nomenon may have significance to the physiologist interested in the administration of a tagged protein.

So far as the practical use of the administration of rabbit thrombin is concerned, it must be pointed out that relatively large amounts of the material were used. The data indicate that in hemophilic individuals even larger amounts would be necessary to reduce the blood coagulation time to normal. Further studies, however, may produce a more potent material which might result in the production of beneficial effects.

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Metabolism and Food Utilization of Riboflavin-Deficient Chicks.

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Riboflavin deficiency, like most dietary deficiencies, lowers the growth rate of animals. This influence has been described for rats by György and his coworkers[1] and for chicks by Lepkovsky and Jukes.[2] The relation of this stunting of growth to the utilization of food energy and food protein is discussed in this paper.

Method. Five respiration trials of 10 days' duration were carried out with 5 groups of 10 chicks. At the age of 7-9 days the chicks were kept on a diet that was considered deficient in riboflavin, since it led to a stunting of growth and that stunting could be prevented by the addition of riboflavin.

At the age of 12 days 10 chicks in each of the 5 trials were placed in an open-air circulating respiration chamber at a temperature of 29 to 30°C. Food and water were kept before the chicks from 8 a.m. to 8 p.m. During the night the chicks had access only to water. The excreta were collected every 12 hours. Food and excreta were analyzed for nitrogen according to Kjeldahl and for energy in the bomb

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1 György, P., Kuhn, R., and Wagner, Jauregg, T., *Klin. Wochenschr.*, 1933, **12**, 1241.

2 Lepkovsky, S., and Jukes, T. H., *Science*, 1935, **82**, 326.

calorimeter. At the end of the 10 days' feeding period in the respiration chamber the chicks were fasted for 24 hours and then their respiratory exchange was again measured over a 12-hour period during the night. A second similar group of chicks was given the same food as the first but Fuller's earth adsorbate of whey was added as riboflavin supplement. The food intake was restricted to that of the deficient chicks. A third group was kept on the flavin supplemented food *ad lib.*, a fourth group was used as a duplicate of the first and a fifth group as a duplicate of the second. The respiratory exchange during fasting was measured also on 5 extra groups of chicks kept under similar conditions as those mentioned. A mean respiratory quotient of 0.724 indicated proper post-absorptive condition.

To check these results we measured in another group of flavin-deficient and control chicks nitrogen and energy balances by paired feeding and carcass analyses in place of the respiration trials. We used a different flavin-deficient basic diet and crystalline riboflavin instead of Fuller's earth adsorbate of whey as vitamin supplement. The chicks were kept on the flavin-deficient diet for several days. At the age of 20 days they were then separated into 3 equivalent groups according to body weight. Ten chicks were killed at the start of the experiment, 10 were continued on the flavin-deficient food *ad libitum*, and 10 others were supplied each with 0.2 mg of riboflavin dissolved in water and pipetted daily into their crops. The food intake of each of these flavin-supplied chicks was restricted to that of its flavin-deficient pairmate. In 7 pairs we were successful to equalize the food intake closely enough for comparison of the results. The mean daily intake of food energy per chick was 47.9 and 44.2 kcal for the flavin-deficient and flavin supplied chicks respectively. The chicks were kept at 30°C throughout the 10-day experiment, at the end of which they were killed and analyzed for energy and nitrogen.

Results. The most common symptom of a qualitative deficiency in food is a decrease in appetite. Such a decrease resulting from flavin deficiency has been observed in rats by Graham and Griffith.[3] Remp and Bing[4] noted that vitamin G-deficient mice consumed only 60% of the amount of food taken in by vitamin G supplied mice. Riboflavin deficiency similarly decreased the appetite of our chicks. During the first 5-day period of the respiration trials the deficient chicks consumed only ⅔ and during the second period only ½ so much as the flavin supplied controls on unlimited food intake.

[3] Graham, C. E., and Griffith, W. H., *J. Nutr.*, 1933, **6**, 195.

[4] Remp, D. G., and Bing, F. C., *J. Nutr.*, 1934, **8**, 457.

