In view of our recently acquired knowledge as to the complex nature of vitamin G, the question arises: Which component of vitamin G determines its low potency in human milk?

The milk of 5 lactating women was analyzed for content of lactoflavin and vitamin B_6. Three of the women gave sufficient amounts of milk for separate tests. The milk of the 2 remaining subjects was pooled and the mixture tested. As with cow's milk, graded doses of the fresh milk were tested, on from 3 to 4 rats at each level. The results are recorded in Table III.

While the vitamin B_6 content of human milk does not differ substantially from that of cow's milk (1 "rat-day dose" in 5 cc., and even less), the lactoflavin potency of cow's milk is on the average about 3 times as high as that of human milk. We have to conclude, therefore, that in human milk the lactoflavin represents the limiting factor. With the exception of one test, in all experiments with human milk the "unit" of lactoflavin could not be reached even when 15 cc. of the milk were given daily.

Under the conditions of the experiments here reported, the low vitamin G potency of human milk, expressed in terms of the whole vitamin G complex, can easily be rectified by adding pure crystalline lactoflavin in sufficient amounts.

8910 C

Growth-Promoting Activity of Lactoflavin Administered Orally and Parenterally.

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Lactoflavin is not only one constituent of the vitamin G (B_2) complex and as such a vitamin, but it is also a pro-ferment, being the prosthetic group of Warburg's yellow enzyme.[1] In the enzyme the lactoflavin is present in the form of a phosphoric acid ester:[2]

Warburg's yellow enzyme $\rightleftarrows$ Lactoflavin + phosphoric acid + protein.

Lactoflavin exists in natural foodstuffs either in the free state or in the ester form, or even in the form of the colloidal protein

[1] Warburg, O., and Christian, W., *Biochem. Z.*, 1932, **254**, 438; *Naturwissensch.*, 1932, **20**, 980; *Biochem. Z.*, 1933, **266**, 377.

[2] Theorell, H., *Biochem. Z.*, 1934, **275**, 37, 344.

compound. Rudy[3] has shown that free lactoflavin can be phosphorylated by the action of intestinal phosphatase. Biologically inactive or less active flavins of the same group lack this property.[4]

Recently Laszt and Verzár[5] demonstrated the interesting fact that when mono-iodoacetic acid was added to a biologically complete synthetic diet, the diet lost its growth-promoting quality, which could, however, be restored by the phosphoric acid ester of lactoflavin, though not by free lactoflavin. They concluded that mono-iodoacetic acid prevents the phosphorylation of the lactoflavin, probably within the intestinal wall, and, in consequence, the cellular synthesis of Warburg's yellow enzyme.

In view of these facts, the question arose, where in the organism the phosphorylation occurs; in particular, whether it is confined to the intestinal tract and, further, whether the phosphoric acid ester of lactoflavin parenterally injected would have a greater effect than free lactoflavin orally administered.

In order to answer these questions, we have compared the physiologic growth-promoting action of free lactoflavin and of pure (synthetic) lactoflavin-5-phosphoric acid* by administering each form orally as well as parenterally. The method used for the feeding experiments was that described previously by us.[6] Slight modifications in the basal diet consisted in the substitution of extracted casein and cornstarch for caseinogen AB Glaxo and rice starch, respectively.

Graded doses were tested generally on 3 rats at each level. The

TABLE I.

Average Values Obtained in Testing the Growth-Promoting Activity of Lactoflavin* by Oral and by Parenteral Administration.

Material Fed	Amt. Given Daily	Aver. Weekly Increase in Wt. of Rats	
		When Lactoflavin Was Orally Administered	When Lactoflavin Was Parenterally Administered
	γ	gm.	gm.
Lactoflavin	15	10	9
	10	9	9
	7.5	7	8.5
Lactoflavin-5-phosphoric acid	13.8	10.5	10
	6.4	7.5	9
	3.2	5	4.5

*Vitamins B_1 and B_6 provided.

[3] Rudy, H., *Naturwissensch.*, 1935, **23**, 286.

[4] Kuhn, R., and Rudy, H., *Naturwissensch.*, 1935, **23**, 286.

[5] Laszt, L., and Verzár, F., *Pflügers Arch. f. d. ges. Physiol.*, 1935, **236**, 693.

* Kindly furnished by Professor R. Kuhn, Heidelberg, Germany.

[6] György, P., *Biochem. J.*, 1935, **29**, 741.

results obtained are recorded in Table I. The values given represent averages.

Conclusions. The growth-promoting effect of lactoflavin or of lactoflavin-5-phosphoric acid is independent of the way in which it is administered, that is, whether orally or parenterally. There are no biological differences, as shown by growth tests, between lactoflavin and lactoflavin-5-phosphoric acid, the rat-day dose for both being about 7 to 10 γ. It can therefore safely be assumed that the phosphorylation of lactoflavin is not only an intestinal but also a general cellular reaction.

8911 C

Vitamin D Deficiency on Concentration of Blood and Tissue Enzymes of the Albino Rat.* V.

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Experimental rickets was produced according to the technique of Steenbock and Black,[1] with the modification of our paired feeding technique, so as to eliminate the influence of the plane of nutrition.[2] The animals were 29 to 55 days of age, and weighed 50 to 58 gm. at the beginning of the experiments. Since the ricketic type of diet employed allows only very small increases of weight, the size of the animals at the time of sacrificing yielded insufficient amount of blood for all the blood serum enzyme determinations; hence, some groups were taken for blood serum amylase and esterase, and others for blood serum phosphatase. Of the total numbers of groups studied, 24 showed by the line tests severe experimental rickets; 4, advanced; 8, moderate; and 2, mild.

There was a total of 658 titrations carried out in this investigation in duplicate, the results of which are summarized in Table I.

It will be noted that no noteworthy changes are apparent in concentration of blood and tissue enzymes in rickets developed in the albino rat compared with enzyme concentrations on the same diet supplemented with vitamin D supplied by irradiation of the ricketic ration.

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* Research paper No. 428, Journal series, University of Arkansas.

1 Steenbock, H., and Black, A., *J. Biol. Chem.*, 1924, **61**, 405.

2 Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 27.