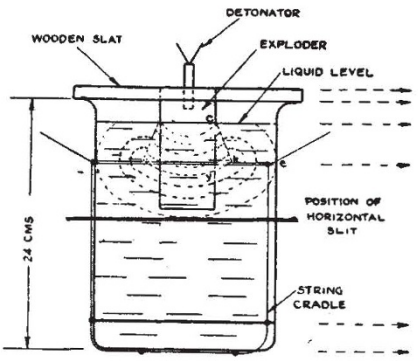

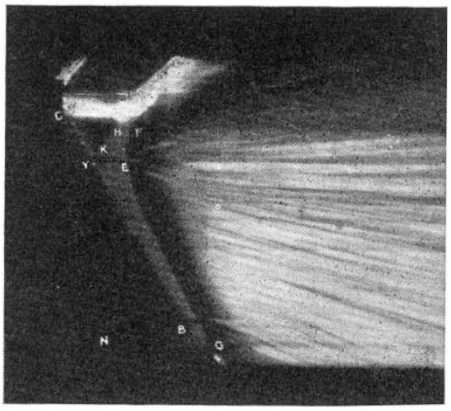

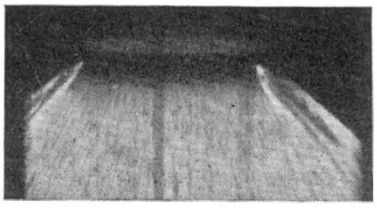

RECORD FOR HORIZONTAL SLIT

of the same film. Use was made in this connexion of adjacent sides of the mirror block. In this manner it was possible to record simultaneously in two directions at right angles. The maximum recording speed was approximately 100 cm. per sec., with a time resolution of 1 microsecond.

A record obtained during the detonation of a 14-lb. charge of the transparent liquid explosive diglycol dinitrate, using a 12-oz. cylindrical tetryl primer, is reproduced herewith. The vertical slit was focused on the axis of the glass beaker used to contain the explosive, while the horizontal slit covered a section slightly below the bottom of the primer. To facilitate interpretation, the sketch of the charge is reproduced to the same linear scale as the records.

Referring to the record from the vertical slit, the flash from the detonator occurs first in time, followed by extensive luminosity from the section of the primer between the underside of the primer support and the level of the liquid. The flame front from this zone, travelling towards the camera, is later deflected upwards by the glass wall of the beaker. The boundary *CYB* on the record indicates the passage of the detonation front, first down the side of the primer and afterwards, with a slight decrease in velocity, down the axis of the beaker. During its passage from *c* to *y* along the primer, the detonation front has spread, to a limited extent only, into the liquid explosive, as sketched on the diagram. This encroachment is indicated by the boundary *CK* on the record.

On a circle concentric with the charge axis, and passing through the point *k*, a new detonation process has originated in the liquid. This has combined with the original wave to form a front of the shape sketched. The upward movement of this front is recorded as the boundary *KH*. When the detonation wave reaches the glass at *e*, luminosity ceases for a few microseconds, before the commencement of burning in air. This corresponds to point *E* on the record. As the expanding front shatters the glass, the boundary *FEG* is developed. From a mathematical analysis of this boundary the shape of the detonation wave can be derived. (The tail *NB* is due partly to reflexion from the convex base of the beaker, and partly to refraction at the curve in which the wall of the beaker meets the base.)

The horizontal slit records nothing until the detonation front in the liquid crosses its field. The curved boundary again gives a key to the shape of the wave, but in this case a correction must be made for refraction in the undetonated liquid. The record as it stands gives the impression that the detonation front has been temporarily contained by the glass!

Using this technique the critical primer/charge diameter ratio necessary to give complete detonation was investigated, and the effect of shaping the primer examined. The horizontal slit was found to be invaluable in the elucidation of the more complex detonation processes associated with shaped primers.

Some of the records obtained suggested that diglycol dinitrate has one or more low orders of detonation of the kind referred to recently by Jones and Mitchell[1]. Unfortunately, the luminosity associated with them was insufficient to record on the fastest film available, and the detonation process had to be reconstructed from the envelope of the after-burning, which was generally sufficiently luminous to record.

This communication is published by permission of the Director of Road Research and of the Chief Scientist, Ministry of Supply, for whom the work was undertaken.

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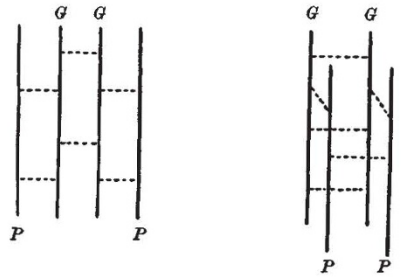
[1] Jones, E., and Mitchell, D., *Nature*, **161**, 98 (1948).

Some Peptides from Insulin

BY oxidation with performic acid, the insulin molecule can be split into its separate polypeptide chains[1]. It has now been possible to prepare two fractions from the oxidized insulin: fraction *A* contains only glycine terminal residues and no arginine, histidine, lysine, phenylalanine or threonine; fraction *B* contains 97 per cent phenylalanine terminal residues and all the amino-acids that are present in insulin. The yield of fraction *A* is more than 25 per cent of the original insulin, indicating that it represents more than one of the peptide chains of the insulin. The yield of fraction *B* is rather less.

