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Effect of CCl_4, Dimethylnitrosamine, and Thioacetamide on Hepatic DPNH and TPNH Cytochrome C Reductase.* (30660)

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Oral administration of carbon tetrachloride (CCl_4) to rats results in an early alteration in the morphology of the endoplasmic reticulum of the liver, a profound decrease in protein synthesis, and an inactivation of glucose 6-phosphatase and aminopyrine demethylating enzyme(1,2,3,10). In contrast the activity of diphosphopyridine nucleotide (DPNH) cytochrome C reductase is increased in CCl_4 poisoning. The following experiments were undertaken to see if a similar increase in activity occurs in poisoning with dimethylnitrosamine (DMNA) and thioacetamide (TIA), both of which have similar effects on liver protein synthesis compared to CCl_4, and to analyze for changes in the triphosphopyridine nucleotide (TPNH) cytochrome C reductase activity(4,5). The experiments were so designed that dosage levels of DMNA and TIA resulted in a depression of amino acid incorporation of similar magnitude to the dose of CCl_4 employed(5).

Materials and methods. Thirty-six male Sprague-Dawley rats weighing 100-250 g were fasted overnight prior to use. CCl_4 was dissolved in an equal amount of mineral oil, and DMNA or TIA in water. The poisons were administered without anesthesia by stomach tube in doses chosen on the basis of preliminary experiments to be equitoxic, producing a similar pattern of histological change and a depression of protein synthesis of about 50% as measured by *in vivo* amino acid incorporation into liver protein. Dosages employed were as follows: 0.25 ml CCl_4/100 g body weight in an equal volume of mineral oil; 20 mg TIA/100 g body weight; 5 mg DMNA/100 g body weight. Controls received mineral oil or water alone. Two hours following administration of CCl_4 or DMNA and 6 hours following administration of TIA the animals were sacrificed by a sharp blow to the head, the body cavity quickly opened, and the liver perfused *via* the aorta with ice-cold 0.85% saline until grossly free of blood. The livers were then isolated, weighed, and minced in 3 volumes of ice-cold 0.15 M sucrose, 0.005 M $MgCl_2$, 0.0025 M KCl, 0.005 M mercaptoethanol, 0.1 M Tris pH 7.3. The minced livers were transferred to homogenizers fitted with teflon pestles, and disintegrated with 8 strokes while the tubes were immersed in an ice bath. The crude homogenates were centrifuged at 0-4°C for 10 minutes at 12,000 *g* in a Servall SS1 centrifuge. The resulting supernatant fluid

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TABLE I. Cytochrome C Reductase Specific Activity.*

	Mean† ± std dev	% of control‡
Control	27.8 ± 4.9	100
CCl_4	40.5 ± 2.9	146
DMNA	25.0 ± 2.0	100
TIA	36.5 ± 2.9	128

* μM of cytochrome C reductase/min/mg protein.
† Mean of 3 or more experiments, each experiment consisting of materials isolated from the pool of 2 rat livers.
‡ Values for DMNA and control were not statistically different.

was transferred to cellulose acetate tubes and recentrifuged in a #40 rotor of a Spinco Model L preparative ultracentrifuge for 60 minutes at 105,000 *g*. The pellets were washed 3 times with the same suspending medium and resuspended to give a final protein concentration of approximately 20 mg/ml, as determined by the method of Lowry *et al*(9).

Cytochrome C reductase activity was measured spectrophotometrically by the following method. To 4 ml silica cuvettes containing 0.3 ml of 1% aqueous cytochrome C (Sigma type III), 0.3 ml 0.1% DPNH or TPNH (Sigma), 0.2 ml 0.1 M sodium azide, 1.2 ml potassium phosphate buffer (pH 7.4) and 1.3 ml distilled water were added aliquots of microsomal suspensions ranging from 5 to 25 μl, depending upon the activity of the suspension. The contents were quickly mixed by inversion, and the change of absorbance of cytochrome C was followed at 550 mμ in a Perkin-Elmer Model 350 recording spectrophotometer against a blank consisting of 0.3 ml of 1.0% cytochrome C and 3.0 ml water. The initial reaction velocity (arbitrarily taken as the change in absorbance during the initial 60 seconds) was taken directly from the instrument record. The μM of cytochrome C reduced per unit time was calculated from the initial reaction velocity using the value $E_{550} = 18.5 \times 10^3$ for reduced minus oxidized cytochrome C(6). The results are expressed as specific activity, numerically equal to μM cytochrome C reduced/minute/mg protein.

