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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The NYU-2 strain of *Plasmodium berghei* was generously supplied by Dr. Meir Yoeli and Dr. Harry Most and was maintained by weekly transfer intraperitoneally in CF1 mice (5). To initiate infection erythrocytes from the hearts of parasitized mice were washed and diluted in saline and injected intravenously in a volume of 0.1 ml. The number of parasitized erythrocytes injected was calculated by performing erythrocyte counts in a bright line hemocytometer and determining the per cent of parasitized erythrocytes on a smear stained with Giemsa stain.

For purposes of bacterial enumeration, the spleen was removed with sterile instruments and homogenized in a teflon tissue grinder (Scientific Glass Apparatus Co., Inc.) with sufficient saline to make a 10% suspension. Serial 100-fold dilutions were made in distilled water and 1 and 0.1 ml aliquots of the dilutions were plated in trypticase soy agar pour plates. The number of viable bacteria per gram of spleen was calculated from colony counts after incubation of the plates for 24 hours at 37°C. One colony from the highest dilution of each spleen was identified as *S. typhimurium*.

Results and discussion. Intravenous injection of 2×10^6 erythrocytes parasitized by *P. berghei* produced infection in all mice as demonstrated by subsequent Giemsa stained smears of circulating erythrocytes. There was 50% mortality in 8-10 days and 100% mortality within 19 days. Death was unusual until the hematocrit fell to levels below 25%.

Intravenous inoculation of 3×10^5 *S. typhimurium* KK produced death in 52% of mice in 14 days; 3×10^6 *S. typhimumium* KK killed 73% in 14 days; and 3×10^7 was lethal for 100% in 9 days.

Concurrent infection produced with these inocula of *P. berghei* and *S. typhimurium* resulted in more rapid death than did infection with either microorganism alone. The enhanced mortality could be demonstrated when salmonella were injected 1 day before or 1 hour to 5 days after inoculation of *P. berghei*.

Figures 1-3 demonstrate representative experiments in which mice were injected with 3×10^6 erythrocytes of which ⅔ were para-

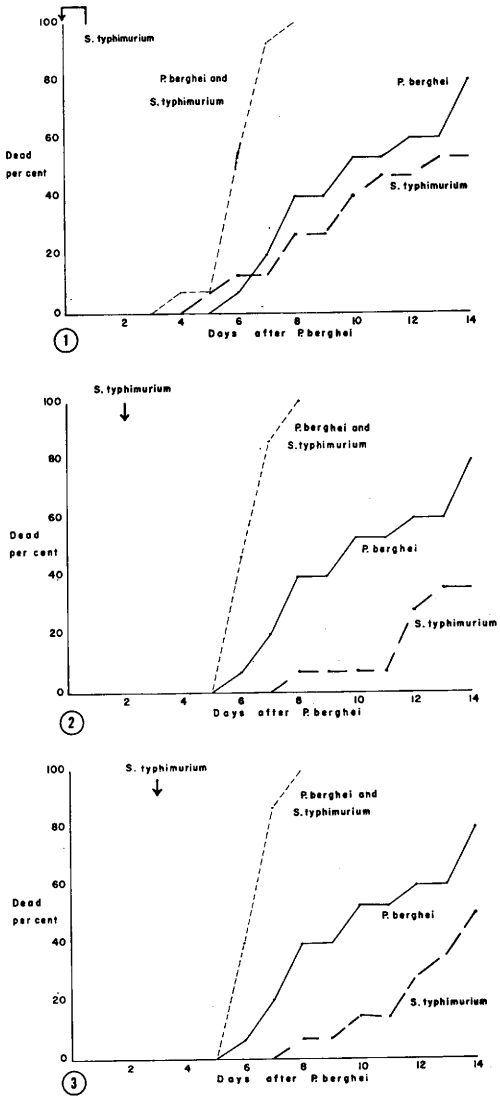

FIG. 1. Cumulative mortality after intravenous injection of 2×10^6 erythrocytes parasitized with *P. berghei* (15 mice), 3×10^6 normal erythrocytes followed in one hour by 5×10^5 *S. typhimurium* (15 mice) or 2×10^6 erythrocytes parasitized with *P. berghei* followed in one hour by 5×10^5 *S. typhimurium* (15 mice).

FIG. 2. Cumulative mortality after intravenous injection of 2×10^6 erythrocytes parasitized with *P. berghei* (15 mice), 3×10^6 normal erythrocytes followed in 2 days by 6×10^5 *S. typhimurium* (14 mice) or 2×10^6 erythrocytes parasitized with *P. berghei* followed in 2 days by 6×10^5 *S. typhimurium* (15 mice).

FIG. 3. Cumulative mortality after intravenous injection of 2×10^6 erythrocytes parasitized with *P. berghei* (15 mice), 3×10^6 normal erythrocytes followed in 3 days by 2×10^5 *S. typhimurium* (14 mice) or 2×10^6 erythrocytes parasitized with *P. berghei* followed in 3 days by 2×10^5 *S. typhimurium* (15 mice).

