the urine but as a precursor (X). The rat, mouse, guinea pig and rhesus monkey did not excrete X or the ketone after being dosed with the drug. In the rabbit, as much as 22% of the dose of amphetamine (10mg./kg.) was excreted as precursor or precursors of the ketone, whereas man excreted only 2–3% and the dog 1%.

The nature of precursor X has been further elucidated. (±)Amphetamine (225mg.) was given to three rabbits over 3 days. The urine was mixed with urine from a rabbit receiving the ^{14}C-labelled drug. Precursor X was separated from the freeze-dried urine by thin-layer chromatography as a pale-yellow solid (20mg.) containing sulphur but practically no nitrogen. On acid hydrolysis it gave benzyl methyl ketone (DNP-hydrazone, m.p. and mixed m.p. 152–153°) and inorganic sulphate. Precursor X was also detected chromatographically in the urine of rabbits receiving [^{14}C]benzyl methyl ketone (1 - phenyl[1 - ^{14}C]propan - 2 - one). When $^{35}SO_4^{2-}$ was given with non-radioactive benzyl methyl ketone, the precursor X located on chromatograms contained ^{35}S. Hydrolysis of precursor X from the urine yielded benzyl methyl ketone (as DNP-hydrazone) and [^{35}S]sulphate (as $Ba^{35}SO_4$).

Gero (1954) reported that benzyl methyl ketone occurs normally in the enol form to the extent of 2·9%. We suggest that precursor X is probably a salt of the sulphate ester of 1-phenylprop-1-en-2-ol, the enol of the ketone, i.e. $C_6H_5 \cdot CH : C(O \cdot SO_3^-) \cdot CH_3$. This structure was supported by nuclear-magnetic-resonance and u.v. spectra.

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The Metabolism of Thiophen and Benzo[*b*]-thiophen

By H. G. Bray and F. M. B. Carpanini (*Department of Physiological Chemistry, University of Birmingham*)

In studies of the metabolic fate of thiophen Heffter (1886), Christomanos (1930) and Chilcote (1945) did not report the identification of any metabolite, although their results suggested that no ethereal sulphate was formed and that the chief metabolite might be a mercapturic acid.

We found that the rabbit excreted about 35% of a dose of thiophen (150mg./kg.) unchanged in expired air and a small amount (about 0·5% of the dose) in faeces. About 40% of the dose was detected in urine as two mercapturic acids, believed to be thienyl 3- and 2-mercapturic acids. No increase in the excretion of glucuronic acid or ethereal sulphate was observed and no evidence of the opening of the ring to form diethyl sulphide, as Christomanos (1930) suggested might occur in the dog, was obtained.

Chilcote (1945) suggested that 2-bromothiophen is metabolized by the rabbit in the same way as is thiophen. In the present investigation, no increase in ethereal sulphate or glucuronic acid was detected and only about 20% of a dose of 2-bromothiophen (120mg./kg.) could be accounted for as mercapturic acid-like compounds in urine. One of these acids is believed to be thienyl 2-mercapturic acid.

Böhm (1941) reported the isolation of a metabolite, believed to be benzothienyl 2-glucuronide, from the urine of rabbits to which benzo[*b*]thiophen had been administered, but we were unable to detect an increase in the excretion of either ethereal sulphates or glucuronic acid by the rabbit dosed with benzo[*b*]thiophen (175mg./kg.). About 80% of the dose appeared in the urine as mercapturic acid-like compounds, four of which could be separated by paper and thin-layer chromatography. One of these has been identified tentatively as benzothienyl 3-mercapturic acid.

A method for the synthesis of thienyl and benzothienyl mercapturic acids from 2-amino-3-chloropropionic acid hydrochloride and the appropriate mercaptothiophen will be described.

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The Metabolism of Cyclohexylamine in Rabbits

By T. H. Elliott, N. Y. Lee-Yoong and Rosaline C. C. Tao (*School of Pharmacy, University of Singapore*)

Bernhard (1937) reported that cyclohexylamine fed to dogs disappears completely. However, in view of the widespread use of sodium cyclamate as a sweetener, and the report by Ohashi (1964) that in the presence of acid and hydrogen peroxide it is hydrolysed to cyclohexylamine, it is of some importance to know the precise metabolic fate of cyclohexylamine.

When orally administered at a dose of 0·2g./kg. to rabbits, cyclohexylamine gives rise to unchanged cyclohexylamine and *N*-hydroxycyclohexylamine in the urine. This has been confirmed by feeding [1-^{14}C]cyclohexylamine to a rabbit (0·17g./kg. or 1·067 μc/kg.), when it was found that 68% of the radioactivity could be recovered from the urine in 60hr.: 13% in the first 10hr., 39% in the next 20hr., and 16% in the last 30hr. The excretion curve showed a small peak at 3hr. and a second larger peak at 32hr. A small amount (0·5%) was eliminated in the breath (0·3% as $^{14}CO_2$ 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.

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

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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 C_{20} or 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.

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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.