Twenty-nine control chicks, that had been on unlimited intake of flavin-supplemented food weighed at the age of 13 days on the average 71 gm apiece and produced during the subsequent fast a mean of 14.8 ± 1.0 kcal of heat per day per chick or 108 kcal per $kg^{3/4}$.† The corresponding mean daily fasting heat production of 45 flavin-deficient chicks with a mean weight of 51 g amounted to only 10.2 ± 0.8 kcal per chick or 95 ± 6 kcal per $kg^{3/4}$. The same low rate of fasting heat production was, however, observed in 19 controls whose food intake during the 10-day trial had been restricted to that of the deficient chicks, whose mean body weight was 56 g and who produced during fast per day 10.1 ± 1.5 kcal of heat per chick or 96 ± 11 kcal per $kg^{3/4}$. Aside from possible influences of decreased food intake flavin deficiency thus did not affect the rate of fasting heat production in chicks. This result is in line with observations of Drummond and Marrian[5] on vitamin B-deficient rats.

The heat production of the chicks during the 10-day feeding period was calculated on the basis of total carbon and nitrogen balances. The flavin supplied controls on unlimited food intake burnt 75% of the metabolizable energy in their food. The mean heat production of the flavin-deficient chicks amounted to 103% of the metabolizable food energy consumed; that of the flavin-supplied controls on restricted food intake to 109%. Flavin deficiency thus did not produce a greater waste of food energy than did a correspondingly low intake of flavin-supplemented food alone.

Protein utilization in contrast was considerably decreased by flavin deficiency. The flavin supplied chicks on unlimited food intake stored 44% of the food nitrogen in their body, the flavin-deficient chicks as little as 25%. Only one-half of this decrease in total efficiency of nitrogen utilization may be accounted for by the decrease in food intake, since the nitrogen gain of the flavin-supplied chicks on restricted food intake amounted to 35% of the food nitrogen. The difference in the total efficiency of nitrogen utilization between flavin-deficient and flavin-supplied chicks on equal food intake, calculated for 5-day periods, has a random probability of 2% and may therefore be regarded as statistically significant.

Flavin deficiency thus decreased the utilization of protein but did not affect the energy utilization. This observation was confirmed by the pair trials with carcass analysis. The main results of this trial are summarized in Table I.

Since body weight as well as food intake among the pairmates

† $Kg^{3/4}$ is the unit of (body weight raised to the ¾ power).

5 Drummond, J. C., and Marrian, G. F., *Biochem. J.*, 1926, **20**, 1229.

TABLE I.
Energy and Protein Utilization of Flavin-Deficient Chicks and Pairfed Flavin Supplied Controls.

Flavin in food	Body wt, g	Total efficiency* of utilization of		Mean partial efficiency† of utilization of	
		Energy %	Protein %	Energy %	Protein %
Deficient	121 ± 5	4.8 ± 1.1	9.8 ± 1.6	44.7 ± 1.0	20.1 ± 1.5
Supplied	128 ± 6	5.1 ± 1.0	24.8 ± 2.0	45.7 ± 0.8	35.8 ± 2.0

*Total efficiency $= \frac{\text{gain}}{\text{food}} \times 100.$

†Mean partial efficiency $= \frac{\text{gain} + \text{basal loss}}{\text{food}} \times 100.$

Basal metabolism measured in previous trials.
Basal N loss assumed to be 2 mg N per kcal. basal metabolism.
[Smuts, D. B., *J. Nutr.*, 1935, **9** (4), p. 427.]

were nearly equalized and since the fasting heat production is not affected by flavin deficiency, it is to be expected that the influence of maintenance requirements on total efficiency does not affect the comparisons. Conclusions based on total efficiency should therefore parallel those based on mean partial efficiency which is essentially independent of maintenance requirement. Table I confirms this expectation. Flavin deficiency had no effect on the utilization of food energy but lowered significantly the utilization of nitrogen. For every 100 kcal gain in energy the flavin-deficient chicks gained 2.3 ± 0.7 g nitrogen; their flavin-supplied controls on equal food intake, 5.1 ± 1.4 g. These controls gained protein while losing body fat.

Summary. Respiration trials with groups of flavin-deficient and flavin-supplied chicks as well as pair trials with carcass analysis indicated that flavin deficiency decreased the utilization of protein but did not affect the utilization of food energy beyond the effect of greatly decreased appetite.

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Cholinesterase in Developing Amblystoma.

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The physiological significance of cholinesterase (ChE) in neuromuscular activity and its importance in the development of behavior