONSUMING SELF-SELECTED DIETS [1]

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In two previous studies (Coons and Schiefelbusch, '32; McKay et al., '42) the calcium and phosphorus intakes of young college women consuming self-selected diets were determined. These intakes averaged 0.93 gm (range of 0.52 to 1.55) and 1.19 gm (range of 0.82 to 1.17) for calcium and phosphorus in the first study and 0.94 gm (range of 0.32 to 2.32) and 1.18 gm (range of 0.66 to 2.13) in the second study. Coons and Schiefelbusch ('32), using 1 gm per day as the standard intake for both calcium and phosphorus, concluded that 10 of the 17 subjects were receiving too little of both elements. McKay et al. ('42) determined the calcium and phosphorus of the feces and urine as well as the daily intake of their subjects. Although approximately 50% of their 124 subjects were in negative calcium balance on less than 1 gm intake, it was concluded that 0.8 gm could maintain calcium equilibrium in these subjects. While only slightly more than a third of these same subjects were in negative phosphorus balance on 1 gm, this amount was stated to be necessary for phosphorus equilibrium. No study of the magnesium met-

abolism of young college women consuming self-selected diets
was found, although Duckworth and Warnock ('42) estimate
the adult woman's magnesium requirement to be 220 mg while
Sherman ('52) gives the average of 150 American dietaries
as 340 mg (range of 140 to 670). In 1946, a long-time metabolic
balance study of young Texas college women consuming
self-selected diets was initiated at North Texas State College.
The present paper is a report on the calcium, phosphorus
and magnesium balances of the subjects participating between
1948 and 1956.

PROCEDURE

The subjects were young college women 17 to 27 years
of age who were living in the Home Management House
Duplex at the time of this study. One hundred and twenty-
nine subjects participated in the calcium, 125 in the phosphorus
and 86 in the magnesium balance studies. They engaged in
the usual home and social activities in addition to their regular
class work during the 5-day (Monday through Friday) periods
reported. They were responsible for planning the menus and
preparing the food to satisfy the appetites of the individual
members of the group. The technique used in collecting the
food and excreta was reported by Holt and Scoular ('48).
In brief, this involved the placing of a serving of each food
from each meal (composite food sample), similar in all re-
spects to that eaten by the college women, in weighed glass
jars at the same time that the subjects were served at the
table. Milk was analyzed separately by removing daily ali-
quots to give one homogeneous sample for analysis. This
permitted each girl to consume the amount desired. A record
was kept of the amount of fluid milk consumed by each girl
each day, and suitable additions were made to each individual's
record of food intake, to include the milk.

Only one series of analyses was made of the carbonated
beverage consumed since the determined values were so small
and only one brand was permitted ad libitum during the 5-day
period. A liter of tap water was evaporated to dryness and
analyzed at the beginning and again near the end of the study.

Carmine was used as the fecal marker for the 5-day periods. Aliquots of both food and feces were obtained for analysis after being weighed and macerated in a Waring Blendor. Aliquots were removed from the 24-hour urine collections which had been made directly into the amber gallon bottles.

The food and fecal aliquots were dried before they were analyzed gravimetrically for calcium, phosphorus and magnesium. Scott's oxalate method (Furman, '39) was used for the determination of calcium, the molybdate method (Furman, '39) for phosphorus and the ammonium phosphate method (Association of Official Agricultural Chemists, '50) was used in determining the magnesium in the filtrate from the calcium determinations. The data from these analyses were used in obtaining the total daily intakes and retentions [3] reported in the present study.

RESULTS AND DISCUSSION

The total calcium intake (food, milk, carbonated beverage and tap water) of the young Texas college women was determined for 645 days, the phosphorus intake for 500 days and the magnesium intake for 430 days. The daily urinary calcium, phosphorus and magnesium were also determined for these women while the fecal calcium, phosphorus and magnesium were determined for the 5-day period and computed to daily values in order to determine the retentions.[3]

The percentage of the intake absorbed [4] by each subject was calculated for calcium, phosphorus and magnesium to see if the subject's ability to absorb these elements might influence the retentions obtained. The average absorption values were 36% of the calcium, 69% of the phosphorus and 74% of the magnesium ingested. Since both the calcium and phosphorus percentages are higher than the usually accepted

[3] Calculated as $\dfrac{\text{total intake minus fecal and urinary output}}{\text{total intake}} \times 100 = \%$ retained.

[4] Calculated as $\dfrac{\text{total intake minus fecal output}}{\text{total intake}} \times 100 = \%$ absorbed.

ones of 30% and 42%, respectively, it may be assumed that the negative retentions reported in the present study are probably due to other factors than the inability to absorb these elements. No reported value for magnesium absorption was available for comparison purposes.

In the two previous reports (Davis and Scoular, '57 and Scoular et al., '57) from this long-time study, the subjects were divided into two groups (group I, 16 to 20 years and group II, over 20 years) for comparison with the National Research Council's ('53) recommended daily allowances for the 16- to 20-year-old girl and for women 25 years of age. This grouping has been used in the present report for the same reason.

Total daily intake. The total daily intakes of calcium, phosphorus and magnesium are given in table 1 for the two groups of subjects together with their retentions. The calcium intake of group I (66 subjects) ranged from 0.50 to 2.40 gm/day as compared to a range of 0.70 to 2.60 gm/day for group II (63 subjects). The intakes of 2 gm or more of calcium and phosphorus were associated with high fluid milk intakes. The NRC's ('53) recommended daily allowances for the 16- to 20-year-old girl (group I) is 1.3 gm and for the 25-year-old woman (group II), 0.8 gm. Fifty per cent of the self-selected diets of group I and 3% of the diets of group II contained less than the recommended amounts of calcium. Furthermore, three times as many negative calcium balances occurred below 1.3 gm as above (20 as compared to 8) in group I, although 13 (38%) of the positive retentions occurred on less than 1.3 gm, and 18 (53%) on intakes above 1.3 gm. The positive retentions on the lower levels of intake suggest that these subjects had been accustomed to lower calcium intakes and that they exhibited the ability to adapt to long continued practices as suggested by Nicolaysen et al. ('53). Further support is given to this theory by the fact that many of these subjects had positive retentions on extremely high levels of intake which implies depleted stores.

TABLE 1

Retentions of calcium, phosphorus, and magnesium distributed for age and average total daily intake

RANGE	CALCIUM				PHOSPHORUS				MAGNESIUM			
	Group I [1] (+)	Group I (−)	Group II [2] ( )	Group II [2] (−)	Group I [1] (+)	Group I [1] (−)	Group II [2] (+)	Group II [2] (−)	Group I [1] (+)	Group I [1] (−)	Group II [2] (+)	Group II [2] (−)
gm	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.	no.
0.10–0.20									2	17	2	5
0.20–0.30										7	2	3
0.30–0.40												1
0.40–0.50												
0.50–0.60												
0.60–0.70	1	3			2		1	1	2	1	4	1
0.70–0.80		3		2	3	3		2	3		5	1
0.80–0.90		2		4	5		5	1	1		3	
0.90–1.00	4	2		2	5		5	4			2	2
1.00–1.10	4	2	1	3	7	5	6	4	1		1	1
1.10–1.20	2	5	4	3	8	2	5	4	2			
1.20–1.30	2	1	4	1	5	1	6	2				
1.30–1.40	3	2	2	1	5		5	4	3		6	
1.40–1.50	1	4	6		3		2	2	2		2	
1.50–1.60	2	1		4			1	1				
1.60–1.70	4	2	2	4								
1.70–1.80	1		3		2		1					
1.80–1.90	5	1		2	1							
1.90–2.00		2		2	3							
2.00–2.10	1		4									
2.10–2.20	1		5									
2.20–2.30			2									
2.30–2.40	2											
2.40–2.50			2		3							
2.50–2.60									1			
Total no.	34	32	35	28	52	11	37	25	17	25	30	14
Per cent	(57%)	(43%)	(56%)	(44%)	(83%)	(17%)	(60%)	(40%)	(40%)	(60%)	(68%)	(32%)
Average/day	1.50	1.14	1.70	1.30	1.17	0.90	1.14	1.02	0.95	0.20	1.02	0.43

[1] Group I composed of girls 16 to 20 years of age.

[2] Group II composed of girls 20 years or more of age.

The average total intake of the subjects in group I with positive calcium balances was lower (1.50 gm) than the 1.70 gm average of those with positive balances in group II. Since the percentages of positive balances were identical (57 and 56%) in the two groups, it implies that the subjects of group II may require more calcium than the amount recommended for women 25 years and over. From the standpoint of intakes and retentions of the present study, group II's need for calcium appears to be similar to that of group I. This is further emphasized by the fact that the negative balances of group II occurred on a higher average total intake (1.30 gm) than in group I (1.14 gm) and that positive retentions occurred on intakes of two or more grams of calcium per day.

The National Research Council ('53) suggests that the phosphorus intake should be about one and one-half times that of the calcium for adults (non-pregnant and non-lactating). Applying this suggestion to group I (63 subjects) and group II (62 subjects) gives 1.95 and 1.20 gm phosphorus, respectively, for comparison with the phosphorus content of these self-selected diets. All of the negative balances (11) in group I occurred below the 1.20 gm level of intake while group II had three negative balances above and 18 below this level. Although the average daily intakes of phosphorus for the subjects in positive balance in both groups are similar (1.17 and 1.14 gm) group I had 83% in positive balance compared with 60% for group II. The younger women (group I) were apparently able to utilize the phosphorus of their diets to a greater extent than the older women (group II).

The total daily intake of magnesium provided by these self-selected diets ranged from 0.16 to 2.55 gm (the highest value from one composite food supply) [5] for both groups. This range exceeds that of 140 to 670 mg given by Sherman ('52) as characteristic of 150 American dietaries. The values obtained in the present study represent 430 days' food supply (group I, 42 subjects and group II, 44 subjects during 5-day balance periods) obtained over a period of three years. In

[5] Cause for this high value was not due to contamination or to any particular food.

group I, 24 of the 25 negative balances and 8 of the 14 in group II occurred on intakes between 0.10 and 0.30 gm magnesium. The positive balances of the two groups occurred on similar average intakes of 0.95 and 1.02 gm, respectively.

Height in relation to calcium, phosphorus and magnesium balances. In the two previous reports of the present long-time study (Davis and Scoular, '57; Scoular et al., '57), both height and weight were used in an attempt to obtain some common reference for comparison of the nutrient intake. In these reports the use of height as a basis for comparison of the caloric and protein intakes of the two groups was found to be better than weight although the young Texas college women were both taller and heavier than the National Research Council's 16- to 20-year olds (group I) and women 25 years of age and over (group II). Consequently, calcium, phosphorus and magnesium (all of which occur in large amounts in the skeleton) intakes are tabulated only as milligrams per centimeter of height for the subjects for the present study in table 2.

The difference between the average milligrams per centimeter intake of the subjects of groups I and II who were in positive balance was small for calcium (9.1 and 10.3 mg/cm) and for phosphorus (8.2 and 7.0 mg/cm) or negligible in the case of magnesium (6.1 and 6.4 mg/cm). The greatest differences existed between the average intakes producing positive and negative calcium balances in each group, namely 2.3 for group I and 2.4 mg/cm for group II. Seventy-eight per cent of the negative calcium balances of group I and 82% of those in group II occurred on intakes below the average intake of those having positive balances. Similarly, 91% of the negative phosphorus balances in group I and 67% in group II occurred on intakes below the average intakes of those in positive phosphorus balances. The magnesium intakes producing positive and negative balances were practically identical for the two groups (6.1 and 1.8 mg/cm and 6.4 and 2.1 mg/cm, respectively).

Simultaneously determined calcium, phosphorus and magnesium balances. Seventy-three of the subjects included in tables 1 and 2 (35 from group I and 38 from group II) participated in the calcium, phosphorus and magnesium balances simultaneously and are reported in table 3. To show any relationship which may exist between the utilization of these

TABLE 2

Retentions of calcium, phosphorus and magnesium distributed for age and average daily intake per centimeter of height

RANGE	CALCIUM				PHOSPHORUS				MAGNESIUM			
	Group I[1] (+)	Group I[1] (-)	Group II[2] (+)	Group II[2] (-)	Group I[1] (+)	Group I[1] (-)	Group II[2] (+)	Group II[2] (-)	Group I[1] (+)	Group I[1] (-)	Group II[2] (+)	Group II[2] (-)
mg/cm	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*
0.0–1.0									1	3	2	4
1.0–2.0									2	17	1	7
2.0–3.0										3		
3.0–4.0		6			4		1	2	3	1	2	
4.0–5.0	1	2		2	6			1	2		7	1
5.0–6.0	4	4		7	10	4	11	9	1		5	1
6.0–7.0	5	6	3	2	13	4	6	5	2	1	2	1
7.0–8.0	4	6	5	4	6	2	9	6	2		4	
8.0–9.0	2	1	7	1	2		7	2	2		4	
9.0–10.0	5	3	3	7	1		2					
10.0–11.0	4	2	2	2	1							
11.0–12.0	4	2	3	3	3	1	1					
12.0–13.0	2		6		3							
13.0–14.0	2		4									
14.0–15.0	1				3				1		1	
15.0–16.0			2		3				1		2	
Total no.	34	32	35	28	52	11	37	25	17	25	30	14
Av. mg/cm	9.1	6.8	10.3	7.9	8.2	6.8	7.0	6.2	6.1	1.8	6.4	2.1

[1] Group I composed of girls 16 to 20 years of age.

[2] Group II composed of girls 20 years or more of age.

elements and the height of the individual, the intakes and retentions are tabulated for height. Schofield et al. ('56) state that they have a report in preparation which describes their taller and heavier 1952–53 subjects who failed to store as much calcium and phosphorus as the 1950–51 subjects on supplemented diets.

There were twice as many (11 as compared to 5) of the "taller" women of group I of the present study in negative calcium balance, but only two in negative phosphorus balance. The distribution of the positive and negative balances of magnesium in group I practically coincided with those for calcium. According to Stearns ('50) this may be accounted for by the fact that the solubility of magnesium is similar to that of calcium.

Group II ingested more calcium but less phosphorus than group I. Ten of the 14 negative calcium balances and 14 of the 16 negative phosphorus balances of group II occurred in the "taller" women. LeBovit and Stiebeling ('57) have suggested changes in applying the 1953 dietary allowances to U. S. population groups which include increasing the average height of women 21 to 34 years of age from 62 to 64 inches, but no change in the calcium allowance for this age group. With this adjustment the number of "taller" women who were in negative balance would be decreased to 7 in negative calcium balance and 9 in negative phosphorus balance. In group II where both the calcium and magnesium intakes were high, the calcium retentions appeared to be unrelated to the magnesium retentions.

There is no unanimity of opinion regarding the effect of the calcium-to-phosphorus ratio upon calcium retentions. In the present study, group II had the higher ratio, 1.45 and a higher percentage (63%) of positive calcium retentions than group I with a ratio of 1.2 and 40% positive retentions. Schofield and others ('56) state that the source of the calcium and phosphorus rather than the level of dietary phosphorus may influence calcium utilization in humans. They found that the same level of mineral ingestion from natural foods was better utilized than that from calcium salt. In the present study, group II consumed more milk, had higher calcium intakes and more positive retentions than group I with less milk.

The reason for the greater ingestion of milk by group II is not known since both age groups are usually included in each 5-day balance period and had access to the same food.

TABLE 3

The simultaneously determined 5-day calcium, phosphorus, and magnesium intakes of young college women consuming self-selected diets, distributed according to age and height with calcium-phosphorus ratios and retentions

GROUP I (BELOW 20 YEARS)

HEIGHT	Calcium				Ca:P ratio	Phosphorus				Magnesium			
	Average	Intake	Balance (+)	Balance (−)	av.	Average	Intake	Balance (+)	Balance (−)	Average	Intake	Balance (+)	Balance (−)
cm	gm/day	mg/cm	no.	no.		gm/day	mg/cm	no.	no.	gm/day	mg/cm	no.	no.
154.9–157.4 (61–62)[1]	1.82 ± 0.02	11.7 ± 0.1	2		1.51 ± 0.20	1.23 ± 0.18	8.0 ± 1.1	2		0.96 ± 0.28	6.2 ± 1.8	2	
157.5–159.9 (62–63)[2]	1.04 ± 0.00	6.6 ± 0.0		1	0.90 ± 0.00	1.16 ± 0.00	7.4 ± 0.0	1		0.20 ± 0.00	1.3 ± 0.0		1
160.0–162.5 (63–64)	1.20 ± 0.44	7.3 ± 2.7	1	3	1.03 ± 0.25	1.35 ± 0.31	8.4 ± 2.1	4		0.79 ± 0.44	4.9 ± 2.6	4	
162.6–165.0 (64–65)[3]	1.20 ± 0.26	7.0 ± 2.0	6	6	1.15 ± 0.39	1.01 ± 0.21	6.3 ± 1.6	9	3	0.50 ± 0.46	3.0 ± 2.8	6	6
165.1–167.5 (65–66)	1.20 ± 0.35	7.2 ± 2.0	3	3	1.18 ± 0.45	1.24 ± 0.23	7.5 ± 2.4	6		0.35 ± 0.20	2.1 ± 1.0	3	3
167.6–170.1 (66–67)	1.36 ± 0.42	8.1 ± 2.5		4	1.16 ± 0.50	1.32 ± 0.35	7.9 ± 2.5	3	1	0.33 ± 0.14	2.1 ± 0.8	3	4
170.2–172.6 (67–68)	1.26 ± 0.58	7.4 ± 3.6	2	1	1.05 ± 0.30	1.13 ± 0.33	6.6 ± 1.4	3		0.43 ± 0.32	2.5 ± 1.9		3
172.7–175.2 (68–69)	1.38 ± 0.00	7.9 ± 0.0		1	1.27 ± 0.00	1.09 ± 0.00	6.3 ± 0.0	1		0.60 ± 0.00	3.3 ± 0.0	1	
175.3–177.7 (69–70)	1.10 ± 0.00	6.2 ± 0.0		1	1.61 ± 0.00	0.68 ± 0.00	3.9 ± 0.0	1		1.33 ± 0.00	14.1 ± 0.0	1	
177.8–180.2 (70–71)	1.38 ± 0.00	7.8 ± 0.0	1		1.15 ± 0.00	1.20 ± 0.00	6.8 ± 0.0		1	0.23 ± 0.00	1.3 ± 0.0		1
av. 165.0 (64.9)	1.29 ± 0.21	7.7 ± 1.3 (14)	(21)		1.20 ± 0.21	1.14 + 0.16	6.9 + 1.1 (30)	(5)		0.58 ± 0.18	3.1 ± 1.1 (17)	(18)	

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GROUP II (OVER 20 YEARS)

HEIGHT	Calcium				Ca:P ratio	Phosphorus				Magnesium			
	Average	Intake	Balance (+)	Balance (−)	av.	Average	Intake	Balance (+)	Balance (−)	Average	Intake	Balance (+)	Balance (−)
cm	gm/day	mg/cm	no.	no.	av.	gm/day	mg/cm	no.	no.	gm/day	mg/cm	no.	no.
152.0–154.8 (60–61)[1]	1.02 ± 0.16	6.6 ± 1.0		2	1.20 ± 0.14	0.87 ± 0.23	5.7 ± 1.5	2		0.89 ± 0.15	5.9 ± 1.0	2	
154.9–157.4 (61–62)	1.30 ± 0.05	8.3 ± 0.2	2		1.38 ± 0.12	0.95 ± 0.12	6.0 ± 0.7	2		0.99 ± 0.36	6.3 ± 2.5	2	
157.5–159.9 (62–63)[2]	1.54 ± 0.17	9.7 ± 1.1	4	2	1.67 ± 0.49	1.01 ± 0.25	6.4 ± 1.6	4	2	0.80 ± 0.30	4.9 ± 2.0	5	1
160.0–162.5 (63–64)	1.49 ± 0.52	9.3 ± 3.1	6	3	1.48 ± 0.32	0.99 ± 0.14	6.1 ± 0.9	4	5	0.82 ± 0.44	6.0 ± 3.7	3	6
162.6–165.0 (64–65)[3]	1.97 ± 0.33	12.6 ± 1.5	3	1	1.39 ± 0.26	1.12 ± 0.10	7.1 ± 0.6	3	1	1.48 ± 0.54	9.0 ± 3.2	4	
165.1–167.5 (65–66)	1.61 ± 0.45	9.9 ± 2.8	3	2	1.35 ± 0.33	1.05 ± 0.19	6.4 ± 1.3	1	4	0.88 ± 0.65	5.3 ± 4.0	3	2
167.6–170.1 (66–67)	1.94 ± 0.25	11.6 ± 1.4	3	1	1.47 ± 0.28	1.06 ± 0.18	6.5 ± 1.2	2	2	1.24 ± 0.64	7.7 ± 4.0	4	
170.2–172.6 (67–68)	2.00 ± 0.10	11.8 ± 0.6	1	1	1.74 ± 0.22	1.16 ± 0.09	6.8 ± 0.6	1	1	0.78 ± 0.52	4.6 ± 3.1	2	
172.7–175.2 (68–69)	1.86 ± 0.29	10.7 ± 1.8	2	1	1.18 ± 0.24	1.18 ± 0.11	6.8 ± 0.4	2	1	1.18 ± 0.90	6.8 ± 5.2	2	1
175.3–177.7 (69–70)	1.67 ± 0.00	9.5 ± 0.0		1	1.62 ± 0.00	1.03 ± 0.00	5.9 ± 0.0	1		0.88 ± 0.00	5.0 ± 0.0	1	
av. 165.0 (64.9)	1.64 ± 0.23	10.0 ± 1.4 (24)	(14)		1.45 ± 0.24	1.04 ± 0.14	6.4 ± 0.9 (22)	(16)		0.99 ± 0.45	6.2 ± 2.9 (28)	(10)	

[1] Height in inches within parentheses.

[2] National Research Council height for the average woman of 25 years or older.

[3] National Research Council height for the 16- to 20-year-old girl.

Although the total calcium of the diet is a deciding factor in its utilization there are other factors which affect it. One of these factors which might apply to self-selected diets is that of day-to-day variation in other nutrients. Protein, which is associated with calcium utilization, was found by Scoular and Davis ('57) to vary from 130 to 500% of the minimum daily intake in a 5-day period. The day-to-day variation of calcium was from 140 to 360% and phosphorus from 120 to 400% of the minimum daily intake in the same period of time. Additional studies, including the day-to-day variation in nutrients, of self-selected diets are under way in this laboratory as a part of the continuing long-time balance study.

SUMMARY

Calcium balances of 129 young college women (645 days), phosphorus balances of 125 women (500 days) and magnesium balances of 86 women (430 days) were determined.

The average total intakes of calcium, phosphorus and magnesium permitting positive balances in group I (16- to 20-year-old girls) were 1.50, 1.17 and 0.95 gm as compared to 1.70, 1.14 and 1.02 gm, respectively, for group II (over 20 years).

Based on height the average total intakes for positive balances were 9.1, 8.2 and 6.1 mg/cm for group I and 10.3, 7.0 and 6.4 mg/cm of calcium, phosphorus and magnesium, respectively, for group II.

The older subjects (group II) drank more milk, had higher calcium intakes and more positive retentions than the younger subjects (group I), while group I consumed more phosphorus and had more positive phosphorus retentions than group II.

Groups I and II consumed the same amounts of magnesium but group I had more negative balances than group II.

In general, more of the negative retentions occurred among the "taller" young women than among those below the average in height in each age grouping.

LITERATURE CITED

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1950 Official methods of analysis, Seventh Edition, Washington.

COONS, C. M., AND A. T. SCHIEFELBUSCH 1932 The diets of college women in relation to their basal metabolism. J. Nutrition, *5:* 459–465.

DAVIS, A. N., AND F. I. SCOULAR 1957 The energy value of self-selected diets consumed by young college women. Ibid., *61:* 289–295.

DUCKWORTH, J., AND G. M. WARNOCK 1942 Magnesium requirements of man in relation to calcium requirements, with observations on the adequacy of diets in common use. Nutrition Abst. and Rev., *12:* 167–183.

FURMAN, N. H. 1939 Scott's standard method of chemical analysis, Fifth Edition. VanNostrand Co., Inc., New York City.

HOLT, F., AND F. I. SCOULAR 1948 Iron and copper metabolism of young women on self-selected diets. J. Nutrition, *35:* 717–723.

LeBOVIT, C., AND H. K. STIEBELING 1957 Applying 1953 dietary allowances to U. S. population groups. J. Am. Dietet. Assoc., *33:* 219–224.

McKAY, H., H. B. PATTON, M. A. OHLSON, M. S. PITTMAN, R. M. LEVERTON, A. G. MARSH, G. STEARNS AND G. COX 1942 Calcium, phosphorus and nitrogen metabolism of young college women. J. Nutrition, *24:* 367–384.

NATIONAL RESEARCH COUNCIL 1953 Reprint and Circular Series no. 302, Washington.

NICOLAYSEN, R., N. EEG-LARSEN AND O. J. MALM 1953 Physiology of calcium metabolism. Physiol. Rev., *33:* 424–444.

SCHOFIELD, F. A., D. E. WILLIAMS, E. MORRELL, B. B. McDONALD, E. BROWN AND F. L. MACLEOD 1956 Utilization of calcium, phosphorus, riboflavin and nitrogen on restricted and supplemented diets. J. Nutrition, *59:* 561–577.

SCOULAR, F., A. N. DAVIS, J. K. PACE, A. B. RANKIN AND G. J. BOSHART 1957 The protein metabolism of young college women consuming self-selected diets. Ibid., *61:* 297–306.

SCOULAR, F. I., AND A. N. DAVIS 1957 The daily variation in determined nutrients of self-selected diets consumed by young college women. Texas J. Science, *9:* 73–83.

SHERMAN, H. C. 1952 Chemistry of food and nutrition, Eighth Edition. Macmillan Company, New York, Chapter XII, 231.

STEARNS, G. 1950 Human requirement of calcium, phosphorus and magnesium. J. Am. Med. Assoc., *142:* 478–485.

NUTRITIONAL IMPROVEMENT OF WHITE FLOUR WITH PROTEIN AND AMINO ACID SUPPLEMENTS [1]

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INTRODUCTION

Osborne and Mendel ('14) and Mitchell and Smuts ('32) showed that the proteins of wheat could be improved by the addition of lysine. Sure ('52, '53, '54) reported that the addition of threonine along with lysine to whole or milled wheat flour had a beneficial effect on growth and protein efficiency in rats.

Rosenberg, Rohdenberg and Baldini ('54) investigated the effect on the growth of rats, of adding lysine, threonine, valine and methionine to a diet in which the protein was derived from white bread which contained 3% non-fat milk solids. From their results they concluded that the only amino acid which was deficient in this commercial white bread was lysine, since no further improvement in growth was obtained by supplementing the diet with other amino acids.

However, Hundley, Ing and Krauss ('56) who explored the possibility of using algae as sources of lysine and threonine in wheat flour and bread diets, observed a growth response to

threonine above that obtained with lysine, in both the cases. The algae served as a good source of threonine but were not a satisfactory source of lysine.

Hutchinson, Moran and Pace ('56a) observed that the protein of whole meal bread promoted a better rate of growth of young rats than that of white bread. They used breads that did not contain any milk solids. They attributed this effect to the lower lysine content of white bread. When white bread was supplemented with lysine, it was no longer inferior to the whole meal bread, in promoting growth in rats. The authors, in a subsequent investigation (Hutchinson et al., '56b) studied the extreme sensitivity of the weanling rat to small changes in the lysine content of diets in which the protein was provided by wheat flour. Their best growth rate of 24 gm/wk. was obtained with a total of 0.5% of lysine in the diet.

One striking observation emerges from these studies, namely, the rate of growth of young rats, with lysine and threonine as supplements to wheat flour, is inferior to that obtained with the same amino acids added to bread which contains about 3% non-fat milk solids. The possibility remains, therefore, that some other amino acids, which are necessary for optimal growth, are limiting in the wheat flour diet. The present investigation was therefore undertaken with a view to improving white flour with amino acid supplements to support a growth rate comparable to that obtained with an adequate synthetic diet.

Since fat accumulated in the livers of rats fed on polished rice (Harper et al., '55; Deshpande et al., '55) this aspect was also studied in the present work.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain, about 21 days old and weighing 40 to 50 gm were used. They were divided into similar groups of 6 animals each and were housed individually in suspended, wide mesh, wire bottom cages. Food was given ad libitum and animals were weighed weekly.

At the end of two weeks the rats were sacrificed for the determination of liver fat. Each animal was stunned and decapitated and the liver was removed and stored at —4° until the determinations were made. For the determination of liver fat, the livers were homogenized in a Potter-Elvehjem homogenizer and dried at 100°. The dried material, after grinding to a fine mesh, was extracted with ether.

