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Relation of Dietary Fat to Riboflavin Requirement of Growing Rats.*

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Studies in this laboratory on the relation of the level of fat, carbohydrate, and protein in the diet to the vitamin requirements of growing animals led to the observation that the feeding of high levels of fat to young rats increased the riboflavin requirement. Modifications of the basal riboflavin-low ration K_{21} described by Wagner, Axelrod, Lipton, and Elvehjem[1] were utilized in studying this effect. Lard was isodynamically substituted for dextrin at two levels so that in ration K_{22} the total fat content was about 25% and in K_{23} about 40%. Choline was added to the extent of 5 mg per 100 g of K_{21} or its isocaloric equivalent in the other rations. Storage of the rations in the refrigerator and daily feeding prevented rancidity.

In the first series albino rats weighing from 60 to 75 g were partially depleted of their riboflavin reserves by placing 12 rats on each of the above rations for a period of 4 weeks. Four animals in each group were then supplemented with 3γ of riboflavin per day, four, with 6γ of riboflavin, and four were continued on the basal ration with no additional supplements. The growth responses of typical animals are illustrated in Fig. 1.

The growth curves show that the feeding of high levels of fat to growing rats increases the requirement for riboflavin and that the higher the fat level, the more noticeable is the effect. The greater loss of hair and extent of dermatitis observed in the rats raised on the high fat diets were even more notable than the weight differences. Rats maintained in a chronic state of deficiency on the high fat rations developed a spastic gait due to a paralytic condition

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[1] Wagner, J. R., Axelrod, A. E., Lipton, M. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1940, **136**, 357.

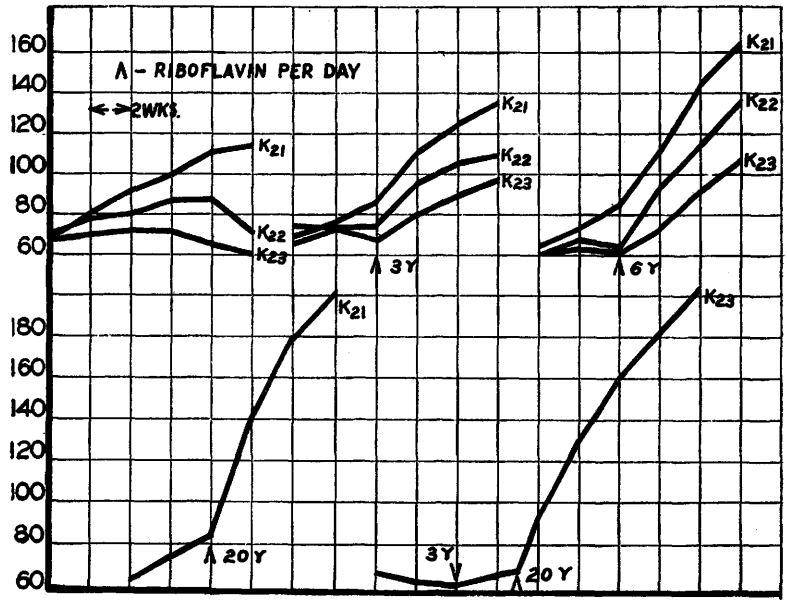

FIG. 1.
Growth curves for rats raised on rations containing different levels of fat and supplemented with different levels of riboflavin.

of the hind legs. This condition could be cured within 2 weeks after the administration of riboflavin. The characteristic high degree of deficiency and the spastic gait developed in a rat by feeding a high level of fat is illustrated in Plate 1. Cataracts similar to those described by Day, Langston, and O'Brien[2] were observed in several of these rats. Animals in the final stages of deficiency when supplemented with 20 or 40γ of riboflavin per day responded remarkably and developed a normal appearance and weight within 3 to 6 weeks. The growth curve of control rats receiving the high carbohydrate diet plus 20γ of riboflavin daily and the curve showing the cure effected by riboflavin in rats depleted on the high fat ration are given in Fig. 1.

Additional evidence supporting interpretation of the above data was gained by subjecting animals to a free choice of both high and low fat-containing rations. During the 10th week of the experiment 3 rats, each of which was receiving a different level of supplemented riboflavin, were selected from each group and fed both the high carbohydrate ration and the ration containing 40% fat. Food consumption records revealed that rats which had been raised on the

[2] Day, P. L., Langston, W. C., and O'Brien, C. S., *Am. J. Ophth.*, 1931, **14**, 1005.

