26. Identification of Vitamin L_1 (Anthranilic Acid).

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In 1934, we⁽¹⁾ demonstrated for the first time the existence of a specific dietary factor of lactation, since designated vitamin L_1 , and the progress made in our investigation on the subject has been communicated to the Imperial Academy from time to time.

Thus in 1936 we^(2, 3) reported that there exists in addition to vitamin L_1 , a second factor necessary for lactation (vitamin L_2), and later⁽⁴⁾ showed that yeast nucleic acid has the action of this second factor. It was obvious, however, that yeast nucleic acid could not be identical with vitamin L_2 , because of the difference in solubility, etc., and we were led to believe that one of the decomposition products of the nucleic acid might be the active substance. In 1942⁽⁵⁾, finally, adenythiomethyl pentose was found to be active as vitamin L_2 . In view of our results with nucleic acid, we concluded that the vitamin L_2 activity may be possessed by a number of chemically related substances and that adenythiomethyl pentose is the first pure substance to be shown to belong to this group.

Having elucidated to the above extent the chemical nature of vitamin L_2 the question before us has been that of the identity of vitamin L_1 , and we believe that this last important question is about to be settled. Our recent experiments established that anthranilic acid is active as vitamin L_1 .

Our interest in anthranilic acid is in a way foreshadowed by the work of Sure⁽⁶⁾, who, re-affirming the existence of a specific lactation factor, suggested that this factor "would seem to contain p-aminobenzoic acid or a

1) Nakahara, W., Inukai, F., and Kato, S.: Proc. 10, 268 (1934); Sci. Pap. Inst. Phys. Chem. Research, 24, 155 (1934).

2) Nakahara, W., Inukai, F., Kato, S., and Ugami, S.: Sci. Pap. Inst. Phys. Chem. Research, 29, 47 (1936).

3) Nakahara, W., Inukai, F., and Ugami, S.: Proc. 12, 289 (1936); Sci. Pap. Inst. Phys. Chem. Research, 31, 42 (1937).

4) Nakahara, W., Inukai, F., and Ugami, S.: Proc. 17, 266 (1941); Sci. Pap. Inst. Phys. Chem. Research, 39, 120 (1941).

5) Nakahara, W., Inukai, F., and Ugami, S.: Proc. 18, 477 (1942); Sci. Pap. Inst. Phys. Chem. Research, 40, 433 (1943).

6) Sure, B.: Science, 94, 167 (1941).

substance of similar physiological properties."

Sure used p-aminobenzoic acid in a very large dose of 10 mg per rat daily, and with this supplement he obtained 60% of lactation efficiency, i. e., 32 of 53 young were reared. Unfortunately, our attempt⁽⁷⁾ to confirm Sure failed completely, and in our hands p-aminobenzoic acid supplement in estimated daily dose of 10 mg per rat permitted only 12 (15.3%) out of 78 young to be reared.

Many possible explanations are conceivable for the discrepancy between the result of Sure and of our own, and among them is the possibility that the sample of p-aminobenzoic acid used by Sure contained o-aminobenzoic acid (anthranilic acid) which may be formed alongside the para-isomer in the synthetical preparation and which might be active as vitamin L₁. Remote as this possibility seemed to us *a priori*, we were determined not to leave a stone unturned.

Experiments were carried out according to the method we previously described, but mention must be made of minor modifications in the composition of the vitamin L₁ deficient basal diet, namely, the use of purified pupa (silk worm pupa) protein 10 g, mixture of cod liver oil and rice germ oil 5 g, and Steenbock's salt mixture 5 g, instead of fish protein, butter and McCollum's salt mixture respectively, other ingredients of the diet being polished rice powder 75 g, and acid earth adsorbate of yeast extract equivalent of 10 g of dried yeast.

Two to three weeks before the expected parturition this diet was supplemented with 5 g of dried sake yeast as the source of vitamin L₂, and also with anthranilic acid, mp. 145° C uncorrected, in the estimated dose of 5 mg per rat daily. In this experiment the lactation efficiency of nearly 70% was obtained.

Paralleling the main experiment in which anthranilic acid was tested, we also carried out some additional experiments replacing anthranilic acid with β -alanine (5 mg per rat daily), inositol (10 mg), or the combination of choline (5 mg), inositol (5 mg), pantothenic acid (1 mg) and p-aminobenzoic acid (5 mg). None of these supplements had any effect on lactation efficiency.

The following table summarizes the results of all these experiments:—

7) Nakahara, W., Inukai, and Ugami, S.: Sci. Pap. Inst. Phys. Chem. Research, 39, 305 (1942).

Vitamin L ₁ deficient diet (modified formula) supplemented with :	No. of females	Total no. of young born	No. of young reared
Anthranilic acid	19	156	109 (69.8%)
β -alanine	7	54	8 (14.8%)
Inositol	7	47	9 (19.1%)
Choline+inositol +pantothenic acid +p-aminobenzoic acid	6	38	3 (7.8%)
No supplement	10	84	22 (26.1%)

The available data based on the vitamin L₁ deficient diet of our old formula may be referred to here as of additional interest. The composition of the diet was: Polished rice powder 75 g, Purified fish protein 10 g, butter 10 g, salt mixture 5 g, acid earth adsorbate of yeast extract, equivalent of 10 g dried yeast, supplemented with dried sake yeast 5 g as the source of vitamin L₂.

Vitamin L ₁ deficient diet (old formula) supplemented with :	No. of females	Total no. of young born	No. of young reared
Liver filtrate (Positive control)	9	86	46 (59.7%)
Anthranilic acid	6	40	23 (57.5%)
p-aminobenzoic acid	9	78	12 (15.3%)
Pantothenic acid	9	69	15 (21.7%)
No supplement	28	220	58 (20.8%)

Though the number of animals was very small for the anthranilic acid group, the result definitely corroborated that on the modified basal diet already described, and again pointed to the vitamin L₁ activity of anthranilic acid.

The conclusion may, therefore, be warranted that anthranilic acid is active as vitamin L₁.

Y. Kotake and co-workers⁽⁸⁾ demonstrated that anthranilic acid is formed from tryptophan in the presence of cat liver tissue *in vitro*, suggesting the possibility that the same thing may occur in the animal body also. However, our vitamin L deficient diet contains nutritionally adequate amount of tryptophan and yet it produces a deficiency which can be prevented by a relatively small amount of anthranilic acid, proving that, even if anthranilic acid is formed within the rat body, the amount must be extremely small—too small

8) Kotake, Y. Studien über den intermediären Stoffwechsel des Tryptophans (Japanisch). Igaku-Soran, p. 226, (1944).

to insure its action as vitamin L_1 . It seems clear that anthranilic acid must be supplied as such to rats in order to show its nutritional effect, and hence there is no real contradiction between Kotake's contention of anthranilic acid formation from tryptophan and our recognition of anthranilic acid as a vitamin.