CCVII. VITAMINS IN RAT'S AND IN GUINEA-PIG'S MILK¹

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THE development of a technique for the satisfactory milking of small laboratory animals [Cox & Mueller, 1937; Temple & Kon, 1937] and the recent progress in the chemical and physical estimation of various vitamins have made it possible to measure several of these factors in the milk of rats and of guinea-pigs.

We have examined the milks of both species for vitamin A and carotene, vitamin B₁, riboflavin and vitamin C. We have also studied the effect of changes in the diet on the levels of these factors in rat's milk.

EXPERIMENTAL

(1) *The guinea-pig*

Guinea-pigs were generally milked about the 4th day of lactation, some being used several times at intervals. For milking in the morning the doe was separated from her young on the previous evening at 5 p.m. Their food consisted of a dry mash, the composition of which is given in Table I. In addition they were allowed unlimited hay and water and about 1 oz. of fresh cabbage daily.

Table I. *Composition of the guinea-pig diet*

Bran	27
Crushed oats	27
Lucerne meal	13
Linseed cake	13
Flaked maize	13
Fish meal	7

Approximately 1% cod liver oil was mixed in immediately before feeding.

Twenty milkings yielded 99.6 ml. of milk, i.e. an average of 5 ml. The best yield from one guinea-pig was 12 ml.

(a) *Vitamin A and carotene.* 15.5 ml. of milk were diluted with 2 vol. of water and the fat was extracted by the method of Gillam *et al.* [1938]. The yield was 1.27 g. or 8.2%. Our usual technique [Gillam *et al.* 1937] showed a content of 4 yellow and 42 blue Moore [1930] units per g. fat, equivalent to 0.143 mg. carotene and 1.08 mg. vitamin A per 100 g. of fat [Bartlett *et al.* 1938].

(b) *Vitamin B₁* was estimated fluorimetrically [Henry *et al.* 1938; Houston & Kon, 1939; Houston *et al.* 1939, 1], both the form which reacts in the Jansen [1936] technique ("free" vitamin B₁) and the total aneurin being measured. In February 1938 a figure of 18.4 I.U./100 ml. was obtained for the "free" form in the pooled milk of 2 guinea-pigs. In October of the same year the readings were 15.4 I.U./100 ml. as "free" aneurin and 22.4 I.U./100 ml. after incubation with pepsin and takaphosphatase (takadiastase of Parke Davis and Co.). These figures are not greatly different from those for cow's milk (Table VII).

¹ Read in part before the Biochemical Society, 11 March 1938 [Houston *et al.* 1938].

When the fluorimetric test was applied to the guinea-pig milk much fluorescent material was found in the aqueous layer after extraction with *isobutanol*. The fluorescence was not removed by incubation with pepsin or with takaphosphatase and it is therefore unlikely that more than a fraction of it was due to cocarboxylase. Similar blue fluorescent compounds are found in products of vegetable origin.

(c) *Riboflavin (lactoflavin)*. The method of Whitnah *et al.* [1937] was used but the readings were taken in the fluorimeter as used by Henry *et al.* [1939]. Estimations carried out in February 1938 showed a content of 86 $\mu\text{g.}/100\text{ ml.}$, a value lower than, but not greatly different from, that for cow's milk (Table VII).

(d) *Vitamin C*. Readings [Kon & Watson, 1936] on milk samples collected in the absence of light from 4 guinea-pigs in February 1938 gave values of 28–31 mg./100 ml., all in the reduced form. These values are very high, more than 10 times the normal for cow's milk (Table VII) and about half that of an average lemon juice. As the reducing substance in guinea-pig's milk is destroyed by light in the usual fashion it is unlikely that it is an artefact. Rasmussen *et al.* [1938] have since reported similar and even higher values for guinea-pig's milk. They found that the vitamin C content is influenced by the diet, and by feeding to a guinea-pig 40 mg. of ascorbic acid daily, beginning 17 days before parturition, brought the concentration in milk to 75 mg./100 ml.

(2) *The rat*

The bulk of the milk for the estimation of the normal vitamin levels was obtained from stock colony rats on the day following weaning, that is on the 23rd day of lactation. Where the effects of dietary changes were studied, milkings were started earlier, generally on the 13th day of lactation, and continued on alternate days. In all cases the doe was separated from the young for 16–18 hr. before milking. 129 milkings were carried out, yielding 173.1 ml. of milk, an average of 1.3 ml. per milking. The best yield was 6.5 ml. An analysis of the milk gave 13.8% fat and 27.9% total solids, in good agreement with the figures of Cox & Mueller [1937]. The diet of the stock colony has been described by Folley *et al.* [1938]. The methods of estimating the various vitamins were the same as for guinea-pig's milk.