The basal ration contained, in per cent, the following ingredients: white flour,[2] 78; corn oil, 5; salts, 4 (Hegsted, Mills, Elvehjem and Hart, '41), 4; choline chloride, 0.15; vitamin mixture, 0.25; and the various supplements of amino acids and proteins as indicated. Dextrin was added to make up to 100%. The vitamin mixture supplied in milligrams/100 gm of the ration: Thiamine HCl, 0.5; riboflavin, 0.5; niacin, 2.5; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12} 0.002 and inositol, 10.0. Fifty milligrams of vitamin C/kg of diet was added to minimize any possible destruction of thiamine (Kandutsch and Baumann, '53). Two drops of halibut liver oil, fortified with vitamins E and K and diluted with corn oil to provide vitamin A, 400 I.U.; vitamin D, 4 I.U.; vitamin E, 4.0 mg and vitamin K (2-methyl-1, 4-naphthoquinone), 0.04 mg, were given orally each week.

RESULTS

From the amino acid composition of white flour (Block and Bolling, '51) it is evident that when the diet contains 78% white flour as the only source of protein, all the essential amino acids except arginine, are provided at levels below the accepted requirements for the growth of the rat (Rose, '37). Since lysine was calculated to be the most limiting amino acid and was shown by Sure ('54) to give some growth response when added singly, and a correspondingly better growth response when added along with threonine to a diet containing whole wheat flour, the effect of supplementing white flour with these amino acids at various levels are recorded. The data summarized in table 1 (exp. 1) show that

[2] Pillsbury's enriched white flour, purchased from the open market, was used.

TABLE 1

Effect of amino acid supplements to enriched white flour diets (all diets contained 78% of white flour)[1]

EXP. NO.	GROUP NO.	SUPPLEMENTS	GROWTH	LIVER FAT
			gm/wk.	*% dry weight*
1	1	......	3.1 ± 0.4	13.9 ± 1.6
	2	0.25% L-lysine	12.2 ± 0.9	16.2 ± 1.3
	3	0.25% L-lysine + 0.22% DL-threonine	12.9 ± 0.6	12.2 ± 0.7
	4	0.5% L-lysine	11.6 ± 1.1	10.4 ± 1.2
	5	0.5% L-lysine + 0.4% DL-threonine	21.3 ± 1.6	13.2 ± 0.7
	6	6% casein	26.3 ± 0.8	11.5 ± 0.7
2	7		3.0 ± 0.6	16.3 ± 1.7
	8	0.9% L-lysine + 0.6% DL-threonine	19.9 ± 0.9	11.1 ± 1.3
	9	6% casein + 0.4% L-lysine + 0.2% DL-tryptophan + 0.4% DL-methionine	33.3 ± 1.9	9.7 ± 0.3
	10	6% fibrin	29.3 ± 1.5	11.6 ± 0.8
	11	6% fibrin + 0.4% L-lysine + 0.4% DL-methionine	35.6 ± 1.3	10.3 ± 0.5
	12	9 essential amino acids[2] = supplements for groups 9 and 11	28.1 ± 1.5	7.8 ± 0.2
3	13	4% L-glutamic acid	2.2 ± 0.3	
	14	4% glutamic acid + 0.4% lysine + 0.5% threonine	18.5 ± 0.8	
	15	4% glutamic acid + 0.4% lysine + 0.5% threonine + 0.15% tryptophan + 0.3% methionine	22.5 ± 1.5	
	16	9 essential amino acids[2]	31.2 ± 1.8	
	17	9 essential amino acids + 4% glutamic acid	32.9 ± 1.5	
	18	Group 17 + 0.2% L-histidine + 0.1% tryptophan + 0.2% DL-methionine	29.4 ± 1.8	
	19	6% fibrin + 0.4% L-lysine + 0.4% methionine	39.1 ± 2.1	
4	20	9.2% glutamic acid	2.7 ± 0.3	
	21	9.2% glutamic acid + 0.5% lysine + 0.4% threonine	19.1 ± 1.4	
	22	9.2% glutamic acid + 0.5% lysine + 0.4% threonine + 0.15% tryptophan + 0.3% methionine	18.4 ± 0.9	
5	23	6% fibrin + 0.4% lysine + 0.4% methionine	38.2 ± 1.7	
	24	9 essential amino acids = supplements for group 23	29.7 ± 1.0	
	25	9 essential amino acids + 7 non-essential amino acids[3] = supplements for group 23	30.1 ± 2.1	

[1] This contained 9.6% protein (N × 6.25), 0.19% L-lysine and 0.26% L-threonine.

[2] The following amino acids were supplied: L-lysine, 0.9%; DL-threonine, 0.6%; DL-tryptophan, 0.3%; DL-methionine, 0.6%; L-histidine, 0.2%; DL-phenylalanine, 0.4%; L-leucine, 0.2%; DL-isoleucine, 0.2%; and DL-valine, 0.6%.

[3] The following non-essential amino acids, in per cent, were supplied: L-tyrosine, 0.32; L-cystine, 0.12; DL-serine, 0.7; L-glutamic acid, 0.82; DL-aspartic acid, 0.64; glycine, 0.3; DL-alanine, 0.22; and L-proline, 0.3

the addition of 0.25% of L-lysine to a diet containing 78% of the flour increased the growth from 3.1 to 12.2 gm/wk. The addition of 0.2% of DL-threonine along with lysine did not further improve the growth. Increasing the level of lysine to 0.5% had no additional effect but when 0.4% of DL-threonine was included along with the higher level of lysine, the growth was increased to 21.3 gm/wk. A still better growth response (26.3 gm/wk.) was obtained when the diet was supplemented with 6% of casein.

In contrast to our previous study with polished rice (Deshpande et al., '55) where it was observed that rats fed on a 90% rice diet developed fatty livers, which were prevented by the addition of lysine, no accumulation of liver fat was observed in any of the groups fed on the white flour, although lysine was still the limiting amino acid for growth.

In the second experiment, groups of rats were fed the white flour supplemented with amino acids or 6% of either casein or fibrin together with amino acids as indicated in table 1. The results show that the highest levels of lysine and threonine (0.9 and 0.6% respectively) did not support quite as good growth as did the intermediate levels (group 5, exp. 1). A significant increase in growth was observed when certain limiting amino acids were provided with either casein or fibrin. These supplements were such that they were calculated to satisfy the requirements of the rat. When, however, a mixture of amino acids was added to meet the requirements (group 12), growth was not as good as when intact protein was included as part of the supplement. The amino acid mixture for group 12 was calculated to provide amounts equal to those provided for groups 9 and 11.

Experiments 3, 4 and 5 were included with a view to obtaining information on growth of rats when glutamic acid was added to diets containing white flour supplemented with various combinations of amino acids. It is evident that neither glutamic acid alone, nor a combination of 7 non-essential amino acids contained in 6% fibrin further improved the growth of rats fed on white flour supplemented with 9

essential amino acids. Maximum growth was obtained consistently in groups fed on the basal diet supplemented with 6% fibrin together with lysine and methionine.

Since the nutritive value of cereal proteins could be improved by adding small quantities of animal proteins, the effect of various animal protein supplements on the growth of rats fed on white flour was studied and the results are shown in table 2.

Of the proteins tested at the 6% level as supplements to white flour, casein, fibrin and beef were most effective; egg al-

TABLE 2

Rate of growth of rats fed white flour supplemented with various animal proteins

GROUP NO.	COMPOSITION OF THE DIET	GROWTH
		gm/wk.
1	78% White flour (basal)	2.2 ± 0.2
2	Basal + 6% casein	30.8 ± 2.5
3	Basal + 6% fibrin	31.5 ± 1.4
4	Basal + 6% egg albumin	21.6 ± 0.8
5	Basal + 6% kidney meal	22.0 ± 1.7
6	Basal + 6% fish meal	21.0 ± 1.9
7	Basal + 6% beef	33.0 ± 1.6
8	Basal + 6% pork	26.6 ± 1.5
9	Basal + 6% gelatin	14.9 ± 0.9
10	Basal + 6% fibrin + 0.4% lysine + 0.4% methionine	38.2 ± 1.7

bumin, kidney meal, fish meal and pork were intermediate, whereas gelatin seemed to be a rather poor supplement. Some further improvement in growth was obtained when additional amino acids were included with either fibrin or casein. So, it seems clear that diets containing white flour supplemented with 6% of a nutritionally well-balanced protein are still deficient in certain amino acids.

The amount of fat in the liver was normal in all groups with various amino acid combinations and hence values for only two experiments are given (table 1). Since a lysine deficiency in rice affected growth as well as liver fat, whereas in the case of white flour fat did not accumulate in the liver

in the presence of a lysine deficiency which depressed growth, it appeared important to investigate why no fatty livers were produced in the present experiments. The obvious difference seemed to be in the level of protein supplied by the white flour. Groups of rats were, therefore, fed on 44.2% of white flour, supplying the same protein level (N × 6.25 = 5.4) as provided by 90% of rice. The effects on growth and liver fat are given in table 3.

TABLE 3

Growth and liver fat in rats fed white flour supplemented with amino acids

GROUP NO.	COMPOSITION OF THE DIET	GROWTH	LIVER FAT
		gm/wk.	*% dry wt.*
1	44.2% Flour [1] + 0.6% DL-threonine (Basal 1)	0.8 ± 0.8	33.4 ± 0.9
2	Basal 1 + 0.3% L-lysine	5.7 ± 1.0	11.4 ± 1.1
3	Basal 1 + 0.6% L-lysine	5.2 ± 0.8	11.7 ± 0.7
4	Basal 1 + 0.9% L-lysine	4.8 ± 1.2	13.0 ± 1.0
5	44.2% Flour + 0.3% L-lysine (Basal 2)	1.3 ± 0.1	13.8 ± 2.5
6	Basal 2 + 0.2% DL-threonine	10.5 ± 1.0	16.1 ± 2.3
7	Basal 2 + 0.4% DL-threonine	9.9 ± 0.5	14.4 ± 1.6
8	Basal 2 + 0.6% DL-threonine	5.7 ± 1.0	11.4 ± 1.0

[1] This contained 0.11% L-lysine and 0.15% L-threonine.

It is clear that when an acute lysine deficiency was created by supplying excess threonine, rats failed to grow and at the same time fat accumulated in the liver. The addition of 0.3% of L-lysine brought the liver fat to normal but a satisfactory growth response was not obtained. Higher levels of lysine had no additional effect on growth. When, on the other hand, a threonine deficiency was created by including 0.3% of lysine in the diet, liver fat remained normal but growth was adversely affected. The addition of 0.2% of DL-threonine improved the growth from 1.3 to 10.5 gm/wk. Higher levels of threonine had a tendency to depress growth.

DISCUSSION

The results confirm the previous observations regarding lysine (Osborne and Mendel, '14; Mitchell and Smuts, '32)

and threonine (Sure, '54; Rosenberg et al., '54) as the most limiting amino acids in a diet consisting entirely of wheat proteins. A growth response superior to that obtained by Sure ('54) with supplements of only lysine and threonine may be due to the higher levels of both of these amino acids, particularly of threonine, used in the present experiments. Hutchinson, Moran and Pace ('56a) reported a somewhat higher average growth rate with only lysine as a supplement than those obtained in the present study. However, this would be expected on the basis of the longer experimental period (4 weeks) they have used.

It is interesting that no abnormal accumulation of liver fat occurred in rats receiving any of the diets that contained 78% of wheat flour. In previous work (Deshpande et al., '55) fatty livers developed in rats fed on diets containing between 80 and 90% of rice. The calculated lysine content of both diets was 0.19%. However, growth was considerably better in the case of rice. When the protein content of the wheat flour diet was dropped to 5.4%, equivalent to that of the rice diet, fatty livers developed. These fatty livers, like those that occurred in rats receiving the rice, could be prevented by lysine supplementation. In the case of rice, the better growth of the rats probably created a greater demand for lysine for general tissue synthesis, so that even though the same amount of lysine was present, less was available for the control of liver fat deposition. In the case of the rats fed on the wheat flour diets growth was poor and thus the demand for lysine was probably less and fatty livers did not develop until the quantity of available lysine was still further decreased. Whether these effects reflect a lower availability of lysine in white flour or whether the differences, in the ratios of other amino acids in relation to lysine in these two cereals are responsible, remains to be determined. It should be noted, however, that the accumulation of liver fat occurred only under extremely strenuous conditions which would probably not be encountered in practical nutrition. However, it is important

to study the role of amino acids under a wide variety of conditions.

In the experiment cited in table 3, the higher level of threonine apparently created an imbalance resulting in a retardation of growth. The higher level of lysine was not more effective than the lower level but it did not create an imbalance. In contrast, in earlier work using rice diets (Deshpande et al., '55) an imbalance was created when the lysine level was increased whereas no imbalance was created by increasing the threonine level.

The growth of animals fed on diets containing a supplement of 6% of intact protein could be further increased by adding certain free amino acids, but 9 essential amino acids had to be added to support a rate of growth equivalent to that obtained with wheat flour supplemented with 6% of fibrin, beef or casein. Less complete combinations of amino acids tested had little effect beyond that obtained with lysine and threonine. Although several of the amino acid mixtures produced a substantial growth response, the finding that a mixture of essential amino acids simulating fibrin was not able to support as good a growth as the intact protein indicates some superiority of intact protein over amino acids as a dietary supplement under the conditions of the present experiments.

SUMMARY

Rate of growth of young rats was increased from 3 to 21 gm/wk when their diet, containing 78% of white flour was supplemented with 0.5% of L-lysine and 0.4% of DL-threonine. Further improvement in growth was obtained only when 7 more essential amino acids were added.

Although lysine was limiting for growth, liver fat did not accumulate when the diet contained 78% of white flour. However, fatty infiltration, which occurred when the flour was fed at a 5.4% protein level, was prevented by a lysine supplement.

Maximum growth was obtained when intact protein formed part of the supplements but growth was not as rapid when

the protein was replaced by equivalent quantities of crystalline essential and non-essential amino acids.

LITERATURE CITED

BLOCK, R. J., AND B. BOLLING 1951 The Amino Acid Composition of Proteins and Foods. 2nd edition; Springfield, Illinois.

DESHPANDE, P. D., A. E. HARPER, F. QUIROS-PEREZ AND C. A. ELVEHJEM 1955 Further observations on the improvement of polished rice with protein and amino acid supplements. J. Nutrition, *27:* 415.

HARPER, A. E., M. E. WINJE, D. A. BENTON AND C. A. ELVEHJEM 1955 Effect of amino acid supplements on growth and fat deposition in the livers of rats fed polished rice. Ibid., *56:* 187.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of chicks. J. Biol. Chem., *138:* 459.

HUNDLEY, J. M., R. B. ING AND R. W. KRAUSS 1956 Algae as sources of lysine and threonine in supplementing wheat and bread diets. Science, *124:* 536.

HUTCHINSON, J. B., T. MORAN AND J. PACE 1956a Nutritive value of the protein of white and whole-wheat bread in relation to the growth of rats. Proc. Royal Soc., B., *145:* 270.

———— 1956b Effect on the growth rate of weanling rats of supplementing the protein of white bread with L-lysine. Nature, *178:* 46.

KANDUTSCH, A. A., AND C. A. BAUMANN 1953 Factors affecting the stability of thiamine in a typical laboratory diet. J. Nutrition, *49:* 209.

MITCHELL, H. H., AND D. B. SMUTS 1932 The amino acid deficiencies of beef, wheat, corn, oats, and soybeans for growth in the white rat. J. Biol. Chem., *95:* 263.

OSBORNE, T. B., AND L. B. MENDEL 1914 Amino acids in nutrition and growth. Ibid., *17:* 325.

ROSE, W. C. 1937 Nutritive significance of amino acids and certain related compounds. Science, *86:* 298.

ROSENBERG, H. R., E. L. ROHDENBURG AND J. T. BALDINI 1954 The fortification of bread with lysine. III. Supplementation with essential amino acids. Arch. Biochem. Biophys., *49:* 263.

SURE, B. 1952 Influence of lysine, valine and threonine additions on the efficiency of the proteins of whole wheat. Arch. Biochem. Biophys., *39:* 463.

———— 1953 Improving the nutritive value of cereal grains. J. Nutrition, *50:* 235.

———— 1954 Protein supplementation. Relative nutritive value of proteins in whole-wheat, and rye and the effect of amino acid supplements. J. Agr. Food Chem., *2:* 1108.

UTILIZATION OF AMINO ACIDS FROM FOODS BY THE RAT

IV. TRYPTOPHAN [1]

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The present study of the quantitative utilization of tryptophan from foods by the rat represents a selected phase of the over-all evaluation of the nutritional quality of food proteins. Earlier work on the quantitative utilization of lysine (Schweigert and Guthneck, '53; Guthneck et al., '53) and methionine (Schweigert and Guthneck, '54) established the validity of the general approach and techniques used in this research. Further refinements of these methods have been used in the present study, and experiments on the composition of the tryptophan-deficient basal diet, the consistency and repeatability of the assays, and other criteria of reliability have been performed. Results are given for the amounts of tryptophan utilized for weight gains and in certain

experiments for the amounts excreted in the feces when foods of plant or animal origin were fed.

EXPERIMENTAL METHODS

Male weanling rats [4] were fed a commercial laboratory chow [5] for one day. Groups of 5 or 7 rats were then equalized by weight, the animals placed in cages with raised screen floors, and started on experiment at 23 days of age, with food and water provided ad libitum. A basal ration deficient in tryptophan was designed to include casein, oxidized with hydrogen peroxide in the presence of formic acid (Toennies, '42; Bennett and Toennies, '42; Lyman et al., '46; Wilkening et al., '47) to destroy all detectable tryptophan, as the major source of protein. The basal ration contained 10% oxidized casein, 4% untreated casein, 4% Salts IV, [6] 4.7% corn oil, 0.3% fish liver oil (2,250 A, 400 D/gm), 0.4% L-cystine, 0.8% DL-methionine, 0.8% L-tyrosine, B vitamins and menadione as required (Schweigert and Guthneck, '53), and sucrose to make 100%. This ration provided 38 mg of tryptophan and 2 mg of niacin per 100 gm of ration. The use of untreated casein provided a basal level of normal, untreated protein in the diet and eliminated the need for large amounts of purified amino acids. Cystine, methionine and tyrosine were added, since these amino acids were destroyed by the oxidation treatment of casein. The tryptophan supplements were added as a 5% mix of L-tryptophan in sucrose. All supplements of L-tryptophan or test foods were included in the rations at the expense of sucrose.

The fresh meat samples were lyophilized, ether-extracted, and ground in a hammer-mill. Paired cuts were roasted by standard procedures, lyophilized, ether-extracted and ground. The soybean meals and nonfat dry milk were used as received, and the rolled oats and split peas were ground in a hammer-mill prior to use. Samples of the foods, oxidized casein, and

[4] Holtzman.
[5] Ralston-Purina.
[6] Hegsted et al. ('41).

untreated casein were analyzed for total nitrogen (macro-Kjeldahl), and the crude protein content (N $\times$ 6.25) calculated. The tryptophan content of the foods was assayed both micro-biologically and chemically, and the results were in good agreement. Samples were hydrolyzed in 4 N NaOH for micro-biological assay, using *Lactobacillus arabinosus* 17-5 (ATCC 8014) as the test organism. The Bates method ('37), as modified by Graham et al., ('47), was used for the colorimetric determination of tryptophan with *p*-dimethylaminobenzalde-hyde. Thirty minutes at 40°C, in the dark, gave maximum color development.

One or more levels of each of the selected foods was added to the basal diet to provide known amounts of tryptophan (20 to 80 mg/100 gm) as calculated from the microbiological assay results. The percentage of tryptophan utilized for growth was calculated on the basis of the rates of gain for groups fed graded levels of L-tryptophan.

Fecal tryptophan excretion was determined in two feeding experiments. Composite collections of the feces of each group were made daily over a three-day period, and the tryptophan content assayed microbiologically, using *Lactobacillus arab-inosus* 17-5 (ATCC 8014) as the test organism. Net food intake of each group was measured (gross intake minus spillage) and the total intake of tryptophan calculated for each group.

Experiments on the composition of the basal ration were conducted, in which variations in the amounts and proportions of oxidized and untreated casein were compared. Additional supplements of niacin and threonine alone and in combination were also tested.

RESULTS AND DISCUSSION

Assay methods. Results obtained with the use of the two tryptophan assay methods were within $\pm$ 5% of the mean values for all foods, and recovery experiments gave good results. The tryptophan/protein ratios were similar for all foods except split peas, and diets providing a chosen level of

tryptophan supplement from the foods were very nearly equivalent in total protein (see table 1).

The rate of gain of 23-day-old male weanling rats fed the basal ration plus excess L-tryptophan (240 mg/100 gm) was rapid and uniform, but it was not equivalent to the growth obtained on feeding the stock laboratory ration. Total gain approximated 50 gm for the 14-day test period, compared with 95 gm for the stock-fed animals. Tests with graded levels

TABLE 1

Percentage of protein in foods (N × 6.25) and percentage of tryptophan in protein of foods

FOOD TESTED [1]	PROTEIN	TRYPTOPHAN IN PROTEIN	
		Microbiological assay	Chemical assay
	%	%	%
Raw beef rib	93.3	1.36	1.37
Roast beef rib	91.9	1.44	1.31
Raw ham (uncured)	88.1	1.32	1.26
Roast ham (uncured)	90.9	1.43	1.30
Raw lamb leg	91.5	1.31	1.22
Roast lamb leg	92.9	1.27	1.20
Unheated soybean meal	48.3	1.26	1.34
Normally processed soybean meal	49.6	1.25	1.27
Overheated soybean meal	49.4	1.22	1.16
Nonfat dry milk	35.2	1.54	1.43
Rolled oats	17.7	1.30	1.41
Split peas	23.8	0.84	0.76

[1] For all meat samples, the values shown were obtained on lyophilized, ether-extracted meat.

of L-tryptophan gave a graded, essentially linear response when the tests were conducted for 7, 11, or 14 days, and the tryptophan requirement approximated 160 mg/100 gm ration (fig. 1). Tests with DL-tryptophan showed that the D-isomer was utilized to the extent of approximately 90%.

The fecal tryptophan excretion data obtained indicated that essentially all of the tryptophan fed was made available for digestion and absorbed by the rat, both in the pure form, and when provided by each of the foods tested. Calculation of the digestibility of tryptophan (100 minus % fecal tryptophan

excretion) gave no indication that tryptophan digestibility varied to any extent for the foods tested. These values ranged from 90% (split peas fed at a high level) to 99% digestibility (L-tryptophan fed in excess). When corrected for fecal tryptophan excretion on the basal ration (no tryptophan supplement added), tryptophan digestibility approximated 100% for all foods, ranging from 94 to 103%.
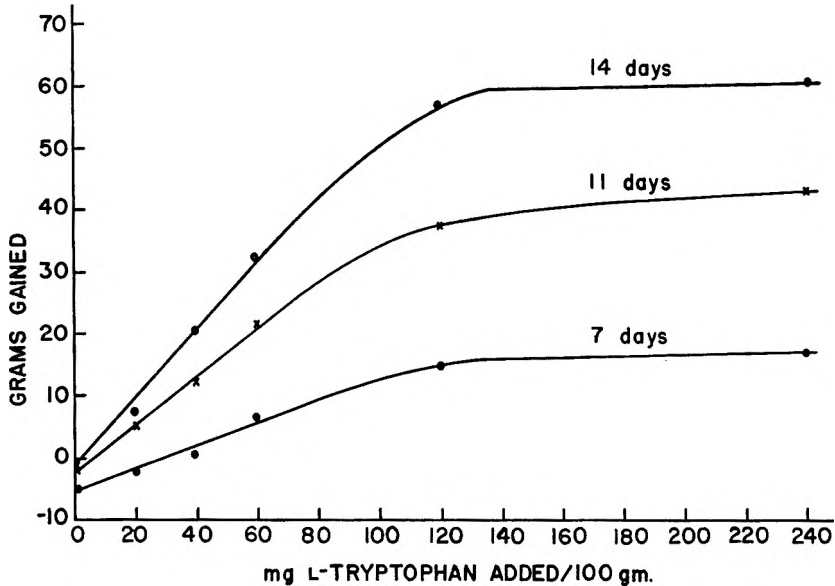

Fig. 1 Rates of gain for groups of male weanling rats fed the tryptophan-deficient basal ration plus graded tryptophan supplements for periods of 7, 11 and 14 days.

Experiments were conducted to ascertain whether modifications in the basal diet would provide increased rates of gain or result in altered tryptophan utilization values, and to give assurance that the basal ration was as complete as possible in all known nutrients. The results indicated that the nutrients other than protein (vitamins, minerals, fats, carbohydrate, and amino acid supplements) supported weight gains equivalent to those observed on the stock ration, when fed with 20 or 30% of untreated casein. It was concluded that the reduced

growth obtained with the basal ration, when supplemented with excess tryptophan, was due to some factor(s) present in the oxidized casein. Although this reduced growth limited the sensitivity of the assay method to some extent, it did not negate the validity of the experimental results obtained with the use of this ration.

In one experiment, the amounts and proportions of oxidized and untreated casein were varied in the presence of excess tryptophan. The results suggested that the desired improvement in growth response could be obtained by inclusion of increased amounts of untreated casein in the ration, but the amount of tryptophan supplied would be increased as well.

It was important to determine whether increased amounts of niacin or threonine or both in the basal diet would result in increased growth response. When the basal ration was fed with 40 or 240 mg of L-tryptophan/100 gm, some improvement in growth response was observed with 3 mg of additional niacin/100 gm, and with 100 mg of DL-threonine/100 gm. In a subsequent test, niacin and threonine were added to the basal ration alone and in combination at various levels, and the ration supplemented with 40 or 240 mg of L-tryptophan/100 gm. Maximum growth response at the 40 mg tryptophan level was obtained with 3 mg of additional niacin + 600 mg of DL-threonine/100 gm ration. At the 240 mg tryptophan level, maximum growth response was observed with 3 mg of additional niacin + 300 mg of DL-threonine (44 gm), and a decrease in growth response occurred when the threonine was increased to 600 mg/100 gm ration (39 gm). The optimum growth response throughout the range of tryptophan supplementation used in the bioassay favored supplements of 3 mg of additional niacin + 300 mg of DL-threonine/100 gm ration.

In the subsequent feeding tests in which several food supplements were included, the basal ration, and the basal ration + 3 mg of niacin + 300 mg of DL-threonine/100 gm were fed. The rations containing supplements of niacin and thre-

onine gave slightly *decreased* growth response, compared to that obtained with the unsupplemented basal ration when tested at the 40- and 80-mg levels of tryptophan supplementation. The results with added niacin and threonine in the basal ration showed decreasing tryptophan utilization with increasing levels of food supplements, an effect which was not observed in any of the feeding trials using the unsupplemented basal ration. This may have resulted from an amino acid imbalance caused by use of the threonine supplement.

TABLE 2

Percentage of tryptophan utilized from several foods after 11- and 14-day experimental periods [1]

FOOD TESTED	TRYPTOPHAN UTILIZED	
	11 days	14 days
	%	%
Raw ham (uncured)	113	103
Roast ham (uncured)	88	86
Unheated soybean meal	124	118
Normally processed soybean meal	125	125
Rolled oats	110	111

[1] Foods added to provide 40 mg of tryptophan/100 gm ration.

Thus, although the preliminary results described above indicated the potential value of adding additional niacin and threonine, these observations were not confirmed. It was concluded that the basal ration as originally designed was the one most satisfactory for determining the tryptophan utilization from foods.

Utilization of tryptophan from foods. The tryptophan utilization results obtained after 11 and 14 days are shown in table 2 for several of the foods studied, at the level of 40 mg of tryptophan/100 gm ration. These results indicate the high consistency observed in these studies for varying experimental periods. Results obtained after 7 days were more variable, and it appears desirable to study the quantitative utilization of tryptophan in the male weanling rat for a period somewhat longer than 7 days.

The results shown in table 3 indicate that the percentage utilization of tryptophan from each of several foods was not dependent upon the level of tryptophan in the diet within the range of 40 to 80 mg/100 gm. Tests of foods at the 20-mg level of tryptophan resulted in greater variability in the

TABLE 3

Percentage of tryptophan utilized when foods were fed at various levels for 14 days

FOOD TESTED	AMOUNT OF DRY FOOD ADDED	TRYPTOPHAN ADDED	TRYPTOPHAN UTILIZED	AVERAGE TRYPTOPHAN UTILIZED
	gm/100 gm	*mg/100 gm*	%	%
Raw ham	3.4	40	103	
(uncured)	5.2	60	109	107 ± 4 [1]
	6.9	80	110	
Roast ham	3.1	40	86	
(uncured)	6.2	80	90	88 ± 3
Unheated soybean	6.6	40	118	
meal	9.9	60	118	119 ± 2
	13.1	80	121	
Normally processed	6.4	40	125	
soybean meal	9.7	60	136	128 ± 8
	12.9	80	122	
Rolled oats	17.4	40	111	
	26.1	60	125	120 ± 8
	34.7	80	124	

[1] Standard deviation.

tryptophan utilization results obtained, with a tendency toward higher values. Tests at this lower level were less accurate, however, due to the low total weight gains achieved, and the large relative effect of small variations in weight.

