AN EFFECT OF CERTAIN NUTRITIONALLY INERT MATERIALS ON THE INCIDENCE OF EXPERIMENTAL DENTAL CARIES [1]

JAMES H. SHAW
Harvard School of Dental Medicine, Boston, Massachusetts

(Received for publication April 9, 1954)

During the past century, a variety of premises have been advanced in the dental literature concerning the relationship of the physical consistency of the diet to the dental caries incidence in human populations. Practically none of these hypotheses have been followed up by investigative studies to determine their validity in man or in laboratory animals. Neumann et al. ('52) conducted an experiment with 266 children under close supervision in an institution. The 81 children in the control group received what was described as a good diet; in addition to the same ration, the 102 children in the experimental group were instructed to chew vigorously on a 6-inch stalk of dried sugar cane once daily; a second control group of 83 was also maintained in which each subject was given, as a supplement to the institutional diet, an aliquot of freshly extracted cane juice equivalent in volume to the juice in one of the stalks chewed by the subjects in the experimental group. After 18 months, the average increase in the number of decayed, missing and filled teeth for the boys in the experimental group was 1.1 in contrast to 2.1 and 2.2 for the first and second control groups, respectively. At present no parallel studies with experimental animals have been

[1] These studies were supported to a large extent by a grant-in-aid from the Sugar Research Foundation, Inc., New York, N. Y.

presented in the literature. The classical studies by Hoppert, Webber and Canniff ('32) on the relation of the particle size of the diet to dental caries incidence cannot be considered to be analogous since the coarse particles initiated carious lesions at least partially through a mechanical injury effect.

The studies which are to be presented in this report were conducted in order to evaluate various methods to increase the amount of mastication by experimental animals. All studies were conducted with modifications of the purified caries-producing diet, which provided the same nutrients but with different physical characteristics.

EXPERIMENTAL

The results of 5 experiments are to be presented. The subjects in the first experiment were cotton rats from our caries-susceptible strain. They were weaned at 14 days of age and distributed among the various groups in which they were maintained for 14 weeks prior to sacrifice. The subjects in the remaining 4 experiments were white rats of our caries-susceptible strain which were weaned at 21 days of age and distributed among the several groups in each experiment. All white rats were desalivated within 10 days of the beginning of the experiment and maintained for 12 weeks thereafter. Throughout these studies a close littermate distribution was followed among the several groups in any experiment.

All animals were maintained in individual cages throughout the experimental periods with ad libitum access to the experimental diets and to drinking water. Weight records were kept on a biweekly basis. At the termination of the experiments, the animals were sacrificed under ether anesthesia and the heads preserved in 95% ethanol. The number of carious molars, the number of carious lesions and the extent of the individual lesions were determined by previously reported criteria (Shaw, Schweigert, Elvehjem and Phillips, '44).

Experiments 1 and 2 were designed to determine whether the increase in mastication occasioned by a simple increase

in the quantity of diet required for normal growth would alter the incidence of carious lesions. This was done by the incorporation into the diet of varying amounts of agar or cellu flour as inert materials which would increase the weight of ration required by a rat per day in order to supply its normal nutritional requirements. Both materials were incorporated into the diet in the form of fine powder.

The first experiment was divided into two parts. The first half was composed of 5 groups and a total of 25 cotton rats. The first group was fed the usual purified, finely divided, caries-producing diet 100 used in these laboratories (Shaw, '47). The cotton rats in the second group received the same diet with a supplement of 10% agar. The subjects in the third, 4th and 5th groups received the same diet with supplements of 10, 35 and 50% cellu flour, respectively. The second half of this experiment consisted of three groups with a total of 44 cotton rats. These subjects received diet 100 with supplements of 10, 75 and 100% cellu flour, respectively.

The second experiment was made up of 4 groups and a total of 23 white rats. The first group received caries-producing ration 100. The remaining groups were fed this diet with supplements of 10, 35 and 50% cellu flour, respectively.

In the third experiment, in which 55 rats were used, a synthetic resin polyvinyl acetate with a molecular weight of 2400 was incorporated into the experimental diets. This material was selected because it could be tolerated physiologically in relatively large amounts. In addition, its physical properties were such that it could be used to produce a mass requiring a large amount of chewing without being brittle or abrasive. In this experiment which was the first in our laboratories to use the polyvinyl acetate or any other resin, the selection was based on its binding properties and not on the basis of any anticipation that this material *per se* would influence the incidence of dental caries in the rat. As in the first two experiments, the first group served as controls and was fed the basic caries-producing ration 100. The sec-

ond group was fed ration 100 with a 20% supplement of the finely powdered, low molecular weight, polyvinyl acetate. This ration was designated as (100 + 20% L-PVA) — no. 1. The ration for the third group had a final composition equivalent to 100 + 20% polyvinyl acetate but was prepared in such a way as to result in a cake with stiff chewy physical properties which was broken up into chunks prior to feeding. This ration was designated as (100 + 20% L-PVA) — no. 2. The latter diet was made by placing 670 gm sucrose, 50 gm corn oil with added fat-soluble vitamins, 40 gm reagent grade nutrient salt mixture, 20 gm whole liver substance and 20 gm 1:20 liver concentrate powder in a food mixer. To this was added 65 ml of water and the entire mass mixed thoroughly. A 240 gm aliquot of casein to which the various members of the B-complex vitamins had been added was introduced into the above mixture. As the final step, 200 gm of low molecular polyvinyl acetate was added in molten form at 150°F. The entire mass was mixed for approximately 30 min. at a temperature in the neighborhood of 130°F. and was then placed to cool in pans in sheets with a thickness of about one inch. At room temperature, this mixture was a hard cake which broke with difficulty and in the fracture faces could be seen large numbers of tenuous thread-like strands of the resin. The general procedure for the preparation of this ration was not based on any scientific design but was an emipirical one which was adopted simply because of the desirability of the end product for the specific purpose.

The 4th experiment with 38 rats was carried out because of unexpected findings in experiment 3 and was composed of 4 groups. The low-molecular weight polyvinyl acetate was incorporated in finely powdered form into ration 100 for the second, third and 4th groups at levels of 5, 10 and 20%, respectively, with the first group being fed ration 100 alone.

The 5th experiment was made up of 5 groups in which were a total of 46 rats. The basal ration was 700 in this case which had the identical composition of ration 100 with the single exception that the "de-vitaminized" casein had been replaced

by an equal amount of crude casein. The use of ration 700 has been shown in a number of experiments in this laboratory to result in an incidence of carious lesions identical to that produced by ration 100. The first group served as controls and received ration 700 only. The second through 5th groups of rats received ration 700 to which had been added either 10% of the low-molecular-weight polyvinyl acetate, 10% of a high-molecular-weight (50,000–100,000) polyvinyl acetate, 10% natural chicle or 10% arochem (an esterification polymer of glycerol, fatty acids and dimerized rosin), respectively. In each case, the material was incorporated into the ration in finely powdered form.

RESULTS

The results of the first experiment are presented in table 1. From these data, it can be concluded that the incorporation of 10% agar or varying amounts of cellu flour from 10 to 100% into caries-producing diet 100 did not alter the incidence of tooth decay in the cotton rat whether measured by the average number of carious molars, the average number of carious lesions or the average extent of carious lesions. The inclusion of 10% agar, 10, 35 or 50% cellu flour into the ration did not alter the rate of growth or the total increase in weight of the cotton rats maintained thereon. When as much as 75 or 100% by weight of cellu flour was added to the ration, the rate of growth of some of the cotton rats was markedly reduced; however the majority of the cotton rats in these groups grew as well as their littermate controls. The average increases in weight for the rats in the groups receiving 75 and 100% supplements of cellu flour were 12 and 18% less, respectively, than for the controls. In all groups receiving an agar or cellu flour supplement, the overall ration consumption increased in proportion to the amount of inert material added until the cotton rats which received ration 100 + 100% cellu flour consumed almost twice as much diet by weight as the controls. Obviously the calories consumed per rat per day by both groups were closely similar.

TABLE 1

Effect of cellu flour on dental caries experience of cotton rats

GROUP NO.	RATION	NO. OF RATS	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS [1]		
			Av.	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]
1	100	5	11.0	0.4		31.6	1.0		103 +	7 +	
2	100 + 10% Agar	8	10.8	0.3		30.9	1.6		97 +	8 +	
3	100 + 10% Cellu flour	4	10.5	0.4		30.0	2.0		93 +	5 +	
4	100 + 35% Cellu flour	4	10.3	0.4		29.8	0.9		94 +	5 +	
5	100 + 50% Cellu flour	4	11.3	0.4		32.3	1.7		114 +	6 +	
Statistical comparison of											
Group 1 vs. 2					0.4			0.4			0.6
Group 1 vs. 3					0.9			0.7			1.2
Group 1 vs. 4					1.2			1.3			1.1
Group 1 vs. 5					0.5			0.4			1.2
6	100 + 10% Cellu flour	14	10.0	0.4		27.9	1.5		93 +	8 +	
7	100 + 75% Cellu flour	15	10.5	0.3		29.5	1.4		107 +	9 +	
8	100 + 100% Cellu flour	15	11.0	0.2		31.3	1.4		117 +	8 +	
Statistical comparison of											
Group 6 vs. 7					1.0			0.8			1.2
Group 6 vs. 8					2.2			1.6			2.2
Group 7 vs. 8					1.4			1.4			0.8

[1] The + sign after each value indicates that these values do not have a strict arithmetical relationship to each other. Each carious lesion was assigned a value from 1 + to 5 + depending on the extent to which the lesions had progressed during the experiment. The extent of carious lesions for each animal is the total of the evaluations of all the carious lesions observed (J. Nutrition, *28:* 333, 1944).

[2] Standard error of means.

[3] *Critical ratio.* The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Wherever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; when the critical ratio is between 2.0 and 2.9, the difference is of borderline significance; when the critical ratio is 3.0 or higher, the difference is highly significant. (Dunning, J. M., J. Dental Research, *29:* 541, 1950.)

The results of the second experiment are recorded in table 2. As in the first experiment, the incorporation of varying amounts of cellu flour from 10 to 50% into the caries-producing diet did not cause any significant change in the incidence of dental caries in the white rat. The average rates of growth and average increases in weight for the several groups were practically identical. Again the quantities of ration consumed increased in proportion to the amount of added cellu flour.

The results of the third experiment are presented in table 3. Both of the rations to which the 20% supplement of the low-molecular-weight polyvinyl acetate had been added caused highly significant decreases in the incidence of dental caries in the desalivated white rat. From these data it is obvious that the different physical forms of the two rations appeared to be unimportant because the small differences between the results for groups 2 and 3 were not statistically significant even at the 5% level. The rates of growth in the control and ration 100 + 20% finely powdered polyvinyl acetate groups were closely similar for both males and females. The rats in the third group which were fed the ration requiring a great deal of mastication attained somewhat higher final weights than their littermates in the first and second groups. Part of this increase in final weight was due to the accumulation in their stomachs of large plastic masses of polyvinyl acetate. Similar but smaller masses had been found in the stomachs of most of the rats receiving the polyvinyl acetate in powdered form. Grossly at autopsy, it appeared that the polyvinyl acetate had been more readily eliminated when fed in powdered form than when incorporated into the ration by the heating described in the experimental procedure.

In the 4th experiment where the low-molecular-weight polyvinyl acetate was incorporated into the caries-producing diet at levels of 5, 10 and 20% (table 4), the data indicate a progressive decrease in the average number of carious molars, the average number of carious lesions and the average extent of carious lesions as the amount of polyvinyl acetate was

TABLE 2

Effect of cellu flour on dental caries experience of white rats

GROUP NO.	RATION	NO. OF RATS	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS [1]		
			Av.	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]
1	100	6	10.1	0.6		18.4	1.5		58 +	5 +	
2	100 + 10% Cellu flour	5	9.6	0.7		17.8	1.8		50 +	4 +	
3	100 + 35% Cellu flour	6	10.0	0.5		18.2	1.3		55 +	3 +	
4	100 + 50% Cellu flour	6	9.8	0.6		18.5	1.0		65 +	3 +	
Statistical comparison of											
Group 1 vs. 2					0.5			0.3			1.3
Group 1 vs. 3					0.5			0.1			0.5
Group 1 vs. 4					0.4			0.1			1.2

[1] See footnote 1, table 1.
[2] Standard error of means.
[3] Critical ratio (see footnote 3, table 1).

TABLE 3

Effect of a low molecular weight polyvinyl acetate on dental caries experience of white rats

GROUP NO.	RATION	NO. OF RATS	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS [1]		
			Av.	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]
1	100	18	11.8	0.5		22.1	1.0		65 +	5 +	
2	(100 + 20% L-PVA) — no. 1 [4]	19	8.4	0.5		12.3	1.0		33 +	4 +	
3	(100 + 20% L-PVA) — no. 2	18	7.2	0.8		11.7	1.7		30 +	6 +	
Statistical comparison of											
Group 1 vs. 2					4.9			7.1			6.4
Group 1 vs. 3					5.3			5.3			4.5
Group 2 vs. 3					1.3			0.3			0.4

[1] See footnote 1, table 1.
[2] Standard error of mean.
[3] Critical ratio (see footnote 3, table 1).
[4] L-PVA = low molecular weight polyvinyl acetate.

increased. The decrease in the average number of carious molars noted between the control group and the group with 5% polyvinyl acetate was highly significant. The decrease in the average number of carious lesions and average extent of carious lesions between the same two groups was statistically significant but not highly significant. The differences between the dental caries experience of the rats in the control group and those in the groups with 10 and 20% low-molecular polyvinyl acetate were highly significant in all cases. The differences between the three groups with added low-molecular-weight polyvinyl acetate were not highly significant in any comparison but the further reductions produced when 20% rather than 5% polyvinyl acetate was used, were statistically significant at the 5% level. The rates of growth and increases in weight produced by all 4 rations in this experiment were indistinguishable from one another for both males and females.

In experiment 5 where the effects of 10% levels of low-molecular-weight polyvinyl acetate, high-molecular-weight polyvinyl acetate, natural chicle, and arochem were compared, each of these materials caused a highly significant reduction in the incidence of dental caries in desalivated rats. On the basis of the arithmetic averages, the low-molecular-weight polyvinyl acetate appeared to be most effective and the high-molecular-weight polyvinyl acetate least effective, with natural chicle and arochem occupying approximately the same intermediate position. The difference between the reductions produced by the two polyvinyl acetate resins was barely significant at the 5% level. The rates of growth and final increase in body weight for the rats in the control group, the group with 10% of the low-molecular polyvinyl acetate and with 10% of a high-molecular polyvinyl acetate were comparable. The rats which received 10% natural chicle or 10% arochem grew slower than the rats in the control group. Their total increase in body weight during the experimental period was about 50% that of the controls.

TABLE 4

Effect of varying amounts of a low molecular weight polyvinyl acetate on dental caries experience of white rats

GROUP NO.	RATION	NO. OF RATS	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS [1]		
			Av.	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]
1	100	9	11.5	0.3		22.4	1.7		74 +	10 +	
2	100 + 5% L-PVA [4]	9	8.9	0.8		15.6	1.8		44 +	7 +	
3	100 + 10% L-PVA	10	8.0	0.5		11.8	1.2		30 +	4 +	
4	100+ 20% L-PVA	10	7.1	0.8		9.5	1.3		23 +	4 +	
Statistical comparison of											
Group 1 vs. 2					3.1			2.7			2.5
Group 1 vs. 3					6.0			5.0			4.1
Group 1 vs. 4					5.2			6.1			4.7
Group 2 vs. 3					0.9			1.7			1.7
Group 2 vs. 4					1.6			2.7			2.6
Group 3 vs. 4					1.0			1.3			1.2

[1] See footnote 1, table 1.

[2] Standard error of mean.

[3] Critical ratio (see footnote 3, table 1).

[4] L-PVA = low molecular weight polyvinyl acetate.

TABLE 5

Effects of a low molecular weight polyvinyl acetate, a high molecular weight polyvinyl acetate, chicle and arochem on the dental caries experience of white rats

GROUP NO.	RATION	NO. OF RATS	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS [1]		
			Av.	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]	*Av.*	*SEM* [2]	*CR* [3]
1	700	12	11.3	0.8		23.4	1.3		75 +	6 +	
2	700 + 10% L-PVA [4]	10	5.6	0.9		7.4	1.5		21 +	5 +	
3	700 + 10% H-PVA [5]	6	8.6	1.3		15.9	3.5		39 +	8 +	
4	700 + 10% Chicle	8	7.1	1.0		11.3	2.4		31 +	8 +	
5	700 + 10% Arochem	10	6.7	0.6		10.0	1.6		29 +	5 +	
Statistical comparison of											
Group 1 vs. 2					4.8			8.0			6.9
Group 1 vs. 3					1.9			1.9			3.6
Group 1 vs. 4					3.4			5.2			4.4
Group 1 vs. 5					4.6			6.5			5.9

[1] See footnote 1, table 1.

[2] Standard error of mean.

[3] Critical ratio (see footnote 3, table 1).

[4] L-PVA = low molecular weight polyvinyl acetate.

[5] H-PVA = high molecular weight polyvinyl acetate.

DISCUSSION

The increase in mastication caused by the inclusion of agar or varying amounts of cellu flour in the purified caries-producing ration did not alter the dental caries incidence in either cotton rats or white rats. Even though as much as twofold increases in the weight of ration consumed were noted, the total amount of mastication caused by these amounts of finely divided rations probably was still fairly low compared to the mastication required by smaller amounts of a hard ration.

The striking reductions in dental caries incidence caused by the two forms of polyvinyl acetate, chicle and arochem cannot be explained at present. These 4 materials show wide differences in chemical composition and physical form. The two materials, low-molecular-weight polyvinyl acetate and chicle, which were the most effective in causing reductions in the dental caries incidence were of such physical form that they were malleable and tacky at body temperatures. In the oral cavity, these materials may have caused appreciable increases in the amount of mastication required by reason of these physical characteristics. However, it is noteworthy that the use of the polyvinyl acetate to form a ration requiring a great deal more chewing to prepare it for swallowing did not result in any lower incidence of dental caries than did the incorporation into the diet of the same amount of polyvinyl acetate in finely powdered form. Hence it would seem on the basis of existing evidence that the low-molecular-weight polyvinyl acetate was not effective because it caused an increase in the amount of mastication or else the smaller increase in mastication required by the tackiness of the small particles of the material at body temperatures was enough to produce the maximum reduction in dental caries incidence that was possible through this pathway.

The results obtained with finely divided arochem and the high-molecular-weight polyvinyl acetate further confuse the issue. Both of these materials at body temperatures maintained a gritty form and gave no evidence of becoming malle-

able or chewy under the circumstances prevailing in the oral cavity. Thus it would seem that neither of these materials would cause any further mastication than a comparable amount of agar or cellu flour. Yet despite this, both materials caused significant reductions in the dental caries incidence of desalivated white rats. In view of this observation, there appears to be ample reason to suspect that all 4 materials were effective in reducing the dental caries incidence through some other route than increased mastication. There are possibilities that these materials produced some type of cleansing action on the tooth surfaces or in the oral cavity as a whole or that they deposited a thin film on the tooth surfaces during the process of mastication which increased the caries resistance of the teeth. Neither of these possibilities has any definite experimental support. The former is postulated on the basis of the observation that the white rats in groups fed these materials had mouths which were distinctly cleaner than those in the control group. The latter is based on the suggestion of a difference in appearance of the teeth themselves as they had a somewhat glossier surface texture although no evidence of a microscopically definable surface coating.

On the basis of present knowledge, there is no reason to postulate that these data can be applied to human beings. Though the low-molecular-weight polyvinyl acetate and natural chicle are common components in chewing gum, the circumstances in these experiments whereby these inert materials were incorporated into the entire food allocation are believed to be considerably different from those resulting from the intermittent use of chewing gum.

SUMMARY

1. The inclusion of 10% agar or varying amounts of cellu flour from 10 to 100% in a caries-producing diet after tooth development was complete did not alter the dental caries incidence in intact cotton rats.

2. The inclusion of 10 to 50% cellu flour in the caries-producing diet after tooth development was complete did not alter the dental caries incidence in desalivated white rats.

3. The inclusion of varying amounts from 5 to 20% of a finely powdered low-molecular-weight polyvinyl acetate in a purified diet caused a much lower incidence of dental caries in desalivated white rats than in littermates fed only the caries-producing diet.

4. The inclusion of 20% polyvinyl acetate in the ration in such a way as to produce a firm cake which required a great deal of mastication did not reduce the dental caries incidence further than the inclusion of 20% polyvinyl acetate in finely powdered form.

5. The addition of 10% of either high-molecular-weight polyvinyl acetate, natural chicle or arochem in a caries-producing diet produced significantly less tooth decay than the diet alone. However, these materials were somewhat less effective than a similar amount of the low-molecular-weight polyvinyl acetate.

These data as a whole do not provide any concrete evidence that the amount of mastication required in the consumption of a purified caries-producing diet is a determining factor in the incidence of dental caries.

ACKNOWLEDGMENT

The author would like to express his indebtedness to Mr. Robert K. Phelan of Taconic Farms, Inc., Germantown, N. Y., for his generosity in obtaining the low-molecular and high-molecular polyvinyl acetates, the arochem and the chicle used in these experiments and for enthusiastic support and counselling.

LITERATURE CITED

HOPPERT, C. A., P. A. WEBBER AND T. L. CANNIFF 1932 The production of dental caries in rats fed an adequate diet. J. Dental Research, *12:* 161.

NEUMANN, H. D., W. LEFKOWITZ AND N. A. DI SALVO 1952 Relation of function to caries rate. Ibid., *31:* 200.

SHAW, J. H. 1947 Carious lesions in cotton rat molars. I. The role of mechanical factors studied by the extraction of antagonistic molars. Ibid., *26:* 47.

SHAW, J. H., B. S. SCHWEIGERT, J. M. MCINTIRE, C. A. ELVEHJEM AND P. H. PHILLIPS 1944 Dental caries in the cotton rat. I. Methods of study and preliminary nutritional experiments. J. Nutrition, *28:* 333.

THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION

XII. THE REQUIREMENT OF ESSENTIAL FATTY ACIDS FOR PREGNANCY AND LACTATION [1,2]

HARRY J. DEUEL, JR., CHARLOTTE ROE MARTIN AND ROSLYN B. ALFIN-SLATER

Department of Biochemistry and Nutrition, School of Medicine, University of Southern California, Los Angeles

(Received for publication April 9, 1954)

There is little information in the literature concerning the requirement of essential fatty acids for reproduction and for lactation. Evans and collaborators ('34a, '34b) reported that normal reproduction was impossible without essential fatty acids in the diet, and that the mothers were unable to suckle their young in the absence of these required nutrients. The addition of saturated fatty acids to the diet failed to alleviate these symptoms (Evans et al., '34b). Quackenbush and coworkers ('42) noted that the essential fatty acid requirement for reproduction is about twice that needed for the cure of dermal lesions produced by a fat-free diet. Linolenic acid was found to be ineffective as a supplement to restore normal reproduction, while there was no significant difference in physiologic activity between linoleic and arachidonic acids.

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Professor Anton J. Carlson of the University of Chicago, of Professor Arthur W. Thomas of Columbia University, and of Dr. H. W. Vahlteich, Dr. Chester M. Gooding, and Dr. Daniel Melnick of The Best Foods, Inc., during the course of these experiments.

We wish to thank Merck and Co., Hoffman-LaRoche, and Lederle Laboratories for the synthetic vitamins used in the basal diet.

[2] Contribution no. 356 of the Department of Biochemistry and Nutrition, University of Southern California.

Insofar as lactation is concerned, it has been generally recognized that fat stimulates this function. For example, Maynard and Rasmussen ('42) reported that a high-fat diet increased milk production in rats and that the mothers gained instead of losing weight. Loosli et al. ('44) demonstrated that the feeding of corn oil improved lactation performance in rats; this effect of fat was traced to the unsaturated fatty acids. It is therefore evident that the type of fat is an important factor in establishing the beneficial action of this foodstuff on lactation. However, in a series of studies by Deuel et al. ('44), no differences in pregnancy or lactation performance were noted in the case of rats fed diets containing 20% of fat, irrespective of whether the fat was corn, cottonseed, olive, peanut, or soybean oil, a vegetable margarine fat, or butter. It is apparent that, at this level of fat intake, the amount of essential fatty acids has not become a limiting factor.

In a later series of tests by Deuel and associates ('47) it was shown that, although some litters were cast by females maintained on fat-low diets, the number of rats per litter was reduced. Although some survived for 21 days, they were inferior in size and weight. In another study by this group (Scheer et al., '47b), it was observed that the total litter weight at 21 days increased progressively when the fat content of the diet was 0, 5, 10, 20, and 40% respectively. In rats which had previously been on a restricted diet, 100% fertility was achieved only when they were subsequently fed on diets containing 20 or 40% of fat (Deuel et al., '47; Scheer et al., '47a, '47b). Kummerow et al. ('52) reported that linoleic acid is the limiting factor in reproduction and lactation.

There would seem to be little doubt that dietary fat is required for successful pregnancy and lactation; the active principle appears to be the essential fatty acids which are present in fat. The object of the present study is to investigate the quantitative aspects of this requirement for the essential acids, both from the standpoint of pregnancy and from that

of lactation, as judged by survival and by the weight gain of the pups at three and 21 days after birth.

EXPERIMENTAL

Two series of tests were carried out, using female rats of the U.S.C. strain. These animals were placed on a fat-free diet when weaned at 21 days. After being maintained for 10 weeks on the fat-free diet, the rats were divided into the several groups listed in tables 1 and 2, and were supplemented from this time as recorded in the tables. They were bred with proven normal male rats from our stock colony which had

TABLE 1

The effect of different levels of cottonseed oil on pregnancy, survival, and growth of young from females on a fat-free diet (series I)

CATEGORY	GROUP NO.						
	1	2	3	4	5	6	7
Cottonseed oil fed daily, *mg*	0	10	40	100	200	400	1000
Females bred	23	24	25	25	25	25	25
Litters cast, *per cent*	83	96	92	100	96	92	100
Litter (at birth)							
Total number of rats	122	196	192	228	210	227	232
Average number per litter	6.4	8.5	8.3	9.1	8.8	9.9	9.3
Litter (at 3 days)							
Total number of rats	0	46	117	162	162	196	175
Total litters represented		9	18	22	21	23	21
Average weight per rat, *gm*	..	4.7	6.1	6.3	6.8	6.4	6.6
Litter (at 21 days)							
Total number of rats	0	9	88	124	114	122	126
Total litters represented		3	16	21	18	20	21
Average weight per rat, *gm*	..	24.6	26.9	29.4	33.4	32.0	29.4
Mortality (0–3 days)							
Total	122	150	75	66	48	31	57
Per cent	100	76	39	29	23	14	25
Mortality (3–21 days)[1]							
Total	..	32	9	11	18	22	14
Per cent	..	77	9	8	14	15	11

[1] Litters were cut to 7 at three days.

previously received a Purina chow diet. During the breeding period, the male rats received only the fat-free diet. The onset of pregnancy was determined from the time of appearance of sperm in the vagina, and from changes in the oestrus

TABLE 2

The effect of different levels of linoleate on pregnancy, survival, and growth of young from females on a fat-free diet (series II)

CATEGORY	GROUP NO.						
	11	12	13	14	15	16	17
Linoleate fed daily, *mg*	0	2.5	5.0	10	20	40	80
Females bred	16	15	16	16	16	16	16
Litters cast, *per cent*	87	93	87	100	100	94	100
Litter (at birth)							
Total number of rats	69	93	96	111	120	108	115
Average number per litter	4.9	6.6	6.8	6.9	7.5	7.2	7.2
Litter (at 3 days)							
Total number of rats	0	0	0	14	68	79	99
Total litters represented	..	..	..	3	11	15	14
Average weight per rat, *gm*	..	..	..	5.0	4.9	5.6	6.5
Litter (at 21 days)							
Total number of rats	0	0	0	..	44	58	77
Total litters represented	..	..	..	..	8	10	13
Average weight per rat, *gm*	..	..	..	..	22.9	25.7	29.2
Mortality (0–3 days)							
Total	69	93	96	97	52	29	16
Per cent	100	100	100	87	43	27	14
Mortality (3–21 days)[1]							
Total	..	..	..	13	21	11	12
Per cent	..	..	..	100	32	16	13

[1] Litters were cut to 7 at three days.

cycle. The males were removed as soon as a successful mating had occurred, and the female rats were then kept in large individual cages. The composition of the basal diet corresponded to that employed by Greenberg et al. ('50).

