of mammary cancer.

The data are presented in Tables I and II.

The results demonstrate that the mammary tumor milk agent may be recovered from spontaneous mammary cancers which developed in mice of the C_{57} black stock and may be propagated in the transplants of these tumors. Two of the tumors arose in mice which had received the agent from their mothers, again demonstrating that females of the C_{57} black strain, at least some sublines, may transmit the agent in the milk.

Summary. Mice of some sublines of the C_{57} black stock may propagate and transmit the mammary tumor agent. The agent may be recovered from spontaneous mammary cancers which developed in mice of the strain and may survive for at least 10 passages of these tumors in mice without the agent.

16259

Effect of Bacterial Endotoxins on Carbohydrate Metabolism of Rabbits.*

ERNEST KUN AND C. PHILLIP MILLER.†

From the Departments of Pharmacology and Medicine, University of Chicago.

The injection into animals of heat-killed cultures of certain bacterial cells has been shown to produce marked hyperglycemia.[1,2] Diphtheria toxin which has been studied more extensively[3] causes a similar rise in blood sugar and depletion of tissue carbohydrate. Some investigators[4,5] have concluded that diphtheria toxin affects metabolism by damaging the liver, causing increased glycolysis and diminished phosphorolysis. The biochemical mechanism by which the metabolites or cellular constituents of bacteria interfere with the metabolism of the infected animal is not well understood.

The experiments described below concern the effects of meningococcal and Salmonella endotoxins on the intermediate carbohydrate metabolism of rabbits. As in experiments already reported,[6] the experimental conditions may be defined as acute endotoxin poisoning. The effect of a lethal dose of endotoxin was studied during the short period of intoxication which terminated in the death of the animal in 1.5-3 hours. It is believed that, under such circumstances, the effect of the toxin is not significantly altered by spontaneous changes in metabolism.

Materials and Methods. The endotoxins were sterile, unpurified preparations made from washed bacterial cells by the method already described.[6] The intravenous dose which was lethal in 2 to 3 hours was 20-50 mg per kg. Rabbits weighing 1.5-3 kg were used. The experimental and control animals were fasted for 24 hours before each experiment. No anesthesia was used. Blood samples were taken by cardiac puncture. Tissue analyses were carried out immediately after the death of the animal. Pairs of rabbits having almost identical weights were kept under the same environmental conditions throughout each experiment. One rabbit in each pair was injected with endotoxin and the other kept as a control and sacrificed when death occurred in the former. In some

* This investigation was undertaken and supported jointly by the U. S. Navy, Office of Naval Research, and the University of Chicago.

† With the technical assistance of Mrs. S. E. Gurnee.

1 Menton, M. L., and Manning, H. M., *J. Med. Res.*, 1924, **44**, 675.

2 Zweckwer, L. T., and Goodell, H., *J. Exp. Med.*, 1925a, **42**, 43; *J. Exp. Med.*, 1925b, **42**, 57.

3 Holmes, E., *Physiol. Rev.*, 1939, **19**, 439.

4 Soskin, S., Allweiss, M. D., and Mirsky, J. A., *Arch. Int. Med.*, 1935, **56**, 927.

5 Taubenhaus, M., and Soskin, S., *J. Clin. Endocrin.*, 1942, **2**, 171.

6 Kun, E., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 197.

instances the animals were sacrificed before death and the same changes were found in the concentration of metabolites and enzyme activities as in the animals which succumbed to endotoxin.

Blood glucose was determined by the method of Nelson,[7] lactic acid according to Barker and Summerson,[8] pyruvic acid as described by Elgart and Nelson,[9] and acid soluble phosphorus by the method of Schneider.[10] Glycogen was determined as glucose.[11]

Results. The results of the analyses for blood sugar, lactic acid, phosphorus and pyruvic acid are shown in Fig. 1, 2, 3, and 4. The graphs were constructed from data obtained from experiments of identical length.

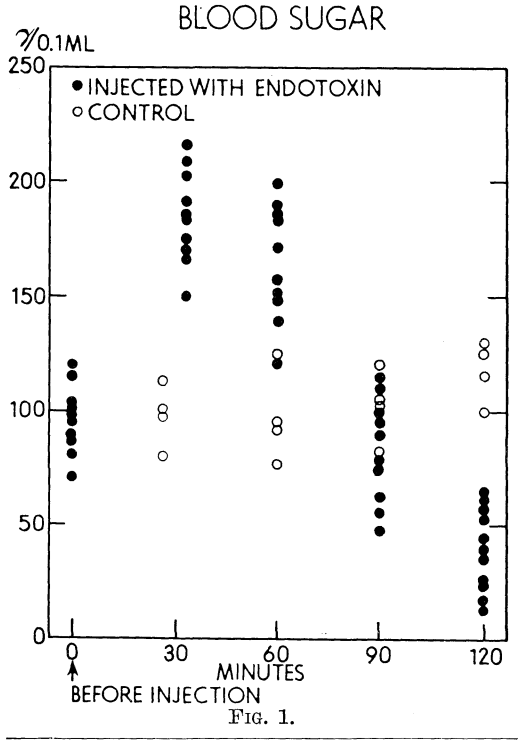

FIG. 1.

