for supplying the monozygous twin sera, and Mr. A. L. Ogden, of this Station, for collecting blood samples in Norfolk and Suffolk.

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[1] Ashton, G. C., *Vet. Rec.*, **69**, 803 (1957).
[2] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[3] Smithies, O., and Poulik, M., *Nature*, **177**, 1033 (1956).

## Purification of β-Glucuronidase from Female Rat Preputial Gland

Because of the enormously high activity of β-glucuronidase in the preputial gland of the female rat[1], this tissue appeared to be a promising source of the purified enzyme. For other tissues employed for this purpose, the specific activity, the degree of purification and the recovery are as follows: ox liver[2] (32,000, 800-fold, 5 per cent), calf spleen[3] (7,900, 1,400-fold, 1 per cent), calf liver[4] (60,000, 9,000-fold, 10 per cent). The specific activity is expressed as μgm. phenolphthalein liberated from phenolphthalein glucuronide by 1 mgm. of protein in 1 hr. at 37° C., and the last two preparations were assayed in presence of deoxyribonucleic acid. A specific activity of 107,000 was observed with the best individual preparation from calf liver[4], and this was claimed to represent a purity of 85 per cent.

Crude preparations of the preputial gland of the female rat have specific β-glucuronidase activities similar to that quoted for the purified enzyme of calf spleen, and much higher specific activities than any yet reported can easily be obtained after four consecutive stages of fractional precipitation. In the experiment summarized in Table 1, twenty-one rats, 4–6 months old, yielded 2·2 gm. preputial gland which was homogenized in water. The homogenate was made 0·1 *M* with respect to acetate buffer, *p*H 5·2, in a final volume of 22 ml. After 2 hr. at 37° C., insoluble material was removed by centrifuging, and the supernatant was fractionated as shown. Precipitation, followed by sedimentation (10 min. at 20,000*g*) after 30 min., and dialysis of the fractions were done at 0° C., but other manipulations were at ordinary room temperature. Solutions were buffered to *p*H 5·2 with 0·1 *M* or 0·01 *M* acetate buffer, and dialysed against the more dilute buffer. Losses in enzyme activity were in part due to the discard of less-pure fractions: there appeared, for example, to be no enzyme inactivation during the final fractionation.

Table 1. One Glucuronidase Unit (G.U.) liberates 1 μgm. Phenolphthalein from 0·00063 *M*-Phenolphthalein β-Glucuronide in 1 hr. at 37° C. and *p*H 5·2 (0·05 *M* Acetate Buffer). Percentages of Saturated Ammonium Sulphate Solution and Ethanol are by Volume. Protein was determined by the Method of Lowry *et al.*[6], employing Bovine Albumin as Standard

| Fraction | G.U./mgm. protein | Recovery, G.U./gm. moist tissue |
|---|---|---|
| Homogenate | 5,850 | 944,000 |
| Supernatant | 11,200 | 979,000 |
| 20–80 per cent sat. ammon. sulphate sol. | 17,700 | 845,000 |
| 20–45 per cent ethanol | 31,000 | 564,000 |
| 33–60 per cent sat. ammon. sulphate sol. | 71,200 | 205,000 |
| 45–50 per cent ethanol | 107,000 | 188,000 |
| | 193,000* | 338,000* |

* Enzyme assay in presence of 0·03 per cent deoxyribonucleic acid.

In agreement with Fishman and his co-workers[5], the enzyme, especially in an advanced state of purity, displayed a reduction in net activity on dilution, and this was reversed by a variety of substances, such as deoxyribonucleic acid, albumin or enzyme inactivated by heat. It should, however, be noted that the figures in Table 1 are not strictly comparable with the measurements of specific enzyme activity quoted by these authors[3,4], since we employed albumin as our reference protein while they used the more chromogenic protein, chymotrypsin[6]. To correct for this, our own results should probably be multiplied by 1·5 throughout.

Since the β-glucuronidase activity of preputial gland of the female rat is under ovarian control[1], this tissue would appear to be ideal for studying the biosynthesis of the active protein.

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[1] Beyler, A. L., and Szego, C. M., *Endocrinol.*, **54**, 334 (1954).
[2] Smith, E. E. B., and Mills, G. T., *Biochem. J.*, **54**, 164 (1953).
[3] Bernfeld, P., and Fishman, W. H., *J. Biol. Chem.*, **202**, 757 (1953).
[4] Bernfeld, P., Nisselbaum, J. S., and Fishman, W. H., *J. Biol. Chem.*, **202**, 763 (1953).
[5] Bernfeld, P., Bernfeld, H. C., Nisselbaum, J. S., and Fishman, W. H., *J. Amer. Chem. Soc.*, **76**, 4872 (1954).
[6] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

## Excretion of Yttrium and Lanthanum Chelates of Cyclohexane 1,2-*Trans* Diamine Tetraacetic Acid and Diethylenetriamine Pentaacetic Acid in Man

The removal of stable or radioactive toxic elements from the human body is of considerable interest[1]. The modification of tissue distribution and excretion of metal ions by chelating agents has been studied in man and animals in several laboratories[2]. Although ethylenediamine tetraacetic acid has been the most effective chelating agent thus far studied, it has the disadvantage that relatively large doses are required in order to effect a significant removal of heavy metal ions, particularly radioactive rare earths. Therefore metal chelates having stability constants higher than the corresponding metal chelates of ethylenediamine tetraacetic acid were investigated for their effectiveness in removal of rare earth elements.

This communication reports observations on the urinary excretion of intravenously administered yttrium and lanthanum chelated with *cyclo*hexane 1,2-*trans* diamine tetraacetic acid[3] (CDTA) and with diethylenetriamine pentaacetic acid[4] (DTPA). The excretion of these metal chelates has been compared with the excretion of the yttrium and lanthanum ethylenediamine tetraacetic acid chelates.

Yttrium and lanthanum chelates of ethylenediamine tetraacetic acid, *cyclo*hexane 1,2-diamine tetraacetic acid and diethylenetriamine pentaacetic acid, using 1·2 : 1 chelate to metal molar ratios, were administered intravenously to man. Yttrium and lanthanum were administered in 1·0-mgm. and 1·5-mgm. quantities labelled with yttrium-90 and lanthanum-140 respectively; they were determined in plasma samples and in aliquots of urine and the