99. *Factor L₂, a Second Dietary Factor for Lactation.*

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The necessity of a hitherto unknown dietary factor for the process of lactation may now be regarded as established. This lactation factor we designated Factor L,¹⁾ and obtained a crude preparation of it by precipitation with barium hydroxide and methanol and then with phosphotungstic acid from beef liver extract, from which vitamin B complex was removed by adsorption with acid earth.²⁾

Working with the factor L deficient diet which was described in our first report³⁾ we later discovered that this diet does not support lactation, even if supplemented with a potent factor L fraction of liver extract, if acid earth adsorbate of yeast extract is substituted for the whole yeast as the source of vitamin B complex. The fact that acid earth adsorbate, which is the vitamin B fraction of yeast extract, cannot support lactation even in the presence of factor L, and that factor L plus whole yeast is required for satisfactory lactation rendered it necessary to assume the existence in whole yeast of a second lactation factor. The duality of the lactation factor being thus brought to light, we re-designated our original factor L from beef liver Factor L₁ and the second factor from yeast Factor L₂, the two together composing Factor L Complex.⁴⁾

Factor L₂ deficiency can be readily produced by feeding female rats on the deficient diet consisting of 75 gr. polished rice powder, 10 gr. fish protein, 10 gr. butter, 5 gr. McCollum's salt mixture, yield from 10 gr. dried yeast of acid earth adsorbate, supplemented with factor L₁ in the form of a potent fraction, usually non-adsorbable fraction, of beef liver extract, in amount corresponding to the yield from 100 gr. fresh liver per 100 gr. of basal diet.

As may be seen from the following table, animals fed on this diet for two weeks or more before parturition showed almost total failure of lactation, in spite of the fact that the diet is known to be adequate for growth of animals, normal pregnancy and parturition. A prolonged feeding on this diet did not prevent the exceptional, very small per cent of successful lactation, which is apparently due to the unavoidable presence of a trace of factor L₂ in the diet.

1) W. Nakahara, F. Inukai, and S. Kato: Proc. **10** (1934), 268; Sci. Pap. I. P. C. R., **24** (1934), 155.

2) W. Nakahara, F. Inukai, and S. Ugami: Proc. **11** (1935), 362; Sci. Pap. I. P. C. R., **28** (1935), 152.

3) W. Nakahara and F. Inukai: Sci. Pap. I. P. C. R., **22** (1933), 22.

4) W. Nakahara, F. Inukai, S. Kato, and S. Ugami: Sci. Pap. I. P. C. R., **29** (1936), 47.

Factor L ₂ deficient feeding before parturition	No. of females	No. of young born	No. of young weaned
14 ~ 21 days	3	22	0 (0.0%)
22 ~ 28 days	11	87	7 (8.0%)
29 ~ 35 days	16	126	2 (1.5%)
36 ~ 42 days	5	36	3 (8.3%)
43 ~ 75 days	2	13	0 (0.0%)
Total	37	284	12 (3.3%)

The same general method of lactation test was adopted for factor L₂ as previously established for factor L₁, but with minor modifications as follows:—Mature virgin female rats were maintained in cohabitation with suitable males for 8 days on the above mentioned diet minus the factor L₁ supplement. After this period of 8 days males were removed and the diet was supplemented with factor L₁ along with the possible source of factor L₂ to be tested. This latter was added in amount corresponding to the yield from 30 gr. dried yeast to 100 gr. diet. In this way all the females received factor L₁ and yeast fraction to be tested for 13 ~ 21 days before parturition, the total factor L₂ deficient feeding being 21 ~ 29 days if the yeast fraction was inactive. Females bearing young were continued on the supplemented diet until either the death or weaning of the young.

We attempted to separate factor L₂ from baker's yeast ("Oriental" brand) by fractionating the latter in the following manner:—

The results of testing each fraction as to its factor L₂ effect according to the above described method were as follows:—

	No. of females	No. of young born	No. of young weaned
Factor L ₂ deficient basal diet	27	284	12 (3.3%)
Supplemented with:			
Unextracted whole yeast	7	40	23 (57.5%)
Extracted yeast residue	7	56	5 (8.9%)
WO ₃ ·2H ₃ PO ₄ precipitate (0.18 gr. per rat daily)	10	80	33 (41.2%)
WO ₃ ·2H ₃ PO ₄ filtrate (0.5 gr. per rat daily)	4	35	15 (42.8%)
AgNO ₃ precipitate (15 mgr. per rat daily)	8	57	20 (35.1%)
AgNO ₃ filtrate (52 mgr. per rat daily)	9	75	0 (0.0%)
HgSO ₄ precipitate	7	56	2 (3.3%)
HgSO ₄ filtrate	6	53	7 (13.2%)

In the above experiments, the phosphotungstic precipitate and filtrate showed about the same degree of factor L₂ effect but the amount of precipitate was much less than that of the filtrate. We therefore interpreted the result as showing that the reagent did not completely precipitate factor L₂ and a small part of it was lost to the filtrate at this stage of separation. This may account for the lack of activity of HgSO₄ fractions of the filtrate, and also for the unmistakable but definitely submaximal factor L₂ effect manifested by the final silver nitrate precipitate. The estimated daily amount per rat of this last fraction was only 15 mgr., however.

From the result of fractionation factor L₂ may be said to behave like a base. In its nutritional rôle, factor L₂ closely parallels factor L₁, and from all the available evidence there is little doubt that factor L₂, like factor L₁, logically comes under the head of vitamins.

Animals depend entirely on dietary supply for both factors, becoming rapidly depleted of both on corresponding deficient diets as evidenced by the failure of lactation, which can be prevented by small amounts of crude preparations carrying these factors. The respective functions of factor L₁ and factor L₂ cannot now be discussed, but both are equally essential to lactation in that one is ineffective without the other.

The details of the present work will soon be published in the Scientific Papers of the Institute of Physical and Chemical Research.