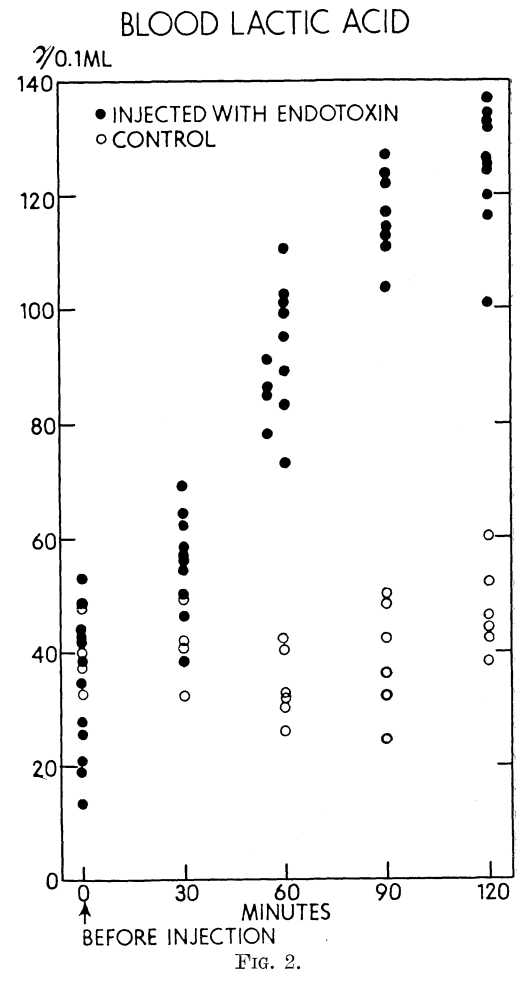

FIG. 2.

Each point represents the blood values of one individual animal.

The results show that the endotoxins caused hyperglycemia which reached its maximum 30 minutes after the injection and was followed by hypoglycemia. Blood lactic acid showed marked increase. Blood phosphorus fell slightly a few minutes after the injection and then rose sharply. The blood pyruvic acid content decreased after the injection of the toxin and remained below the normal level.

The results of the tissue analyses are given in Fig. 5, in which the liver and muscle glycogen, lactic acid and pyruvic acid of normal animals are compared with the results obtained from animals injected with meningococcal endotoxin. (Eight pairs of rabbits were used for glycogen, 5 pairs for tissue

[7] Nelson, N., *J. Biol. Chem.*, 1944, **153**, 375.

[8] Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, **138**, 535.

[9] Elgart, J. S., and Nelson, N., *J. Biol. Chem.*, 1941, **138**, 443.

[10] Schneider, W., *J. Biol. Chem.*, 1945, **161**, 293.

[11] Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, Burgess Publishing Company, 1945.

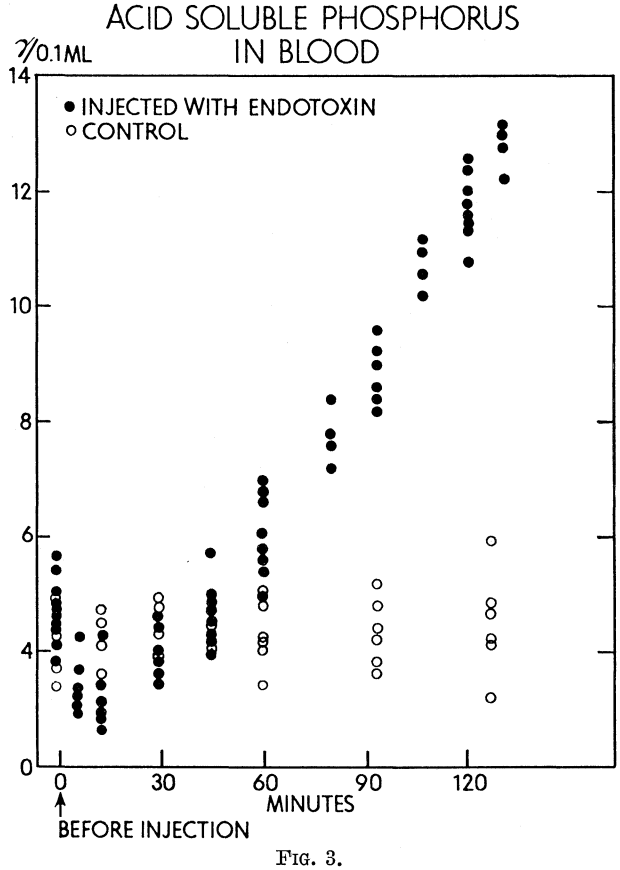

FIG. 3.

lactic acid and 4 pairs for tissue pyruvic acid determinations.)

