Further investigations on sex differences in androgen sulphate formation have been carried out with mice. It was found that mice exhibit similar *in vivo* and *in vitro* sex differences. The methods used in determining the androgen sulphates formed in the whole-animal and liver-slice experiments were described by Lewis (1968).

The reason for these sex differences is not known. Roy (1958) showed that there is no difference in the synthesis of phenyl sulphate by preparations from livers of male and female rats. Weisburger, Grantham & Weisburger (1964) reported that male rats excrete more of the administered carcinogen *N*-hydroxy-*N*-2-fluorenylacetamide as a sulphate than do female rats. In contrast, Rao & Taylor (1965) found that liver homogenates from female rats formed more cold-acid-hydrolysable metabolites of progesterone than homogenates from male rats.

These results are consistent with the suggestion (Roy, 1958) that the sex differences in steroid sulphate formation may be due to differing amounts of steroid sulphotransferases rather than in the enzymes required to synthesize adenosine 3'-phosphate 5'-sulphatophosphate.

In the present investigation no sex difference was found between the sulphate-activating system prepared from particle-free supernatants of male and female rat livers (De Meio, Wizerkaniuk & Schreibman 1955) in the enzymic synthesis of dehydroepiandrosterone sulphate (Roy, 1956). A sex difference was observed when it was found that the steroid sulphotransferase system prepared from particle-free supernatant of female rat liver (Roy, 1960) was more active than the system prepared from male liver in the enzymic synthesis of androsterone sulphate, epiandrosterone sulphate and dehydroepiandrosterone sulphate.

These results and others suggest that the observed sex differences in androgen sulphate formation, at least in rats, may be due to differing amounts or activities of steroid alcohol sulphotransferases in male and female livers.

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The High-Molecular-Weight Ribonucleic Acid Species of Soya-Bean Mitochondria

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Much work in recent years has indicated that mitochondria contain the mechanism for protein synthesis, but that the genetic information they contain may apply only to mitochondrial protein formation (Gibor & Granick, 1964). The existence of ribosomes in mitochondria has been reported (e.g. Howell, Loeb & Tomkins, 1964). Ribosomes have also been demonstrated in chloroplasts, such ribosomes having a smaller size than those of the cytoplasm (Boardman, Francki & Wildman, 1965). Further, the protein-synthesis systems of both mitochondria and chloroplasts are more sensitive to inhibition by chloramphenicol than is the cytoplasmic protein-synthesis system (Clark-Walker & Linnane, 1966; Eisenstadt & Brawerman, 1964). There is some reason therefore to suspect conformational differences between ribosomes from mitochondria and those from the cytoplasm.

We have used polyacrylamide-gel electrophoresis to separate and compare the high-molecular-weight RNA species from the mitochondria and cytoplasmic ribosomes of soya-bean (*Glycine max*) seedlings. Seedlings were grown in the dark, in vermiculite moistened with potassium [32 P]phosphate. Mitochondria and cytoplasmic ribosomes were isolated from the hypocotyls, and the 32 P-labelled RNA was obtained by the 4-aminosalicylate-phenol-cresol extraction method of Kirby (1965). The RNA samples were subjected to electrophoresis through 2.4% polyacrylamide gels, 3 H-labelled *Escherichia coli* RNA being used as marker. After electrophoresis the gels were scanned at 266 m μ to obtain extinction profiles, and 1 mm. sections were taken for simultaneous radioactivity counting of 32 P and 3 H.

Since, for RNA, a linear relationship appears to exist between electrophoretic mobility and the logarithm of molecular weight (Bishop, Claybrook & Spiegelman, 1967), estimates of the molecular size of the mitochondrial and cytoplasmic RNA species were possible. Base compositions of the prominent components were also obtained, by the method of Hayashi & Spiegelman (1961).

Electrophoretic separation showed the cytoplasmic ribosome RNA to possess two major RNA species, presumably corresponding to the 25s and 16s ribosome components of the plant. The base

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compositions of these species agreed with the data on soya-bean ribosome components reported by Ingle, Key & Holm (1965). The mitochondrial preparation, however, revealed two RNA species intermediate in size between the two major cytoplasmic ribosomal RNA components. The more prominent of the mitochondrial components was found to have a relatively low GC content.

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Specific Assay of Glycosulphatase Acting on Sulphate Esters of D-Glucose and of D-Galactose and on Mixed Substrates

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The oxidation of D-glucose by D-glucose oxidase from *Aspergillus niger* forms the basis of colorimetric methods for the determination of this sugar in biological fluids (Ogston, 1948). D-Galactose oxidase from *Dactylium dedroites*, another enzyme of high specificity, is used in the assay of D-galactose in a similar procedure (Cooper, Smith, Bacila & Medina, 1959; Roth, Segal & Bertole, 1965).

These procedures have been adapted to the assay of glycosulphatase to determine, specifically the activity against sulphate esters of D-glucose or D-galactose, and are particularly useful in mixed-substrate experiments. It has been shown that in each case 6-O-sulphate esters of D-glucose and D-galactose and sulphate ions do not interfere in the assay, and for liberation of hexose in the range 2–100 µg./ml. linear calibration curves are obtained. By using the two methods with D-glucose 6-O-sulphate and D-galactose 6-O-sulphate respectively as substrates, the activities and pH-activity curves

obtained were in good agreement with those obtained by the pH-stat method (Lloyd & Stuart, 1966; Lloyd & Fielder, 1967). The enzyme used was a partially purified glycosulphatase from *Patella vulgata* (Lloyd & Stuart, 1966). For this enzyme acting on the 6-O-sulphate esters of D-glucose and D-galactose respectively at pH 6.0, the following values were obtained: optimum substrate concentration, 0.04, 0.04 M; activity, 3295, 4035 units; K_m , 1.86, 28.3 mM. When, in the hexose oxidase methods of assay, *Patella* glycosulphatase was incubated with D-glucose 6-O-sulphate (0.04 M) together with D-galactose 6-O-sulphate (0.04 M) the following activities were recorded: 2729 (D-glucose oxidase assay) and 460 (D-galactose oxidase assay). These data suggest that a single enzyme is responsible for the desulphation of both sugars.

The view that a single enzyme is acting on both substrates is supported by other data including constancy in the ratio of activities for different preparations of the enzyme and identical pH optima, and by studies on some inhibitors.

Difficulties accompanying the use of D-glucose 6-O-sulphate as substrate for *Patella* glycosulphatase have been indicated earlier (Lloyd & Fielder, 1967). Although there is evidence that there are at least three sulphatases present in the initial extract from the viscera of *Patella vulgata* that act on carbohydrate sulphates, it is clear that the 6-O-sulphates of D-glucose and D-galactose are alternative substrates for the glycosulphatase under investigation.

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Desulphation of Chondroitin Sulphate by an Enzyme from *Patella vulgata*

By P. F. LLOYD and R. J. FIELDER (*Department of Chemistry, University College of North Wales, Bangor*)

The viscera of *Patella vulgata* contains a glycosulphatase that is active against monosaccharide mono- and poly-sulphates (Lloyd, Lloyd & Owen, 1962; Lloyd & Stuart, 1966). Recently it has been shown that there is also present an enzyme that liberates inorganic sulphate from chondroitin