

There is evidence from work on kidney metabolism of an effect of inositol on phospholipids. Thus Handler<sup>6</sup> has found that administration of inositol to rats fed lipogenic diets increases the incidence and severity of renal hæmorrhages, while Patterson, Keevil and McHenry<sup>7</sup> have obtained evidence which may indicate that increased turnover of kidney phospholipid accompanies reduction of these hæmorrhages.

We would like to thank Prof. B. S. Platt for his interest in this work.

M. BLEWETT  
I. G. CAMPBELL  
JUNE OLEY

Human Nutrition Research Unit,  
Medical Research Council,  
National Hospital, Queen Square,  
London, W.C.1.  
March 31.

<sup>1</sup> Handler, P., *J. Biol. Chem.*, **162**, 77 (1946).

<sup>2</sup> Forbes, J. C., *Proc. Soc. Exp. Biol. and Med.*, **54**, 89 (1943).

<sup>3</sup> Beveridge, J. M. R., and Lucas, C. C., *J. Biol. Chem.*, **157**, 319 (1945).

<sup>4</sup> Taugog, A., Entenman, C., Fries, B. A., and Chaikoff, I. L., *J. Biol. Chem.*, **155**, 19 (1944).

<sup>5</sup> Fisher, R. A., "Statistical Methods for Research Workers" (Oliver and Boyd, 1944).

<sup>6</sup> Handler, P., *J. Nutrition*, **31**, 621 (1946).

<sup>7</sup> Patterson, J. M., Keevil, and McHenry, E. W., *J. Biol. Chem.*, **153**, 489 (1944).

### Crystalline Derivatives of the Polymyxins and the Identification of the Fatty Acid Component

FIVE antibiotic polypeptides, polymyxins *A*, *B*, *C* and *E* (discovered in these Laboratories<sup>1</sup>) and polymyxin *D* (described by the American Cyanamid Company<sup>2</sup>), and readily differentiated by their amino-acid components and their behaviour on the paper chromatogram<sup>3</sup>, have been isolated from the products of the growth of different strains of *B. polymyxa* (*B. aerosporus*).

Research in these Laboratories has been directed chiefly to polymyxins *B* and *E*, both of which have yielded crystalline naphthalene- $\beta$ -sulphonates, from which, by repeated recrystallization and regeneration, highly active materials have been isolated. The use of naphthalene- $\beta$ -sulphonic acid has been found to be an essential step in the purification of these polymyxins, and has now been instrumental in the isolation of crystalline polymyxin *B* hydrochloride and crystalline polymyxin *E* neutral sulphate.

Following a private communication from the American Cyanamid Company that acid hydrolysis of polymyxin *D* liberated a fatty acid isomeric with pelargonic acid, it was found that acid hydrolysis of all polymyxins yielded an optically active fatty acid, C<sub>9</sub>H<sub>18</sub>O<sub>2</sub>, identical in each case and readily characterized as the amide and *p*-bromobenzylthiuronium

salt<sup>4</sup>. The constitution of the acid has now been determined as *d*-6-methyloctan-1-oic acid (I). Degradation of the methyl ester according to the scheme represented below gave *d*-3-methylpentan-1-oic acid (II), identified by comparison of the melting points of the amide and *p*-bromobenzylthiuronium salt with authentic samples derived from the synthetic acid obtained by resolution of the quinine salt of the racemic 3-methylpentan-1-oic acid.

An absolute proof of the identity is now being sought by the synthesis of *d*-6-methyloctan-1-oic acid (I). Full details will be reported elsewhere.

S. WILKINSON

Chemical Division,  
Wellcome Research Laboratories,  
Beckenham, Kent.  
April 19.

<sup>1</sup> Brownlee, G., and Jones, T. S. G., *Biochem. J.*, **43**, xxv (1948).

<sup>2</sup> Stansly, P. G., Shepherd, R. G., and White, H. J., *Bull. Johns Hop. Hosp.*, **81**, 43 (1947).

<sup>3</sup> Jones, T. S. G., *Biochem. J.*, **43**, xxvi (1948).

<sup>4</sup> Catch, J. R., Jones, T. S. G., and Wilkinson, S., *Ann. N.Y. Acad. Sci.*, **51**, 917 (1949).

### Crystallizable Recombined Hæm-Globin from Human Red-Cell Hæmoglobin

THE preparation of globin from hæmoglobin by removal of the hæm with acid has long been known<sup>1</sup>, but it has become increasingly clear that such globin preparations, when recombined with hæm, give a product differing from the original hæmoglobin in some properties such as spectral absorption<sup>2</sup> and solubility. Gralén showed<sup>3</sup> that although in the ultracentrifuge recombined hæm-globin gave results similar to those of the original horse hæmoglobin, electrophoresis showed up differences.

We have prepared from human red-cell hæmoglobin a globin which will recombine spontaneously with hæm to give a product more closely resembling the original hæmoglobin than any so far described, in that spectral absorption differences have been eliminated, its solubility is of the right order, and it can be crystallized in forms normal to human adult hæmoglobin.

We have observed that in globins prepared by the usual methods a prominent spectral absorption band due to tryptophan appears at wave-lengths varying between 289.0  $\mu$  and 290.3  $\mu$ . This band normally appears at 288.0  $\mu$  in free tryptophan in aqueous solution, but is shifted to 291.0  $\mu$  in most proteins, including human adult red-cell hæmoglobin. The wave-length of this band provided a most valuable criterion in following the globin preparation, and for its observation the moving-plate spectrographic method of Holiday<sup>4</sup> proved indispensable. Fractionation of the crude globin solution by 50 per cent saturation with ammonium