Results. Table I shows a comparison of the effects of CCl_4, TIA, and DMNA on microsomal DPNH cytochrome C reductase activity. No difference in TPNH reduction was found in preparation of livers from CCl_4, DMNA, or TIA poisoned animals.

Discussion. CCl_4, DMNA, and TIA have in common an early and profound effect on the protein synthetic capacity of rat liver. The first two substances are associated with an alteration of the morphology of the rough endoplasmic reticulum within the first 2 hours of intoxication, whereas TIA poisoning produces such changes only later (6-8 hours) in the course of the poisoning.

At these times (2 hours in CCl_4 and DMNA intoxication, and 6 hours in TIA intoxication) there is a detectable decrease of similar magnitude in amino acid incorporation into microsome protein *in vivo* and *in vitro* using cell fractions derived from the endoplasmic reticulum(3,4,5,7,8). Concomitantly there is a decrease in the glucose 6-phosphatase and aminopyrine demethylating enzyme activity(1). The only known enzyme with increased activity is DPNH cytochrome C reductase, which Lombardi and Recknagel found to have an activity 1.3 × greater in CCl_4 poisoned animals than in controls(1). Our results confirm their finding, and indicate that TIA has a lesser activating influence and DMNA has none at times during which protein synthesis is depressed to a similar degree. Furthermore, none of the poisons tested had any effect on TPNH cytochrome C reductase with the dosage range employed.

The mechanism by which this increased activity occurs in CCl_4 poisoning is not clear. Direct attack upon the membranes seems unlikely since addition of CCl_4 to homogenates did not increase enzymic activity and since solubilization of the enzymic activity by deoxycholate treatment of the microsomes revealed a similar ratio of activities in livers from treated and control animals.

The alteration in protein synthesis and reductase activities do not appear to be linked, and suggest separate, and possibly independent, effects of these poisons. Furthermore, the difference between CCl_4 and TIA poisoning on DPNH and TPNH cytochrome C reductase activities further points out that these enzymes may have quite different roles in cellular homeostasis.

Summary. The effects of CCl_4, DMNA, and TIA poisoning of liver DPNH and TPNH cytochrome C reductase were measured. CCl_4 and to a lesser extent TIA increased these enzyme activities, whereas DMNA had no effect, even though protein synthesis had been markedly depressed by all these poisons.

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Influence of *Plasmodium berghei* Infection on Susceptibility to Salmonella Infection.* (30661)

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Previous studies(1,2) have shown that intramuscular injection of phenylhydrazine hydrochloride or intravenous administration of anti-mouse-erythrocyte serum, heterologous erythrocytes or antibody-coated homologous erythrocytes markedly increases the susceptibility of mice to infection caused by *Salmonella typhimurium, Escherichia coli* or *Staphylococcus aureus*. Other studies(3) have demonstrated that mice of the NZB/Bl strain with naturally occurring autoimmune hemolytic anemia are more susceptible to infection with *S. typhimurium* than NZB/Bl mice without autoimmune hemolysis. The hypothesis has been advanced that phagocytosis of heterologous or altered homologous erythrocytes by reticuloendothelial cells impairs the capacity of these cells to kill ingested bacteria.

Malaria is another condition associated with hemolysis. Furthermore salmonella bacteremia has been described as a complication of malaria in man, and there is evidence to suggest a reduction in resistance to salmonellosis in human malaria(4). The present studies were undertaken to investigate the effect of *Plasmodium berghei* infection on susceptibility of mice to salmonella infection.

Methods. Male Swiss mice (CF1 strain, Carworth Farms, New City, N. Y.) weighing 20 to 24 g were used in all experiments. Hematocrit values were determined by a micro-hematocrit method(1) in heparinized capillary tubes, 75 mm in length and 1.2 to 1.4 mm in diameter. Approximately 0.03 ml of blood was collected from the retroorbital venous plexus and centrifuged for 3 minutes at 12,500 rpm in a microhematocrit centrifuge (Clay-Adams, Inc.). Hematocrit values were read directly from the tubes using a micro-capillary reader (International Equipment Co.).

The *Salmonella typhimurium* strain KK(1) was used in all experiments. Stock cultures were maintained by storing aliquots of an 18-hour culture in trypticase soy broth at —20°C. Inocula for infecting mice were prepared by subculturing an aliquot of the stock culture in trypticase soy broth. After incubation at 37°C for 18 to 22 hours, the bacteria were washed 3 times in pyrogen-free saline solution and then diluted in saline for injection. The dilutions of bacterial suspensions were inoculated intravenously in a volume of 0.1 ml.

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