The greatest difference between the control rabbits and those injected with endotoxin was found in the glycogen content of liver and muscle, particularly in the former, as the livers of the animals poisoned by endotoxin contained only traces of glycogen.

The lactic acid content of the liver and muscle in the endotoxin treated rabbits was markedly increased, whereas the pyruvic acid content was diminished.

It is well established that in mammalian tissue under normal circumstances glycogen is broken down to pyruvate which is further oxidized through various channels. If the aerobic conditions in tissues are altered in some way, accumulation of lactate and inorganic phosphate occurs.[12] Our observations on these constituents in the blood and tissues of our experimental animals suggested, therefore, that the injection of the bacterial endo-

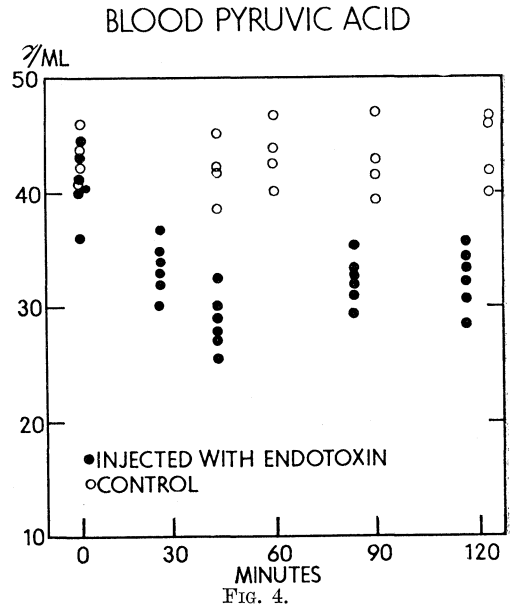

FIG. 4.

[12] Barron, E. S. G., *Advances in Enzymology*, 1943, **3**, 149.

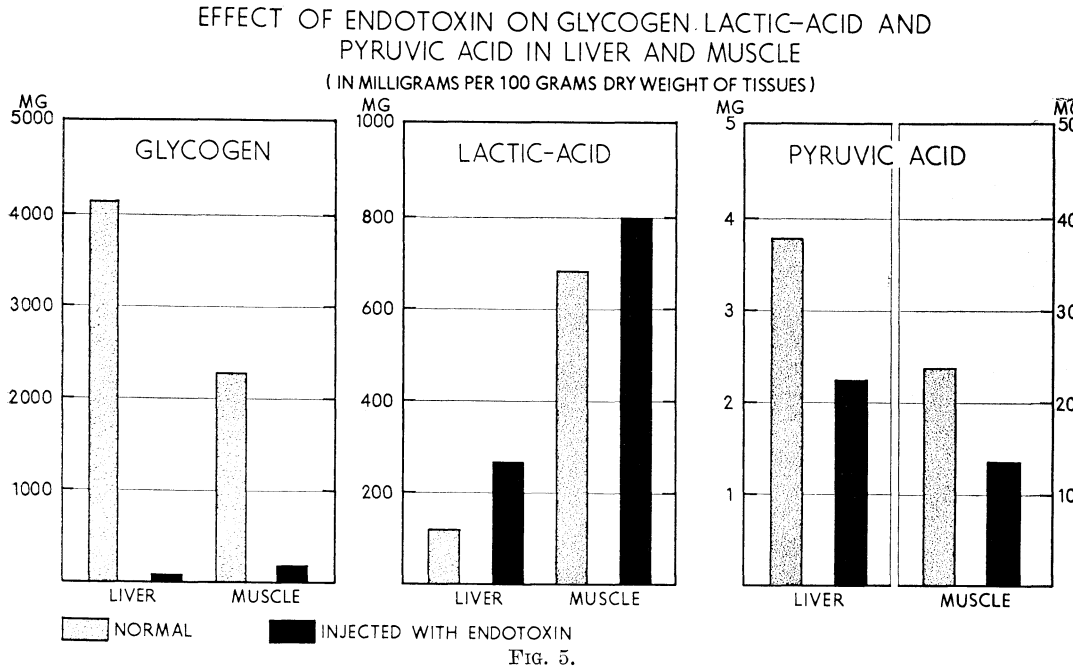

FIG. 5.

TABLE I.
Succinic Dehydrogenase Activity of the Liver and Muscle of Rabbits Injected with Endotoxin.