The two series of experiments were carried out in a similar manner except that, in series I, the supplement used was

cottonseed salad oil while, in series II, methyl linoleate (Hormel) was employed as the source of the essential fatty acids.

RESULTS AND DISCUSSION

The results of series I are recorded in table 1, while those of series II are summarized in table 2.

Tables 1 and 2 offer proof that dietary fats are not required by the female for conception, for the continuance of the development of the fetuses, and for parturition. Thus, in series I (group 1) 83% of the pregnant females cast a litter while, in series II (group 11) the figure was 87.5%. However, many of the pups were born dead, and none of those which were alive at birth survived as long as three days.

Although dietary fats or essential fatty acids are not required for the completion of intrauterine growth, they are required for the survival of the pups for the first critical three-day period. Up to a certain level, survival is related quantitatively to the intake of cottonseed oil or of essential fatty acids by the mother. Thus, in the case of cottonseed oil (table 1), although a partial survival of the young was noted in the groups receiving 10 and 40 mg of cottonseed oil daily, at a level of 200 mg of supplement daily, the best average weight was noted at three days, whereas the greatest proportion of survival was found when the mothers were supplemented with 400 mg of cottonseed oil daily. In series II, no survival of the young was obtained until a daily dosage of 10 mg of methyl linoleate was given. The optimum survival in series II, which compared favorably with that obtained in series I, was noted when the daily dosage level of 80 mg of linoleate was given.

The requirement of essential fatty acids for lactation is reflected in the percentage of survival, in the average body weight, and in the total number of animals in the litters at 21 days after birth. In series I, the optimum results insofar as lactation is concerned were achieved when the dosage level of cottonseed oil was 100 to 200 mg daily (50 to 100 mg linoleate, assuming that cottonseed oil is approximately 50%

linoleic acid). When these levels were exceeded (400 mg or 1000 mg daily), neither the average weight per rat at 21 days nor the total number of rats or litters reaching 21 days was significantly altered. In the tests in which methyl linoleate was employed, these results were confirmed; group 17, in which the individual rats received 80 mg of methyl linoleate daily, yielded results comparable to the optimum recorded for cottonseed oil.

This requirement for pregnancy and lactation is at least twice that necessary for optimum growth and clearing of the dermal deficiency symptoms in the female rat (Quackenbush et al., '42).

SUMMARY

In studies designed to determine whether or not fats are necessary for successful pregnancy and lactation and, if so, in what amounts, the following conclusions were reached:

1. Dietary fat is not required by the female rat for conception or for the completion of pregnancy when the diets are otherwise complete.

2. However, fat is required in the diet of the mother to insure the survival of the pups after birth. Although, in some cases, daily doses of cottonseed oil as low as 10 mg insured the survival of the pups for three days, as much as 200 mg of cottonseed oil were required per day for optimum results.

3. The optimum daily requirement of cottonseed oil for lactation, as determined by the weight of the young at 21 days, was found to be between 100 mg and 200 mg.

4. The constituents of the fat responsible for the survival of the young and for satisfactory lactation appear to be the essential fatty acids.

LITERATURE CITED

DEUEL, H. J., JR., E. MOVITT AND L. F. HALLMAN 1944 Studies on the comparative nutritive value of fats. IV. The negative effect of different fats on fertility and lactation. J. Nutrition, *27:* 509.

DEUEL, H. J., JR., E. MESERVE, E. STRAUB, C. HENDRICK AND B. T. SCHEER 1947 The effect of fat level of the diet on general nutrition. I. Growth, reproduction, and physical capacity of rats receiving diets containing various levels of cottonseed oil or margarine fat ad libitum. Ibid., *33:* 569.

EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY 1934a Vital need of the body for certain unsaturated fatty acids. IV. Reproduction and lactation upon fat-free diets. J. Biol. Chem., *106:* 431.

———— 1934b Vital need of the body for certain unsaturated fatty acids. V. Reproduction and lactation upon diets containing saturated fatty acids as their sole source of energy. Ibid., *106:* 441.

GREENBERG, S. M., C. E. CALBERT, E. E. SAVAGE AND H. J. DEUEL, JR. 1950 The effect of fat level of the diet on general nutrition. VI. The interrelation of linoleate and linolenate in supplying the essential fatty acid requirement in the rat. J. Nutrition, *41:* 473.

KUMMEROW, F. A., H. P. PAN AND H. HICKMAN 1952 The effect of dietary fat on the reproductive performance and the mixed fatty acid composition of fat-deficient rats. Ibid., *46:* 489.

LOOSLI, J. K., J. F. LINGENFELTER, J. W. THOMAS AND L. A. MAYNARD 1944 The role of dietary fat and linoleic acid in the lactation of the rat. Ibid., *28:* 81.

MAYNARD, L. A., AND E. RASMUSSEN 1942 The influence of dietary fat on lactation performance in rats. Ibid., *23:* 385.

QUACKENBUSH, F. W., F. A. KUMMEROW AND H. STEENBOCK 1942 The effectiveness of linoleic, arachidonic, and linolenic acids in reproduction and lactation. Ibid., *24:* 213.

SCHEER, B. T., J. F. CODIE AND H. J. DEUEL, JR. 1947a The effect of fat level on the diet on general nutrition. III. Weight loss, mortality, and recovery in young adult rats maintained on restricted calories. Ibid., *33:* 641.

SCHEER, B. T., D. F. SOULE, M. FIELDS AND H. J. DEUEL, JR. 1947b The effect of fat level on the diet on general nutrition. II. Growth, mortality, and recovery in weanling rats maintained on restricted calories. Ibid., *33:* 583.

THE EFFECT OF FAT LEVEL OF THE DIET ON GENERAL NUTRITION

XIII. THE EFFECT OF INCREASING DOSAGES OF X-IRRADIATION ON THE PROTECTIVE ACTION OF FAT ON RADIATION INJURY [1,2]

AMBER L. S. CHENG, ROSLYN B. ALFIN-SLATER AND HARRY J. DEUEL, JR.

Department of Biochemistry and Nutrition, School of Medicine, University of Southern California, Los Angeles

Although diet plays an insignificant role in the survival of rats subjected to single high doses of x-irradiation, the nature of the food ingested may alter the susceptibility of rats when they are exposed to repeated sublethal doses of x-irradiation. Cheng et al. ('52) found that, under the latter conditions, rats receiving a diet containing as small an amount as 2% of cottonseed oil (Wesson oil) exhibited a resistance to x-irradiation which was statistically greater than that shown by animals on a fat-free regimen. In a later study in this laboratory (Deuel et al., '53), it was found that the survival time after x-ray injury was increased when ethyl linoleate in amounts as small as 10 mg per day were fed to rats on fat-free diets. More recently, it has been observed that the survival time of male rats, as judged by the intervals at which an LD_{25}, an LD_{50}, or an LD_{75} were reached, or by the average length of survival, was progressively improved when ethyl linoleate was given in doses of 10, 50, or 100 mg daily (Cheng et al., '54).

[1] This work was carried out under a contract between the University of Southern California and the Atomic Energy Commission (no. AT (11-1)-113).

[2] Contribution number 366 of the Department of Biochemistry and Nutrition, University of Southern California.

In the current tests, the authors wished first to determine at what levels of x-irradiation the beneficial effects of dietary fat on survival could be noted. A second objective was to develop a procedure for studying x-irradiation injury which would involve a minimum number of exposures to x-rays and, at the same time, would permit the therapeutic effect of fat to be exhibited. The employment of this technic would simplify the experiments and render possible a more exact evaluation of the time relations of exposure to the application of therapy. In the third place, the present tests attempted to determine how long a preliminary feeding period, using the experimental diets, is required after weaning for the protective effect of fat to be demonstrable. Finally, an answer was sought to the question as to whether or not commercial casein can replace vitamin-test casein in the fat-free diet without loss of the protective effect of fat.

EXPERIMENTAL PROCEDURE AND RESULTS

A total of 33 series of tests were carried out on male rats; each series was conducted as a unit, and consisted of two or three groups of test animals, which received the fat-low diets A and/or B and the diet containing 15% cottonseed oil (C). The detailed composition of the diets is given in table 1.

The rats were placed on the diets under investigation immediately after weaning at 21 days of age. The mothers had previously been receiving the Sherman B diet. The young rats were prefed on the several diets for three to 8 weeks, after which they were exposed to x-rays at various levels of dosage. Groups 1 to 5 received 300 *r* per week over a period of one to 5 weeks, respectively, giving a total of 300 *r*, 600 *r*, 900 *r*, 1200 *r*, or 1500 *r* in the several groups. Groups 11 to 13 received 400 *r*, 800 *r*, or 1200 *r* during one to three weeks; finally groups 21 and 22 were irradiated with 500 *r* or 1000 *r* over one or two weeks. The irradiation was carried out in a manner similar to that employed in our earlier tests (Cheng et al., '52). The results on these rats are recorded in condensed form in table 2. In another series of tests, which

are recorded in table 3 in more detail than the earlier tests, the rats all received 800 *r*, either in two doses of 400 *r* each, spaced at intervals of 4 or 8 days, or in 4 doses of 200 *r* each, given on 4 successive days. The rats used were from the U.S.C. strain. These data are summarized in tables 2 and 3. The rats were kept under observation for 84 days after the last x-irradiation had been given. Animals still living at this time were considered to have a survival time of 84 days.

TABLE 1

Composition of diets

COMPONENT [1]	DIET A	DIET B	DIET C
	%	%	%
Casein, vitamin-test [2]	20.00	....	....
Casein, commercial	....	20.00	24.00
Cottonseed oil [3]	....	....	15.00
Sucrose	70.68	70.68	51.68
Cellulose [4]	4.00	4.00	4.00
Salt mixture [5]	4.00	4.00	4.00
Water-soluble vitamin mixture [6]	0.32	0.32	0.32
Fat-soluble vitamin mixture [7]	1.00	1.00	1.00

[1] The folic acid was furnished through the kindness of Lederle Laboratories, and the biotin by the Hoffmann-LaRoche Co. The remaining B vitamins were given us through the courtesy of Merck and Co.

[2] General Biochemicals Inc., Chagrin Falls, Ohio.

[3] Refined, winterized cottonseed oil.

[4] "Solka-floc" obtained from the Mefford Co., Los Angeles.

[5] Mixture of L. G. Wesson (Science, *75:* 339, 1932). Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

[6] The water-soluble mixture had the following composition in per cent: choline chloride, 67.51; thiamine hydrochloride, 2.43; riboflavin, 0.92; pyridoxine hydrochloride, 0.91; calcium pantothenate, 2.02; niacin, 2.02; *i*-inositol, 16.85; folic acid, 0.34; biotin, 0.08; vitamin B_{12}, 0.01; ascorbic acid, 6.74; and menadione, 0.17.

[7] The fat-soluble vitamin mixture was composed of the following vitamins made up to 100 ml with propylene glycol: Napsol, 8.72 gm (vitamin A: 100,000 U.S.P. units per gram and vitamin D: 20,000 I.U. per gram), obtained from the National Oil Products Co., Harrison, N. J.; and mixed tocopherols, 25.7 gm (Distillation Products, Inc., Rochester, N. Y.).

TABLE 2

The survival time of male rats receiving x-irradiation weekly at levels of 300 r up to 5 times, of 400 r up to 3 times, and of 500 r up to 2 times

GROUP NO.	DIET	NUMBER OF EXPOSURES	PERIOD ON DIET PRIOR TO X-IRRADIATION					
			3 weeks		5 weeks		8 weeks	
			No. of rats	Average survival	No. of rats	Average survival	No. of rats	Average survival
300 *r* once weekly								
				days		*days*		*days*
1	A	1	..	...	..	...	10	59.8
	B		..	...	14	79.7	10	72.2
	C		..	...	14	84	10	84.0
2	A	2	16	49.1	..	...	12	59.4
	B		14	57.2	15	63.6	11	68.0
	C		14	59.0	14	76.8	10	66.3
3	A	3	18	45.4	..	...	13	15.2
	B		18	24.6	14	48.1	12	46.7
	C		14	34.7	15	63.5	12	53.2
4	A	4	19	22.8	..	...	12	16.3
	B		19	13.6	14	48.1	12	22.4
	C		13	29.9	15	63.5	10	54.0
5	A	5	6	4.8	..	..	11	6.0
	B		6	8.2	13	11.5	11	17.4
	C		13	20.2	14	16.3	11	31.5
400 *r* once weekly								
11	A	1	14	75.3	..	..	10	53.9
	B		14	66.7	14	84.0	10	60.0
	C		14	79.6	14	84.0	10	84.0
12	A	2	17	29.9	..	...	12	35.5
	B		17	40.6	16	49.5	12	49.1
	C		14	49.3	16	56.4	10	84.0
13	A	3	11	15.1	..	...	15	12.8
	B		12	17.4	14	47.1	12	21.8
	C		14	20.1	14	41.6	12	32.1
500 *r* once weekly								
21	A	1	14	54.4	..	...	..	...
	B		14	65.2	14	68.3	..	...
	C		13	81.9	14	78.9	..	...
22	A	2	14	22.3	..	...	..	...
	B		14	26.3	19	38.4	..	...
	C		14	47.9	17	34.1	..	...

TABLE 3

The LD_{25}, LD_{50}, and LD_{75} and the average survival time of male rats receiving fat-low diets containing vitamin-test casein (A), commercial casein (B), or commercial casein with 15% cottonseed oil (C), and which received 800 r

GROUP NO.	PREVIOUS PERIOD ON DIET	DIETS FED	RADIATION — 800 r		NO. OF RATS	LD (DAYS)			AVERAGE SURVIVAL TIME
			Each dosage	Elapsed period		25%	50%	75%	
	weeks		*r*	*days*					*days*
31	3	B	400	8	16	10	33	>	49.5
		C			16	12	>	>	56.4
32	3	B	400	4	15	12	36	64	42.6
		C			15	14	>	>	64.9
33	3	B	200	4	15	17	>	>	59.3
		C			15	18	>	>	63.6
34	3.5	B	400	4	15	10	13	22	26.5
		C			15	21	>	>	57.8
35	5	A	400	8	12	9	21	58	35.5
		B			12	9	49	>	49.1
		C			10	>	>	>	84.0
36	8	A	400	4	17	9	13	37	29.9
		B			17	11	16	>	40.6
		C			14	11	42	>	49.3

DISCUSSION

The superior survival of rats receiving the fat diet was noted under practically all the experimental conditions listed under table 2. The only exceptions were group 2 (8-week group), group 3 (3-week group), group 13 (5-week group) and group 22 (5-week group). In these cases, the results were erratic. In all other 23 series of tests listed in table 2, as well as in all 6 series of experiments given in table 3, the fat-fed rats survived longer than did those on the low-fat diets (A or B).

Although commercial casein contains only minimal quantities of essential fatty acids, the survival time was, in practically every case, considerably extended in those groups which received diet B (commercial casein) as contrasted with those receiving diet A (vitamin-test casein). However, the survival time of the rats receiving diet C (commercial casein plus 15% cottonseed oil) was generally considerably greater than that of the animals on the commercial casein diet devoid of fat.

The extended survival of the rats on the high-fat diet over that of the animals on the fat-free regimens was apparent irrespective of whether they had been receiving the diets for three, 5, or 8 weeks previous to exposure to x-irradiation. Moreover, rats which had received the diets for only three weeks before x-irradiation (and hence were only 6 weeks old) exhibited the protective effect of fat as well as did adult animals.

In the tests listed in table 3, in which 800 *r* was the total dosage of x-irradiation, groups 32, 34, and 36, in which the x-irradiations were given 4 days apart, yielded results which were consistently as satisfactory in demonstrating the protective effect of fat against x-irradiation as were any other series of tests we have conducted. It may be desirable to employ this procedure in the future because of its simplicity, and because of the shorter interval over which the x-irradiation is administered.

SUMMARY

1. The present tests confirmed the earlier experiments in demonstrating the protective effects of dietary fat against x-irradiation in a variety of doses and given at several time intervals.

2. The rats on the test diets for only three weeks after weaning exhibited the protective effects of fat against x-irradiation as satisfactorily as did rats which had received the test diets for as long as 8 weeks after weaning.

3. The survival time of rats receiving a commercial casein diet which was otherwise fat-free was considerably longer than that of rats on a fat-free diet containing vitamin-test casein. However, the period of survival was prolonged by an additional interval when cottonseed oil was also added to the diet.

4. A simple procedure for studying the effect of diet on protection from x-irradiation involves prefeeding the weanling rats for three weeks on the test diets, followed by exposure of the animals to two 400 *r* doses of x-rays at intervals of 4 days.

LITERATURE CITED

CHENG, A. L. S., G. D. KRYDER, L. BERGQUIST AND H. J. DEUEL, JR. 1952 The effect of fat level of the diet on general nutrition. IX. The relationship of radiation injury in the rat to the fat content of the diet. J. Nutrition, *48:* 161.

CHENG, A. L. S., M. RYAN, R. B. ALFIN-SLATER AND H. J. DEUEL, JR. 1954 The effect of fat level of the diet on general nutrition. XI. The protective effect of varying levels of ethyl linoleate against multiple sublethal doses of x-irradiation in the rat. J. Nutrition, *52:* 637.

DEUEL, H. J. JR., A. L. S. CHENG, G. D. KRYDER AND M. E. BINGEMANN 1953 The protective effect against x-irradiation of methyl linoleate in the rat. Science, *117:* 254.

ORGAN WEIGHTS AND OBESITY IN MICE[1]

SAMUEL H. WAXLER AND MARILYN ENGER
Department of Pharmacology and Therapeutics, Standford University School of Medicine, San Francisco, California

(Received for publication November 9, 1953)

The experimental production of obesity in mice by a single injection of gold thioglucose was described by Brecher and Waxler ('49). Measurements of food intake showed that the food consumption of those animals which became obese exceeded that of the controls (Waxler and Brecher, '50). This obesity can be readily regulated as gold-treated animals, given a diet identical in caloric value with that of their controls, maintain the same weight levels. Autopsy findings and chemical analyses of total body lipids, proteins, water and ash indicated that these weight gains were primarily due to an increase in adipose tissue. There was also, however, a definite increase in body protein in the obese mice.

An increase in size and weight of several organs in the obese animals was evident on gross observation. It was therefore thought desirable to determine the degree of change of the individual organs and to ascertain whether the lipids could wholly account for this change or whether there was some enlargement of the organ itself. Similarly the experiment was extended to ascertain the effect of dieting on the organs of previously obese animals.

EFFECT OF EXPERIMENTALLY INDUCED OBESITY ON ORGAN SIZE

Method. A pure line strain of C_3H male mice with known birth dates were used in this experiment. At 5 months of age,

[1] This investigation was supported by a grant-in-aid from the National Cancer Institute, National Institutes of Health, Public Health Service, Bethesda, Maryland.

the animals were divided into two groups, and those in one group were given a toxic dose of gold thioglucose [2] intraperitoneally. Survivors of the LD_{50} dose of gold were then ear-tagged, weighed and set aside to be observed at intervals in comparison with the control group (Waxler et al., '53). The mice were fasted a day prior to injection to enhance their tolerance to the drug (Waxler and Brecher, unpublished data). The animals were housed in groups of 8 per cage and were allowed free access to Purina Laboratory chow and water throughout the experiment. At 9 months of age the animals were weighed and autopsied. The following organs were removed and weighed rapidly on a torsion balance: brain, heart, lung, liver, spleen, kidney, thymus, testes, femurs and adrenals. After obtaining the wet weights, the organs were dried for 24 hours at 70°C. and reweighed. The organs were subsequently defatted with a petroleum ether-ethyl ether mixture, dried and then weighed for the third time.

Results. Table 1 gives the data on 12 obese and 24 control mice. The obese mice weighed from 46 to 55 gm compared with the controls of 27 to 38 gm. The wet, dry and defatted weights of the 10 organs are listed.

THE EFFECT OF REDUCTION IN BODY WEIGHT ON ORGAN SIZE

In the first phase of this experiment we showed that there was a definite increase in the weight of various organs with an increase in total body weight. The weights of the dried and defatted organs indicated that this increase was due to more than just adipose tissue, and that some enlargement had occurred to account for the heavier organs.

Method. Male C_3H mice of similar ages were used in this experiment. At 6 months of age one group of mice was given a toxic dose of gold thioglucose and allowed to become obese. Four months later 14 obese animals with an average weight of 50 gm were paired with 14 controls averaging 30.8 gm. With-

[2] The gold thioglucose was generously supplied by Schering Corp., Bloomfield, New Jersey.

TABLE 1 •

Organ weights of control and obese C_3H male mice

		WET WEIGHT		DRY WEIGHT		DEFATTED WEIGHT	
		mg	*Per cent change*	*mg*	*Per cent change*	*mg*	*Per cent change*
Liver	Control	2115.9 ± 210.5 [1]		634.0 ± 49.9 [1]		596.6 ± 46.4 [1]	
	Obese	3077.9 ± 457.4	45	1059.7 ± 230.1	67	863.3 ± 168.2	43
Spleen	Control	145.5 ± 29.3		32.2 ± 5.6		31.7 ± 6.6	
	Obese	221.2 ± 20.4	52	51.6 ± 4.8	60	50.5 ± 15.1	59
Thymus	Control	16.0 ± 4.2		3.0 ± 0.8		2.5 ± 0.7	
	Obese	22.6 ± 5.3	41	6.4 ± 3.4	113	3.3 ± 0.8	32
Adrenals	Control	4.3 ± 0.6		1.0 ± 0.2		0.7 ± 0.3	
	Obese	5.8 ± 1.5	35	1.7 ± 0.8	70	1.1 ± 0.3	57
Heart	Control	139.8 ± 15.4		32.4 ± 2.7		31.6 ± 2.8	
	Obese	152.3 ± 17.1	9	38.3 ± 4.3	18	36.5 ± 4.3	15
Lung	Control	175.2 ± 16.6		35.3 ± 4.2		32.9 ± 3.7	
	Obese	181.4 ± 30.0	8	45.0 ± 1.7	27	36.3 ± 5.3	10
Femurs	Control	123.5 ± 14.5		81.6 ± 5.4		81.0 ± 5.3	
	Obese	134.3 ± 23.6	8	89.2 ± 3.5	9	85.7 ± 10.3	5
Kidney	Control	632.7 ± 84.9		129.5 ± 12.7		127.2 ± 13.3	
	Obese	616.7 ± 70.4	0	137.1 ± 16.4	5	130.5 ± 16.0	2
Testes	Control	178.2 ± 13.4		27.5 ± 2.8		27.0 ± 2.4	
	Obese	170.9 ± 15.2	0	29.2 ± 4.2	7	27.2 ± 3.1	0.7
Brain	Control	455.6 ± 14.2		101.0 ± 4.8		71.8 ± 5.1	
	Obese	405.8 ± 13.4	0	102.0 ± 8.9	0.9	65.8 ± 4.4	0

[1] Standard deviation.

holding of food from these obese mice brought them down to the weights of their controls. Subsequently, both groups were maintained for the next three months at similar weights by paired-feeding at which time the animals were autopsied and

TABLE 2

Organ weights of control and previously obese mice

		WET WEIGHT	DRY WEIGHT	DEFATTED WEIGHT
		mg	*mg*	*mg*
Liver	Control	1867.7 ± 167.4 [1]	573.4 ± 37.7 [1]	527.2 ± 29.8 [1]
	Reduced	1674.7 ± 170.9	538.7 ± 49.2	461.1 ± 45.3
Spleen	Control	116.0 ± 33.3	25.7 ± 7.2	24.5 ± 7.1
	Reduced	147.4 ± 40.4	32.5 ± 9.7	31.1 ± 9.3
Thymus	Control	12.6 ± 4.3	2.1 ± 0.6	1.5 ± 0.5
	Reduced	12.7 ± 3.9	2.5 ± 0.96	1.8 ± 0.8
Adrenals	Control	5.1 ± 0.6	0.9 ± 0.4	0.5 ± 0.1
	Reduced	5.0 ± 0.7	0.9 ± 0.4	0.5 ± 0.1
Heart	Control	157.2 ± 15.2	34.5 ± 3.4	32.8 ± 2.4
	Reduced	148.1 ± 11.0	31.8 ± 1.4	30.4 ± 2.3
Lung	Control	178.2 ± 18.5	33.9 ± 2.7	30.7 ± 1.5
	Reduced	189.5 ± 22.2	39.9 ± 1.2	33.1 ± 4.2
Femur	Control	139.0 ± 12.4	84.0 ± 5.6	80.8 ± 5.4
	Reduced	124.8 ± 18.3	75.3 ± 6.2	72.4 ± 6.0
Kidney	Control	704.7 ± 25.5	146.7 ± 15.2	140.2 ± 14.5
	Reduced	570.2 ± 24.0	116.7 ± 18.5	110.8 ± 15.5
Testes	Control	176.2 ± 12.2	26.5 ± 2.5	25.3 ± 2.4
	Reduced	170.2 ± 13.2	26.6 ± 2.0	24.8 ± 1.8
Brain	Control	429.7 ± 11.5	98.5 ± 9.6	66.1 ± 6.0
	Reduced	399.2 ± 22.0	90.1 ± 7.0	62.4 ± 4.9

[1] Standard deviation.

organs removed for wet, dry and defatted weights. At this time the previously obese mice averaged 34.5 gm and the controls, 33.7 gm.

Results. Table 2 lists the various organs and weights in the two groups of mice, the controls and the previously obese mice. With this reduction in body weight in the injected mice

from an average of 50 gm to that of 34.5 gm, there is a concomitant decrease in organ weights. When comparison is made between the obese animals (table 1) and the reduced animals of table 2, one will note the marked decrease which has occurred during the period of body weight reduction.

DISCUSSION

Gross examination showed that the livers were markedly enlarged in the obese animals. The livers of these animals were almost 50% heavier than those of the controls. This increased weight was also evident in the dry and defatted weights. The spleen and the thymus likewise showed an enlargement which was obvious and was reflected in the percentage increase in the weights of the wet, dry and defatted organs. The increase in weight of the lungs and hearts in the obese animals was of a lesser order. Of interest was the increase of the femurs of the obese animals over that of the controls. The weights of the adrenals showed a definite change, but with such small organs it was difficult to ascertain whether or not these were of a valid nature. The three organs which did not show any degree of change were the kidneys, testes and brain. In the obese animals the brain weighed less than that of the control animals.

The organ weights of reduced, previously obese, mice are in all cases much lower than those of the obese mice fed ad libitum. Generally these organ weights were quite close to those of their controls, and in some instances the control organs were heavier than those found in the reduced mice.

SUMMARY

1. The organs of obese animals tend to be heavier than those of control mice, and this increase in weight is apparent in the wet, dry and defatted state. This increase in organ weight of obese animals cannot be accounted for by adipose tissue alone.

2. Mice which were made experimentally obese were subsequently reduced to the weight of controls. These animals

were then maintained by pair-feeding at the weight level of their controls. The organ weights of these reduced animals were of the same order of magnitude as those of the controls.

LITERATURE CITED

BRECHER, G., AND S. H. WAXLER 1949 Obesity in albino mice due to single injections of gold thioglucose. Proc. Soc. Exp. Biol. and Med., *70:* 498.

WAXLER, S. H., AND G. BRECHER 1950 Obesity and food requirement in albino mice following administration of gold thioglucose. Am. J. Physiol., *162:* 428.

———— Unpublished data.

WAXLER, S. H., P. TABAR AND L. R. MELCHER 1953 Obesity and the time of appearance of spontaneous mammary carcinoma in C_3H mice. Cancer Research, *13:* 276.

