

that time our knowledge on the subject has been greatly extended, and it now appears that this lactation factor may well be considered as a new vitamin.^{2,3}

A diet can be formulated which is entirely adequate except for lactation. We found, for example, a mixture of polished rice powder, 75 gr, fish protein, 10 gr, butter, 10 gr, McCollum's salt mixture, 5 gr, adequately supplemented with acid earth adsorbate of yeast extract (vitamin B complex), to be just such a diet. On this diet, in spite of the excellent growth of young rats, normal pregnancy and parturition, rats fail to rear the young at the first as well as at the second births.

In order to secure normal lactation, two hitherto unidentified substances must be added to the diet, namely, factor L_1 and factor L_2 . That these two are different substances was proved by the fact that a large amount of the one can not substitute for the other. The two factors are equally essential in that one is without effect in the absence of the other.

We separated factor L_1 from beef liver extract by removing vitamin B complex by adsorption on acid earth at pH 3-4, precipitating from the non-adsorbable fraction with $Ba(OH)_2$ and methanol, removing water-soluble matter (glycogen) from the precipitate, and finally precipitating from the aqueous solution with $WO_3 \cdot 2H_3PO_4$. This crude preparation proved active in the estimated daily amounts of less than 50 mg per rat.

Factor L_2 was obtained from baker's yeast by

similarly removing from the extract vitamin B complex by adsorption on acid earth, precipitating from the filtrate with $WO_3 \cdot 2H_3PO_4$ and again with $AgNO_3$ and $Ba(OH)_2$. This preparation was effective in daily amounts of 15 mg per rat.

Beef liver does not contain factor L_2 , while baker's yeast seems to be devoid of factor L_1 .

The exact physiological role of L-factors is as yet far from being clear, but we recently found that if the lactation mechanism is established at the first birth in the presence of L-factors, these latter are no longer needed for the second lactation in so large an amount as is absolutely necessary for the first lactation. It may be that these factors have specific relation to the first establishment or maturation of the lactation mechanism.

From the point of view of nutritional study, the lactation factors in question must be regarded as vitamins, since there is no doubt that animals depend entirely on dietary supply for the substances, which we know must be effective in very minute amounts. We, therefore, propose to call them vitamin L_1 and vitamin L_2 , together constituting vitamin L complex.

WARO NAKAHARA
FUMITO INUKAI
SABURO UGAMI

THE INSTITUTE OF PHYSICAL
AND CHEMICAL RESEARCH
TOKYO

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE VIVI-DIFFUSION APPARATUS

A VIVI-DIFFUSION apparatus which has been found satisfactory for the rapid removal of non-protein nitrogen from the circulating blood is shown in Fig. 1. In contrast to the vivi-diffuser described by Abel and coworkers¹ this apparatus (a) uses a ready-made membrane, (b) is simple in design, (c) has provision for the stirring of the dialyzing fluid and (d) is equipped with a constant temperature mechanism.

A metal can (36 × 30 cm) contains a hollow, weighted, removable, spiralled core (31 × 20 cm) on which is wound 20 feet of $\frac{3}{8}$ inch size Visking sausage casing.² This length of casing holds approximately 600 cc of blood. The ends of the casing are moistened

and fitted over glass tubes, A and V, tied with light cord, and are then clamped with rubber-lined clamps (C). Tubes A and V are sealed into the can by means of rubber stoppers. Tube A is connected by means of rubber tubing to the arterial canula; tube B is similarly connected to the venous canula.

An electric heater (H), a thermostat (T) and an electric stirrer (N) are fitted in the can cover. These devices are so constructed that they can be dropped into place after the cover has been attached to the can.

The dialyzing fluid consists of 0.7 per cent. sodium chloride solution (14 liters) kept at a temperature of 38° C. During operation the venous end of the membrane is kept at the same level as the venous canula.

Normal and nephrectomized dogs anesthetized with nembutal were used to test the efficiency of the apparatus in removal of non-protein nitrogen compounds from the blood. Heparin was used as an anti-coagulant. Blood was taken from the carotid artery and returned to the jugular vein. When small dogs were

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

³ W. Nakahara, F. Inukai and S. Ugami, *Proc. Imp. Acad.*, 11: 362, 1935; 12: 289, 1936; *Sci. Pap. Inst. Phys. Chem. Research*, 28: 31, 1935; 31: 42, 1937.

¹ J. J. Abel, L. G. Rowntree and B. B. Turner, *Jour. Pharmacol. and Exp. Therap.*, 5: 275-316, 1914.

