Comparison of SDHG activity of rabbit cadaver kidneys with and without perfusion after varying periods of warm ischemia

	Non-perfused				Perfused			
					Rheomacrodex		Saline	
Ischemia time (h)	0	2	4	6	0	1	0	1
Mean	0.295	0.240	0.174	0.089	0.201	0.139	0.081	0.038
Number	6	10	6	6	9	6	5	6
Standard deviation (Sd. Dv.)	0.037	0.054	0.035	0.010	0.031	0.010	0.013	0.014
Standard error of Mean (S.E.M.)	0.015	0.017	0.014	0.001	0.010	0.004	0.005	0.006
Relative percentages	100	80	58	27	68	47	27	13

The results are expressed as µl/min/mg. S.D. and S.E.M. are included.

measure of pH by COUCH[9] and DMOCHOWSKI[10]; a method for measuring oxygen tension and consumption in the Spinner flask cell culture, as suggested by COHEN[11]; the oxygen electrode of BAUTISTA[12]; and, the measure of SDHG activity by LANNON[13].

In the present study utilizing rabbit cadaver kidneys at various ischemia times with and without perfusion, SDHG activity was used as an index of viability. Sufficient precision was obtained utilizing small amounts of tissue so as to be practicable and yield quantitative results within a brief period. The present work revealed SDHG activity to decrease with increasing periods of warm ischemia. This is, thus, in accordance with previous reports of a decreased viability and therefore a decreased suitability for transplantation[14–22]. Furthermore, perfusion with Dextran-40 or saline also decreased SDHG activity. The reason for the latter is not known and will be the subject for further studies where the SDHG method seems of value for the testing of optimal perfusion conditions.

Conclusions. 1. Prolonged warm ischemia decreases the SDHG activity of the kidney. 2. Perfusing solutions further decrease the SDHG activity, normal saline more than Rheomacrodex. 3. The measurement of SDHG activity in kidney homogenates appears to be useful in determining cadaver kidney viability.

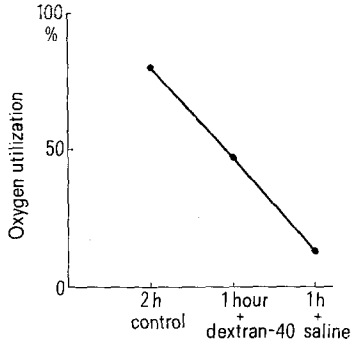

Fig. 3. Succinic dehydrogenase activity of the rabbit kidney after warm ischemia and perfusion with Dextran-40 or saline.

Zusammenfassung. Succinase-Dehydrogenase-Aktivität, als praktischer Index für die Lebensfähigkeit von Nierenrinden-Homogenaten wurde in der Warburgapparatur mit der Mikrowaage gemessen. Verlängerte Wärme-Ischämie vermindert die Fähigkeit der Sauerstoffverwertung und setzt die Lebensfähigkeit herab.

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Lipid Peroxidation in Dietary Liver Necrosis

Dietary liver necrosis in the rat[1,2] constitutes a suitable experimental model for the study of the pathogenesis of non-toxic cellular necrosis in vivo. The condition is produced about 28 to 30 days after feeding the rat a diet deficient in vitamin E and selenium. Supplementation of the diet with either one or both factors completely prevents the onset of necrosis. A prenecrotic period, lasting about 3 weeks, preceeds the development of

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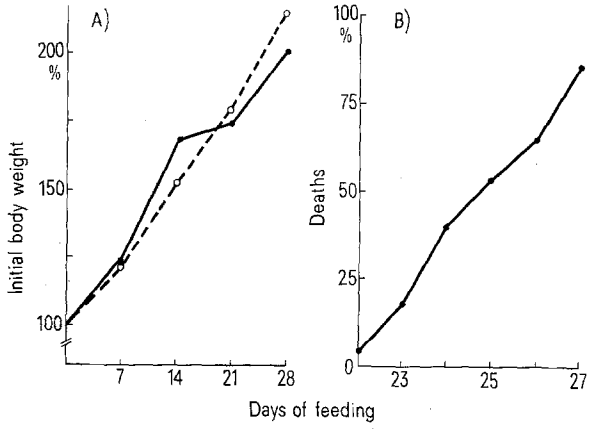

Fig. 1. A) growth curves: ●—●, necrogenic diet; o---o, supplemented diet. Each point is the mean value of 5 rats. B) Percentage of deaths among 40 rats fed the necrogenic diet.