THE EFFECT OF DIETARY MOLYBDENUM UPON GROWTH, HEMOGLOBIN, REPRODUCTION AND LACTATION OF RATS[1]

MAX A. JETER[2] AND GEORGE K. DAVIS
Department of Animal Husbandry and Nutrition, Florida Agricultural Experiment Station, Gainesville

(Received for publication December 10, 1953)

The toxic effect of small quantities of molybdenum in feed for cattle has been described by Ferguson et al. ('38) and by Britton and Goss ('46). In subsequent studies, others (Fairhall et al., '45; Neilands et al., '48; Singer, '49; Gray and Ellis, '50) have shown the toxicity of molybdenum for laboratory animals. The therapeutic effect of copper upon molybdenosis has been demonstrated in cattle, sheep and laboratory animals. (Ferguson et al., '40, '43; Neilands et al., '48; Singer, '49; Gray and Ellis, '50; Comar et al., '49.) Although anemia is a common symptom of excess molybdenum in cattle and sheep, varying results have been reported relative to the effect of dietary molybdenum upon the hemoglobin in rats. The extent to which molybdenum toxicity may affect reproduction has not been established. Since the completion of this study, the adverse effect of molybdenum upon spermatogenesis of young dairy bulls has been reported by Thomas and Moss('51).

The present investigation was conducted to extend observations of the effect of molybdenum upon growth and hemoglobin in rats and to study the effect upon reproduction.

[1] Florida Agricultural Experiment Station Journal Series, no. 236.

[2] Present address: Indiana Farm Bureau Cooperative Association, Indianapolis, Indiana.

EXPERIMENTAL

The Long-Evans strain of rats was used throughout the study. Animals were housed in wire cages in a room maintained at 80°F. The basal diet was a mineralized milk-sucrose mixture of the following composition: Whole milk powder, 3418 gm; sucrose, 3381 gm; ferrous sulfate, 0.136 gm; manganous sulfate, 0.06 gm; thiamine hydrochloride, 0.023 gm. The copper content by analysis was 1.78 p.p.m. and molybdenum was less than 1 p.p.m. In order to vary the copper and molybdenum content of the diets, copper was added as $CuSO_4 \cdot 5H_2O$ and molybdenum was added as $NaMoO_4 \cdot 2H_2O$ (table 1). At the time of breeding, the iron and manganese content of the diets was increased to a level optimum for reproduction. Diets were fed ad libitum and food consumption determined periodically. Pyrex distilled water was supplied.

Growth measurements were made by weekly weights from weaning to 11 weeks of age. Hemoglobin was determined at 4, 7, 10 and 13 weeks of age using the acid hematin method. Reproductive studies were initiated after completion of the growth experiments. Fertility studies involved mating of the experimental males and females, and in cases of apparent infertility, the rats were bred to proved fertile rats on a stock diet. Histological sections were made of the testes from infertile males. The number of litters produced, size and weight of litter, loss of weight of lactating females and weight of litters at weaning were criteria of gestation and lactation performance. New-born litters were reduced to 6 when they exceeded that number. In order to determine the effect of molybdenum upon estrus cycle, 4 females were fed a ration containing 700 p.p.m. of molybdenum and vaginal smears were made over a 5-week period by the technique of Long and Evans ('22).

RESULTS AND DISCUSSION

Growth. A summary of the growth data is presented in table 1. Eighty parts per million of molybdenum in the diet did not significantly retard the gain in weight when 20 p.p.m. of

copper was included in the ration. When the copper content of the diet was decreased to 5 p.p.m., the growth of male rats was retarded at all levels of molybdenum. The growth of females was not retarded on the ration of 20 p.p.m. of molybdenum, but was decreased at 80 p.p.m. of molybdenum and 5 p.p.m. of copper. The difference in the growth rate of the male and female rats receiving molybdenum in the ration indicates a sex difference in tolerance to molybdenum.

TABLE 1

Growth and hemoglobin concentration of rats receiving varying levels of molybdenum

RATION		NUMBER OF RATS	SEX	AVERAGE GAIN AT 11 WEEKS
Mo	Cu			
p.p.m.	*p.p.m.*			*gm*
< 1	20	4	♂	163
		4	♀	
80	20	4	♂	135
		4	♀	
< 1	5	8	♂	176
		8	♀	128
20	5	4	♂	140 [1]
		4	♀	117
80	5	8	♂	147 [1]
		8	♀	105 [1]
140	5	4	♂	80 [2]
		4	♀	85 [1]

[1] Growth retardation significant at 5% level.
[2] Growth retardation significant at 1% level.

Hemoglobin. Hemoglobin concentration which was determined at intervals during the experimental period was not affected by the presence of molybdenum in the diet. The values obtained were within the range of that of normal rats on a stock ration. The absence of anemia is in contrast to the anemia in ruminants caused by dietary molybdenum.

Other symptoms. Achromotrichia followed by varying degrees of alopecia was present in the groups receiving 80 and

140 p.p.m. of molybdenum, but this condition was not observed in all of the rats in each group. Depigmentation was occasionally observed in the rats receiving 20 p.p.m. of molybdenum.

Fertility and gestation. The small number of litters from the females receiving 80 and 140 p.p.m. of molybdenum (table 2) was the result of male infertility. There was no evidence that fertility in females was affected. Of 8 male rats which received 80 and 140 p.p.m. of molybdenum from weaning,

TABLE 2

Reproduction and growth of nursing young of females receiving varying levels of molybdenum

RATION		NO. OF MATINGS	NO. OF LITTERS	AVERAGE BIRTH WT. OF YOUNG [1]	NO. DEAD AT BIRTH	NO. DYING BEFORE 21 DAYS	AVERAGE WT. OF YOUNG AT 21 DAYS
Mo	Cu						
p.p.m.	*p.p.m.*			*gm*			*gm*
< 1	20	4	4	4.83	..	..	25.1
80	20	4	4	5.01	..	..	24.2
< 1	5	8	8	5.21	0	7	32.7
20	5	8	8	4.77	0	13	29.3
80	5	4 [2] 4 [3]	1 4	4.72	1	6	28.3
140	5	4 [2] 4 [3]	1 4	5.07	11	9	23.8

[1] Taken from weight of entire litter.

[2] Males received molybdenum from weaning.

[3] Males had not received molybdenum from weaning.

only two proved to be fertile. When the apparently infertile males were bred to proved stock females, they did not sire litters. Histological examination of the testes from these males showed varying degrees of seminiferous tubule degeneration, while testes from rats which had received less than 1 p.p.m. of molybdenum were normal.

The data for the gestation studies (table 2) do not indicate any detrimental effect of molybdenum upon gestation. All females were heavier on the day after parturition than at the time of breeding, but there were no differences in gains which

could be attributed to molybdenum content of the diet. Mature virgin female rats showed irregular estrus cycles after receiving the rations containing 700 p.p.m. molybdenum for 10 days. Similar female rats on the same ration without molybdenum had normal estrus.

Lactation. The measurement of lactation performance indicates a reduced milk yield from rats consuming molybdenum (table 3). When female rats were raised from weaning on diets containing molybdenum or were placed on these diets

TABLE 3

Lactation performance of female rats receiving dietary molybdenum

	MOLYBDENUM IN DIET (P.P.M.)			
	< 1	20	80	140
Number of litters nursed	5	5	3	4
Percentage loss of weight in mothers [1]	8.40	7.34	16.21	19.83
Loss of weight per young nursed (gm)	3.57	2.71	7.28	7.30
Weaning weight (gm)				
Males	33.9	27.8	28.1	24.7
Females	32.5	27.8	28.4	22.9

[1] Size of litters was 5 or 6 rats.

at breeding, the loss of weight during lactation, and the weaning weights of the litters indicated poor lactation. A determination of the food consumption during the second week of lactation indicated normal food intake, and demonstrated that a low food intake could not account for the poor lactation. An increased secretion of molybdenum into the milk remains a possibility and may account for low weaning weights of the litters.

SUMMARY

Molybdenum as sodium molybdate was fed to weanling rats and to mature rats in order to study the effect of molybdenum upon growth and reproduction. The gain in weight of both

sexes was retarded, but males were affected to a greater extent than females. Seventy-five percent of the males which were fed from weaning on a diet containing 80 and 140 p.p.m. of molybdenum were sterile. Limited histological examination showed some testicular degeneration. Fertility and gestation in females were not affected by the quantities of molybdenum fed, but there was some interference with normal lactation.

LITERATURE CITED

BRITTON, J. W., AND H. GOSS 1946 Chronic molybdenum poisoning in cattle. J. Am. Vet. Med. Assn., *108:* 176.

COMAR, C. L., L. SINGER AND G. K. DAVIS 1949 Molybdenum metabolism and interrelationships with copper and phosphorus. J. Biol. Chem., *180:* 913.

FAIRHALL, L., R. C. DUNN, N. E. SHARPLESS AND E. A. PRITCHARD 1945 Toxicity of molybdenum. U. S. Public Health Bull., *293:* 1–36.

FERGUSON, W. S., A. H. LEWIS AND S. J. WATSON 1938 Action of molybdenum in nutrition of milking cattle. Nature, *141:* 553.

———— 1940 The teart pastures of somerset, cause of teartness and its prevention. Jealott's Hill Research Station, Bull. no. 1.

———— 1943 The teart pastures of somerset. I. The cause and cure of teartness. J. Agr. Sci., *33:* 44.

GRAY, LOUISE F., AND G. N. ELLIS 1950 Some interrelationships of copper, molybdenum, zinc, and lead in the nutrition of the rat. J. Nutrition, *40:* 441.

LONG, J. A., AND H. M. EVANS 1922 The Oestrus Cycle in the Rat and Its Associated Phenomena. Memoirs University of California 6.

NEILANDS, J. B., F. M. STRONG AND C. A. ELVEHJEM 1948 Molybdenum in the nutrition of the rat. J. Biol. Chem., *172:* 431.

SINGER, L. 1949 The Metabolism of Phosphorus, Copper and Molybdenum and Their Interrelationships in the Animal Organism. Ph.D. Dissertation, University of Florida.

THOMAS, J. W., AND S. MOSS 1951 The effect of orally administered molybdenum on growth, spermatogenesis and testes histology of young dairy bulls. J. Dairy Sci., *34:* 929.

THE EFFECT OF WATER RESTRICTION ON THE FOOD INTAKE AND FOOD EFFICIENCY OF GROWING RATS

E. W. CRAMPTON AND L. E. LLOYD
Department of Nutrition, Macdonald College, McGill University, Quebec, Canada

(Received for publication April 2, 1954)

Since water is the largest single constituent of all living matter its importance in vital body processes has long been recognized. Excellent reviews dealing with water metabolism (Adolph, '33, '47), with water requirements of different animal species (Leitch and Thomson, '44; Bruce, '50), and with the effects of both water deprivation and water excesses on body function (Adolph, '47a) have appeared during the past few years. None of these, however, have made mention of the effect of water restriction on growth and food utilization in young animals. The type of food eaten has a direct effect on the water requirement, and the mineral content is particularly important in this respect. Adolph himself ('33) points out that while one calorie of protein in food requires 3 ml of water for the elimination of the urea and sulfate formed from it, 65 ml are needed for each gram of ash. This explains why, in spite of its high free-water content, there is a body water deficit to the extent of some 43 ml for each 100 cal. of milk consumed. At Wisconsin, Rupel ('29) showed that calves given skimmed milk as the only beverage ate only half the quantity of hay and grain that was eaten by calves given the same quantity of milk but provided with water twice daily. The water deficit arising from the feeding of milk alone also resulted in a 24% decrease in daily gain.

The experiments outlined in this report have been conducted over a period of 4 years, and were designed to deter-

Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Journal Series no. 348.

mine the effect of a restriction of water intake on the rate of gain, food intake and food utilization of growing rats.

EXPERIMENTAL

Animals. A total of 164 white rats, ranging in age from 4 to 8 weeks, were used in 4 separate tests. Within each test the rats were paired as to sex and initial weight, and housed individually in wire-bottomed cages. All tests were of two weeks duration.

Water allowance. One rat of each pair, chosen at random, was restricted in water intake to approximately one-half that of the pair mate which was allowed water ad libitum. All rats received water ad libitum for the first 24 hours and the quantity consumed was recorded as a basis for the initial restricted allowances which began on the second day. Subsequently, the restricted allowance was based on the previous day's ad libitum intake of the appropriate pair mate. The rats on the ad libitum water intake were provided with an inverted-bottle watering device calibrated as to volume, while the restricted animals received once daily their allowance of water in an open container.

Diet. The rats were given the regular stock colony diet in use in this laboratory. The proximate analysis of the diet was as follows: total ash 8, crude protein 25, ether extract 3, crude fibre 3 and nitrogen-free extract 49%.

Records. The rats were weighed to the nearest whole gram at the start of the test, at the end of the first week and at the end of the second week. Food consumption records were obtained at the end of the first and the second weeks.

Water consumption was recorded daily for each rat.

RESULTS AND DISCUSSION

The average data from this study are shown in table 1.

The immediate effect of restricting the water intake of growing rats was to reduce substantially the voluntary intake of food and consequently to inhibit gain in body weight.

TABLE 1

The effect of water restriction on the food intake, gain and food efficiency of growing rats.

TEST NO.	NO. OF RAT PAIRS	AV. INITIAL WEIGHT		AV. WATER INTAKE			AV. FOOD INTAKE			AV. GAIN			AV. GAIN/FOOD RATIOS		
		Water ad libitum	Water restricted	Water ad libitum	Water restricted	Extent of restriction	Water ad libitum	Water restricted	Extent of reduction	Water ad libitum	Water restricted	Extent of reduction	Water ad libitum	Water restricted	Extent of reduction
		gm	*gm*	*ml*	*ml*	%	*gm*	*gm*	%	*gm*	*gm*	%	*gm/ 100 gm*	*gm/ 100 gm*	%
1	12	88	83	286	140	51	225	171	24	68	35	49	30	21	30
2	10	103	97	275	133	52	212	169	20	50	28	44	24	17	29
3	30	52	51	297	139	53	183	118	36	72	32	56	39	27	31
4	30	86	84	325	159	51	205	145	29	58	26	55	28	18	36
Mean						52			27			51			31

Coefficient of variability: food intake = 12%; gain = 23% of the means.
Percentage reduction necessary for significance at P = 0.05 and n = 82: food intake = 4%; gain = 7% of the means.

The greatest and least reductions in average food intake were found for the youngest and oldest rats respectively. These data suggest that the age of the animal (presumably relative physiological age) may affect the extent of the effect of water restriction on food intake. Of particular interest was the fact that the food efficiency of all rations, expressed as gain/food ratios, was reduced by over 30% when water intake was restricted.

The marked adverse effects of limited water intake on the efficiency of the diet in permitting growth has apparently not heretofore been widely recognized.

LITERATURE CITED

ADOLPH, E. F. 1933 The metabolism and distribution of water in body and tissues. Physiol. Rev., *13:* 336.

———— 1947 Water metabolism. Ann. Rev. of Physiol., *9:* 381.

———— 1947a Physiology of man in the desert. Interscience Publishers, Inc., New York.

BRUCE, H. M. 1950 The water requirement of laboratory animals. J. Animal Technicians Assn. 1, no. 3, 1.

LEITCH, I., AND J. S. THOMSON 1944 The water economy of farm animals. Nutr. Abstr. and Rev., *14:* 197.

RUPEL, I. W. 1929 Raising the dairy calf. Wisc. Agr. Expt. Sta. Bull., no. 404.

IMPROVING THE NUTRITIVE VALUE OF FLOUR

VI. A COMPARISON OF THE USE OF SOYA FLOUR AND WHEAT GERM [1]

BEULAH D. WESTERMAN, BESS OLIVER AND EVELYN MAY
Department of Foods and Nutrition, Kansas Agricultural Experiment Station, Manhattan

(Received for publication January 9, 1954)

Wheat germ and soya flour are two naturally-occurring substances containing considerable quantities of the B vitamins and essential amino acids, which are known to be deficient in wheat flour. Considerable work has been reported on the supplementation of wheat flour with soya protein (Jones and Divine, '44; Carlson, Hafner and Hayward, '46; Volz, Forbes, Nelson and Loosli, '45), but little attention has been given to soya flour as a source of B vitamins. Ofelt, Smith and Mills ('54) found that soya flour used in bread doughs did not impair loaf volume or crumb character.

Wheat germ also could be used to supply B vitamins and essential amino acids. Westerman, Roach and Stone ('52) showed that the addition of defatted wheat germ to enriched and non-enriched flour produced an increase in growth rate, reproduction and lactation performance and storage of pantothenic acid in the livers of albino rats.

The purpose of these experiments was to compare the nutritive value of wheat germ and soya flour as a supplement to wheat flour, using as criteria the effects upon rate of growth, reproduction and lactation and deposition of B vitamins in the liver and tissues of rats.

[1] Contribution 174, Department of Home Economics. This research was supported by Purnell Funds.

PROCEDURE

Albino rats, 24 days of age and weighing between 45 and 50 gm were placed on a vitamin B-free diet in order to deplete their bodies of these vitamins. After 10 to 14 days the animals were divided into 8 groups of 10 rats each, with equal distribution as to sex and litter mates. Diets consisting of natural foods similar to those consumed by human beings were prepared according to the methods described previously by Westerman, Linn, Templeton and Wells ('49). Enriched and non-enriched flour made up the cereal component of the diet and furnished approximately 52% of the total calories. The wheat germ had been defatted and toasted. The soya flour called "Nutrisoy" had been heated during processing to improve the nutritive value of the protein. These substances were added to the flour at a 3% level, as the standards set for bread allow the use of 3% soya flour in the formula. The diets contained the following variables: (I) enriched flour with 3% wheat germ; (II) non-enriched flour with 3% wheat germ; (III) enriched flour with 3% soya flour; (IV) non-enriched flour with 3% soya flour; (V) enriched flour with the addition of both 3% soya flour and 3% wheat germ; (VI) enriched flour only; (VII) non-enriched flour only; and (VIII) stock diet of Purina laboratory chow.

The rats were weighed weekly for a 12-weeks period. They were mated and carried through three periods of pregnancy and lactation. The young were used in a second generation growth test while the first generation animals were sacrificed and their muscle tissues and livers analyzed for thiamine, riboflavin, niacin and pantothenic acid. In order to report the data on a dry fat-free basis, fat and water determinations were made also.

RESULTS AND DISCUSSION

The nutritive value of the diets was calculated and the results are shown in table 1. The amounts of iron and of the B vitamins were lowest in those diets containing the non-

enriched flour, i.e., II, IV, and VII. Protein was slightly lower in those without added soya flour or wheat germ.

The total average weight gains of the first generation animals over the 12-weeks period are shown in table 2. The rats receiving diet VII made the smallest weight gains of the entire group. The addition of 3% wheat germ to the non-enriched flour, (diet II), brought about an increase in growth

TABLE 1

Nutritive value of diets

Based on the daily food consumption of some human beings

	FOOD	PROTEIN	IRON	THIAMINE	RIBOFLAVIN	NIACIN	PANTOTHENIC ACID
		gm	*mg*	*mg*	*mg*	*mg*	*mg*
I	Enriched + 3% wheat germ	66.9	15.3	3.55	1.95	20.5	1.52
II	Non-enriched + 3% wheat germ	66.9	8.2	2.12	1.34	12.2	1.52
III	Enriched + 3% soya flour	68.2	15.3	3.29	1.91	20.4	1.56
IV	Non-enriched + 3% soya flour	68.2	8.2	1.86	1.30	12.1	1.56
V	Enriched + 3% soya flour + 3% wheat germ	72.1	15.3	3.62	1.99	21.1	1.64
VI	Enriched flour	63.0	15.3	3.22	1.87	19.8	1.45
VII	Non-enriched flour	63.0	8.2	1.79	1.26	11.5	1.45

rate which was statistically significant ($P < .05$) for the females but a non-significant increase for the males. When 3% soya flour was added, (diet IV), there was a non-significant increase for the females but a significant increase ($P < .01$) for the males. Evidently the B vitamins and amino acids, particularly lysine and valine in the wheat germ and soya flour, were helpful in providing for an increase in growth. However, the difference in weight gain between the animals receiving diets II and IV, were non-significant, indicating

that soya flour and wheat germ, when added to non-enriched flour at the 3% level, aided in producing the same beneficial results.

None of the animals receiving non-enriched flour gained as much as those on the stock diet. The males on diet VIII gained 34 gm more than those on diet II, a significant difference ($P < .05$). Differences in weight gains by the females on the two diets were non-significant. The average gains made by the rats on diet VIII were significantly greater ($P < .05$) for both sexes than those on diet IV, and were significantly greater for both females ($P < .01$) and males ($P < .001$) than those on diet VII. While wheat germ and soya flour were beneficial in promoting growth, when added to non-enriched flour these diets did not promote as good growth as the stock diet.

The gains made by the females on diets I and III were not statistically significant when compared with those on diet VI. However the males on diet I ($P < .001$) and those on diet III ($P < .05$) made a significant increase over those on diet VI. The differences in weight gains between the animals receiving diets I and III were not significant. Wheat germ and soya flour seem to be equally beneficial when used with enriched flour. The rats receiving both wheat germ and soya flour in the diet did not show a significant increase in growth over those receiving enriched flour alone. The male animals on diet I showed a highly significant increase ($P < .01$) over those on diet V. Other differences between animals on diets I, III and V were non-significant. These data seem to indicate there may be more value in the addition of either wheat germ or soya flour alone to flour rather than both at the same time.

The male animals, which grew to a larger size than the females, showed greater differences in weight gains. The males on diet I made a significantly greater gain ($P < .01$) than those on diets II, IV and V, thus indicating that the addition of 3% wheat germ to enriched flour provided for an increase in the growth rate above that of the animals receiving non-

TABLE 2

Average total weight gains

First generation.[1] Twelve-week period

	I ENRICHED + 3% WHEAT GERM	II NON-ENRICHED + 3% WHEAT GERM	III ENRICHED + 3% SOYA FLOUR	IV NON-ENRICHED + 3% SOYA FLOUR	V ENRICHED + 3% SOYA FLOUR + 3% WHEAT GERM	VI ENRICHED FLOUR	VII NON-ENRICHED FLOUR	VIII STOCK DIET
	gm	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
♀	172	167	173	159	169	163	151	175
♂	334	288	311	291	298	282	263	322
	Second generation.[2] Ten-week period							
♀	145	127	151	148		112	98	
♂	261	197	258	249		158	115	

[1] Test of significance: II > VII at 5% level for females. IV > VII at 1% level for males. II and IV differences non-significant. VIII > II at 5% level for males. VIII > IV at 5% level for both sexes. VIII > VII at 1% level for females, at 0.1% level for males. I > VI at 0.1% level and I > III at 5% level for males. I > V at 1% level for males. I > II, IV and V at 1% level for males. I > VI and VII at 1% level for males. III > VI at 5% level and III > VII at 0.1% level for males. I and III differences non-significant.

[2] Test of significance: VI > VII at 5% level for males. II > VII at 5% level for both sexes. IV > VII at 1% level for both sexes. I and III > VI at 1% level for both sexes. Differences between I and III non-significant.

enriched flour plus 3% wheat germ or 3% soya flour. The weight gains on diets VI and VII were significantly less ($P < .001$) than those on diet I.

For the males the addition of wheat germ to both enriched and non-enriched flour was highly beneficial. The addition of 3% soya flour, as in diet III, allowed a significant increase ($P < .05$) in growth over diet VI and a very highly significant increase ($P < .001$) over diet VII. Non-significant differences were found between the weight gains of the animals on diets I and III, indicating that while the addition of either wheat germ or soya flour was significantly beneficial, there was no statistically significant difference between the two supplements.

At the end of the growth test the animals were bred and the young used for a second generation growth test. The results are shown in table 2. The animals on diet VII made the least gains during the 10-week period. The males on diet VI made a significantly greater ($P < .05$) gain than those receiving diet VII. The gain made by the females on diet VI was not significantly greater than that made by those on diet VII. When wheat germ was added to the non-enriched flour, the gains made by both the males and the females were significantly greater ($P < .05$) than for those on diet VII. With soya flour added the gains made by both sexes were significantly greater ($P < .01$) than for those on diet VII thus indicating that the addition of either wheat germ or soya flour to non-enriched flour was beneficial in promoting growth in the second generation animals. The increase of 52 gm by the males on diet IV over that of those on diet II was highly significant. The increase of 21 gm by the females on diet IV in comparison with diet II was significant, showing that with both sexes the addition of soya flour to the enriched wheat flour increased the growth rate above that of those receiving wheat germ. When wheat germ and soya flour were added to enriched flour, the differences in growth rate for the rats on these diets were non-significant for both sexes. Evidently the B vitamins in the soya flour were responsible for the

increased growth of the animals receiving non-enriched flour for when such flour was enriched, the growth of those receiving wheat germ and soya flour was the same. This is also shown by the non-significant differences in growth between the animals on diets III and IV.

Both sexes on diet I made highly significant increases ($P < .01$) over those on diet VI. Significant increases were also made by both sexes on diet III, indicating again that the addition of either soya flour or wheat germ was beneficial in increasing growth rate. Differences between the animals on diets I and III were non-significant showing that, when added to enriched flour, wheat germ and soya flour are about equal in benefiting growth.

The livers were removed from the first generation rats and analyzed for thiamine, riboflavin, pantothenic acid and niacin. Table 3 shows the average vitamin contents of the livers. The analysis of variance showed that differences due to diets were produced only for thiamine and riboflavin and no significant differences were indicated for pantothenic acid and niacin content. The animals receiving diet VI stored a significantly greater ($P < .01$) amount of thiamine than those on diet VII. The differences in storage of the other three vitamins were non-significant between these two groups.

The addition of wheat germ to non-enriched flour, diet II, resulted in a significant increase in thiamine storage ($P < .01$) over diet VII while the addition of soya flour, diet IV, did not produce a significant increase in thiamine storage. Diets III, VI and VIII produced the greatest storage of thiamine in the livers. These amounts were significantly greater ($P < .05$) than those in livers of animals on diet I.

As for the storage of riboflavin, the animals receiving the stock diet stored the largest amounts of vitamin in the livers followed by those on diet III. Rats receiving non-enriched flour alone stored the least. When soya flour was added there was a very highly significant increase in storage ($P < .001$). With the addition of wheat germ, there was a significant increase ($P < .05$). Evidently wheat germ or soya flour when

TABLE 3

Vitamin content

Data reported on a dry fat-free basis. Liver [1]

DIET NO.	DESCRIPTION	THIAMINE	RIBOFLAVIN	PANTOTHENIC ACID	NIACIN
		μg/gm	*μg/gm*	*μg/gm*	*μg/gm*
I	Enriched flour + 3% wheat germ	14.7	60.8	115.6	447.1
II	Non-enriched flour + 3% wheat germ	14.7	63.8	105.4	431.7
III	Enriched flour + 3% soya flour	20.5	68.1	93.7	460.1
IV	Non-enriched flour + 3% soya flour	11.4	62.3	106.2	436.4
VI	Enriched flour	19.5	59.3	50.9	404.5
VII	Non-enriched flour	6.0	46.0	81.2	428.3
VIII	Stock diet	18.8	71.8	121.8	441.9
	Muscle tissues [2]				
I	Enriched flour + 3% wheat germ	5.2	10.8	25.2	241.4
II	Non-enriched flour + 3% wheat germ	6.5	10.1	23.7	236.8
III	Enriched flour + 3% soya flour	6.1	8.4	21.5	272.1
IV	Non-enriched flour + 3% soya flour	4.3	9.5	20.4	259.9
VI	Enriched flour	6.7	10.9	26.3	274.1
VII	Non-enriched flour	4.0	11.4	30.6	263.6
VIII	Stock diet	8.0	9.9	20.6	262.1

[1] Test of significance: Thiamine: II and VI > VII at 1% level. IV and VI no significant differences. III, VI and VIII > I at 5% level. Riboflavin: II > VI at 5% level. IV > VI at 0.1% level. Differences between I, III and VI non-significant. Pantothenic acid and niacin showed no significant differences.

[2] Test of significance: Thiamine: VIII > VII at 1% level. VI > VII at 5% level. II > IV and VII at 1% level. I, III and VI no significant differences. Pantothenic acid: VII > IV and VIII at 1% level. VI > II at 1% level. I, II, III, VI and VII no significant differences. No significant differences for riboflavin and niacin.

added singly to non-enriched flour provide good sources of riboflavin for storage purposes. Enriched flour provided for good storage of this vitamin and the addition of either wheat germ or soya flour did not increase the storage a statistically significant amount.

