action of hydrolytic enzymes and the rest by some unknown non-enzymic components capable of causing some lysing.

Certain levels of inhibition by 3 metal coordination compounds and the antioxidant (Table IV), although of not very large magnitude under the present experimental conditions, suggests that metal-catalyzed lipid peroxidation could also be a possible reaction leading to hemolysis. Tsen and Collier(5) showed that lipid peroxidation can be a dominant reaction leading to hemolysis of erythrocytes. Tissue fractions high in iron give increased catalysis of lipid peroxidation(6). Liver lysosomes are known to contain relatively large amounts of ferritin. Analysis of some of our preparations of lysosomes showed that they contained 2 mg iron, .1 mg copper and .03 mg manganese per gram protein.

The evidence obtained here also suggests that other hemolytic agents of lipoprotein nature contribute to the hemolytic activity of the lyososomes. Charge interactions or binding between the reactive groups on the membranes of both lysosomes and the erythrocytes may play an important role in the lysing process. Recently, a similar hemolytic factor has been extracted from necrotic portions of the hamster tumor(7) and had characteristics similar to those observed in the present study. It is apparent that, under the experimental conditions described, the factors contributing to hemolysis can possibly be ascribed to the following: lysosomal hydrolytic enzymes, lysosomal lipoprotein membrane, and lysosome heavy metals catalyzing lipid peroxidation. Further investigation into this complex of reactions requires further knowledge of the chemical composition and the structure of lysosomes.

Summary. Study has been made of the hemolytic activity of the isolated rat liver lysosomes. This activity, specific to lysosomes, was in proportion to their concentration in the reaction system. Under the experimental conditions described the hemolysis could be related to: lysosomal hydrolytic enzymes, the lysosome lipoprotein membrane and other factors such as lysosome heavy metals which may catalyze lipid peroxidation.

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Effects of 3,5-Dimethylisoxazole (U-21221) on Fat Metabolism. (29888)

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It was previously demonstrated that 3,5-dimethylisoxazole produced a marked effect on glucose metabolism(1). Since insulin is capable of depressing plasma free fatty acid (2) and since FFA may inhibit glucose utilization(3,4), it was of interest to study the effects of U-21221 on FFA. This paper describes the action of U-21221 on FFA metabolism and the possible relationship of this effect to observed changes in glucose metabolism.

Methods. Plasma FFA and blood sugar. Intact rats weighing 140-150 g were used following overnight fast. Animals used in evisceration studies weighed 180-200 g and were fasted overnight. Eviscerations were done as previously described, using spinal transectomized rats(1). In some studies on intact

rats, animals were anesthetized (cyclopal*) 10 minutes before bleeding; while in other experiments animals were under cyclopal anesthesia during the entire 2 hours of experiment. Compound was administered orally to intact rats in .5 ml of CMC Vehicle(5) after subcutaneous injection of 100 mg glucose. U-21221 was given subcutaneously to eviscerated rats which were infused with i.v. glucose at 40 mg/100 g/hr in .9% saline. FFA were determined by method of Dole(2) and blood sugars on AutoAnalyzer on blood taken from posterior vena cava.

Oxidation of palmitate-1-C^{14}. Male rats weighing 165 g were used following overnight fast. Palmitate-1-C^{14} (1.5 μC) was injected intraperitoneally 30 minutes after oral treatment with U-21221. Animals were placed in glass metabolic units and expired CO_2 collected and counted as previously described (6).

Specific activity of FFA. Male rats weighing 175-180 g were used following overnight fast. Palmitate-1-C^{14} (1.0 μC) was injected intraperitoneally immediately after treatment orally with U-21221. Animals were bled 2 hours later from the posterior vena cava while under cyclopal anesthesia. FFA were determined(2) and the extracted FFA were counted by liquid scintillation. Specific activity (SA) is expressed as CPM/μE FFA.

Results. 3,5-Dimethylisoxazole depressed blood sugar and plasma free fatty acids in intact rats which were under barbiturate anesthesia for the final 10 minutes of the experiment. In animals under barbiturate anesthesia during the entire 2 hours of study, U-21221 had no effect on blood sugar but depressed plasma FFA levels. No change in blood sugar but depression of plasma FFA concentration was observed in spinal transected eviscerated rats injected with U-21221 (Table I). Oxidation of palmitate-1-C^{14} to $C^{14}O_2$ by intact rats was not significantly altered by treatment with U-21221 (Fig. 1). Specific activity (CPM/μE) of FFA was increased in animals treated with the isoxazole (Table II). Time response curves of blood sugar and FFA show that concentration of plasma FFA was depressed by 30 minutes while blood sugar level was not lowered until 2 hours (Fig. 2).