An evaluation of the repeatability of the results obtained in these experiments may be made from the data shown in table 4. Most of the results are in good agreement, with raw beef and unheated soybean meal showing the greatest variation between the results obtained in experiment 1 and subsequent experiments. Although the reasons for these variations are not apparent, the standard growth curve in the

first experiment was obtained using 0, 20, 40, 60, 120, and 240 mg of L-tryptophan/100 gm ration. Thus the shape of the standard curve was not defined by experimental results in the region between 60- and 120-mg tryptophan supplement levels (see fig. 1). The weight gains observed for those foods fed at the 80 mg level of tryptophan supplementation were near the asymptotic portion of the growth curve and small differences in gain resulted in large differences in the calculated percentage utilization of tryptophan. All subsequent experiments included L-tryptophan at 80- and 100-mg levels as well, to provide additional information on the shape and configuration of the curve between the 60- and 120-mg levels.

TABLE 4

Comparison of tryptophan utilization values observed in repeat experiments [1]

FOOD TESTED	EXP. 1	EXP. 2	EXP. 6-a	EXP. 6-b [2]
Raw beef	68	. . .	112	104
Roast beef	82	. . .	108	89
Unheated soybean meal	152	118	. . .	. . .
Normally processed soybean meal	138	125	116	123
Rolled oats	107	111	111	104

[1] Foods added to provide 40 mg of tryptophan/100 gm ration.

[2] Basal ration supplemented with 3 mg of niacin + 300 mg of DL-threonine/100 gm.

It was also important to determine the recovery of tryptophan when pure L-tryptophan was fed to provide 40 mg/100 gm ration in addition to an equivalent amount provided by each of several foods. Total tryptophan utilization values for L-tryptophan + test food of 95, 100, 111 and 103% were obtained for raw ham (uncured), unheated soybean meal, normally processed soybean meal, and rolled oats, respectively. These results indicate that tryptophan is readily utilized to support growth of the male weanling rat, whether obtained as the purified amino acid or as it occurs naturally in these food proteins.

Table 5 summarizes the values observed for the utilization of tryptophan from the foods tested in these studies, ranging

from 75% for split peas to 132% for unheated soybean meal. These results indicate that tryptophan from fresh and cooked meats, milk, legumes, and cereal foods is very well utilized to support growth in the male weanling rat. Values for the utilization of tryptophan were much higher than those observed in the earlier studies of lysine and methionine utilization (Guthneck et al., '53; Schweigert and Guthneck, '54). For example, percentage values for lysine, methionine, and tryptophan were 79, 87, and 95 respectively, for raw beef; 48, 49, and 132, for unheated soybean meal; and 70, 77, and

TABLE 5

Summary of tryptophan utilization from test foods by the rat

FOOD TESTED	TRYPTOPHAN UTILIZED
	%
Raw beef rib	95
Roast beef rib	93
Raw ham (uncured)	107
Roast ham (uncured)	83
Raw lamb leg	88
Roast lamb leg	94
Unheated soybean meal	132
Normally processed soybean meal	121
Overheated soybean meal	78
Nonfat dry milk	85
Rolled oats	117
Split peas	75

117, respectively, for rolled oats. The high utilization of tryptophan from unheated soybean meal is in marked contrast to the low value observed with the chick (20%) (Schweigert, '48). The results obtained with the rat in the present study were confirmed in repeat experiments, and led to the conclusion that tryptophan is essentially 100% utilized from foods which have not been subjected to cooking or heat-processing. In such foods, tryptophan utilization values ranged from 75 to 94% for the foods studied.

Tryptophan utilization values greater than 100% have been observed in several instances in these studies. The figure of

107% obtained for raw ham (uncured) is within the range of experimental error of the chemical and microbiological tryptophan assay methods and rat bioassay methods employed. The figures of 132, 121 and 117% observed for unheated soybean meal, normally processed soybean meal and rolled oats, respectively, suggest that the assay results for the tryptophan content of these foods may have been low. Other studies have shown that the presence of carbohydrates in foods may result in some destruction of tryptophan during hydrolysis of the sample, resulting in low values for tryptophan content. The use of alkali and cysteine is designed to minimize these losses in the hydrolysis procedures for microbiological analyses. Since the chemical and microbiological assay values agree, however, it is concluded that appreciable destruction of tryptophan did not occur during preparation of the samples for the microbiological assays. In the chemical method, the sample is merely solubilized by mild heating in alkali. The tryptophan values for the meats studied appear reliable, and the small amounts of carbohydrate present would not be expected to result in low values for the tryptophan assays. If the tryptophan assay results for the unheated and normally-processed soybean meals and rolled oats were too low by 10 to 15%, "true" tryptophan utilization values for these foods would lie close to 100%. It is also possible that tryptophan may be more effectively utilized from these food proteins than from L-tryptophan supplements.

The possibility that net synthesis or destruction of tryptophan by microörganisms in the intestinal tract might affect the results was considered. If this did occur, its effect was negligible in terms of the tryptophan utilization results obtained, in view of the consistency of the results for foods fed at several levels. Similarly, the possibility that such organisms might respond differently to tryptophan obtained from food proteins than to the pure amino acid was also considered, but the results of the recovery experiment indicate that any such differential response was of negligible effect on the tryptophan utilization values observed.

There is some indication that tryptophan is less available from overheated soybean meal than from unheated or normally processed soybean meal. A similar effect was noted in the studies of lysine utilization (Guthneck et al., '53). There was no tendency toward increased fecal excretion of tryptophan for the cooked or overheated foods. The effect of processing may also explain the lower tryptophan utilization values observed for nonfat dry milk and split peas than for some of the other foods.

A reliable assay for the determination of the utilization of tryptophan in foods and feeds has been developed in the course of these studies, using the techniques described. While more expensive and time-consuming than the chemical and microbiological methods commonly employed, it possesses certain advantages, such as elimination of the problems involved in assays for tryptophan in foods high in carbohydrates, and provision of a basis for evaluating the effects of cooking or processing on the availability of the tryptophan present, for the support of growth.

SUMMARY

Experiments were conducted to provide a test procedure for the quantitative evaluation of the utilization of tryptophan from foods using the male weanling rat. The method developed is of short duration and provides an adequate range of response to tryptophan supplementation of the diet. A tryptophan-deficient basal ration composed of 10% of oxidized casein + 4% of untreated casein, supplemented with cystine, methionine, and tyrosine, was used. The weight gain of rats fed the basal ration plus graded levels of L-tryptophan or known amounts of tryptophan in foods, was used as the criterion for determining the quantitative utilization of tryptophan from those foods. The repeatability of the results using different levels of the test foods, after varying experimental periods, and in independently repeated experiments gives support to the validity of the methods employed. Attempts to obtain improved growth response with supplements

of niacin and threonine resulted in decreasing tryptophan utilization values as the amount of test food was increased.

The percentage of tryptophan utilized for growth of the male weanling rat when fed raw and roast beef, ham and lamb, nonfat dry milk, overheated soybean meal, and split peas ranged from 75 to 107%. Tryptophan utilization results for unheated and normally-processed soybean meals, and rolled oats ranged from 117 to 132%. The results suggest that cooking or heat processing may result in reductions in the availability of tryptophan to support growth. The percentage of ingested tryptophan excreted in the feces ranged from 1 to 10%.

The high values observed for tryptophan utilization in these studies gain added significance when compared to those observed in the earlier studies on lysine and methionine. The ranges in the observed amino acid utilization values for the foods tested were 49 to 98% for lysine, 48 to 83% for methionine, and 75 to 132% for tryptophan.

LITERATURE CITED

BATES, R. W. 1937 A rapid method for the quantitative determination of tryptophan. J. Biol. Chem., 119: vii.

BENNETT, M. A., AND G. TOENNIES 1942 A nutritional assay of casein modified by the action of hydrogen peroxide and formic acid. Ibid., 145: 671–677.

GRAHAM, C. E., E. P. SMITH, S. W. HIER AND D. KLEIN 1947 An improved method for the determination of tryptophan with p-dimethylamino-benzaldehyde. Ibid., 168: 711–716.

GUTHNECK, B. T., B. A. BENNETT AND B. S. SCHWEIGERT 1953 Utilization of amino acids from foods by the rat. II. Lysine. J. Nutrition, 49: 289–294.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of the chick. J. Biol. Chem., 138: 459.

LYMAN, C. M., O. MOSELEY, S. WOOD AND F. HALE 1946 Note on the use of hydrogen peroxide-treated peptone in media for the microbiological determination of amino acids. Arch. Biochem., 10: 427–431.

SCHWEIGERT, B. S. 1948 Availability of tryptophan from various products for growth of chicks. Ibid., 19: 265–272.

SCHWEIGERT, B. S., AND B. T. GUTHNECK 1953 Utilization of amino acids from foods by the rat. I. Methods of testing for lysine. J. Nutrition, 49: 277–288.

SCHWEIGERT, B. S., AND B. T. GUTHNECK 1954 Utilization of amino acids from foods by the rat. III. Methionine. Ibid., *54:* 333-344.

TOENNIES, G. 1942 The oxidative conversion of casein into protein free of methionine and tryptophan. J. Biol. Chem., *145:* 667-670.

WILKENING, M. C., B. S. SCHWEIGERT, P. B. PEARSON AND R. M. SHERWOOD 1947 Studies on the requirement of the chick for tryptophan. J. Nutrition, *34:* 701-713.

POTENTIATING
EFFECTS OF MATERIALS OF PLANT AND ANIMAL ORIGIN ON SYMPTOMS OF HYPERVITAMINOSIS A IN THE RAT [1]

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In recent years increasing interest has been shown in the study of hypervitaminosis A, the intoxication resulting from excessive intake of vitamin A. The subject is of interest not only because hypervitaminosis A has been observed in man but also because the condition serves as a useful tool for gaining insight into the metabolic behavior of vitamin A, particularly in regard to its interaction with other nutrients *in vivo* (Nieman and Obbink, '54). Available data indicate that the prolonged ingestion of massive doses of vitamin A results in growth retardation, spontaneous fractures, paralysis and other toxic manifestations in the immature rat, the severity of symptoms and their rapidity of onset being proportional to the dosage of vitamin A administered (See Rodahl, '50, and Nieman and Obbink, '54, for review of the literature). In the present communication data are presented indicating that alfalfa and other materials of plant and animal origin contain a factor or factors, apparently distinct from any of the known

nutrients, which significantly increase the toxic manifestations of hypervitaminosis A in immature rats fed massive doses of this vitamin.

PROCEDURE

A series of experiments was designed to study the effects of the addition of alfalfa and other materials of plant and animal origin on symptoms of hypervitaminosis A in immature rats fed a purified ration containing a massive but relatively non-toxic dose of vitamin A. The basal diet employed in these experiments consisted of sucrose, 66%; casein,[2] 24%; salt mixture,[3] 5%; and corn oil, 5%. To each kilogram of the above diet were added the following crystalline vitamins: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; vitamin B_{12}, 150 µg; 2-methyl-naphthoquinone, 5 mg; choline chloride, 2 gm; vitamin A, 5000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins and supplements to be tested were added in place of equal amounts of sucrose (table 1). The rats were housed in metal cages with raised screen bottoms (two or three animals per cage) and were provided with water and food ad libitum. Diets were made up weekly and stored under refrigeration when not in use. The animals were fed daily and all food not consumed 24 hours after feeding was discarded. These measures were employed to minimze oxidative changes in the diet. The feeding was continued for 6 weeks or until death, whichever occurred first. For all of the experiments female rats of the Wistar strain were selected at 21 to 23 days of age and an average body weight of approximately 43 gm (range 38 to 50 gm). In each experiment one group was fed the basal diet; the remaining groups were fed the basal diet with the supplements listed in table 1.

[2] Vitamin-free Test Casein, General Biochemicals, Inc., Chagrin Falls. Ohio.
[3] Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

RESULTS

*Experiment 1. Effects of graded levels of vitamin
A acetate and vitamin A palmitate on symptoms
of hypervitaminosis A in the rat*

In agreement with earlier findings (Rodahl, '50) the ingestion of massive doses of vitamin A resulted in a significant retardation in growth, the occurrence of spontaneous bone fractures in the legs and other manifestations of hypervitaminosis A toxicity including death. The severity and rapidity of onset of symptoms were proportional to the dosage of vitamin A administered. The ingestion of rations supplemented with 2.5 million U. S. P. units of vitamin A per kilogram of diet (either as the acetate or the palmitate) resulted in a slight retardation in growth but other manifestations of hypervitaminosis A toxicity including the occurrence of grossly detectable spontaneous leg fractures and paralysis were lacking. With the exception of a slight reduction in size these animals appeared grossly normal in all respects and were indistinguishable from rats fed the basal diet. The ingestion of diets supplemented with 3 or 3.75 million U.S.P. units of vitamin A per kilogram of diet (either as the acetate or the palmitate) resulted in a more pronounced retardation in growth and the occurrence of grossly detectable spontaneous leg fractures or paralysis or both in approximately 50% of the animals in these groups. The ingestion of diets supplemented with 5 million U.S.P. units of vitamin A per kilogram of diet (either as the acetate or the palmitate) resulted in a 100% incidence of leg fractures (and paralysis) and the death of 15 of the 16 rats in these groups within an experimental period of 6 weeks. No significant difference was observed at the various levels of supplementation between rats fed vitamin A acetate and those administered a comparable amount of vitamin A palmitate.

*Experiment 2. Potentiating effect of alfalfa meal
on symptoms of hypervitaminosis A in the rat*

The findings indicate that supplements of alfalfa meal when fed at a 20% level in the diet resulted in a striking increase in

TABLE 1

Comparative effects of alfalfa meal and other supplements on symptoms of hypervitaminosis A in the rat [1,2]

SUPPLEMENTS FED WITH BASAL DIET	NUMBER OF ANIMALS	INITIAL WEIGHT	GAIN IN BODY WEIGHT FOLLOWING DAYS OF FEEDING		PER CENT SURVIVING	RATS WITH FRAC-TURES [3]	AVERAGE TIME OF ONSET OF FRAC-TURE [3]
			21	42			
		gm	gm	gm		%	days
EXPERIMENT 1							
None	8	42.4	79 (8)	136 (8)	100	0	
2.5 million units vitamin A acetate/kg of diet	8	42.6	69 (8)	114 (8)	100	0	
3 million units vitamin A acetate/kg of diet	8	43.0	57 (8)	99 (8)	100	37.5	24
3.75 million units vitamin A acetate/kg of diet	8	43.0	52 (8)	78 (7)	87.5	62.5	22
5 million units vitamin A acetate/kg of diet	8	42.8	22 (3)	. .	0	100	11
2.5 million units vitamin A palmitate/kg of diet	8	42.7	69 (8)	109 (8)	100	0	
3 million units vitamin A palmitate/kg of diet	8	43.1	62 (8)	106 (8)	100	50	26
3.75 million units vitamin A palmitate/kg of diet	8	43.0	54 (8)	91 (6)	75	50	26
5 million units vitamin A palmitate/kg of diet	8	43.0	20 (4)	37 (1)	12.5	100	10
EXPERIMENT 2							
None	6	44.0	74 (6)	134 (6)	100	0	
2.5 million units vitamin A palmitate/kg of diet	6	43.5	64 (6)	108 (6)	100	0	
3 million units vitamin A palmitate/kg of diet	6	43.5	64 (6)	102 (6)	100	50	26
2.5 million units of vitamin A palmitate/kg of diet plus the following supplements:							
20% alfalfa meal, lot 1	6	43.7	42 (6)	58 (6)	100	100	22
20% alfalfa meal, lot 2	6	43.5	33 (6)	58 (6)	100	100	16
5% dried alfalfa juice	6	43.6	61 (6)	98 (6)	100	67	26
15% alfalfa residue [4]	6	43.5	45 (6)	74 (6)	100	100	22
2.5 million units vitamin A acetate/kg of diet	7	43.4	72 (7)	117 (7)	100	0	
3 million units vitamin A acetate/kg of diet	7	43.5	57 (7)	99 (7)	100	43	24
2.5 million units of vitamin A acetate/kg of diet plus the following supplements:							
20% alfalfa meal, lot 1	7	43.4	49 (7)	85 (6)	86	100	18
20% alfalfa meal, lot 2	7	43.2	44 (7)	70 (7)	100	100	17
5% dried alfalfa juice	7	43.3	62 (7)	104 (7)	100	71	23
15% alfalfa residue [4]	7	43.5	59 (7)	96 (7)	100	100	26
EXPERIMENT 3							
None	8	42.2	73 (8)	129 (8)	100	0	
2.5 million units vitamin A palmitate/kg of diet	8	42.7	62 (8)	107 (8)	100	25	26
3 million units vitamin A palmitate/kg of diet	8	43.0	57 (8)	92 (8)	100	50	23

TABLE 1 *(continued)*

Comparative effects of alfalfa meal and other supplements on symptoms of hypervitaminosis A in the rat [1,2]

SUPPLEMENTS FED WITH BASAL DIET	NUMBER OF ANIMALS	INITIAL WEIGHT	GAIN IN BODY WEIGHT FOLLOWING DAYS OF FEEDING		PER CENT SURVIVING	RATS WITH FRACTURES [3]	AVERAGE TIME OF ONSET OF FRACTURE [3]
			21	42			
		gm	*gm*	*gm*	*%*		*days*
2.5 million units of vitamin A palmitate/kg of diet plus the following supplements:							
20% alfalfa meal, lot 3	8	42.0	40 (8)	51 (8)	100	100	17
20% defatted alfalfa meal [5]	8	42.3	46 (8)	58 (8)	100	100	21
B vitamins, C and K [6]	8	43.2	55 (6)	97 (6)	75	50	14
Vitamins A, D, and E [7]	8	43.4	54 (8)	96 (8)	100	37.5	24
10% casein [8]	8	42.2	60 (8)	105 (8)	100	50	24
10% cellulose [9]	8	43.0	55 (8)	106 (8)	100	37.5	21
5% corn oil	8	43.0	57 (7)	108 (7)	87.5	25	17
2.5% salt mixture [10]	8	42.3	63 (8)	111 (8)	100	25	17
Combined supplements [11]	8	42.0	60 (8)	103 (8)	100	50	21
2.5% alfalfa ash	8	42.5	64 (8)	115 (8)	100	12.5	29
Aureomycin HCl [12]	8	42.6	62 (8)	95 (8)	100	75	22
Mixed tocopherols [13]	8	42.4	59 (8)	114 (8)	100	25	23
EXPERIMENT 4							
None	8	42.3	72 (8)	121 (8)	100	0	
20% alfalfa meal, lot 4	8	42.0	77 (8)	119 (8)	100	0	
2.5 million units vitamin A palmitate/kg of diet	12	43.0	64 (12)	107 (12)	100	0	
3 million units vitamin A palmitate/kg of diet	8	43.0	54 (8)	94 (8)	100	12.5	22
2.5 million units of vitamin A palmitate/kg of diet plus the following supplements:							
5% alfalfa meal, lot 4	7	42.7	42 (7)	73 (7)	100	57	19
10% alfalfa meal, lot 4	7	43.0	43 (7)	66 (6)	86	86	16
20% alfalfa meal, lot 4	8	42.8	26 (8)	36 (3)	37.5	100	10
20% rye grass	7	42.5	32 (7)	60 (3)	43	86	15
20% orchard grass	7	42.9	28 (7)	66 (2)	29	86	17
20% wheat grass	7	43.0	32 (7)	49 (5)	71	100	21
20% fescue grass	7	42.6	44 (7)	60 (5)	71	86	19
20% oat grass	7	43.0	44 (7)	59 (7)	100	86	24
10% desiccated liver N.F.	8	42.2	45 (8)	80 (7)	87.5	100	14
10% liver residue	7	42.8	57 (7)	76 (7)	100	86	23
2.5% liver concentrate N.F.	7	43.0	52 (7)	90 (6)	86	71	14
10% yeast [14]	8	43.0	62 (8)	110 (8)	100	62.5	22
2.5% Vigofac [15]	7	43.0	45 (6)	76 (5)	71	71	13
5% tuna meal	8	42.6	64 (8)	105 (8)	100	0	
5% tuna solubles	8	42.8	52 (8)	96 (8)	100	25	24

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

[2] The alfalfa samples were kindly provided by Dr. S. Tenkoff of Nutrilite Products, Inc., Buena Park, California. The dehydrated rye gass, orchard grass, wheat grass, fescue grass and oat grass were obtained from the National Chlorophyll and Chemical Company, Lamar, Colorado. The desiccated liver N.F., liver concentrate N.F. and liver residue were provided by Dr. David Klein of Wilson Laboratories, Chicago, Ill. Dr. E. Geiger of the Van Camp Sea Foods Company, Terminal Island, California, supplied the tuna meal and tuna solubles (50% solids). The vitamin A supplements employed in the present experiment were synthetic vitamin A acetate in corn oil (1 million U.S.P. units/gm) and synthetic vitamin A palmitate in corn oil (1 million U.S.P. units/gm), obtained from Hoffman-La Roche, Inc., Nutley, New Jersey.

[3] Data were based only on animals with grossly detectable leg fractures sufficiently marked to result in dragging or disuse of the limb.

[4] The water-washed alfalfa pulp remaining after the extraction of the juice.

[5] Alfalfa meal was extracted for 15 hours with n-hexane followed by an 8-hour extraction with 80% acetone-water solvent. The carotenoid content of the resulting meal was 0.15 μg per gram.

[6] The following vitamins were added per kilogram of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; paraaminobenzoic acid, 400 mg; inositol, 800 mg; vitamin B_{12}, 150 μg; and 2-methyl-naphthoquinone, 5 mg.

[7] 5000 U.S.P. units of vitamin A, 500 U.S.P. units of vitamin D_2, and 100 mg alpha-tocopherol acetate per kilogram of diet.

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

[9] Solka-floc BW 200, Brown and Co., Berlin, New Hampshire.

[10] Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

[11] 10% cellulose, 5% corn oil, 2.5% salt mixture and the vitamin supplements indicated in footnotes 7 and 8.

[12] 100 mg aureomycin HCl per kilogram of diet.

[13] The mixed tocopherols were supplied at a level of 500 I.U. per kilogram of diet. The product employed was Vitamin E, Type 4-50 Concentrate (assaying 372 I.U. per gram), Distillation Products Industries, Rochester, New York.

[14] Primary Dried Yeast, Strain no. 200, Anheuser, Busch, Inc., St. Louis, Missouri.

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

the toxic manifestations of hypervitaminosis A in the imma-
ture rat. These effects were manifest not only by the increased
growth retardation of animals fed alfalfa meal in conjunction
with the vitamin A supplement but also by the high incidence
of leg fractures in the alfalfa groups (100%) as compared to
the 0% incidence in animals fed a similar diet with the alfalfa
meal omitted. Dried alfalfa juice when fed at a 5% level in
the diet and alfalfa residue when incorporated at a 15% level
in the diet were also active in potentiating symptoms of hyper-
vitaminosis A in the rat. The effects obtained with the latter
supplements were less pronounced, however, than those
obtained with the whole alfalfa meal. The potentiating effects
of alfalfa meal and the alfalfa fractions on symptoms of
hypervitaminosis A in the rat were demonstrable both when
vitamin A acetate and vitamin A palmitate were employed as
sources of excess vitamin A.[4]

*Experiment 3.   Comparative effects of alfalfa meal
and supplements of the known nutrients on
symptoms of hypervitaminosis A
in the rat*

In agreement with observations made in experiment 2,
supplements of alfalfa meal when fed at a 20% level in the
diet resulted in a striking potentiation of the symptoms of
hypervitaminosis A in the rat. Defatted (carotenoid-free)
alfalfa had a similar potentiating effect. In contrast to the
results obtained above, supplements of all the known vitamins,
salt mixture, cellulose, protein in the form of casein, or fat in
the form of corn oil, either when fed alone or with one another,

[4] Preliminary data indicate that the reduced growth increment of rats fed the
diets containing alfalfa meal was associated with a reduced food intake as
compared to that of rats fed the diets containing a similar amount of vitamin A
but with the alfalfa omitted. Since the vitamin A was incorporated in the diet,
this meant that a reduction in food intake was associated with a reduction in the
amount of vitamin A ingested. The higher incidence of fractures in rats fed the
alfalfa-containing diets occurred despite the fact that the total amount of vitamin
A ingested by these animals was less than that of rats fed similar diets with the
alfalfa meal omitted.

had little if any potentiating effect. Alfalfa ash at a level corresponding to the amount provided by 20% alfalfa meal in the diet and mixed tocopherols at a level of 500 I.U. per kilogram of diet were also without significant effect. Aureomycin HCl at a level of 100 mg per kilogram of diet had little if any effect on weight increment but did appear to increase the incidence of leg fractures. The potentiation of the symptoms of hypervitaminosis A resulting from supplementation with alfalfa meal does not appear to be due to the ingestion of vitamin A precursors (carotenoids) in this material. This is indicated by the fact that the defatted carotenoid-free alfalfa meal was just as active as unextracted alfalfa meal in potentiating the symptoms of hypervitaminosis A in the present experiment. Furthermore, the severity of symptoms (i.e., growth retardation and incidence of leg fractures) obtained in animals fed the alfalfa supplements was significantly greater than that of animals fed the diet supplemented with 3 million U.S.P. units of vitamin A palmitate per kilogram of diet in spite of the fact that the latter ration contained 425,000 and 500,000 units more of vitamin A per kilogram of diet than the unextracted and defatted alfalfa meal rations, respectively.

Experiment 4.   Comparative effects of alfalfa meal and other materials of plant and animal origin on symptoms of hypervitaminosis A in the rat

In agreement with previous finding supplements of alfalfa meal resulted in a striking increase in the toxic manifestations of hypervitaminosis A in the immature rat. The effects obtained were proportional to the alfalfa content of the ration. A supplement of 5% alfalfa meal significantly increased the growth retardation and incidence of leg fractures of rats fed the basal diet supplemented with 2.5 million U.S.P. units of vitamin A palmitate per kilogram of diet as compared to the results obtained on a similar ration with the alfalfa meal omitted. The effects obtained were less marked, however, than those which occurred at the 10% level of supplementation;

and the latter in turn were less marked than those obtained with the diet containing 20% alfalfa meal. The alfalfa meal employed in this experiment (lot 4) appeared to be more potent in accentuating symptoms of hypervitaminosis A than the alfalfa meals tested in experiments 1 and 2. This is indicated by the greater growth retardation and higher incidence of mortality of rats fed the diet containing 20% alfalfa meal, lot 4, as compared to the growth retardation and incidence of mortality of rats fed a similar level of the other alfalfa meal samples. These findings suggest that different batches of alfalfa meal may vary significantly in their capacity to potentiate symptoms of hypervitaminosis A.

In addition to alfalfa meal a number of other materials of both plant and animal origin were also active in potentiating symptoms of hypervitaminosis A in the immature rat. Dehydrated rye grass, orchard grass, wheat grass, fescue grass and oat grass when fed at a 20% level in the diet were as active as an equal amount of alfalfa meal in this respect. Desiccated liver when fed at a 10% level in the diet also showed significant activity. Both the water-insoluble liver residue and the water-soluble liver concentrate were active in this regard. A product derived from fermentation sources [5] when fed at a 2.5% level in the diet also exhibited significant activity. Tuna solubles at a 5% level in the diet also appeared to have some activity although less than the supplements indicated above. Yeast at a 10% level in the diet had no effect on weight increment but did increase the incidence of fractures. Tuna meal at a 5% level in the diet was without significant effect. In view of the marked growth retardation obtained with alfalfa meal when fed at a 20% level in the ration together with 2.5 million U.S.P. units of vitamin A per kilogram of diet, tests were conducted to determine whether this amount of alfalfa meal would depress growth when fed in a comparable diet with the excess vitamin A omitted. The findings indicated that the addition of alfalfa meal, lot 4, at a 20% level to the basal diet was without deleterious effect on either weight increment or

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

gross appearance. Rats fed the latter ration appeared normal in all respects and were indistinguishable grossly from the rats fed the basal diet.