Animals receiving diet VI stored the least amount of pantothenic acid in the livers, followed by those on diet VII and diet III. Those on diets VIII and I stored the largest amount. The storage of niacin showed no striking differences due to diet.

The average vitamin content of the muscle tissue is shown in table 3. The analysis of variance showed that differences due to diet in the deposition of thiamine were very highly significant ($P < .001$) and of pantothenic acid were highly significant ($P < .01$) while the differences for riboflavin and niacin were non-significant. Animals receiving the stock diet had the largest amounts of thiamine, in their tissues while those receiving non-enriched flour had the least. This difference was highly significant ($P < .01$). Those receiving enriched flour stored a significantly greater amount ($P < .05$) than those on diet VII. When wheat germ was added to non-enriched flour the average amount stored was significantly greater ($P < .01$) than those on diets VII and IV. The differences in tissue storage for thiamine by the rats receiving enriched flour alone or with wheat germ or soya flour added were not significant. The addition of either wheat germ or soya flour to enriched flour did not increase the deposition of thiamine in the tissues above that of enriched flour alone. The addition of wheat germ to non-enriched flour produced about the same amount of storage of this vitamin in the tissues as enriched flour but the addition of soya flour to non-enriched flour did not increase the tissue storage. Analysis showed that soya flour contained less thiamine, 9.1 μg/gm, than wheat germ, 24.2 μg/gm, which may have influenced the amount deposited in the tissues.

The differences between the amount of pantothenic acid deposited in the muscle tissues of the animals on diets I, II,

III, VI and VII were non-significant. The differences between those on diet VII and those on diets IV and VIII were highly significant ($P < .01$) and the difference between those on diets VII and II was significant ($P < .01$). These data do not show any striking effects of the addition of wheat germ or soya flour upon the deposition of this vitamin in muscle tissue.

The animals on diets I, II, III, IV, VI and VII were carried through three periods of reproduction and lactation. The females on diet I produced 94 young and weaned 40 of these at 21 days. Those on diet II produced 40 young and weaned 9. Diet III females had 73 young, weaning 19, while those on diet IV had 87 young, weaning 29. Those on diet VI had 46, weaning 12 and those on diet VII had 20, weaning 8. The average loss of weight by the females during the lactation period was 44, 19, 38, 47, 30 and 16 gm, respectively, on the different diets. The small loss by those on diets II and VII probably is due to the smaller number of young raised.

It may be noted that the animals on diet I weaned 42% of the young born. Those on diet II produced fewer young and raised only 22% of these. Evidently the vitamins in the enriched flour were responsible for this difference. However the added wheat germ did help to increase the number of young produced over those on diet VII. The females on diet IV produced a greater number of young and raised more to maturity than those on diet III. Both groups with soya flour added, produced and weaned more young than those on diet VI with only enriched flour.

The addition of either wheat germ or soya flour to enriched and non-enriched flour aided in increasing the number of young produced. Wheat germ gave the best results when added to enriched flour and soya flour the best results when added to non-enriched flour.

SUMMARY

Comparisons were made of the value of adding soya flour or wheat germ to enriched and non-enriched flour. Results

indicated that both were significantly beneficial in promoting growth in rats when added to non-enriched flour but neither supported as good growth as the stock diet. When added to enriched flour these materials supported as good growth as the stock diet. No significant differences were found in rats receiving enriched flour plus wheat germ as against those receiving enriched flour plus soya flour in either the first or second generation. There was some evidence that soya flour when added to non-enriched flour aided in promoting better growth in the second generation than did wheat germ.

Differences in storage of the B vitamins in the livers were significant for thiamine and riboflavin but not for pantothenic acid and niacin. Rats receiving non-enriched flour alone stored the least thiamine and riboflavin. Those with enriched flour plus soya flour in the diet stored the largest amount of these vitamins. Significant differences in amounts of thiamine deposited in the muscle tissues were found. The addition of wheat germ or soya flour to enriched flour did not increase the deposition of thiamine above that of enriched flour alone. The addition of wheat germ to non-enriched flour increased deposition of this vitamin up to that of enriched flour.

ACKNOWLEDGMENTS

The authors express their appreciation to the VioBin Corporation for the wheat germ and to Archer-Daniels-Midland Co. for the soya flour.

LITERATURE CITED

CARLSON, S. C., F. H. HAFNER AND J. W. HAYWARD 1946 Effect of soya flour and non-fat dry milk solids in white bread on the nutritional quality of the protein as measured by three biological methods. Cereal Chem., *23:* 305–317.

JONES, D. B., AND J. P. DIVINE 1944 The protein nutritional value of soybean, peanut and cottonseed flours and their value as supplements to wheat flour. J. Nutrition, *28:* 41–49.

OFELT, C. W., K. SMITH AND J. M. MILLS 1954 Baking behavior and oxidation requirements of soy flour. II. Commercial defatted soy flour. Cereal Chem., *31:* 23–28.

VOLZ, F. E., R. M. FORBES, W. L. NELSON AND J. K. LOOSLI 1945 The effect of soy flour on the nutritive value of the protein of white bread. J. Nutrition, *29:* 269–275.

WESTERMAN, B. D., D. R. LINN, F. TEMPLETON AND R. I. WELLS 1949 Improving the nutritive value of flour. III. The use of enriched and non-enriched flour in diets similar to those consumed by certain low-income groups in South Carolina. Ibid., *38:* 421–434.

WESTERMAN, B. D., F. ROACH AND M. STONE 1952 Improving the nutritive value of flour. V. The effect of the use of defatted wheat germ. Ibid., *47:* 147–158.

THE UTILIZATION OF ALFALFA AND ALFALFA FIBER FRACTIONS BY GROWING RATS

JAMES H. MEYER
Animal Husbandry Department, University of California, Davis

(Received for publication April 12, 1954)

Investigations of the use of alfalfa in the rations of simple-stomached species have been concerned mainly with alfalfa as a source of vitamins or unidentified factors. Little attention has been paid to the protein and energy furnished by alfalfa. The work of Schmidt and Lagneau ('32) with swine indicated that alfalfa nitrogen was 40 to 50% digestible but the organic matter only 28 to 37% digestible. Mitchell and Hamilton reported a poor utilization of alfalfa fiber by pigs on low-protein rations, while Forbes and Hamilton ('52) found a higher utilization of the fiber of alfalfa by pigs on a higher protein ration. Tscherniak ('36) reported that alfalfa meal fiber was indigestible by poultry. Fraps and Carlyle ('41) in work with growing chickens found the total digestible nutrients of alfalfa leaf meal to be only 18.4%.

In general, fiber, cellulose, and hemicellulose disappear to a variable extent when they pass through the digestive tract of swine (Forbes and Hamilton, '52; Hvidsten and Homb, '47; Mitchell and Hamilton, '33; Trautman and Asher, '42; Woodman and Evans, '47), man (Hummel et al., '43; Williams and Olmsted, '36), and most other simple-stomached animals (Mangold, '34). The data do not show the extent of the actual utilization of the apparently digested fiber.

The experiments reported herein were designed to investigate the utilization of alfalfa and certain alfalfa fiber fractions by the use of paired-feeding techniques, nitrogen balances, and digestibility.

EXPERIMENTAL

Three fiber fractions were isolated from an alfalfa meal and fed to growing rats. An extracted fraction was prepared by extracting alfalfa three times with a hot ethyl alcohol-benzene solution, then three times with hot water. This residue was then dried at room temperature. Holocellulose was prepared from alfalfa by the method of Wise et al. ('46). This

TABLE 1

Basal rations

	LOW PROTEIN	HIGH PROTEIN
	gm	*gm*
Sucrose	76.6	51.6
Casein [1]	10.0	35.0
Cottonseed oil [2]	5.0	5.0
Salts IV [3]	6.0	6.0
Vitamin mix [4,5]	0.2	0.2
Choline	0.2	0.2
Liver powder, 1: 20 [6]	2.0	2.0
Total	100.0	100.0

[1] Vitamin-free test casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

[2] Wesson oil.

[3] Phillips and Hart, J. Biol. Chem., *109:* 657, 1935.

[4] The vitamin mix supplied the following vitamins in milligrams per 100 gm of the ration: thiamine 0.6, riboflavin 0.6, pyridoxine 0.4, niacin 4.0, calcium pantothenate 4.0, folic acid 0.05, biotin 0.02, and inositol 20.0.

[5] Ample amounts of vitamins A, D, E, and K were supplied weekly by oral administration.

[6] General Biochemicals, Inc., Chagrin Falls, Ohio.

method uses sodium chlorite to remove part of the lignin and protein, leaving cellulose and hemicellulose in a fraction called holocellulose. The method of Ellis et al. ('46), with certain modifications, was used to isolate lignin. The modifications were the adaptation of the method to a larger scale and the elimination of the pepsin digestion.

Two rations were used — a low-protein ration for experiment 1 and a high-protein ration for experiment 2. The rations are shown in table 1. It should be noted that the vitamins, liver

powder, and the minerals were added in large enough quantities to insure adequate intakes of these constituents by all groups.

The following 6 treatments were used at each protein level: lot 1, basal; lot 2, 38% alfalfa; lot 3, 30% extracted alfalfa; lot 4, 21% holocellulose; lot 5, 8% lignin; and lot 6, 21% holocellulose and 8% lignin. The above are percentages of the total ration on an oven-dry basis and the fiber fractions are equal to an amount isolated from 38% alfalfa. One member of each litter of 6 was assigned to each treatment. All members of a litter were fed the same amount of basal ration consumed by the rat on the alfalfa ration (lot 2). The various fiber fractions were consumed in addition to the basal ration. The moisture content of the rations was determined weekly, and appropriate adjustments were made to insure equal basal ration intake and the proper fiber fraction intake.

Male, weanling, inbred Long-Evans rats were used. They were housed in screen-bottomed cages for the first 7 days. Metabolism cages designed from the description of Harned et al. ('49) were then used for the 21-day collection and metabolism study. The Kjeldahl method was used for the nitrogen determination. The method of Ellis et al. ('46) was used for lignin analysis. The analysis of variance as described by Snedecor ('46) was used as a test of significance. The method of least significant difference was used to determine differences between treatments and the controls.

RESULTS AND DISCUSSION

Table 2 shows some analyses of the fiber fractions. The isolation procedures used did not remove all of the protein from the holocellulose and lignin. All of the lignin remained in the extracted alfalfa and lignin fractions. About 80% of the lignin was removed when the holocellulose was isolated.

The results of the growth and digestion trials are shown in table 3. These experiments were designed to pair feed all rats within a litter of 6 the same amount of basal ration plus the designated fiber fraction. Additional weight gain over and above the controls fed the basal ration alone would be due to

the contribution of the fiber. When the low-protein ration was used additional weight gains were noted for rats fed alfalfa, extracted alfalfa, and holocellulose. No additional weight gains were observed for the rats fed lignin.

Rats fed alfalfa and extracted alfalfa as a supplement to the high protein ration showed a significant growth increase in comparison with the controls. The addition of holocellulose and of lignin to the high protein ration did not result in increased weight gains over these obtained for the controls fed

TABLE 2

Analysis of alfalfa fiber fractions

	ALFALFA	EXTRACTED ALFALFA FRACTION	HOLOCELLULOSE FRACTION	LIGNIN FRACTION
Percentage of original alfalfa	100	71	45	14
Protein, % [1]	17	16	3	15
Percentage of original alfalfa protein remaining	100	66	7	12
Lignin, % [1]	10.9	11.2	4.8	78.1
Percentage of original alfalfa lignin remaining	100	103	19	100

[1] Oven dry basis.

the basal ration. The utilization of the holocellulose with the two protein levels does not agree. Since the utilization occurred on the low protein ration, the presence of incompletely extracted protein, 3%, may have been responsible for the growth increase.

Any additional weight gain observed in the rats of the alfalfa groups in comparison with those fed the extracted alfalfa would be due to the soluble constituents of the alfalfa. There was a differential response to the alfalfa extract for the two protein levels. On the low protein ration the rats fed the

CATEGORY OF INTEREST	GROWTH											
	Low protein						High protein					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
	Basal	Alfalfa	Extracted alfalfa	Holo-cellulose	Lignin	Holo-cellulose lignin	Basal	Alfalfa	Extracted alfalfa	Holo-cellulose	Lignin	Holo-cellulose lignin
Alfalfa fraction (oven dry basis) %	0	38	30	21	8	29	0	38	30	21	8	29
Rats per group	5	5	5	5	5	4	4	4	4	4	4	4
Average initial wt., gm	65	64	61	65	65	60	68	66	65	66	66	65
Average gain in 4 weeks, gm	29	59 [2]	45 [2]	36 [2]	29	33 [3]	112	149 [2]	118 [3]	111	107	113
Average gain due to fiber fraction, gm	..	30.0	16	7	..	4	..	37	6	..	..	..
Food consumption, gm	206	354	294	263	223	280	293	488	417	373	318	398
	DIGESTION TRIAL PERIOD [1]											
Organic matter consumed basal ration, gm	156	156	156	156	156	156	211	211	211	211	211	211
Organic matter consumed alfalfa fraction, gm	..	97	63	41	15	58	..	138	89	57	20	77
Organic matter digested, gm	151	182 [2]	158 [2]	156 [2]	152	160 [2]	203	251 [2]	214 [2]	207 [2]	203	208 [2]
Organic matter alfalfa fraction digested, [4] %	..	31	12	14	9	15	..	34	12	8	..	7
Lignin digested, %	41	0	—3	—22	7	6	58	10	6	8	6	11

[1] Twenty-one-day collection period.

[2] Indicates a highly significant difference from lot 1 within the respective protein level.

[3] Indicates a significant difference from lot 1 within the respective protein level.

[4] Calculated by difference.

extracted alfalfa gained 15 gm more than the controls, and the rats fed alfalfa gained 14 gm more than those fed extracted alfalfa. This would indicate that the protein from the alfalfa extract and extracted alfalfa were used for about the same weight gain. A different response occurred in the animals fed the high-protein rations. The rats fed the extracted alfalfa gained 6 gm more than those fed the basal ration, while the rats fed alfalfa gained 32 gm more than those fed extracted alfalfa. It would appear from this that the extracted alfalfa was more valuable as a source of nitrogen than as a source of energy.

The consumption and apparent digestion of organic matter are also shown in table 3. A larger amount of organic matter was digested by the rats fed alfalfa, extracted alfalfa and holocellulose than by those fed the basal ration. This indicates an apparent utilization of these fractions. There was no significant increase in the amount of organic matter utilized by the rats on the lignin fraction.

The percentage of the alfalfa or fiber fraction digested was calculated by difference. As might be expected, a great drop in the digestibility of the organic matter occurred when the alfalfa was extracted. There was no significant difference between the digestibility of holocellulose and that of extracted alfalfa. Protein level did not influence digestibility except for the lignin groups. Here an increase occurred when the low-protein ration was fed.

Lignin digestibility was determined by analysis of the food and feces according to the method of Ellis et al. ('46). When the lignin digestibility of the rations containing lignin was calculated, inconsistent coefficients of digestibility were observed. Consistent, small amounts of lignin were found in the food and feces of the rats fed the basal ration. These amounts of lignin are artifacts since obviously there is no true lignin in the basal rations.

Table 4 presents the data for the nitrogen balance studies. The rats fed alfalfa and extracted alfalfa apparently absorbed more nitrogen than did the controls. The addition of holocel-

TABLE 4

Effect of alfalfa fiber fraction on nitrogen balance [3]

	NITROGEN CONSUMED	NITROGEN APPARENTLY ABSORBED	FIBER NITROGEN APPARENTLY ABSORBED	NITROGEN RETAINED	FIBER NITROGEN RETAINED
	gm	*gm*	*gm*	*gm*	*gm*
LOW PROTEIN:					
Lot 1 Basal	2.63	2.22	...	1.13	...
Lot 2 Alfalfa	5.03	3.53 [1]	1.31	1.61 [1]	0.48
Lot 3 Extracted alfalfa	4.37	2.69 [1]	0.47	1.40 [1]	0.27
Lot 4 Holocellulose	2.91	2.15 [1]	— 0.07	1.27 [1]	0.14
Lot 5 Lignin	2.90	2.18 [1]	— 0.04	1.15	0.02
Lot 6 Holocellulose lignin	3.20	2.03 [1]	— 0.19	1.16	0.03
HIGH PROTEIN:					
Lot 1 Basal	12.58	11.79	...	4.30	...
Lot 2 Alfalfa	16.02	13.45 [1]	1.66	4.84 [2]	0.53
Lot 3 Extracted alfalfa	14.84	12.24 [1]	0.45	4.89 [2]	0.58
Lot 4 Holocellulose	12.63	11.12 [1]	— 0.67	4.00	— 0.30
Lot 5 Lignin	13.02	11.70	— 0.10	4.07	— 0.23
Lot 6 Holocellulose lignin	13.17	11.37 [1]	— 0.44	4.85 [2]	0.55

[1] Indicates a highly significant difference from lot 1 within the respective protein level.

[2] Indicates a significant difference from lot 1 within the respective protein level.

[3] Twenty-one-day collection period.

lulose to the ration decreased the apparent nitrogen absorption at both protein levels, while lignin decreased absorption only on the low-protein level. Mitchell ('24), Duckworth and Godden ('35), Woodman and Evans ('47), and Forbes and Hamilton ('52), have reported increases in fecal nitrogen when fiber was included in the rations of rats and pigs. They suggest that this is due to increased fecal metabolic nitrogen. It is apparent in our experiments that when holocellulose and lignin, either singly or in combination, were added to the rations, the nitrogen excretion increased over and above that in the controls. Adolph and Wu ('34) have reported no difference in the true digestibility of protein fed with high fiber rations.

A certain amount of nitrogen was retained by the rats from the alfalfa fiber fractions fed with the low-protein rations. The rats fed alfalfa had the highest nitrogen retention, followed by the animals fed extracted alfalfa and then those fed holocellulose. These differences from the controls were highly significant. No significant increase in nitrogen retention in comparison with the controls was noted for the rats fed lignin and lignin plus holocellulose. In general, the nitrogen retention closely confirms the weight gains. This indicates that these fiber fractions, with the exception of lignin, supply nitrogen that can be utilized by the rats.

The high protein ration was fed to determine the energy value of the fiber. In this case, a high correlation of nitrogen retention with body weight gain would not be expected, since adequate nitrogen was supplied by the basal ration. A large increase in weight gains was noted for the rats fed alfalfa but only small increments for those fed extracted alfalfa. Nitrogen retention, however, was the same for both groups. This indicated a good utilization of the nitrogen from the extracted alfalfa but a poor utilization of that from extracted alfalfa for weight gain. Utilization of holocellulose and lignin for weight gain with the high protein ration was *nil*. Holocellulose and lignin fed alone did not increase nitrogen retention. For some unexplained reason, the feeding of holocellulose plus lignin caused an increase in nitrogen retention.

The nitrogen retention confirms the observation that extracted alfalfa was valuable as a source of nitrogen but not as a source of energy, while the soluble constituents of alfalfa are valuable as a source of both energy and nitrogen. With the low-protein ration, nitrogen retention for the rats fed the extracted alfalfa was 0.27 gm more than that of the controls, while the alfalfa-fed rats retained 0.21 gm more than those fed extracted alfalfa. Calculations show a 15% retention of the nitrogen from extracted alfalfa, 31% from the soluble constituents.

In experiments with rats Duckworth and Godden ('35) reported a lowered nitrogen balance when fiber was added to rations. Food consumption decreased and as a consequence nitrogen intake was lower when fiber was added to diets. This may account for their lowered nitrogen balance. We observed no significant decrease in nitrogen retention with fiber additions.

SUMMARY

Experiments were designed to test the utilization of alfalfa and three alfalfa fiber fractions — namely, extracted alfalfa, holocellulose, and lignin — through the use of paired-feeding techniques, nitrogen balances, and digestibility. Alfalfa lignin was not utilized for weight gains or nitrogen retention but was digested to a small extent only when fed in conjunction with low-protein rations. Alfalfa holocellulose was not utilized for weight gains or nitrogen retention when fed with a high-protein ration but did support a small weight increase and nitrogen retention when fed with a low-protein ration. From 8 to 14% of the holocellulose was apparently digested.

Alfalfa extracted with water and a benzene-alcohol mixture was utilized to a very small extent as an energy source; however, its nitrogn was utilized. Approximately 12% of the organic matter of the extracted alfalfa was apparently digested. In comparison, the soluble constituents of alfalfa were important for weight gain and nitrogen retention. The soluble constituents were from 69 to 75% digested.

LITERATURE CITED

ADOLPH, W. H., AND M. WU 1934 The influence of roughage on protein digestibility. J. Nutrition, *7:* 381.

DUCKWORTH, J., AND W. F. GODDEN 1935 The influence of dietary fibre on secretory activities of the alimentary tract. Observations on foecal phosphatase excretion and calcium and nitrogen balances of rats. Biochem. J., *35:* 16.

ELLIS, G. H., G. MATRONE AND L. A. MAYNARD 1946 A 72 percent sulfuric acid method for the determination of lignin and its use in animal nutrition studies. J. Animal Sci., *5:* 285.

FORBES, R. M., AND T. S. HAMILTON 1952 The utilization of certain cellulosic materials by swine. Ibid., *11:* 480.

FRAPS, G. S., AND E. C. CARLYLE 1941 Productive energy of corn meal, alfalfa leaf meal, dried buttermilk, casein, cottonseed meal and tankage as measured by production of fat and flesh by growing chickens. Texas Agr. Exp. Sta. Bull., 600.

HARNED, B. K., R. W. CUNNINGHAM AND E. R. GILL 1949 A metabolism cage for small animals. Science, *109:* 489.

HUMMEL, F. C., M. C. SHEPHERD AND I. G. MACY 1943 Disappearance of cellulose and hemicellulose from the digestive tracts of children. J. Nutrition, *25:* 59.

HVIDSTEN, H., AND T. HOMB 1947 A survey of cellulose and Beckmann-treated straw as a feed. Inst. Animal Nutr., Royal Agr. College of Norway, Rept. No. 62, pp. 14.

MANGOLD, E. 1934 The digestion and utilisation of crude fibre. Nutr. Abst. Rev., *3:* 647.

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

MITCHELL, H. H., AND T. S. HAMILTON 1933 True and apparent digestibility of oat hulls and alfalfa meal by swine with special reference to the ability of swine to digest cellulose and crude fiber. J. Agr. Res., *47:* 425.

SCHMIDT, J., AND E. LAGNEAU 1932 Verdauungsversuche an Schweinen mit Luzerneheumehl verschiedener Mahlung. Ztschr. f. Schweinezucht, *39:* 820. Nutr. Abst. Rev., *2:* 694.

SNEDECOR, G. 1946 Statistical methods applied to experiments in agriculture. Iowa State College Press, Ames.

TRAUTMAN, A., AND T. ASHER 1942 Über die Verdauung künstlicher und pflanzlicher zellulose Proparate im Blenddarm des Schweines. Biedermanns Zentralhl. (B) Tierernahrung, *14:* 353.

TSCHERNIAK, A., 1936 Über die Verdauung der Zellwandbestandteile des Futters (lignin, pentosane, cellulose, und rahfaser) durch das haushuhn. Ibid., *8:* 408.

WILLIAMS, R. D., AND W. H. OLMSTED 1936 The effect of cellulose, hemicellulose and lignin on the weight of the stool: A contribution to the study of laxation in man. J. Nutrition, *11:* 443.

WISE, L. E., M. MURPHY AND A. A. D'ADDIECO 1946 Chlorite holocellulose, its fractionation and bearing on summative wood analyses and on studies on the hemicelluloses. Paper Trade J., *122:* 35.

WOODMAN, H. E., AND R. E. EVANS 1947 The nutritive value of fodder cellulose from wheat straw. I. Its digestibility and feeding value when fed to ruminants and pigs. J. Agr. Sci., *37:* 202.

QUANTITATIVE INTERRELATIONSHIPS BETWEEN THE EFFECTS OF IODINE AND THIOURACIL ON THYROID FUNCTION[1]

D. R. HIGHLEY, H. E. PARKER AND F. N. ANDREWS
Departments of Biochemistry and Animal Husbandry, Purdue Agricultural Experiment Station, West Lafayette, Indiana

(Received for publication February 25, 1954)

The modifying influence of iodine upon the goitrogenicity of thiouracil has been studied by Astwood ('43), Mackenzie ('47), McGinty ('49) and others. However, there is a need for more information concerning the quantitative interrelationships between thiouracil and the iodine intake of the animal.

There has been a tendency to minimize or overlook the importance of dietary iodine in studies concerning the activity of the goitrogens.

In an effort to re-emphasize the importance of dietary iodine in the response to thiouracil and to develop a more quantitative evaluation of the interrelationships between thiouracil and ingested iodine these experiments were undertaken.

EXPERIMENTAL

In experiment 1, 160 weanling, male white rats of the Wistar Strain were divided into 16 groups (table 1) and placed in individual wire cages. An incomplete block, balanced lattice design was used to facilitate adjustment of litter difference. The rats were fed diets containing 20, 100, 400, and 1600 parts per billion (p.p.b.) of iodine for 6 weeks and during the 5th and 6th weeks the diets at each iodine level were

[1] Journal Paper no. 779 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

supplemented with thiouracil at the 4 levels indicated in table 1. At the end of the 6th week, the animals were sacrificed by a blow on the head. Thyroid and adrenal glands were excised and weighed. One thyroid from each animal was reserved for histological examination. The remaining thyroids were grouped according to treatment, dried to constant weight at 100°C. and analyzed for total iodine.

In experiment 2, the effect of the iodine level in the diet prior to and during thiouracil feeding on the response to thiouracil was determined using 90 weanling male rats treated as shown in table 2. Analysis of data from experiment 1 had shown that litter difference caused only slight variations in response, therefore no effort was made in this experiment to adjust for litter difference. Autopsy was performed in the same manner as in experiment 1.

The percentage composition of the diet used in the experiments was as follows: ground yellow corn, 58.6; wheat germ,[2] 35.0; minerals, 4.0; cottonseed oil, 2.0; and DL-methionine, 0.4. Vitamin additions in mg per 100 gm of diet were as follows: riboflavin, 1.0; thiamine hydrochloride, 0.75; nicotinic acid, 5.0; *i*-inositol, 30.0; pyridoxine hydrochloride, 1.0; choline chloride, 200; biotin, 0.01; folic acid, 0.01; calcium pantothenate, 5.0; *p*-amino benzoic acid, 30.0; 2-methyl-1,4-naphthoquinone, 0.50; B_{12}, 0.002; α-tocopherol, 25.0; vitamin A acetate, 0.05; and calciferol, 0.005. The mineral mixture was prepared as described by Parker et al. ('51), from compounds known to be low in iodine. This diet was found to contain 20 p.p.b. of iodine using the combustion procedure described by Godfrey et al. ('51) and the catalytic method of Chaney ('40). The latter procedure was modified by replacing the complicated iodine trap with a simple distillation apparatus and gently evaporating the distillate to a convenient volume for analysis. Additional dietary iodine levels were obtained by adding potassium iodide to the feed.

[2] Partially defatted; Viobin Corporation, Monticello, Illinois.