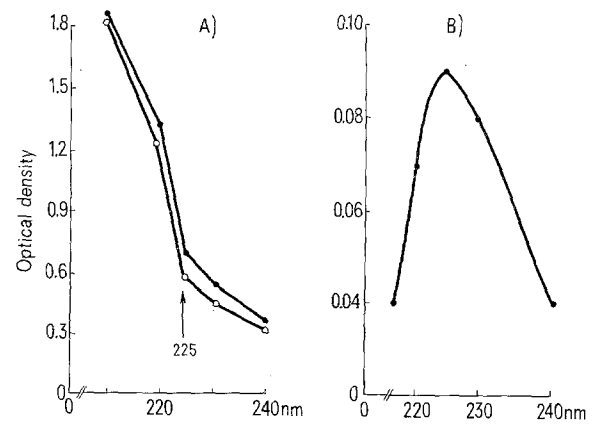

Fig. 2. A) UV-spectra for microsomal lipids at 21 days: ●—●, necrogenic diet; ○—○, supplemented diet. Each point is the mean value of 5 readings. B) Difference spectrum.

cellular necrosis. The slow progression of the disesae allows a sequential study of the earlier alterations and the subsequent evolution.

Several hypotheses have been advanced in order to explain the pathogenic mechanism of this cellular necrosis. It has been suggested[3,4] that lipid peroxidation of biomembranes is the original cause of cellular injury. This is perhaps one of the most attractive but controversial hypotheses. Considering that the role of lipid peroxidation in dietary liver necrosis is still a matter of debate, we decided to investigate this question in the prenecrotic period.

Material and methods. Diene conjugation absorption was used as a direct test for the presence of lipid peroxides produced in vivo[5]. The method consists in measuring the optical density of samples containing the lipids extracted from subcellular membranes. When lipid peroxidation has occurred, the absorption spectrum between 220 and 280 nm shows a peculiar difference from that of control samples. A distinctive feature of the difference spectrum is the absorption peak at 230–235 nm, which could be used for quantitative estimate[6].

Weanling male Wistar rats, were fed a selenium-vitamin E deficient diet similar to that formulated by SCHWARZ[7]. Members of the control groups received the basal diet supplemented with DL-α-tocopherol acetate and sodium selenite, at concentrations of 3 mg (1 mg = 1 IU) and 0.36 mg per 100 g of diet, respectively. Food was offered on individual paired feeding basis in order to reduce differences in food intake. Growth and survival time were used as parameters to test the efficiency of the necrogenic diet (Figure 1).

Animals from both groups were killed at 7, 14 and 21 days. The livers were pooled and submitted to cell fractionation by differential centrifugation. Mitochondrial and microsomal fractions were isolated by the conventional methods. Plasma membrane fractions were obtained by the method proposed by EMMELOT et al.[8]. The whole procedure was carried out at 4 °C and EDTA (final concentration: 0.003 *M*), was added to all media in order to prevent oxidation of lipids during the extraction procedures. Total lipids from each fraction were extracted in Folch under N_2 atmsophere and diluted in methanol to a final concentration of 1 mg/ml. Optical density readings were taken between 220 and 280 nm. The results were expressed as the difference spectra between values from deficient and supplemented rats. 6 experiments were carried out with this method.

Results. Total lipids extracted from the subcellular fractions did not show quantitative differences between the 2 groups. At 7 days, no difference existed between readings in the lipids of the mitochondrial and microsomal fractions. At 14 and 21 days, the difference spectra of mitochondrial and microsomal lipids peaked at wavelengths other than that of diene conjugation. In only 2 experiments, the peaks of the microsomal fractions were near 235 nm. Further experiments consistently showed atypical curves (Figure 2).

At 7 and 14 days, there was no spectrum difference in the samples containing the plasma membrane lipids. At 21 days, the difference spectrum between lipids of the plasma membrane fraction presented a typical curve of diene conjugation with a peak absorption at 235 nm (Figure 3). The extent of lipid peroxidation measured in

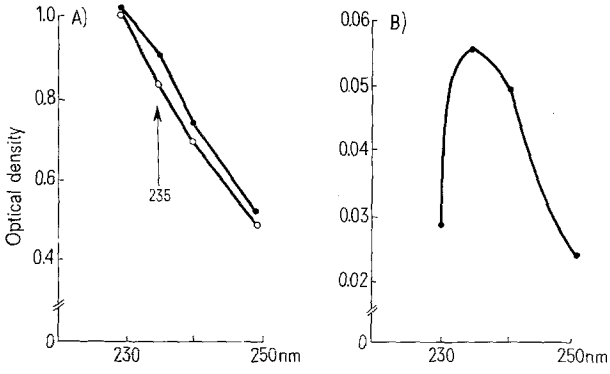

Fig. 3. A) UV-spectra for plasma membrane lipids at 21 days: ●—●, necrogenic diet; ○—○, supplemented diet. Each point is the mean value of 5 readings. B) Difference spectrum.

