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Quantitative Estimation of Lactoflavin and of Vitamin B_6 in Cow's Milk and in Human Milk.

PAUL GYÖRGY. (Introduced by Henry J. Gerstenberger.)

From the Babies and Childrens Hospital and the Department of Pediatrics, School of Medicine, Western Reserve University, Cleveland.

It has been shown[1] that vitamin G as needed by the rat contains at least 2 major components: lactoflavin and vitamin B_6, the lack of the latter being associated with specific skin lesions ("rat pellagra" or, rather, "rat acrodynia"[2]).

By feeding to rats a vitamin B-free diet complemented by vitamin B proper, the antineuritic factor (B_1), and lactoflavin, or by vitamin B_1 and a vitamin B_6 concentrate, it was possible to obtain quantitative data[2, 3] concerning the distribution of lactoflavin and vitamin B_6 in different foodstuffs. For the estimation of the lactoflavin values, the basal vitamin B-free diet was supplemented by crystalline (or highly purified) vitamin B_1 (3 international units daily) plus vitamin B_6 concentrate. For the estimation of vitamin B_6 values, vitamin B_1 was similarly provided but supplemented by pure lactoflavin (10 γ daily). The vitamin B_6 concentrate consisted of a yeast preparation such as may be obtained according to the method of Peters, *et al.*,[4] by adsorption of a yeast extract on charcoal and subsequent elution with alcohol containing hydrochloric acid. It has been found that 1 cc. of this so-called Peters' eluate is equivalent to about 1 "rat-day dose" of vitamin B_6.

The values for lactoflavin were determined by means of the growth test, skin symptoms being too irregular and frequently insufficiently obvious. The "rat-day dose," taken as provisional unit, was defined as the minimum quantity of the substance which would give rise to a gain in weight of about 10 (9 to 11) gm. per week for at least 4 weeks.

The values for vitamin B_6 were based directly on the curative effect against dermatitis. The "rat-day dose," or provisional unit for estimation of vitamin B_6, therefore, was defined as the minimum quantity of the substance that would cause healing of the specific dermatitis.

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1 György, P., Kuhn, R., and Wagner-Jauregg, T., *Klin. Wchnschr.*, 1933, **12**, 1241; György, P., *Nature*, 1934, **133**, 498; György, P., *Biochem. J.*, 1935, **29**, 741.

2 Birch, T. W., György, P., and Harris, L. J., *Biochem. J.*, 1935, **29**, 2830.

3 György, P., *Biochem. J.*, 1935, **29**, 760.

4 Kinnersley, H. W., O'Brien, J. R., Peters, R. A., and Reader, V., *Biochem. J.*, 1933, **27**, 225.

Average values for distribution of lactoflavin and vitamin B_6 in cow's milk tested on several occasions in the spring and summer of 1935 in Cambridge, England,[3] are shown in Table I.

TABLE I.

Average Values for Distribution of Lactoflavin and Vitamin B_6 in Cow's Milk.*

Daily Dose of Cow's Milk	Tests for Lactoflavin (Vitamins B_1 and B_6 provided): Aver. Weekly Increase in Wt. of Rats	Tests for Vitamin B_6 (Vitamin B_1 and Lactoflavin provided): Aver. Weekly Increase in Wt. of Rats	Healing of Specific Dermatitis
cc.	gm.	gm.	
15	—	14	Yes
10	12	13	"
5	4	5	Mostly none

*Tests made in Cambridge, England.

These tests were repeated, in the winter and spring months of 1935-36 in Cleveland. The technic described was employed, as well as the same vitamin B_1, B_6 and lactoflavin supplements* used in the previous experiments in England.

The basal diet given the rats was as follows:

	%
Extracted casein	18
Cornstarch	68
Butterfat (melted and filtered)	9
Salt mixture (McCollum 185)	4
Cod liver oil	1

TABLE II.