TABLE 1

The influence of the dietary iodine level upon the response to thiouracil

GROUP [1]	TREATMENT			AVERAGE GAIN PER WEEK		DRY THYROID WEIGHTS PER 100 GM BODY WT.[3]	THYROID IODINE (DRY WEIGHT BASIS)	ADRENAL WEIGHT (FRESH BASIS)	
	Iodine in diet	Thiouracil		First 4 weeks	Last 2 weeks [2]			% Body weight	Total
		In diet	Per 24 hours						
	µg/kg	%	*mg/kg B.W.*	*gm*	*gm*	*mg*	%	*mg*	*mg*
1	20	0.000	0.00	38.6	41.0	2.87	0.0032	12.24	34.86
2	20	0.005	3.14	35.9	38.9	4.07	0.0028	12.77	33.19
3	20	0.025	15.29	37.4	17.7	4.52	0.00095	13.13	30.20
4	20	0.100	59.42	34.9	12.4	4.90	0.00062	13.81	27.91
5	100	0.000	0.00	37.6	37.9	1.50	0.077	11.80	32.40
6	100	0.005	3.13	35.2	40.0	2.11	0.012	12.46	32.95
7	100	0.025	15.12	35.4	34.6	3.97	0.0033	11.96	30.45
8	100	0.100	61.68	34.9	21.8	4.34	0.0018	13.01	28.16
9	400	0.000	0.00	36.8	43.8	1.28	0.240	14.56	40.63
10	400	0.005	3.25	34.7	41.0	1.69	0.054	13.81	34.35
11	400	0.025	15.95	36.5	38.1	2.64	0.0067	13.09	35.38
12	400	0.100	63.76	38.6	25.8	3.80	0.0034	11.11	28.08
13	1600	0.000	00.00	37.7	38.7	1.45	0.272	12.57	34.45
14	1600	0.005	3.29	38.4	42.6	1.50	0.076	12.35	34.98
15	1600	0.025	14.38	34.9	42.5	2.04	0.0198	12.12	33.81
16	1600	0.100	66.66	36.7	27.8	3.03	0.0088	11.71	29.81

[1] Each group consisted of 10 rats.

[2] Least significant difference: 5% level 5.14, 1% level 6.79.

[3] Least significant difference: 5% level 0.57, 1% level 0.75.

TABLE 2

The effect of iodine intake before and during thiouracil feeding upon the response to thiouracil

GROUP [1]	TREATMENT				AVERAGE GAIN PER WEEK		DRY THYROID WEIGHT PER 100 GM BODY WEIGHT	THYROID IODINE (DRY WEIGHT BASIS)	ADRENAL WEIGHT (FRESH BASIS)	
	Iodine		Thiouracil							
	First 4 weeks	Last 2 weeks	First 4 weeks	Last 2 weeks	First 4 weeks	Last 2 weeks			% Body weight	Total
	µg/kg	*µg/kg*	*%*	*%*	*gm*	*gm*	*mg*	*%*	*mg*	*mg*
1	Killed at weaning						2.92	0.088		
2	400	0	0.0	0.0	36.93		1.67	0.192		
3	400	20	0.0	0.1	33.00	31.10	3.59	0.0016		
4	400	400	0.0	0.1	35.22	28.95	3.48	0.0023		
5	400	1600	0.0	0.1	35.15	31.95	3.00	0.0067		
6	20	20	0.0	0.1	33.78	14.30	5.28	0.00066	11.66	24.18
7	20	400	0.0	0.1	33.75	14.40	4.75	0.0019	11.91	25.66
8	20	20	0.1	0.1	9.70	— 1.35	10.72	0.0008	18.78	15.00
9	400	400	0.1	0.1	11.62	1.60	9.99	0.0036	17.22	15.44

[1] Each group consisted of 10 rats.

RESULTS

The growth rate of the rats was not affected by the iodine content of the diet when the thiouracil level was 0.005% or less (table 1). Increasing the iodine level to 400 p.p.b. counteracted the growth reducing effect of 0.025% thiouracil, but 1600 p.p.b. of iodine did not completely neutralize the effect of 0.1% thiouracil. The addition of thiouracil caused an increase in thyroid size and a decrease in thyroid iodine concentration of rats at all levels of iodine; however, thiouracil affected thyroid size and iodine content more at the 100 and 400 p.p.b. levels of iodine than at low or high iodine levels.

It was found that the mathematical relationship developed by Bargeton et al. ('49) that thyroid weight (mg % body weight) was proportional to the log log of the dose of thiouracil (mg/kg body weight/day) was not valid at all dietary iodine levels (table 1). This relationship exists when the iodine level in the diet is relatively low (20 to 400 p.p.b.). At higher iodine levels (400 to 1600 p.p.b.) the thyroid weight appears to be proportional to the log of the dose of thiouracil.

The thyroid glands from all rats fed the lowest level of iodine contained scant amounts of colloid, the epithelial cells were columnar, and hypertrophy and hyperplasia were evident. The histological appearance of the thyroids of rats receiving thiouracil at all levels of iodine resembled that of iodine-deficient rats. Columnar epithelial cells, hypertrophy and hyperplasia were observed in the thyroids of rats at the highest level of iodine when as little as 0.005% thiouracil was included in the diet.

While there were no significant differences in adrenal weights at different dietary levels of iodine or thiouracil in trial 1, certain trends were observed. Increasing the thiouracil level tended to decrease the adrenal weight (mg% body weight) at the two higher levels of iodine, but at the two lower levels of iodine an increase in dietary thiouracil appeared to cause an increase in adrenal weight. When 0.1% thiouracil was administered for 6 weeks to rats fed 20 or 400 p.p.b. of iodine a significant increase in adrenal weight was observed

(table 2). Histological examinations of adrenal sections failed to reveal any definite cytological differences in either the medulla or cortex due to thiouracil treatment.

The iodine level in the diet of the rats before the addition of thiouracil had a pronounced effect on the growth response to thiouracil (table 2). When weanling rats were fed an iodine-deficient diet for 4 weeks and then fed thiouracil for two weeks the rate of growth (during the latter two weeks) was greatly reduced regardless of the iodine content of the thiouracil-containing diet. Rats receiving 0.1% thiouracil for 6 weeks grew poorly and were cretinoid in appearance.

The increase in thyroid size and decrease in thyroid iodine caused by thiouracil were partially counteracted by increased iodine levels before or during thiouracil feeding.

DISCUSSION

The comparative thyroid enlargement and decreased iodine content of the thyroid of rats caused by thiouracil were dependent upon the level of iodine in the diet. These effects were greatest near the minimum iodine requirement of the normal rat which was found to lie between 100 and 400 p.p.b. of iodine.

That thiouracil exerted its growth-reducing activity through its action on the thyroid gland is indicated by the fact that the growth-reducing influence was depressed when the intake of iodine was increased. As would be expected iodine intake prior to thiouracil feeding modified the growth response to thiouracil. Growth retardation caused by thiouracil was overcome by increased dietary iodine provided the thiouracil content of the diet did not exceed 0.025%.

When the dietary level of iodine was near the minimum iodine requirement of the rat, thyroid enlargment caused by thiouracil appeared to be proportional to the log log of the dose of thiouracil. This is in agreement with the findings of Bargeton et al. If, however, the iodine content of the diet is increased, this relationship no longer exists, emphasizing

the importance of iodine intake when the potency of a goitrogen is being investigated.

Increased adrenal size observed after 6 weeks of thiouracil feeding was not in agreement with the findings of Zarrow and Money ('49), or Freedman and Gordon ('50). These conflicting results are interesting when viewed in terms of a trend observed in experiment 1. When the iodine intake was below the iodine requirement of the rat, increased thiouracil appeared to cause adrenal enlargement, but when the minimum iodine requirement was exceeded, decreased adrenal size was observed with increased thiouracil. It should be emphasized that these changes were small but this approach might help explain adrenal atrophy obtained in long term thiouracil feeding experiments with animals fed commercial feeds that were relatively high in iodine.

SUMMARY

In quantitative studies of the effect of goitrogens at different iodine levels it was found that the synthetic goitrogen, thiouracil, exerted a maximum effect on the thyroid glands of rats when the iodine level in the diet was near the minimum level required to prevent thyroid enlargement in the normal rat. High dietary iodine decreased, but did not prevent, the effects of thiouracil on thyroid size, histology, and iodine content.

It was found that the mathematical relationship between thyroid size and the dose of thiouracil changed with alterations in the dietary iodine level. Increasing the iodine intake completely counteracted the effect of thiouracil on growth only at the lower levels of thiouracil. Thiouracil appeared to exert its growth-reducing effect by way of the thyroid gland, at least at the lower levels used.

The iodine level in the diet prior to the feeding of thiouracil had a pronounced effect on the response to thiouracil. When thiouracil was fed for 6 weeks, adrenal enlargement was observed. This is in contrast to previous reports, and may have been due to differences in the iodine level of the diet.

LITERATURE CITED

ASTWOOD, E. B. 1943 The chemical nature of compounds which inhibit the function of the thyroid gland. J. Pharmacol., *78:* 79.

BARGETON, D., C. KRUMM-HELLER AND F. DE FOMBELLE 1950 Quantitative study of the hypertrophy of the thyroid provoked by thiouracil derivatives. Compt. rend. soc. biol., *144:* 650.

CHANEY, A. L. 1940 Improvements in determination of iodine in blood. Ind. Eng. Chem., Anal. Ed., *12:* 179.

FREEDMAN, H. H., AND A. S. GORDON 1950 Effects of thyroidectomy and thiouracil on adrenal weight and ascorbic acid. Proc. Soc. Exp. Biol. Med., *75:* 729.

GODFREY, P. R., H. E. PARKER AND F. W. QUACKENBUSH 1951 Polarographic determination of iodine in water, soil and plant material. Anal. Chem., *23:* 1850.

MACKENZIE, C. G. 1947 Differentiation of the antithyroid action of thiouracil, thiourea, and P.A.B.A. from sulfonamides by iodide administration. Endocrinology, *40:* 137.

MCGINTY, D. A. 1949 Iodine absorption and utilization under the influence of certain goitrogens. Ann. N. Y. Acad. Sci., *50:* 403.

PARKER, H. E., F. N. ANDREWS AND F. W. QUACKENBUSH 1951 Studies on the iodine requirements of white rats during growth, pregnancy and lactation. J. Nutrition, *44:* 501.

ZARROW, M. X., AND W. L. MONEY 1949 Involution of the adrenal cortex of rats treated with thiouracil. Endocrinology, *44:* 345.

THE INFLUENCE OF FLAVONOID COMPOUNDS ON THE NASAL EXCRETION OF A RED PIGMENT BY RATS SUBJECTED TO STRESS CONDITIONS[1,2]

MANUEL SCHREIBER AND CONRAD ARNOLD ELVEHJEM
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

(Received for publication March 29, 1954)

INTRODUCTION

In a preliminary study by Collins, Schreiber, Niño-Herrera and Elvehjem ('52), and in a more detailed report by Collins, Schreiber and Elvehjem ('53), the influence of high and low relative humidity conditions upon young albino rats fed vitamin-deficient diets was described.

The animals maintained at 90% relative humidity developed within a period of one to three weeks, a red-stained fur about the head and body. This condition was nearly non-existent in animals maintained below 50% relative humidity. The greatest extent and incidence of this phenomenon occurred among the rats receiving diets deficient in riboflavin, pantothenic acid or pyridoxine, and to a lesser degree among those fed a vitamin A-deficient diet.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

[2] We are indebted to the Eastern Regional Research Laboratory, BAIC-ARA, USDA, Philadelphia, Pa., for the quercetin, rutin, and quercitrin; to the Sunkist Growers Inc., Research Department, Ontario, California, for the hesperetin, hesperidin, naringenin, naringin and hesperidin-methyl-chalcone; to Abbott Laboratories, North Chicago, Ill., for the penicillin and streptomycin; and to the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., for the aureomycin.

When illuminated with ultraviolet light, the nasal exudate gave a red fluorescence similar to that of the porphyrin pigments arising from the Harderian glands of rats subjected to a vitamin A deficiency or to acetylcholine-induced chromodacryorrhea, or "bloody tears." The extent of production of chromodacryorrhea, using the method devised by Tashiro, Smith, Badger and Kezur ('40), was not influenced by the humidity conditions or the vitamin deficiencies studied.

TABLE 1

Composition of complete diet

	Casein (vitamin free)	18%	
	Sucrose	73%	
	Cottonseed oil	5%	
	Salts IV [1]	4%	
	Vitamins (mg/100 gm of diet):		
Thiamine·HCl	0.4 [2]	Folic acid	0.2
Riboflavin	0.3	Vitamin B_{12}	0.002
Pyridoxine·HCl	0.2	Choline chloride	100.0
Nicotinic acid	2.5	β-Carotene	0.5
Ca Pantothenate	2.0	Calciferol	0.005
Inositol	10.0	α-Tocopherol	3.0
Biotin	0.01	Menadione	1.0

[1] Hegsted, Mills, Elvehjem and Hart ('41).

[2] Fed initially at 0.2 mg/100 gm of diet. Changed to 0.4 mg/100 gm of diet in experiment 400 et seq.

Since flavonoid compounds have been reported by a number of workers to possess beneficial action for the alleviation of a variety of pathological conditions including the reduction of capillary fragility and permeability while other investigators have reported negative results (see compilations on the subject by Couch, '48, and Couch, Krewson and Naghski, '51), it was decided to study the effect of the flavonoid compounds on the red pigment excretion.

EXPERIMENTAL

Male weanling albino rats of the Sprague-Dawley strain were given a complete diet (table 1) and B-vitamin-deficient

diets prepared by omitting the appropriate vitamin. Ad libitum feeding of the diets and water administration by bottle were the general procedures employed with exceptions noted. Supplements were incorporated into the diets.

The animals were housed in individual raised screen-bottom cages and were maintained in chambers at a controlled relative humidity of approximately 90% and a temperature of 24 to 26°C. Calibrated instruments were used to check the experimental conditions.

In order to evaluate the extent of the red pigment excretion, an arbitrary rating system was devised: (0.0) = no observable red pigment; (1.0) = slight excretion; (2.0) = moderate excretion; and (3.0) = severe red pigment excretion.

All experiments were of three weeks duration. Water consumption records were kept in every experiment. The results of this study appear in table 2.

RESULTS

The purpose of experiment 100 was to determine whether the flavonols — rutin, quercetin, and quercitrin — when incorporated jointly into riboflavin- or pyridoxine-deficient diets possessed activity in preventing the nasal excretion of the red pigment. The inclusion of all three flavonols in the deficient diets prevented the discharge of pigmented exudate and altered water consumption (see table 2, experiment 100).

The following objectives were studied in experiment 200: the excretion-preventive activity of the flavonols at levels approaching those of the vitamins; the effect of feeding thiamine-, choline-, and niacin-deficient diets; and the effect of food or water restriction, or both, using a complete diet. Food and water consumption data in earlier experiments revealed an increasing self-restriction by animals fed deficient diets with progressive depletion of vitamin stores. Restricted levels were based on food and water consumption values obtained with the riboflavin deficiency, namely, 4 gm of food and 4 ml of water/rat/day.

TABLE 2

Nasal excretion of red pigment and water consumption by rats under controlled conditions of humidity and temperature and fed vitamin-deficient diets supplemented with flavonoid compounds. (Experimental conditions: Relative humidity, 90%; temperature, 25°C. All experiments were of three weeks duration.)

EXP. NO.	GROUP NO.	DIETS	WT. GAIN (3 WKS.)	EXTENT,[1] INCIDENCE OF RED PIGMENT EXCRETION		AV. H_2O INTAKE/ RAT/DAY
			gm			*ml*
100	101	Complete	94	(0.2)	3/6[2]	7.3
	102	Complete — riboflavin	13	(1.8)	6/6	5.1
	103	Same as 102 + 0.25% of each of 3 flavonols[3]	15	(0.1)	1/6	3.6
	104	Complete — pyridoxine	35	(1.2)	6/6	4.8
	105	Same as 104 + 0.25% of each of 3 flavonols	28	(0.1)	1/6	5.8
200	201	Complete	92	(0.1)	1/4	8.4
	202	Complete (4 ml H_2O/day by cup)	62	(0.6)	3/4	4.0
	203	Complete (4 gm food/day)	22	(0.2)	2/4	6.5
	204	Complete (4 ml H_2O by cup and 4 gm food/day)	21	(0.2)	2/4	4.0
	205	Complete — riboflavin	11	(2.0)	4/4	4.2
	206	Same as 205 + 0.1% rutin	11	(0.7)	2/4	6.5
	207	Same as 205 + 0.1% quercetin	11	(0.4)	1/4	4.7
	208	Same as 205 + 0.1% quercitrin	8	(2.7)	4/4	4.3
	209	Same as 205 + 0.05% of each of 3 flavonols[3]	12	(0.6)	1/4	5.6
	210	Same as 205 + 0.005% of each of 3 flavonols	15	(1.7)	2/2	4.9
	211	Same as 210 + 0.25% vitamin C	7	(2.5)	2/2	5.0
	212	Complete — thiamine	12	(1.2)	3/3	5.2
	213	Complete — choline	88	(0.6)	2/2	9.0
	214	Complete — niacin	62	(0.3)	1/2	
300	301	Complete	104	(0.0)	0/4	10.2
	302	Complete (2 ml H_2O/day by cup)	47	(2.4)	4/4	2.0
	303	Same as 301 + 0.25% quercitrin	110	(0.1)	1/4	9.7
	304	Same as 301 — thiamine	15	(2.4)	4/4	4.6
	305	Same as 304 + 0.25% quercetin	14	(0.2)	2/4	5.2
	306	Complete — riboflavin	13	(2.0)	3/3	4.6
	307	Same as 306 + 0.1% quercetin	15	(0.2)	1/4	3.9
	308	Same as 306 + 0.25% quercetin	13	(0.1)	1/4	4.5
	309	Complete — pyridoxine	39	(1.6)	3/3	5.5
	310	Same as 309 + 0.25% quercetin	41	(1.5)	4/4	5.4
	311	Complete — pantothenate	48	(1.4)	4/4	8.0
	312	Same as 311 + 0.25% quercetin	46	(0.1)	1/4	9.7
	313	Same as 311 + 0.25% quercitrin	43	(0.2)	2/4	8.9

EXP. NO.	GROUP NO.	DIETS	WT. GAIN (3 WKS.)	EXTENT,[1] INCIDENCE OF RED PIGMENT EXCRETION		AV. H_2O INTAKE/ RAT/DAY
			gm			*ml*
500-A [4]	501	Complete	104	(0.3)	3/4	10.8
	502	Complete (2 ml H_2O/day)	45	(2.1)	4/4	2.0 [5]
	503	Same as 502 + 0.25% quercetin	45	(0.0)	0/4	2.0
	504	Same as 502 + 0.25% quercitrin	42	(0.5)	3/4	2.0
	505	Same as 502 + 8 mg quercitrin [6]	43	(1.2)	4/4	2.0
	506	Complete — riboflavin	13	(2.2)	3/3	4.4
	507	Same as 506 + 0.25% quercetin	12	(0.3)	2/4	4.7
	508	Same as 506 + 0.02% salicylic acid	18	(1.5)	4/4	6.1
	509	Same as 506 + 8 mg rutin [6]	10	(0.9)	3/4	4.6
	510	Same as 506 + 8 mg quercitrin [6]	17	(0.9)	4/4	8.8
	511	Same as 506 + 0.02% penicillin	13	(1.5)	4/4	4.6
	512	Same as 506 + 0.02% streptomycin	15	(1.8)	4/4	4.6
500-B [7]	513	Complete	103	(0.0)	0/4	12.0
	514	Complete + 0.25% rutin	107	(0.0)	0/4	13.6
	515	Complete + 0.25% quercitrin	96	(0.0)	0/4	14.4
	516	Complete + 0.25% quercetin	104	(0.0)	0/4	13.0
	517	Complete (2 ml H_2O/day)	18	(0.5)	4/4	2.0 [8]
	518	Same as 517 + 0.25% rutin	21	(0.2)	3/4	2.0
	519	Same as 517 + 0.25% quercitrin	20	(0.5)	3/4	2.0
	520	Same as 517 + 0.25% quercetin	19	(0.2)	3/4	2.0
	521	Complete (1 ml H_2O/day)	—9	(0.9)	4/4	1.0
	522	Complete (3 ml H_2O/day)	39	(0.2)	2/4	3.0
	523	Complete (4 ml H_2O/day)	54	(0.1)	1/4	4.0
700	701	Complete	101	(0.1)	1/4	10.8
	702	Complete (2 ml H_2O/day)	41	(1.8)	4/4	2.0 [9]
	703	Same as 702 + 0.25% quercetin	40	(0.0)	0/4	2.0
	704	Same as 702 + 0.25% rutin	43	(0.2)	2/4	2.0
	705	Same as 702 + 0.25% quercitrin	42	(0.4)	3/4	2.0
	706	Same as 702 + 0.25% hesperetin	40	(1.4)	4/4	2.0
	707	Same as 702 + 0.25% naringenin	39	(1.9)	4/4	2.0
	708	Same as 702 + 0.25% hesperidin	39	(1.5)	4/4	2.0
	709	Same as 702 + 0.25% naringin	38	(0.7)	4/4	2.0
	710	Same as 702 + 0.25% hesperidin-methyl-chalcone	38	(1.6)	4/4	2.0
	711	Same as 702 + 0.1% menadione	39	(1.0)	4/4	2.0

[1] Extent is based on the total number of animals in each group.

[2] Denominators indicate the number of animals/group.

[3] Three flavonols include: quercetin, rutin and quercitrin.

[4] Thiamine level was increased from 0.2 to 0.4 mg/100 gm of diet in this experiment et seq.

[5] Groups 502-5 received water in cups.

[6] Injected intraperitoneally each day.

[7] Experiment 500-B conducted in animal room: relative humidity, approx. 60%; temperature, 24–26°C.

[8] Groups 517-23 received water in cups.

[9] Groups 702–11 received water in cups.

Only the water-restrictive treatment with animals fed the complete diet provided an indication of increased pigment excretion; food- and food-plus-water-restriction did not (groups 201–4). Quercetin and rutin at a level of 0.1% demonstrated good but incomplete activity with the riboflavin deficiency, whereas, 0.1% quercitrin was ineffective. A 0.05% level of all three flavonols also reduced the extent of pigmentation; however, a 0.005% level with or without ascorbic acid supplementation gave no protective action. The thiamine deficiency and to lesser extents the choline and niacin deficiencies paralleled the excretion phenomena witnessed in the initial B-vitamin deficiency studies.

To check the possibility of an antagonistic action of quercitrin (see groups 205 and 208) and quercetin's efficacy with other B-vitamin deficiencies, experiment 300 was conducted. The inclusion of quercitrin in the complete diet did not induce excretion (groups 301 and 303). In the pantothenate-deficient diet it was protective (groups 311 and 313). One-quarter per cent quercetin was effective with the thiamine-, riboflavin-, or pantothenate-deficient diets but not so with the pyridoxine deficiency. When water was restricted to 2 ml /day to animals of group 302 receiving the complete diet, a moderate-to-severe extent of red pigment was observed and the growth rate was less than 50% of their control. There were thus two ways of producing the nasal excretion or red pigment extensively in the high relative humidity environment, by the feeding of B-vitamin-deficient diets or the use of water-restrictive measures with the complete diet.

Experiment 400, not tabulated here, revealed that with the pyridoxine deficiency 1% quercetin or 0.25% of each of the three flavonols fed simultaneously prevented the nasal discharge of pigment. Good protection was again obtained by adding 0.25% quercetin to the thiamine-, riboflavin-, or pantothenate-deficient diets as in experiment 300.

Up to this time the flavonols had been incorporated into the various diets. Although the urine of animals fed these compounds had acquired a color similar to that of the flavonols,

thus indicating absorption, the bulk of these compounds could conceivably be excreted in the feces (changed or unchanged) and therefore perform their primary action in the gastrointestinal tract by altering or modifying the flora or the waste products of these organisms. Two measures were adopted to test this hypothesis. The first was to by-pass the tract by injecting the flavonols. Unfortunately, these compounds were practically water- and fat-insoluble but could be made soluble in water with alkali; quercetin completely dissolved at pH 10.9, rutin at 8.5 and quercitrin at 8.0. Because of the large quantity of alkali required to render these substances (especially quercetin) soluble only alkaline solutions of rutin and quercitrin could be used for intraperitoneal injections. The second measure to test the hypothesis was to feed antibiotics to see if the results obtained with the flavonols could be achieved with antibiotics. Salicylic acid, reported to have pharmacological properties similar to the flavonoid compounds, was also assayed.

Restricting water to 2 ml/day to animals fed the complete diet produced a moderate extent of red pigment excretion (groups 501–2). When 0.25% quercetin was given to a similarly-treated group, complete protection occurred; quercitrin was almost as active. Intraperitoneal injection of quercitrin gave only partial protection, but the large alkaline content of each daily dose obscured the interpretation of the results. The same situation was encountered with the animals of groups 509 and 510 which were fed the complete-riboflavin diet. Here also partial protection was obtained with rutin or quercitrin by the injection route; salicylic acid, penicillin, or streptomycin were of little value when given in addition to the riboflavin-deficient diet.

Experiment 500-B was run concurrently with 500-A but was performed in our animal room at a lower and more variable relative humidity. All groups in this experiment were fed the complete diet. Animals of groups 514–16 consumed more water when given any one of the three flavonols but grew approximately at the same rate as their control, group 513,

and exhibited no sign of pigmentation. Animals of groups 518–20, supplied 2 ml of water/day, also gained about the same weight as their control, group 517, and, like their control, showed only the very slightest indication of pigment excretion. Inspection of water cups provided no evidence of washed-off pigment. By decreasing the daily water allotment in 1 ml gradations, a progressively lower growth rate was obtained (see weight gain of groups 513, 523, 522, 517, and 521). Only when 1 ml of water/rat/day was supplied to the rats of group 521 could a slight extent of nasal discharge of pigment be produced in an environment of 60% relative humidity.

A comparison of the weight gain and pigment excretion data for the two groups receiving 2 ml of water/rat/day (group 502 maintained at 90% and group 517 at 60% relative humidity) dramatized the great influence of humidity when water was the limiting nutrient. The group maintained at 90% relative humidity grew at a rate 150% faster and excreted considerably more pigment than did their counterpart at 60% relative humidity. (This particular work has been repeated on a subsequent occasion.) Groups 501 and 513, given access to water ad libitum, grew similarly and displayed minimal pigmentation but differed somewhat in their water consumption, a consequence of the different humidity environments.

The ratios of thymus weight to body weight and of kidney weight to body weight of the animals in all groups of experiment 500-A and 500-B have been calculated; however, only those of key groups are presented in table 3. Inspection of the ratios for those groups which received graded amounts of water revealed that as the water allotment was reduced the ratio of thymus weight to body weight declined progressively, whereas the ratio of kidney weights to body weight increased (see values for groups 513, 523, 522, 517, and 521 in table 3). Note also the ratios for groups 502 and 517 referred to above.

The acquisition of 5 additional flavonoid compounds — hesperetin, hesperidin, naringenin, naringin, and hesperidin-methyl-chalcone — permitted the testing of their excretion-preventive properties. In the first trial (experiment 600) inconclusive results were obtained because the (negative) control group displayed erratic pigmentation; however, 0.04%

TABLE 3

Ratios of thymus weight to body weight and of kidney weights [1] to body weight of key groups [2] in experiments 500-A and 500-B

GROUP NO.	DIETS	$\frac{\text{THYMUS WEIGHT}}{\text{BODY WEIGHT}} \times 1000$	$\frac{\text{KIDNEY WEIGHTS}}{\text{BODY WEIGHT}} \times 100$
501 [3]	Complete (H_2O ad libitum)	4.87 (4.45–5.55) [4]	0.98 (0.96–1.04) [4]
502	Complete (2 ml H_2O/day)	3.56 (3.29–3.81)	1.10 (1.04–1.16)
503	Same as 502 + 0.25% quercetin	4.06 (3.86–4.16)	1.07 (1.01–1.18)
504	Same as 502 + 0.25% quercitrin	3.99 (3.11–4.61)	1.11 (1.07–1.13)
506	Complete — riboflavin	1.06 (0.83–1.63)	1.28 (1.18–1.34)
507	Same as 506 + 0.25% quercetin	1.85 (1.19–2.77)	1.23 (1.12–1.30)
513 [5]	Complete (H_2O ad libitum)	4.72 (4.17–5.17)	0.98 (0.91–1.05)
523	Complete (4 ml H_2O/day)	3.67 (3.48–4.00)	1.10 (1.06–1.18)
522	Complete (3 ml H_2O/day)	3.40 (3.25–3.69)	1.15 (1.11–1.18)
517	Complete (2 ml H_2O/day)	2.98 (2.75–3.10)	1.33 (1.28–1.44)
521	Complete (1 ml H_2O/day)	0 [6]	1.64 (1.56–1.70)

[1] Both kidneys used to determine kidney weights.
[2] Animals sacrificed at the end of the three-week experiment.
[3] Groups 501 to 507, inclusive, maintained at 90% relative humidity.
[4] Figures in parentheses refer to range of values for each group.
[5] Groups 513 to 523, inclusive, maintained at 60% relative humidity.
[6] Complete involution of thymus glands in this group.

aureomycin and 0.04% penicillin were of no benefit. Consequently, a re-examination of the efficacy of all the flavonoid compounds at hand was conducted in experiment 700 using the water restriction technique of alloting to animals 2 ml of water/day with the complete diets. Although 0.001% menadione was routinely added to all diets (see table 1), its structural similarity to that of the flavonoids suggested a trial at a higher level, e.g., 0.1%.

