simultaneously. Kidneys and brain were macroscopically similar to controls.

N-Nitrosodiphenylamine was rapidly removed from liver and kidneys with diphenylamine as an important degradation product (Table 1). The disappearance rates were rather similar in both tissues.

The injections caused decreased glutathione concns in liver after 4 and 24 hr while an increased concn was seen after 48 hr (Table 2). Glutathione peroxidase activity was increasingly inhibited during the follow-up period together with succinate dehydrogenase activity (Table 2).

Liver microsomal cytochrome P-450 and the related ethoxycoumarin *O*-deethylase activity were decreased after 4 hr and restored to control ranges later on (Table 3). Cytochrome b_5 was below control after 48 hr while cytochrome *c* reductase activity increased transiently above the control range after 24 hr (Table 3). Epoxide hydrolase activity increased time-dependently above the respective control ranges (Table 3).

Cerebral glutathione concn was below the control range after 24 hr (Table 2). Succinate dehydrogenase activity was smaller than in controls after 48 hr while glutathione peroxidase activity was above the control range throughout the study (Table 2).

Discussion

Our analyses for the *N*-nitrosodiphenylamine burden indicate a rapid transformation to diphenylamine. Its metabolism by the cytochrome P-450 complex could explain the decrease in the cytochrome concn as a nitroso complex with the prosthetic haem is formed in the process leading to destruction of the haemochrome [4].

The mitochondrial succinate dehydrogenase activity was analyzed to shed light on the consequences of the postulated uncoupling of oxidative phosphorylation by the nitrosamine. Succinate dehydrogenase is coupled to the respiratory chain [15], and the decrease in its activity might reflect mitochondrial damage. For unknown reasons, this process seems to be aggravated at later follow-up times with low *N*-nitrosodiphenylamine concns.

Glutathione is not known to participate in the primary denitrosation. However, an unidentified secondary product or an event related to the mitochondrial effects could have contributed to the decrease in the nonprotein sulfhydryl groups. In this respect, it is interesting to note that epoxide hydrolase activity increased in the liver microsomes in a later follow-up, a finding associated, for example, with structural changes in the membrane [16].

The decrease in the hepatic glutathione peroxidase activity and the enhanced activity in the brain remain largely unexplained. However, cyanide anion is known to inhibit the enzyme by catalyzing the removal of the prosthetic combine with organic selenide, and, furthermore, the glutathione peroxidase activity decreased in liver after 48 hr with minimal amounts of *N*-nitrosodiphenylamine still present.

In conclusion, rats dosed with a large intraperitoneal dose of *N*-nitrosodiphenylamine showed delayed biochemical effects including early inhibition of hepatic drug-oxidizing activity followed by considerable mitochondrial and microsomal membrane effects 48 hr after the dose with almost no nitrosamine or metabolite detectable any more.

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Androstenedione stimulation of ouabain-sensitive $^{86}Rb^+$ influx into human red blood cells *in vitro* [$(Na^+–K^+)$ATPase *in situ*]

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Most of the steroid hormones tested either do not change or inhibit the *in vitro* influx of $^{86}Rb^+$ into human RBC, which is an index of $(Na^+–K^+)$ATPase activity *in situ* [1]. Only some testosterone preparations display stimulating activity caused [2] by an impurity with an R_f of 0.67 in TLC (the testosterone R_f is 0.48). In the present study the

Table 1. Comparison of GC retention times of the R_f 0.67 zone and androst-4-ene-3,17-dione standard (methylene units)

	Standard androstenedione	R_f 0.67 zone
Underivatised MO	25.17	25.16
syn form	26.28	26.26
anti form	26.32	26.34

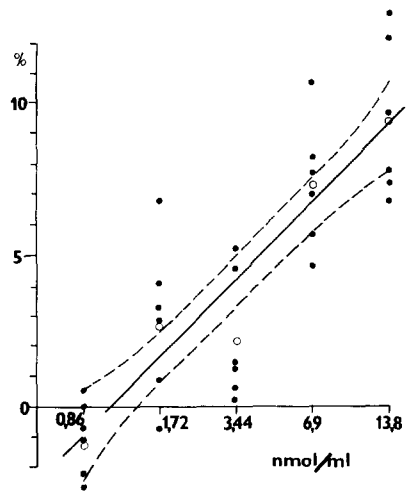

Fig. 1. Androstenedione stimulation of $^{86}Rb^-$ influx into RBC. Individual values (small full dots), means (open dots); computed regression line ($y = 3.6693 \ln x - 10.1875$, $r = 0.8459$, $P < 0.01$); 95% confidence limits (broken lines). x = concn of androst-4-ene-3,17-dione (nmoles/ml) and y = % of stimulation of $^{86}Rb^+$ influx into RBC *in vitro*.

impurity was identified by GC-mass spectrometry (GC-MS) as androst-4-ene-3,17-dione; authentic androstenedione actually stimulates (Na^+–K^+)ATPase activity *in situ*.

23.6 mg commercial testosterone (Merck, batch No. 70152927) was dissolved in methanol, applied to a thin-layer plate (Merck Kieselgel 60 F_{254}, 0.25 mm) and chromatographed in chloroform–ethanol–water (92:8:0.5). The R_f 0.67 zone (detected in u.v. light, 254 nm) was eluted with methanol and the extract was dried. About 0.2 mg of the impurity was used for GC-MS. GC-MS was performed on an OV-1-coated open tubular column (temp programme 2.5°/min from 200 to 280°) connected to a Varian MAT 731 mass spectrometer with a Varian SpectroSystem 200 data system. The accelerating voltage was 8 kV and the ionising voltage 70 eV. Retention times, expressed as methylene unit values were obtained by co-injection with a mixture of *n*-alkanes into a Becker 410 gas chromatograph with column and operating conditions as earlier. The mass spectra of the free and derivatised form match those of the androst-4-ene-3,17-dione standard; the same applies to the GC retention times [methylene units (Table 1)].

Androstenedione puriss. CHR (Koch-Light) was dissolved in methanol and serially diluted samples were prepared with physiological saline at final concns of 0.36–13.6 nmoles/ml incubation mixture. (Na^-–K^-)ATPase activity *in situ* was determined by measuring $^{86}Rb^-$ uptake by human RBC *in vitro* by Lowenstein's method [3, 4]; the results are shown in Fig. 1. Androstenedione produced dose-dependent stimulation of influx, but when influx was inhibited by ouabain (200 nmoles/ml) (up to 35% inhibition), none of the given androstenedione concns stimulated it (data not shown).

The physiological significance of this action of androstenedione is unknown. Androstenedione is present in human blood, however, and $^{86}Rb^-$ influx/stimulating activity was recently observed in the blood of patients with low-renin essential hypertension [5]. This type of hypertension is presumably caused or modulated by an unidentified steroid hormone. It should be added, however, that the concns of androstenedione used in our *in vitro* studies are much higher than those in human blood.

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Enhanced serum binding of propranolol and oxprenolol and microsomal enzyme induction by rifampicin in the dog

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There have recently been a number of reports concerning the increased binding of drugs to α_1-acid glycoprotein (α_1-AGP) when microsomal enzymes are induced by anti-epileptic drugs such as phenobarbital and phenytoin in man [1], the dog [2, 3] and the rat [4].

Rifampicin stimulates drug metabolism in man and in