TABLE I. Hematocrit Values in Mice with Malaria and/or Salmonella Infection.*

	Mice injected with erythrocytes containing *P. berghei*						Mice injected with normal erythrocytes followed in 1 hr by 5×10^5 *S. typhimurium* IV	
			6×10^6 *S. typhimurium* IV		2×10^5 *S. typhimurium* IV			
Days after erythrocytes	Mice alive	Mean hematocrit (%)	Mice alive	Mean hematocrit (%)	Mice alive	Mean hematocrit (%)	Mice alive	Mean hematocrit (%)
1	15/15	44.0	15/15	50.3	15/15	45.7	15/15	46.5
2	15/15	46.3	15/15	46.0	15/15	42.0	15/15	43.7
3	15/15	48.3	15/15	51.3	15/15	45.8	15/15	44.7
4	15/15	45.0	15/15	51.3	15/15	45.3	15/15	46.8
5	15/15	32.7	15/15	41.3	15/15	34.3	14/15	45.3
6	14/15	29.0	8/15	29.3	9/15	28.7	13/15	43.0
7	12/15	25.7	2/15	24.0†	2/15	20.5†	13/15	42.3
8	9/15		0/15		0/15		11/15	40.7

* Unless otherwise designated each hematocrit value is the mean of hematocrits from 3 mice.
† Mean of hematocrits from the 2 surviving mice.

sitized with *P. berghei* or with 3×10^6 erythrocytes from normal mice. Groups of 14-15 mice that had received normal erythrocytes or parasitized erythrocytes were challenged with $2\text{-}6 \times 10^5$ *S. typhimurium* 1 hour, 2 days or 3 days after injection of the erythrocytes. A control group of 15 mice was injected with parasitized erythrocytes but was not challenged with salmonella. Hematocrit values were determined daily for 3 different mice in each group. It is clear from Fig. 1-3 and from other experiments that in each instance the mice with both malaria and salmonella infection died more rapidly than the mice with either malaria or salmonella infection.

The more rapid rate of death seemed to be related to enhancement of the salmonella infection and not to enhancement of malaria as determined by the parameter of anemia. Table I demonstrates survival rates and mean hemotocrit values for the following groups: a) mice injected with *P. berghei* and not challenged with *S. typhimurium;* b) mice injected with *P. berghei* and challenged 2 days later with *S. typhimurium;* c) mice injected with *P. berghei* and challenged 3 days later with *S. typhimurium;* and d) mice injected with normal erythrocytes and challenged 1 hour later with *S. typhimurium.*

The fall in hematocrit was slight in mice with only *S. typhimurium* infection (Group d) during the 8 days of observation, and in fact the mean hematocrit value was normal (45.3%) on the fifth day of observation. The decrease in hematocrit was more marked in mice with *P. berghei* infection and mean values reached 32.7-41.3% on the fifth day of malarial infection. However, there was no consistent difference in mean hematocrit values between mice with both malaria and salmonella infection and mice with only malaria. By the sixth day of malarial infection 13 of 30 mice also infected with salmonella were dead as compared with 1 of 15 mice with only malaria. However, the hematocrit values of the survivors were equivalent for each group. Therefore the presence of salmonella infection did not enhance malaria as measured by the parameter of anemia.

In the experiments demonstrated in Fig. 1-3 and additional experiments, spleens from 1-3 mice of each group infected with salmonella were removed within 30 minutes after death and the numbers of salmonella determined. In each instance more than 10^8 salmonella were present per gram of spleen. Previous studies in this laboratory(2) have demonstrated that mice become moribund and die from infection with *S. typhimurium*

KK when titers in the spleen reach 10^8 per gram. Spleens of animals infected only with *P. berghei* were bacteriologically sterile.

These studies indicate that concurrent infection in mice with *P. berghei* and *S. typhimurium* is more rapidly fatal than either infection alone. The decreased survival time seems to be related to enhancement of salmonella infection and not to enhancement of malaria as the mice died with a lethal salmonella infection but with hematocrit values generally above the level at which mouse malaria was fatal.

Summary. Concurrent infection in mice with *P. berghei* and *S. typhimurium* was more rapidly fatal than either infection alone. There was no consistent difference in mean hematocrit values between mice with both malaria and salmonella infection and mice with only malaria. Spleens removed from mice dying with *P. berghei* and *S. typhimurium* infection demonstrated more than 10^8 salmonella per gram of spleen. Therefore the decreased survival time seemed to be related to enhancement of salmonella infection and not to enhancement of malaria as the mice died with a lethal salmonella infection but with hematocrit values above the level at which mouse malaria was fatal.

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Depression of Liver Affinity for Iron with Iron Overload.* (30662)

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When animals were fed a diet containing ethionine, there was an increase in the amount of iron absorbed into the body, and an increase in storage of iron in the liver(1). When *in vitro* studies were carried out in which liver slices were exposed to an iron-containing solution, it was shown that the liver from animals maintained on this ethionine-supplemented diet for 1 week had a greater affinity for the iron in the solution than did the control animals(2). It was suggested that the affinity may play a role in influencing the deposition of the iron in the liver and promoting the absorption from the gastrointestinal tract. From these experiments, it was not clear whether the change in affinity was caused by the ethionine, with secondary iron deposition, or whether the presence of iron caused the increase of the liver's affinity for the iron, leading to further iron deposition. The present experiment was designed to determine whether iron loading would, in itself, cause an alteration in liver affinity when the metabolic influence of ethionine was not introduced.

Materials and methods. Sprague-Dawley male albino rats weighing approximately 200 and 250 g were used. All animals were fed a synthetic diet of the following composition: glucose, 66.7 g; casein, 18 g; salt mixture, 4 g(3); corn oil, 11 g; choline chloride, 0.3 g. Each 100 g of diet was supplemented with approximately 60 USP units of vit. A and 1 USP unit of vit. D. Crystalline vitamins were added in the following amounts per 100 g of diet: thiamine chloride, 400 μg; pyridoxine hydrochloride, 400 μg; riboflavin, 800

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