LXXXVII. INVESTIGATIONS ON THE VITAMIN B₂ COMPLEX.

II. THE DISTRIBUTION OF LACTOFLAVIN AND OF THE “PELLAGRA-PREVENTING FACTOR” (VITAMIN B₆) IN NATURAL PRODUCTS OF ANIMAL ORIGIN.

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(Received January 31st, 1935.)

THE isolation of lactoflavin as one of the components of the vitamin B₂ complex and the separation of the actual “pellagra-preventing factor”—provisionally termed vitamin B₆ [György, 1934; 1935]—made it possible to test natural products for their content of lactoflavin and vitamin B₆ by biological experiments. All the statements in the literature regarding the natural distribution of vitamin B₂ need re-examining, since the complex nature of vitamin B₂ was not formerly realised. This criticism applies to our own earlier communication [György *et al.*, 1934, 1] in which the quantitative data concerned only the total vitamin B₂ complex and not its individual components.

The example of egg-white illustrates the gross errors to which disregard of this complexity may lead. Egg-white is commonly regarded as a natural product rich in vitamin B₂ [Chick and Roscoe, 1929]. As we have pointed out in the preceding paper, this assertion holds good only for lactoflavin, and not for the “pellagra-preventing substance”. As source of lactoflavin 3 ml. of fresh egg-white provide 1 rat day dose, whilst the vitamin B₆ content is too small to be demonstrable by biological methods.

Miss Chick has kindly pointed out to me that she always used egg-white in the cooked or dried state. Since the possibility had to be considered that the “pellagra-preventing substance” might be more easily absorbed from boiled than from unboiled egg-white, we repeated the experiments with boiled egg-white. However, we were unable to produce the slightest therapeutic effect in eight animals suffering from “pellagra”, even with 3–10 g. of boiled egg-white; three animals died during treatment and the others could only be saved and eventually cured by the administration of active preparations.

Using the experimental technique described below, we determined the lactoflavin and vitamin B₆ contents of various natural products of animal origin. In the case of lactoflavin the titre was determined by means of the growth test, the skin symptoms being too irregular and frequently insufficiently obvious; the titre of vitamin B₆ was determined by its effect on the skin changes. In estimating the lactoflavin values, crystalline (or highly purified) vitamin B₁ (3–4 pigeon units), and B₆, in the form of Peters's eluate from bakers' yeast (equivalent to 10 g. of fresh bakers' yeast), were added to the diet. The additions to the diet for the elimination of vitamin B₆ were again vitamin B₁ and, in this instance, pure lactoflavin (10 γ daily) or egg-white (3–5 ml. daily).

Later on lactoflavin was generously placed at my disposal by my colleagues Kuhn and Wagner-Jauregg, while vitamin B₁ and Peters's eluate were supplied

through the kindness of the I.G. Farbenindustrie Wuppertal-Elberfeld (Germany). The Peters's eluate used displayed a constant vitamin B₆ value, so that the experimental results obtained were strictly comparable. The Peters's eluate originally employed was prepared by myself.

A large part of this work was devoted to investigations of muscle tissue of various origins, since we had previously found [György *et al.*, 1934, 1] striking differences in the total contents of vitamin B₂ complex in red and white poultry muscle, and in heart-muscle, veal and beef. In the chicken, dark muscle was found to be richer in total vitamin B₂ than white muscle. On the other hand, these earlier experiments with the diet of Bourquin and Sherman, showed that ox heart muscle possessed the highest content of vitamin B₂ and skeletal muscle the lowest, the value for veal being intermediate between the two.

