Table 2. Effect of Extracellular Level of L-Phenylalanine on Distribution and Flux of Free Phenylalanine and Leucine in Hepatic Tissue

Initial extracellular phenylalanine (μM)	Final phenylalanine concentration Liver (μmole/g)	Medium (μmole/ml.)	Final leucine concentration Liver (μmole/g)	Medium (μmole/ml.)	Flux Phenylalanine (mμmole/g/min)	Leucine
2	0·035	0·028	0·109	0·080	0·32	1·10
50	0·045	0·048	0·098	0·078		
155	0·141	0·115	0·122	0·082	14·34	0·75

Liver slices were incubated in the presence of 0·5 μc. each of uniformly labelled ^{14}C-phenylalanine and ^{14}C-leucine (16 μM) and varying amounts of non-labelled phenylalanine. Amino-acid content was determined in liver and medium after 60 min incubation using the Beckman model 120 automatic amino-acid analyser. Flux of free amino-acid was calculated over the 1·5–3 min period. Each value is a single determination on extracts combined from 3 incubations. Normal hepatic levels of amino-acid were 0·085 ± 0·005 μmole phenylalanine per g and 0·206 ± 0·017 μmole leucine per g of tissue. Radioactivity of amino-acids was measured directly on the column effluent of the amino-acid analyser employing a Packard scintillation flow detector.

utilization. Thus, the amount of ^{14}C-leucine remaining after incubation of hepatic tissue for 60 min in the presence of 155 μM phenylalanine was reduced about 15 per cent compared to that remaining in similar experiments with 50 μM phenylalanine.

These observations indicate that the degradative and synthetic aspects of protein metabolism were both stimulated in surviving hepatic tissue when the initial extracellular concentration of a single essential amino-acid was elevated. Secondary changes in uptake and distribution of other amino-acids between extracellular and intracellular liver compartments did not appear to be correlated with the alterations in protein turn-over. Information is not available to ascertain whether the primary effect of alterations in amino-acid levels on protein turn-over was on protein synthesis or protein breakdown.

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Betty M. Hanking *
Sidney Roberts

Department of Biological Chemistry,
School of Medicine,
and the
Brain Research Institute,
University of California Center
for the Health Sciences, Los Angeles.

* Postdoctoral Trainee, Mental Health Training Program (5 *T*1 *MH*–6415), U.S. Public Health Service.

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Rapid Spectrophotometric Micromethod for Determination of Histaminase Activity

It is known that in biological systems histamine is broken down by histaminase[1]. In this reaction oxygen is consumed and hydrogen peroxide is formed. A manometric method of measurement[2] is based on the oxygen consumption and a chemical method[3,4] on the production of hydrogen peroxide. In the latter method indigo is added to the incubation mixture and the discoloration of this mixture by the hydrogen peroxide formed is a measure of the enzyme activity. This is due to the peroxidatic activity of catalase mostly present in the biological fluids or tissue extracts under test. The disadvantages of the chemical method are the long incubation period of 24 h and the necessity of at least 1 ml. of serum in the case of histaminase determinations in blood.

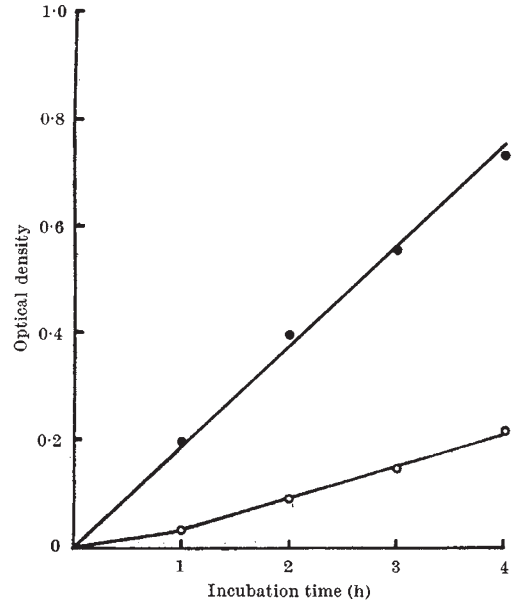

Fig. 1. Increase in optical density at 470 mμ of an incubation mixture of 1 ml. total volume, containing the phosphate buffer, *p*H 7·2, 0·1 ml. of rabbit plasma, 20 μg peroxidase, 50 μg ortho-dianisidine and 10^{-2} M histamine dihydrochloride (upper curve). The lower curve is related to a mixture without added histamine dihydrochloride

In our experiments with small animals this amount of serum makes it impossible to estimate histaminase activity repeatedly in the blood of the same animal. Therefore, we needed a more sensitive method, which was obtained by using ortho-dianisidine instead of indigo as a hydrogen donor. With this reagent it is possible to start the incubation with a colourless mixture. The increase in optical density during incubation is a measure of the histaminase activity. Moreover, we added peroxidase to the incubation mixture to be independent of the peroxidatic activity of the sample to be tested.

The reagents used were: (1) 0·1 M phosphate buffer, *p*H 7·2; (2) 2×10^{-1} M histamine dihydrochloride solution; (3) 4 mg of peroxidase (Boehringer) dissolved in 10 ml. of distilled water; (4) a solution of ortho-dianisidine in 96 per cent alcohol (1 mg/ml.).

An incubation mixture of 1 ml. containing the buffer, 0·05–0·1 ml. of the test sample and 0·05 ml. of the reagents 2, 3 and 4 was prepared on ice in a 10-mm light pass cell of a Beckman spectrophotometer. After measuring the optical density at 470 mμ the mixture was incubated at 25° C. The intensity of the resulting colour was determined at intervals of 1 h.

With this method it is possible to determine histaminase activity in samples of 0·05–0·1 ml. of plasma within a period of 4 h. The slope of the upper curve in Fig. 1 shows the increase in optical density of an incubation mixture to which 0·1 ml. of rabbit plasma had been added. The lower curve relates to a mixture with the same amount of rabbit plasma but without added histamine (reagent 2) and demonstrates that there is still some increase in optical density. This is most likely due to the presence of histamine in the sample of plasma. This may indicate that this method is also suitable for determining histamine. In this case the substrate instead of the enzyme will be the limiting factor in the reaction.

P. N. Aarsen
A. Kemp

Department of Pharmacology,
University of Amsterdam.

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