

**Table 1.** Female rats were injected subcutaneously at 0 and 1.5 h with 100 mg ethionine. Control animals were given corresponding injections of 0.9 % NaCl solution. At 2 h the rats were given an intraperitoneal injection of a tracer dose of either methyl-<sup>14</sup>C-methionine or hydroxyethyl-<sup>14</sup>C-choline. At 6 h the rats were killed by decapitation. The specific activity of choline in the choline-containing phospholipids in the liver and plasma was determined after extraction with chloroform-methanol (2:1) and subsequent hydrolysis of the phospholipids. The specific activities given as counts/min/mg choline represents the average of 5 animals.

Precursor	Source of choline isolated	Specific radioactivity of phospholipid choline isolated	
		Control	Ethionine-treated
<sup>14</sup> C-choline	Liver phospholipid	19 300	15 000
	Plasma-phospholipid	14 670	7 570
<sup>14</sup> CH <sub>3</sub> -methionine	Liver phospholipid	33 500	17 600
	Plasma phospholipid	25 140	8 410

is the same with both precursors, although reduced as compared to this relation in the controls. This shows a delayed equilibration of the liver and plasma lecithins in the ethionine treated rats.

The plasmaphospholipid-choline content of the ethionine treated rats was reduced to approximately 50 % of the controls, while the liver phospholipid-choline content was unchanged (not shown). This shows an inhibited excretion of lecithin from the liver in the ethionine treated rats.

Altogether these results show that the anti-lipotropic action of ethionine mainly must be due to interference with the synthesis of the protein part of the plasmalipoproteins in the liver. This conclusion is in agreement with the fact that choline has no effect on the fatty liver in ethionine treated rats.

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## Free Radicals in the Reaction of Alloxan with Glutathione and Ascorbic Acid

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It has been postulated<sup>1</sup> that the diabetogenic action of alloxan is produced by an inactivation of essential sulphhydryl enzymes. This theory is based on the observation that the prior injection of large doses of either glutathione or cysteine protected animals from the action of alloxan. Further, *in vitro* experiments have shown that alloxan reacts with sulphhydryl compounds<sup>2</sup>. These reactions are considered to involve a reduction of alloxan to dialuric acid.

With the technique of electron spin resonance (ESR) it has been found that free radicals are formed from alloxan in reactions with sulphhydryl compounds and ascorbic acid.

*Experimental.* The ESR spectra were obtained by a Varian 100 kc. spectrometer. All experiments were performed with aqueous solutions at ambient room temperature.

*Results.* (1) Free radicals were obtained when alloxan (0.01 M) was dissolved in phosphate

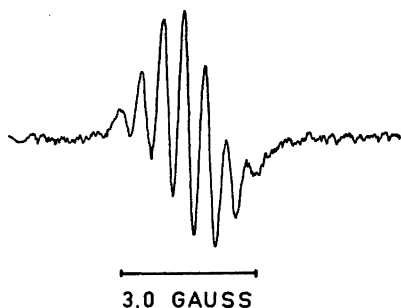


Fig. 1. ESR spectrum of free radicals derived from alloxan (0.01 M). Glutathione (0.0075 M). Phosphate buffer pH 7.4.

or barbiturate buffers together with either glutathione, cysteine hydrochloride or ascorbic acid. No radicals could be detected in buffer solutions containing only alloxan (0.01 M). (2) The maximum yield of radicals was obtained at pH 7.6. Radicals could be detected in the pH range between 4.6 and 8.6. (3) The radical concentration was found to increase with increasing amounts of glutathione. The maximum yield was obtained with a mole ratio of glutathione to alloxan equal to 0.75. (4) The radical concentration decreased slowly with time. The decay was more rapid in an alkaline medium than in an acid one (50 % decomposition at pH 8.4: 6 min, at pH 6.9: 18 min). (5) The ESR spectra of the radicals exhibited a hyperfine structure consisting of seven equally spaced lines with a splitting of about 0.4 gauss. (Fig. 1). The extreme width of the spectra was 3.0 gauss. The intensity ratio was rather close to 1:4:8:10:8:4:1. Identical spectra were obtained in the reaction of alloxan with either glutathione, cysteine hydrochloride or ascorbic acid. (6) When dialuric acid (0.02 M, pH 6.2) was oxidized by an equimolar amount of potassium ferricyanide free radicals were formed which exhibited a spectrum identical with that obtained from alloxan.

These results indicate that an intermediate free radical is formed in the redox-reactions of the system dialuric acid-alloxan. The hyperfine structure is tentatively ascribed to the interaction of the odd electron with two nitrogen nuclei and two protons assuming equal

coupling constants of the nitrogen nuclei and the protons.

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## Intestinal Uptake of Micellar Solutions of Fatty Acids and Monoglycerides

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Mixed micelles of taurodeoxycholate, mono-olein and oleic acid in the concentration of 2.4, 0.3 and 0.6 m equiv/l, respectively, at pH 6.3 have been incubated with intestinal slices from rat and hamster. The cellular uptake and incorporation into higher glycerides of  $^3\text{H}$ -1-mono-olein and/or  $^{14}\text{C}$ -oleic acid have been determined by the examination of the activity present in the slices following incubation.

In the case of the  $^{14}\text{C}$ -oleic acid or  $^3\text{H}$ -mono-olein, approximately 60 % of the initial activity was taken up by the intestinal slice after 1 h incubation. Enzymatic hydrolysis of the 1-mono-olein was observed. In experiments in which both  $^{14}\text{C}$ -oleic acid and  $^3\text{H}$ -1-mono-olein were included in the micellar solution a parallel uptake of the two isotopes was found. The predominate glyceride containing activity isolated from the intestinal wall following the incubation with either labeled oleic acid or mono-olein was found to be triglyceride. The uptake by the intestinal slice would appear to be enzymatically independent, since slices incubated at 37° and 0°C showed a similar uptake as well as those which had been heat denaturated prior to incubation. These two latter preparations, however, did not show an appreciable acylation. The relationship of the above findings to the overall absorptive process of fats by the intestine will be discussed.