We repeated these latter experiments, using the new method in which the animals received, to begin with, only vitamin B₁ and the meat concerned in addition to the basal diet. After an initial increase, which, with the meat supplements, continued for 6–8 weeks, the weight became stationary. At this stage of the experiment a third supplement was added, one group receiving lactoflavin and the other vitamin B₆ in the form of Peters's eluate, whereupon a fresh increase in weight set in. We obtained the following mean weekly figures (in g.) for the two periods (a) with vitamin B₁ alone, (b) vitamin B₁ *plus* lactoflavin or vitamin B₆:

Meat fed	Daily amount (g.)	Weekly increase in weight in g.	
		(a) With vit. B ₁	(b) After additional administration of lactoflavin
Heart muscle	0.75	6	3
Veal	2	8	7
Beef	2	3	4
		(a) With vit. B ₁	(b) After the addition of vit. B ₆
Heart muscle	0.75	8	11
Veal	2	10	8
Beef	3	6	12

Our earlier conclusions were thus fully supported by these preliminary experiments, which also yielded fresh information. In order to produce the standard rate of growth, heart muscle, at all events in the doses given, required the addition of vitamin B₆ rather than of lactoflavin. At first sight the same conclusion appeared to hold good for beef also, but the employment of different quantities of meat (2 and 3 g. respectively) in the two groups did not allow a definite comparison to be made.

Clearer results were obtained in experiments in which two groups of animals were given lactoflavin and Peters's eluate respectively, together with vitamin B₁ from the commencement, before supplementary feeding with meat was begun.

As determined by the growth test 0.75 g. heart muscle, 2 g. veal or 3 g. beef contain 1 rat day dose of lactoflavin. The results are thus of the same order as those obtained previously with the experimental methods which have since been superseded. There were no marked differences between the three kinds of meat in respect of their contents of vitamin B₆.

In their curative effects upon "pellagra-like" dermatitis 0.75 g. heart muscle, 0.6–0.75 g. of veal or 1 g. beef are of equal value.

The values for lactoflavin are much higher in veal, and even more so in beef, than the values for vitamin B₆. For these meats, therefore, lactoflavin represents the limiting factor. In this respect, striated skeletal muscle differs widely from

egg-white, which as we have already seen, is practically free from vitamin B₆ and comparatively rich in lactoflavin.

	Daily amount (g.)	With vits. B ₁ + B ₆	With vit. B ₁ + lactoflavin	
		weekly increase in weight in g.	Weekly increase in weight in g.	Healing of pellagra-like dermatitis
Meat fed				
Heart muscle	1	12	9	Yes
	0.75	11	6	Yes
	0.5	6	3	No
Veal	3	12	12	Yes
	2	10	9	Yes
	1	—	7	Yes
	0.75	1	6	Yes
	0.5	—	5	Inconstant
Beef	3	12	9	Yes
	2	5	8	Yes
	1.5	5	7	Yes
	1	—	5	Yes
	0.75	—	5	No
	0.5	—	3	No

Fish muscle lies right at the other end of the series; salmon (fresh and frozen), herring and haddock were investigated.

	Daily amount (g.)	With vits. B ₁ + B ₆	With vit. B ₁ + lactoflavin	
		weekly increase in weight in g.	Weekly increase in weight in g.	Healing of pellagra-like dermatitis
Fish muscle fed				
Fresh salmon	3	0	—	—
	2	2	—	—
	1	1	8	Yes
	0.75	—	9	Yes
	0.5	—	8	Yes
Frozen salmon	1	—	8	Yes
	0.5	—	7	Yes
Herring	3	6	—	—
	2	4	10	Yes
	1	2	10	Yes
	0.5	—	6	Yes
Haddock	3	2	—	—
	2	1	9	Yes
	1	1	—	—
	0.75	—	9	Yes
	0.5	—	3	Yes

It will be seen that fish muscle is inadequate as a source of lactoflavin even in doses of 3 g. daily; only in the case of herring is there a slight increase of growth and even this, despite the high dosage, falls far short of the standard amount. In view of these results, the lactoflavin content of fish muscle must be extraordinarily small. In contrast to this however fish muscle is remarkable for its richness in vitamin B₆. The therapeutic dose of all the varieties of fish investigated was in the region of 0.5 g. daily, or even less. Fish muscle is the only natural product which we have so far investigated, which is practically free from lactoflavin, and is at the same time rich in vitamin B₆. In the absence of artificial preparations of lactoflavin and of vitamin B₆, egg-white and fish muscle may therefore be used as sources of lactoflavin and vitamin B₆ respectively in biological experiments. Frozen fish contains the same quantity of "pellagra-preventing substance" as fresh fish.