Average Values for Distribution of Lactoflavin and Vitamin B_6 in Certified and Pasteurized Cow's Milk.*

Material Fed	Amt. Given Daily	Tests for Lactoflavin (Vitamins B_1 and B_6 provided): Aver. Weekly Increase in Wt. of Rats	Tests for Vitamin B_6 (Vitamin B_1 and Lactoflavin provided): Aver. Weekly Increase in Wt. of Rats	Healing of Specific Dermatitis
	cc.	gm.	gm.	
Raw certified	10	15.5	13	Yes
cow's milk	5	10	7.5	"
	3	6	1	No
Ordinary	10	13	12	Yes
pasteurized	5	8.5	7	"
cow's milk	3	5	3	No

*Tests made in Cleveland.

* Kindly furnished by I. G. Farbenindustrie, Germany, through the courtesy of Winthrop Chemical Company, Inc., New York.

In the tests for vitamin B_6, cornstarch and extracted casein were replaced by sugar and Merck's unextracted casein, respectively. Graded doses of fresh milk (raw certified and ordinary pasteurized) were tested on from 4 to 6 rats at each level. The results are summarized in Table II.

When comparison is made between the values for lactoflavin and vitamin B_6 found in the milks tested in Cambridge and in Cleveland, it is seen that the "unit" (rat-day dose) for lactoflavin is distinctly lower for the Cleveland milk (5 cc.) than for the Cambridge milk (10 cc.); while for vitamin B_6 the level is at 5 to 10 cc. in each case, with the Cleveland milk somewhat favored.

The lactoflavin values for certified milk in Cleveland were slightly higher than those for pasteurized milk in Cleveland, but no conspicuous quantitative differences could be detected in the vitamin B_6 content of these milks.

Earlier workers[5] have established the fact that vitamin B_1 in cow's milk and vitamin G in human milk are the limiting factors for rats.

TABLE III.
Average Values for Distribution of Lactoflavin and Vitamin B_6 in Human Milk.

Material Fed	Amt. Given Daily	Tests for Lactoflavin (Vitamins B_1 and B_6 provided): Aver. Weekly Increase in Wt. of Rats	Tests for Vitamin B_6 (Vitamin B_1 and Lactoflavin provided): Aver. Weekly Increase in Wt. of Rats	Tests for Vitamin B_6 (Vitamin B_1 and Lactoflavin provided): Healing of Specific Dermatitis
	cc.	gm.	gm.	
Human milk I	15	15	—	—
	10	5	12	Yes
	5	4	4	"
	3	2	—	—
" " II	15	7	—	—
	10	4	—	—
	5	2	6	Yes
	3	—	—1	No
" " III	15	6	—	—
	10	5	8	Yes
	5	5	6	"
	3	—	4	"
" " IV	15	7	—	—
(Mixture)	10	5	11.5	Yes
	5	5	6	"
Average of I-IV	15	8	—	—
	10	4.5	11	Yes
	5	5	5	"
	3	2	1.5	Inconstant

[5] Macy, I. G., Outhouse, J., Graham, A., and Long, M. L., *J. Biol. Chem.*, 1927, **73**, 189; Outhouse, J., Macy, I. G., Brekke, V., and Graham, A., *Ibid.*, p. 203; Sherman, H. C., and Axtmayer, J. H., *Ibid.*, 1927, **75**, 207; Hunt, C. H., and Krauss, W. E., *Ibid.*, 1928, **79**, 733; Macy, I. G., and Outhouse, J., *Am. J. Dis. Child.*, 1929, **37**, 379; Donelson, E., and Macy, I. G., *Am. J. Physiol.*, 1932, **100**, 420; Samuels, L. T., and Koch, F. C., *J. Nutrition*, 1932, **5**, 307.

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.

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Growth-Promoting Activity of Lactoflavin Administered Orally and Parenterally.

PAUL GYÖRGY. (Introduced by Henry J. Gerstenberger.)

From the Babies and Childrens Hospital and the Department of Pediatrics, School of Medicine, Western Reserve University, Cleveland.

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.