TABLE I. Effect of a Barbiturate (Cyclopal) or Evisceration on Blood Sugar and Free Fatty Acids Response of Rats to U-21221.

Treatment	Rats	Dose (mg/kg)	Blood sugar (mg%)		FFA (μE/l)
		Without cyclopal			
Control	5	—	66		725
U-21221	6	.2	49†		516*
U-21221	6	1.0	41†		270†
		With cyclopal			
Control	5	—	69		815
U-21221	5	.2	79		506*
U-21221	6	1.0	69		335†
		Eviscerated			
			0 hr	2 hr	
Control	12	—	73	136	590
U-21221	12	25	74	126	272†

* P = <.05. † P = <.001.

Discussion. It was demonstrated previously that U-21221 depressed blood sugar of intact and alloxan diabetic rats and increased glucose oxidation by intact rats(1), thus exhibiting several activities similar to those pro-

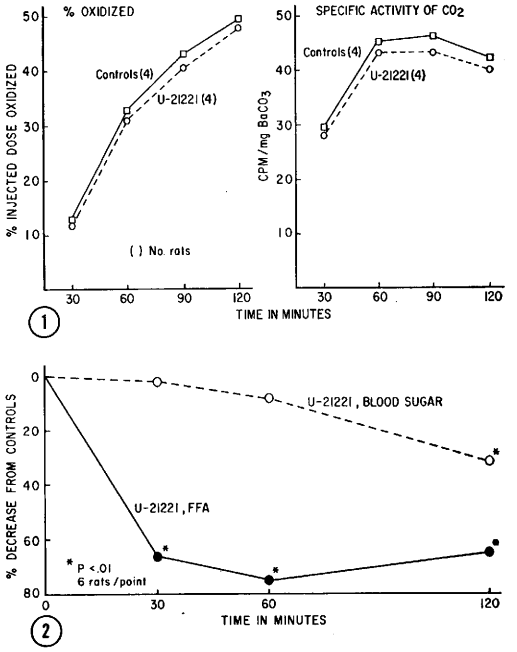

FIG. 1. U-21221 (1 mg/kg) on oxidation of palmitate-1-C^{14} by fasted intact rats.

FIG. 2. Effect of U-21221 on the concentration of glucose and free fatty acids in the blood of intact rats.

* 5-(1-Cyclopenten-2-yl)-5-allylbarbituric acid sodium.

TABLE II. U-21221 on Specific Activity of Plasma Free Fatty Acids.

Treatment	No.	Dose (mg/kg)	Blood sugar (mg%)	FFA	
				μE/l*	CPM/μE†
Controls	5	—	82	952	.70
U-21221	5	.5	76	240‡	1.80‡

* Microequivalents/liter.
† Counts per minute/microequivalent.
‡ $P < .01$.

duced by insulin. Since insulin has an effect on FFA(2), it was of interest to study effects of U-21221 on FFA. Data reported here show that the isoxazole also depresses FFA. Observation showing that U-21221 does not alter oxidation of palmitate-1-C^{14} and increased specific activity of circulating FFA after injection of labeled palmitate suggests that the mechanism of U-21221 depression of FFA was due to decreased release from the depots.

The finding that FFA were depressed prior to change in blood sugar suggests that blood sugar change might be due to a compensatory increased glucose utilization due to reduction of FFA as an energy source or removal of the inhibitory effect of FFA on glucose utilization(3,4). However, this is probably an over simplification of the mechanism of action of U-21221 on blood sugar since FFA were depressed in barbiturate-treated intact rats following treatment with isoxazole without a decrease in blood sugar. These results suggest that there is either no relationship of the FFA and blood sugar effects of U-21221 or that barbiturates and evisceration are able to specifically block the action of U-21221 on blood sugar.

Summary. 3,5-Dimethylisoxazole was shown to decrease FFA, increase specific activity of plasma FFA after injection of labeled palmitate but to have no effect on palmitate oxidation. These observations were interpreted to mean that U-21221 lowered FFA as a result of decreased release from depots. Depression of FFA occurred prior to the fall in blood sugar in intact rats treated with U-21221. The isoxazole caused a depression of FFA in barbiturate-treated intact rats and in eviscerated rats without producing depression of blood sugar. These results were interpreted to mean that either the effects of U-21221 on FFA and blood sugar were independent of each other or that barbiturates and evisceration can specifically block action of U-21221 on blood sugar.

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Action of Aldosterone and Ouabain on O_2 Consumption and Short-Circuit Current of Toad Bladder.* (29889)

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It has been established that aldosterone is capable of stimulating active sodium (Na^+) transport across the isolated urinary bladder of the toad(1-4). The exact mechanism

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