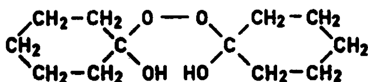
Signs for about 30 % of the structure factors were determined by a computer procedure based on the Cochran—Douglas method[4] (programmed in SPS for IBM-1620 by the author). The corresponding Fourier maps could easily be interpreted, and the final R-values arrived at after least squares refinements were about 10 % for each projection. The final electron density maps (Figs. 1 and 2) show considerable overlapping and one cannot expect the final atomic positions to be very accurate. A three-dimensional analysis is therefore now being undertaken.

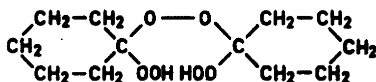
Fig. 3 shows the three chair formed rings of the molecule.

The corresponding compounds obtained from cycloheptanone (space group $P2_1/c$, $Z = 2$), cyclooctanone and cyclododecanone are also being studied, and the investigation will be extended to some other organic peroxides namely

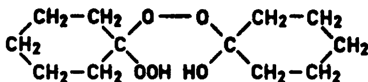
a) 1,1′-dihydroxycyclohexanylperoxide-1,1′



b) 1,1′-dihydroperoxycyclohexanylperoxide-1,1′



c) 1-hydroperoxycyclohexanyl-1′-hydroxycyclohexanyl-peroxide-1,1′



(space group $P2_1/c$, $Z = 4$)

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Received May 13, 1964.

Crystalline β-Glucuronidase

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Several papers have appeared on the preparation of β-glucuronidase. Bernfeld *et al.*[1] in 1953 claimed that the pure enzyme had an activity of about 1 × 10^6 Units per mg. Recently Fialkow *et al.*[2] reported an activity of 1.4 × 10^5 while Plapp *et al.*[3] reported a value of about 2.2 × 10^5 Units per mg for a fraction obtained by zone electrophoresis. In all cases only small amounts of enzyme were available. The protein determination was made according to the method of Lowry *et al.*[4]

As starting material beef liver was chosen. One kg of fresh liver contains about 6 × 10^6 Units. The liver was ground in a blendor and extracted overnight in the cold with 3 times its weight of either water or acetate buffer pH 5, 0.1 M. From this crude extract there are many ways to arrive at a product containing about 500—1000 Units per mg protein. Repeated ammonium sulphate fractionation, treatment according to Tsuchihashi [5] as in the preparation of ADH [6] or fractionation with alcohol at a low temperature. In the present paper the first method was used.

When the above mentioned purity is reached two or three more ammonium sulphate fractionations at pH 5 give a product of about 4000—5000 Units per mg. The solution is now dialyzed against 0.1 M acetate buffer, pH 5, until practically all sulfate is removed. Some loss of enzyme usually occurs. The more concentrated the solution is, the smaller is the loss. A heavy precipitate of inactive protein formed during the dialysis is removed by centrifugation.

Next step is an alcohol fractionation to get rid of the sticky brown colored material that usually is found in liver extracts. At pH 4.2 all enzyme is precipitated with about 8—10 % alcohol (v/v) at 0 to +2°C, while at pH 7.8 the enzyme is not precipitated until 30—50 % alcohol is reached.

This behaviour gives a wide latitude for chosing conditions during the alcohol fractionation. More than two successive fractionations should not be made as this gives rise to losses of enzyme.

Addition of alcohol up to 5 % to the dialyzed solution usually precipitates a black colored inactive protein. The solution is then again dialyzed, this time against a pyrophosphate buffer, pH 7.8, until most alcohol is removed. At this pH more inactive protein could be precipitated by adding as much as 20 % alcohol, and the alcohol is subsequently removed by dialysis against acetate buffer 0.1 M, pH 5. At this step the purity is about 10 000 – 15 000. From now on only ammonium sulphate fractionation was used. Fractionation at pH 4.2 and at pH 5 yields a product with 20 000 – 30 000 Units per mg. Careful fractionation at pH 6 then raises the purity of the enzyme to about 50 000 – 60 000 with a fairly good yield.

The pH is adjusted to 7.8 and the enzyme comes down first on addition of ammonium sulphate. Twice crystallized the activity was about $1.3 - 1.7 \times 10^5$ Units per mg, A_{280} for 1 mg per ml is 1.4. When the crystals are dissolved in water and dialyzed against distilled water the enzyme crystallizes, see Fig. 1.

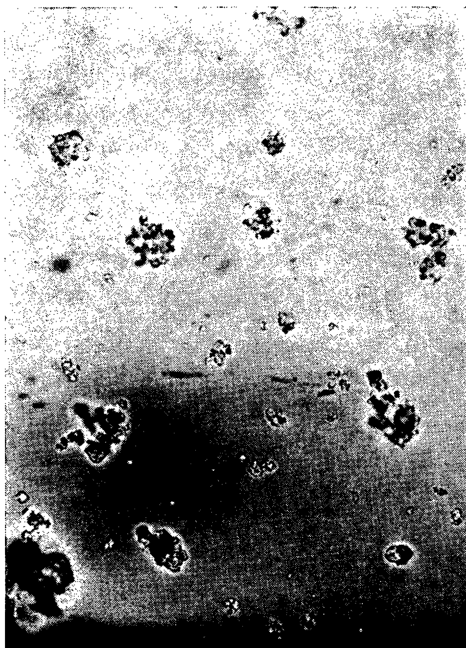

Fig. 1. Crystalline β-glucuronidase (×400).

Stability. The enzyme is stable from about pH 3.7 to 8. It can be frozen and thawed several times without loss. Dialysis slowly inactivates the enzyme. In ammonium sulphate solutions the enzyme has been kept for months at zero degrees without loss of activity.

This work was supported by a grant from *Statens Medicinska Forskningsråd* and from *Institutet för Maltdrycksforskning*. It is a pleasure to acknowledge the valuable assistance of Bo Fallman and Hugo Gschwind.

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Received June 9, 1964.

The Influence of Dextran on the Precipitin Reaction

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Although various polymers, such as dextran, gelatin, polyvinylalcohol, and polyvinylpyrrolidone have been used to increase the sensitivity of immunoreactions *in vitro* (*cf. e.g.* Ref.[1]) the role of the polymers in the reactions has not been elucidated. Recently Laurent [2,3] discussed the steric interaction between polysaccharides and proteins. He showed that this interaction decreases the solubility of the proteins; the larger the protein, the larger the effect. Such a steric interaction could account for the effect of polymers in immunoreactions. The interaction should then be most pronounced in the region of antigen excess, where it is believed that high-molecular weight antigen-antibody complexes exist in solution.[4]

Stahman and Matthews [5] showed that small amounts of polylysine increased the