XCV. STUDIES ON THE VITAMIN B COMPLEX

I. THE OCCURRENCE OF THE RAT ANTI-DERMATITIS FACTOR (VITAMIN B₆) IN FISH AND FISH PRODUCTS

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(Received 8 February 1938)

It was first shown by Goldberger *et al.* [1926] that the vitamin B complex consisted of two factors, the anti-beriberi factor and a factor which was necessary for growth and prevented a pellagra-like dermatitis in rats. In Goldberger's opinion this factor was identical with a vitamin which prevented pellagra in human beings and was called P-P (pellagra-preventing) factor. This factor was afterwards called vitamin G. It was also assumed by Goldberger *et al.* [1928] that this factor was identical with a factor which prevented black-tongue in dogs and rabbits. Later investigations showed, however, that it was difficult to produce the pellagra-like dermatitis regularly in rats. The vitamin G content of foods was therefore determined biologically, not by its pellagra-preventing effect, but by its stimulating effect on growth. Aykroyd [1930] later demonstrated that this vitamin G or B₂, as it was named by English authors, was present in foods which were constituents of typical pellagra-producing diets. This discrepancy was explained by the finding that vitamin G (or B₂) consisted of two components, lactoflavin and a further supplementary factor [György *et al.* 1933]. Several workers demonstrated later that lactoflavin could not cure the pellagra-like dermatitis in rats [György, 1935; Chick *et al.* 1935; Harris, 1935]. The rat dermatitis-preventing factor was termed vitamin B₆ by György. Later Birch *et al.* [1935] and Dann [1936] demonstrated that the P-P factor was neither lactoflavin nor vitamin B₆. Vitamin B₆ was found to differ from the P-P factor in chicks [Lepkovsky *et al.* 1936]. The distribution of vitamin B₆ was studied by Birch & György [1936] who found that fish and wheat germ were good sources.

The present paper deals with the occurrence of vitamin B₆ in different fish and fish products.

As a standard of reference we used Peters' eluate, placed at our disposal by the I. G. Farbenindustrie A. G., Wuppertal/Elberfeld. 0.2 ml. of this eluate was sufficient to cure dermatitis in rats. Peters' eluate was also prepared in this laboratory according to the original description by Kinnersley *et al.* [1933]. 1 ml. of this preparation corresponded to 10 g. baker's yeast and 0.5 ml. cured dermatitis in rats.

We used in these experiments the diet described by György [1935]. Our second method of purifying the casein was that used by Elvehjem *et al.* [1936] in their work on pellagra.

EXPERIMENTAL

Young rats weighing 40–50 g. and about 4 weeks old were placed on the following diet:

	%
Casein, purified	18
Rice starch	68
Butter fat	9
Salt mixture (Osborne & Mendel)	4
Cod liver oil	2

In addition the rats received daily 6 γ vitamin B₁ (cryst. Hoffmann-La Roche) and 10 γ synthetic lactoflavin (Hoffman-La Roche). Some animals received 2 ml. raw egg-white daily as a source of lactoflavin.

Purification of the casein. In the first experiment we extracted the casein with 70 % alcohol. Later the casein was dissolved in NH₄OH and precipitated with HCl.

1st method. 3 kg. casein (techn.) were extracted with c. 7 l. hot 70 % ethyl alcohol for $\frac{1}{2}$ hr. After filtration the extraction was repeated with fresh alcohol. This procedure was repeated until the extract showed no yellow fluorescence when examined in ultraviolet light (lactoflavin-free). The casein was dried at 45–50° and ground.

2nd method. 10 kg. casein (techn.) were dissolved in c. 170 l. H₂O with 750 ml. NH₄OH (sp. gr. 0.88). The solution was acidified with HCl ($pH=4.5$). The precipitated casein was filtered through gauze and the procedure was repeated three times. The final product was dried at 45–50° and ground to a fine powder.

The rats were kept in separate cages with a screen bottom to prevent coprophagy. The rats were weighed twice a week.