	Liver		Muscle	
	Normal control	Endotoxin	Normal control	Endotoxin
Meningococcal endotoxin	88.4	28.0	16.2	6.0
	60.1*	40.2*	15.1*	6.5*
	68.9	29.7	24.2	8.4
	80.0	16.0	26.1	8.7
	65.4*	20.4*	19.3*	7.1*
	72.4*	31.0*	29.2*	6.9*
Salmonella endotoxin	62.4	26.1	19.6	5.2
	79.7	15.1	17.1	7.1
	72.4*	20.0*	25.3*	8.0*
	80.1	17.8	22.0	9.2

* These values were obtained from animals which were sacrificed before death from the toxin.

toxins might have produced a state of anoxia in their tissues.

In order to learn more about the mechanism of the tissue anoxia caused by endotoxin, rabbits were injected with meningococcal and Salmonella endotoxin‡ as described above and the cytochrome oxidase and succinic-dehydrogenase activity of their muscle and liver were determined by the Warburg technic as described by Schneider and Potter.[13] In these enzyme assays phosphate buffer (pH 7.4) was used instead of H_2O for the preparation of tissue homogenates. The importance of this technical detail will be discussed elsewhere. A significant inhibition of succinic dehydrogenase activity was demonstrable. Cytochrome oxidase was not affected (see Table I).

It is interesting to note that the endotoxins

‡ The endotoxin of *Salmonella aertrycke* was prepared in the same way as meningococcal endotoxin.

[13] Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, 1943, **149**, 217.

of both Salmonella and meningococcus produced similar effects not only in their inhibition of succinic-dehydrogenase but also on the carbohydrate metabolite concentration of tissues. Since the enzyme inhibition occurred in a few minutes after the injection of the endotoxin, it is reasonable to assume that the inhibition of this enzyme activity plays an important role in the mechanism responsible for the changes in carbohydrate metabolite concentration in the poisoned rabbit tissues. The mechanism of this enzyme inhibition and its effect on carbohydrate metabolism is being investigated in detail.

Summary. The intravenous injection of meningococcal or Salmonella endotoxin into rabbits produced increase in blood glucose, lactic acid and inorganic phosphorus. This was followed by hypoglycemia which could be observed before the death of the animal. Liver and muscle glycogen decreased while lactic acid content of the tissues increased. The pyruvic acid content of blood and tissues showed a significant decrease. Succinic dehydrogenase in both muscle and liver was markedly inhibited. Cytochrome oxidase activity was not affected.

The Na-ascorbate used in the enzyme assays was generously supplied by Van Patten Pharmaceutical Company, Chicago.

16260

Passive Cellular Transfer of the Tuberculin Type of Hypersensitivity.*

ABRAM B. STAVITSKY.† (Introduced by D. H. Campbell.)

From the Gates and Crellin Laboratories, California Institute of Technology, Pasadena.‡

Chase[1] recently reported that the tuberculin type of hypersensitivity was transferred passively to normal guinea pigs by injection of the cells of peritoneal exudate, lymph nodes or spleen of guinea pigs sensitized to tuberculin by the injection of heat-killed tubercle bacilli. The passively conferred sensitivity was transient, lasting about 5 days. This is a significant achievement as the failure passively to transfer tuberculin sensitivity has been adduced as a basic distinction between the tuberculin and the anaphylactic or Arthus types of hypersensitivity.[2] It was hoped that, if confirmed, the passive cellular transfer of tuberculin hypersensitivity might provide a method suitable for the elucidation of the underlying chemical factors in the induction and development of this peculiar allergic phenomenon. The repetition of Chase's studies was therefore undertaken.§

Materials and Methods. The materials and methods were essentially those used by Chase[1] in his experiments. White albino guinea pigs of both sexes were sensitized to tuberculin by subcutaneous injection into the groin of heat-killed human tubercle bacilli suspended in mineral oil[3,4,5] and aquafor. Usually 2.5 mg of dried bacillary growth was suspended in a total inoculum of 1 ml for each animal. Five to 9 weeks later, when cutaneous sensitivity to

* Supported by a grant from the Rockefeller Foundation for research in immuno-chemistry.

† Present address: Institute of Pathology, Western Reserve University, Cleveland, Ohio.

‡ Contribution No. 1153.

1 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

2 Rich, A. R., *The Pathogenesis of Tuberculosis*, Springfield, Charles C. Thomas, 1944.

§ Since these confirmatory studies have been completed another group of workers reported successful repetition of Chase's experiments. Cummings, M. M., Hoyt, M., and Gottshall, R. Y., *Pub. Health Rep.*, 1947, **62**, 994.

3 Freund, J., Casals-Ariet, J., and Hosmer, E. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 509.

4 Freund, J., Casals-Ariet, J., and Genghof, D. S., *J. Immunol.*, 1940, **38**, 67.

5 Freund, J., and Gottshall, R. Y., *Arch. Path.*, 1942, **34**, 73.