

When orally administered at a dose of 0.2 g./kg. to rabbits, cyclohexylamine gives rise to unchanged cyclohexylamine and *N*-hydroxycyclohexylamine in the urine. This has been confirmed by feeding [ $^{14}\text{C}$ ]cyclohexylamine to a rabbit (0.17 g./kg. or 1.067  $\mu\text{C}/\text{kg.}$ ), when it was found that 68% of the radioactivity could be recovered from the urine in 60 hr.: 13% in the first 10 hr., 39% in the next 20 hr., and 16% in the last 30 hr. The excretion curve showed a small peak at 3 hr. and a second larger peak at 32 hr. A small amount (0.5%) was eliminated in the breath (0.3% as  $^{14}\text{CO}_2$  and 0.2% as ethanol-soluble material). By isotope-dilution experiments, 45% of the administered dose was shown to be excreted in the urine as unconjugated cyclohexylamine, 0.2% as *N*-hydroxycyclohexylamine in conjugated form, and 2.5% as cyclohexanone oxime, which is probably an artifact arising from the glucuronide of *N*-hydroxycyclohexylamine in the hydrolysis procedure.

Thin-layer chromatography of all fractions of the radioactive urine in butanol-acetic acid-water (4:1:5, by vol.) revealed the presence of one metabolite ( $R_F$  0.53) giving a positive reaction with naphtharesorcinol, and two metabolites ( $R_F$  0.37 major and  $R_F$  0.59 minor) both giving a positive reaction with ninhydrin, the minor one corresponding in position to cyclohexylamine.

Bernhard, K. (1937). *Hoppe-Seyl. Z.* **248**, 256.

Ohashi, T. (1964). *J. pharm. Soc. Japan*, **84**, 563.

### Changes in the Fatty Acids of the Reproductive Tissues of Male Rats in Essential Fatty Acid Deficiency

By J. A. CARNEY and D. L. WILLIAMS (*Department of Biochemistry, University of Liverpool*)

It has long been known that one of the major symptoms of essential fatty acid (EFA) deficiency in rats is a lack of reproductive capacity (Burr & Burr, 1929, 1930). There have, however, been few reported studies about the effects of EFA deficiency on the lipids of the reproductive glands.

A group of ten male weanling rats was maintained on a low-EFA diet consisting of a semi-synthetic basal fat-free diet supplemented with 3% by weight of butterfat. A second group was given a normal-EFA diet consisting of the above diet in which the butterfat was replaced by maize oil. Five rats from each group were killed after 2 months and the remainder were killed after 4 months. The fatty acid composition of the phospholipid and triglyceride fractions of the testes was determined.

In both lipid fractions the proportion of  $\omega-6$  fatty acids, which are formed only from dietary

EFA, fell substantially in the rats given the low-EFA diets, particularly in the first 2 months. This decline was caused mainly by a decrease in the contents of arachidonic acid (20:4,  $\omega-6$ ) and docosapentaenoic acid (22:5,  $\omega-6$ ) in the phospholipids and of linoleic acid and 22:5,  $\omega-6$  acid in the triglycerides. The fall was balanced by a rise in the proportion of  $\omega-9$  fatty acids, particularly oleic acid.

In a second experiment 12 rats were given a fat-free diet for 10 months. The diet of six of these rats was supplemented by 3% of maize oil. The remainder received no supplement. The fatty acid composition of the phospholipid and triglyceride fractions of the testes, seminal vesicles and prostate glands of the animals was determined. The fall in the proportion of  $\omega-6$  acids in the testes of the rats given the fat-free diet was much more marked than in the first experiment, and was counterbalanced by a rise in the proportion of the  $\omega-7$  as well as in that of the  $\omega-9$  acids.

The phospholipid fractions of the prostate gland and seminal vesicles of rats given the maize-oil-supplemented diet contained very large amounts of  $\omega-6$  acids, particularly arachidonic acid. In the corresponding fractions of the rats given the fat-free diet almost all of these  $\omega-6$  acids were replaced by  $\omega-7$  and  $\omega-9$  acids. There were only trace amounts of  $\text{C}_{20}$  or  $\text{C}_{22}$  acids in the triglyceride fraction from both of the groups of rats.

The large amounts of 22:5,  $\omega-6$  and 20:4,  $\omega-6$  acids in the lipids of the testis and of 20:4,  $\omega-6$  acid in the phospholipids of the seminal vesicles and the prostate gland suggest an important role for these polyunsaturated acids in the reproductive tissues.

Burr, G. O. & Burr, M. M. (1929). *J. biol. Chem.* **82**, 345.

Burr, G. O. & Burr, M. M. (1930). *J. biol. Chem.* **86**, 587.

### Sex Differences in Androgen Sulphate Formation in Rats and Mice

By D. A. LEWIS (*Pharmacology Group, Bath University of Technology*)

Particle-free supernatants of female rat and mouse liver homogenates have been reported to be more active in conjugating dehydroepiandrosterone with sulphuric acid than similar preparations from male animals (Roy, 1958). Lewis (1968) showed that female rats excrete larger amounts of administered androsterone, epiandrosterone, dehydroepiandrosterone or testosterone as sulphuric acid conjugates than do male rats. It was also shown that liver slices from female rats conjugated the four androgens to a greater extent than males.

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.

De Meio, R. H., Wizerkaniuk, M. & Schreibman, I. (1955). *J. biol. Chem.* **213**, 439.

Lewis, D. A. (1968). *Biochem. J.* **106**, 497.

Rao, L. G. S. & Taylor, W. (1965). *Biochem. J.* **96**, 172.

Roy, A. B. (1956). *Biochem. J.* **63**, 294.

Roy, A. B. (1958). *Biochem. J.* **68**, 519.

Roy, A. B. (1960). *Biochem. J.* **74**, 49.

Weisburger, E. K., Grantham, P. H. & Weisburger, J. H. (1964). *Biochemistry*, **3**, 808.

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

By R. BAXTER\* and D. H. L. BISHOP (introduced by P. W. TRUDGILL) (*Departments of Agronomy and Microbiology, University of Illinois, Urbana, Ill., U.S.A.*)

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 [<sup>32</sup>P]phosphate. Mitochondria and cytoplasmic ribosomes were isolated from the hypocotyls, and the <sup>32</sup>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, <sup>3</sup>H-labelled *Escherichia coli* RNA being used as marker. After electrophoresis the gels were scanned at 266 mμ to obtain extinction profiles, and 1 mm. sections were taken for simultaneous radioactivity counting of <sup>32</sup>P and <sup>3</sup>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

\* Present address: Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth.