of drinking water; for group B isocaloric sucrose replaced ethanol in the drinking water; group C received 30% ethanol in the drinking water and the basic diet was supplemented by 1% choline; for group D isocaloric sucrose replaced ethanol in the drinking water and the basic diet was supplemented by 1% choline; group E received the choline supplemented diet *ad lib.* and water *ad lib.* All animals except those in group E were pair-fed with group A.

After 10–12 weeks biopsy specimens of liver were taken and sections prepared for light-microscopy. Subcellular fractions were prepared by homogenization in 0.25 M-sucrose and differential centrifugation. The 8000g fraction and the microsomal pellet from 100mg of liver were incubated separately in air for 30min in phosphate buffer, pH 7.4, with added [³H]oestradiol and containing 0.3mM-NADPH. The mixture was extracted twice with ethyl acetate and a sample of this extract was taken for counting of radioactivity. Protein was precipitated from the ethyl acetate-extracted aqueous material by addition of trichloroacetic acid and centrifugation. A sample of the supernatant was taken for counting of radioactivity. The protein precipitate was treated by the procedure of Jellinek, Lazier & Copp (1965) and its radioactivity counted. Thus the percentages of ³H in the lipid-soluble, water-soluble and protein-bound fractions were assessed. Livers from groups A and B showed a marked decrease in the formation of water-soluble and protein-bound metabolites, and a corresponding increase in lipid-soluble material. This increase was not due to unmetabolized precursor. Morphologically all animals in groups A and B showed fatty change of liver cells. No change was noted in the groups receiving 1% choline. Some parenchymal fibrosis was noted in both of the choline-deficient groups. The results indicate that the inability to form water-soluble metabolites is related to the degree of fibrosis.

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Metabolism *in vitro* of [4-¹⁴C]4-Androstene-3,17-dione by Livers from Normal and Carbon Tetrachloride-Poisoned Rats

By F. M. MARTIN and M. E. BEARY (introduced by M. G. HARRINGTON). (*Department of Biochemistry, University College, Dublin 2, Irish Republic*)

4-Androstene-3,17-dione is metabolized in mature male rat hepatocytes in two distinct ways: (1) the C-4-C-5 double bond and the 3- and 17-oxo groups are readily reduced; (2) microsomal mixed-function

oxidases catalyse hydroxylation of this steroid (Dorfmann & Ungar, 1965).

Sasame, Castro & Gillette (1968) demonstrated that carbon tetrachloride impairs the hepatic microsomal enzymes that oxidize drugs by destroying cytochrome P-450. It has been suggested that the same enzymes hydroxylate steroids (Conney, 1967). Beary (1967) found a decrease in the activity of the microsomal system that metabolizes [4-¹⁴C]4-androstene-3,17-dione to polar metabolites.

In the present work the effect of carbon tetrachloride on 4-androstene-3,17-dione reduction was investigated.

[4-¹⁴C]4-Androstene-3,17-dione was incubated with the 9000g supernatant fraction of liver homogenates from normal, 6h- and 24h-carbon tetrachloride-poisoned rats. Supernatant from 100mg of liver was incubated for 5min at 37°C in phosphate buffer, pH 7.4, with added NADPH.

In incubations with normal supernatant all the metabolites were polar hydroxylated compounds. When oxidized cytochrome c was added to this system hydroxylation was inhibited by 68±5% and most of the metabolites were reduced forms. These reduced metabolites were separated into four fractions by using t.l.c. The principal components of these fractions were identified by g.l.c. on 3% QF-1 and 3.3% XE-60 columns as epiandrosterone, androsterone, testosterone and aetiocholanolone.

The amount of unmetabolized substrate [expressed as % of radioactivity eluted from the t.l.c. plate ± s.e.m. (no. of animals)] increases on treatment with carbon tetrachloride: normal, 9.6±1.4 (8); 6h-carbon tetrachloride-poisoned, 29.4±3.1 (6); 24h-carbon tetrachloride-poisoned, 54.2±2.6 (6). The residual hydroxylating activity was quickly destroyed by the carbon tetrachloride: normal, 30.8±2.4 (8); 6h-carbon tetrachloride-poisoned, 10.9±1.4 (6); 24h-carbon tetrachloride-poisoned, 6.5±0.9 (8). Microsomal epiandrosterone formation was gradually impaired: normal, 30.2±2.2 (8); 6h-carbon tetrachloride-poisoned, 22.5±3.0 (6); 24h-carbon tetrachloride-poisoned, 6.7±1.2 (8). The fraction containing androsterone was less affected: normal, 14.5±1.6 (8); 6h-carbon tetrachloride-poisoned, 15.4±1.1 (6); 24h-carbon tetrachloride-poisoned, 8.9±0.7 (8). The formation of the soluble metabolite aetiocholanolone increased: normal, 11.9±1.2 (8); 6h-carbon tetrachloride-poisoned, 17.6±1.8 (6); 24h-carbon tetrachloride-poisoned, 16.2±0.5 (8); as did testosterone formation: normal 2.8±0.4 (8); 6h-carbon tetrachloride-poisoned, 3.9±0.3 (6); 24h-carbon tetrachloride-poisoned, 7.1±0.1 (8).

