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

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In a previous communication (Martin & Beary, 1971) we described the effect of carbon tetrachloride poisoning on the enzymes associated with the reduction of tracer amounts of [4-¹⁴C]4-androstene-3,17-dione by the 9000g supernatant fraction of male-rat liver homogenates. This communication describes the continuation of this work: the reductive metabolism of [4-¹⁴C]4-androstene-3,17-dione *in vitro* by isolated microsomal fractions and soluble enzyme fractions obtained from livers of normal and carbon tetrachloride-poisoned rats.

Microsomal fractions were obtained by centrifugation at 200 000g for 30 min of the supernatant obtained after centrifugation of the liver homogenate at 9000g for 10 min. The microsomal pellet was resuspended in phosphate buffer, pH 7.4. The 200 000g supernatant constituted the soluble enzyme fraction.

[4-¹⁴C]4-Androstene-3,17-dione was incubated with the microsomal and soluble fractions from normal, 3h-, 6h- and 24h-carbon tetrachloride-poisoned rats. Microsomal fraction (or soluble fraction) obtained from 100mg wet wt. of liver was incubated with 0.1 μ Ci of substrate (specific radioactivity 0.6 mCi/mmol) for 5 min at 37°C in phosphate buffer, pH 7.4, with an NADPH-regenerating system added. Oxidized cytochrome c (final concn. 80 μ M) was added as an inhibitor of microsomal steroid hydroxylases (Orrenius, 1968). The microsomal reduced metabolites were separated into three main fractions by using t.l.c. The principal components of these fractions were tentatively identified by g.l.c. as epiandrosterone, androsterone and testosterone.

Under normal conditions epiandrosterone was the major reduced metabolite, accounting for at least five times the radioactivity of either androsterone or testosterone. After treatment with carbon tetrachloride for 3h the ability to reduce the substrate under our experimental conditions was noticeably decreased. Epiandrosterone formation was decreased to one-third that of the normal amount. Androsterone and testosterone formation was increased over the normal values after 3h- and 6h-carbon tetrachloride poisoning. After 24h poisoning the overall microsomal reducing ability was greatly diminished.

The main soluble metabolite formed was aetiocholanolone. A small amount of testosterone was also formed. At 24h after carbon tetrachloride administration aetiocholanolone was still formed in large amounts.

The relatively highly labile nature of the microsomal epiandrosterone-forming enzyme system and the relative resistance of the soluble reducing enzyme systems to carbon tetrachloride poisoning reflected the results obtained in our homogenate studies (Martin & Beary, 1971).

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Effect of Hypophysectomy and Growth Hormone Replacement on the Composition of Canine Aorta

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An increased incidence of atherosclerosis is found in patients with diabetes mellitus or hypothyroidism, and removal of the hypophysis has been advocated in some cases of proliferative retinitis. These clinical observations prompted us to investigate the effects of lack of hypophysial hormones on the cells, fibres and ground substance of the aortic wall.

Ten young dogs were surgically hypophysectomized and maintained for 3 months post-operatively in our animal colony with eight age-matched controls. Four of the hypophysectomized dogs received bovine growth hormone (0.2 mg/kg body wt. per day) for the 3 weeks immediately before they were killed. Aortae were removed immediately after the animals were killed, divided into thoracic and abdominal segments, dried and defatted. Cellularity of the aorta was assessed by DNA measurement, and collagen and elastin were determined as recommended by Jackson & Cleary (1967). Glycosaminoglycans were solubilized by proteolytic digestion of the tissue, isolated by precipitation with cetylpyridinium chloride and resolved into individual glycosaminoglycans by electrophoresis on cellulose acetate (Breen, Weinstein, Andersen & Veis, 1970). All results are expressed as means $\pm$ S.E.M. per g dry wt. of defatted tissue. The differences quoted are significant at least at the 5% level.