Quercetin, rutin, and quercitrin fed at a level of 0.25% to the rats of groups 703 to 705, respectively, were effective in keeping the extent of red pigment excretion at a minimum. The animals of group 709, receiving naringin, were partially protected, whereas, hesperetin, hesperidin, naringenin, and hesperidin-methyl-chalcone were practically inactive. Menadione supplied at the 0.1% level to the rats of group 711 gave rather poor protection.

DISCUSSION

Graffin ('41) observed that the Harderian gland secreted porphyrin. Prior to this, earlier investigators had established the fact that this gland synthesized and stored porphyrin. Figge and Atkinson ('41) attempted to correlate the extent of porphyrin excretion with the size and degree of fluorescence of the Harderian gland. Ablation of this gland by McElroy, Solomon, Figge and Cowgill ('41) and by Figge and Solomon ('42) prevented the accumulation of the red pigment in rats fed a pantothenic acid-deficient diet.

By the maintenance of an atmosphere with a constant high relative humidity the two types of nutritional stresses studied in this laboratory were responsible for the production of a uniform, extensive amount of red pigment excretion. The imposition of either the environmental or nutritional stresses in the absence of the other yielded practically no pigmentation during the three-week periods of experimentation. The mechanism whereby high relative humidity was instrumental in insuring consistent excretion remains unsolved. Selye's belief ('50) that simultaneous exposure to several systemic stressors can produce a summated effect, might explain the coupling of the environmental stress (high relative humidity) with the nutritional stresses to induce the nasal discharge of pigment.

Smith ('32) reported that rats denied food but given water ad libitum exhibited no red-stained fur; however, when deprived of water but given food ad libitum, the animals developed what was called "blood-stained" wrists. The mouth,

nose, and eyes were also stained. We have performed this type of experiment in our animal room at a relative humidity of approximately 37% using a complete (synthetic) diet. The extent of pigmentation was considerably less than that observed when the animals were maintained at high humidity and given water-restrictive of vitamin-deficient diets. Smith and Sprunt ('35) and Smith ('42) related the red staining of paws and whiskers of rats to dehydration effects. Figge and Atkinson ('41) observed that water deprivation or limitation induced porphyrin incrustations in rats supplied with unlimited amounts of food. Although there was a tendency for animals receiving less water to develop the condition more rapidly than those receiving a larger allotment, these workers found little correlation between the absolute volume of water ingested and the extent of pigment excretion. This finding is in agreement with the observations of Collins et al. ('53), yet in the present study a reduction in the restricted water ration from 4 to 2 ml/rat/day increased the extent of discharge of red pigment (see groups 202 and 302, table 2). It was also noticed in the initial study that when animals were fed the B vitamin-deficient diets and maintained at the different humidities, those which were exposed to an atmosphere of 90% relative humidity excreted the most pigment and drank the least amount of water. On the other hand animals given 1 ml of water/day and kept at a relative humidity of 60% developed only half the extent of pigmentation of those rats which received 2 ml of water/day in 90% relative humidity (see groups 521 and 502, table 2). Furthermore, the addition of the active flavonoids to various diets was instrumental in minimizing or preventing the discharge of red pigment but provided no consistent effect on water consumption. Lemberg and Legge ('49) have reviewed the porphyrin metabolism of the Harderian gland and have correlated the porphyrin excretion resulting from B vitamin deficiencies with that resulting from water restriction. There is, therefore, ample evidence to suggest a link of the excretion

phenomenon with a disturbance in water metabolism. The relationship does not appear to be direct or consistent.

The action of the flavonols in minimizing or preventing the discharge of red pigment might be explained by any of the following: (1) prevention of permeability alterations in the nasolacrymal duct of the Harderian gland, (2) correction of the disturbance in water metabolism with subsequent stabilization of a balance, (3) prevention of vascular leakage in the respiratory tract. Whether the flavonols can influence the synthesis, concentration, or distribution of the porphyrins must also be considered a possibility.

The fact that the intraperitoneal injection of rutin or quercetin provided partial protection (groups 505, 509, and 510) would suggest that these flavonols could function systemically, although they might be excreted into the tract following injection and perform their action there. (Examination of the collecting tubules of the kidneys of stock animals injected intraperitoneally with these compounds revealed evidence of their presence). Inasmuch as levels of the flavonols in excess of 0.1%, and 0.25%, in the case of the pyridoxine deficiency, were necessary to demonstrate excretion-preventative action, it appeared that they were active systemically; the relatively non-aqueous, non-lipid solubility of these compounds would necessitate their use in amounts considerably larger than those of the vitamins to insure adequate absorption. The ineffectiveness of feeding penicillin, aureomycin, or streptomycin favors the view that the flavonols were active in regions other than the gastro-intestinal tract.

Of what value these flavonoid compounds are to animals subjected to environmental and nutritional stresses other than to prevent or minimize the excretion of red pigment cannot be stated at this time. However, healthy animals receiving adequate diets with water supplied ad libitum, and maintained in a "normal" environment did not excrete the pigment to any extent. It is not inconceivable to assume that the mechanism blocking the nasal discharge of red pigment could also serve as a barrier by denying invasive and toxic agents a

portal of entry. Consequently, it might be necessary to give consideration to the possible requirement or need of animals, at least the albino rat, for the active flavonoid compounds or other substances with similar properties when dealing with stress situations, intentional or otherwise.

SUMMARY

1. A nasal excretion of a red pigment was observed in young male albino rats maintained at approximately 90% relative humidity for one to three weeks and fed (a) a diet deficient in either thiamine, riboflavin, pyridoxine, or pantothenate, or (b) a complete diet with a limited water allotment.

2. The discharge of pigment was essentially prevented by (a) reducing the relative humidity below 70%, or (b) incorporating into the vitamin-deficient or water-restrictive diets the active flavonoid compounds, quercetin or rutin. Quercitrin and naringin were somewhat less effective, whereas hesperetin, hesperidin, naringenin, and hesperidin-methylchalcone possessed little activity.

3. A large excess of menadione or the intraperitoneal injection of alkaline solutions of rutin or quercitrin partially decreased the extent of pigmentation; penicillin, streptomycin, aureomycin, salicylic acid, and ascorbic acid were practically inactive.

4. It is suggested that rats may require flavonoid compounds under conditions of stress.

LITERATURE CITED

COLLINS, R. A., M. SCHREIBER AND C. A. ELVEHJEM 1953 The influence of relative humidity upon vitamin deficiences in rats. J. Nutrition, *49:* 589.

COLLINS, R. A., M. SCHREIBER, H. NIÑO-HERRERA AND C. A. ELVEHJEM 1952 Importance of relative humidity in vitamin deficiency. Fed. Proc., *11:* 438 (abst.).

COUCH, J. F. 1948 Selected references to the literature on rutin, vitamin P, flavonols and related subjects. Bur. Agr. Ind. Chem., ARA-USDA, *AIC — 192.*

COUCH, J. F., C. F. KREWSON AND J. NAGHSKI 1951 Chemistry, pharmacology, and clinical application of rutin. Ibid., *AIC — 291.*

FIGGE, F. H. J., AND W. B. ATKINSON 1941 Relation of water metabolism to porphyrin incrustations in pantothenic acid-deficient rats. Proc. Soc. Exp. Biol. Med., *48:* 112.

FIGGE, F. H. J., AND K. SOLOMON 1942 Prevention of porphyrin incrustations on pantothenic acid-deficient rats by Harderian gland ablation. J. Lab. Clin. Med., *27:* 1495.

GRAFFIN, A. L. 1941 Porphyrin-secreting Harderian gland of the albino rat. Anat. Rec., *79:* 25 (abst.).

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.

LEMBERG, R., AND J. W. LEGGE 1949 Hematin Compounds and Bile Pigments. Interscience Publishers, Inc., New York, N. Y., p. 584.

MCELROY, L. W., K. SOLOMON, F. H. J. FIGGE AND G. R. COWGILL 1941 On the porphyrin nature of the fluorescent "blood caked" whiskers of pantothenic acid deficient rats. Science, *94:* 467.

SELYE, H. 1950 The Physiology and Pathology of Exposure to Stress. Acta, Inc., Medical Publishers, Montreal, Canada, p. 51.

SMITH, S. G. 1932 A new symptom complex in vitamin G-deficiency in rats. Proc. Soc. Exp. Biol. Med., *30:* 198.

———— 1942 Red staining of paws and whiskers as in vitamin B_2-complex-deficient rats after dehydration. Ibid., *49:* 691.

SMITH, S. G., AND D. H. SPRUNT 1935 Pathological skin changes in the tail of the albino rat on a diet deficient in vitamin G. J. Nutrition, *10:* 481.

TASHIRO, S., C. C. SMITH, E. BADGER AND E. KEZUR 1940 Chromodacryorrhea, a new criterion for biological assay of acetylcholine. Proc. Soc. Exp. Med. Biol., *44:* 658.

THE EFFECT OF AUREOMYCIN ON GROWTH AND PROTEIN UTILIZATION IN THE RAT

MARION E. BERRY AND CECILIA SCHUCK
Department of Foods and Nutrition, Purdue University, Lafayette, Indiana

(Received for publication May 13, 1954)

Several publications have appeared which suggest a protein-sparing action of dietary antibiotics in pigs (Catron et al., '52), turkey poults (McGinnis, '51), and chicks (Macklin et al., '52). As a result of their studies with chicks Anderson et al. ('52) concluded that penicillin enhanced protein utilization but did not alter the protein requirements. Similar conclusions have been drawn by Biely et al. ('52) and Johnson ('52). A review of this aspect of antibiotic action reveals considerable difference of opinion in the interpretation of similar sets of experimental data when based on growth or food and protein efficiency ratios.

Vijayaraghaven et al. ('52) postulated that the growth-promoting effect of aureomycin depends on the biological value of the dietary protein employed. They noted that the majority of positive reports on the action of aureomycin have been obtained, as with their studies using mice, on diets containing plant proteins. The authors considered that antibiotics may promote the development of a microflora which enhances the availability or intestinal synthesis of critical amino acids. Lack of response to aureomycin when proteins or protein mixtures of high biological value (e.g., casein, peanut meal, calf meal) were fed, indicated to them that a favorable effect of aureomycin did not occur because amino acids were not limiting factors in these proteins. Although 5 antibiotics tested improved 90% rice diets for rats, only

two of these antibiotics were effective in improving the rice rations when supplemented with lysine and threonine (Pecora, '53).

On the other hand, Biely et al. ('52) concluded that with a better balance of amino acids a greater response to antibiotics was possible in chicks than with rations deficient in some amino acid. Antibiotics had little or no stimulatory effect on growth with rations in which the limiting factor was either lysine or tryptophan. Similarly, Bloss et al. ('53) found that aureomycin was ineffective in counteracting the tryptophan deficiency of experimental rations for pigs. Jones and Combs ('51) reported that chicks responded with improved growth when aureomycin was added to a diet suboptimal in tryptophan, but not in the case of a diet suboptimal in lysine. Penicillin enhanced the growth response from added methionine with a diet low in this amino acid but did not spare methionine in the basal diet alone.

A close similarity of nitrogen retention in antibiotic-supplemented and control animals, in spite of improved growth and protein efficiency ratios by the supplemented groups, has been noted in rats (Black and Bratzler, '52; Pecora, '53), pigs (Braude and Johnson, '53), and dogs (Arnrich et al., '52). This raises a question regarding the composition of gains induced by antibiotics. Several publications (Becker et al., '53; Beeson, '52; Bowland et al., '51; Huang and McCay, '53; Perry et al., '53) have indicated increased fat deposition with decreased protein content of pigs fed antibiotics. Others (Catron et al., '52; Bennison et al., '51; Jensen et al., '52), however, were unable to verify this observation. Wilson et al. ('53) found that fat deposition was not increased when the addition of aureomycin accelerated growth, but only when a high-protein ration was used without stimulation of growth. Recent studies by Hartsook and Johnson ('53) showed that with methionine and cystine at levels bordering on the inadequate, the inclusion of terramycin enhanced weight gains chiefly by increasing fat deposition in rats, the efficiency of nitrogen utilization being reduced. When adequate or excess

methionine or cystine was present, the efficiency of nitrogen utilization was increased, and carcass fat deposition did not increase as fast as with the diet bordering on inadequacy with respect to these amino acids.

Observations of body composition following antibiotic administration have not been limited to studies with swine. Arnrich et al. ('52) concluded that extra weight gains of dogs fed purified diets supplemented with aureomycin were due solely to increased fat deposition. Microscopic observations of some rats receiving antibiotics showed a slight increase in tissue fat and glycogen as compared with the controls (Pecora, '53). Black and Bratzler ('52) have also reported a trend toward a higher fat content of the bodies of rats when streptomycin was added to their rations. Similar results were reported when turkeys were fed penicillin (Saxena et al., '53).

Since reports in the literature concerning the interrelation of dietary proteins and antibiotics are somewhat variable, further study of this aspect of antibiotic action seemed desirable. This investigation deals with the effect of the biological value of the dietary protein, amino acid supplementation, and varying levels of protein in the diet on the response of young rats to aureomycin.

EXPERIMENTAL

Weanling male albino rats were housed individually in elevated wire-bottom cages. Rats from the Purdue Biochemistry colony were used in the preliminary experiments, and those from a Wistar-Purdue strain for the remainder of the study. The minimum length of the experimental period was 4 weeks; some groups were continued for an additional one or two weeks. Data are presented only for the 4-week periods since essentially the same results were obtained for the longer periods. The diets and water were fed ad libitum. Rats were weighed twice weekly, while their food intake was determined once a week. Aureomycin hydrochloride, when used, was added to basal diets at a level of 10 mg %.

Sources of dietary protein were casein, cottonseed meal, or soybean meal. The composition of the various diets is given in table 1. An attempt was made to have equivalent amounts of fat and fiber in the 15% protein diets by the addition of Wesson oil and "Alphacel" in suitable amounts to the soybean meal and casein diets.

TABLE 1

Percentage composition of experimental diets

	SOYBEAN MEAL	COTTONSEED MEAL			CASEIN	
	%	%	%	%	%	%
15% protein	31.4	37.1	..	..	15.0	..
18% protein	..	..	44.6	..	..	..
21% protein	..	..	..	52.0	..	..
9% protein	..	..	..	..	..	9.0
Sucrose	53.8	52.4	44.9	37.5	68.1	74.1
Wesson oil	2.1	..	..	..	2.4	2.4
Mazola	5.6	5.6	5.6	5.6	5.6	5.6
"Alphacel"	2.2	..	..	..	4.0	4.0
Salt mixture [1]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture [2]	0.4	0.4	0.4	0.4	0.4	0.4
Liver extract Conc. 1: 20	0.5	0.5	0.5	0.5	0.5	0.5

[1] Wesson, L. G. Science, *75:* 339, 1932.

[2] Vitamin mixture: thiamine HCl 250 mg, pyridoxine HCl 250 mg, riboflavin 500 mg, niacin 500 mg, calcium pantothenate 2.5 gm, para-aminobenzoic acid 5 gm, inositol 15 gm, choline 150 gm, folic acid 10 mg, biotin 50 μg, menadione 125 mg, alpha-tocopherol 5 gm, oleum percomorphum 25 gm.

Amino acid supplementation was designed to raise the level of deficient amino acids to that used by Ramasarmer and co-workers ('49) in their optimum essential amino acid mixture for the growing rat. The composition of the amino acid mixtures is indicated at the bottom of table 3.

From the report of the Bureau of Biological Research, Rutgers University ('46–'50) on the amino acid composition of casein, it was calculated that 9 gm of casein should supply 0.3 gm of methionine. Accordingly, 0.3 gm of DL methionine was added per 100 gm of the 9% casein rations to supply a total of 0.6% methionine, the amount recommended by Wo-

mack and Rose ('41) and Ramasarmer et al. ('49). It was thought that this amount of methionine would be sufficient to satisfy the requirement for both methionine and cystine.

In addition to observations on weight gains, 5-day nitrogen metabolism studies were conducted on selected groups of rats during the second, third, or 4th week of the experimental period. All nitrogen determinations were made by the boric acid modification of the macro-Kjeldahl method.

TABLE 2

Average weight gains on protein fed at 15% level with and without aureomycin supplementation

SOURCE OF PROTEIN	GROUP NO.	NO. OF RATS	INITIAL WT.	WT. GAIN	GAIN RELATIVE TO CONTROLS
			gm	*gm*	%
Soybean meal	1 C	4	40	143	
	1 S	4	40	177	124
Cottonseed meal	2 C	4	40	44	
	2 S	4	40	49	111
Casein	3 C	4	42	158	
	3 S	4	42	157	99

C = control. S = aureomycin supplemented.

DISCUSSION OF RESULTS

In a preliminary experiment, a study was made of the degree of growth stimulation induced by aureomycin when (a) casein, (b) cottonseed meal, or (c) soybean meal supplied 15% protein in the diet. The results of this study are presented in table 2. Rats fed 15% casein diets did not respond with increased growth when aureomycin was given. When the two plant products were used in the diet, however, aureomycin was effective in stimulating growth. These findings are in accord with those of Vijayaraghaven et al. ('52) as a result of similar studies with mice.

When compared at the end of 4 weeks, the stimulatory effect of aureomycin was more pronounced at the 15% protein level with soybean-meal diets than with the cottonseed-

meal diets which did not support a good rate of growth. It was decided to increase the level of protein in the cottonseed-meal diets to 18 and 21% in order to promote better growth. Taking the gain of the controls as 100%, aureomycin added to 18% protein diets produced a gain of 134% during a period of 4 weeks (table 3) as compared with one of 111% when used as a supplement to the 15% protein rations (table 2). Similarly, when added to 21% protein rations, aureomycin produced a gain of 119%. Supplementing the 15% protein level of the cottonseed meal rations with amino acids thought to be deficient markedly improved the growth rate. Aureomycin added to the amino acid-supplemented rations caused a gain 38% greater than in the control group. These results bring to mind the report of Biely et al. ('52) who suggested that a greater response to antibiotics was possible with a better balance of amino acids.

The addition of aureomycin to soybean-meal diets (15% protein) supplemented with AAM 1[1] elicited less response than its addition to the same rations without amino acid supplementation. It is conceivable that the greater maturity of these rats may have influenced results. The possibility also exists that this amino acid mixture actually produced an amino acid imbalance, in which case these results would be consistent with the hypothesis of Biely et al. (op. cit.) concerning the importance of amino acid balance in antibiotic growth studies. A comparison of growth and protein efficiency data (table 3) of rat groups 8 (15% protein) and 9 (15% protein plus AAM 2) indicates that AMM 2 was at least partially effective in counteracting the deficiencies of the soybean rations. The response to aureomycin in these two groups was nearly the same.

The difference in growth rate of groups 1 (table 2) and 8 (table 3), both fed 15% soybean-protein rations and having the same average starting weights, may be due to two factors. The preliminary study with groups 1, 2, and 3 was carried out in the spring, while the remainder of the experimental

[1] See footnotes to table 3 for composition of the three amino acid mixtures used.

TABLE 3

Average weight gains and protein intake with and without aureomycin supplementation

PROTEIN LEVEL	GROUP NO.		NO. OF RATS	INITIAL WT.	WT. GAIN	GAIN RELATIVE TO CONTROLS	PROTEIN INTAKE	P. E. RATIO
				gm	*gm*	*%*	*gm*	
Cottonseed-meal diets								
15%	11	C	4	40	85		40.0	2.12
+ AAM3 [1]		S	4	40	117	138	49.2	2.35
18%	5	C	6	47	99		48.8	2.02
		S	6	47	133	134	59.6	2.23
21%	6	C	4	47	122		65.6	1.87
		S	4	47	145	119	71.0	2.04
Soybean-meal diets								
15%	8	C	3	40	110		44.6	2.46
		S	3	40	136	124	50.0	2.72
15%	9	C	4	40	138		48.5	2.85
+ AAM2 [2]		S	4	40	168	122	57.7	2.91
15%	4	C	4	59	163		56.6	2.89
+ AAM1 [3]		S	4	59	175	107	57.2	3.07
Casein diets								
9%	7	C	4	58	33		14.6	2.29
		S	4	58	44	133	17.5	2.49
9%	12	C	2	47	31		14.0	2.11
		S	2	47	47	151	20.0	2.37
9% + 0.3% DL-methionine	13	C	5	47	57		20.8	2.70
		S	5	47	89	156	25.3	3.50

C = control.

S = aureomycin-supplemented.

P. E. ratio = gm gain/gm protein intake.

[1] Amino acid mixture 3 = DL-methionine 0.37, L-lysine 0.47, DL-phenylalanine 0.12 gm per 100 gm ration.

[2] Amino acid mixture 2 = DL-methionine 0.36, L-lysine 0.16, DL-phenylalanine 0.16, L-histidine 0.14 gm per 100 gm ration.

[3] Amino acid mixture 1 = DL-valine 0.70, DL-methionine 0.50, L-lysine 0.05, L-histidine 0.20, DL-threonine 0.60 gm per 100 gm ration.

work was performed in the summer months during which extremely warm and humid weather prevailed. Since the laboratory was not air-conditioned, the weather conditions may have influenced food consumption and growth. Secondly, a difference in the strain of rats may have been a factor since the strain used for the preliminary study was not available for the subsequent experiments.

The fact that improving the nutritive value of cottonseed or soybean rations, either by increasing the protein level or by supplementation with limiting amino acids, did not cancel the stimulatory effect of aureomycin on growth suggests that the antibiotic does not function by increasing the availability or intestinal synthesis of these amino acids.

To test this hypothesis further, the protein level of the casein rations was lowered to 9% where methionine or cystine is a limiting factor. Contrary to expectations, aureomycin did enhance growth under these conditions, with both groups of rats studied (7 and 12), although it had no effect with 15%-casein rations, as seen in table 2. The effect appeared to be more pronounced with the less mature animals. At first appraisal, these results seemed to oppose the findings obtained with the plant protein rations in that the antibiotic elicited a response when casein was fed at a level known to be inadequate with respect to amino acid content, but not when a higher level, supplying adequate amounts of amino acids, was used. In an attempt to elucidate the problem, 0.3% DL methionine was added to the 9%-casein diet of another group of animals. Improved growth and food efficiency ratios indicated that the supplementary methionine improved the quality of the 9%-casein rations. But since aureomycin produced an even greater response when added to the methionine-supplemented rations, it would seem that some factor or factors other than an amino acid deficiency was involved in the response of casein rats to aureomycin. A possible explanation is that aureomycin spares some unidentified growth factor in casein (but absent in the plant proteins used) which is limiting at the 9% level. That antibiotics may spare the require-

ment for an unidentified growth factor in casein, has also been suggested by Scott and Jensen ('52) as a result of their studies with turkeys.

The influence of auroemycin on nitrogen metabolism is indicated in table 4. It is interesting to note the increased

TABLE 4

Influence of aureomycin on nitrogen utilization
(Av. N intake and retentions)

PROTEIN LEVEL	GROUP NO.		NO. OF RATS	NITROGEN INTAKE	APPARENT DIGESTIBILITY	NITROGEN RETENTION		
						gm	% of N intake	% of N absorbed
				gm				
				Cottonseed-meal diets				
15% + AAM3	11	C	3	1.14	82.5	0.65	57.0	69.1
		S	3	1.48	84.5	0.80	54.1	64.0
18%	5	C	3	2.03	79.3	0.98	48.3	60.9
		S	3	2.57	83.7	1.13	44.0	52.6
21%	6	C	3	2.79	79.6	1.32	47.3	59.5
		S	3	3.18	83.1	1.34	42.1	50.8
21%	10	C	2	1.36	77.2	0.63	46.1	59.8
		S	2	1.45	81.4	0.63	42.9	53.0
				Soybean-meal diets				
15%	1 & 8	C	5	0.82	82.9	0.51	62.2	75.0
		S	5	0.96	87.5	0.61	63.6	73.5
15% + AAM2	9	C	4	1.00	83.0	0.71	71.0	85.6
		S	4	1.26	85.7	0.90	71.3	83.4
15% + AAM1	4	C	4	2.01	83.3	1.24	61.6	74.0
		S	4	1.95	86.8	1.21	62.0	71.4

percentage of absorbed nitrogen which is retained as a result of the addition of AAM 2 and AAM 3 to soybean and cottonseed rations, respectively. This gives further evidence that these amino acid mixtures improved the amino acid balance of the rations to which they were added.

The antibiotic consistently improved the apparent digestibility of cottonseed and soybean proteins in all dietary groups

tested regardless of the age of the rats. This is in accord with the findings of Huang and McCay ('53) who reported increased protein digestibility coefficients when pigs were fed terramycin. Black and Bratzler ('52), however, found no observable effect on apparent digestibility of protein when streptomycin was added to rations for rats. Although the larger animals consumed more nitrogen and retained larger amounts, the depression in the percentage of absorbed nitrogen with aureomycin supplementation was similar to that of the smaller rats. It is interesting to note the studies cited by Braude et al. ('53) in which it was found that chicks receiving penicillin had thinner intestinal walls than the unsupplemented controls. An effect of this nature might well result in more efficient absorption of nutrients.

In contrast to the beneficial effect of aureomycin on protein digestibility, the antibiotic appeared to impair nitrogen utilization following absorption from the digestive tract. This effect was particularly pronounced in rats fed the various cottonseed diets; with these animals the favorable influence on digestibility was more than offset by the greater amount of nitrogenous products excreted in the urine. In rats fed soybean-meal diets, the positive effect of auroemycin on digestibility more nearly balanced the negative effect on utilization subsequent to absorption.

The antibacterial effects of a number of antibiotics have been explained on the basis of their action as competitive inhibitors in enzyme systems essential for the life of the bacterial cell. A number of these same enzyme systems are also important in the cells of the host organism. One suggestion advanced to explain the selective action of antibiotics is that selective permeability prevents antibiotics from reaching the critical enzyme in the animal cell. If, however, small amounts of the antibiotic were able to penetrate the host cells and were able to exert a minor inhibitory effect, impaired protein utilization by the host could result. This might be an explanation for the results obtained in this study — namely, an adverse effect of aureomycin on protein utili-

zation in the body tissues. Of particular interest are studies which have suggested that antibiotics interfere with protein synthesis in bacterial cells (Hahn and Wisseman, '51; Work, '52). That antibiotics may also affect enzymatic processes in mammalian tissues is indicated by a number of studies, for example, those of Van Meter et al. ('52) using liver homogenates.

In group 6, fed cottonseed-meal diet and in groups 1, 8, and 9 fed soybean diets, weight gains of aureomycin-supplemented rats during the 5-day metabolism periods, considered as percentage relative to controls, were greater than the relative gains of body nitrogen. These data imply a smaller gain of nitrogen per unit of body weight gained, when rats were fed aureomycin. Several studies cited previously have indicated decreased protein content and increased percentage of body fat as a result of antibiotic administration. In two groups of rats, 5 and 11, fed cottonseed-meal diets, the average percentage weight increases induced by aureomycin were in close relationship to the average percentage gains of body nitrogen. It appears that in these two groups, the increased food consumption of supplemented animals supplied enough extra protein to support gains of approximately normal composition with respect to nitrogen, in spite of the impaired net utilization of nitrogen. Since this study exhibits somewhat inconsistent results regarding this aspect of antibiotic action more information is needed before drawing definite conclusions concerning the composition of the weight gains induced by aureomycin in rats.

The data indicate that protein efficiency ratios bear little relation to the efficiency of protein utilization as judged by nitrogen metabolism studies. Since the weight gains induced by antibiotics are of questionable composition, it is considered that nitrogen metabolism studies serve as more reliable criteria of protein utilization than protein efficiency ratios. The chief limitation of the latter is the assumption, inherent in the method, that all weight increase is of equal composition.

