pancreata were minced together for each incubation, and the pooled mince was divided into three equal aliquots. One aliquot served as the control, one was incubated with STH, and one with DNP. The ^{14}C labeled amino acids were added to the incubation mixture and the tissues were incubated for 4 hours. Insulin activity was extracted from each tissue and medium and concentrated on Sephadex G-50 columns. Assay for ^{14}C, IRI, and ILA indicated that the greatest amount of IRI and ILA was found in the fraction from the column that eluted in the same position as crystalline insulin. This fraction in the control and STH tissues had an appreciable amount of ^{14}C. It is concluded that the duct-tied pancreas preparation is a useful model to study insulin biosynthesis. The presence of STH did not alter the ^{14}C incorporation or the recovery of IRI or ILA in this preparation.

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The Effects of 6-Thioguanine and Endotoxin on Experimental St. Louis and Japanese B Encephalitis Virus Infections in Chiroptera* (32828)

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Variations in the susceptibility of different species of insectivorous bats to experimental infection with certain strains of arthropod-borne viruses and the failure of susceptible species to show overt signs of encephalitis even when high levels of virus were demonstrable in brains and other tissues have been reported (1). These observations prompted additional studies designed to determine factors which might alter the response of resistant species or precipitate symptoms and death in susceptible species. Experiments with gravid bats demonstrated that the physiological stress of pregnancy did not alter the course of infection with strains of Japanese B encephalitis (JBE) or St. Louis encephalitis (SLE) viruses (2). In a correlated study it was shown that administration of cortisone did not increase the susceptibility of *Tadarida b. mexicana* to a strain of SLE virus of low infectivity for this bat species (Sulkin S. E., Sims, R. A., and Allen, R., unpublished observation). The increased body temperature and metabolic rate resulting from the maintenance of bats at 37°C caused a more rapid

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and intense viremia with JBE virus but no signs of encephalitis or evident damage to tissues was observed (3).

Bacterial endotoxin has been shown under various circumstances to alter the susceptibility of mice to certain bacterial and viral infections (4–6) and 6-thioguanine, a radiomimetic compound, is known to suppress resistance of mice to viral infection (6). This report presents preliminary data concerning the influence of endotoxin and thioguanine on the course of experimental JBE and SLE virus infections in two species of insectivorous bats. Since environmental temperature influences the course of experimental arbovirus infection in bats (3) experiments were conducted at 24 and 37°C.

Materials and Methods. The source and history of the OCT–541 strain of JBE virus and the Flicker Bird-55 strain of SLE virus used in this study appear elsewhere (1). Little brown bats *(Myotis l. lucifugus)* and Mexican free-tailed bats *(Tadarida b. mexicana)* were maintained in the laboratory at 24 or 37°C as described previously (1, 3).[1] Bats to be maintained at 37°C were adapted to that temperature for a period of 3 days prior to the initiation of the experiment.

Commercial preparations of 6-thioguanine (2-amino-6-mercaptopurine) were obtained from Calbiochem. The compound was suspended in 0.1 *M* phosphate buffered saline (PBS), pH 7.0, and was dissolved as the pH was raised slowly to 9.5–10.5 with 4 *N* NaOH. The minimum toxic dose was determined by inoculating bats intraperitoneally with 0.5 ml of varying concentrations of the compound. A sublethal dose of 0.12 mg/gm of body weight was administered 3 days prior to inoculation with JBE or SLE virus. Dried, purified preparations of the endotoxin (lipopolysaccharide) of *Salmonella abortus equi* were obtained from Difco laboratories. This material was dissolved in a volume of distilled water to a concentration of 250 μg/ml. Bats were given 5 μg of endotoxin/gm of body weight intraperitoneally 1 day before administration of JBE or SLE virus. Control groups of bats which received virus alone were included.

Animals held at 24°C were sacrificed on day 7 after subcutaneous inoculation of 150 weanling mouse intracerebral LD_{50}; those held at 37°C were sacrificed on the third day postinoculation. Brain, spleen, lung, and liver were harvested for virus assay. The presence of virus in bat tissues was determined by the intracerebral inoculation of 20% suspensions in PBS, pH 7.8, into groups of weanling white Swiss mice.

Results. Table I shows the effects of pretreatment with endotoxin or thioguanine on experimental SLE and JBE virus infection in *Tadarida b. mexicana.* In bats inoculated with SLE virus and maintained at 24°C, pretreatment with either substance significantly affected the course of the infection. In the control group there were few animals with virus demonstrable in any tissue at time of harvest. After treatment with either endotoxin or thioguanine, however, all tissues in the majority of the bats tested were positive, with the exception of brain tissue of animals which received thioguanine. In most instances the levels of virus present in the tissues of treated bats were higher as evidenced by the higher percentage of mice succumbing as compared to the untreated control group. The influence of endotoxin and thioguanine on SLE infection in bats maintained at 37°C was not as marked as observed at 24°C. Thioguanine induced increased multiplication of virus in spleen, lung, and liver, whereas endotoxin influenced the demonstration of virus in only spleen and lung tissue. Neither substance had any effect on growth of SLE virus in brain tissue at this temperature.

