Ca^{2+} and was inhibited by EDTA, cysteine and serum. EDTA-sensitive activity on insoluble tendon collagen and casein was also elicited by trypsin. Trypsin-activated bone collagenase chromatographed on Sephadex with an apparent molecular weight of 105 000 and was slightly retarded with respect to the latent enzyme.

Some culture fluids contained a non-competitive thermostable collagenase inhibitor of high molecular weight (at least 120 000). The latent enzyme is, however, unlikely to be a simple combination of active collagenase with this inhibitor for the following reasons: (1) mixtures of enzyme and inhibitor are not reactivated by trypsin; (2) the decrease in molecular weight accompanying activation is much too small; (3) up to 100-fold dilution caused no activation whatsoever of latent enzyme; (4) on exposure to trypsin the inhibitor is apparently destroyed before significant activation of latent enzyme has occurred.

However, considerably more information is needed on this inhibitor and on the activation phenomenon before it can be stated that, as our results suggest, collagenase is released as an inactive proenzyme that is activated by limited proteolysis. In any case it appears that regulation of extracellular collagenase activity is achieved by complex interactions involving the production of latent enzyme, of activating enzymes (possibly via lysosomes) and of protein inhibitors.

This work was supported by Grant no. 1209 from the Belgian Fonds de la Recherche Scientifique Médicale.

Gross, J. (1970). In *Chemistry and Molecular Biology of the Intercellular Matrix*, vol. 3, p. 1623. Ed. by Balazs, E. A. London: Academic Press (Inc.) Ltd.

Nagai, Y., Lapiere, C. M. & Gross, J. (1966). *Biochemistry*, Easton, 5, 3123.

An Altered Erythrocyte Acetylcholinesterase Associated with Hereditary Muscular Dystrophy in Mice

By P. K. DAS, R. L. WATTS and D. C. WATTS.
(Department of Biochemistry, Guy's Hospital Medical School, London S.E.1, U.K.)

When the cholinesterases from the blood of normal and dystrophic Bar Harbor mice are compared the plasma pseudocholinesterases (EC 3.1.1.8) appear to be similar whereas the erythrocyte acetylcholinesterases (EC 3.1.1.7) show a number of differences: in the dystrophic mice both plasma and erythrocyte cholinesterase activities are about half the normal values.

Blood from normal and dystrophic mice was centrifuged to separate the plasma, which was used for investigation of pseudocholinesterase activity.

The erythrocytes were used to prepare erythrocyte 'ghosts' (Dodge, Mitchell & Hanahan, 1963), from which acetylcholinesterase was solubilized with 0.5% Triton X-100 in 67mM-sodium phosphate buffer, pH 7.4, the debris being centrifuged off.

In the assay method described by Das & Liddell (1970) acetylcholinesterase was found to hydrolyse acetylthiocholine but not butyrylthiocholine. Comparison of the Michaelis curves for the normal and 'dystrophic' enzymes showed that $V_{\max}$ was reached when the acetylthiocholine concentrations were 0.18 and 0.45mM respectively, yielding corresponding $V_{\max}/2$ values of 0.04 and 0.1mM. Lineweaver-Burk plots gave K_m values of 0.06 and 0.19mM for normal and 'dystrophic' respectively, the difference from $V_{\max}/2$ values reflecting the effect of inhibition by excess of substrate.

The two enzymes were also affected to different extents by several inhibitors. Eserine when added to the assay mixture to give a final concentration of $0.5 \mu\text{M}$ has less effect on normal acetylcholinesterase activity but inhibited the 'dystrophic' enzyme by about 50%. Inhibition values obtained with other inhibitors were for the normal and 'dystrophic' preparations respectively: with $5 \mu\text{M}$ -compound C51 [1,5-bis-(4-allyldimethylammonium phenyl)-pentan-3-one dibromide] 90 and 78%; with 6% butanol, 23 and 52%. Other inhibitors tested, 0.3mM-sodium fluoride, $0.01 \mu\text{M}$ -compound RO2-0683 [dimethylcarbamate of (2-hydroxy-5-phenylbenzyl)trimethylammonium bromide], 0.1mM-dibucaine hydrochloride and 10mM-nialamide hydrochloride, inhibited both enzymes to the same extent within the limits of experimental error.

Electrophoresis of normal mouse acetylcholinesterase on starch gels at pH 8.6 with a tris-citrate/borate buffer system (Watts & Moreland, 1970) revealed three or four enzymically active components. 'Dystrophic' preparations contained only one or two with slightly different electrophoretic mobility.

Criteria similar to those reported here, taken in conjunction with family or population studies (reviewed by Harris, 1970), have been used as evidence for genetic variants of the pseudocholinesterases. Because of the complexity of expression of hereditary muscular dystrophy a similar inference for acetylcholinesterase must be treated with caution until better characterization of the purified enzyme is possible.

The pseudocholinesterase of plasma from both normal and dystrophic mice was equally active towards acetylthiocholine and butyrylthiocholine. No significant differences could be detected with the inhibitors mentioned above, but electrophoresis revealed an additional band of low electrophoretic mobility in dystrophic-mouse plasma. This minor component probably originates in muscle.