(a) *Vitamin A and carotene*. From 19.1 ml. of milk obtained from 17 rats in January 1938, 2.23 g. of fat were extracted which contained 36 Moore blue units of vitamin A per g. or 0.92 mg. of vitamin A per 100 g. Calculated on milk this is rather higher than the value for cow's milk (Table VII). The fat was quite colourless and contained no measurable carotene, which indicates that the rat is, like the goat, more efficient than the cow in converting the pigment into vitamin A.

(b) *Vitamin B₁*. Results of tests are summarized in Table II.

Table II. *Estimation of vitamin B₁ in rat's milk*

Date	No. of rats	Day of lactation	Yield of milk ml.	Vitamin B ₁ (i.u./100 ml.)	
				"Free"	Total
27. i. 38	7	23rd	14.2	70.5	—
21. vi. 38	2	17th	3.0	44.8	—
21. vi. 38	2	17th	4.9	41.7	—
31. x. 38	3	23rd	6.1	55.3	77.8
8. v. 39	4	15th	6.0	33.3	53.1
9. v. 39	4	15th	6.0	45.3	49.1
			Mean	48.4	60.0

Only the "free" vitamin B₁ was at first estimated. Both it and the total vary fairly considerably but are in any case 3-4 times higher than in cow's milk (Table VII). The figures have a tendency to rise with progressing lactation.

The effects of diets deficient in vitamin B₁ and of feeding large quantities of the factor were also tested. The secretion of aneurin in milk during vitamin B₁ deficiency is shown in Table III.

Table III. *Vitamin B₁ content of milk secreted by rats receiving diets deficient in vitamin B₁*

Exp. no.	No. of rats	Date	Day of lactation	Days on deficient diet	Yield of milk ml.	Vitamin B ₁ (i.u./100 ml.)	
						"Free"	Total
1*	2	21. vi. 38	17th	0	4.9	41.7	—
		23. vi. 38	19th	2	1.0	20.6	—
		25. vi. 39	21st	4	2.7	22.9	—
2†	4	9. v. 39	14th	0	6.0	45.3	49.1
		11. v. 39	16th	2	1.9	27.3	32.1
		17. v. 39	22nd	8	6.0	Trace	Trace

* Other members of the vitamin B complex supplied as autoclaved yeast.

† Other members of the vitamin B complex supplied as autoclaved whey.

It is evident from these figures that the secretion of vitamin B₁ into the milk drops rapidly when little or none is present in the diet. This is in agreement with previous experience [cf. Hartwell, 1925].

The effect of feeding relatively large doses of crystalline aneurin to lactating rats receiving the stock diet is summarized in Table IV. The aneurin, dissolved in a few drops of water at pH 5, was fed by pipette.

Table IV. *The vitamin B₁ content of milk of rats receiving massive doses of aneurin*

Exp. no.	No. of rats	Date	Day of lactation	Days on experiment	Total additional vitamin B ₁ fed, i.u. per rat	Yield of milk ml.	Vitamin B ₁ in milk i.u./100 ml.	
							"Free"	Total
1	2	21. vi. 38	17th	0	0	3.0	44.8	—
		23. vi. 38	19th	2	333	2.0	40.6	—
		25. vi. 38	21st	4	666	1.8	39.1	—
2	5	8. v. 39	15th	0	0	6.0	33.3	53.1
		10. v. 39	17th	2	530	2.9	43.2	47.1
		12. v. 39	19th	4	1060	4.8	43.6	54.1
		16. v. 39	23rd	8	2120	4.9	40.2	47.1

It is surprising to find that the massive doses fed did not raise the vitamin B₁ level of the milk, especially as the effect of cutting down the supply was so definite. This may mean that the vitamin B₁ content of rat's milk is under physiological control and is not allowed to rise beyond the level already reached on the stock diet. The possibility of an effect similar to that reported by Perla & Sandberg [1939] is by no means excluded.

(c) *Riboflavin (lactoflavin)*. One assay in January 1938 on the pooled milk of 5 rats on the 23rd day of lactation gave a value of 407 µg./100 ml. Another in June of the same year gave for the combined milk of 2 rats on the 17th day of lactation double this value, 807 µg./100 ml. Data in Table V show that the feeding to stock rats of additional riboflavin definitely increases the level of this factor in milk and it is possible that the variation was due to fluctuations

in riboflavin content of the stock diet, especially of such a component as beef liver. To test the effect of increased intake, pure riboflavin dissolved in a small quantity of water was fed by pipette (Table V).