DISCUSSION

The findings indicate that supplements of alfalfa and other succulent plants accentuated the symptoms of hypervitaminosis A in immature rats fed massive but relatively non-toxic doses of this vitamin. Desiccated liver, yeast, a product derived from fermentation sources and aureomycin HCl also showed some activity in this regard. In contrast to the above, supplements of all the known nutrients, either when fed alone or with one another, had little if any potentiating effect. These findings suggest that alfalfa and the other materials indicated above contain a factor (or factors) apparently distinct from any of the known nutrients which significantly increases the toxic manifestations of hypervitaminosis A in immature rats fed massive doses of this vitamin. No data are available to indicate the mechanism whereby the factor (or factors) indicated above exerts its physiologic effects. The possibility that alfalfa and the other active materials contain antioxidants that prevent the destruction of vitamin A in the diet and hence leave more of the vitamin present for absorption appears to be ruled out by chemical tests which indicate that no detectable destruction of vitamin A occurred in the purified basal diet supplemented with 2.5 million U. S. P. units of vitamin A palmitate per kilogram of ration after 30 days of refrigeration. Under conditions of the present experiment diets were made up at weekly intervals and were consumed within 10 days of the time they were made. In addition, no significant difference in response occurred between rats fed the above ration and animals fed an identical diet which was made up daily immediately before feeding. Furthermore, if an antioxidant effect were involved, one might have anticipated that the mixed tocopherols when fed at a level of 500 I. U. per kilogram of diet would have been active in potentiating symptoms of hypervitaminosis A. Such was not the case. The possibility that alfalfa and the other active materials contain

an antioxidant (or antioxidants) with specialized follow-through activity which protected vitamin A from oxidation in the intestinal tract or the animals' tissues, however, has not been excluded. In this regard, Deuel et al. ('56) have observed that DPPD (N, N[1]-diphenyl-p-phenylenediamine) was extremely active in potentiating symptoms of hypervitaminosis A under conditions comparable to those employed in the present experiment. In contrast to the effects obtained with DPPD, 6-ethoxy-2, 2, 4-trimethyl-1, 2-dihydroquinoline,[6] or alpha-tocopherol acetate were without significant effect. In more recent work the antioxidants DBH (2, 5-di-tert-butylhy-droquinone) and BHT (2, 6-di-tert-butyl-p-cresol) were also found to be without effect.[7] The mechanism whereby DPPD accentuates symptoms of hypervitaminosis A has not been established. Since other antioxidants did not behave in a similar manner, it is possible that the effects obtained with DPPD may have been due to some property other than its antioxidant activity.

The symptoms of hypervitaminosis A exhibited in the present experiment by rats fed supplements of alfalfa meal and other materials appeared to be identical in appearance, time of onset, severity and incidence to those of rats fed the purified basal diet supplemented with amounts of vitamin A considerably in excess of the amounts present in the above rations. These findings suggest that supplements of alfalfa meal (and other materials) may have promoted an increased absorption or utilization (or both) of vitamin A. That unidentified factors exist in natural foodstuffs which promote utilization of vitamin A has been reported by a number of investigators. Palm kernel meal, coconut cake, acetone-extracted herring roe (Tainsh and Wilkinson, '39; Gridgeman et al., '40), soybean lecithin (Slanetz and Scharf, '43, '45), yeast (Patrick and Morgan, '43) and fish solubles (Harms et al., '56a, b) have all been shown to increase vitamin A utiliza-

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[6] Santoquin, Monsanto Chemical Co., St. Louis, Mo.

[7] Cox, R. P., R. B. Alfin-Slater, H. J. Deuel, Jr. and B. H. Ershoff. Unpublished data.

tion in experimental animals. A similar effect has been reported for aureomycin (Murray and Campbell, '55a, b). That at least two separate factors exist in the above supplements would seem to be indicated by the observation that the yeast factor is soluble in fat solvents (Patrick and Morgan, '43) whereas the fish factor is water-soluble (Harms et al., '56b). Whether alfalfa and the other materials which potentiated symptoms of hypervitaminosis A in the present experiment would increase the absorption or utilization of vitamin A when the latter is ingested in suboptimal amounts has not been determined. It is of interest, however, that both yeast and aureomycin which were effective in increasing the absorption or utilization of suboptimal amounts of vitamin A (Patrick and Morgan, '43; Murray and Campbell, '55a, b) resulted in a significant increase in the incidence of fractures in rats fed a massive but relatively non-toxic dose of vitamin A under conditions of the present experiment.

SUMMARY

Immature rats were fed a purified ration containing a massive but relatively non-toxic dose of vitamin A. Supplements of alfalfa meal and other succulent plants resulted in a significant potentiation of the symptoms of hypervitaminosis A. Both the dried alfalfa juice and the water-washed pulp remaining after the extraction of the juice were active in this regard. Desiccated liver, yeast, a product derived from fermentation sources [8] and aureomycin HCl also showed activity. In contrast to the above, supplements of all the known nutrients had little if any potentiating effect.

LITERATURE CITED

DEUEL, H. J., JR., R. P. COX, R. B. ALFIN-SLATER AND B. H. ERSHOFF 1956 Comparative effects of DPPD and other antioxidants on hypervitaminosis A in the rat. Fed. Proc., *15*: 548.

GRIDGEMAN, N. T., H. LEES AND H. WILKINSON 1940 Effect of the basal diet on the biological assay of vitamin A. J. Trans. Soc. Chem. Ind., *59*: 120.

[8] See footnote 5, p. 534.

HARMS, R. H., A. A. CAMP B. L. REED, E. L. GRANT, B. G. CREECH AND J. R. COUCH
     1956a   Eviden:e for a substance in fish solubles which enhances vitamin
     A storage in chick livers. Poultry Sci., *35*: 285.
HARMS, R. H., B. L. REED AND J. R. COUCH   1956b   Further studies on a factor
     obtained from condensed fish solubles which enhances vitamin A storage
     in chick livers. Ibid., *35*: 1254.
MURRAY, T. K., AND J. A. CAMPBELL   1955a   The effect of aureomycin on the
     apparent utilization of vitamin A by the ovariectomized rat. J.
     Nutrition, *57*: 89.
————— 1955b   Effect of aureomycin on liver storage of vitamin A, and on
     growth, depletion and survival time of rats. Ibid., *57*: 101.
NIEMAN, C., AND H. J. KLEIN OBBINK   1954   The biochemistry and pathology of
     hypervitaminosis A. Vitamins and Hormones, *12*: 69.
PATRICK, H., AND C. L. MORGAN   1943   An unidentified nutrient required for
     proper utilization of dl-alpha-tocopherol by the chick. Science, *98*: 434.
RODAHL, K.   1950   Hypervitaminosis A in the rat. J. Nutrition, *41*: 399.
SLANETZ, C. A., AND A. SCHARF   1943   The effect of phosphatides on utilization of
     vitamin A and carotene. Proc. Soc. Exp. Biol. Med., *53*: 17.
————— 1945   Effect of soybean phosphatides on vitamin A metabolism.
     J. Nutrition, *30*: 239.
TAINSH, P., AND H. WILKINSON   1939   Biological standardization of vitamin A.
     Chemistry and Industry, *58*: 1051.

NUTRIENTS AFFECTING THE VITAMIN B_{12} REQUIREMENT OF CHICKS [1]

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It has been recognized from the time of the early studies with vitamin B_{12} that the requirement for this vitamin may be influenced by other nutrients in the diet. Schaefer and co-workers ('49) reported that choline could lower the vitamin B_{12} requirement of chicks from hens receiving a standard breeder diet. Thus, these chicks did not have low vitamin B_{12} stores. Briggs et al. ('50) reported that the growth of nondepleted chicks was stimulated by supplemental choline or methionine in the absence of vitamin B_{12}. Sunde et al. ('51) observed vitamin B_{12}-sparing effects with choline and methionine in chicks fed a peanut oil meal basal ration. Machlin et al. ('52) observed similar sparing effects with methionine in chicks fed a vitamin B_{12}-free corn-soybean oil meal diet. Some workers, however, have failed to obtain a sparing effect upon the vitamin B_{12} requirement with methionine (Jukes and Stokstad, '51; Titus et al., '55); it appears that a sparing effect might have occurred if higher supplemental levels of methionine had been used. Early studies of the "animal protein factor," which are pertinent to the general topic of vitamin B_{12} activity as affected by constitution of the diet, have been reviewed by Briggs et al. ('50).

[1] A preliminary report of some of these data was given at the annual meeting of the American Institute of Nutrition, Atlantic City, April 12–16, 1954.

In previous reports from this laboratory it was shown that the vitamin B_{12} deficiency and requirement in non-depleted chicks could be markedly enhanced by increasing the fat content of a corn-soybean oil meal diet (Spivey et al., '54; Fox et al., '56). Attempts to produce a vitamin B_{12} deficiency in nondepleted chicks by increasing the fat content of purified diets containing casein were unsuccessful[2]. In the present studies, the high-fat corn-soybean oil meal diet has been employed to evaluate the vitamin B_{12} sparing activity of nutrients in the purified diets.

EXPERIMENTAL PROCEDURE

Female New Hampshire chicks from a commercial hatchery were distributed into groups of 6 chicks each at one day of age. The chicks were maintained in standard electrically heated batteries with screen wire floors. Feed and water were supplied ad libitum; records of feed intake during the 4-week period were kept. The chicks were weighed at weekly intervals and the 4-week weight at the termination of the experiments was the chief criterion used in evaluating the response to dietary treatment.

Diet C30 was used in these experiments. This vitamin B_{12}-free diet has the following composition per kilogram: soybean oil meal 350 gm, ground yellow corn 375 gm, glucose 10 gm, corn oil 5 gm, lard 200 gm, chick salts A[3] 60 gm, riboflavin 8 mg, vitamin D_3 0.02 mg, and 2-methyl-1,4-naphthoquinone 1 mg. The small amounts of glucose and corn oil served as vehicles for the vitamins. Incorporation of other nutrients into the diet was made by their substitution for an equivalent weight of corn.

Since we had been unable to modify a purified casein diet to produce a vitamin B_{12} deficiency in chicks, the noncarbohydrate portion of purified chick diet C2 (Fox et al., '55) was incorporated into the corn-soybean oil meal diet in order to evaluate the vitamin B_{12}-sparing activity of these

[2] Unpublished data.
[3] Briggs et al. ('52).

components. The C2 concentrate [4] (diet C2 less carbohydrate and vitamin B$_{12}$) was substituted for corn in diet C30; various incomplete C2 concentrates were also fed, as indicated in table 1. The amino acid mixture AA5,[5] which was used in some experiments, was formulated to simulate the amino acid composition of casein.

In some experiments the chicks were decapitated and the livers, pooled by experimental group, were assayed for vitamin B$_{12}$ with *Lactobacillus leichmannii* by the U. S. P. procedure ('55).

<div align="center">RESULTS</div>

The data in table 1 are from one experiment that demonstrates the typical effects observed upon supplementing the basal corn-soybean oil meal diet with vitamin B$_{12}$ and various ingredients of the C2 diet. Chicks receiving the basal diet alone grew very poorly during the 4-week period, utilized the diet inefficiently, and had low stores of vitamin B$_{12}$ in the liver. The addition of 100 μg of vitamin B$_{12}$ per kilogram of diet improved growth, feed efficiency, and elevated the concentration of vitamin B$_{12}$ in the liver. Inclusion of 219 gm of C2 concentrate (which contained no vitamin B$_{12}$) per kilogram of basal diet C30 markedly improved growth and feed efficiency but did not raise the concentration of vitamin B$_{12}$ in the liver. The C2 concentrate, from which methionine was omitted,

[4] When the C2 concentrate was incorporated into diet C30, it furnished the following per kilogram of diet: casein 100 gm, gelatin 40 gm, DL-methionine 1.5 gm, corn oil 20 gm, chick salts A 30 gm, glucose 18.5 gm (carrier for the B vitamins), thiamine hydrochloride 4 mg, riboflavin 4 mg, calcium pantothenate 10 mg, choline chloride 1000 mg, nicotinic acid 50 mg, pyridoxine hydrochloride 4 mg, *d*-biotin 0.15 mg, pteroylglutamic acid 1.5 mg, vitamin A acetate 1.5 mg, vitamin D$_3$ 0.01 mg, α-tocopherol acetate 5 mg, and 2-methyl-1,4-naphthoquinone 0.5 mg.

[5] When amino acid mix AA5 was incorporated in the diet at a level of 5%, it contributed the following quantities of amino acids in grams per kilogram of diet: DL-alanine 1.56, L-arginine hydrochloride 1.61, DL-aspartic acid 2.57, L-cystine 0.22, L-glutamic acid 9.65, glycine 0.88, L-histidine hydrochloride 1.26, DL-isoleucine 5.45, DL-leucine 4.16, L-lysine hydrochloride 3.45, DL-methionine 1.50, DL-phenylalanine 2.06, DL-serine 2.31, DL-threonine 4.03, L-tryptophan 0.59, L-tyrosine 2.90, and DL-valine 5.80.

TABLE 1

Vitamin B$_{12}$ sparing effect of constituents of purified diet C2

(6 chicks per group)

GROUP NUMBER	SUPPLEMENT PER KILOGRAM BASAL DIET C30	MEAN 4-WEEK WEIGHT AND S.E.	LIVER WEIGHT	LIVER VITAMIN B$_{12}$	FEED EFFICIENCY
		gm	*gm*	*mμg/gm*	*gm gain/gm diet*
1	None	175 ± 15	4.5	30	0.44
2	Vitamin B$_{12}$, 100 μg	310 ± 16[1]	7.0	700	0.56
3	C2 conc.,[2] 219 gm	409 ± 19[1]	9.7	25	0.70
4	C2 conc, 218 gm (no methionine)	358 ± 18[1]	9.6	19	0.63
5	C2 conc, 190 gm (no methionine or B vitamins)	222 ± 18	6.9	39	0.59
6	C2 conc, 151 gm (no vitamins A, D, E, K or gelatin)	369 ± 39[1]	9.4	32	0.63
7	C2 conc, 219 gm + vitamin B$_{12}$, 100 μg	381 ± 18[3]	8.8	..	0.68

[1] Mean weight significantly higher (P = < 0.01) than the basal group receiving no vitamin B$_{12}$ (group 1).

[2] The C2 concentrate was composed of the noncarbohydrate constituents of purified diet C2 at levels equal to those in 0.5 kg of C2 diet; no vitamin B$_{12}$ was added. The C2 concentrates below were the same except with the indicated omissions.

[3] Mean weight significantly higher (P = < 0.05) than the control group receiving vitamin B$_{12}$ (group 2).

caused an increase in growth; however, the 4-week weight of this group was poorer than that of chicks receiving the complete C2 concentrate. When both methionine and the B vitamins (including choline) were omitted from the C2 concentrate, growth was not increased significantly over that of the basal group. The omission of the fat-soluble vitamins and gelatin from the supplemental C2 concentrate did not significantly alter the good growth supported by the complete concentrate. The data thus far indicate that methionine and one or more of the B vitamins were the chief constituents in the diet C2 that spared vitamin B_{12}.

The simultaneous incorporation of vitamin B_{12} and the complete C2 concentrate into the basal diet resulted in a very marked improvement of growth. It has been reported previously (Fox et al., '56) that 100 µg of vitamin B_{12} per kilogram of diet supported a maximal growth response with this diet; therefore, the improved growth with both supplements over that with only vitamin B_{12} indicated that this basal corn-soybean oil meal diet was somewhat deficient in nutrients other than vitamin B_{12} for optimal growth of the young chick.

In table 2 data are presented from 5 series of experiments on the vitamin B_{12}-sparing capacities of individual nutrients and combinations of nutrients present in the C2 concentrate. The column headed ''Average'' is the mean weight and standard error of the total number of chicks from all series that were fed the indicated supplement. Since all supplements were not included in each series, evaluation of the average values must be tempered by the data of the specific series involved. Mortality in all groups was negligible. When the fat-soluble vitamins, the complete B vitamin mix, or the B vitamin mix minus choline was incorporated in the basal diet, there was no improvement in growth. The addition of 0.2% choline chloride always resulted in mean weight gains. These weight increases were not statistically significant on the basis of comparisons within each series; however, upon consideration

TABLE 2

Vitamin B_{12} sparing effect of B vitamins, casein, and amino acids

(6 chicks per group)

GROUP NUMBER	SUPPLEMENT PER KILOGRAM BASAL DIET C30	MEAN 4-WEEK WEIGHT AND S.E.					
		Series 48	Series 64	Series 66	Series 71	Series 79	Average
		gm	*gm*	*gm*	*gm*	*gm*	*gm*
1	None	170 ± 17	164 ± 28	194 ± 26	198 ± 16	215 ± 26	187 ± 9
2	Vitamin B_{12}, 100 µg	289 ± 21[1]	320 ± 22[1]	329 ± 19[1]	320 ± 18[1]	323 ± 12[1]	316 ± 7[1]
3	Vitamins A, D, E, K[2]	174 ± 25	172 ± 23	174 ± 25			173 ± 16
4	B vitamins[3]	236 ± 28					236 ± 28
5	B vitamins (no choline)[4]		146 ± 17	172 ± 27			160 ± 17
6	As 5 + vitamin B_{12}, 100 µg		294 ± 17				294 ± 17
7	Choline, 2 gm	202 ± 34	198 ± 27	252 ± 28	217 ± 26	280 ± 18	231 ± 12[1]
8	As 7 + vitamin B_{12}, 100 µg		329 ± 14				329 ± 14
9	Cystine, 3 gm	160 ± 18	170 ± 16				165 ± 12
10	Methionine, 1.5 gm	320 ± 34[1]	310 ± 20[1]	325 ± 32[1]	308 ± 18[1]	304 ± 34	313 ± 13[1]
11	As 10 + vitamin B_{12}, 100 µg		356 ± 38				356 ± 38
12	Methionine, 1.5 gm + choline, 2 gm		298 ± 32[1]	352 ± 43[1]	296 ± 28[5]		315 ± 18[1]
13	As 12 + vitamin B_{12}, 100 µg			387 ± 12[6]			387 ± 12[6]
14	Casein, 50 gm	226 ± 29	260 ± 19[5]	206 ± 15	244 ± 20	296 ± 14[5]	244 ± 10[1]
15	Casein, 50 gm + choline, 2 gm		287 ± 15[1]	355 ± 16[1]	324 ± 21[1]	299 ± 18[5]	316 ± 9[1]
16	AA5[7] 50 gm		260 ± 25	232 ± 34	234 ± 33	232 ± 24	240 ± 14[1]
17	AA5, 50 gm + choline, 2 gm				307 ± 13[1]	297 ± 22[5]	302 ± 12[1]
18	AA5, 50 gm (no methionine)[8]			103 ± 17[5]	140 ± 10[1]	125 ± 11[1]	123 ± 8[1]
19	As 18 + vitamin B_{12}, 100 µg			336 ± 26			336 ± 26
20	AA5, 50 gm (no methionine) + choline, 2 gm			193 ± 23	232 ± 22	149 ± 16	191 ± 13

[1] Mean weight significantly different (p = < 0.01) from the basal group receiving no vitamin B_{12} (group 1).

[2] Fat soluble vitamins added at the levels present in one kilogram of diet C2.

[3] B vitamins (no vitamin B_{12}) added at the levels present in one kilogram of diet C2.

[4] B vitamins added as above except that choline was omitted.

[5] Mean weight significantly different (p = < 0.05) from the basal group receiving no vitamin B_{12} (group 1).

[6] Mean weight significantly different (p = < 0.05) from the control group receiving vitamin B_{12} (group 2).

[7] Amino acid mix AA5 was based on the amino acid composition of casein.

[8] Amino acids added as above except that methionine was omitted.

of all 5 series together choline did cause a significant increase in weight gain.

The addition of 0.15% methionine to the basal diet caused marked improvement of growth, which was as good as that with vitamin B_{12}. Data obtained from feeding levels of methionine below 0.15% down to 0.05% were not presented in table 2 since the increases in growth were quite variable. These results may be related to slight variations in the methionine content of crude materials in the diet. Supplementary cystine was completely without effect upon growth of vitamin B_{12}-deficient chicks. The effect of feeding the combination of choline and methionine was little different from the result due to methionine alone. Feeding 5% of casein, which should contribute approximately 1.5 gm of methionine per kilogram of diet, did not improve the growth of vitamin B_{12}-deficient chicks to the same extent as this level of free methionine. In two of the 5 series the casein caused significantly improved growth; however, the combination of casein and choline supported growth that in general was superior to that attained with either alone.

The amino acid mix AA5, whose composition was based on the amino acid content of casein, stimulated growth of vitamin B_{12}-deficient chicks in a manner similar to that of the same level of casein. Again, the combination with choline was superior to either alone. Omission of methionine from the amino acid mix caused a marked depression of growth of the vitamin B_{12}-deficient chicks, but feeding vitamin B_{12} with the methionine-free amino acid mix resulted in growth similar to that of the control group receiving vitamin B_{12} alone. The addition of 0.2% choline chloride at least partially overcame the growth depression caused by the methionine-free amino acid mix.

Many of the supplements that supported improved growth in the deficient chicks were also fed in the presence of vitamin B_{12}. The growth increments caused by the various supplements in the presence of vitamin B_{12} were much smaller than the growth increments of the supplements seen in the absence

of vitamin B_{12}. Only in the case of methionine plus choline (group 13) was growth with the supplement plus vitamin B_{12} significantly better than that of the vitamin B_{12} control group. However, these are data from only one series.

DISCUSSION

From the results presented, it is clear that a number of factors affect vitamin B_{12} deficiency and requirement in non-depleted chicks. The failure to produce a vitamin B_{12} deficiency in chicks fed a purified diet containing casein was due solely to the presence of nutrients which decreased the chick's dietary requirement for vitamin B_{12}.

The data also show that the only nutrients in the purified diet that significantly spared vitamin B_{12} were methionine and choline. It would appear, therefore, that increasing the fat content of the corn-soybean oil meal diet created a dietary demand that could be satisfied with either vitamin B_{12} or additional methionine or partially satisfied with additional choline. It is of interest that the same nutrients that have been reported by other workers (as reviewed above) as sparing the vitamin B_{12} requirement of chicks are the same nutrients that spared vitamin B_{12} in the presence of high fat.

The primary effect of high fat under these dietary conditions was probably to increase the methionine requirement. Baldini and Rosenberg ('55) demonstrated that the methionine requirement of young chicks increased with increasing percentage of fat in the diet. Since choline had a relatively small sparing effect in the present experiments, it appears that a deficiency of preformed methyl groups was not a significant factor. The role of vitamin B_{12} in the synthesis and transfer of methyl groups has been recently reviewed by Arnstein ('55) and this general area of vitamin B_{12} function will be studied further under the dietary conditions employed in the present investigation.

The superiority of free methionine over that in casein for supporting good growth seems to be due to a methionine-depressing action of the other amino acids present in casein

rather than to limited availability of the methionine. The amino acid mix AA5, which was based on the composition of casein, elicited growth responses under various conditions comparable to those with the intact protein. In addition, the same amino acid mix minus methionine depressed growth below that obtained with the basal diet alone. This nutritional stress is comparable to that of high fat in that either methionine or vitamin B_{12} could overcome the effect. The growth depression observed with the methionine-free amino acid mix may be due to an amino acid imbalance involving specific amino acids, or it may be a non-specific increase in methionine requirement associated with increased protein in the diet.

The combination of choline with casein or the complete amino acid mix was similar to the equivalent amount of free methionine fed alone. Under these conditions, choline may have been counteracting the suppression of methionine by the other amino acids present, or it may have been exerting some other beneficial effect unrelated to the amino acid content of the diet.

Diet C30 is limiting chiefly in methionine; therefore, when determining the vitamin B_{12} potency for the chick of crude concentrates or compounds related to vitamin B_{12}, it is necessary to keep the methionine content of the material being assayed at a minimum. On the other hand, it is desirable to limit the methionine content of the diet to the lowest possible level consistent with good growth in order to obtain the greatest growth stimulation upon addition of vitamin B_{12}.

SUMMARY

The effect of certain constituents of purified diets upon the vitamin B_{12} requirement of chicks fed a high fat corn-soybean oil meal diet has been studied. It is concluded that:

1. The only nutrients having a significant vitamin B_{12}-sparing activity were methionine and choline. Methionine at a level of 0.15% completely replaced vitamin B_{12}, whereas addition of 0.2% choline chloride to the diet resulted in only a small sparing effect.

2. Free methionine was more effective in sparing vitamin B_{12} than an equivalent amount of methionine present in casein (5% of the diet) or in an amino acid mix based on the amino acid composition of casein.

3. This same amino acid mix without methionine markedly suppressed growth of chicks below that of the group receiving no vitamin B_{12}. The addition of vitamin B_{12} to the basal diet containing the methionine-free amino acid mix supported optimal growth. Thus, a greater response to vitamin B_{12} was obtained under these conditions.

ACKNOWLEDGMENT

LITERATURE CITED

ARNSTEIN, H. R. V. 1955 The function of vitamin B_{12} in animal metabolism. In ''The Biochemistry of Vitamin B_{12},'' Cambridge University Press, London, pp. 92–108.

BALDINI, J. T., AND H. R. ROSENBERG 1955 The effect of productive energy level of the diet on the methionine requirement of the chick. Poultry Sci., *34:* 1301.

BRIGGS, G. M., E. G. HILL AND M. J. GILES 1950 Vitamin B_{12} in all-plant rations for chicks and sparing activity of methionine and choline. Ibid., *29:* 723.

BRIGGS, G. M., M. R. SPIVEY, J. C. KERESZTESY AND M. SILVERMAN 1952 Activity of citrovorum factor for the chick. Proc. Soc. Exp. Biol. Med., *81:* 113.

FOX, M. R. S., L. O. ORTIZ AND G. M. BRIGGS 1955 Toxicity of ethionine in the young chick. J. Agric. Food Chem., *3:* 436.

———— 1956 Effect of dietary fat on the requirement of vitamin B_{12} by the chick. Proc. Soc. Exp. Biol. Med., *93:* 501.

JUKES, T. H., AND E. L. R. STOKSTAD 1951 Studies of vitamin B_{12}, choline and related factors in the diets of chicks. J. Nutrition, *43:* 459.

MACHLIN, L. J., C. A. DENTON AND H. R. BIRD 1952 Supplementation with vitamin B_{12} and amino acids of chick diets containing soybean or cottonseed meal. Poultry Sci., *31:* 110.

SCHAEFER, A. E., W. D. SALMON AND D. R. STRENGTH 1949 Interrelationship of vitamin B_{12} and choline. II. Effect of growth of the chick. Proc. Soc. Exp. Biol. Med., *71:* 202.

SPIVEY, M. R., G. M. BRIGGS AND L. O. ORTIZ 1954 Effect of diets high in fat or protein on vitamin B_{12} deficiency in non-depleted chicks. Ibid., *85:* 451.

SUNDE, M. L., P. E. WAIBEL, W. W. CRAVENS AND C. A. ELVEHJEM 1951 A relationship between antibiotics, vitamin B_{12} and choline and methionine in chick growth. Poultry Sci., *30*: 668.

THE PHARMACOPEIA OF THE UNITED STATES OF AMERICA, FIFTEENTH REV. 1955 p. 835.

TITUS, H. W., J. H. BRUMBAUGH AND A. L. MEHRING, JR. 1955 Evaluation of the effect of additions of vitamin B_{12}, DL-methionine, and procaine penicillin, singly and in combination, to corn-soybean diets for young growing chicks. Poultry Sci., *34*: 167.

UTILIZATION OF FOOD FOR WEIGHT MAINTENANCE AND GROWTH

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The problems of how food is utilized under various conditions have been discussed by nutritionists for many years. Practically every worker in this field has made contributions to these questions. A new facet of the old problem was revealed in studies of the food requirements for weight maintenance and growth of rats kept at constant weight by restricted feeding. As previously shown (Kaunitz et al., '56a, b), measurements of food and water intake and organ weights of such animals disclosed the existence of certain adaptive mechanisms. It therefore seemed worth while to study food utilization for weight maintenance and growth of rats of various ages and weights after different periods of food restriction. The findings are discussed in this paper.

PROCEDURE

The male albino rats used for the experiments were drawn from a homogeneous colony known to us for 15 years. When they were 20 days old and weighed 45 to 50 gm, they were placed on a complete stock diet containing 30% protein. They were weighed at 24 and at 28 days, and, at the latter time, those of suitable weights (usually about two-thirds of the total) were divided into matching groups of 8 animals whose average weights at 24 and again at 28 days were identical. When these groups were permitted to eat freely, their average

weights and food consumptions differed by only a few grams during the subsequent weeks.