Not even the highest doses of fish muscle were able to exercise any favourable influence on the skin manifestations met with in cases of deficiency of lactoflavin [György, 1935]. The animals were refractory both as regards growth and the healing of the skin manifestations. The addition of lactoflavin to only 1 g. of fish muscle gave rise to a marked increase in weight which continued for weeks with undiminished intensity (Fig. 1). The impression was gained that the requirement of lactoflavin was lower in the presence of Peters's eluate + fish muscle than in experiments with Peters's eluate alone.

Fig. 1. Salmon in doses of 1 g. (beginning at A) is unable to supplement Peters's eluate + vitamin B₁. Lactoflavin (10γ daily) added at B.

By means of separate estimations of lactoflavin and vitamin B₆ we were able to confirm our previous conclusions [György *et al.*, 1934, 1], regarding the greater biological value of the red muscles of the leg of chickens as compared with the white muscles of the breast.

	Daily amount (g.)	With vits. B ₁ + B ₆ weekly increase in weight in g.	With vit. B ₁ + lactoflavin	
			Weekly increase in weight in g.	Healing of pellagra-like dermatitis
Meat fed Leg of chicken	3	11	13	Yes
	2	6	—	—
	1	4	8	Yes
Breast of chicken	3	8	12	Yes
	2	3	—	—
	1	2	4	Yes

As in the case of striated mammalian muscle (beef and veal), poultry meat contains relatively more "pellagra-preventing" substance than lactoflavin. In the latter case however the difference does not amount to much, especially if the results of the growth tests are compared. Healing of pellagra-like dermatitis is obtained with red muscle of the leg as well as with white breast meat, smaller doses being required for this purpose than are necessary to produce the standard growth in animals fed with lactoflavin-free supplements, but in receipt of Peters's eluate. The red muscle is richer than the white, in lactoflavin especially, but also, according to the growth test, in vitamin B₆.

The contents of lactoflavin and of pellagra-preventing substance (vitamin B₆) of the smooth muscle of the chicken's gizzard are lower than those of striated muscle including the white muscle of poultry. The "rat day dose" of lactoflavin

is equivalent to 3–4 g., and the antidermatitic, therapeutic, “rat day dose” of vitamin B₆ is equivalent to 2 g. of gizzard muscle. Daily doses less than 2 g. are unable to bring about healing of the pellagra-like dermatitis.

Among the viscera, mammalian liver (beef) was examined for its contents of lactoflavin and vitamin B₆. Liver is the organ which is richest in vitamin B₆ and lactoflavin. The “rat day dose” for lactoflavin was found to be present in 0.2–0.3 g., and for the “pellagra-preventing” factor in 0.3 g. daily. Thus the liver, like the heart and unlike striated and smooth muscle, appears to contain more lactoflavin than vitamin B₆.

Compared with mammalian liver, fish liver is very poor both in lactoflavin and in pellagra-preventing substance. The small lactoflavin content of fish liver had previously been established by means of a chemical, or fluoroscopic, method by Kuhn *et al.* [1934], and also by Euler and Adler [1934, 1, 2]. These authors agree that the lactoflavin content of cod liver is 0.5γ per g. fresh weight (in mammalian liver 15.9γ). According to the present biological investigations the lactoflavin “rat day dose” of cod-liver is over 3 g. per day. Such biological tests are preferable to chemical analysis, among other reasons because there is the possibility that mixtures of active and inactive flavins are present in all natural products, between which purely chemical analysis is, at present, unable to distinguish. The average weekly gain in weight with this dose of 3 g. amounted to 6 g. In contrast, bovine liver in doses 1/10 of these, *i.e.* 0.3 g. daily, caused an average increase in weight of 12 g. weekly. This comparison constitutes the most impressive proof of the poverty of fish liver in lactoflavin. The smallest therapeutic dose which will cure dermatitis is about 2 g. of cod liver daily, with which dose the effect on the weight curve is just noticeable.

Cow's milk contains about the same amount of lactoflavin as of vitamin B₆, the “rat day dose” for both components of the vitamin B₂ complex being equivalent to about 10 ml. These investigations were performed in the summer months.