Table V. *Effect on the level in milk of feeding of additional riboflavin to lactating stock rats*

No. of rats	Date	Day of lactation	Days on experiment	Total additional riboflavin fed $\mu\text{g. per rat}$	Yield of milk ml.	Riboflavin in milk $\mu\text{g./100 ml.}$
2	21. vi. 38	17th	0	0	3.0	807
	23. vi. 38	19th	2	1000	2.5	1318
	25. vi. 38	21st	4	2000	2.0	1426

The feeding of riboflavin nearly doubled the already high content of the milk. Rat's milk is a richer source of this factor than cow's milk (Table VII) but we have found up to 800 $\mu\text{g./100 ml.}$ in the colostrum of Shorthorn cows [Houston *et al.* 1939, 2].

(d) *Vitamin C.* Rat's milk contains very little vitamin C. A sample of 8.4 ml. collected in January 1938 in the absence of light from 6 rats (23rd day of lactation) contained 0.25 mg./100 ml. of reduced and 0.39 mg./100 ml. of total ascorbic acid. The corresponding figures in June of the same year obtained on a 4 ml. sample from 2 rats on the 17th day of lactation amounted to 0.31 mg./100 ml. for both reduced and total ascorbic acid, showing that the reversibly oxidized form was not present. This is only about one-eighth of the amount found in cow's milk. The level is not increased by feeding liberal quantities of the crystalline substance dissolved in a few drops of water, as can be seen from Table VI.

Table VI. *Effect of feeding of ascorbic acid to lactating rats on the vitamin C content of milk*

No. of rats	Date	Day of lactation	Days on experiment	Total additional vitamin C fed mg. per rat	Yield of milk ml.	Vitamin C in milk mg./100 ml.	
						Reduced	Total
2	21. vi. 38	17th	0	0	4.0	0.31	0.31
	23. vi. 38	19th	2	50	1.5	0.32	0.33
	25. vi. 38	21st	4	100	0.5	—	<0.3

The last figure is very doubtful owing to the very small yield of milk on that day. There is no doubt, however, that the feeding of 100 mg. of ascorbic acid to each of 2 rats had no effect on the level of the substance in the milk. In this respect the finding of Longenecker *et al.* [1939] that rats excrete in the urine only a small quantity of vitamin C when fed as much as 100 mg. of the pure substance is of interest. It is generally agreed that the rat does not require preformed vitamin C in the diet and both the deficiency of the substance in the normal milk and the lack of response to the feeding of large quantities can be readily understood on teleological grounds. One might be tempted to suggest a similar explanation of the relatively enormous concentration of vitamin C in the milk of the guinea-pig, but one should remember that the young of this species begins to nibble solid food almost immediately after birth. Whatever the explanation, the striking fact remains that in guinea-pig's milk there is 70 times as much vitamin C as in the milk of the rat.

(3) *Comparison of rat's, guinea-pig's and cow's milk*

In Table VII we have compared the vitamin values found by us for rat's and guinea-pig's milks with those for cow's milk.

Table VII. *Comparison of the concentrations of certain vitamins in rat's, guinea-pig's and cow's milks*

Milk	Fat %	Vitamin A mg./100 ml.	Carotene mg./100 ml.	Vitamin B ₁ I.U./100 ml.	Riboflavin mg./100 ml.	Vitamin C mg./100 ml.
Rat	14.0	0.13	Not measurable	50-80	0.4-0.8	0.4
Guinea-pig	8.0	0.09	0.011	20	0.085	29.0
Cow (Shorthorn)	3.8	0.02-0.07	0.006-0.03	12-20	0.1-0.15	2.5

SUMMARY

1. The following values were obtained by physical and chemical methods for the concentration per 100 ml. of vitamin A, carotene, vitamin B₁, riboflavin and vitamin C respectively in rat's and guinea-pig's milks, viz. rat: 0.13 mg.; not measurable; 50-80 I.U.; 0.4-0.8 mg.; 0.4 mg.; guinea-pig: 0.09 mg.; 0.011 mg.; 20 I.U.; 0.085 mg.; 29.0 mg.

2. Compared with cow's milk rat's milk is richer in vitamin B₁ and riboflavin but contains much less vitamin C.

3. Guinea-pig's milk on the contrary contains relatively enormous quantities of vitamin C, about half the amount present in an average lemon juice.

4. Feeding large quantities of riboflavin to lactating rats definitely increased the concentration of this factor in milk but generous administration of vitamin C and of vitamin B₁ was without effect. On the other hand the vitamin B₁ content of rat's milk dropped sharply when a vitamin B₁-deficient diet was fed.

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