ether and absolute alcohol. After drying in the air, the sections were treated with periodic acid and Schiff's reagent. Controls were prepared as described above.

Glycogen was demonstrated by both methods in the epididymal epithelium of several species, though in different amounts and different regions of the organ. In some regions moderate amounts were present in the secretion products. In globules lying free in the lumen, the glycogen was often less prominent or absent, especially in those intermingled with the spermatozoa. In frozen-dried material the reaction was diffusely distributed within the secretion globules, and stronger in their periphery. After chemical fixation small, heavily coloured granules were seen.

In the control sections a faint red colour persisted, and the stereocilia were generally still dark red.

McLeod[4] has shown that glycolysis and motility of human spermatozoa are produced by glycogen as well as by glucose and fructose under anaerobic conditions. The glycogen secretion reported here may therefore represent a mode of 'nutrition' of epididymal spermatozoa during the long time required for their passage through the organ.

A detailed description of the pictures observed in the different species will be published elsewhere.

LENNART NICANDER

Department of Anatomy and Histology,
Veterinärhögskolan,
Stockholm.
Aug. 10.

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Use of *p*-Chloromercuribenzoic Acid in the Determination of Ascorbic Acid in the Presence of Sulphydryl Compounds

IN certain circumstances hydrogen sulphide, cysteine and related sulphydryl compounds react with 2 : 6-dichlorophenol-indophenol (indophenol) and thus may interfere with the determination of ascorbic acid using this reagent. In view of the known ability of *p*-chloromercuribenzoic acid to combine with sulphydryl compounds[1], the possibility of using this reagent to suppress such interference has been examined.

Results indicate that this reagent does not itself react with indophenol nor does it affect the determination of ascorbic acid in pure solution, using a colorimetric procedure based on the decolorization of excess indophenol. Under similar conditions the addition of *p*-chloromercuribenzoic acid has been found to suppress completely the decolorization of indophenol by hydrogen sulphide, cysteine and glutathione respectively.

The reagent has been used in the following manner : 1 ml. of *p*-chloromercuribenzoic acid solution (200 mgm./100 ml. in 0·05 *N* sodium hydroxide) was added to 3 ml. of standard ascorbic acid solution (0·0–2·0 mgm./100 ml.), or test solution, in 1·8 per cent (w/v) metaphosphoric acid solution. Because of the low solubility of *p*-chloromercuribenzoic acid at this *p*H (about 1·5) most of the excess reagent was precipitated. The solution containing the precipitate was allowed to stand for 5 min. and then centrifuged. To 2 ml. of the supernatant fluid was added 0·5 ml. sodium citrate solution followed by 1 ml. indophenol solution (4 mgm./100 ml.) and the optical density of the resulting colour determined 30 sec. after the addition of the dye using a Unicam spectrophotometer *S.P.* 600 at a wave-length of 520 mμ. The concentration of the sodium citrate used was such as to bring the final *p*H to 3·5. With solutions of ascorbic acid in 1·8 per cent (w/v) metaphosphoric acid a concentration of 7 per cent (w/v) was required.

It is suggested that *p*-chloromercuribenzoic acid may be of value in increasing the specificity of the indophenol reaction applied to the determination of ascorbic acid in biological material containing sulphydryl compounds. It offers advantages over mercuric acetate[2] for the removal of cysteine and certain other sulphydryl compounds in that subsequent treatment with hydrogen sulphide, to remove the excess mercuric ions and to convert the dehydroascorbic acid formed to ascorbic acid, is not required. It thus avoids a procedure which itself introduces several sources of error[3,4] and does not permit the determination of ascorbic acid in the presence of dehydroascorbic acid.

The effect of *p*-chloromercuribenzoic acid on the determination of the 'ascorbic acid' content of human plasma is being studied and it is hoped to publish results shortly.

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J. A. OWEN
B. IGGO
D. B. HORN

Department of Clinical Chemistry,
University, Edinburgh.
Aug. 9.

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A New Mixed Disulphide: Penicillamine–Cysteine

As part of a study of skin sensitization to penicillin and its derivatives, we have been investigating the behaviour of penicillamine (β,β′-dimethylcysteine). Since D-penicillamine proved capable of eliciting allergic skin responses[1], it was expected that it would react with epidermal proteins[2], possibly through cystine or cysteine residues. Accordingly, it was of interest to determine whether this sulphydryl compound reacts with cystine or cysteine. It was found that a reaction occurs readily with cystine with the formation of a mixed disulphide which is here designated penicillamine–cysteine.

A typical experiment consisted of mixing 1 mgm. of D-penicillamine and 2 mgm. of L-cystine in 0·4 ml. of 0·5 *M* ammonium chloride at *p*H 7·4. Aliquots were analysed by ascending chromatography on paper with phenol–water as solvent. A new ninhydrin-positive compound was found, $R_F = 0 \cdot 41$, about midway between cystine, $R_F = 0 \cdot 2$, and penicillamine, $R_F = 0 \cdot 7$. The new compound gave a positive nitroprusside reaction only after reduction with alkaline cyanide solution. It yielded cysteic acid and penicillamine sulphonic acid after oxidation with

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