Endotoxin and thioguanine also influenced experimental JBE virus infection in *T. mexicana* maintained at 24°C although less significantly than in experiments with SLE virus since all five untreated control bats showed evidence of virus multiplication in liver tissue at time of sacrifice. Endotoxin increased the number of animals with positive brain tissue, whereas thioguanine had no effect on the demonstration of virus in the brain but did induce an increase in virus multiplication

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TABLE I. Effects of Pretreatment with Endotoxin or Thioguanine on the Distribution of Virus in Tissues of *Tadarida b. mexicana* Held at 24°C or 37°C following Subcutaneous Inoculation with St. Louis or Japanese B Encephalitis Virus.[a]

Virus inoculated	Treatment	Tissues from bats held at 24°C[b]				Tissues from bats held at 37°C[c]			
		Brain	Spleen	Lung	Liver	Brain	Spleen	Lung	Liver
St. Louis encephalitis (Flicker Bird-55)	Endotoxin	5[d](68)[e]	5 (52)	3 (94)	5 (60)	0	3 (54)	2 (40)	0
	Thioguanine	1 (20)	5 (68)	4 (80)	5 (94)	0	3 (44)	3 (20)	5 (100)
	None	0	1 (20)	1 (20)	1 (60)	1 (20)	0	0	1 (60)
Japanese B encephalitis (OCT-541)	Endotoxin	4 (30)	2 (70)	1 (100)	4 (95)	0	0	0	3 (15)
	Thioguanine	0	5 (56)	5 (52)	4 (65)	1 (20)	0	0	2 (80)
	None	0	1 (20)	0	5 (64)	0	0	0	5 (64)

[a] Inoculum was approximately 150 weanling mouse intracerebral LD_{50}.

[b] Harvested on seventh day after inoculation.

[c] Harvested on third day after inoculation.

[d] Number of bats, out of five tested, with virus in tissue.

[e] Percentage of mice succumbing when inoculated with positive tissues. When 20% or less succumbed, virus identified by serum neutralization tests in HKC cultures.

TABLE II. Effects of Pretreatment with Endotoxin or Thioguanine on the Distribution of Virus in Tissues of *Myotis l. lucifugus* Held at 24°C or 37°C following Subcutaneous Inoculation with St. Louis or Japanese B Encephalitis Virus.[a]

Virus inoculated	Treatment	Tissues from bats held at 24°C[b]				Tissues from bats held at 37°C[c]			
		Brain	Spleen	Lung	Liver	Brain	Spleen	Lung	Liver
St. Louis encephalitis (Flicker Bird-55)	Endotoxin	3[d](67)[e]	3 (74)	3 (94)	5 (100)	1 (20)	0	0	5 (100)
	Thioguanine	4 (45)	4 (60)	4 (80)	5 (80)	0	0	0	0
	None	0	0	0	0	0	0	0	0
Japanese B encephalitis (OCT-541)	Endotoxin	5 (88)	5 (80)	5 (84)	5 (88)	3 (47)	2 (30)	3 (47)	2 (30)
	Thioguanine	1 (20)	2 (30)	3 (20)	3 (40)	2 (60)	1 (20)	3 (47)	2 (50)
	None	0	0	0	0	0	0	0	0

[a] Inoculum was approximately 150 weanling mouse intracerebral LD_{50}.

[b] Harvested on seventh day after inoculation.

[c] Harvested on third day after inoculation.

[d] Number of bats, out of five tested, with virus in tissue.

[e] Percentage of mice succumbing when inoculated with positive tissues. When 20% or less succumbed, virus identified by serum neutralization tests in HKC cultures.

in spleen and lung tissue. Infection in bats maintained at 37°C was not significantly affected by either substance. Only liver tissue contained JBE virus in quantity regardless of whether the animals had been treated or not.

Table II presents data from experiments concerned with the influence of endotoxin and thioguanine on SLE and JBE virus infection in another bat species, *Myotis l. lucifugus*. The results obtained with animals held at 24°C were quite striking. Neither virus could be detected in any tissue from control animals sacrificed 7 days postinoculation. Pretreatment with either endotoxin or thioguanine, however, resulted in a high degree of involvement of all tissues tested from the majority of the bats sacrificed at this time. Endotoxin rendered animals particularly susceptible to JBE infection, virus being present in every tissue tested from all 5 bats. Treatment with either substance appeared to increase the susceptibility of *Myotis* held at 37°C to JBE virus to approximately the same degree observed in experiments at 24°C and

endotoxin had a marked effect on the growth of SLE virus in liver tissue of this species held at the higher temperature. Thioguanine, on the other hand, failed to induce a demonstrable effect on SLE virus infection in *Myotis* maintained at 37°C.