² From the Visking Corporation, 4311 Justine St., Chicago, Ill.

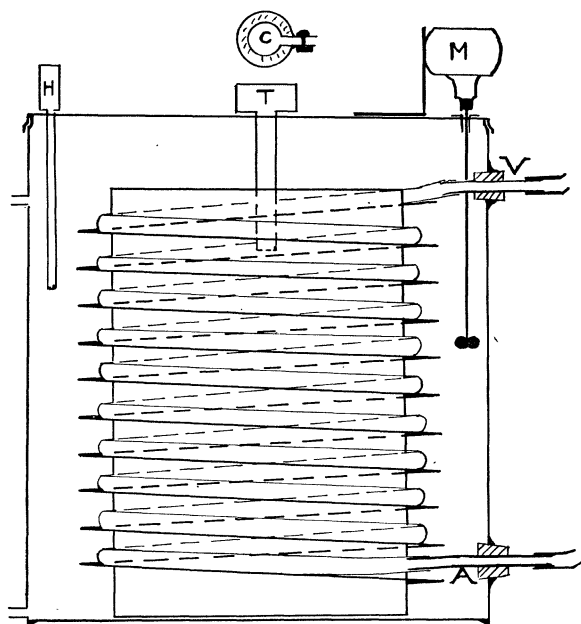


FIG. 1.

used the apparatus was first filled with blood taken from another dog. With dogs weighing 20 kilos or more this was not done. Typical results obtained are shown in Table 1.

TABLE 1

Expt. No.	Blood level at start mg/100 cc		Grams removed in two hours	
	Urea nitrogen	Non-protein nitrogen	Urea nitrogen	Non-protein nitrogen
(1) Nephrec- tomized ..	237	270	3.74	4.11
(2) Nephrec- tomized ..	203	255	3.26	3.62
(3) Normal ..	14	22	0.23	0.31
(4) " ..	12	27	0.24	0.34

F. W. BERNHART

UNIVERSITY OF MINNESOTA
MEDICAL SCHOOL

LUCITE NOT A SUBSTITUTE FOR CANADA BALSAM WHEN MOUNTING MICROSCOPE SLIDES¹

LUCITE² is a crystal-clear methyl methacrylate polymer which has a refractive index nearly the same as glass and is readily soluble in dioxan. Dioxan³ has proven useful as a dehydrating agent for the preparation of sections and objects to be mounted for observation with the microscope. Thus with the Lucite in dioxan solution it should be possible to mount the preparations as soon as they are dehydrated. In fact,

¹ From the Marine Biological Laboratory, Chemical Room.

² Formerly called Pontalite; manufactured by du Pont.

³ H. W. Mossman, *Stain Tech.*, 12: 147, 1937.

the Lucite hardens rapidly and the mounts are firm enough for use in about an hour after they are mounted on a slide under a cover glass. After a time the Lucite dries and contracts and draws air bubble channels under the cover glass. When no cover glass is used, successful, stained smear preparations have been made.⁴ Our own preparations, mounted in Lucite dissolved in acetone, amyl acetate, ethyl acetate or dioxan with no cover glass, show much less fading at the end of five months than those mounted under a cover glass.

Unfortunately, the Lucite dissolved in dioxan bleaches many of the more important stains used in microscopy which, in the order of least faded to completely faded, are the following: basic fuchsin, methylene blue, eosin, Heidenhain's iron haematoxylin, Ehrlich's haematoxylin, acid fuchsin and light green (all aqueous solutions or standard formulae). Dissolving the Lucite in other solvents (acetone, amyl acetate, ethyl acetate) did not prevent the fading. The decolorizing of the stained sections takes place in from a few days to five months. Furthermore, the sections are less well cleared than they are in balsam or damar. Clearing is very poor also when the sections are mounted with no cover glass, but the use of an immersion oil then clears the sections fairly well.

Lucite is unsatisfactory as a mounting medium for microscope slides for other than temporary use, because the Lucite decolorizes the stained sections and in drying forms air-bubble channels which spoil the preparation mechanically. When no cover glass is used the fading is much less rapid.

OSCAR W. RICHARDS

RESEARCH SECTION, SPENCER LENS Co.,
BUFFALO, N. Y.

JAY A. SMITH

DEPARTMENT OF ZOOLOGY,
JOHNS HOPKINS UNIVERSITY

⁴ B. F. Skiles and C. E. Georgi, *SCIENCE*, 85: 367, 1937.

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