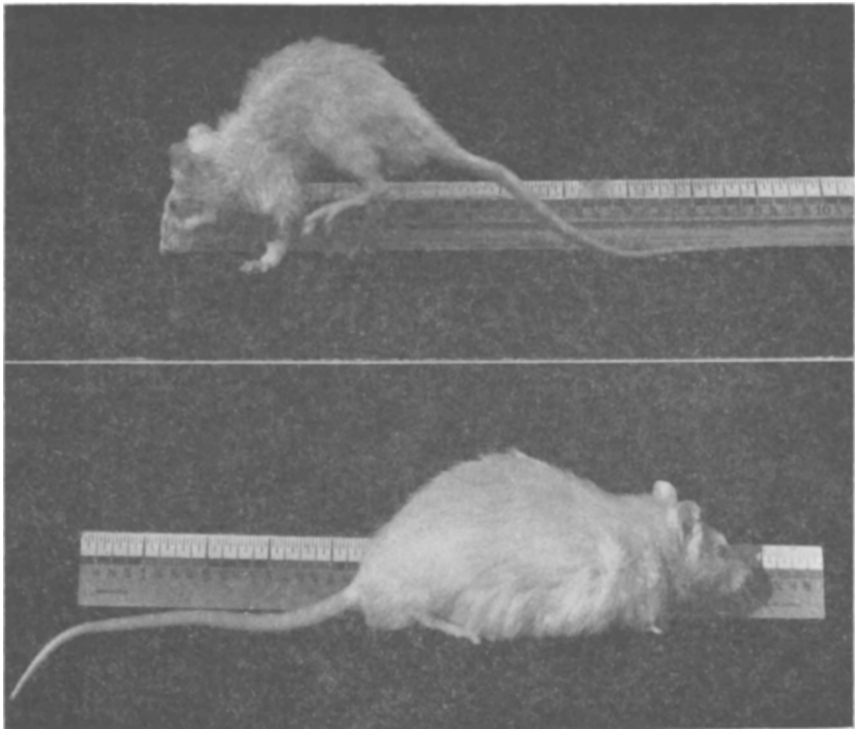

PLATE 1.

The upper photograph shows a rat raised for 18 weeks on the ration containing 40% fat plus 3 γ of riboflavin per day. Weight: 66 g. Below is the same animal 4 weeks later after having been fed 40 γ of riboflavin daily during this period. Weight: 205 g.

high fat rations selected the high carbohydrate ration in preference to the high fat ration with almost complete neglect of the latter. A corresponding weight increase resulted. Though rats which had been receiving the high carbohydrate diet selected the high fat ration during the first 3 or 4 days of the experiment, they gradually reversed their choice until they were consuming only the high carbohydrate ration. These animals were then given only the high fat ration and a retardation of growth followed. That the selection was not influenced by rancidity or the greasy texture of the high fat ration was shown by the preference of normal rats for the fatty diet.

A second series of albino rats weighing from 30 to 40 g were partially depleted for 2 weeks on rations K_{21} and K_{22} in which the choline and vitamin B_6 had been doubled and crystalline pantothenic acid added at the level of 500γ per 100 g ration. At the end of the

depletion period the rats which had been fed the high fat ration weighed less than those which had been placed on the high carbohydrate ration. Various levels of riboflavin were then added, namely, 0, 6, 18, and 54γ per day. The final weights of the animals after being on experiment for 7 weeks and the gains in weight during the 5-week supplementation period are given in Table I. Each figure represents an average of 3 rats. Rats receiving no added riboflavin attained a higher weight plateau level when fed the high carbohydrate diet than when fed the high fat diet as is shown by the final weights and gains in weight per week. All animals supplemented with 6γ of riboflavin daily showed a weight response to the vitamin but rats fed the high fat ration showed a smaller weekly weight increase and a correspondingly lower final weight. The differences between rats receiving the high and low fat rations plus 18γ of riboflavin daily were not large but might indicate that this level of riboflavin is not quite adequate for the support of maximum growth of animals fed the high fat ration. Both rations supported good growth in rats receiving 54γ of riboflavin daily.

From a number of possible explanations for the antagonistic effect of fat on riboflavin, two deserve special consideration; namely, (1) increasing the level of fat in the diet may alter the intestinal flora so that less than normal bacterial synthesis, or more than normal bacterial destruction of riboflavin occurs, or (2) riboflavin may be directly concerned with fat metabolism or phosphorylation. As yet no experimental evidence is available supporting definite conclusions regarding the mechanism involved. Further work in this direction is in progress at this laboratory.

TABLE I.

Response of Rats Raised on High and Low Fat Rations to Various Levels of Added Riboflavin.

	Micrograms of riboflavin per day			
	0		6	
Ration	Final wt, g	Gain* per wk, g	Final wt, g	Gain* per wk, g
K_{21}	84	4.7	112	11.5
K_{22}	61	2.3	86	8.7
	Micrograms of riboflavin per day			
	18		54	
Ration	Final wt, g	Gain* per wk, g	Final wt, g	Gain* per wk, g
K_{21}	180	26.2	185	26.9
K_{22}	166	23.3	193	29.5

*Based on the 5-week supplementation period.

In summary, it has been demonstrated that increasing the fat level in a riboflavin-low ration has a corresponding deleterious effect on the growth of young rats, and that the administration of adequate amounts of riboflavin completely corrects this deficiency.

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Reductions in Blood Pressures of Renal Hypertensive Dogs by Hog Renin.*†

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A number of attempts to reduce the blood pressures of renal ischemic hypertensive dogs have been reported recently. Most of these have been unsuccessful, although a few investigators have obtained results showing some promise. Thus Davis and Barker[1] reported that potassium sulphocyanate reduced the blood pressure of renal hypertensive dogs. Mansfield, *et al.*,[2] found that nephro-splenopexy but not nephro-omentopexy caused a more or less permanent decrease in the blood pressure of dogs rendered hypertensive by the Goldblatt technic. Similar results were later reported by Cerqua and Samaan.[3] Obviously methods involving the production of an increase in the collateral circulation to the ischemic kidney have at best a limited applicability to the therapy of essential and malignant hypertension in the human since the ischemia in the clinical condition is ordinarily due to arteriosclerosis rather than narrowing of the renal arteries. Katz and coworkers[4] reported that transplanted autolyzing kidney tissue reduced the blood pressure of renal hypertensive dogs but Goldblatt[5] was unable to confirm this

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1 Davis, L., and Barker, M. H., *Ann. Surg.*, 1939, **110**, 1016.

2 Mansfield, J. S., Weeks, D. M., Steiner, A., and Victor, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 708.

3 Cerqua, S., and Samaan, A., *Clin. Sc.*, 1939, **4**, 113.

4 Rodbard, S., Katz, L. N., and Sokolow, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 360.

5 Goldblatt, H., personal communication.