Discussion and Summary. The results presented show that treatment of bats with either endotoxin or 6-thioguanine prior to inoculation with JBE or SLE virus affects the course of infection in these animals. At a given harvest time, when little virus was demonstrable in the tissues of control animals, tissues from most bats pretreated with either substance showed evidence of viral multiplication. This increase in susceptibility was much more marked in bats maintained at 24°C than in those held at 37°C where, in some instances, no change in the response to virus was observed. The apparent difference in the effect of these two substances on experimental arbovirus infection in bats maintained at 24°C versus animals held at 37°C could possibly be due to variations in the metabolism of the compounds at the different temperatures. Although treatment with endotoxin or thioguanine increased the multiplication of JBE and SLE virus in various tissues of the bat, no overt signs of encephalitis were observed in any of the treated animals during the course of the experiments.

Both 6-thioguanine and endotoxin are believed to influence the susceptibility of animals to bacterial and viral infections through action on the reticuloendothelial system. As already mentioned, 6-thioguanine is a radiomimetic compound and, hence, has effects similar to those of X-rays which are known to injure the phagocytic mechanism (7, 8). Although macrophages from X-rayed animals are still capable of ingesting infectious agents (9–11), the power to destroy the agents is lost and multiplication within the phagocytic cells can occur, furthering the infection (12, 13). Mims' suggestions concerning the role of macrophages in viral pathogenesis (14, 15) are important in light of the above mentioned observations. If macrophages are made to support multiplication of a virus which they would otherwise destroy, a successful infection may be established where it could not be normally. Endotoxin also affects the reticuloendothelial system (4) but the nature of the effect is more complicated. A single large dose of endotoxin reduces the ability of the macrophages to ingest whereas several doses cause first an inhibition then a stimulation of the reticuloendothelial system (16). Another factor in the effect of endotoxin is the presence of a pyrogenic substance which could have affected the response of the bat to virus at the different environmental temperatures.

Regardless of the exact mechanism of the effects of endotoxin and thioguanine, both do affect the reticuloendothelial system and the response of the bat to experimental infection with JBE or SLE virus. It may be inferred, then, that the success of viral infections in the bat, as in the mouse, may depend to a great extent on the response of the reticuloendothelial system to the agent. Results obtained in studies concerned with early sites of multiplication of JBE and SLE virus in experimentally infected bats (Middlebrooks, *et al.*, unpublished observations) support this hypothesis. It was found that the liver rapidly removed injected virus from the blood and that this organ was a prime site for viral multiplication. In all cases virus apparently multiplied originally in the vascular endothelium and macrophages.

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Enterohepatic Circulation and Conversion of Protoporphyrin to Bile Pigment in Man* (32829)

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The literature prior to 1950 on the intestinal formation and absorption of porphyrins was reviewed in relation to an earlier detailed study of this problem (1). The collected evidence for and against absorption of copro- or protoporphyrin was inadequate and indecisive. The study referred to failed to reveal any increase of urinary coproporphyrin after feeding of either of the coproporphyrin isomers, or of hemoglobin, or meat. None of these earlier studies excluded absorption and re-excretion in the bile or possible conversion to other derivatives. This would relate especially to protoporphyrin which is not excreted in the urine. Appropriate studies in subjects with bile fistulae were not available. More recently, Aziz (2) fed protoporphyrin through an inlying duodenal tube in an individual having a complete external bile fistula following operation for carcinoma of the pancreas. The administered protoporphyrin was unaccounted for on the basis of serial determinations of protoporphyrin in bile samples collected after the feeding. In the meanwhile London and co-workers (3) had shown that protoporphyrin ^{15}N given intravenously in a normal dog was converted to stercobilin but whether *via* preliminary heme formation in the liver was not determined. Quite recently (4) we have reported that protoporphyrin ^{14}C is promptly converted to hepatic heme, thence to bilirubin, after intravenous administration in bile fistula dogs. A part of the injected protoporphyrin was excreted in the bile as such, together with labeled bilirubin.

Recently clinical improvement has been reported following cholestyramine therapy in patients with porphyria cutanea tarda (5) and with erythropoietic protoporphyria (6). This stimulated our interest to seek the existence of an enterohepatic circulation (EHC) of protoporphyrin. It was believed that the problem could only be solved by the use of suitably labeled protoporphyrin (Proto-). The above-mentioned conversion of Proto-^{14}C to bilirubin in dogs (4) provided the basis for the present study. If Proto-^{14}C administered intraduodenally in man is absorbed, some fraction of the absorbed porphyrin should be converted to bile pigment in the liver and would then be detected as stercobilin ^{14}C in the feces.

Material and Methods. The study was carried out on one of us (G.I.), a normal male subject, age 37, in excellent health. Proto-^{14}C was prepared as described previously (4) from a duck red cell hemolysate system incubated with 0.1 mC of δ-aminolevulinic acid-4-^{14}C (ALA-^{14}C), see Table I. The hemoglobin Proto- dimethyl ester was isolated from duck hemoglobin by Grinstein's method (10). The free erythrocyte porphyrins in the preliminary acetone wash of the hemoglobin powder (10) were fractionated as described in a previous publication (4). The crystalline free Proto- fraction (125 μg, 178.3 × 10^6 DPM/mg) was added to enrich the benzene solution of the crystalline (Hb) Proto- (5.22 mg, 1.75 × 10^6 DPM/mg). The mixture was chromatographed

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