At 29 days of age, the rats were placed in single unit cages with wire bottoms and were given the experimental diet, which consisted of 30% casein, 54% dextrose, 10% lard, 4% salts, 2% cellulose, and liberal amounts of all known vitamins. Measurement of food intakes, restricted and unrestricted, was begun at this time. The freely eating animals were given weighed food cups having an inner metal plate with a small hole and an outer cover. Any spilled food was replaced.

The animals on restricted intake were weighed daily on a scale accurate to one gram and given measured amounts of food (weighed on a scale accurate to 0.1 gm) just sufficient to keep their weights constant within ± 2 gm. On the first day of food restriction, an amount of food corresponding to roughly 6 to 8% of the body weight was fed (with heavier animals receiving the lower percentage). We soon learned to compensate for the small daily fluctuations in body weight by changing the food intake (by approximately 0.5 to 1.0 gm of food for each gram of body weight change).

As previously discussed for other species (Rubner, '02; Cowgill, '28), the maintenance requirements are influenced by the environmental temperature. In our experiments, it was observed in one series that a decline of 10°F. in the average weekly temperature increased the requirements of animals on restricted intakes of diets differing widely in composition by approximately 25%. We have encountered similar effects in the 15 experimental series on restricted intakes which we have studied in the last three years. Although these findings are incidental as far as the main subject of this paper is concerned, they deserve consideration if comparisons are made between groups.

The experiments were intended to yield the food requirements for weight maintenance and growth at different ages and body weights after varying periods of restricted food intake. For this purpose, studies on two experimental series

TABLE 1

Average food requirements of male rats for maintenance of body weight and growth at different ages and body weights and after different periods of restricted food intake

GROUP[1]	AGE	PREVIOUS RESTRICTION	BODY WT.	Maintenance requirement		FIRST 3 DAYS OF REALIMENTATION				
				Per gm body wt. per wk.	Total for 3 days (calculated)[2]	Total food consumed	Increase in body wt.	Requirement per gm increase	Maintenance requirements for successive weeks of restriction	
	wks.	wks.	gm	gm	gm	gm	gm	gm	gm	
					Series I					
1	9	4	97 ± 3[a]	0.37	17.2 ± 1.2	38.1 ± 1.9	23 ± 4	0.91	0.51, 0.42, 0.38, 0.37	
2	9	3	122 ± 9	0.38	21.9 ± 1.4	44.7 ± 6.0	26 ± 5	0.84	0.49, 0.36, 0.37	
3	9	2	151 ± 17	0.39	27.1 ± 3.2	44.7 ± 7.0	22 ± 5	0.80	0.44, 0.39	
4	9	1	177 ± 13	0.43	34.2 ± 2.7	48.7 ± 4.4	19 ± 4	0.76	0.43	
5	9	0	214 ± 12	0.37	33.2[4]	43.2 ± 2.9	10 ± 4	1.00	...	
					Series II					
1	4	1	61 ± 2	0.51	15.1 ± 0.8	29.8 ± 1.7	16 ± 3	0.93	0.51	
4	5	1	95 ± 6	0.50	21.6 ± 1.4	32.6 ± 2.6	11 ± 2	1.00	0.50	
3	6	1	109 ± 6	0.46	22.2 ± 1.1	29.5 ± 2.4	7 ± 3	1.04	0.46	
2	13	1	180 ± 15	0.40	31.9 ± 2.8	43.7 ± 5.1	12 ± 3	0.98	0.40	
1	13	2	167 ± 13	0.33	24.5 ± 1.8	36.5 ± 4.2	11 ± 3	1.09	0.40, 0.33	
6	13	4	154 ± 15	0.32	22.3 ± 2.1	40.9 ± 6.8	16 ± 4	1.16	0.44, 0.37, 0.30, 0.32,	
5	13	6	128 ± 14	0.33	19.7 ± 2.0	39.6 ± 6.5	19 ± 3	1.05	0.41, 0.40, 0.36, 0.33,	
									0.31, 0.33	

[1] Eight animals per group.

[2] Obtained by multiplying the average body weight for the three-day period by 3/7 of the food requirement per gram of body weight as observed in the preceding week.

[a] Average ± standard deviation.

[4] Calculated by second method described in the text.

with 5 and 6 groups of 8 rats each were carried out, the results of which are summarized in table 1.

In the first series, one group was restricted immediately when the experiment began, and the restriction was continued for 4 weeks (group 1, column 1). The next group was restricted one week later (group 2), and the subsequent groups were begun on successive weeks (groups 3 and 4). One group was never restricted (group 5). After the first group had been restricted for 4 weeks and the 4th group, for one week, all groups were permitted to eat freely. In the second series, the duration of food restriction, as well as the age and body weight at which it was started, were varied as indicated in table 1.

<div align="center">RESULTS</div>

Requirements for weight maintenance. The basis for the calculation of the weekly requirements for weight maintenance was the measured food intake of the rats whose weight had been kept constant. In calculating the weekly requirements, which were expressed in grams of food per gram of body weight, a correction was made for the minor differences in body weight between the beginning and the end of the week. As an approximation, one gram was added to or subtracted from the weekly intake for each gram of body weight lost or gained (see requirements for weight increment described below). The average weekly intake divided by the average body weight at the beginning of the week gave the desired value.

It has been shown previously (Quimby, '48; Kaunitz et al., '56a) that the food requirements for weight maintenance decline steeply when the food restriction is prolonged. Keys ('50) observed this to be accompanied by a decline in basal metabolic rate. The decline in food intake also occurred in the current experiments, where the weekly requirements declined from 0.51 to 0.37 gm of food per gram of body weight for the group in the first experimental series which had been kept on restricted intake for 4 weeks and whose maintenance

weight was 97 gm and from 0.41 to 0.33 gm for the group in the second series which was kept at 128 gm for 6 weeks.

When the animals were restricetd for one week at different ages and weights, the requirements for the maintenance of one gram of body weight declined with increasing body weight. This is evident from the data from the second series, where the average body weight increased from 61 to 180 gm (column 4) and the maintenance requirements per gram of body weight decreased from 0.51 to 0.40 (column 5). This was also true for the animals in the first series when the requirements were calculated after one week of food restriction at different weight levels. The average body weight varied, among 4 groups, from 95 to 177 gm, and the maintenance requirements decreased from 0.51 to 0.43 gm.

The decreasing maintenance requirements for higher body weights are in agreement with many studies of the relation of food requirements to body surface in that heavier animals have a relatively smaller surface. It is not probable that the differences in age in these experiments were of consequence because animals restricted for a short period earlier in the experiments and having, thus, comparatively reduced body weights had requirements similar to those of younger animals of comparable weights which had eaten freely throughout the experiment.

Requirements for weight increase. For the determination of the requirements for weight increase, the groups which had been restricted for varying periods of time and then permitted to eat freely were used. The weight gain and food consumption of only the first three days of realimentation were used because, on the one hand, the period was long enough for accurate measurements and, on the other hand, was short enough to permit the assumption that the basic requirement for the maintenance of one gram of body weight would not have changed with the return to "luxury" food consumption. The rats' maintenance requirements for this three-day period were calculated by multiplying the requirement at the lower body weight, as known from the previous week's restriction,

by the average body weight for the three-day period, because
this was the average amount of body tissue which they had
to maintain, and then correcting for a three-day period instead
of one week. This requirement for weight maintenance was
then deducted from the total food consumption for the three-
day period and the result divided by the weight increase to
give the amount of food utilized for each gram of body weight
gained.

This can be illustrated by an example from series I. For
group 1, the weekly requirements for weight maintenance at
the end of the restricted period were 0.37 gm per gram of
body weight per week or a total of 35.8 gm. The animals
weighed, at the beginning of the three-day period, 97 gm and
they gained 23 gm. Their average weight for the period,
therefore, was 108.5, which, when multiplied by 0.37, indicated
maintenance requirements of 40.1 gm for one week or 17.2 gm
for the three-day period. When this was deducted from 38.1
gm (their total food consumption for the period), the re-
mainder of 20.9 gm was the amount of food they had used
for growth. This divided by the weight increase of 23 gm
gave a factor of 0.91 gm of food needed for each gram of body
weight gained. From the data presented in table 1, it can
be seen that the food requirements for one gram of body weight
increase ranged from 0.76 to 1.00 in the first series. Although
the body weights varied from 97 to 214 gm and the previous
period of food restriction from zero to 4 weeks, there was
probably no significant difference in the requirements for the
building of new substance.

In the second series, the requirements for growth varied
only from 0.93 to 1.16 gm of food per gram of weight increase
although the determinations were carried out on animals rang-
ing in weight from 61 to 180 gm and in age from 4 to 13 weeks
and although the previous periods of food restriction varied
from one to 6 weeks.

The food requirements for growth could also be obtained
by deducting the weight maintenance requirements of re-
stricted groups from the total food intake of freely growing

groups. The total requirements for weight maintenance during the first week of food restriction of two well-matched groups whose restriction had begun on successive weeks were averaged because the similarity in the requirements of different groups made it permissible to assume that the value thus obtained would be approximately that of any well-matched freely eating group of the same series over the same weight range. This was now deducted from the total food intake of the freely eating group, and the difference was divided by the gain in body weight to give the amount required for one gram of weight increase. The values obtained by this procedure varied, with one exception, from 0.75 to 1.25 gm of food for one gram of body weight increase and were in fair agreement with the data obtained by the first method. In the table, only the values obtained by the first method are given with the exception of the value for group 5, series I, which was calculated by the second method.

When considering errors in the determination of the requirements for weight maintenance and for weight increase, it must be kept in mind that the calculation of the latter is based on three variables, namely, weight maintenance requirements, weight gain, and total food consumption. This contributes to greater variability in the values for weight increase requirements than in those for weight maintenance requirements because the latter are merely based on the determination of the daily food requirements. For this reason and also because we have noted considerable constancy in 15 experimental series with several groups in each one, differences of as little as 5 to 10% are usually significant in the determination of weight maintenance requirements. In the determination of the requirement for weight increase, the data indicated that, in all probability, only differences exceeding 25% are significant. Direct statistical analyses of the weight increase requirements are difficult in view of the above-noted three variables.

DISCUSSION

In studies of food utilization, investigators sometimes divide the total food consumption by the weight gain during a certain period and relate the value obtained to the efficiency of food utilization. However, inasmuch as the emphasis is usually placed on utilization for growth, errors may arise from the failure to distinguish the two aspects of food utilization — that for weight maintenance and that for the building of new tissue.

The capacity of the animal to lower its maintenance requirements during reduced food intake should also be taken into consideration in experiments involving paired feeding or paired weighing. If differences in average body weights between two groups of pair-fed animals are noted, one is not entitled to conclude, as is so frequently done, that the differences are due to variations in the requirements for weight gain. This was illustrated in paired-feeding experiments with rats fed a diet containing either oxidized or fresh fat (Kaunitz et al., '55). The animals on fresh fat paired to those on oxidized fat grew better, which first led us to believe that the food utilization of the former was better. However, the animals on oxidized fat ate freely but in such small amounts that their paired mates living on fresh fat were fed like animals on a restricted diet. Furthermore, by direct measurement, we later found that the requirements for weight maintenance over a protracted period were much higher among the animals fed oxidized fat (Kaunitz et al., '56b, c). Therefore, the difference in growth on the same food intake between the two groups could have resulted from the ability of the animals on fresh fat to adapt more easily to a restricted intake and, thus, to spare more food for growth. Similar considerations must be made in analyzing results from paired weighing techniques.

SUMMARY

1. Food requirements for weight maintenance and weight increase were determined by measurements of the food intake

of rats kept at constant weight by restricted feeding of a complete, purified diet and of the food intake and weight increase of well-matched rats permitted to eat freely of the same diet.

2. The requirements for the maintenance of one gram of body weight were found to be influenced by the duration of food restriction, the body weight, and the room temperature but hardly by the age of the animals. Continued food restriction led to a reduction of about 30% in the weight maintenance requirements.

3. Food requirements for weight increase did not seem to be influenced by the above factors. Approximately one gram of food was necessary to build one gram of body substance under widely different conditions.

4. The implications of these findings for studies of food utilization and paired weighing and paired feeding techniques are discussed.

LITERATURE CITED

COWGILL, G. R. 1928 The energy factor in relation to food intake: experiments on the dog. Am. J. Physiol., *85*: 45.

KAUNITZ, H., C. A. SLANETZ AND R. E. JOHNSON 1955 Antagonism of fresh fat to the toxicity of heated and aerated cottonseed oil. J. Nutrition, *55*: 577.

KAUNITZ, H., C. A. SLANETZ, R. E. JOHNSON AND J. GUILMAIN 1956a Influence of diet composition on calorie requirements, water intake and organ weights of rats during restricted food intake. Ibid., *60*: 221.

KAUNITZ, H., C. A. SLANETZ, R. E. JOHNSON, H. B. KNIGHT, D. H. SAUNDERS AND D. SWERN 1956b Biological effects of the polymeric residues isolated from autoxidized fats. J. Am. Oil Chem. Soc., *33*: 630.

KAUNITZ, H., C. A. SLANETZ, R. E. JOHNSON, J. GUILMAIN, H. B. KNIGHT, D. H. SAUNDERS AND D. SWERN 1956c Nutritional properties of the molecularly distilled fractions of autoxidized fats. J. Nutrition, *60*: 237.

KEYS, A. 1950 *The Biology of Human Starvation.* Univ. of Minnesota Press, Minneapolis.

QUIMBY, F. H. 1948 Food and water economy of the young rat during chronic starvation and recovery. J. Nutrition, *36*: 177.

RUBNER, M. 1902 *Energiesetze*, Berlin.

THE NON-ESSENTIALITY OF FLUORINE
IN NUTRITION [1,2,3]

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Fluorine occurs naturally in virtually all foods and drinking water and it is present in the bodies of all higher animals. Even though the element is of practical significance in the partial protection of teeth from dental caries, conclusive studies have not been reported concerning its essentiality in nutrition.

Sharpless and McCollum ('33) investigated the essentiality of fluorine for rats using a semipurified diet containing casein, starch, butterfat, yeast, and salts. The femurs of the animals contained about 150 µg of fluorine per gram at 120 days of age, indicating that appreciable amounts of the element remained in the diet. Under these conditions there was no indication that fluorine is essential.

Phillips, Hart and Bohstedt ('34) fed rats a mineralized milk diet reported to contain only 0.1 to 0.2 ppm of fluorine. Nevertheless, at 140 days of age the animals contained several hundred micrograms of the element. Evans and Phillips ('39)

reinvestigated the problem using the same type of mineralized milk diet, and reported that the bones of their animals contained some 10 to 15 micrograms of fluorine per gram, regardless of the age of the animals. There were no indications that the element is essential in nutrition. Muhler ('51, '54) has reported on the storage of fluorine in the femurs of rats fed diets which contained not more than 0.1 ppm of fluorine. The fluorine concentration in the bones was maximal at about 80 days of age. At that time the femurs of the females contained about 23 ppm of fluorine, and those of the males contained about 39 ppm. Unfortunately the purified diet would not sustain reproduction.

McClendon ('44) and McClendon and Gershon-Cohen ('53) have reported that fluorine is definitely an essential element. Rats were raised on a diet based principally on the use of corn and sunflowers. The plants were grown by culture in rain water which was reported to contain only 0.002 to 0.004 ppm of fluorine. The control animals received a diet compounded of ordinary soil grown crops. No data were given on the fluorine content of either the diet or the animals. The "deficient" animals were a great deal smaller and had a much higher incidence of caries than the control group. McClendon and Gershon-Cohen ('54) have also claimed that periodontoclasia is characteristic of fluorine deficiency.

The object of the present investigation was to prepare a substantially fluorine-free diet and observe the growth, reproduction, and general well-being of rats on it through several generations. The deficient animals were to be compared with controls given small amounts of sodium fluoride in the drinking water.

EXPERIMENTAL

Analytical methods. The analytical procedure for the determination of fluorine was a modification of the method of Smith and Gardner ('48). As used here, the method was sensitive for the determination of no less than 1.0 µg of fluorine. For this reason it was necessary to employ large

samples of material when the concentration of fluorine was very small.

In the analysis of samples containing large amounts of salts a single Willard and Winter ('33) distillation did not suffice to separate all the fluorine from the elements which interfere in the determination. Savchuck and Armstrong ('51) had already noted the necessity of employing a double distillation procedure for the determination of fluorine in some cases. The technique employed here was to collect 200 ml of distillate during the primary distillation and then return this to the rinsed still for evaporation to near dryness with highly purified calcium oxide (Weddle and Maurer, '54). A second distillation was then run. Samples were ashed in silica or platinum dishes, first over a Meker burner and then for 8 hours at 550 to 600°C. in a muffle furnace fitted with a silica lining to prevent contamination.

Alkaline and acid phosphatase were determined according to the procedure of King and Armstrong ('34).

Experimental diet. The diet was composed of casein, 18; DL-methionine, 0.5; corn oil, 15; vitamin mixture, 1; salts, 4; and corn starch, 61.5%. The composition of the salt mixture was, in grams, sodium chloride, 293; monopotassium phosphate, 817; calcium oxide, 488; magnesium sulfate, 120; ferric sulfate, 138; manganese sulfate, 9; zinc sulfate, 3; cupric sulfate, 1; potassium iodide, 2; molybdic oxide, 2. The vitamin mixture contained thiamine hydrochloride, 1400 mg; inositol, 1400 mg; calcium pantothenate, 700 mg; pyridoxine hydrochloride, 700 mg; riboflavin, 1400 mg; nicotinic acid, 1400 mg; folic acid, 350 mg; menadione, 350 mg; vitamin B_{12}, 14 mg; biotin, 2 mg; choline chloride, 42 gm; corn starch carrier, 302 gm. The vitamins were all the purest crystalline materials available commercially and were used without further purification. Each 15 gm of corn oil was fortified with 2500 units of vitamin A and 360 units of vitamin D added as percormorph oil, and 15 mg of α-tocopherol.

All equipment used in the purification, storage, compounding and dispensing of the diet was cleaned by boiling in either

a 10% NaOH solution or a H_2SO_4 cleaning solution. After this treatment the equipment was rinsed thoroughly in redistilled water. The latter was obtained by distilling ordinary distilled water from an alkaline permanganate solution in all-pyrex stills.

Purification of dietary components. Commercial cornstarch was washed by stirring and filtering 600 gm batches three times with 3% HCl and then three more times with redistilled water. The starch was finally dried by washing it on the filter with three portions of acetone redistilled slowly from fluorine-free calcium oxide (Weddle and Maurer, '54). The acetone was allowed to evaporate before the starch was bottled for future use.

The commercial DL-methionine was recrystallized 4 times from hot redistilled water and dried by vacuum desiccation.

Commercial corn oil was purified in pint lots by extracting it continuously for one week with hot redistilled water in a suitably designed liquid-liquid extractor. After being cooled, vitamins A, D and E were added, and the oil was stored at 4°C. in clean pyrex glass-stoppered bottles.

The most troublesome dietary component was the casein. It was extremely difficult to obtain a product which was both low enough in fluorine content and of the proper physical character for inclusion in the diet. Briefly, the purification procedure used consisted of dissolving the casein in dilute NaOH and filtering it. The casein was then precipitated over a 12- to 18-hour period with a mixture of 2 parts of 1.0 N acetic acid and 1 part of 0.5 N HCl. The precipitation was concluded when the material had reached pH 4.7. Vigorous stirring was used during the precipitation. The mixture was allowed to settle under refrigeration and then the casein was filtered off and washed 4 times with redistilled water. Dehydration was accomplished by washing and filtering three more times with acetone redistilled from fluorine-free calcium oxide. After the final acetone wash the casein was turned into clean glass trays and the acetone volatilized beneath infrared heat lamps. It was essential that the casein be stirred fre-

quently for the two hours required to evaporate all traces of acetone.

Sodium chloride, monopotassium phosphate, and cupric sulfate were purified by multiple recrystallizations from redistilled water. Calcium oxide was prepared according to the procedure of Weddle and Maurer ('54), and contained no more than 0.2 ppm of fluorine. Magnesium, ferric, zinc, and manganous salts were purified by procedures involving recrystallizations from redistilled water and ignition to red heat with sulfuric acid. Molybdic acid was dissolved with the aid of NH_4OH, filtered, reprecipitated with HCl, and ignited to red heat. Potassium iodide was purified by fractional recrystallizations from redistilled absolute ethanol.

General care of animals. The animals were housed individually in special round metabolism cages mounted on pyrex cake pans. The cages were constructed entirely of stainless steel, and were fabricated by techniques which eliminated the use of welding and soldering fluxes containing fluorine. The bottoms were 8 inches in diameter and made of 3-mesh stainless steel wire. The excreta required for analysis were collected on filter paper circles,[5] which had an especially low fluorine content. The caged animals were kept in a special air-tight chamber supplied with air washed with water. This was necessary owing to the presence of some fluorine at all times in unfiltered or unwashed air. The temperature and humidity were controlled at approximately 25°C. and 70%, respectively. All fluorine-containing materials except fluoridized water for the control animals were rigorously excluded from the room.

The experimental animals were obtained in the following manner: a pair of weanling albino rats of the Wistar strain was obtained from a dealer and placed on a stock corn diet (Muhler and Day, '51) and redistilled water. The diet contained only about 0.6 ppm of fluorine. When sexually mature, the animals were mated and their pups in turn were raised to maturity on the corn diet and then transferred to clean cages,

[5] CS and S filter paper no. 470.

provided with the highly purified diet and mated. The litters from this mating were designated as first generation animals. Thereafter the highly purified diet was used exclusively. In each generation a few rats were carefully selected to serve as controls. All the animals were weaned at 25 days of age. The controls were given water containing 2 ppm of fluorine as sodium fluoride. Owing to space limitations in the animal chamber a number of the litters were destroyed at weaning.

All the animals were weighed by difference, in their cages, every 5 days from weaning until they reached 100 days of age. Thereafter the females were weighed only occasionally owing to pregnancy and lactation. Growth data were obtained on the males as long as they were allowed to survive.

RESULTS

One hundred and ten animals representing 4 generations were conceived, born, and weaned on the fluorine-deficient diet. All the mated females became pregnant and they gave birth to live pups. Thus fluorine deficiency did not seem to impair this aspect of reproduction. However, only approximately one-half of the pups born were successfully weaned. Controls given fluorine also failed to wean more than about one-half of their pups; hence it is improbable that this defect is attributable to the deficiency of fluorine. All of the losses may have been due to imbalances in the diet other than fluorine deficiency or to the type of cages used because the growth rate of the survivors was not affected by the provision of fluorine in the drinking water.

A summary of the representative growth data is given in table 1. As shown in the table, 18 fluorine-deficient males and females were compared with 9 males and females given fluorine. There was no indication of difference between the fluorine-deficient and fluorine-supplemented males. Superficial inspection of the data suggests that the control females may have received some growth stimulus from the fluorine, but the average initial weights of these animals was a little higher than those of the experimentals. Thus the slight addi-

TABLE 1

Growth of fluorine-deficient rats compared with controls given sodium fluoride[1]

GENERATION AND SEX		FLUORINE ADDED	AGE IN DAYS										
			25	35	45	55	65	75	85	95	105	120	150
1	M	—	66	119	176	237	294	319	312	348	358	374	454
1	M	—	. .	101	151	213	261	288	284	313	324	353	380
1	M	+	56	96	160	215	268	317	307	347	355	375	395
1	M	+	. .	128	184	215	270	304	311	322	325	338	363
1	F	—	62	107	142	159	178	199	205	209	213	224	. . .
1	F	—	58	100	138	158	178	198	193	. . .	. . .	221	. . .
1	F	—	36	103	. . .	171	185	197	212	214	. . .	. . .	. . .
1	F	+	54	100	134	153	178	192	231	241	242	246	250
2	M	—	51	110	. . .	222	253	286	298	320	330	325	357
2	M	—	63	128	172	244	280	311	327	345	352	374	395
2	M	+	59	116	166	229	270	295	308	324	340	385	405
2	F	—	52	104	138	158	177	185	185	196	. . .	. . .	. . .
2	F	—	45	94	119	136	155	178	166	170	197	. . .	. . .
2	F	—	42	94	130	164	186	212	226	234	. . .	. . .	259
2	F	—	37	84	119	143	160	178	188	200	. . .	. . .	220
2	F	—	56	97	129	145	. . .	185	187	195	211	227	221
2	F	—	46	91	135	145	164	172	180	178	193	. . .	220
2	F	+	55	98	126	150	173	189	194	204	214	. . .	. . .
2	F	+	54	99	136	. . .	183	194	195	199	203	. . .	. . .
2	F	+	45	94	134	160	178	196	196	200	214	. . .	258
3	M	—	49	108	143	202	250	285	300	295	273	. . .	. . .
3	M	—	50	115	161	227	276	309	307	313	310	. . .	. . .
3	M	—	42	110	158	238	283	310	320	332	355	. . .	. . .
3	M	+	53	117	173	220	275	287	300	314	289	. . .	. . .
3	F	—	46	93	117	138	150	173	170	170	180	. . .	. . .
3	F	—	46	100	123	154	161	180	180	180	170	. . .	. . .
3	F	+	50	102	132	160	171	190	200	206	205	. . .	. . .
Mean Males (7)		—	54	113	160	226	271	301	307	324	329	356	392
Males (4)		+	56	114	170	220	271	301	306	326	327	366	388
Fem. (11)		—	48	97	129	152	169	187	190	195	194	. . .	. . .
Fem. (5)		+	52	99	132	156	176	192	203	210	215	. . .	. . .

[1] The weights of the animals are given in grams.

tional growth may have been due only to appreciably imperfect matching of the females. The growth of most of the animals was observed for 150 days but two pairs, one deficient and one supplemented in each pair, were kept on the diet 325 days. All 4 of the latter were in good condition and their weights remained essentially alike throughout the long period of observation. All the fluorine-deficient animals had sleek coats and, in all respects, they appeared to be in good condition. They could not be distinguished from the controls which were given fluorine.

Owing to the apparent importance of phosphatase in the growth and metabolism of bone it was supposed that this principal site of fluorine in the body might undergo some change in phosphatase activity if fluorine deficiency really has any metabolic effect on the bones. Accordingly the activity of both alkaline and acid phosphatase was determined not only in bone but in liver and kidneys. The results are summarized in table 2. The analyses were made on 10 different pairs of rats from 105 to 325 days of age. In two pairs both animals were on the fluorine-free regimen but in each of the others one received fluorine from weaning and the other did not. The experimental error was large, especially in the determination of alkaline phosphatase. Certainly there was no indication of significant differences attributable to the deficiency in fluorine.

The teeth of the animals were examined with the aid of a dental probe. There was no evidence of gross caries or other dental defects. The diet was not conducive to impaction and it contained no fermentable sugar; thus the results indicate that fluorine is dispensable in the maintenance of sound teeth if cariogenic factors such as high concentrations of sugar are not operative.

Because the diet and the environment of the rats appeared to be essentially fluorine free, a dependable measure of the possible fortuitous acquirement of the element was through the determination of fluorine in the tissues. Table 3 presents the results of fluorine determinations on animals at various

ages. No distinction was made according to generation, since the pups in the first generation, as well as in subsequent generations, were free of detectable amounts of fluorine until they were over 10 days of age. Such fluorine as was present in the animals must therefore have been accumulated from

TABLE 2

Phosphatase activity of tissues of deficient and supplemented animals

AGE	SEX	FLUORINE	ALKALINE PHOSPHATASE [1]			ACID PHOSPHATASE [1]		
			Kidney	Liver	Bone	Kidney	Liver	Bone
days								
105	M	—	183	. .	86	15	18	10
105	M	+	155	1.2	63	14	16	13
125	F	—	198	0.4	61	20	14	9
125	F	+	149	0.8	38	15	13	18
130	F	—	198	0.9	61	14	17	12
130	F	+	190	2.6	82	17	20	16
145	F	—	207	0.6	66	15	12	10
145	F	—	299	0.7	29	17	12	4
145	M	—	94	0.1	78	20	14	. .
145	M	+	122	0.6	81	22	16	. .
153	F	—	291	1.1	. .	25	18	. .
153	F	+	184	1.2	. .	24	16	. .
160	F	—	235	1.0	66	27	13	8
160	F	+	140	1.0	62	22	9	7
190	F	—	252	1.2	55	22	15	9
210	M	—	183	1.2	51	25	11	6
325	M	—	120	0.6	38	24	12	9
325	M	+	150	1.1	35	21	12	8
325	F	—	326	0.5	46	26	15	6
325	F	+	167	0.5	51	17	12	5
Av.		—	210	0.70	62	21	15	9
S.d.			59	0.38	15	4.8	2.2	1.7
Av.		+	174	0.92	55	19	14	10
S.d.			28	0.28	19	4.0	3.3	5.3

[1] Milligrams of phenol liberated from disodium phenylphosphate per hour per gram of tissue.