The compositions of control aortae differed in the thoracic and abdominal segments. Elastin, DNA and chondroitin sulphate were significantly higher in the thoracic aorta, and collagen and dermatan

sulphate were significantly lower. In the thoracic aorta hypophysectomy increased the DNA content from 6.0 ± 0.1 to 7.4 ± 0.6 mg/g; growth hormone treatment had no effect. Collagen content was not changed by hypophysectomy, but the elastin content increased from 357 ± 13 to 400 ± 14 mg/g; growth hormone administration returned the elastin content towards normal. Removal of the hypophysis had no effect on the hyaluronic acid content, but all of the sulphated glycosaminoglycans were markedly decreased: heparan sulphate from 2.3 ± 0.1 to 1.6 ± 0.1 μ mol uronic acid/g, dermatan sulphate from 3.4 ± 0.2 to 2.7 ± 0.1 μ mol uronic acid/g and chondroitin sulphate from 6.9 ± 0.6 to 3.7 ± 0.3 μ mol uronic acid/g. Growth hormone treatment returned the concentrations of all three sulphated glycosaminoglycans to control values.

The significance of these findings in relation to vascular diseases will be discussed.

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Comparative Metabolism of Nicotinamide and Nicotinic Acid in Mice

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It is suggested that, in the mouse, metabolism is designed for the utilization of nicotinamide as a precursor of NAD and that the Preiss-Handler pathway (Preiss & Handler, 1958) functions primarily for the purpose of converting nicotinic acid into nicotinamide. It also appears that deamidation of nicotinamide to nicotinic acid is not a prerequisite to either its absorption from the gut or to its utilization as a precursor of NAD.

Adult female C3HeB/FeJ mice were injected intraperitoneally with $[7\text{-}^{14}\text{C}]$ nicotinamide or $[7\text{-}^{14}\text{C}]$ nicotinic acid at a dose of $0.9 \mu\text{mol/animal}$. Mice fed on Purina chow *ad libitum* ingest approximately this same quantity of the vitamin per day. At intervals from 5 to 90 min after injection animals were killed and tissues were analysed for labelled nicotinamide coenzymes and precursors by paper chromatography.

In all the tissues examined more label was found in NAD after $[7\text{-}^{14}\text{C}]$ nicotinamide injection than after $[7\text{-}^{14}\text{C}]$ nicotinic injection. Nicotinic acid was rapidly cleared from all tissues, whereas nicotin-

amide persisted for 90 min. This finding agrees with observations by Chaykin, Dagani, Johnson & Samli (1965) and Chaykin, Dagani, Johnson, Samli & Battaile (1965). These authors found that nicotinamide is ordinarily the only form of the vitamin found in mice. In liver, after $[7\text{-}^{14}\text{C}]$ nicotinic acid injection, label in nicotinic acid rapidly disappeared. At 5 min label appeared primarily in desamido-NAD and NAD. By 10 min the concentrations of these compounds had fallen with a concomitant increase in labelled nicotinamide. By 20 min 90% of the total liver radioactivity was in the form of nicotinamide.

With regard to the metabolism of the vitamin in mice, a further question remained: namely, in what form does the vitamin pass the gut? When nicotinamide was administered, either by stomach tube or by direct injection into the intestine, the only form of the vitamin detectable in blood was nicotinamide. By contrast, 5 min after $[7\text{-}^{14}\text{C}]$ nicotinic acid administration by the same routes, 30% of the radioactivity added to the blood during passage through the intestine was in the form of nicotinamide. Subsequent passage through the liver resulted in the removal of nicotinic acid from the blood and its replacement by nicotinamide.

These findings suggest that metabolism in mice is adapted to the utilization of nicotinamide and that nicotinic acid is converted into nicotinamide for this purpose.

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The Chemical Nature of Bound Nicotinic Acid

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It has been reported that the bound nicotinic acid of cereals is incorporated in a polysaccharide (Kodicek & Wilson, 1960) or in a polypeptide (Das & Guha, 1960). To investigate further the nature of bound nicotinic acid, experiments were carried out in which bound nicotinic acid was extracted in high yield from wheat bran by a mild chemical procedure. When wheat bran was extracted with aq. 50% (v/v) ethanol (after the method of Christianson, Wall, Dimler & Booth, 1968) at 0°C for 3 h, 60% of the total nicotinic acid was brought into solution. On dialysis 90% of this nicotinic acid