Daily dose of milk (ml.)	With vits. B ₁ + B ₆ weekly in- crease in weight in g.	With vit. B ₁ + lactoflavin	
		Weekly in- crease in weight in g.	Healing of pellagra-like dermatitis
15	—	14	Yes
10	12	13	Yes
5	4	5	Mostly not

If, in unsuccessful treatments with 5 ml. cow's milk, Peters's eluate be substituted, a marked increase in weight sets in which exceeds the increase which usually occurs with this dose of Peters's eluate (Fig. 2). The preliminary feeding of milk therefore appears to reinforce the combined actions of lactoflavin and vitamin B₆ (Peters's eluate) on growth, much in the same way as was observed with fish muscle.

The investigations of Warburg and Christian [1932; 1933] have shown that in the oxidation of hexosemonophosphoric acid by the “yellow oxidation-ferment” (Warburg), a co-ferment from horse's red blood corpuscles plays an important rôle. After the identity of the coloured component of this flavoprotein [György *et al.*, 1934, 2] with lactoflavin had been established, it became of importance to submit horse's red blood corpuscles to biological examination to test their possible activity in the second missing component, that is, the pellagra-preventing substance vitamin B₆. However, 2 ml. of an emulsion of erythrocytes obtained by centrifuging freshly drawn horse blood were shown to be free from the missing supplementary factor (vitamin B₆). In addition to this the

content of lactoflavin was also very low. In the same way, we were unable to obtain either the slightest lactoflavin effect or pellagra-preventing effect with 2 ml. of horse serum. We were also unable to find any biologically active lactoflavin in the lens of the eye, even on administering as much as 8 g. of tissue daily.

Fig. 2. 5 ml. milk do not contain sufficient pellagra-preventing substance to supplement vitamin B and lactoflavin (—). At ↑ Peters's eluate (equivalent to 10 g. fresh bakers' yeast) is substituted for the milk. The other weight curve (----) shows the effect of 10 ml. milk in supplementing vitamin B₁ + lactoflavin.

The numerical results of these investigations on the contents of lactoflavin and vitamin B₆ of various natural animal products may be summarised in the two tables following:

1 "rat day dose" of lactoflavin is contained in g.		1 "rat day dose" of vit. B ₆ is contained in g.	
Liver (beef)	0.2	Liver (beef)	0.3
Beef heart muscle	0.75	Salmon	0.5
Veal	2	Herring	0.5
Beef muscle	3	Haddock	0.5
Leg of chicken	3	Veal	0.75
Breast of chicken	3-4	Heart muscle (beef)	0.75
Chicken's gizzard	3-4	Beef muscle	1.0
Egg-white	3	Leg of chicken	1.0
Milk	10 ml.	Breast of chicken	1.0
Horse serum	>2	Chicken's gizzard	2.0
Horse erythrocytes	>2	Fish liver (cod)	2.0
Fish liver (cod)	>3	Milk	10.0 ml.
Salmon muscle	>3	Horse serum	>2.0
Herring	>3	Horse erythrocytes	>2.0
Haddock	>3	Egg-white	>10.0
Lens of eye	>8		

While the lactoflavin values accepted in the above table are based on the results of the growth test, the titre values for the "pellagra-preventing substance" (vitamin B₆) are based on the antidermatitic therapeutic effects of the products examined for "rat pellagra". The values for lactoflavin and vitamin B₆ thus obtained are not therefore, strictly speaking, absolutely comparable and they hold good only for the method employed. However, these figures may be regarded as relatively correct.

SUMMARY.

Results are given of quantitative estimations of the vitamin B₆ and lactoflavin activities of foodstuffs of animal origin. It has been shown that various products differ in their absolute and relative contents of lactoflavin and vitamin B₆. Fish muscle (herring, salmon and haddock) is a rich source of the "antipellagra" factor but is practically devoid of lactoflavin. On the other hand, egg-white despite its high content of lactoflavin has no antipellagra activity.

Further, results are given for the lactoflavin and vitamin B₆ contents of the striated muscles of ox, calf and chicken, the heart muscle of ox, chicken gizzard, liver and milk.

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