TABLE 3

Fluorine content of individual unsupplemented animals

AGE	TOTAL FLUORINE [1]		FLUORINE CONCENTRATION	
	Males	Females	Males	Females
days	*µg*	*µg*	*µg/gm ash*	*µg/gm ash*
Whole carcasses				
30	3.0		1.6	
30	1.8		1.2	
45		1.0		0.2
50		1.5		0.4
55	2.5		0.6	
60		4.2		1.0
65	2.9		0.5	
65	8.4		1.1	
65		7.0		1.2
70	17.8		3.0	
75	7.0		1.2	
75		4.8		0.1
85	23.2		2.6	
85		3.8		0.6
95		6.2		1.0
100	10.2		0.9	
105		6.0		0.8
105		8.5		1.2
110		8.0		1.1
120	7.5		0.7	
145	28.5		2.3	
145		8.0		1.6
150		12.4		1.8
Both femurs only				
105	2.2		3.2	
145		0.0		0.0
150		0.0		0.0
160		0.0		0.0
165	4.0		4.5	
190		0.0		0.0
195		4.5		8.8
300		2.8		4.7
325	1.8		1.9	
325		1.4		2.4

[1] The analytical method was sensitive for no less than 1.0 µg of total fluorine. The absolute error in the method was equivalent to about 0.5 µg of fluorine.

the diet, the drinking water, and the environment. A few fluorine determinations were made on the femora of older animals for purposes of comparison. It should be noted that even at 160 days of age the fluorine content of both femora was often too small to be measured. Even the rats that had been on the diet 325 days had only approximately 2 ppm fluorine in the femora. The whole carcasses, including skin and hair, or femora of over 33 rats on the fluorine-deficient regimen were analyzed. The results indicated considerable variability in the amounts of fluorine present but in only two or three cases were the amounts high enough to suggest that less than maximum care had been exercised to prevent appreciable contact with the element.

DISCUSSION

Under the extremely rigorous conditions of this study fluorine was not found to have any influence on the growth and well-being of rats. There were not even any grossly detectable dental defects. Thus it is justifiable to conclude that under some conditions fluorine may not have any value in nutrition or even in the maintenance of dental health. Concerning dental caries, it should be noted that the experimental diet was sugar free and it was not conducive to impaction of food in the fissures of the teeth. Thus, on the basis of the current views regarding dental caries (Muhler, Hine and Day, '54), fluorine would not be expected to play an important role in the protection of the teeth from decay under the conditions of this experiment.

Several attempts were made to analyze the compounded diet for fluorine, but the content was apparently so low that the analytical method would not measure it accurately. Therefore, the amount of fluorine which accumulated in the experimental rats became an important criterion of the success in producing a diet and environment extremely low in fluorine.

When the total amount of fluorine is so extraordinarily small, probably of greater importance is the amount that is utilizable. If it is assumed that a 150-day-old animal con-

taining 10 µg of fluorine acquired all of the fluorine from the diet, and that the animal ate at least 1500 gm, then the concentration of utilizable fluorine in the diet may have been about 0.007 ppm. Apparently this level is far less than has been achieved in any other controlled studies so far reported.

SUMMARY

By means of exhaustive purification procedures it was possible to prepare a diet which proved to be nutritionally adequate for the maintenance of experimental rats through three generations and the beginning of the 4th generation when the experiment was terminated. The diet was estimated to contain no more than 0.007 ppm of utilizable fluorine.

Rats maintained on this diet, but receiving 2 ppm of fluoride in their drinking water, did not show significant improvements in health or weight gain over similar animals receiving the same diet and redistilled water.

Alkaline and acid phosphatase determinations on the kidneys, livers and bones of deficient and supplemented animals showed no differences which could be attributed to the fluorine supplementation.

The teeth of both supplemented and deficient animals appeared to be sound and without gross evidences of decay or defects.

The investigation has demonstrated that under the rigorous experimental conditions employed, fluorine is not a dietary essential. Thus its value in the body is apparently limited to the promotion of resistance to dental caries.

ACKNOWLEDGMENTS

LITERATURE CITED

EVANS, R. J., AND P. H. PHILLIPS 1939 A new low fluorine diet and its effect upon the rat. J. Nutrition, *18:* 353.

KING, E. J., AND A. R. ARMSTRONG 1934 Convenient method for determining serum and bile phosphatase activity. Canad. Med. Assn. J., *31:* 376.

McCLENDON, J. F. 1944 Fluorine is necessary in the diet of the rat. Federation Proc., *3:* 94.

McCLENDON, J. F., AND J. GERSHON-COHEN 1953 Trace element deficiencies. Water-culture crops designed to study deficiencies in animals. J. Agr. Food Chem., *1:* 464.

——— 1954 The effect of fluorine-free food on dental and periodontal structures as revealed by Roentgen studies. Am. J. Roentgenol. Radium Therapy, Nuclear Med., *71:* 1017.

MUHLER, J. C. 1951 Fluorine in relation to specific problems of medicine and biology. Ph.D. Thesis, Indiana University.

——— 1954 Retention of fluorine in the skeleton of the rat receiving different levels of fluorine in the diet. J. Nutrition, *54:* 481.

MUHLER, J. C., AND H. G. DAY 1951 Effect of stannous fluoride in food and drinking water on caries prevention in rats on high sucrose and coarse corn diets. Ibid., *44:* 413.

MUHLER, J. C., M. K. HINE AND H. G. DAY 1954 Preventive Dentistry, C. V. Mosby Co., St. Louis, Mo.

PHILLIPS, P. H., E. B. HART AND G. BOHSTEDT 1934 The influence of fluorine ingestion upon the nutritional qualities of milk. J. Biol. Chem., *105:* 123.

SAVCHUCK, W. B., AND W. D. ARMSTRONG 1951 Metabolic turnover of fluorides by the skeleton of the rat. Ibid., *193:* 575.

SHARPLESS, G. R., AND E. V. McCOLLUM 1933 Is fluorine an indispensable element in the diet? J. Nutrition, *6:* 163.

SMITH, F. A., AND D. E. GARDNER 1948 Fluoride studies. U. S. Atomic Energy Commission Bull. No. A.E.C.D.-2161.

WEDDLE, D. A., AND R. L. MAURER 1954 Method for preparation of fluorine-low calcium oxide. Anal. Chem., *26:* 584.

WILLARD, H. H., AND O. B. WINTER 1933 Volumetric method for determination of fluorine. Ind. Eng. Chem. Anal. Ed., *5:* 7.

STUDIES ON THE PROTEIN
QUALITY OF HIGH-OIL, HIGH-PROTEIN CORN

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Several recent studies have been made on the nutritive value of high-protein corn. Flynn, Zuber, Leweke, Grainger and Hogan ('54) and Sauberlich, Chang and Salmon ('53) reported that the percentages of lysine and tryptophan in the protein decreased with the increase in the protein content of corn. The decrease in the relative amounts of these essential amino acids is usually explained on the basis that in high-protein corn the poorer quality zein protein contained in the horny endosperm increases at a faster rate than the high quality protein of the germ portion of the corn.

Mitchell, Hamilton and Beadles ('52) found that for rats the biological value of the protein decreased considerably as the protein content of the corn increased. Eggert, Brinegar and Anderson ('53) concluded that high-protein corn was superior to low-protein corn when fed to pigs; however, the protein quality of low-protein corn was found to be better than that of high-protein corn. Ross, Carigus, Hamilton and Earley ('54) found that the high- and medium-protein corns gave better weight gain and wool production in lambs. Schulz and Thomas ('49) fed the germ and endosperm from waxy and starchy inbred corns of different genetic constitution to rats for determination of a biological value based on nitrogen balance experiments. No highly significant differences were noted in biological values among these germ and endosperm samples.

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The purpose of this study was to compare the protein quality of regular corn to experimental high-oil corn samples. In addition to the whole corns themselves, the grits and germ components of the regular and experimental corns were also fed to determine differences in protein quality. Amino acid determinations were made of grits and germ to compare the growth obtained with amino acid patterns.

<div align="center">EXPERIMENTAL</div>

A special high-oil corn, a special three-way cross hybrid, and a regular yellow corn were studied. The special corns were experimental samples which were bred for their high oil contents.[1] The grits and germ used in the rat-feeding tests were separated by a commercial dry-milling process.[2] Smaller samples of grits and germ were obtained for amino acid analyses by soaking the corn in distilled water at 40°C. for 24 hours, followed by a separation of the bran, grits and germ by hand. This process was used to give a better separation than the commercial dry-milling process. The grits and germ were then dried in the vacuum oven for 16 hours at 50°C., after which amino acid analyses were made. Amino acid analyses were done by the method of Henderson and Snell ('48). The protein, fat and moisture contents of the various corn samples are given in table 1.

For the feeding tests the corn samples were pulverized in a Fitzpatrick mill. Vitamins and minerals, which are essential for the rat, were blended with the various corn samples in a Hobart mixer to make one group of diets (group I). In a second group of diets (II) constarch was added to the special corn samples to adjust the protein to a calculated level of 7.5%. Corn oil was also added to give a calculated level of 6% fat. A third group of diets (III) was made in which the corn germ and grits samples were similarly adjusted to equal levels of protein and fat. Diets (group IV) were also

[1] Funk Brothers Seed Company, Bloomington, Illinois.
[2] Corn Mill Division of General Foods Corporation, Kankakee, Illinois.

TABLE 1

Analysis of corn samples[1]

SAMPLE	PROTEIN [2]	FAT [2]	MOISTURE
	%	%	%
High-oil corn	13.0	8.6	10.2
Three-way cross corn	11.5	7.0	11.5
Regular yellow corn	9.3	5.1	14.0
High-oil corn grits	11.8	2.3	11.6
Regular corn grits	8.2	1.2	9.7
High-oil corn germ	13.1	27.5	6.7
Regular corn germ	14.6	20.7	2.3

[1] Values for grits and germ obtained from commercial dry-process separation.

[2] Calculated on dry basis.

TABLE 2

Composition of test diets

DIET	RATION COMPONENTS						
	Corn	Mineral [1] mix	Corn-starch	Corn oil	Dex-trose	Alpha-cel	Vitamin [2] mix
	%	%	%	%	%	%	%
Group I							
High-oil corn	95.8	4.0	...	..	...	..	0.2
Three-way cross	95.8	4.0	...	..	...	..	0.2
Regular corn	95.8	4.0	...	..	...	..	0.2
Group II							
High-oil corn	64.5	4.0	30.3	1.0	...	..	0.2
Three-way cross	73.9	4.0	20.5	1.4	...	..	0.2
Regular corn	94.2	4.0	...	1.6	...	..	0.2
Group III							
Regular corn germ	81.7	4.0	6.0	8.1	...	..	0.2
High-oil corn germ	95.8	4.0	...	..	...	..	0.2
Regular corn grits	90.9	4.0	...	4.9	...	..	0.2
High-oil corn grits	64.7	4.0	27.1	4.0	...	..	0.2
Group IV							
Nitrogen-free	...	4.0	...	6.0	87.8	2.0	0.2
High-oil	95.8	4.0	...	..	...	..	0.2
Regular corn	93.9	4.0	...	1.9	...	..	0.2

[1] U.S.P. Salt Mix XIV.

[2] Vitamins added per 1,000 gm of each of the diets: 2 methyl-naphthoquinone, 2 mg; thiamine hydrochloride, 12 mg; pyridoxine hydrochloride, 20 mg; biotin, 0.3 mg; vitamin B_{12}, 1 mg; folic acid, 0.9 mg; calcium pantothenate, 50 mg; nicotinic acid, 90 mg; inositol, 200 mg; p-aminobenzoic acid, 300 mg; riboflavin, 50 mg; choline chloride, 1,000 mg; dl-atocopherol diacetate, 100 mg. These vitamins were diluted to 2 gm with cornstarch before addition to the diets.

made for a protein digestibility determination of the high-oil and regular corn sample, using the method developed by Mitchell ('24) in which a correction was made for the metabolic nitrogen in the feces obtained from feeding a "nitrogen-free" diet. The composition of the test diets is given in table 2.

Weanling 28- to 29-day-old Wistar albino rats were used as test animals.[3] Six rats were fed each test diet for 4 weeks. The animals were housed in individual raised-bottom wire cages in an air-conditioned room kept at $75 \pm 2°F$. Distilled water was given ad libitum to all animals. No restriction was placed on the food consumption in group I. Daily food intakes were adjusted in groups II and III to give equal food consumption where the diets were being compared at the same protein level. Three drops of cod liver oil were given by medicine dropper twice a week to supply vitamin A and vitamin D.

RESULTS

The body weight gain and protein efficiencies obtained from this study are given in table 3 and the "P" values are listed in table 4. It is shown that when the samples were fed ad libitum (group I) without adjusting the protein levels, both of the higher protein corns gave significantly better growth and protein efficiency than the regular corn (all P values < 0.05). However, when the same samples were fed at equal protein levels (group II) the experimental corns gave slight increases in body weight gain and protein efficiency, which were not significant (all P values > 0.1).

When the grits from the regular corn were compared with the high-oil corn grits at a 7% protein level, there were no significant differences in growth and protein efficiency (P > 0.05). On the other hand, at a 12% protein level the regular corn germ gave better body weight gain (P < 0.05) and much better protein efficiency (P < 0.01) than the high oil variety. A digestibility determination showed that the high-oil sample had a protein digestibility value of 91% as compared to 87% for regular corn.

[3] Carworth Farms, New City, Rockland County, New York.

TABLE 3

Summary of test data

DIET	PROTEIN CONTENT OF DIET	AVERAGE FOOD EATEN	AVERAGE PROTEIN EATEN	AVERAGE BODY WEIGHT GAIN	AVERAGE PROTEIN EFFICIENCY [1]
	%	gm	gm	gm	
Group I					
High-oil corn	10.8	264.9	28.6	38.6 ± 4.2 [2]	1.33 ± 0.08 [2]
Three-way cross corn	9.8	275.7	27.3	37.8 ± 3.1	1.39 ± 0.07
Regular corn	8.4	254.8	21.4	19.1 ± 4.3	0.89 ± 0.18
Group II					
High-oil corn	7.3	181.8	13.3	6.7 ± 1.7	0.51 ± 0.13
Three-way cross corn	7.5	183.1	13.7	9.4 ± 2.2	0.69 ± 0.14
Regular corn	7.7	181.9	14.0	5.5 ± 1.7	0.40 ± 0.12
Group III					
Regular corn germ	11.8	254.5	30.0	64.5 ± 5.2	2.15 ± 0.08
High-oil corn germ	12.1	248.3	30.0	52.1 ± 1.9	1.74 ± 0.05
Regular corn grits	6.9	185.8	12.8	2.3 ± 1.0	0.18 ± 0.08
High-oil corn grits	7.1	180.7	12.8	0 ± 0.6	. . .

[1] Protein efficiency calculated as grams gain in weight per gram of protein eaten.
[2] Standard error of mean.

TABLE 4

Significance of means

DIETS COMPARED	BODY WEIGHT GAIN		PROTEIN EFFICIENCY	
	"t" value	P	"t" value	P
Group I				
High-oil vs. regular corn	3.24	< 0.01	2.31	< 0.05
Three-way cross vs. regular corn	2.99	< 0.02	2.39	< 0.05
Group II				
High-oil vs. regular corn	0.50	> 0.1	0.62	> 0.1
Three-way cross vs. regular corn	1.33	> 0.1	1.37	> 0.1
Group III				
Regular corn germ vs. high-oil corn germ	2.24	< 0.05	4.02	< 0.01
Regular corn grits vs. high-oil corn grits	1.97	> 0.05	1.03	> 0.1

TABLE 5

Amino acid contents of regular and high-oil corn

AMINO ACIDS	AMINO ACID CONTENT OF GRITS AND GERM								AMINO ACID CONTENT OF REGULAR AND HIGH-OIL CORN SUPPLIED FROM THE GERM AND GRITS COMPONENTS [1]			
	Corn grits				Corn germ				Regular corn		High-oil corn	
	Regular		High-oil		Regular		High-oil					
	In grits [2]	In protein	In grits [2]	In protein	In germ [2]	In protein	In germ [2]	In protein	In corn [3]	In protein [4]	In corn [3]	In protein [4]
	%	%	%	%	%	%	%	%	%	%	%	%
Arginine	0.28	3.0	0.40	3.1	1.61	8.3	1.45	8.6	0.39	4.1	0.51	4.1
Histidine	0.24	2.6	0.32	2.4	0.50	2.6	0.47	2.8	0.25	2.6	0.31	2.5
Isoleucine	0.42	4.5	0.60	4.6	0.66	3.4	0.62	3.7	0.41	4.3	0.54	4.4
Leucine	1.32	14.1	1.83	14.0	1.14	5.8	0.98	5.8	1.19	12.4	1.53	12.4
Lysine	0.28	3.0	0.33	2.5	1.35	6.9	1.29	7.7	0.37	3.9	0.44	3.6
Methionine	0.17	1.8	0.22	1.7	0.23	1.2	0.21	1.3	0.16	1.7	0.20	1.6
Phenylalanine	0.37	3.9	0.47	3.6	0.24	1.2	0.23	1.4	0.32	3.3	0.39	3.1
Tryptophan	0.05	0.5	0.08	0.6	0.23	1.2	0.20	1.2	0.06	0.6	0.09	0.7
Threonine	0.36	3.8	0.43	3.3	0.73	3.7	0.64	3.8	0.37	3.9	0.42	3.4
Valine	0.55	5.9	0.80	6.1	1.14	5.8	0.96	5.7	0.56	5.8	0.75	6.1
Cystine	0.31	3.3	0.30	2.3	0.50	2.6	0.50	3.0	0.30	3.1	0.30	2.4
Protein [5]	9.39	..	13.1	..	19.5	..	16.8	..	..	..	..	..

[1] These values derived from amino acid analyses of germ and grits and do not include any bran component.

[2] All calculated on a dry-basis.

[3] Calculated as grams of amino acid per 100 gm of dry corn.

[4] Calculated as grams of amino acid from germ and grits per 100 gm of protein, assuming no protein from the bran component.

[5] Nitrogen × 6.25.

Table 5 lists the amino acid contents of the germ and grits from the high-oil and the regular corn. These analyses were made on the hand-separated samples. Calculations based on the percentage composition of corn grits showed that the high-oil sample had greater amounts of all the amino acids than the regular grits sample except cystine. The regular corn germ had higher levels of all the amino acids except cystine than the high-oil corn germ. On a percentage of protein basis the high-oil grits did not contain as much lysine as the regular corn grits. On the other hand the high-oil corn germ contained more lysine than the regular corn grits.

After the whole corn samples were separated by hand, it was found that on a dry basis the regular corn contained 80.9% grits and 10.3% germ, whereas the high-oil sample yielded 76.0% grits and 14.3% germ. Table 5 lists the amino acid composition of the regular and high-oil samples supplied by the grits and germ when the amino acid values were combined in the proper proportions according to yield. These calculations do not include the amino acids supplied by the bran portions which were not analyzed. It was shown that the high-oil sample contained higher levels of all the amino acids listed, except cystine, than the regular corn sample when the calculations were based on grams of amino acid per 100 gm of corn. On a percentage of protein basis, the high-oil sample had lower amounts of histidine, methionine, lysine, phenylalanine, threonine and cystine than the regular corn.

DISCUSSION

The growth and protein efficiency data obtained by feeding the whole corn samples indicated that the high-oil sample was significantly better than that from the regular corn when protein levels were not adjusted. The values in table 5 do not include the amino acid content of the bran portion of the corn which was removed and discarded in the separation of the germ and grits. The bran furnished very little of the protein content of the corn; therefore, it is probable that these

values would not differ very much if the bran were included. The data indicate that, on a whole corn basis, the high-oil sample had greater amounts of all the essential amino acids, including lysine and threonine. Therefore, it might be expected that the high-oil variety would give equal or better protein efficiency than the regular variety when fed on a whole corn basis. Table 5 also shows that with the exception of threonine and cystine, the amino acid composition of the protein from the high-oil sample approximates that of the regular corn. The protein digestibility was found to be higher in the high-oil sample than the regular corn. Since lysine and tryptophan are considered the limiting amino acids of corn, it might be expected that on an equal protein basis, the high-oil sample would not show poorer protein quality than the regular corn. This was confirmed by the feeding results reported in table 3, in which the high-oil corn showed slightly better but insignificant growth and protein efficiency than the regular corn when both were adjusted to equalize the protein levels.

When the feeding tests of the grits and corn samples are compared with the amino acid analyses in table 5, it must be remembered that the samples used in the rat assay were separated by a commercial dry-mill process, and this, no doubt, does not give as clear-cut a separation as the hand-separated samples which were used for the amino acid analyses. In the dry-milling process the corn is steeped in warm water before degermination, a step which might also affect the amino acid quality of the samples. The high-oil grits were shown to be somewhat lower in lysine than the regular corn grits on a percentage of protein basis (2.5 vs. 3.0%). From the lower lysine content it might be expected that the high-oil grits would not result in as high a protein efficiency as that obtained by the regular grits. The feeding tests have shown that the regular grits did result in slightly better protein efficiency and growth. However, these differences were not significant.

The regular corn germ gave significantly better growth and protein efficiency than the high-oil sample when both were fed at a 12% protein level. This may not have been expected,

since the amino acid data in table 5 indicated that the lysine content of the regular germ was lower than that of the high-oil sample (6.9 vs. 7.7%), while the threonine and tryptophan contents were approximately the same on a percentage of protein basis. Other factors, such as differences in digestibility of protein and availability of amino acids, the "balance" of all, including the non-essential amino acids, and components in the samples other than protein, may all affect the growth and protein efficiency. However, with the exception of the germ samples, good correlation was obtained between rat growth and amino acid analyses in this study.

SUMMARY

1. Two high-oil, high-protein corn varieties (13.0% protein with 8.6% oil and 11.5% protein with 7.0% oil) gave significant increases in growth and protein quality over that obtained from regular corn (9.3% protein with 5.1% oil) in ad libitum feeding with rats.

2. When the protein levels were adjusted to 7.5%, no significant differences in growth or protein quality were shown by the high-oil corns over the regular corn.

3. On an equal protein level of 7%, grits from one of the high-oil samples (8.6% oil) did not shown significant differences in growth or protein efficiency when compared to a sample of regular corn grits. When fed at 12% protein levels, the germ from regular corn gave significantly better growth and protein efficiency than did the high-oil variety.

4. Microbiological amino acid analyses correlated rather well with rat growth tests. However, a corn germ sample containing high oil resulted in poorer growth and protein efficiency than regular corn germ which seemed to have a poorer amino acid pattern including a lower level of lysine.

LITERATURE CITED

EGGERT, R. G., M. J. BRINEGAR AND C. R. ANDERSON 1953 The quality of protein of normal- and high-protein corn for growing swine. J. Animal Sci., *12*: 282.

FLYNN, L. M., M. S. ZUBER, D. H. LEWEKE, R. B. GRAINGER AND A. G. HOGAN 1954 Relation between protein content of corn and concentration of amino acids and nicotinic acid. Cereal Chem., *31*: 217.

HENDERSON, L. M., AND E. E. SNELL 1948 A uniform medium for determination of amino acids with various micro-organisms. J. Biol. Chem., *172*: 15.

MITCHELL, H. H. 1924 A method of determining the biological value of protein. Ibid., *58*: 873.

MITCHELL, H. H., T. S. HAMILTON AND J. R. BEADLES 1952 The relationship between the protein content of corn and the nutritional value of the protein. J. Nutrition, *48*: 461.

ROSS, C. V., U. S. CARRIGUS, T. S. HAMILTON AND E. B. EARLEY 1954 Comparing high-, medium-high-, and low-protein corn for fattening lambs. J. Animal Sci., *13*: 433.

SAUBERLICH, H. E., W.-Y. CHANG AND W. D. SALMON 1953 The amino acid and protein content of corn as related to variety and nitrogen fertilization. J. Nutrition, *51*: 241.

SCHULZ, J. A., AND B. H. THOMAS 1949 The biological value of the proteins of corn germ and endosperm wet-milled experimentally. Cereal Chem., *26*: 67.

EFFECT OF VITAMIN B_{12} AND AUREOMYCIN SUPPLEMENTS ON VITAMIN B_{12} LIVER STORES AND ON THE DEVELOPMENT OF ANEMIA IN GASTRECTOMIZED RATS [1,2]

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Several investigators (Chow et al., '55; Watson and Florey, '55; Nieweg et al., '56) have studied the assimilation of radioactive vitamin B_{12} by gastrectomized rats and have concluded that the vitamin is not absorbed from the intestinal tract in these animals.

In the present report, the amount of vitamin B_{12} stored in the livers of gastrectomized rats has been measured. Since the values obtained were low when compared with those of non-operated rats fed the same diet, it became of particular interest to study the effect of oral and injected supplements of vitamin B_{12} on the anemia which invariably develops in gastrectomized rats (Bussabarger and Jung, '36). Furthermore, since the antibiotic Aureomycin (chlortetracycline) has been found to increase the amount of free vitamin B_{12} in the intestine of rats (Peterson et al., '53) and to increase the growth rate of several species of animals (Jukes, '55), the

effect of adding Aureomycin to the diet of gastrectomized rats was also tested. Especially pertinent is the finding of Toon and Wangensteen ('50) that the anemia produced in rats with a blind intestinal loop was corrected by the administration of Aureomycin.

The common experience of investigators working with gastrectomized rats has been that these animals develop an anemia and lose weight during the post-operative period (Jung, '33; Bussabarger and Jung, '36). In the presence of iron salt supplementation, the anemia is usually normocytic and does not respond to folic acid, B-complex vitamins, liver extract, or protein hydrolysates (Higgins et al., '47; Balfour et al., '50). Neutralized human gastric juice, however, was reported to be an anti-anemia agent (Balfour et al., '50).

In the study reported here, the anemia developing in the gastrectomized rat was not affected by vitamin B_{12} given orally or injected, but it was corrected by the inclusion of Aureomycin as a diet supplement.

METHODS

Male rats of the Long-Evans strain were selected for operation when they weighed approximately 150 gm. The gastrectomy was performed according to the procedure of Balfour et al. ('50). The animals were then placed on a semi-synthetic diet of the following percentage composition: sucrose 73; purified casein 18; salt mixture [4] 4; corn oil 4; cod liver oil 4; with added vitamins (milligrams per kilogram of ration): thiamine chloride 4; riboflavin 8; pyridoxine hydrochloride 4; calcium pantothenate 25; niacin 40; 2-methyl naphthoquinone 4; choline chloride 1000. For selected groups, dietary supplements of vitamin B_{12}, folic acid or Aureomycin were given. Vitamin B_{12} was also given with a source of intrinsic factor and in some experiments it was injected. Animals not regaining their pre-operative weight within 30 days were discarded from the experiment. A like number of non-operated

[4] Hubbell, Mendel and Wakeman ('37).

animals were simultaneously placed on the same dietary regimen and served as controls for the experimental procedures. The animals were sacrificed after 90 days on the experimental diet. Liver tissue was removed and analyzed for vitamin B_{12} using *L. leichmannii* ATCC 4797 (Wright et al., '48).

RESULTS

Table 1 shows the values obtained when the liver tissue of gastrectomized rats was analyzed for vitamin B_{12}. In two separate experiments the range and average values show that the vitamin B_{12} content was approximately 10% of the amount present in liver tissue of non-operated control animals. This is evidence that a 90-day period on this dietary regimen results in a depletion of the vitamin B_{12} liver content of the gastrectomized rat.

TABLE 1

Vitamin B_{12} in liver tissue of gastrectomized and control rats

GROUP	NO. OF RATS	GASTRECTOMIZED RATS μg B_{12} per gm liver		CONTROL RATS μg B_{12} per gm liver	
		Range	*Average*	*Range*	*Average*
I	6	4–20	13	130–250	166
II	7	13–26	23	109–215	190

A moderate reduction in hemoglobin concentration was found in gastrectomized rats at the end of the experimental period (table 2) when values were compared to those of control animals fed the same unsupplemented diet. The addition of vitamin B_{12} to the diet had no effect on hemoglobin values. Similar results were obtained when a hog gastric mucosal concentrate of intrinsic factor [5] was fed in conjunction with vitamin B_{12} and also when vitamin B_{12} was injected or given orally with a folic acid supplement. In experiments where Aureomycin was added to the diet, hemoglobin values were increased to the normal range. This effect of Aureomycin

[5] Bifacton Sec, obtained through the courtesy of Dr. K. J. Thompson of Organon, Inc., Orange, New Jersey.

occurred whether the antibiotic was added alone or together with folic acid and vitamin B_{12}.