P. K. D. is indebted to the Muscular Dystrophy Group of Great Britain and R. L. W. to the Medical Research Council for maintenance grants. We are also grateful to the Medical Research Council for providing the Cary spectrophotometer used in this work.

- Das, P. K. & Liddell, J. (1970). *J. med. Genet.* **7**, 351.
 Dodge, J. T., Mitchell, C. & Hanahan, D. J. (1963). *Archs Biochem. Biophys.* **100**, 119.
 Harris, H. (1970). *The Principles of Human Biochemical Genetics*, p. 109. Amsterdam and London: North-Holland Publishing Co.
 Watts, D. C. & Moreland, B. M. (1970). In *Experiments in Physiology and Biochemistry*, p. 21. Ed. by Kerkut, G. A. London and New York: Academic Press.

Identification in Urine of a Low-Molecular-Weight Highly Polar Glycopeptide Containing Cysteinyl-galactose

By C. J. LOTE and JACQUELINE B. WEISS. (*Rheumatism Research Centre, University of Manchester, Manchester M13 0JJ, U.K.*)

Several distinct types of glycopeptide linkages are known. These include the β -glycosidic bond of *N*-acetylglucosamine to the amide group of asparagine (Marshall & Neuberger, 1964), the *O*-glycosidic link of serine or threonine (Anderson *et al.* 1964) and the β -glycosidic linkage between galactose and the hydroxyl group of hydroxylysine (Butler & Cunningham, 1966). To date no linkage has been described in which the thiol group of cysteine is involved, although complex glycosides of 1-thio-D-glucose are common in mustard oils (Horton & Hutson, 1963).

We have recently isolated, from normal human urine, an octapeptide in which two galactose residues are *S*-glycosidically linked to cysteine.

Urine was concentrated 300-fold by a method designed to protect labile groups (Weiss & Steven, 1968; C. J. Lote & J. B. Weiss, unpublished work), and applied to a Sephadex G-15 column. The fraction corresponding to a 900–1000 molecular weight was further analysed on a split-stream peptide analyser. Of the 36 peptides separated one appeared to have a covalently bonded sugar. Galactose was the only sugar detected in this peptide by g.l.c. For each 2mol of galactose amino acid analysis revealed 1mol each of aspartic acid, serine, cysteine, glutamic acid, glycine and alanine and 2mol of histidine. Sequential analysis by the dansyl-Edman technique (Beale, 1969) indicated the following structure:

Cys-Glu-His-Ser-His-Asp-Gly-Ala

Alkaline borohydride reduction indicated that serine was not involved in the glycopeptide bond.

Prolonged digestion of the peptide with leucine aminopeptidase (EC 3.4.1.1) at pH 8.4 released a single component, which on mild acid hydrolysis was shown to contain only cysteine and galactose. No galactose was detected in the remaining portion of the peptide.

We conclude that the galactose is linked glycosidically to the sulphur atom of cysteine. A thiazolidine-type structure is excluded by the availability of the amino group of cysteine for dansylation and by the lability of the bond to mild acid hydrolysis. In view of the extreme polarity of the peptide adjacent to the thioglycoside its function as a binding or carrier site cannot be excluded, and we are currently investigating the possibility that this peptide may be part of a membrane glucose-carrier system.

This work was supported in part by a grant from the Nuffield Foundation.

- Anderson, B., Seno, N., Sampson, P., Riley, J. G., Hoffman, P. & Meyer, K. (1964). *J. biol. Chem.* **239**, rc2716.
 Beale, D. (1969). In *Chromatographic and Electrophoretic Techniques*, vol. 1, p. 189. Ed. by Smith, I. London: William Heinemann (Medical Books) Ltd.
 Butler, W. T. & Cunningham, L. W. (1966). *J. biol. Chem.* **241**, 3882.
 Horton, D. & Hutson, D. H. (1963). *Adv. Carbohydr. Chem.* **18**, 123.
 Marshall, R. D. & Neuberger, A. (1964). *Biochemistry, Easton*, **3**, 1596.
 Weiss, J. B. & Steven, F. S. (1968). *Nature, Lond.*, **217**, 661.

A Sensitive Method for the Detection of Metabolites of Dianabol in Urine in Man

By A. M. LAWSON* and C. J. W. BROOKS. (*Department of Chemistry, University of Glasgow, Glasgow W.2, U.K.*)

The mass-spectrometric technique of single-ion monitoring has been investigated as a rapid, sensitive and quantitative method for the detection of major human metabolites of the anabolic drug Dianabol (methandienone; 17 β -hydroxy-17 α -methylandrostan-1,4-dien-3-one).

Single-ion monitoring is the monitoring of a single preselected mass number by mass spectrometry so that a response is obtained from any compound giving a fragment of that mass value. Detection of a characteristic ion at the correct retention time for a particular compound affords a good basis for identification.

* Present address: Clinical Research Centre, Watford Road, Middx. HA1 3UJ, U.K.