The provitamin hypothesis may be valid for mammals, but it is incorrect for the hen.

TORBEN K. WITH.

Rømersgade 9 III,
Copenhagen, K.

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Microbiological Assay of Amino-Acids with *Leuconostoc mesenteroides P.60*

DUNN *et al.*[1,2,3] have described four different media, varying in their amino-acid contents, for the microbiological assay of amino-acids by means of the lactic organism *Leuconostoc mesenteroides P*.60, and consider that one of these media (medium *D*) is suitable for the assay of lysine and histidine. A re-investigation of medium *D* has shown that in our hands it is inadequate and acid production is poor. Apparently this has also been found by Dunn *et al.*, for in their third communication[3] they recommend the use of 0·05 *N* sodium hydroxide solution instead of 0·1 *N*. In a fourth paper, Dunn *et al.*[4] describe the assay of phenylalanine with a further modification of the medium, in which a still weaker standard solution (0·028 *N*) is used for titrating the acid produced, although in the assays the contents of six tubes were combined and titrated with 0·111 *N* sodium hydroxide.

We have found that a medium containing the following concentrations of amino-acids gave excellent results, with high acid production, equivalent to 14–16 ml. of 0·1 *N* sodium hydroxide.

dl-Alanine	1 gm.	*dl*-Lysine monohydrochloride	250 mgm.
l-(+)Arginine monohydrochloride	250 mgm.	*dl*-Methionine	100 ,,
dl-Aspartic acid	800 ,,	*dl*-Norleucine	100 ,,
l-(−) Cystine	100 ,,	*dl*-Norvaline	100 ,,
l-(+) Glutamic acid	500 ,,	*dl*-Phenylalanine	100 ,,
Glycine	100 ,,	*l*-(−)Proline	100 ,,
l-(−)Histidine monohydrochloride	100 ,,	*dl*-Serine	100 ,,
dl-Isoleucine	200 ,,	*dl*-Threonine	500 ,,
l-(−)Leucine	100 ,,	*dl*-Tryptophan	100 ,,
		l-(−)Tyrosine	100 ,,
		dl-Valine	200 ,,

These amounts are sufficient to make up 1 litre of medium. The other constituents are added in the same concentrations as in medium *D* of Dunn *et al.*

It was further found that folic acid was not an essential nutrient for this organism, although its presence did cause slight stimulation. The remaining vitamin supplements recommended by Dunn *et al.* were found to be adequate. With this modified medium we have been able to assay the following amino-acids: methionine, lysine, phenylalanine, aspartic acid and proline, using *L. mesenteroides P*.60. Details of the assays will be published elsewhere.

E. C. BARTON-WRIGHT.

Ashe Laboratories, Ltd.,
120–122, Victoria Street,
S.W.1.

W. B. EMERY.
F. A. ROBINSON.

Glaxo Laboratories, Ltd.,
Greenford, Middx.
Feb. 25.

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Assay of the Biological Value of a Protein by its Effect on Liver Cytoplasm

IT has previously been shown[1] that the amount of cytoplasm present in the liver is dependent both on the quality and quantity of the protein of the diet. Liver cytoplasm may be determined either by estimating the sum of the protein, phospholipin and nucleic acid contents of the liver or by estimating the non-glycogen non-lipid liver solids. It was suggested at the time that the determination of liver cytoplasm by means of the non-glycogen non-lipid solids may lend itself to a simple and rapid assessment of the biological value of a protein. Since then, investigations have been in progress in this laboratory with the view of developing a suitable method. The most important results obtained from a large number of tests have already been reported[2,3], and a detailed paper is being prepared for publication.

In a communication just published, Harrison and Long[4] also followed up the suggestion made by us in 1944 and fully confirmed the data given in our previous papers on casein, egg albumin, zein and gelatin, and extended them to lactalbumin, gliadin, to casein supplemented by methionine or cystine and to whole egg and whole liver proteins. Their method is based on the regeneration of the protein (total N) of the rat's liver following a forty-eight hours fast.

Two methods have been developed in this laboratory during the last year: in the first, the rats are transferred from the stock diet to the test diet for one week, thus measuring the ability of the protein being tested to *maintain* the non-glycogen non-lipid liver solids (labile liver cytoplasm); in the second method, the rats are first given a protein-free diet for four days, during which period their livers are completely deprived of their labile cytoplasm, and then transferred to the test diet for another four days, thus measuring the ability of the protein being tested to *form* labile liver cytoplasm. The results obtained by the two methods are not significantly different.

One fact emerged from these experiments at an early stage[2]. The amount of labile liver cytoplasm depends not only on the quantity and quality of the dietary protein but also on the quantity of food consumed. It was found that unless food corresponding to not less than 26–27 cal./100 gm. body-weight is consumed daily, the maximal effect of the dietary protein on liver protein or cytoplasm is not obtained. In Harrison and Long's experiments, the calorie intakes of the rats fell considerably short of this level. This would account for the fact that they obtained no regeneration of liver protein on 10 per cent casein or lactalbumin diets, while in our experience, with a sufficient calorie intake, considerable amounts of labile liver cytoplasm are formed on a 4·5 per cent casein diet. It is often impossible to increase the protein intake beyond 10 per cent or thereabout, either because the proteins are not readily available in high concentration, as is the case with the mixed flour proteins, or because they are so little palatable, as for example certain protein hydrolysates, that the rats will not take them in concentrations higher than 10 per cent.

We have found that the amount of non-glycogen non-lipid solids (labile cytoplasm) present in the liver is directly proportional to the logarithm of the protein intake. It is thus possible to construct straight regression lines for the different proteins. The regression lines for egg albumin and casein are almost identical, while that for zein has approximately the same slope but lies at a much lower level of liver cytoplasm. Reduction of the calorie intake to 75 per cent decreases the 'biological value' of casein to about that of zein supplemented by tryptophan or that of the mixed soya bean proteins, while reduction to half brings the regression line for casein down to the level of that of unsupplemented zein.

We have used our methods for the assay of protein hydrolysates prepared for therapeutic purposes and for the evaluation of the amount of tryptophan necessary for supplementing acid-hydrolysed casein. Further, the biological values of the protein of 70, 80 and 85 per cent extraction flour have been assayed: a suggestive but not significant difference was found between the 70 and 85 per cent extraction flours when the values were calculated in terms of nitrogen ingested. When, however, the lower digestibility of the 85 per cent extraction flour was taken into account, its biological value became just significantly higher than that of the 70 per cent extraction flour. These results are in general agreement with those of Henry and Kon[5], who used the 'balance sheet' method, and with those of Chick, Copping and Slack[6], who used the 'growth method'.

An investigation into the part played by the individual essential amino-acids in the maintenance and formation of labile liver cytoplasm is in progress in this laboratory.

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H. W. KOSTERLITZ.
ROSA M. CAMPBELL.

Physiology Department,
University of Aberdeen.
Feb. 14.

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In vitro Grafts

IN 1935, Gautheret[1] published an account of *in vitro* grafts he had obtained between fragments of cambium of *Populus nigra*, *Salix caprea* and other trees, excised and cultured on artificial media. In attempting to repeat this work, I encountered some difficulties when tissues freshly removed from the plant were employed. With cambial cultures of longer standing, however, union *in vitro* was obtained easily. Fig. 1 *a* shows two excised fragments of cambium from *Vinca rosea* as they appeared after two weeks culture on nutrient agar. Regeneration of callus was controlled by the natural polarity of the fragment. After these two fragments had been placed in close contact for three weeks, they were found to be firmly united (Fig. 1 *b*). The union was accompanied by an increased proliferation in other portions of the excised fragment.

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