The sulphur contents of the two fractions were kindly determined by Mr. M. W. Rees, who found



that fraction *A* contains four cysteic acid residues per molecule of molecular weight 2,900, and fraction *B* contains two per molecule of molecular weight 4,100. From these results it is possible to formulate a number of possible structures for the insulin submolecule. Thus, if we assume that it can be regarded as four parallel peptide chains, then only two structures are possible, as shown in the accompanying diagram, where the full lines represent the peptide chains and the broken lines the —S—S— bridges. The chains marked *G* have glycine terminal residues and those marked *P* phenylalanine terminal residues. It is assumed that the two glycyl chains have equal cysteic acid contents, which is probable from their similar properties. Other structures are possible if the chains cannot be regarded as parallel.

In order to carry further the investigation of the structure of these peptides, the products of the partial hydrolysis of their 2 : 4-dinitrophenyl-derivatives were studied. Using this method, it is possible to separate from a complex partial hydrolysate a relatively simple mixture of dinitrophenyl-peptides originating from the terminal peptides of the molecule. The isolation of a number of such peptides from an enzymic digest of insulin has already been reported by Woolley[2]. In the present work, acid hydrolysis has been used and the dinitrophenyl-peptides fractionated by partition chromatography on silica gel. The order of the amino-acids in them was determined by subsequent partial hydrolysis.

In a preliminary experiment with horse globin, the following peptides were isolated in a crystalline form and their structure determined: *H*1, 2 : 4-dinitrophenyl-valyl-leucine; *H*2, 2 : 4-dinitrophenyl-valyl-glutamyl-leucine; *H*3, the amide of *H*2.

This demonstrates that the six open peptide chains of horse globin[3] are not identical but consist of at least two different types, one with the terminal peptide valyl-leucine and one with the terminal peptide valyl-glutaminyl-leucine.

The method was then applied to the above fractions of oxidized insulin. From a partial hydrolysate of the dinitrophenyl-derivative of fraction *A* the following peptides were separated: *A*1, 2 : 4-dinitrophenyl-glycyl-isoleucine; *A*2, 2 : 4-dinitrophenyl-glycyl-isoleucyl-valine; *A*3, 2 : 4-dinitrophenyl-glycyl-isoleucyl-valyl-glutamic acid; *A*4, the peptide containing glutamic acid, leucine, serine and O-2 : 4-dinitrophenyl-tyrosine, which has been reported by Woolley[2]. Its structure has not yet been determined.

Fraction *A* thus consists of at least two separate chains with different terminal peptides. It is interesting to note that these chains, though not identical, are very similar in that they have no basic amino-acids, threonine or phenylalanine, are not easily separated from one another and have the same molecular weight and electrophoretic mobility.

Using fraction *B* the following peptides were detected: *B*1, 2 : 4-dinitrophenyl-phenylalanyl-valine; *B*2, 2 : 4-dinitrophenyl-phenylalanyl-valyl-aspartic acid; *B*3, 2 : 4-dinitrophenyl-phenylalanyl-valyl-aspartyl-glutamic acid; *B*4, ε-2 : 4-dinitrophenyl-lysyl-alanine; *B*5, threonyl-(ε-2 : 4-dinitrophenyl)-lysyl-alanine.

No evidence has yet been obtained of a second chain in this fraction.

The method of determining the structure of these peptides can be illustrated by considering the peptide *A*3. Using quantitative paper chromatography[4], it was shown that there was one molecule of isoleucine, valine and glutamic acid per molecule of 2 : 4-dinitrophenyl-glycine. While no great accuracy is claimed for this method as used here, it is sufficient to indicate without any doubt the number of residues present. On further partial hydrolysis of peptide *A*3, the peptides *A*1 and *A*2 and 2 : 4-dinitrophenyl-glycine were produced. From the composition of these peptides the order of the amino-acids could then be deduced, and it was confirmed by the identification of isoleucyl-valine in partial hydrolysate of peptide *A*2, by the method of Consden, Gordon and Martin[5].

In addition to peptide *A*4, Woolley has isolated from insulin a number of 2 : 4-dinitrophenyl-glycyl peptides in which threonine occurred not far from the terminal residue. We have not yet found evidence of these peptides in the present work in any of the main fractions of oxidized insulin; but it may be that they are not extracted under the procedure employed. It is possible, therefore, that there may be at least three different glycyl chains in insulin, though it is difficult to reconcile this with the present picture of the structure of insulin.

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May 24.

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[2] Woolley, D. W., *Federation Proc.*, **7**, 200 (1948).
[3] Porter, R. R., and Sanger, F., *Biochem. J.*, **42**, 287 (1948).
[4] Polson, A., Mosley, V. M., and Wyckoff, R. W. G., *Science*, **105**, 603 (1947).
[5] Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **41**, 590 (1947).

"17-Ketosteroid II" Isolated from Urine; its Reaction with Hydrochloric Acid

IN an earlier communication[1] we announced the isolation of a new 17-ketosteroid in the urine of a girl with adenoma of the adrenal cortex. This compound, which we designated "17-ketosteroid II", is converted in presence of HCl into a mixture of $C_{19}H_{27}OCl$ and dehydro-*iso*-androsterone. We have now studied this reaction in more detail.

In the first place we were able to prove that the compound $C_{19}H_{27}OCl$ is identical with the 3-chloro-Δ^5-androstenone-17 isolated by Butenandt and Dannenbaum[2] from human male urine. This compound can be synthesized by the method of Wallis and Fernholz[3] by the action of phosphorus pentachloride on dehydro-*iso*-androsterone. We purified the product thus obtained by means of chromatographic analysis. The melting point was 153·5–155·5° C. (corr.).

We then added 5 per cent aqueous hydrochloric acid to a solution of pure 17-ketosteroid II in ethanol; under these conditions the latter reacts immediately even at room temperature. The reaction mixture consists of about 80 per cent of $C_{19}H_{27}OCl$