No evidence could be obtained from assaying liver tissue for vitamin B_{12} that Aureomycin feeding increased the stores of this vitamin. Table 3 shows the amount of vitamin B_{12} in

TABLE 2

Comparison of hemoglobin values in gastrectomized and control rats on various diet supplements

DIET SUPPLEMENT PER 100 GM	NO. OF RATS	GASTRECTOMIZED RATS gm % Hb		CONTROL RATS gm % Hb	
		Range	Average	Range	Average
. . . .	7	10.1–13.5	12.4	15.5–16.8	16.0
Vitamin B_{12}, 2 µg	7	9.8–13.1	11.9	14.9–16.6	15.8
Vitamin B_{12}, 2 µg + Bifacton Sec,[1] 1 gm	6	10.1–12.4	11.9	15.1–17.8	16.5
Vitamin B_{12}, 0.5 µg, injected daily	8	10.2–13.9	12.4	15.1–16.4	16.2
Vitamin B_{12}, 2 µg + folic acid, 500 µg	5	6.0–12.1	10.2	14.7–17.0	15.5
Vitamin B_{12}, 2 µg + folic acid, 500 µg + Aureomycin, 50 mg	7	14.3–16.8	15.7	15.3–16.6	15.8
Aureomycin, 50 mg	8	13.9–16.0	15.2	14.1–16.4	15.7

[1] A hog gastric mucosal concentrate of intrinsic factor, obtained through the courtesy of Dr. K. J. Thompson of Organon Inc., Orange, New Jersey.

TABLE 3

Vitamin B_{12} in liver tissues of gastrectomized and control rats on diets containing Aureomycin

DIET SUPPLEMENT PER 100 GM	GASTRECTOMIZED RATS µg B_{12} per gm liver		CONTROL RATS µg B_{12} per gm liver	
	Range	Average	Range	Average
None	10–41	26	120–225	176
Aureomycin, 50 mg	4–26	11	130–400	260
Aureomycin, 50 mg + 2 µg B_{12} + 500 µg folic acid	3–31	18	50–250	130

livers of antibiotic-fed gastrectomized rats to be below normal values and similar to values obtained for unsupplemented gastrectomized rats (table 1). Other workers (Peterson et al., '53; Chow et al., '53) likewise found no increase in the storage of vitamin B_{12} in antibiotic-fed animals.

DISCUSSION

The reduction in vitamin B_{12} liver content occurring in gastrectomized rats can be explained on the basis that the vitamin is not absorbed from the intestinal tract in the absence of intrinsic factor (Chow et al., '55; Watson and Florey, '55; Nieweg et al., '56). The extremely low values obtained after 90 experimental days are unexpected considering the difficulties encountered in producing a vitamin B_{12} deficiency in the normal weanling rat. The depletion of vitamin B_{12} in gastrectomized rats apparently has no effect on blood cell production, since Aureomycin-fed animals have normal hemoglobin values associated with low vitamin B_{12} liver stores. This would confirm the conclusion of other workers (Jukes and Williams, '54) that a vitamin B_{12} deficiency does not greatly affect the hemopoietic process in the rat.

The results of these experiments show that the anemia occurring in gastrectomized rats on an adequate iron intake can be corrected by Aureomycin feeding. The treatment for this anemia is therefore similar to that for the anemia in the blind intestinal loop syndrome where Toon and Wangensteen ('50) found antibiotic supplements to be an effective therapy.

Although no adequate explanation is at hand, it would appear that Aureomycin could act, presumably through its effect on intestinal microflora, either to enhance the production of some unknown hemopoietic factor or to inhibit the formation of a hemolytic toxin.

SUMMARY

Ninety days after operation, the vitamin B_{12} liver content of gastrectomized rats was found to be greatly reduced when

compared to that of non-operated animals. The moderate anemia present in the gastrectomized animals could not be corrected by giving vitamin B_{12} either orally or by injection. Vitamin B_{12} administered in conjunction with folic acid or a hog stomach preparation as a source of intrinsic factor likewise had no effect in preventing the development of the anemia. However, hemoglobin values were found to be within the normal range in gastrectomized rats fed a supplement of Aureomycin. There was no apparent increase in vitamin B_{12} liver stores of the antibiotic-fed gastrectomized animals.

LITERATURE CITED

BALFOUR, D. C., JR., G. M. HIGGINS AND K. A. WOODS 1950 A factor in neutralized human gastric juice which prolongs survival of gastrectomized rats. Proc. Staff Meet., Mayo Clin., 25: 434.

BUSSABARGER, R. A., AND F. T. JUNG 1936 Dietary and hematologic studies after total gastrectomy in the rat. Am. J. Physiol., 117: 59.

CHOW, B. F., R. L. DAVIS AND S. DAVIS 1953 The effect of antibiotics and the composition of diets on fecal vitamin B_{12} and biotin. J. Nutrition, 49: 657.

CHOW, B. F., J. K. QUATTLEBAUM, JR. AND C. ROSENBLUM 1955 Effect of intrinsic factor concentrated on vitamin B_{12} absorption by gastrectomized rats. Proc. Soc. Exp. Biol. Med., 90: 279.

HIGGINS, G. M., D. J. INGLE AND O. R. JONESON 1947 The effect of synthetic *Lactobacillus casei* factor on the blood changes induced by gastrectomy in the rat. J. Lab. Clin. Med., 32: 635.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, 14: 273.

JUKES, T. H., AND W. L. WILLIAMS 1954 Pathology of vitamin B_{12}. The Vitamins, 1: 489.

JUKES, T. H. 1955 Antibiotics in Nutrition. Medical Encyclopedia, New York.

JUNG, F. T. 1933 Anemia produced by gastrectomy in the rat. Am. Gastro. Assoc., 36: 237.

NIEWIG, H. O., A. ARENDS, E. MANDEMA AND W. B. CASTLE 1956 Enhanced absorption of vitamin B_{12} in gastrectomized rats by rat intrinsic factor. Proc. Soc. Exp. Biol. Med., 91: 328.

PETERSON, G. E., E. C. DICK AND K. R. JOHANSSON 1953 Influence of dietary aureomycin and carbohydrate on growth, intestinal microflora and vitamin B_{12} synthesis of the rat. J. Nutrition, 51: 171.

TOON, R. W., AND O. H. WANGENSTEEN 1950 Anemia associated with blind intestinal segments and its prevention with aureomycin. Proc. Soc. Exp. Biol. Med., 75: 762.

WATSON, G. M., AND H. W. FLOREY 1955 The absorption of vitamin B_{12} in gastrectomized rats. Brit. J. Exp. Path., *36*: 479.
WRIGHT, L. D., H. R. SKEGGS AND J. W. HUFF 1948 The ability of thymidine to replace vitamin B_{12} as a growth factor for certain Lactobacilli. J. Biol. Chem., *175*: 475.

THE EFFECT OF FOLIC ACID ON THE USE OF GLYCINE BY THE TURKEY POULT

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Folic acid has been found to be essential for serine synthesis in microorganisms (Holland and Meinke, '49) and rat liver (Plaut et al., '50). Totter et al. ('50) found that livers of folic acid-repleted chicks were capable of converting glycine to serine more rapidly than deficient chicks. Naber et al. ('52) showed that glycine was more toxic for chicks fed a ration deficient in folic acid than for those fed a supplemented ration. Machlin et al. ('52) found folic acid and vitamin B_{12} to be effective in counteracting glycine toxicity in chicks. More recent work has shown that a folic acid derivative, tetrahydrofolic acid, serves as a single carbon carrier in the conversion of glycine to serine in microorganisms, rats and pigeons (Blakley, '54; Elwyn et al., '55; Kisliuk and Sakami, '55; and Alexander and Greenberg, '55). The same mechanism may operate in turkey poults since the livers of poults deficient in folic acid showed a greater reduction in the ability to incorporate the alpha carbon of glycine into the beta carbon of serine than into the alpha position of serine (Vohra et al., '56).

Naber et al. ('56) were unable to simulate a toxicity of glycine in chickens by feeding compounds related to glycine, including oxalic acid, glyoxylic acid and serine. Dietary glycine did not depress the folic acid content of the tissues and folic acid did not depress the glycine level in the blood.

Cervical paralysis has been described as a symptom of a folic acid deficiency in poults by Richardson et al. ('45), Jukes et al. ('47) and Lance and Hogan ('48). The rations used in

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these studies contained 10.8 and 10% of gelatin respectively which supplied fairly large amounts of glycine.

In the present study, 6 experiments were conducted to determine whether the level of folic acid can influence the tolerance for glycine in poults and whether cervical paralysis is a symptom of the toxicity of glycine or of a deficiency of folic acid.

EXPERIMENTAL

Poults used in experiments 1, 2, 4 and 5 were hatched from Broad Breasted Bronze turkey hens which had been maintained on a ration deficient in folic acid (Kratzer et al., '56). Hatchability of the breeder hens was slightly reduced at the time these experiments were conducted, indicating that the stores of the vitamin in the poults were reduced from that in poults from hens fed a normal ration. Poults used in experiments 3 and 6 were from hens fed a practical breeder ration. The poults for all experiments were fed a folic acid-low ration for about a week before they were divided into comparable groups and started on the experiment.

The basal ration used in experiments 1, 2 and 3 contained the following per 100 gm: soybean protein,[1] 30.0 gm; cellu flour, 3.0 gm; soybean oil, 3.5 gm; dicalcium phosphate, 3.0 gm; calcium carbonate, 2.5 gm; salt mixture (Kratzer et al., '49), 2.5 gm; DL-methionine,[2] 0.35 gm; d-alpha tocopheryl acetate concentrate (44 units/gm), 0.2 gm; dimethylaminoethanol, 0.2 gm; dry vitamin A (10,000 units/gm), 0.1 gm; dry vitamin D_3 (1,500 units/gm), 0.1 gm; homocystine, 0.1 gm; niacin, 10.0 mg; aureomycin[2] (92% pure), 5.0 mg; calcium pantothenate, 3.0 mg; thiamine chloride, 1.0 mg; riboflavin, 1.0 mg; pyridoxine HCl, 1.0 mg; menadione, 1.0 mg; biotin, 0.04 mg; vitamin B_{12},[2] 1.0 µg; and corn starch to equal 100 gm. Additions of glycine, DL-serine, and betaine·HCl were made at

[1] Drackett assay protein, C-1. The Drackett Products Company, Cincinnati, Ohio.

[2] We are indebted to Merck and Company, Rahway, N. J., Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.; and The Dow Chemical Company, Midland, Michigan for kindly donating vitamins and amino acids used in this work.

the expense of cornstarch. In experiments 4 and 5, 38% of acetone-extracted fishmeal replaced the soybean protein, dicalcium phosphate and calcium carbonate. In experiment 6, 30% of acid-washed casein and 0.6% of L-arginine replaced the soybean protein.

The poults were housed in electrically heated batteries with raised wire floors and were weighed and examined at frequent intervals.

TABLE 1

The effect of glycine, serine, betaine and folic acid upon the growth of poults deficient in folic acid [1]

SUPPLEMENT	LEVEL	PER CENT DAILY GAIN (IN %) AND SURVIVAL					
		No folic acid			Folic acid (10 mg/kg)		
		Exp. 1	Exp. 2	Av.	Exp. 1	Exp. 2	Av.
	%						
None	..	8.7 4/4 [2]	7.1 4/5	7.9 8/9	8.7 4/4	9.2 5/5	9.0 9/9
Glycine	4.0	1.9 2/4	4.1 4/5	3.0 6/9	9.7 4/4	8.1 5/5	8.9 9/9
DL-Serine	5.6	9.7 4/4	8.7 4/5	9.2 8/9	10.1 4/4	8.6 4/5	9.4 8/9
Betaine·HCl	0.5	7.7 4/4	8.3 4/5	8.0 8/9	9.6 4/4	8.6 5/5	9.1 9/9

[1] Duration — 9 and 10 days in experiments 1 and 2 respectively.

[2] $\frac{\text{Number of survivors}}{\text{Initial number}}$

RESULTS

In experiment 1 and 2 (table 1) glycine in the absence of folic acid caused a marked depression in growth. Three of the 9 birds in the glycine supplemented groups exhibited cervical paralysis which was very similar to that described by Richardson et al. ('45), and no cervical paralysis was noted in any of the other groups. Folic acid prevented the growth depressions as well as the cervical paralysis caused by glycine. DL-Serine at an equimolar level caused neither a growth depression nor cervical paralysis. Betaine·HCl, although used at a lower level than glycine, was not growth depressing, nor did it improve growth above the control rations. This indicates that the basal ration contained adequate methyl groups for growth, although it contained homocystine and dimethylethanolamine in an attempt to provide a low level of available methyl groups.

Poults from hens fed normal rations were used in the third experiment (table 2) and were fed the experimental rations for 24 days. There was only a slight depression in growth with 4% of glycine. Cervical paralysis was increased as glycine was added to the ration. No growth depression or cervical paralysis was noted when folic acid was added to the ration. The low mortality and less severe growth depression with glycine compared to that in other experiments probably indicates greater stores of folic acid in the poults used.

TABLE 2

Effect of glycine and folic acid on growth, survival and incidence of cervical paralysis of poults fed diets containing soybean protein (experiment 3)[1]

GLYCINE	FOLIC ACID	DAILY GAIN	SURVIVAL [2]	CERVICAL PARALYSIS
%	mg/kg	%		% incidence
0	0	6.1	7/7	0
2	0	6.0	7/7	14
4	0	5.6	5/7	71
0	10	6.2	7/7	0
2	10	6.0	7/7	0
4	10	6.2	7/7	0

[1] Duration — 24 days.

[2] $\dfrac{\text{Number of survivors}}{\text{Initial number}}$

In experiments 4 and 5 (table 3) an attempt was made to lower the amount of folic acid in the basal ration by substituting extracted fish meal for the soybean protein. The commercial soybean protein is reported to contain 2.5 μg of folic acid per gram (Anonymous, '55) while fish meal has been found to be a very poor source of the vitamin (Lillie and Briggs, '47). Depleted chicks were used in these trials. Excessive mortality was observed in the basal group of experiment 4 and there were no survivors when 2% of glycine was fed. Only one bird in each of these groups exhibited cervical paralysis, probably because the birds were so severely depleted of folic acid that they died before showing the symptoms. Essentially similar results were observed in experiment 5, even with a low level of folic acid added to the basal ration.

The glycine content of the basal ration was reduced in experiment 6 (table 4) by the use of casein and arginine as a source of amino acids. This basal ration contained 0.1 to 0.2% of glycine, by calculation. Normal poults were used and were continued on the experiment for 34 days. Growth was reduced and mortality increased by the addition of glycine to the basal. Cervical paralysis was noted in 4 of the 6 birds in the group without glycine and folic acid. When glycine was added the poults died sooner than in the control group and there were

TABLE 3

Effect of glycine and folic acid on growth, survival, and incidence of cervical paralysis of poults fed diets containing fish meal

DURATION	GLYCINE	FOLIC ACID	DAILY GAIN	SURVIVAL [1]	CERVICAL PARALYSIS
days	%	mg/kg	%		% incidence
			Experiment 4		
10	0	0	5.5	5/10	10
	2	0	. .	0/10	10
	0	10	8.3	9/10	0
	2	10	7.9	7/10	0
			Experiment 5		
9	0	0.4	7.5	1/5	0
	2	0.4	. .	0/5	0
	0	10.4	7.4	5/5	0
	2	10.4	8.8	4/5	0

[1] Number of survivors
    Initial number

TABLE 4

Effect of glycine and folic acid on growth, cervical paralysis, and survival of poults fed a low-glycine diet (experiment 6)

GLYCINE	FOLIC ACID	19-DAY DAILY GAIN	CERVICAL PARALYSIS	SURVIVAL [1]	
				19 days	34 days
%	mg/kg	%			
0	0	5.4	4/6	3/6	0/6
4	0	2.6	1/6	1/6	0/6
0	10	6.7	0	4/6	4/6
4	10	6.9	0	6/6	5/6

[1] Number of survivors
    Initial number

fewer cases of cervical paralysis; with folic acid present no cases of cervical paralysis were observed.

DISCUSSION

Glycine caused a depression of growth in poults which was similar to its effect in chicks (Machlin et al., '52; Naber et al., '52). Growth could be improved by a high level of folic acid in the diet. DL-Serine at an equimolar level was not growth depressing. However, it is not known whether the D form of serine can be utilized by the poult. The level used (equivalent to 4% glycine) was great enough so that a growth depression should have been noted, even if the L form were the only one utilized.

Liver homogenate studies (Vohra et al., '56) have shown that serine can be formed from glycine in turkeys by a mechanism similar to that in other species (Blakley, '54; Elwyn et al., '55; Kisliuk and Sakami, '55; Alexander and Greenberg, '55). We would expect folic acid to be converted to tetrahydrofolic acid which could transfer a hydroxymethyl group originally derived from the alpha carbon of glycine to become the beta carbon of serine. An excess of glycine could either overload the system so that free glycine would accumulate or it could react with tetrahydrofolic acid to the extent that the available supply of folic acid would be limited. Naber et al. ('56) showed that in chicks, a deficiency of folic acid caused no increase in glycine in the blood. They also showed that there was no decrease in folic acid in the tissues of chicks fed toxic levels of glycine. These data suggest that the growth depression is not caused by an accumulation of glycine but by a lack of folic acid for other functions when excessive amounts are used in converting glycine to serine. Higher levels of dietary folic acid are effective in counteracting the toxic effects of glycine.

The basal rations used by Richardson et al. ('45) Jukes et al. ('47) and Lance and Hogan ('48) in studying a deficiency of folic acid contained, by calculation, approximately 2.5, 2.0 and 2.5% of glycine respectively, while the basal diets with

soybean protein, in the present studies, contained approximately 1.2% of glycine. These are rather high levels of glycine in comparison to a requirement of 0.9% (Kratzer and Williams, '48a) and might suggest that it is essential for the production of cervical paralysis. In experiment 6, however, it was shown that cervical paralysis could be produced in poults fed a ration low in glycine, and is thus actually a symptom of a folic acid deficiency. This fact gives further support to the theory that glycine is toxic by creating a folic acid deficiency.

The cervical paralysis seems to be a symptom of a chronic deficiency since poults which were depleted when hatched and fed an extremely deficient ration showed less cervical paralysis than poults fed higher levels of folic acid or less glycine. This is analogous to a deficiency of pantothenic acid in which early mortality may prevent the development of typical symptoms (Kratzer and Williams, '48b).

SUMMARY

In poults fed rations low in folic acid, glycine caused depressed growth, increased mortality and cervical paralysis. The effects could be prevented by supplementing the rations with folic acid. DL-Serine at an equimolar level had no adverse effect upon the birds. Poults fed a ration deficient in glycine as well as folic acid also developed cervical paralysis. This indicates that the cervical paralysis is a result of a deficiency of folic acid rather than an excess of glycine.

LITERATURE CITED

ALEXANDER, N., AND D. M. GREENBERG 1955 Studies on the biosynthesis of serine. J. Biol. Chem., *214:* 821.

ANONYMOUS 1955 Technical bulletin on Drackett assay protein C-1. Drackett Products Co., Cincinnati, Ohio.

BLAKLEY, R. L. 1954 The interconversion of serine and glycine: role of pteroylglutamic acid and other cofactors. Biochem. J. *58:* 448

ELWYN, D., A. WEISSBACH, S. S. HENRY AND D. B. SPRINSON 1955 The biosynthesis of choline from serine and related compounds. J. Biol. Chem., *213:* 281.

HOLLAND, B. R., AND W. W. MEINKE 1949 The serine requirement of Streptococcus faecalis R as a function of the basal medium. Ibid., *178:* 7.

JUKES, T. H., E. L. R. STOKSTAD AND M. BELT 1947 Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. Pteroylglutamic acid, riboflavin, niacin and inositol. J. Nutrition, *33:* 1.

KISLIUK, R. L., AND W. SAKAMI 1955 A study of the mechanism of serine biosynthesis. J. Biol. Chem., *214:* 47.

KRATZER, F. H., AND D. E. WILLIAMS 1948a The glycine requirement of young poults. J. Nutrition, *35:* 315.

———— 1948b The pantothenic acid requirement of poults for early growth. Poultry Sci., *27:* 518.

KRATZER, F. H., D. E. WILLIAMS AND B. MARSHALL 1949 The sulfur amino acid requirements of turkey poults. J. Nutrition, *37:* 377.

KRATZER, F. H., P. N. DAVIS AND B. J. MARSHALL 1956 The folic acid requirements of turkey breeder hens. Poultry Sci., *35:* 711.

LANCE, B. G., AND A. G. HOGAN 1948 Vitamin Bc, inositol, and nicotinic acid in the nutrition of the turkey. J. Nutrition, *36:* 369.

LILLIE, R. J., AND G. M. BRIGGS 1947 Biological assay of folic acid activity in common feedstuffs. Poultry Sci., *26:* 289.

MACHLIN, L. J., A. H. LANKENAU, C. A. DENTON AND H. R. BIRD 1952 Effect of vitamin B_{12} and folic acid on growth and uricemia of chickens fed high levels of glycine. J. Nutrition, *46:* 389.

NABER, E. C., E. E. SNELL AND W. W. CRAVENS 1952 The effect of folic acid on glycine toxicity in the chick. Arch. Biochem. Biophys., *37:* 158.

NABER, E. C., W. W. CRAVENS, C. A. BAUMANN AND H. R. BIRD 1956 The relation of dietary supplements and tissue metabolites to glycine toxicity in the chick. J. Nutrition, *60:* 75.

TOTTER, J. R., B. KELLEY, P. L. DAY AND R. R. EDWARDS 1950 The metabolism of glycine by folic acid-deficient chick liver homogenates. J. Biol. Chem., *186:* 145.

PLAUT, G. W. E., J. J. BETHEIL AND H. A. LARDY 1950 The relationship of folic acid to formate metabolism in the rat. Ibid., *184:* 795.

RICHARDSON, L. R., A. G. HOGAN AND H. L. KEMPSTER 1945 Requirement of the turkey poult for vitamin Bc. J. Nutrition, *30:* 151.

VOHRA, P., F. H. LANTZ AND F. H. KRATZER 1956 The effect of folic acid and vitamin B_{12} on the synthesis of serine and choline from glycine in the liver of young turkey poults. J. Biol. Chem., *221:* 501.

THE ADDITION OF NON-IONIC SURFACE-ACTIVE AGENTS OF THE POLYOXYETHYLENE TYPE TO THE DIET OF THE HAMSTER, THE MOUSE AND THE DOG [1]

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The non-ionic surface-active agents, such as the sorbitan and polyoxyethylene derivatives of fatty acids, have a number of attractive uses in food technology, particularly as emulsifiers. It is important, therefore, to establish the effects of these compounds, in amounts proposed for technological purposes, on the nutrition and health of the consumer. Data presented in the literature can be interpreted to mean that these compounds should not be classified as substitutes for fats but rather be considered as food additives, compounds which could be used in relatively small amounts whenever or wherever they develop technological advantage.

One of the most difficult tasks presented to food technologists is the determination of safety, particularly of compounds that are not recognized as normal to foods and work on this problem has been done by Bourke and Fitzhugh, '53; Chow et al., '53; Culver et al., '51; Harris et al., '50; Jones et al., '48; Krantz et al., '51; Schweigert et al., '51; Wang et al., '50; Allison et al., '52. The task is difficult, in part, because any substance, whether it be classified as inert, as a food,

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or as a food additive, may appear to be harmless, beneficial, or toxic according to the experimental design and concentrations used. The pathologies associated with toxicity may be a result of an increase in abnormalities associated with the growth and aging of a colony of laboratory animals, or they may be specific for the compound added to the food, or a combination of the two.

One error that is sometimes made in determining the safety of a group of compounds such as the emulsifiers is to treat them as if all members of the group had uniform effects on the living system. Even related compounds such the polyoxyethylene stearates Myrj [R]45 and Myrj 52 which differ only in the number of polyoxyethylene units in the molecule may be absorbed and metabolized entirely differently. The following experiments involve these two emulsifiers together with a polyoxyethylene sorbitan monostearate (Tween [R]60).

THE EXPERIMENTAL DESIGN

Essentially the same experimental design was used with three different species of animals, the hamster, the mouse and the dog. The emulsifiers Myrj 45[2], Myrj 52[2] and Tween 60[2] were fed in several concentrations, but the amounts were usually small and did not replace essential dietary components, so that nutritional imbalances were to a large extent avoided. The compounds were fed throughout the youth of the hamsters and mice and into adulthood, the experiments being terminated before senility itself caused a high degree of pathological changes. The experimental design employed involved measurement of growth and food efficiencies, a search for pathological conditions specific to the compound fed, and the determination of changes in incidence of pathology common to the colony of animals used in the test. Normal biological growth is an integrated process where the whole animal and

[2] Produced and furnished by Atlas Powder Compahy; chemically designated, respectively, as follows: polyoxyethylene (8) stearate, polyoxyethylene (40) stearate and polyoxyethylene (20) sorbitan monostearate.

all of its parts increase in weight or volume in a definite pattern. This pattern of growth was determined by measuring not only the weight of the whole animal but also some of its parts. The process of growth is a function of food intake and its utilization, a function which was expressed as food efficiency. Abnormalities were detected by histopathological techniques.

TABLE 1

Basal diets

INGREDIENT	gm	INGREDIENT	mg/1000 gm of dry diet
		Diet A	
Casein (vitamin-free)	180	Thiamine	10
Sucrose	134	Riboflavin	20
Dextrose	202	Pyridoxine	10
Dextrin	140	Vitamin K	15
Lard	241	Niacin	80
Salt mixture [1]	40	Pantothenic acid	80
Agar	33	Inositol	200
Cod liver oil	20	Folic acid	0.5
Liver powder	10	Biotin	0.5
	1000	Alpha-tocopherol	40 units
		PABA	80
Water	1400	Choline	2000
		Ascorbic acid	2
	2400	Vitamin A	40,000 units
		Vitamin D	4,000 units
		Diet B	
Casein (vitamin-free)	250	Thiamine	2.0
Sucrose	. . .	Riboflavin	1.6
Dextrose	366	Nicotinic acid	16.0
Dextrin	187	Calcium pantothenate	13.0
Lard	153	Pyridoxine	1.0
Salt mixture [1]	17	Choline	1000.0
Agar	27	2-Methyl-naphthoquinone	0.0006
	1000	Alpha-tocopherol	30.0
		Biotin	0.6
Water	1400	Folic acid	0.6
		Vitamin A	55,000 units
	2400	Vitamin D	11,000 units

[1] Wesson ('32).

THE DIET

Diet A, recorded in table 1, was used in some of the hamster and all of the mouse experiments. Both species of animals have been maintained on this diet from weaning to a year of age or more with no evidence of abnormalities except the development of obesity in some mice in old age an obesity attributed to the high caloric density of the diet. A commercial preparation [3] was also fed to hamsters to protect them from a disease called by us "Wet Spot." Dogs were fed a synthetic diet closely resembling diet A, called diet B (table 1). (See Allison, Wannemacher and Migliarese, '54).

EXPERIMENTS WITH HAMSTERS

Preliminary work with a synthetic diet. While developing the vitamin requirements of the hamster, diet A (table 1) was fed with roughly one half the recorded vitamins. Four groups of 12 animals each were fed this low vitamin diet to which 0, 1, 2.5 or 5% of Myrj 52 (dry basis) was added. All groups of animals grew well initially. Many, however, reduced their food intake markedly after 66 days, and two animals in the 5% Myrj 52 group died. Thiamine deficiency was suspected because of symptoms similar to those of polyneuritis. Increasing all vitamins to the concentrations in diet A (to avoid other possible deficiencies) was accompanied by improved appetite and return to normal growth. From then on until approximately 200 days animals in all groups received this diet and were in good condition. At this time 6 control animals and 4 animals from the group fed 5% Myrj 52 were autopsied. There was some evidence of fatty accumulation in the liver of animals receiving the Myrj 52, otherwise no gross or microscopic pathology was observed. The remaining animals in all groups lived for approximately one year, at which time a severe epidemic of "Wet Spot" caused deaths in all groups. Attempts to repeat these experiments failed because of the prevalence of "Wet Spot" in all animals, including the controls.

[3] Purina Fox Chow.

Diet and the disease "Wet Spot".   It is possible that many experiments where synthetic diets are fed to hamsters may be complicated by the disease "Wet Spot" which is characterized primarily by a wet posterior end, with bloody diarrhea and inanition. A private communication from Ben-Menachem at Hebrew University, Jerusalem, suggests that the disease may be similar to the obscure and fatal "Wet Tail" referred to by Hindle (see Worden, '47). Hamsters fed diet A grew and were in excellent condition provided they were free from this disease. Exposure to "Wet Spot" was impossible to avoid, however, and occurred when animals were placed in cages that had contained sick animals or that were adjacent to sick animals.