On this diet growth ceased after 2–4 weeks and some of the rats lost weight. The first sign of dermatitis developed after 3 $\frac{1}{2}$ weeks in some of the rats. After 4 weeks 57 % of the rats showed dermatitis. After 6 $\frac{1}{2}$ weeks the dermatitis developed in all rats to a greater or less degree.

The rats received the substance to be tested in addition to the basal diet when the symptoms of dermatitis were pronounced. Usually the dermatitis increased in severity during the first days of treatment when the substances tested were low in vitamin B₆, and in cases with very severe dermatitis no sign of recovery could be observed until after 2–3 weeks.

The dermatitis developed as severely and rapidly in rats receiving pure lactoflavin as in those receiving raw egg-white as a source of lactoflavin. As several authors have described an egg-white syndrome in rats, this result is of special interest, and will be dealt with in a subsequent paper.

RESULTS

A summary of the results is given in Table I. It was found that the time necessary for complete healing of the dermatitis varied according to the severity of the original condition.

Varying amounts of anti-dermatitis factor were found in the products tested. Cod liver was a very rich source, 0.25 g. a day being sufficient to cure even a severe dermatitis. Thus cod liver is at least as rich in vitamin B₆ as Peters' eluate, and may prove a better source for preparing concentrates than baker's yeast. It is of interest to note that cod flesh was not as rich in the factor as flounder.

Table I. *Rat anti-dermatitis factor (vitamin B₆) in various products*

Product tested	Amount fed g.	No. rats	Time for healing of dermatitis weeks	Av. gain in wt., g.	
				in 4 weeks	in 6 weeks
Peters' eluate	0.4 ml.	1	3½	29	49
	0.2 ml.	3	3-6	18	29
Cod liver, raw	1.0	3	3-3½	40	57
	0.5	3	2-4	25	38
	0.25	4	2-6	24	28
Cod roe, canned	1.5	1	3	33	—
	1.0	2	1 no improvement in 3½ weeks; 1 cured in 3 weeks	17*	—
	0.5	3	3-4	32	38
Cod flesh, raw	0.5	4	1 died after 4 weeks; 3 no improvement after 2½ weeks	30	44
	2.0	3	2-4	0†	—
	2.0	2	2½	10	—
Flounder flesh, raw	2.0	2	2½	40	56
	1.0	2	3-4	33	—
Herring milt, canned	1.0	2	Not cured in 5 weeks	17	—
Kippered herrings, canned	1.0	4	4-6	21	31
Herring sardines, canned	1.0	4	3-5	26	38
Brisling sardines, canned	2.0	1	4	34	—
	1.0	2	2-6	23	39
Crab, canned	0.5	4	2 died after 2½ weeks; 2 no improvement after 3 weeks	-13†	—
	2.0	2	3½	7	—

* In 3½ weeks.

† In 2½ weeks.

As the canned products tested were effective in curing the dermatitis, it must be concluded that canning does not destroy the factor.

It will be seen from Table I that some of the products tested had a greater growth-promoting effect than others. Thus growth did not occur when crab and cod flesh were added to the basal diet, though the dermatitis was cured. On the other hand, the animals grew when herring milt was added, but the dermatitis was not cured. There would seem to be at least two factors necessary for normal development of the rat, an anti-dermatitis factor and a growth factor, in addition to vitamins B₁ and B₂ (lactoflavin). Edgar & Macrae [1937] found two factors, an eluate and a filtrate factor, necessary for the growth of rats in addition to vitamins B₁ and B₂. Lepkovsky *et al.* [1936] also found two factors, which seem to be identical with the factors described by Edgar & Macrae. Further studies of the occurrence and distribution of these two factors will be reported in a subsequent paper.

SUMMARY

Several fish products were tested as sources of the rat anti-dermatitis factor (vitamin B₆). Cod liver was found to be a very good source.

The authors are indebted to Messrs Hoffmann-La Roche et Cie, Basel, for furnishing us with a sample of pure synthetic vitamin B₁.

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