Thus it is shown that carbon tetrachloride affects the major reductive pathways of the microsomal fraction.

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An Intact Muscle Preparation Suitable for Studies of the Metabolic Effects of Electrical Stimulation

By E. B. FERN, V. M. PAIN and K. L. MANCHESTER.*
(Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.)

Studies of the metabolic effects of stimulation on mammalian muscle have usually been carried out on whole hind limbs, either stimulated *in situ* or in perfused preparations. Changes in concentrations of metabolites in the plasma or perfusate from such systems can only indicate the response of a group of muscles, so that information on individual muscles is limited to that obtained by excision and analysis after stimulation. We have designed a system whereby an isolated muscle preparation may be electrically stimulated while allowing the incubation mixture to be readily sampled.

The system is basically that described by Pain & Manchester (1970) with modifications, and was developed for studies of the metabolism of the extensor digitorum longus muscle from young female rats. Each muscle is attached to an electrode and incubated at 37°C in 2 ml of Krebs-Ringer bicarbonate buffer continuously gassed with O₂ + CO₂ (95:5). The other electrode is immersed in the medium. Constant tension is applied to the muscle during the passage of electric stimuli, the voltage, duration and frequency of which may be varied to suit a particular type of muscle. Additions to the medium or withdrawal of samples can easily be carried out throughout the incubation period.

When extensor digitorum longus muscles of 30 mg or less were incubated in this system for 60 min, tissue weights were unaltered, and the concentration of K⁺ in the intracellular fluid was maintained at 88% (resting) and 76% (stimulated) of that in zero-time controls. With larger muscles we observed increases in tissue weight and extracellular fluid and decreases in K⁺ and ATP concentrations, these changes being particularly pronounced in stimulated muscles. Probably the O₂ supply becomes critical in muscles of this size, and it is likely that this would also be the case for

muscles with a higher proportion of aerobic red fibres.

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The Physiological Role of Taurine

By L. K. KACZMAREK and A. N. DAVISON. (*Medical Research Council Membrane Biology Research Group, Department of Biochemistry, Charing Cross Hospital Medical School, London WC2N 4HH, U.K.*)

In a previous communication (Kaczmarek, Agrawal & Davison, 1970) we showed that taurine is present in nerve-ending particles isolated from rat brain, together with its synthesizing enzyme, cysteinesulphinate decarboxylase. In both distribution and properties this enzyme closely resembles glutamate decarboxylase, which is responsible for the synthesis of γ -aminobutyrate, a compound believed to act as a neuroinhibitor (Davison, 1956; Agrawal, Davison & Kaczmarek, 1971). We have now extended the work to a study of the uptake of taurine by cortical tissue, as well as its release into extracellular space on stimulation. Rat brain-cortex slices were first incubated for 30 min in the presence of radioactive taurine. The slices were transferred to cells that were perfused with oxygenated taurine-free Krebs-Ringer medium. After an initial washout of taurine from the extracellular space a slow loss of radioactivity from the tissue was observed. On the application of rectangular electrical pulses to the tissue, the efflux of radioactivity was considerably enhanced, a result that has been found for γ -aminobutyrate by Srinivasan, Neal & Mitchell (1969). If such a process is of physiological significance and taurine is released from nerve endings to exert an inhibitory influence on the postsynaptic membrane, it should be accompanied by a mechanism for the termination of its action. This may be by re-uptake into the tissue, as has been proposed for the cessation of γ -aminobutyrate action (Iversen & Neal, 1968). We have found that slices of rat brain cortex will efficiently take up radioactive taurine at low concentrations (0.5 μ M) from Krebs phosphate medium. This work adds support to the suggestion from iontophoretic evidence (Curtis & Watkins, 1965) that taurine may be considered as a possible neurotransmitter, but much further work will be necessary to verify this hypothesis.

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* Present address: Department of Biochemistry, University of the West Indies, Mona, Kingston 7, Jamaica.