SUMMARY

Young albino rats were used to study the effect of the addition of aureomycin to diets containing casein, cottonseed meal and soybean meal as sources of protein. Observations were made on growth responses and protein utilization.

A growth-stimulating effect of aureomycin was observed when soybean meal and cottonseed meal supplied protein at the 15% level. The effect was less marked with the cottonseed meal at this level than when the protein level was raised to 18%. Aureomycin was ineffective when added to casein at the 15% level but brought about increased weight gains with casein at the 9% level.

Supplementation of the casein at the 9% level and the soybean and cottonseed-meal proteins at the 15% level with the amino acids considered limiting factors resulted in some increase in growth, but there was further improvement on the addition of aureomycin. This suggests that the aureomycin functioned in a manner beyond that of increasing the availability of the limiting amino acids in the proteins studied.

Nitrogen metabolism studies indicated that aureomycin improved the apparent digestibility of the cottonseed- and soybean-meal proteins, but impaired utilization following absorption. This effect was evident to such a degree in rats receiving cottonseed-meal diets that nitrogen retention expressed both in terms of per cent of ingested nitrogen and absorbed nitrogen was actually depressed in antibiotic-supplemented animals.

ACKNOWLEDGMENT

The cottonseed meal and soybean meal used in this study were kindly furnished by Purina Mills, Lafayette, Indiana, and the aureomycin by Lederle Laboratories, American Cyanimide Company, New York.

LITERATURE CITED

ANDERSON, G. W., J. D. CUNNINGHAM AND S. J. SLINGER 1952 Effect of protein level and penicillin on growth and intestinal flora of chickens. J. Nutrition, *47*: 175.

ARNRICH, L., E. M. LEWIS AND A. F. MORGAN 1952 Growth of dogs on purified diets plus aureomycin and/or vitamin B_{12}. Proc. Soc. Exp. Biol. Med., *80:* 401.

BECKER, D. E., C. R. ADAMS, S. W. TERRILL AND R. J. MEADE 1953 The influence of heat treatment and solvent upon the nutritive value of soybean oil meal for swine. J. Animal Sci., *12:* 107.

BEESON, W. M. 1952 Summary of swine nutrition discoveries. Feedstuffs, *24,* 44: 32.

BENNISON, R. W., D. CATRON AND H. M. MADDOCK 1951 Effect of antibiotics and vitamin B_{12} on the pantothenic acid requirement of growing-fattening swine. J. Animal Sci., *10:* 1038.

BIELY, J., B. E. MARCH AND D. F. SMITH 1952 The effect of feeding antibiotics on the carbohydrate and protein requirements of the chick. Poultry Sci., *31:* 863.

BLACK, A., AND J. W. BRATZLER 1952 The effects of a vitamin B_{12} supplement, vitamin B_{12}, and streptomycin on the metabolism of the rat. J. Nutrition, *47:* 159.

BLOSS, R. E., R. W. LUECKE, J. A. HOEFER, F. THORP, JR. AND W. N. MCMILLEN 1953 Supplementation of a corn-meat and bone scrap ration for weanling pigs. J. Animal Sci., *12:* 102.

BOWLAND, J. P., S. E. BEACOM AND L. W. MCELROY 1951 Animal protein factor and antibiotic supplementation of small grain rations for swine. Ibid., *10:* 629.

BRAUDE, R., AND B. C. JOHNSON 1953 Effect of aureomycin on nitrogen and water metabolism in growing pigs. J. Nutrition, *49:* 505.

BRAUDE, R., S. K. KON AND J. W. G. PORTER 1953 Antibiotics in nutrition. Nutr. Abstr. Rev., *23:* 473.

BUREAU OF BIOLOGICAL RESEARCH, RUTGERS UNIVERSITY 1946–1950 Cooperative Determinations of the Amino Acid Content and the Nutritive Value of Six Selected Protein Food Sources. New Brunswick, N. J., p. 15.

CATRON, D. V., A. H. JENSEN, P. G. HOMEYER, H. M. MADDOCK AND G. C. ASHTON 1952 Re-evaluation of protein requirements of growing-fattening swine as influenced by feeding an antibiotic. J. Animal Sci., *11:* 221.

HAHN, F. E., AND C. L. WISSEMAN, JR. 1951 Inhibition of adaptive enzyme formation by antimicrobial agents. Proc. Soc. Exp. Biol. Med., *76:* 533.

HARTSOOK, E. W., AND B. C. JOHNSON 1953 Effects of dietary terramycin and methionine supplements on fat and protein gains in weanling rats. J. Nutrition, *51:* 205.

HUANG, T., AND C. M. MCCAY 1953 The effect of terramycin on the growth and body composition of pigs. Ibid., *50:* 129.

JENSEN, A. H., G. C. ASHTON, H. M. MADDOCK, P. G. HOMEYER AND D. V. CATRON 1952 Effects of antibiotic and lysine supplementation on different protein levels for growing-fattening swine. J. Animal Sci., *11:* 767.

JOHNSON, E. L. 1952 Providing vitamin B_{12}, antibiotic and unknown growth factor activity for chick diets. Poultry Sci., *31:* 955.

JONES, H. L., AND G. F. COMBS 1951 Effect of antibiotics on the dietary requirement for methionine, lysine, tryptophan, and unidentified growth factors. Ibid., *30:* 920.

MACHLIN, L. J., C. A. DENTON, W. L. KELLOGG AND H. R. BIRD 1952 Effect of dietary antibiotic upon feed efficiency and protein requirement of growing chickens. Ibid., *31:* 106.

MCGINNIS, J. 1951 The effect of antibiotics on the nutritional requirement of turkeys and chicks. Ibid., *30:* 924.

PECORA, L. J. 1953 Effect of antibiotics on rats fed amino acid-supplemented rice diets. J. Nutrition, *49:* 621.

PERRY, T. W., W. M. BEESON AND B. W. VOSTEEN 1953 The effect of an antibiotic or a surfactant on the growth and carcass composition of swine. J. Animal Sci., *12:* 310.

RAMASARMER, G. B., L. M. HENDERSON AND C. A. ELVEHJEM 1949 Purified amino acids as a source of nitrogen for the growing rat. J. Nutrition, *38:* 177.

SAXENA, H. C., L. G. BLAYLOCK, W. J. STADELMAN, J. S. CARVER AND J. MCGINNIS 1953 Effect of penicillin on growth, feed efficiency and fattening of turkeys. Poultry Sci., *32:* 721.

SCOTT, M. L., AND L. S. JENSEN 1952 The effect of antibiotics upon the requirement of turkeys for unidentified vitamins. Ibid., *31:* 986.

VAN METER, J. C., A. SPECTOR, J. J. OLESON AND J. H. WILLIAMS 1952 Study of vitro action of aureomycin on oxidative phosphorylation in animal tissues. Fed. Proc., *11:* 301.

VIJAYARAGHAVEN, P. K., E. A. MURPHY AND M. S. DUNN 1952 The effect of aureomycin on the growth of mice. Arch. Biochem. and Biophysics, *36:* 127.

WILSON, G. D., J. E. BURNSIDE, R. W. BRAY, P. H. PHILLIPS AND R. H. GRUMMER 1953 Pork carcass value as affected by protein level and supplementation with aureomycin and vitamin B_{12}. J. Animal Sci., *12:* 291.

WOMACK, M., AND W. C. ROSE 1941 The partial replacement of dietary methionine by cystine for purposes of growth. J. Biol. Chem., *141:* 375.

WORK, T. S. 1952 The biochemistry of antibiotics. Ann. Rev. Biochem., *21:* 431.

DENTAL CARIES IN THE ALBINO RAT ON HIGH SUCROSE DIETS CONTAINING DIFFERENT AMOUNTS OF ALUMINUM

WINFREY WYNN AND JOHN HALDI

Department of Physiology, Division of Basic Sciences in the Health Services, Emory University, Emory University, Georgia

(Received for publication May 21, 1954)

It has been shown (Wynn, Haldi, Shaw and Sognnaes, '53) that two high-sucrose diets, which we have designated as the Emory and Harvard diets, differing from each other in some respects but containing identical amounts of sugar, show a pronounced difference in cariogenicity. In searching for a possible single factor which might account for this difference, it was noted that a very small amount of aluminum had been incorporated in the salt mixture of the Emory diet whereas none had been added to the Harvard diet. As the Emory diet was much less cariogenic than the Harvard diet, the present experiments were undertaken to determine whether additions of this trace element to the diet might serve somewhat like the fluoride ion in giving protection to the teeth against dental caries. In this connection it may be of interest to note that Pieruccini ('47, cited by McConnell, '52) has suggested that aluminum can substitute for calcium and phosphorus in the structure of apatite. This type of substitution, says McConnell, is not particularly surprising when considered in the light of the demonstration by Yoder and Keith ('51) of complete replacement of silicon by aluminum in synthetic yttrium garnetoid, although substitution of AlO_4 for SiO_4 was previously supposed to be very limited in orthosilicates.

It has been shown by Hove, Elvehjem and Hart ('38) that if aluminum is needed in the nutrition of the rat during its

most rapid period of growth, 1 μg per day will satisfy this requirement. Conversely, no deleterious action upon growth, reproduction or general well-being of animals has been observed when relatively large amounts of aluminum have been added to the diet. In the experiments of Myers and Mull ('28) 4 generations of rats were fed 2 mg of aluminum in the form of potassium aluminum sulfate per rat daily in addition to the stock diet. No abnormalities were noted on autopsy and all animals appeared healthy. Similar results were obtained by McCollum, Rask and Becker ('28). They found that aluminum compounds in the diet in concentrations as high as 600 p.p.m of the element aluminum exerted no noticeably deleterious action on growth, reproduction, or general well-being as judged by external appearance and by autopsy. In view of these observations on aluminum requirements of the rat and the effects of a relatively large intake, it was deemed unnecessary to extend our studies beyond the condition of the teeth. Notation was nevertheless made of the amount of food consumed and of the weekly change in body weight.

EXPERIMENTAL

Albino rats of the Wistar strain were selected at weaning in groups of 4 of the same sex from the same litter and placed on 4 rations that were identical in composition except for their aluminum content. The basic ration consisted of 64% sucrose, 20% casein, 8% fat, 4% yeast and liver extract, 4% salt mixture and all the known essential vitamins in adequate amounts. The composition of the salt and vitamin mixtures is given elsewhere (Haldi and Wynn, '52). The 4 rations will be referred to as rations A, B, C and D, respectively. No aluminum was added to ration A. To ration B there was added the same amount of aluminum (0.16 p.p.m.) as in the ration fed in our previous experiments (Haldi and Wynn, '52; Wynn, Haldi, Shaw and Sognnaes, '53), whereas 12.5 and 125 times this amount was added to rations C and D, respectively. These amounts of aluminum (2 p.p.m. and

20 p.p.m.) may appear to be small, but it should be borne in mind that similarly small amounts of fluoride afford some protection to the teeth against dental caries.

There were two sets of experiments, one on non-desalivated and the other on desalivated animals. In the experiment on the non-desalivated animals, 14 rats were placed on each of the 4 rations. They were fed ad libitum for 180 days and at the end of this period were sacrificed and the teeth examined under a dissecting microscope for the evaluation of dental caries.

In the second experiment the major salivary glands were removed when the animals had been fed the rations 120 days. In this experiment there were 10 animals on each ration. They were continued on these rations 60 days longer and were then sacrificed and the teeth examined for caries. The method of desalivation has been described elsewhere (Haldi, Wynn, Shaw and Sognnaes, '53).

Carious lesions were graded in accordance with the criteria followed in our earlier study (Haldi and Wynn, '52). The total score for the animals on each ration was derived by adding the products that were obtained by multiplying the number of lesions of each grade by the grade itself; the average score by dividing the total score by the number of teeth. This latter value is particularly helpful in comparing the carious destruction of the teeth on the 4 rations.

RESULTS

The number of carious lesions and the caries score for the non-desalivated rats are presented in table 1 and for the desalivated rats in table 2. The males and females have been grouped together as we have found that there is no sex difference in caries susceptibility in our colony of albino rats.

Comparison of the data in tables 1 and 2 shows that desalivation resulted in both an increase in the average number of carious teeth per animal and in the severity of the lesions.

The differences in the total scores on the 4 rations were relatively small both in the non-desalivated and desalivated

animals. Statistically, these differences were not significant. The critical ratio, for example, was less than one in the case of the greatest difference which was between the non-desalivated animals on rations to which were added aluminum in

TABLE 1

Carious lesions and caries score of non-desalivated albino rats on rations containing various amounts of aluminum

ALUMINUM ADDED TO RATION	TOTAL NO. TEETH	TOTAL NO. CARIOUS TEETH	TOTAL NO. CARIOUS LESIONS	NO. OF LESIONS Grade 1	2	3	4	TOTAL SCORE	AV. SCORE
				LOWER MOLARS					
None	84	32	38	21	15	2	0	57	0.68
0.16 p.p.m.	84	33	39	25	10	2	2	59	0.70
2.0 p.p.m.	84	31	39	24	10	3	2	61	0.73
20 p.p.m.	84	31	39	28	7	4	0	54	0.64
				UPPER MOLARS					
None	84	4	4	4	0	0	0	4	0.05
0.16 p.p.m.	84	5	6	6	0	0	0	6	0.07
2.0 p.p.m.	84	2	2	2	0	0	0	2	0.02
20 p.p.m.	84	3	5	5	0	0	0	5	0.06

TABLE 2

Carious lesions and caries score of desalivated albino rats on rations containing various amounts of aluminum

ALUMINUM ADDED TO RATION	TOTAL NO. TEETH	TOTAL NO. CARIOUS TEETH	TOTAL NO. CARIOUS LESIONS	NO. OF LESIONS Grade 1	2	3	4	TOTAL SCORE	AV. SCORE
				LOWER MOLARS					
None	60	26	46	22	10	9	5	89	1.48
0.16 p.p.m.	60	30	46	19	14	11	2	88	1.47
2.0 p.p.m.	60	28	43	14	15	12	2	88	1.47
20 p.p.m.	60	25	45	13	19	12	1	91	1.52
				UPPER MOLARS					
None	60	10	14	12	2	0	0	16	0.27
0.16 p.p.m.	60	12	15	11	3	1	0	17	0.28
2.0 p.p.m.	60	13	20	15	5	0	0	20	0.33
20 p.p.m.	60	12	16	10	4	2	0	24	0.40

the amounts of 2 and 20 p.p.m. It may therefore be concluded that the addition of aluminum to the Emory diet was not the factor responsible for making it less cariogenic than the Harvard diet.

Incidental to these observations on the teeth it is of interest to note that there was no perceptible difference in the general physical condition of the animals on the 4 rations. The average weight of the 4 groups of males at the conclusion of the experiment was 302, 304, 301 and 307 gm, respectively; and of the females, 191, 194, 190 and 193 gm. The initial average weight of the groups at the beginning of the experiment did not vary by more than 1 or 2 gm. Although the animals were allowed to eat ad libitum the amount of food consumed by the different groups was remarkably uniform. The average daily consumption by the 4 groups of males was 11.2, 11.5, 11.2 and 11.3 gm, respectively, and of the females, 8.7, 8.9, 8.8 and 8.7 gm. Obviously, appetite and growth were not affected by the addition of aluminum to the rations in the amounts used in these experiments.

SUMMARY AND CONCLUSIONS

These experiments were undertaken to determine whether aluminum, like fluoride, when added to the diet in small amounts may offer some protection against dental caries.

No significant differences were found in the number of carious molars or in the caries scores as a result of the addition of aluminum in the amounts used in this study, either in non-desalivated or desalivated animals.

It may be concluded therefore that the difference in cariogenicity of two diets containing the same amount of sucrose but with different amounts of aluminum, as reported previously, is not due to the small amount of aluminum that was added to the salt mixture of one of these diets. The explanation of the lower cariogenicity of one of the high sucrose diets must be sought elsewhere.

The addition of aluminum up to 20 p.p.m. had no effect on appetite or growth of the animals.

ACKNOWLEDGMENT

The authors wish to express their appreciation for the support given by grants-in-aid from the National Institute of Dental Health (D136-C) and the Sugar Research Foundation, Inc.

LITERATURE CITED

HALDI, J., AND W. WYNN 1952 Cariogenicity of sugar containing diets. J. Nutrition, *46:* 425.

HALDI, J., W. WYNN, J. H. SHAW AND R. F. SOGNNAES 1953 The relative cariogenicity of sucrose when ingested in the solid form and in solution by the albino rat. Ibid., *49:* 295.

HOVE, E., C. A. ELVEHJEM AND E. B. HART 1938 Aluminum in the nutrition of the rat. Am. J. Phys., *123:* 640.

MCCOLLUM, E. V., O. S. RASK AND J. E. BECKER 1928 A study of the possible role of aluminum compounds in animal and plant physiology. J. Biol. Chem., *77:* 753.

MCCONNELL, D. 1952 The crystal chemistry of carbonate apatites and their relationship to the composition of calcified tissues. J. Dent. Res., *31:* 53.

MYERS, V. C., AND J. W. MULL 1928 The influence of the administration of aluminum upon the aluminum content of the tissues, and upon the growth and reproduction of rats. J. Biol. Chem., *78:* 605.

WYNN, W., J. HALDI, J. H. SHAW AND R. F. SOGNNAES 1953 Difference in the caries-producing effects of two purified diets containing the same amount of sugar. J. Nutrition, *50:* 267.

YODER, H. S., AND M. L. KEITH 1951 Complete substitution of aluminum for silicon. Am. Mineral, *36:* 519.

PROTEIN METABOLISM IN THE PREGNANT RAT[1]

GEORGE H. BEATON, JOYCE BEARE, MI HEH RYU AND E. W. McHENRY
Department of Public Health Nutrition, University of Toronto
Toronto, Canada

ONE FIGURE

(Received for publication May 10, 1954)

It has been known for many years that pregnant women normally exhibit a markedly positive nitrogen balance. An adequate explanation of the mechanism has not been established. To permit tissue studies not possible in humans, we have conducted studies on protein metabolism in pregnant rats.

Poo et al. ('40) reported that the amount of protein retained by maternal rat tissues during gestation varies with the protein content of the diet. Pike et al. ('54) found that the amount of protein retained by pregnant rats was influenced by the caloric intake, provided that protein content of the diet was adequate. Morse and Schmidt ('44) reported balances of -1 to $+2$ gm of protein for the entire period of pregnancy in mature rats if the loss of fetal protein was considered. The present experiments have confirmed these findings and demonstrated the simultaneous appearance of certain other metabolic disturbances.

EXPERIMENTAL

To obtain pregnant rats, female Wistar strain animals, weighing from 250 to 300 gm, were housed with males for two and a half days. Following breeding, the pregnant rats and comparable non-pregnant controls were placed in in-

[1] This study was made possible by a grant from the Department of National Health and Welfare of the Government of Canada.

dividual cages and provided, ad libitum, with a prepared fox chow.[2] During nitrogen balance studies, the animals were housed in individual metabolism cages and fed ground fox chow ad libitum. Collections of feces and urine were made daily and pooled for three-day periods.

In experiment I, 117 rats, with an initial average body weight of 258 gm, were used. Animals were killed in groups of 12 over a two-day period such that there were groups for the 5th, 8th, 12th, 14th, 16th, 18th, 20th and 22nd days of gestation and on the 4th and 9th days post partum. In both experiments I and II, the young were removed at parturition. Nine pregnant and two non-pregnant rats, having an initial average body weight of 269 gm, were used in experiment II for a continuous nitrogen balance study throughout gestation. Six pregnant and 6 non-pregnant rats, having an initial average body weight of 290 gm, were killed on the 17th day of gestation in experiment III; 8 pregnant and 7 non-pregnant rats, having an initial average body weight of 279 gm, were killed on the 16th day of gestation in experiment IV. In experiment V, 6 pregnant and 8 non-pregnant rats, having an initial average body weight of 251 gm, were killed over a period of 7 days, through the last week of gestation. It should be noted that all the times specified are the times from the middle of the mating period to the middle of the killing period.

All of the animals were killed by stunning and decapitation following a 20-hour fast. Blood was collected from the neck and heparinized; livers were removed, weighed and immediately prepared for enzyme assays. Analytical procedures were as follows: blood urea, Archibald ('45); blood amino nitrogen, Frame et al. ('43); blood hemoglobin, Collier ('44); blood packed cell volume by the standard procedure. Nitrogen determinations were carried out by a standard macro-Kjeldahl procedure; the values were multiplied by 6.25 to obtain the

[2] Master Fox Breeder Ration, Toronto Elevators Limited.

crude protein	— 20%
crude fat	— 3.5%
crude fiber	— 5%

protein content. A modified Liebermann procedure, as described by Gavin and McHenry ('40), was employed for the estimation of total crude fatty acids. Tissue moisture was determined by drying aliquots of tissue in aluminum dishes (A.O.A.C.) for 5 hours in an electric oven at 105°C.

Liver urea formation was determined as the Q_{urea} of Krebs and Henseleit ('32), defined as the number of microliters of urea-CO_2 formed per milligram dry tissue per hour. The procedure of Gornall and Hunter ('43), as modified by Caldwell and McHenry ('53a), was employed. Activity of the aspartic-glutamic transaminase in liver homogenates was estimated by the procedure of Tonhazy et al. ('50); activity of the alanine-glutamic transaminase was estimated by this procedure as modified by Caldwell and McHenry ('53b). Results are expressed as the Q_T^{10}, defined as the microliters of pyruvate-CO_2 formed per milligram wet tissue per hour. Arginase activity of liver homogenates was estimated by the method of Van Slyke and Archibald ('46) as adapted to liver tissue by Liener and Schultze ('50). Arginase activities were expressed as the micromols of urea formed per 100 mg wet tissue per hour.

RESULTS AND DISCUSSION

Changes in body, fetal and liver weight and liver moisture

The rats used in experiment I were weighed initially and before fasting. After killing or at parturition the fetal weights were also determined. Liver weights and moistures were determined after killing. The experimental findings are shown in table 1.

Fetal growth was markedly increased after the 15th day of gestation. Maternal body weight gains paralleled the increases in total fetal weight until parturition when the maternal weight rapidly fell toward the non-pregnant level. Liver weight and moisture also increased after the 15th day of gestation. The increase in liver weight can not be accounted for by water retention alone.

Carcass composition

The decapitated carcasses from experiment I were completely eviscerated, pooled by groups, frozen and passed through a power grinder. Analyses were carried out on

TABLE 1

Gross changes during and after gestation in the rat

TIME OF GESTATION	GROUP	NO. OF RATS	AV. BODY WT. GAIN	AV. INDIVIDUAL FETAL WT.	AV. TOTAL FETAL WT.	AV. LIVER WT. (mean ± S.D.)	AV. LIVER MOISTURE (mean ± S.D.)
days			*gm*	*gm*	*gm*	*gm*	*%*
5	pregnant	6	12	...	...	7.1 ± 0.2	69.8 ± 0.3
	control	6	8			6.7 ± 0.4	69.9 ± 0.9
8	pregnant	3	13	0.03	0.3	7.4 ± 0.4	69.5 ± 0.8
	control	9	13			6.7 ± 0.9	69.2 ± 0.4
12	pregnant	3	28	0.05	0.6	8.4 ± 0.5	69.8 ± 0.4
	control	9	12			7.8 ± 1.2	69.3 ± 0.7
14	pregnant	4	30	0.12	1.0	8.9 ± 0.5	69.7 ± 0.6
	control	8	7			7.7 ± 1.1	69.6 ± 0.6
16	pregnant	6	45	0.65	5.8	10.5 ± 0.8	70.5 ± 0.3
	control	6	5			7.3 ± 0.8 diff.[3]	69.2 ± 0.8 diff.[2]
18	pregnant	5	78	1.76	19.4	10.7 ± 0.6	70.6 ± 0.7
	control	7	6			7.5 ± 0.6 diff.[3]	69.3 ± 0.6 diff.[3]
20	pregnant	5	96	4.54	45.4	9.8 ± 0.5	70.4 ± 0.6
	control	6	8			7.0 ± 1.1 diff.[3]	69.7 ± 0.3 diff.[1]
22	pregnant	6	78	5.86	53.1	9.3 ± 0.9	70.9 ± 0.8
	control	6	8			6.8 ± 0.5 diff.[3]	69.6 ± 0.7
Post partum							
4	pregnant	6	22	...	...	8.3 ± 0.8	70.0 ± 0.6
	control	6	7			7.3 ± 1.3	70.0 ± 0.7
9	pregnant	6	24	...	..	8.1 ± 0.8	69.2 ± 0.5
	control	4	11			6.7 ± 1.1	70.4 ± 1.0

[1] Statistical significance at the 5% level.
[2] Statistical significance at the 2% level.
[3] Statistical significance at the 1% level.

samples of these, and the results are shown in table 2, expressed as the net changes in grams of the carcass constituents, the 5th day of gestation being taken as the initial point.

Initially there was an accumulation of total crude fatty acids in the maternal carcass and then, at the time when fat storage decreased, there was an increase in protein. This change was coincident with the commencement of rapid growth

TABLE 2

Changes in carcass constituents in pregnant and in control rats [1]
(Calculated in grams per animal)

TIME OF GESTATION	PREGNANT [2]			CONTROL [2]		
	Fat	Protein	Moisture	Fat	Protein	Moisture
days						
8	+ 2.1	+ 1.7	+ 6.2	+ 1.9	— 2.4	— 0.5
12	+ 4.5	+ 2.4	+ 7.5	+ 3.3	+ 0.4	+ 2.5
14	+ 6.5	+ 3.2	+ 11.8	+ 3.5	+ 2.7	+ 0.5
16	+ 5.7	+ 2.4	+ 7.0	+ 1.4	+ 1.0	— 0.2
18	+ 1.5	+ 7.0	+ 13.8	+ 3.7	— 1.4	+ 0.7
20	+ 0.4	+ 4.9	+ 11.9	+ 1.5	— 0.8	+ 3.1
22	— 0.5	+ 0.1	+ 3.4	— 0.3	— 2.2	— 0.2
Post partum						
4	— 0.4	+ 2.6	+ 4.7	+ 1.7	+ 2.7	— 1.0
9	+ 2.4	+ 2.0	+ 7.0	+ 1.2	+ 1.1	+ 4.5

[1] The analyses reported in this table were carried out on pooled samples of ground, decapitated, eviscerated carcasses.

[2] The initial compositions in grams per carcass of the two groups were as follows:
Pregnant: fat 19.8; protein 37.6; moisture 101.2.
Control: fat 17.8; protein 40.5; moisture 102.0.

by the fetuses. After the initial storage of protein, it too decreased, presumably being transferred to the rapidly growing fetuses. This might suggest an inter-relationship of fat and protein metabolism, the fat being catabolized to provide energy and to spare the protein when protein storage was desirable. Water was retained throughout gestation but was rapidly excreted following parturition.

It should be noted that the proportions of protein, fat and carbohydrate in the food intake were constant throughout

pregnancy. However, the intake of food varied, depending on free-will use of the animal. Generally, the food use was greater in the second half of pregnancy. This increased consumption might explain extra protein retention, but does not explain the decrease in fat storage. The alterations in nutrient

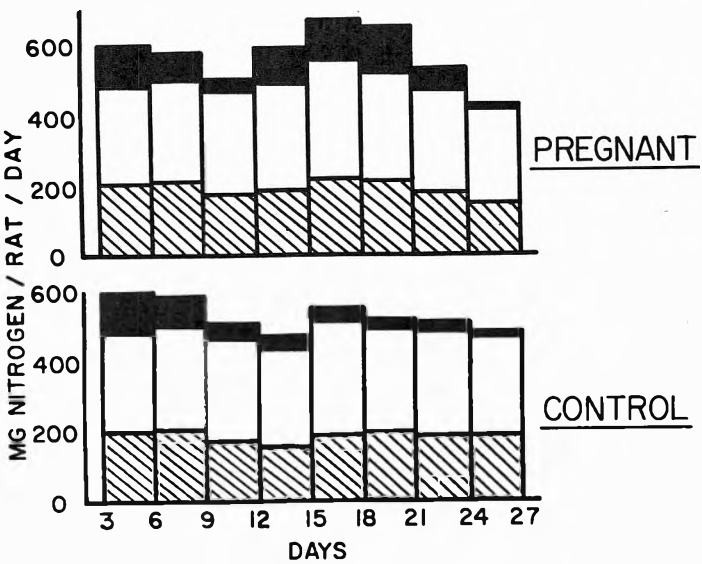

Fig. 1 Nitrogen balance studies during gestation. The bars represent the total nitrogen intake; the shaded portions represent the fecal nitrogen; the blank portions represent the urinary nitrogen; and the black portions represent the retention. It should be noted that in this experiment, the average period of gestation was 21.5 days. The average fetal nitrogen per litter was found to be 787 mg.

retention, not explicable on the basis of food consumption, are likely consequent to an alteration in metabolism.