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this fraction showed a low mean delta value: E 1cm/1% = 0.55.

Discussion. The concept of a purely biological antioxidant activity of vitamin E, and an associated function of selenoamino acids as free radicals scavengers and peroxide descomposers, has suggested lipid peroxidation in vivo as the original alteration in the pathogenesis of dietary liver necrosis[3,4]. Cellular membranes would be damaged, since they are largely composed by polyunsaturated fatty acids.

A large part of the argument in support of the lipid peroxidation hypothesis derives either from experiments in vitro on the properties of antioxidants, or comparative studies with other experimental models, such as radiation damage and ageing processes. A protective action of antioxidants compounds like DPPD, replacing the vitamin E in the diet, has been advocated as direct evidence for the lipid peroxidation mechanism[9]. However, studies conducted by other authors failed to confirm the alternative action of vitamin E and structurally different antioxidants as related to prevention of lipid peroxidation[10,11]. It is interesting to note that in other non-toxic cellular necrosis, like renal necrosis in choline deficient rats, in which lipid peroxidation in vivo has been demonstrated, DPPD leads to a decrease of the renal lesions while vitamin E fails to exert a similar protective action[12]. Critical examinations of the lipid peroxidation hypothesis have concluded that the peroxide content in rat liver is not altered by the addition of vitamin E to the diet[11].

The significance of peroxides detected by the widely used reaction of the thiobarbituric acid (TBA) with malonaldehydes, has been seriously objected to as evidence of the existence of lipid peroxidation in living tissues. It is presently believed that malonaldehyde is metabolized in vivo through mitochondrial pathways, and therefore the TBA reaction would depend on peroxides formed in vitro during the procedure[6].

The results reported here, obtained by the method of detection of diene conjugates, indicate that there is no evidence of lipid peroxidation during the different stages of the prenecrotic period, except for the plasma membrane fraction at 21 days. Since it has been previously demonstrated[13] that the plasma membrane of liver cells presents enzymatic alterations at 14 days, this positive result must be considered an expression of a late alteration, unrelated to the causal mechanism of induction of the cellular injury. Furthermore, the existence of microscopic necrotic changes in some of the livers in the prenecrotic period cannot be excluded. Slight contamination of the microsomal fractions with plasma membrane might account for the atypical curves observed in some experiments.

The present results stress the need for alternative explanations. Mild lipoperoxidation damage comes too late in the sequence of events leading to cellular necrosis to account for its pathogenesis.

Zusammenfassung. Es wird festgestellt, dass der Einfluss von Lipidperoxyden nicht für die Entstehung gewisser Formen der Lebernekrose verantwortlich gemacht werden können.

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Identification of Two New Metabolites of Caffeine in the Rat Urine

During our recent studies[1] on the metabolism of caffeine-^{3}H in the rat, we reported the isolation of the following metabolites from the chloroform-methanol (9:1) extract[2] of the urine: theophylline (1.2%), theobromine (5.1%), paraxanthine (8.8%) and trace amounts of 1, 3, 7-trimethyluric acid and 3-methyluric acid. In addition, two unidentified metabolites, A (11.4%) and B (1.3%), were isolated. The present communication deals with the structure elucidation of these 2 new metabolites of caffeine.

The thin-layer chromatographic (TLC) and spectral (IR, UV and mass) characteristics of the isolated metabolites A and B were found to be markedly different from those of the known mono-, di-, and trimethyl derivatives of xanthine and uric acid[1,3]. The major metabolite A appeared to be a polar compound. It readily dehydrated to caffeine under TLC and gas chromatographymass spectrometric (GC column: 1%-OV-17, temperature 190 °C) conditions and as such it is difficult to isolate this metabolite in pure form. We have assigned structure I (1, 3, 7-trimethyldihydrouric acid) to the metabolite A, primarily on the basis of the mass spectra of the metabolite [peaks at *m/e:* 212 (M^+), 194 ($M-H_2O$), 184 (M-CO, *m** 159.5), 169 (184-CH_3, *m** 155), 142 (169-HCN, *m** 119.5) and 109 (194-CH_3NCO and CO)] and its ditrimethylsilyl derivative II [peaks at *m/e:* 356 (M^+) and 341 ($M-CH_3$)]. Proton nuclear magnetic resonance analysis ($CDCl_3$ solvent) indicated that in solution, metabolite A appears to be in equilibrium with its open-chain, *N*-formyl analog III. About 25% caffeine (IV) was also found to be present in the solution (Scheme I). Oxidation at the 8 position of the purine ring to yield 8-hydroxy derivatives has been previously observed in the rat with guanine-3-oxide[4] and purine[5] itself.

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