Since "Wet Spot" was seldom found in animals fed the commercial "Fox Chow," variations were made in diet A to include this and certain natural foodstuffs. Addition of 30% of "Fox Chow," yeast, or alfalfa meal to diet A reduced the incidence of "Wet Spot," and reduced mortality from close to 100% to under 50%. Decreasing the fat in diet A by one-half, to approximate more nearly the low fat content (3%) of "Fox Chow," did not protect the animals from the disease entirely, but delayed its onset. Much work needs to be done to characterize the disease and the protective effect of foods, but these preliminary observations are mentioned at this time since the disease may be prevalent in other colonies of hamsters. Our work was continued using "Fox Chow" as the basal diet.

Surface-active agents in the "Fox Chow" diet.   Two experiments were carried out with the same general experimental pattern. Weanling hamsters were divided at random into groups of 12 animals each, the groups having the same average body weight, and fed "Fox Chow" alone or "Fox Chow" containing varying amounts, in terms of percentage weight, of the surface-active agents Myrj 45, Myrj 52, Tween 60, or 1% of vegetable fat. In one experiment, hamsters weighing approximately 27 gm were divided into 4 groups fed "Fox Chow" containing 0, 2.5, 5 or 10% of Myrj 52, respectively.

The other experiment carried out a year later and in triplicate (experiments 19, 20 and 21) contained 8 groups, a "Fox Chow" control and groups fed "Fox Chow" plus 1% vegetable fat (in one of the triplicates a duplicate "Fox Chow" control group was substituted for the 1% fat group), 1% and 5% of Myrj 45, 1% and 5% Myrj 52, and 1% and 5% of Tween 60. The "Fox Chow" with 1% of vegetable fat was considered as another control group, to include the effects of altering the texture and caloric value of the "Fox Chow," to approximate some of the non-specific changes that might be associated with addition of surface-active agents. Experiment 20 was started one month following experiment 19 and experiment 21 three months following experiment 20. This division was made to determine the validity of one experimental design repeated at different times and using 12 animals in each group.

For the first 6 weeks of the experimental period the amount of food consumed by each group in the triplicate experiment was accurately measured so that food efficiency (grams weight gained per gram of food consumed) could be calculated for the period of most rapid growth of the animals. All animals were weighed twice weekly at first, then at weekly and monthly intervals. At the end of 12 or 13 months of maintenance on the control and experimental diets all animals were autopsied and heart, liver, kidneys and spleen weighed. Duplicate samples of lymph node, thyroid, lung, heart, kidney, liver, adrenal, spleen, stomach, jejunum, duodenum, ileum, colon, cecum, bladder and testis were fixed in Bouin's solution or in 10% formalin, sectioned and examined for histopathology. Body weights and organ weights of animals in each experiment, at autopsy, were subjected to statistical tests for significance.

Body weight gain, food efficiency and organ weights. No difference was observed among any of the groups, individually or when averaged, in body weight gained or in grams of weight gained per gram of food consumed (food efficiency) over the first 6 weeks (see table 2). The average weight of all groups

TABLE 2

Food efficiency and body weights in hamsters fed "Fox Chow" or "Fox Chow"
containing Myrj 45, Myrj 52, Tween 60 or a vegetable fat

DIET	FOOD EFFICIENCY [1] FIRST 6 WEEKS	BODY WEIGHT [2] MONTHS ON DIET			
		0	3	6	12
		gm	*gm*	*gm*	*gm*
Experiment 17					
Fox Chow	. . .	27	76	87	101
Myrj 52, 2.5%	. . .	26	79	98	108
Myrj 52, 5%	. . .	27	79	95	100
Myrj 52, 10%	. . .	27	81	94	101
Experiment 19					
Fox Chow	0.20	50	98	110	106
Vegetable fat, 1%	0.25	43	90	103	94
Myrj 45, 1%	0.26	52	96	110	99
Myrj 45, 5%	0.19	52	90	103	102
Myrj 52, 1%	0.22	52	93	103	96
Myrj 52, 5%	0.21	53	92	109	102
Tween 60, 1%	0.24	53	97	111	108
Tween 60, 5%	0.19	53	98	110	103
Experiment 20					
Fox Chow	0.19	40	100	103	101
Vegetable fat, 1%	0.19	40	96	100	104
Myrj 45, 1%	0.20	40	107	104	105
Myrj 45, 5%	0.20	40	100	104	108
Myrj 52, 1%	0.20	40	98	104	101
Myrj 52, 5%	0.18	40	94	99	96
Tween 60, 1%	0.19	40	97	99	94
Tween 60, 5%	0.19	40	98	101	104
Experiment 21					
Fox Chow A	0.16	49	100	107	104
Fox Chow B	0.16	49	94	108	104
Myrj 45, 1%	0.16	49	98	104	101
Myrj 45, 5%	0.16	49	98	99	90
Myrj 52, 1%	0.17	49	97	113	103
Myrj 52, 5%	0.14	49	87	97	90
Tween 60, 1%	0.16	49	95	110	107
Tween 60, 5%	0.16	49	97	109	104

[1] Grams weight gained per gram of food consumed.
[2] Twelve animals in each dietary group.

decreased at 12 months in experiment 19, a decrease which was significantly greater than that of the "Fox Chow" controls in the group fed the diet containing 1% of vegetable fat (see table 3). No difference between the "Fox Chow" control and the vegetable fat control groups was observed, however, in experiment 20. Loss in body weight at 12 months was also observed in experiment 21 where the loss was significantly greater than that of the controls in animals fed 5% of Myrj 45 and 5% of Myrj 52. It is doubtful, however, that these losses in weight in the older animals are associated with the addition of surface-active agents *per se* since it was not consistent in the three experiments and occurred also in one of the vegetable fat control groups. With one exception, the weights of the heart, liver, kidneys, and spleen relative to body weight were not altered from the controls by the presence of Myrj 45, Myrj 52 or Tween 60 in the diet (see table 3). The one exception was an average liver weight which was significantly larger ($p \leq 0.05$) than that of the "Fox Chow" control in animals fed 5% of Myrj 45 in experiment 20. This larger liver was not observed in experiments 19 or 21.

If the data obtained from the three groups of animals in the triplicate experiment are combined into one set, these differences disappear except for one instance. The average weight of the groups fed 5% of Myrj 52 is still significantly less than that of the "Fox Chow" controls at 12 months but not significantly lighter than the control animals fed 1% of vegetable fat. This can be interpreted to mean that the occasional differences noted while feeding groups of 12 animals, not observed when considering the 36 as a group, may be statistically significant because of a different distribution of unknown stresses in the triplicate experiment. Even though all experiments were done in the same animal room under as similar conditions as could be established, a stress such as a respiratory infection could occur asymmetrically with respect to distribution of groups in the cages and with respect to time. Care must be taken, therefore, in the interpreta-

TABLE 3

Average body weight and organ weights of hamsters fed control and experimental diets for 12 to 13 months

DIET	NO. ANIMALS	BODY WEIGHT	HEART	LIVER	KIDNEY	SPLEEN
		gm	*gm*	*gm*	*gm*	*gm*
Experiment 17 (Started 3/19/52)						
Fox Chow (Control)	9	101	0.403	3.93	0.767	0.078
Myrj 52, 2.5%	11	108	0.430	3.99	0.815	0.089
Myrj 52, 5%	8	100	0.476	3.68	0.859	0.110
Myrj 52, 10%	11	101	0.400	3.91	0.851	0.081
Experiment 19 (Started 2/16/53)						
Fox Chow (Control)	11	106	0.398	4.28	0.899	0.076
Vegetable fat, 1%	12	94 [1]	0.357 [1]	3.80	0.752 [1]	0.067
Myrj 45, 1%	10	99	0.377	4.05	0.869	0.079
Myrj 45, 5%	12	102	0.380	4.24	0.893	0.070
Myrj 52, 1%	11	96	0.401	3.84 [1]	0.911	0.071
Myrj 52, 5%	11	103	0.387	4.04	0.886	0.072
Tween 60, 1%	12	108	0.414	3.99	0.967	0.076
Tween 60, 5%	12	103	0.396	3.94	0.955	0.072
Experiment 20 (Started 3/23/53)						
Fox Chow (Control)	12	101	0.413	3.42	0.815	0.082
Vegetable fat, 1%	10	104	0.411	3.45	0.724	0.076
Myrj 45, 1%	10	105	0.460	3.73	0.810	0.079
Myrj 45, 5%	10	108	0.403	4.23 [1,2]	0.841	0.072
Myrj 52, 1%	11	101	0.398	3.58	0.796	0.075
Myrj 52, 5%	11	97	0.376	3.49	0.776	0.073
Tween 60, 1%	12	94	0.379 [1]	3.37	0.776	0.074
Tween 60, 5%	9	104	0.397	3.61	0.870	0.083
Experiment 21 (Started 6/11/53)						
Fox Chow (Control)	11	104	0.420	3.91	0.824	0.109
Fox Chow (Control)	12	105	0.418	3.97	0.831	0.101
Myrj 45, 1%	11	101	0.404	3.63	0.780	0.085
Myrj 45, 5%	11	90 [1]	0.375 [1]	3.50	0.733 [1]	0.086
Myrj 52, 1%	12	103	0.410	3.74	0.874	0.092
Myrj 52, 5%	10	90 [1]	0.383	3.65	0.786	0.086
Tween 60, 1%	12	107	0.432	3.96	0.822	0.103
Tween 60, 5%	12	104	0.413	3.92	0.839	0.098

[1] Significant at $P \leqq 0.05$ confidence level.

[2] Only weight significantly different from control (at $P \leqq 0.05$ confidence level) when values expressed per 100 gm body weight.

tion of so-called "significant differences" where asymmetry of stresses or other variables may exist.

Pathology. The data recorded in table 4 demonstrate that mortality was low in all groups of hamsters and was not altered by the presence of any of the surface-active agents in the diet. The histological examination of all the tissues at the end of one year was performed by a pathologist without knowledge of the diet fed the animal from which the tissues were taken. The report stated "All tissues examined were within normal limits except the following." Following this statement was a report of pathological diagnosis in terms of incidence in each group of hamsters such as 1/11 (i.e. one out of 11 of the hamsters in a certain group) had chronic interstitial nephritis. The results in each experiment were discussed and conclusions were drawn.

Significant pathology of three types was observed. The first was a sub-clinical colony infection characterized by mild to moderate generalized lymphoid hyperplasia, focal parenchymal hepatitis with perivascular cuffing of portal veins which rarely progressed to granulomatous degeneration in the liver, fibrosis of the spleen, and atrophy of the adrenal cortex, especially the zonae fasciculata and reticularis. The frequency of occurrence of this pathology was 32% in the controls and 28% in the hamsters receiving diets containing surface-active agents. Comparison of data between individual groups demonstrated that this pathology was not correlated with the presence or absence of surface-active agents.

The second type of pathology observed was mild testicular atrophy. In the year-old animals the incidence was above that in the controls in one experiment where hamsters were fed 5 and 10% of Myrj 52 (see table 4). The incidence was not increased, however, in triplicate experiments where animals were fed 5% of Myrj 52. Grossly, the incidence of testicular atrophy with "brown discoloration" was observed to be higher in the animals fed 10% of Myrj 52 for one year than in the controls.

The third type of pathology was concerned with the kidneys. This pathology could be divided into a primary type characterized by the formation of hyaline casts in the renal tubules and a secondary type characterized by hyaline tubule casts accompanied by chronic interstitial nephritis. The data indicate that whenever diarrhea was present the incidence of casts and chronic interstitial nephritis generally increased

TABLE 4

Pathologies in hamsters fed "Fox Chow" or "Fox Chow" containing vegetable fat, Myrj 45, Myrj 52, or Tween 60

DIET	NUMBER ANIMALS	DEAD AT 1 YEAR	DIARRHEA	MILD TESTICULAR ATROPHY	CASTS [1]	CIN [1]
Fox Chow	36	2	—	1	9	2
Vegetable fat, 1%	24	2	—	1	8	1
Myrj 45, 1%	36	5	—	1	4	1
Myrj 45, 5%	36	3	—	0	12	0
Myrj 52, 1%	36	3	—	1	10	3
Myrj 52, 5%	36	3	+	0	17	5
Tween 60, 1%	36	0	—	0	11	0
Tween 60, 5%	36	3	+	1	18	6
Fox Chow	12	3	—	1	2	0
Myrj 52, 2.5%	12	1	—	1	4	1
Myrj 52, 5%	12	3	+	4	8	1
Myrj 52, 10%	12	1	+	7	10	6

[1] Casts and chronic interstitial nephritis (CIN) in kidney.

significantly above the controls and other groups fed surface-active agents without resulting diarrhea (see table 4). Diarrhea was obvious in animals fed 5 and 10% of Myrj 52 and 5% of Tween 60 and absent in animals fed 1 or 2.5% of Myrj 52, 1% of Tween 60 and 1 or 5% of Myrj 45.

In order to investigate further the cause of increased kidney pathology in hamsters fed higher concentrations of Myrj 52 and Tween 60, 4 groups of 10 young male hamsters each were fed, ad libitum, diets of "Fox Chow" alone or "Fox Chow" containing 5% of Myrj 45, 5% of Myrj 52, or 5% of Tween 60 for one week. For the next three days, while the animals were fed the same diets, food and water intakes were measured

and feces collected quantitatively. Slight diarrhea in the hamsters fed Myrj 52 and Tween 60 rendered the fecal collections not completely accurate. Analysis of food and feces for oxyethylene content [4] made possible calculation of the percentage of ingested oxyethylene excreted in the feces. Twenty-four per cent of the ingested oxyethylene of Myrj 45 was excreted in the feces in contrast to 110% for Myrj 52 and 96% for Tween 60. Animals fed ''Fox Chow'' drank approximately 6.7 ml of water per day while those fed 5% of Tween 60 drank 8.1 ml per day. Thus complete non-absorption of the ingested molecules is associated with increased water intake and diarrhea. The resulting water imbalance may in turn be related to the increased incidence of casts and chronic interstitial nephritis in animals fed 5% or more of Myrj 52 and Tween 60.

EXPERIMENTS WITH MICE

The effect of surface-active agents on growth and maintenance of mice was determined in order to reveal possible species differences in reaction. These animals have dietary requirements similar to those of the hamster, and they are not susceptible to the disease ''Wet Spot.''

Experimental plan. In each experiment, groups of 10 to 12 weanling mice, male or female, were fed diet A either alone or with concentrations of from 2.5 to 10% (dry basis) of the surface-active agents Myrj 45, Myrj 52 or Tween 60. Daily food intake was measured for the first three weeks. Twice-weekly, weekly and then twice-monthly body weights were also determined. In one experiment, female mice were maintained on a diet containing Myrj 52 at concentrations from 2.5 to 15%, from weaning through gestation and lactation. The animals were maintained on experiment for periods varying from three months to over a year. At the termination of the experiment all animals were autopsied, and the same

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[4] These analyses were done by Dr. Charles Smullin of The Central Research Laboratory of Atlas Powder Company.

organs weighed and tissues sectioned, stained and examined for histopathology as in the case of the hamsters.

Environmental factors. High mortality was observed in early experiments in groups of mice fed diet A containing 15% of Myrj 52 or Tween 60. This high mortality was concurrent with an erythema and loss of hair. Data were obtained to suggest that these conditions were due to direct skin contact with the experimental diets and resulting cannibalism. Although similar effects were produced by the control diet alone when it was rubbed onto the skin of the animals, the

TABLE 5

Average body weight and organ weights of mice fed various amounts of Myrj 45, Myrj 52 or Tween 60 in diet A for 3 to 4 months [1]

DIET	NO. ANIMALS	BODY WEIGHT	HEART	LIVER	KIDNEYS	SPLEEN
		gm	*gm*	*gm*	*gm*	*gm*
Diet A (Control)	13	38.7	0.156	1.61	0.526	0.213
+ Myrj 45, 5%	9	41.6	0.182	1.70	0.645	0.235
+ Myrj 45, 10%	12	42.3	0.176	1.83	0.605	0.225
+ Myrj 52, 2.5%	11	43.4	0.160	1.74	0.537	0.236
+ Myrj 52, 5%	10	40.3	0.163	1.68	0.554	0.251
+ Myrj 52, 10%	10	40.3	0.168	1.64	0.538	0.184
+ Tween 60, 2.5%	6	42.1	0.186	1.82	0.576	0.196
+ Tween 60, 5%	9	41.2	0.173	1.68	0.552	0.202
+ Tween 60, 10%	9	42.0	0.161	1.92	0.550	0.234

[1] There is no difference, at the $P \leq 0.05$ confidence level, between the average body weights and organ weights of any of the experimental groups and the controls.

condition was aggravated when the food contained the surface-active agents. Lowering of mortality and improvement of the skin condition occurred when wood shavings were kept in the wire or metal-bottom cages in which the mice were housed and when food was available only from overhead wire baskets. Therefore all subsequent experiments were conducted in this fashion.

Body weight gain and organ weights. There were no outstanding differences in average body weights of animals fed the various experimental diets over periods of from three to 4 months, nor did the organ weights of these animals

determined on autopsy at three to 4 months differ (table 5). Those mice fed Myrj 45 seemed to have slightly larger kidneys, but kidney weights, expressed per 100 gm of body weight, did not differ significantly, at $P \leqq 0.05$ confidence level, from the controls.

Mice fed 10 or 15% of Myrj 52 or Tween 60 developed *pruritis ani* and diarrhea, with no associated pathology. There were no gastrointestinal disturbances in animals fed 10% or more of Myrj 45.

Gestation and lactation. Two and one half to 15% of Myrj 52 added to the control diet caused no deviation from the normal pattern of gestation and lactation in female mice

TABLE 6

Female mice fed various amounts of Myrj 52 from weaning through the first gestation and lactation [1]

DIET	NUMBER OF MICE BRED	AVERAGE IN LITTER	AVERAGE BIRTH WT.
			gm
Diet A (Control)	8	7.9	1.655
Myrj 52, 2.5%	8	9.1	1.515
Myrj 52, 5%	9	9.0	1.444
Myrj 52, 10%	8	8.0	1.607
Myrj 52, 15%	7	10.6	1.511

[1] There is no difference, at the $P \leqq 0.05$ confidence level, between the litter size and birth weight of any of the experimental groups and the controls.

maintained on these diets from weaning on (table 6). This was true despite some gastrointestinal disturbances at the higher concentrations as indicated above.

Pathology. In the experiments lasting three to 4 months all of the tissues examined from animals fed the three surface-active agents Myrj 45, Myrj 52 and Tween 60 were normal with the exception of those described below. A sub-clinical colony infection present in 11 of the mice in the control group, fed diet A alone, was characterized by generalized lymphoid hyperplasia, focal parenchymal hepatitis with occasional to many intranuclear inclusion bodies and tubular nephritis. The latter was accompanied by tubule cast formation and

mononuclear perivascular cuffing in the bladder, which approximated early cystitis. Eight of the 9 mice fed 5% and 9 of the 12 fed 10% of Myrj 45 showed signs of the generalized colony infection. Two mice fed 5% of Myrj 45 and none in the 10% Myrj 45 group had marked infiltration of the liver, with intranuclear inclusion bodies. No pathological conditions attributable to the presence of Myrj 45 in the diet were evident. Five of the 11 mice fed 2.5%, 6 of the 10 fed 5%, and 8 of the 10 fed 10% of Myrj 52 demonstrated pathology that was associated in whole or in part with the colony infection. There were no abnormalities attributable to the feeding of Myrj 52. In the experiments involving Tween 60, 5 of the 6 mice fed 2.5% of this surface-active agent, 7 of the 9 fed 5% and 7 of the 9 fed 10% had evidence of the colony infection. Two animals, one fed 2.5% and one fed 10% of Tween 60, evidenced a mild gastritis. There were no pathological conditions specific for Tween 60.

EXPERIMENTS WITH BEAGLES

Weanling beagle puppies were fed an agar gel diet B (table 1) containing 0, 5, or 10% (dry basis) of Myrj 45, Myrj 52 or Tween 60. The basal diet, containing 25% of casein, has been used for a number of years to grow and maintain dogs. Protein efficiencies are recorded in table 7. More data are needed to determine the variation in protein efficiencies of beagle puppies but these values are of the same order of magnitude as those determined previously on this diet in our laboratories, and the variation is small when it is considered that each value represents a single animal. None of the animals developed diarrhea on any of these diets. One animal fed 5% of Myrj 45 for approximately one year and two receiving 10% of Myrj 45 for 40 weeks were autopsied and the same group of tissues listed for hamsters and mice were examined for histopathology. No significant pathology attributable to the feeding of Myrj 45 was found. Two dogs, one male and one female, receiving 5% of Myrj 52 were autopsied at the end

of 9 months. No significant pathology was found. All other dogs, including those fed Tween 60, were kept on the diets for a year or more and since they were in good condition they are being used for other nutritional experiments.

TABLE 7

Gain in body weight, nitrogen intake, and protein efficiency in beagle puppies fed basal diet, and the same diet plus various amounts of Myrj 45, Myrj 52 or Tween 60 in the 14 weeks after weaning

DOG NO.	DIET	WEIGHT INCREASE	NITROGEN INTAKE	PROTEIN EFFICIENCY
		kg	*kg*	*kg wt. gain/kg N eaten*
	Males			
1	Basal diet	5.5	0.60	9.2
2	Basal diet	6.1	0.66	9.2
3	Basal diet	5.5	0.58	9.5
4	Basal diet	6.3	0.68	9.3
5	+ Myrj 52, 5%	5.4	0.53	10.2
6	+ Myrj 52, 10%	5.2	0.62	8.4
7	+ Myrj 45, 10%	5.9	0.56	10.5
8	+ Myrj 45, 10%	5.7	0.55	10.3
9	+ Tween 60, 5%	7.6	0.76	10.0
10	+ Tween 60, 5%	4.4	0.49	8.9
11	+ Tween 60, 10%	7.8	0.79	9.8
	Females			
12	Basal diet	3.8	0.49	7.5
13	Basal diet	5.5	0.67	8.1
14	+ Myrj 52, 5%	3.9	0.52	7.5
15	+ Myrj 52, 5%	4.9	0.66	7.4
16	+ Myrj 52, 10%	4.9	0.55	8.9
17	+ Tween 60, 10%	3.6	0.53	6.8

DISCUSSION

The experiments involving hamsters emphasize the necessity of feeding a diet that protects the animal from severe effects of variable stresses such as colony infections. Such uncontrolled stresses can sweep through the colony at random so that significant differences may be observed between groups, differences that are a function of the nature of the stress and not of the experimental variable. It was found impossible to use a synthetic diet for the experiments involving hamsters

because of the lack of protection by the diet against a disease we have called "Wet Spot." Partial protection could be obtained by adding alfalfa meal, certain yeasts or "Fox Chow" to the synthetic diet. For that reason, "Fox Chow" was used as the basal diet. Even with some asymmetry of stresses between groups may be one explanation of the fact that in triplicate experiments some significant differences (P $\leqq$ 0.05) occurred in one member of the triplicate which disappeared when the data were considered as a whole.

The addition of Myrj 45, Myrj 52 or Tween 60 at varying concentrations to the diet of hamsters, mice or dogs did not alter growth or food efficiency. Myrj 45 did not produce diarrhea in any of the animals or any abnormalities that could be attributed to this surface-active agent *per se*. The possibility that 5% of Myrj 45 could be considered the upper limit for feeding surface-active agents to hamsters might be argued because in a single experiment there was a significant fall in body weight in year-old animals below the "Fox Chow" control, a significance, however, that disappeared when triplicate experiments were analyzed. A reduction in growth and food efficiencies was reported by Schweigert et al. ('50) for hamsters fed 5 and 15% of Myrj 45 in place of an equivalent amount of lard in a semi-synthetic diet. The gastrointestinal disturbances found by these authors in hamsters fed Myrj 45 and the accompanying pathologies (see Wang et al., '50) were not observed in any of our animals. Possibly there were added stresses in their experiments, such as a nutritional imbalance due to substitution of Myrj 45 for lard. Recently Krehl, Cowgill and Whedon ('55) concluded from their studies on the effects of polyoxyethylene esters in the diet of the rat and the cat that Myrj 45 up to 20% of the diet, or the oxyethylene moiety at a level of 6%, had no deleterious effect.

Diets containing 5% of Myrj 52 or Tween 60 did produce diarrhea in hamsters. Higher concentrations of these surface-active agents up to 10 and 20% were necessary to produce diarrhea in mice or dogs. Increase in the incidence of kidney casts and of chronic interstitial nephritis occurred in hamsters

fed 5% or more Myrj 52 or Tween 60 in "Fox Chow." Chow et al. ('53) reported that a semi-synthetic diet supplemented with 5% of Tween 60 produced diarrhea and growth retardation in rats. This same emulsifier, or Myrj 52, could be fed by them in a soybean meal basal diet even at the 15% level without any observable toxic effects. These observations, together with those presented here, suggest that the abnormal effects associated with the feeding of Myrj 52 and Tween 60 may be the result, at least in part, of non-absorption of the oxyethylene moiety from the gut and complete excretion in the feces. Absorption of roughly three quarters of the oxyethylene portion of the Myrj 45 molecule may have prevented diarrhea and associated kidney pathology in animals fed this compound. Only those compounds produced pathological kidney conditions that, at the concentrations fed, caused diarrhea. Perhaps the higher incidence of mild testicular atrophy observed in hamsters fed 10% of Myrj 52 for one year could be associated with the stress of long-continued diarrhea. The incidence of this mild testicular atrophy was higher in animals fed 5% of Myrj 52 than in the controls in one experiment but was not higher than the controls in three other experiments. These results indicate that 5% of Myrj 52 is near the upper limit for feeding this surface-active agent to hamsters. No other histopathologies were observed while feeding Myrj 52 or Tween 60 to hamsters.

No histopathologies specific for Myrj 45, Myrj 52 or Tween 60 were observed in mice, results which indicate that hamsters are more susceptible than mice to the effects of diarrhea or other stresses associated with these agents.

REFERENCES

ALLISON, J. B., H. L. ROSENTHAL AND A. H. MILLS 1952 Effects of nonionic surface active agents on the growth of animals. Fed. Proc., 11: 435.

ALLISON, J. B., R. W. WANNEMACHER, JR. AND J. F. MIGLIARESE 1954 Diet and the metabolism of 2-aminofluorene. J. Nutrition, 52: 415.

BOURKE, A. R., AND O. G. FITZHUGH 1953 High and low levels of emulsifiers on rat intestinal flora. Fed. Proc., 12: 302.

CHOW, B. F., J. M. BURNETT, C. T. LING AND L. BARROWS 1953 Effects of basal diets on the response of rats to certain dietary non-ionic surface-active agents. J. Nutrition, 49: 563.

CULVER, P. J., C. S. WILCOX, C. M. JONES AND R. S. ROSE, JR. 1951 Studies in the intermediary metabolism of certain polyoxyethylene derivatives in man. Recovery of the polyoxyethylene moiety from urine and feces following ingestion of polyoxyethylene (20) sorbitan monooleate and polyoxyethylene (40) monostearate. J. Pharm. Exp. Therap., 103: 377.

HARRIS, R. S., H. SHERMAN AND W. W. JETTER 1950 Effect of surface active agents when fed in diets of rats. Fed. Proc., 9: 361.

JONES, C. M., P. J. CULVER, G. D. DRUMMEY AND A. E. RYAN 1948 Modification of fat digestion in the digestive tract by the use of an emulsifying agent. Ann. Int. Med., 29: 1.

KRANTZ, J. C., JR., P. J. CULVER, C. J. CARR AND C. M. JONES 1951 Sugar alcohols. XXVIII. Toxicologic, pharmacodynamic and clinical observations on Tween 80. Bull. Med. Sch. Univ. of Md., 38 (2).

KREHL, W. A., G. R. COWGILL AND A. D. WHEDON 1955 Nondeleterious effects of polyoxyethylene esters in the nutrition of rats and cats. J. Nutrition, 55: 35.

SCHWEIGERT, B. S., B. H. McBRIDE AND A. J. CARLSON 1950 Effect of feeding polyoxyethylene monostearates on growth rate and gross pathology of weanling hamsters. Proc. Soc. Exp. Biol. Med., 73: 427.

WANG, H. B., B. H. McBRIDE AND B. S. SCHWEIGERT 1950 Histological manifestations of feeding polyoxyethylene monostearate to weanling hamsters. Ibid., 75: 342.

WESSON, L. G. 1932 A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. Science, 75: 339.

WORDEN, A. A., EDIT. 1947 The U. F. A. W. Handbook on the Care and Management of Laboratory Animals. Balliere, Tindall and Co., London.

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