incubated aerobically at 35° C., there occurred a rapid formation of *epi*testosterone, together with smaller amounts of testosterone. This phenomenon was not observed in plasma separated from corpuscles, nor in washed blood corpuscles resuspended in saline.

The present investigation has demonstrated by means of direct chemical determination of steroids that androgenic substances are secreted by the bovine testis into the blood stream already at a very early age. *In vitro* studies by Slaunwhite and Samuels[5] have indicated the following sequence of biosynthetic reactions in rat testicular tissue: Progesterone → 17α-hydroxyprogesterone → androstenedione → testosterone. Our results suggest that the conversion of androstenedione to testosterone may be a rate-limiting reaction in the elaboration of testosterone by the immature bovine testis. A transition to a secretion in which testosterone is predominant would seem to precede the appearance of pubertal changes in this species.

This study is now being extended to other species of farm animals, and in the adult ram, for example, both testosterone and androstenedione were shown to be present in the spermatic vein blood in proportions similar to those found in the bull.

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[1] David, K., Dingemanse, E., Freud, J., and Laqueur, E., *Hoppe-Seyl. Z.*, **233**, 281 (1935).
[2] Hooker, C. W., *Amer. J. Anat.*, **74**, 1 (1944).
[3] Mann, T. R. R., "Recent Progr. Hormone Res.", **12**, 353 (1956).
[4] Bush, I. E., *Biochem. J.*, **50**, 370 (1952).
[5] Slaunwhite, jun., W. R., and Samuels, L. T., *J. Biol. Chem.*, **220**, 341 (1956).

Acid Mucopolysaccharides of Normal Urine

The occurrence of at least one acid mucopolysaccharide in normal urine has repeatedly been reported[1-3]. It was found by various authors[1-3] to behave chromatographically and electrophoretically like chondroitin sulphate *A*. Separation and staining, however, were unsatisfactory.

We recently described[4] a convenient and highly specific staining method for acid mucopolysaccharide in which use was made of a 1 per cent solution of alcian blue (Gurr or Harleco) in a 9 : 1 mixture of acetic acid and water (5 min.), followed by alternate washings with acetic acid and tap water. The intensely blue acid mucopolysaccharide spots stand out on a pure white background, and as little as 0·5 μgm. of heparin or 2·0 μgm. of chondroitin sulphate *A* can be detected. Scanning allows quantitative estimations, since the uptake of dye appears to be proportional to the acid mucopolysaccharide concentration over a wide range. A correction factor is nevertheless required to allow for different specific dye uptakes by different acid mucopolysaccharides.

By using an ultrafiltration and dialysis method, to be described elsewhere, it was possible to concentrate normal urines up to 3,000 times without any denaturation of their colloids. Electrophoresis on Whatman No. 1 filter paper in a veronal–acetate buffer of *p*H 8·6 and ionic strength 0·1, using 3 V./cm. for 12 hr., resulted in very sharp separation of three different acid mucopolysaccharides, which migrated ahead of the albumin peak. The fastest and most abundant peak migrated about twice as fast as albumin, and at exactly the same speed as commercial chondroitin sulphate *A*. This is presumably the substance described by other authors[1-3]. The second peak, which was usually though not always smaller than the first, had a mobility of about 1·5 times greater than albumin. The third and smallest peak was about 1·1 times faster than albumin. None of these substances could be stained by protein dyes.

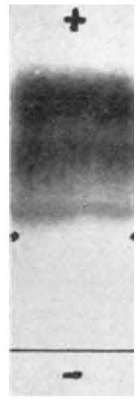

Fig. 1. Urinary acid mucopolysaccharides separated by electrophoresis at *p*H 8·6 and stained with alcian blue. The albumin(unstained) is indicated by arrows

All three appeared to have some anticoagulant activity of the heparin type, which casts considerable doubt on their identification as chondroitin sulphates *A* or *C*, which are known to be devoid of anticoagulant properties. On the other hand, commercial alpha-heparin samples ('Liquaemin', Roche) was found to migrate distinctly faster than the fastest urinary acid mucopolysaccharide peak.

The identification of these three substances must await further investigation, which is now in progress in our laboratory.

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[1] Kerby, G. P., *J. Clin. Invest.*, **33**, 1168 (1954).
[2] Hamerman, D., Hatch, F. T., Reife, A., and Bartz, K. W., *J. Lab. Clin. Med.*, **46**, 848 (1955).
[3] Di Ferrante, N., and Rich, C., *J. Lab. Clin. Med.*, **48**, 491 (1956).
[4] Heremans, J. F., and Vaerman, J. P., *Clin. Chim. Acta*, **3**, 430. (1958).

Persistence of Cholesterol-4-^{14}C in the Central Nervous System

Recent work from this laboratory[1,2] has shown that once cholesterol-4-^{14}C is incorporated into the central nervous system of developing rabbits and chickens it remains with little change for more than a year. It seems likely that this radioactive cholesterol is incorporated into the myelin sheath, but there remains the possibility that once in the central nervous system the cholesterol undergoes internal turnover, being broken down and resynthesized *in situ*. This communication presents evidence to show that the radioactive carbon atom, even after 18 months, remains in its original position in the cholesterol molecule. In these experiments cholesterol extracted from the central nervous system with chloroform–methanol (2 : 1) was isolated by fractionation on an alumina column. The cholesterol was converted to cholestenone by an Oppenauer oxidation[3]. Next the radioactive cholestenone was ozonized