LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. They cannot undertake to return, or to correspond with the writers of, rejected manuscripts intended for this or any other part of NATURE. *No notice is taken of anonymous communications.*

IN THE PRESENT CIRCUMSTANCES, PROOFS OF "LETTERS" WILL NOT BE SUBMITTED TO CORRESPONDENTS OUTSIDE GREAT BRITAIN.

NOTES ON POINTS IN SOME OF THIS WEEK'S LETTERS APPEAR ON P. 982. CORRESPONDENTS ARE INVITED TO ATTACH SIMILAR SUMMARIES TO THEIR COMMUNICATIONS.

Analysis of Protein by Means of Deuterium-containing Amino-Acids

As is well known, the analysis of amino-acids giving no colour reactions is very difficult as most of the methods known lead to inevitable losses. This difficulty can, however, be overcome in the following way.

To the hydrolysed protein, of which the leucine content for example is to be determined, is added a known amount of leucine labelled by introduction of deuterium in the (C—H) position. After mixing, some leucine is isolated from the mixture by the usual methods.

The next step is the determination of the deuterium content of the leucine mixture isolated. Where the protein does not contain leucine, the leucine sample recovered should be pure 'heavy' leucine; while from the protein containing normal leucine a mixture of 'heavy' and normal leucine is recovered.

From the deuterium content of the leucine mixture isolated we can calculate the proportion in which the 'heavy' leucine added is diluted by the leucine originating from the protein.

The mixture of amino-acids which is the result of the usual acid hydrolysis consists (with the exception of glycine) of optically active forms, whereas the usual methods for synthesis of amino-acids containing deuterium leads to racemic mixtures. It is necessary therefore, either to perform a resolution of the synthetic product into the *d*- and *l*-forms, or to racemize the amino-acid to be estimated in the hydrolysate. The last path, being by far the least expensive, is followed here.

Deuterium-containing *dl*-leucine was prepared by heating *l*-leucine with 33 per cent sulphuric acid containing, say, 7–10 per cent D_2O in a sealed pyrex tube to 170° for twenty-four hours[1].

After removal of SO_4^{--} and repeated evaporation of the water and re-dissolving in pure water, all deuterium in combination with oxygen and nitrogen is removed, and a portion of deuterium is left which cannot be removed by boiling water. As the leucine is now optically inactive, as was determined in two cases, the stable linked deuterium has obviously entered the molecule during the racemization and is linked to the α-carbon. Under the treatment mentioned, the destruction of leucine did not exceed two per cent. The determination of the deuterium content of combustion water from the leucine is performed by the gradient-tube method of Linderström-Lang[2].

3·0 gm. dry hæmoglobin was hydrolysed and racemized by treatment with 33 per cent sulphuric acid in a sealed ampoule at 170°. The hydrolysate was mixed with 0·392 gm. 'heavy' leucine. Sulphuric acid was removed, the amino-acid mixture dried and powdered. The powder was extracted with dry propionic acid (method of Przylecki and Kasprzyk) which extracts the hexon bases, proline and part of the leucine. Propionic acid was removed and the leucine isolated as copper salt and purified by recrystallization from water. The excess density of the water formed by combustion of the 'heavy' leucine was 518 parts in a million, that of the leucine mixture isolated 225 parts in a million. The amount of 'heavy' plus normal leucine was:

$$0{\cdot}392 \times \frac{518}{225} = 1{\cdot}262 \text{ gm.}$$

The amount of leucine originally present in the protein was

$$1{\cdot}262 - 0{\cdot}392 = 0{\cdot}870 \text{ gm.},$$

which corresponds to 29 per cent of the hæmoglobin.

A second estimation gave 30 per cent. An estimation of the leucine content of gelatine gave 8·5 per cent.

These results should, of course, be regarded as examples only. It should be possible to adopt the method for a series of amino-acids, for example, alanine, valine, isoleucine and aspartic acid, for which to my knowledge no quite reliable estimation methods are known.

HANS H. USSING.

Laboratory of Zoophysiology,
University of Copenhagen.
Nov. 3.

[1] Ussing, H. H., NATURE, 142, 399 (1938).
[2] Linderström-Lang, K., Jacobsen, O., and Johansen, G., *C.R. Lab. Carlsberg* (Ser. Chim.), 23, 17 (1930).

A Mucolytic Enzyme in Testis Extracts

WE have recently described a polypeptide, isolated from peptic protein hydrolysates, which is chemotactic to leucocytes and increases the permeability of capillaries[1]. It is probably closely related to Menkin's 'leukotaxine'[2]. As a result of this work we became interested in another permeability-increasing substance, known as the 'spreading factor'[3]. This substance, which is present in large amounts in testis extracts and certain bacterial filtrates, is a protein and is active in dilutions so low as 10^{-8}. It acts by increasing the permeability of the dermal layer of the skin, as demonstrated by the flattening of the wheal and the rapid spread of intradermally injected solutions of dyes, bacterial toxins and particulate matter in suspension.

So far, nothing has been known about the mechanism of the action of the spreading factor. From its properties, it seemed to us likely that it was an enzyme acting on some substrate in the skin, causing