Nitrogen balance

In experiment II a record of the nitrogen retention was kept throughout gestation as shown in figure 1. At parturition, the fetuses were removed, pooled by litters and analysed for total nitrogen. The difference between the average nitrogen retentions in the pregnant and control groups was found to

be statistically significant at 12½ days ($t = 2.26$; significant at 5% level) and at 19½ days ($t = 3.58$; significant at 1% level). A significant difference between the average nitrogen retention of pregnant and control groups was also observed at the 14th day in experiment III (pregnant = 140 mg/rat/day, control = 87 mg/rat/day; $t = 2.60$, significant at the 5% level).

Blood constituents

An examination of the blood constituents of the animals used in experiment I revealed a marked hemodilution and a fall in blood amino nitrogen during the last week of gestation, as is shown in table 3. Blood urea, which was also measured, showed no change except at parturition when there was an elevated level. (Average blood urea value for all determinations = 35.2 mg%.) Parsons ('30) reported that the unfasted blood urea did not change during gestation in the rat. The observation of hemodilution is in agreement with the findings of Bond ('48), who reported an increase in blood volume in the pregnant rat; he observed that there was an increase in the total circulating erythrocytes, but that this failed to increase as rapidly as did the blood volume. The increased packed cell volume on the 9th day post partum could be explained on the basis of this observation of an increased total circulating erythrocyte count, since a decrease in blood volume to the normal level would give a higher than normal erythrocyte count until they also decreased. The decreased blood amino nitrogen might be a reflection of the increased utilization of amino acids by the growing fetuses.

The rate of urea production in liver slices

In experiment I it was found that there was a consistent lowering of the Q_{urea} from the 15th day of gestation until parturition, returning to the non-pregnant level at parturition; however, due to a high individual variation, statistical significance was not obtained. For brevity these results will not be presented in detail. This lowered Q_{urea} in the last week

TABLE 3

Blood constituent levels in pregnant and in control rats

TIME OF GESTATION	GROUP	PACKED CELL VOLUME	HEMOGLOBIN	BLOOD AMINO NITROGEN
		(mean ± S.D.)	(mean ± S.D.)	(mean ± S.D.)
days		*%*	*gm/100 ml*	*mg %*
5	pregnant	51.0 ± 4.2	12.0 ± 1.2	9.5 ± 0.7
	control	50.9 ± 3.7	12.7 ± 1.0	9.1 ± 0.8
8	pregnant	50.5 ± 0.9	11.7 ± 0.7	10.9 ± 1.3
	control	50.7 ± 4.7	12.1 ± 1.5	11.3 ± 1.3
12	pregnant	45.7 ± 1.4	11.2 ± 0.5	11.2 ± 2.6
	control	46.6 ± 3.7	11.8 ± 1.2	11.4 ± 1.4
14	pregnant	44.4 ± 1.6	11.2 ± 0.4	8.6 ± 1.8
	control	47.3 ± 2.2 diff.[1]	12.5 ± 0.9 diff.[1]	10.2 ± 1.9
16	pregnant	47.3 ± 4.3	11.6 ± 0.5	7.9 ± 0.6
	control	48.4 ± 3.8	12.0 ± 0.4	9.1 ± 0.8 diff.[2]
18	pregnant	42.0 ± 4.0	10.9 ± 0.5	7.4 ± 1.3
	control	48.7 ± 2.4 diff.[2]	12.0 ± 1.0 diff.[1]	8.3 ± 0.8
20	pregnant	41.0 ± 4.3	10.3 ± 0.8	7.1 ± 0.2
	control	46.4 ± 1.8 diff.[1]	12.5 ± 0.7 diff.[3]	9.0 ± 2.2
22	pregnant	39.5 ± 5.9	10.5 ± 1.3	9.1 ± 1.9
	control	46.7 ± 2.3 diff.[2]	12.0 ± 0.9 diff.[1]	8.2 ± 0.5
Post partum				
4	pregnant	48.0 ± 4.3	12.6 ± 1.1	11.0 ± 1.7
	control	51.2 ± 4.6	13.3 ± 0.8	10.4 ± 1.9
9	pregnant	54.9 ± 4.6	14.1 ± 1.3	8.4 ± 0.6
	control	48.3 ± 3.9 diff.[1]	12.9 ± 1.4	9.2 ± 0.7

[1] Statistical significance at the 5% level.

[2] Statistical significance at the 2% level.

[3] Statistical significance at the 1% level.

of gestation had been consistently noted in preliminary experiments in our laboratory. In experiment III it was found that pregnant rats killed on the 17th day of gestation had a significantly lower Q_{urea} than comparable non-pregnant controls (pregnant $= 3.04 \pm 0.72$, control $= 5.01 \pm 0.71$; $t = 4.75$, significant at the 1% level).

No change in liver arginase activity was observed when determinations were carried out in experiment IV (pregnant $= 57 \pm 15$, control $= 64 \pm 16$). This is in agreement with the results of Folley and Greenbaum ('47), who could show no change during gestation except on the 15th day when they

TABLE 4

The effect of additions of ornithine and of citrulline on the Q_{urea} [1] of pregnant and of control rats

ADDITION	Q_{urea} [1]	
	Pregnant	Control
	(mean ± S.D.)	*(mean ± S.D.)*
None	3.04 ± 0.42	3.51 ± 0.63
DL-ornithine hydrochloride	8.19 ± 1.45	9.18 ± 1.68
DL-citrulline	8.22 ± 1.35	8.66 ± 1.79

[1] Defined as microliters of urea-CO_2 formed per milligram dry tissue per hour.

observed a lowered activity. Lightbody et al. ('41) also reported no change in liver arginase activity per milligram of tissue during gestation.

In experiment V an attempt was made to find the cause of the lowered Q_{urea} observed in pregnant rats. Ornithine and citrulline were added to the medium in the determination of Q_{urea} in amounts that had been shown by Gornall and Hunter ('43) to give maximal stimulation of urea production in normal fasted rats. Sufficient slices were prepared from each liver for 6 determinations of Q_{urea}. Duplicate estimations were made without additions, in the presence of added DL-citrulline (31.2 mg%), and in the presence of added DL-ornithine hydrochloride (30.0 mg%). It should be noted that equimolar

amounts of the two additives were used. Simultaneous determinations of liver arginase activity were carried out. The results are shown in table 4. A lowered Q_{urea} was observed in the pregnant group although statistical significance was not attained. No difference in liver arginase activity was noted (pregnant = 57.4 ± 9.9, control = 54.2 ± 8.8) and on the basis of individual results, there was no apparent correlation between the Q_{urea} and liver arginase activity. Among the individual results, it was also noted that even livers exhibiting

TABLE 5

Liver alanine-glutamic transaminase activity in pregnant and in control rats

TIME OF GESTATION	ALANINE-GLUTAMIC TRANSAMINASE [1]		SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEANS
	Pregnant	Control	
days	*(mean ± S.D.)*	*(mean ± S.D.)*	*%*
5	41.5 ± 7.7	42.3 ± 7.6	
8	31.0 ± 12.2	40.9 ± 10.7	
12	39.0 ± 7.0	38.0 ± 12.3	
14	43.3 ± 8.3	53.3 ± 18.5	
16	26.3 ± 7.4	36.5 ± 7.1	5
18	14.8 ± 2.2	33.0 ± 4.0	1
20	18.2 ± 7.8	36.8 ± 9.0	1
22	12.7 ± 7.1	40.7 ± 7.8	1
Post partum			
4	23.1 ± 10.5	27.8 ± 8.4	
9	36.5 ± 5.1	38.0 ± 5.5	

[1] Defined as microliters pyruvate-CO_2 formed per milligram wet tissue per hour.

a very low initial Q_{urea} demonstrated a stimulated Q_{urea} as high as other livers exhibiting a higher initial Q_{urea}. It would seem therefore that there was no abnormality of the enzymes of the ornithine cycle since no difference between the groups was observed in the stimulation following the addition of either catalyst. The decrease in the rate of urea formation is likely secondary to the increased utilization of amino acids for protein synthesis. Although there may have been a reduced amount of ornithine and citrulline present in the livers of the pregnant rats, sufficient enzyme activity was present to permit marked stimulation by the addition of ornithine or citrulline.

Liver transaminase activity

In experiment I, no change was observed in the aspartic-glutamic transaminase activity at any time; the alanine-glutamic transaminase showed a significantly lowered activity during the last week of gestation, with a rapid return to the non-pregnant level following parturition, as is shown in table 5.

In experiment III both transaminases were estimated in livers and kidneys; there was a lowering of alanine-glutamic transaminase activity in the livers of pregnant rats (pregnant $= 38 \pm 6.6$, control $= 47 \pm 16.8$) but no change was observed in the kidney (pregnant $= 14 \pm 3.5$, control $= 13 \pm 4.9$); no differences were observed between the two groups in the aspartic-glutamic transaminase of either liver (pregnant $= 97 \pm 13.8$, control $= 98 \pm 12.1$) or kidney (pregnant $= 61 \pm 13.9$, control $= 62 \pm 11.0$).

Discussion of changes occurring during and after gestation

The observations reported above are summarized as follows:—

During 0–15 days of gestation.

1. Slight increase in total body weight.
2. Small increase in fetus weight.
3. Small increase in liver weight.
4. Total crude fatty acids accumulate and water retention occurs in the maternal carcass.
5. Slightly greater retention of nitrogen than in the non-pregnant rat.

After 15 days gestation up to parturition.

1. Marked increase in total body, fetus and liver weights and liver moisture.
2. Sharp fall in the fat stores and a simultaneous increase in protein stores with a subsequent gradual fall; continued water retention.
3. Greatly increased retention of nitrogen.

4. Hemodilution and lowered blood amino nitrogen.
5. Lowered Q_{urea} in liver slices.
6. Lowered alanine-glutamic transaminase.

At and following parturition.

1. An initial rise in blood urea and a rise in Q_{urea} to the non-pregnant level.
2. Immediate rise in blood amino nitrogen to the non-pregnant level.
3. Gradual return of other changes to the non-pregnant level.

All of the changes noted were sharply accentuated following the 15th day of gestation. This appears to be a critical point in the total period of pregnancy in the rat. Some factor, probably hormonal, may control these alterations in metabolism. In view of the apparent interrelationship of fat and protein metabolism as evidenced by the changes in carcass constituents, it is interesting to note that it has been recently suggested by Greenbaum ('53) that growth hormone may exert its primary effect on fat metabolism with a secondary effect on protein metabolism. It has been known for many years that the administration of this hormone promotes nitrogen retention. Experiments in our laboratory (Beaton et al., '53) have demonstrated that the injection of growth hormone into normal male rats can cause a lowered Q_{urea} and a decrease in liver alanine-glutamic transaminase activity. It may be that growth hormone plays an important role in controlling the metabolic alterations which have been observed in the pregnant rat.

SUMMARY

1. During the first two weeks of pregnancy in the rat, marked storage of fat and water occurs in the maternal carcass while there is little fetal growth.

2. At about the 15th day of gestation, a definite decrease in fat stores occurs and the retention of protein increases. Accompanying protein retention there are decreases in blood amino nitrogen, in hepatic alanine-glutamic transaminase and in the rate of urea formation in liver slices.

3. It is suggested that growth hormone may be implicated in the alteration in metabolism observed at about the 15th day of gestation.

LITERATURE CITED

ARCHIBALD, R. M. 1945 Colorimetric determinations of urea. J. Biol. Chem., *157:* 507.

BEATON, G. H., G. OZAWA, J. R. BEATON AND E. W. MCHENRY 1953 Effect of anterior pituitary growth hormone on certain liver enzymes. Proc. Soc. Exp. Biol. Med., *83:* 78–.

BOND, C. F. 1948 The nature of the anemia of pregnancy in the rat. Endocrinology, *43:* 180.

CALDWELL, E. F., AND E. W. MCHENRY 1953a Vitamin B_6 and urea formation. Arch. Biochem. Biophys., *44:* 396.

———— 1953b Studies on vitamin B_6 and transamination in rat liver. Ibid., *45:* 97.

COLLIER, H. B. 1944 The standardization of blood haemoglobin determinations. Canad. Med. Assoc. J., *50:* 550.

FOLLEY, S. J., AND A. L. GREENBAUM 1947 Changes in the arginase and alkaline phosphatase contents of the mammary gland and liver of the rat during pregnancy, lactation and mammary involution. Biochem. J., *41:* 261.

FRAME, E. G., J. A. RUSSELL AND A. E. WILHELMI 1943 The colorimetric estimation of amino nitrogen in blood. J. Biol. Chem., *149:* 255.

GAVIN, G., AND E. W. MCHENRY 1940 The B vitamins and fat metabolism. III. The effects of vitamin B_6 upon liver and body fat. Ibid., *132:* 41.

GORNALL, A. G., AND A. HUNTER 1943 The synthesis of urea in the liver with special reference to citrulline as an intermediary in the ornithine cycle. Ibid., *147:* 593.

GREENBAUM, A. L. 1953 Changes in body composition and respiratory quotient of adult female rats treated with purified growth hormone. Biochem. J., *54:* 400.

KREBS, H. A., AND K. HENSELEIT 1932 Untersuchungen über die Harnstoffbildung im Tierkörper. Ztschr. f. physiol. Chem., *210:* 33.

LIENER, I. E., AND M. O. SCHULTZE 1950 Liver arginase activity as related to blood urea in acute uremia of new-born rats. J. Biol. Chem., *187:* 743.

LIGHTBODY, H. D., E. WITT AND A. KLEINMAN 1941 Variations in arginase concentration in livers of white rats caused by thyroxine administration. Proc. Soc. Exp. Biol. Med., *46:* 472.

MORSE, L. M., AND C. L. A. SCHMIDT 1944 Nitrogen balances on rats during pregnancy and lactation. Ibid., *56:* 57.

PARSONS, H. T. 1930 Urea concentrations in the blood of the rat in relation to pregnancy and lactation on diets containing varying concentrations of protein. J. Biol. Chem., *88:* 311.

PIKE, R. L., H. B. SUDER AND M. L. ROSS 1954 The influence of protein and energy intakes upon nitrogen retention in the pregnant rat. J. Nutrition, *52:* 297.

Poo, L. J., W. Lew, D. D. Lee and T. Addis 1940 Protein anabolism in the organs and tissues of pregnant rats at different levels of protein consumption. Ibid., *19:* 505.

Tonhazy, N. E., N. G. White and W. W. Umbreit 1950 A rapid method for the estimation of the glutamic-aspartic transaminase in tissues and its application to radiation sickness. Arch. Biochem., *28:* 36.

Van Slyke, D. D., and R. M. Archibald 1946 Gasometric and photometric measurement of arginase activity. J. Biol. Chem., *165:* 293.

EFFECT OF ASCORBIC ACID ON RATS DEPRIVED OF PANTOTHENIC ACID DURING PREGNANCY[1, 2]

GLADYS EVERSON,[3] LOIS NORTHROP, NAM YOUNG CHUNG AND ROBERT GETTY

Nutrition Laboratory, Home Economics Research Department, Iowa Agricultural Experiment Station, and Department of Veterinary Anatomy, Iowa State College, Ames

(Received for publication May 12, 1954)

An interesting effect of large amounts of ascorbic acid in delaying the onset of pantothenic acid deficiency has been reported by Daft ('51) and Daft and Schwarz ('52). More recently this work has been continued by Hundley and Ing ('53) who have found that a number of compounds structurally related to ascorbic acid also stimulate growth of young rats deprived of pantothenic acid. Glucuronolactone, which was a growth stimulant when given orally, was ineffective if given parenterally. To date there is no satisfactory explanation as to how ascorbic acid and certain other 6 carbon compounds "spare" the need for pantothenic acid.

At the time of the first report from the National Institutes of Health, studies underway in this laboratory permitted the testing of the value of ascorbic acid when rats were fed synthetic rations containing varying amounts of pantothenic acid during the gestation period.

[1] Journal Paper No. J-2409 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1213. A preliminary report appears in Federation Proc., *12*, 413 (1953).

[2] Financial support from the Department of Health Education and Welfare, Public Health Service, National Institutes of Health (G-3198) is gratefully acknowledged.

[3] Present address: Department of Home Economics, University of California, Davis, California.

In the present paper we wish to report the effect of including 2% ascorbic acid in the diet upon the reproductive performance of the rat and upon certain chemical and histochemical findings in the young at birth when the maternal diet is lacking in pantothenic acid.

TABLE 1

Composition of the synthetic ration

Vitamin-free casein	25%
Dextrose	55%
Swiftening [1]	15%
Salts [2]	5%
Vitamin mix fed daily per rat	
Choline chloride	10 mg
Inositol	5 mg
p-aminobenzoic acid	100 μg
2-methyl-Napthoquione	250 μg
Thiamine hydrochloride	150 μg
Riboflavin	100 μg
Niacin	500 μg
Biotin	2.5 μg
Folic acid	6 μg
Pyridoxine hydrochloride	50 μg
Vitamin B_{12}	0.5 μg
Fat soluble vitamins	
Cod liver oil:	9 U.S.P. units vitamin D
	90 U.S.P. units vitamin A
Alpha tocopherol	750 μg

[1] An hydrogenated fat made by Swift and Company, Chicago, Ill., which contains both animal and vegetable fat.

[2] Richardson and Hogan, J. Nutrition, *32:* 459, 1946.

EXPERIMENTAL

Young adult female rats from an inbred colony of the Wistar strain were selected for the study. These animals received the customary stock ration from weaning age until the end of first pregnancies. When it was established that the first litters were normal, the young were sacrificed and the females were immediately transferred to one of the following groups:

Group I (35 females) received a synthetic ration plus a vitamin mix which contained all known factors except ascorbic acid and pantothenic acid.

Group II (17 females) received the same basal ration plus 100 μg of calcium pantothenate daily.

Group III (16 females) received the same ration as group I plus 2% ascorbic acid.

The synthetic diet is given in table 1.

Immediately following the birth of the second litters and before the young had an opportunity to nurse, the newborn were removed from the cage and their weight and sex were determined. Any evidence of abnormalities was recorded. The young were then distributed into several groups for subsequent chemical or histological study.

Total pantothenic acid activity of the blood of the young at birth, of the tissues of the newborn, and of the maternal liver and carcass was determined by the microbiological procedure using *Lactobacillus arabinosus* as the test organism. All tissues were treated with chicken liver enzyme and intestinal phosphatase to release bound forms of the vitamin. Serum ascorbic acid was determined in the young at birth by the Lowry, Lopez and Bessey ('45) microadaptation of the dinitrophenylhydrazine method of Roe and Kuether ('43). Alkaline phosphatase values for the serum of the young were measured by the method of Bessey, Lowry and Brock ('46). Pyruvic acid concentrations of the blood of the young at birth were established using the micromethod of Tsao and Brown ('50). Histological sections were prepared of the adrenal, tibia, duodenum and liver of the young at birth and acid and alkaline phosphatase activities of these tissues were demonstrated by the method of Gomori ('39, '41) as modified by Deane and Dempsey ('45).

RESULTS AND DISCUSSION

A distinct difference in the reproductive performance of the females as well as chemical findings in the tissues of the mother and the young reveal that ascorbic acid in amounts

TABLE 2

Reproductive performance and chemical findings in tissues of rats fed diets varying in ascorbic acid and pantothenic acid [1]

GROUP NO.	RATION	NO. OF YOUNG PER LITTER	BIRTH WEIGHT	PANTOTHENIC ACID CONTENT OF BLOOD OF YOUNG	PANTOTHENIC ACID CONTENT OF TISSUES			ALKALINE PHOSPHATASE OF SERUM OF YOUNG	ASCORBIC ACID OF SERUM OF YOUNG	BLOOD PYRUVIC ACID OF YOUNG
					YOUNG AT BIRTH	MATERNAL LIVER	MATERNAL CARCASS			
			gm	*μg/100 ml*	*μg/gm*	*μg/gm*	*μg/gm*	*nitrophenol units*	*mg/100 ml*	*mg/100 ml*
I	*Synthetic diet* No calcium pantothenate (35 rats)	4.2	3.7	295 (108–447) (20)	6.7 (5.1–7.6) (25)	40.7 (38.6–43.6) (10)	4.4 (3.2–5.0) (10)	12.0 (7.0–16.9) (20)	4.12 (2.47–6.00) (20)	2.79 (0.78–6.33) (23)
II	*Synthetic diet* 100 μg calcium pantothenate No ascorbic acid (17 rats)	7.8	4.6	502 (320–678) (27)	15.8 (12.6–24.8) (25)	54.6 (45.0–62.4) (10)	5.5 (4.8–6.0) (10)	11.8 (7.7–15.8) (40)	4.58 (3.19–7.31) (42)	1.04 [2] (0.29–1.88) (23)
III	*Synthetic diet* No calcium pantothenate 2% ascorbic acid (16 rats)	4.8	4.3	542 (496–609) (25)	14.2 (10.0–23.0) (38)	60.3 (52.0–72.5) (10)	4.4 (4.0–4.6) (10)	10.7 (7.6–15.4) (38)	5.30 (3.86–7.31) (38)	0.65 (0.16–2.21) (23)

[1] Average values listed first; range given below; third value indicates number of animals examined in each group.

[2] Values for females receiving the stock ration providing 500 μg of pantothenic acid daily.

to supply 2% of the ration has a "sparing" effect upon pantothenic acid in the case of the rat (see table 2). Litters born to females receiving a synthetic diet lacking in both pantothenic acid and ascorbic acid were always poor while young born to females receiving ascorbic acid were usually superior in appearance. The average birth weight of the young of females receiving neither pantothenic acid or ascorbic acid was 3.7 gm, with an average litter size of 4.2 young. The presence of ascorbic acid increased the birth weight of the young slightly although the litter size was still small. The presence of 100 μg of calcium pantothenate in the ration per day allowed the birth of larger litters composed of heavier young.

Pantothenic acid concentrations of the blood, found to be approximately 500 μg per 100 ml for young of stock females at birth, were maintained when the females received only 100 μg of the vitamin per day (Everson, Northrop, Chung, Getty and Pudelkewicz, '54). The complete withdrawal of the vitamin, however, caused a lower blood pantothenic acid concentration in the young. This drop was prevented when the maternal diet included 2% ascorbic acid.

Total tissues of the newborn averaged 6.7 μg of pantothenic acid per gram (normal moisture) when the maternal diet contained no pantothenic acid and no ascorbic acid. The addition of either 2% ascorbic acid or 100 μg of calcium pantothenate per day more than doubled the quantity of pantothenic acid present in the tissues of the young at birth. Similarly the presence of ascorbic acid resulted in a higher concentration of pantothenic acid in the hepatic tissue of the female than when both vitamins were lacking.

The accumulation of pyruvic acid in the young delivered by females receiving no dietary source of pantothenic acid was not observed when ascorbic acid was included in the ration.

While no structural differences were found in the adrenal, tibia, duodenum, or liver of newborn rats which could be attributed to a withdrawal of pantothenic acid from the diet

of the mother during pregnancy, histochemical differences were noted in the adrenal of young produced by females restricted in both pantothenic acid and ascorbic acid (Chung, Northrop, Getty and Everson, '54). The concentration of alkaline phosphatase was less intense in this tissue when both vitamins were omitted from the maternal diet. The addition of ascorbic acid to the ration of the mother protected against this change.

SUMMARY AND CONCLUSIONS

Studies conducted on albino rats during reproduction have revealed that 2% ascorbic acid in a synthetic ration lacking in pantothenic acid had a beneficial effect upon reproductive performance. The presence of the ascorbic acid increased the birth weight of the young slightly. Pantothenic acid concentrations in the tissues of the young at birth and of the mother were higher when ascorbic acid was included in the diet. Blood values for pantothenic acid were comparable to those of stock young when 2% ascorbic acid was mixed with the ration while young born to females deprived of both pantothenic acid and ascorbic acid possessed lower concentrations.

Newborn rats produced by females receiving neither pantothenic acid or ascorbic acid also exhibited some rise in blood pyruvic acid. The addition of 2% ascorbic acid to the diet prevented the accumulation of pyruvic acid and the resulting values were more like those found in young of stock females.

A single variation from the normal was encountered in histological studies of 4 tissues of the young at birth — that of a decrease in alkaline phosphatase activity of the adrenal when the ration of the female contained neither pantothenic acid or ascorbic acid. When ascorbic acid was incorporated into the ration the alkaline phosphatase activity was much like that found in the adrenals of healthy stock young.

The data reveal that in each case where a definite sign of pantothenic acid deficiency was established, ascorbic acid lessened the severity of the deficiency and blood and tissue findings were more nearly like those of normal young. In

several instances the protection afforded by the ascorbic acid was equal to that of 100 μg of calcium pantothenate.

LITERATURE CITED

BESSEY, O. A., O. H. LOWRY AND M. J. BROCK 1946 A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem., *164:* 321.

CHUNG, N. Y., L. NORTHROP, R. GETTY AND G. EVERSON 1954 Effect of varying the intake of calcium pantothenate of rats during pregnancy. II. Histological and Histochemical studies of the liver, adrenal, duodenum and tibia of the young at birth. J. Nutrition, *54:* 97.

DAFT, F. S. 1951 Effect of vitamin C on pantothenic-deficient rats. Fed. Proc., *10:* 380.

DAFT, F. S., AND K. SCHWARZ 1952 Prevention of certain B vitamin deficiencies with ascorbic acid or antibiotics. Ibid., *11:* 200.

DEANE, H. W., AND E. W. DEMPSEY 1945 The localization of phosphatases in the golgi region of intestinal and other epithelial cells. Anat. Rec., *92–93:* 401.

EVERSON, G., L. NORTHROP, N. Y. CHUNG, R. GETTY AND C. PUDELKEWICZ 1954 Effect of varying the intake of calcium pantothenate of rats during pregnancy. I. Chemical findings in the young at birth. J. Nutrition, *53:* 341.

GOMORI, G. 1939 Microtechnical demonstration of phosphatase in tissue sections. Proc. Soc. Exp. Biol. Med., *42:* 23.

———— 1941 The distribution of phosphatase in normal organs and tissues. J. Cell. and Comp. Physiol., *17:* 71.

HUNDLEY, J. M., AND R. B. ING 1953 Prevention of pantothenic acid deficiency with glucuronolactone and other compounds related to ascorbic acid. Fed. Proc., *12:* 417.

LOWRY, O. H., J. A. LOPEZ AND O. BESSEY 1945 The determination of ascorbic acid in small amounts of blood serum. J. Biol. Chem., *160:* 609.

ROE, J. H., AND C. A. KUETHER 1943 The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. Ibid., *147:* 399.

TSAO, M. U., AND S. BROWN 1950 Pyruvic acid determination: A micromethod. J. Lab. and Clin. Med., *35:* 320.

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

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

Nominations may be made by anyone. Nominations for the 1955 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1955.

Chairman, Nominating Committee:

DR. FLOYD S. DAFT
Institute of Arthritis and Metabolic Disease
National Institutes of Health
Bethesda, Maryland

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1955 Award and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers during the previous calendar year, but the Jury of Award may recommend that it be given for important contributions made over a more extended period of time not necessarily including the previous calendar year. The award is usually given to one person, but if in their judgment circumstances and justice so dictate, the Jury of Award may recommend that it be divided between two or more collaborators in a given research. The Jury may also recommend that the award be omitted in any given year if in its opinion the work submitted does not warrant the award. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award. Employees of the Borden Company are not eligible for this honor.

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

Chairman, Nominating Committee:

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