Intraperitoneal Injections of India Ink and Ox Bile and the Blood Lactic Acid in Rats.* (18205)

ROBERT M. HILL AND ENID K. RUTLEDGE.
(With the technical assistance of Evelyn Campbell)
From the Departments of Biochemistry and Pathology, University of Colorado School of Medicine, Denver.

We have shown(1,2) that the intraperitoneal injection of certain particulate and large molecular substances lowers the body-temperature in white rats, and that this fall in body-temperature seems to be associated with the removal of these substances from the peritoneal cavity by the reticulo-endothelial system. Further, it appears that the fall in body-temperature can occur without shock as shown by the small coincident hematocrit and plasma protein changes(2). However, it seemed advisable to investigate further the possible role of shock in the production of hypothermia following the intraperitoneal injection of these substances. For this purpose we compared the changes in blood lactic acid after intraperitoneal injection of India ink (a representative of the particulate substances, which cause hypothermia) with the changes following intraperitoneal injection of ox bile, which is known to produce shock(2).

Experimental. The methods of injection, the technic of measurement of body-temperature, and the care of the animals have been described previously(1). Young adult white rats were used in all instances. Higgins India Ink was used as purchased. Ox bile was used as received from the slaughter house. Blood lactic acid was determined by the method of Barker and Summerson(3) as modified by LePage(4). The rats were kept in individual wire cages at room temperature (25°-27°C) throughout the experiment. Except as indicated the experiments were performed with the rats under pentothal-sodium anesthesia in order to avoid the possibility that any observed rise in blood lactic acid might have been caused by excitement. Preliminary procedures were carried out in the following order:

High colonic temperatures were measured, a sample of blood for lactic acid analysis was taken by clipping the tip of the tail, and, whenever pentothal sodium was used, it was injected subcutaneously immediately thereafter. Twenty minutes later, in order, the temperature was taken again, another sample of blood was collected, and the experimental substance, India ink or ox bile, was injected intraperitoneally. The rats were then placed in individual wire cages. Body-temperatures were measured and blood-samples were drawn for lactic-acid determination at intervals throughout the experiment.

Results. In all of the figures, the numbers of the individual rats are shown at the end of the curves so that each lactic-acid curve can be compared with its corresponding body-temperature curve.

Fig. 1 shows the results of intraperitoneal injection of 2 cc of India ink without anaesthesia. With these rats, as in the other experiments (Fig. 2, 3, 4) there is a strong tendency to high blood lactic acids in the first sample. Presumably this is a reaction to the excitement of the first handling in the morning although the excitement was not otherwise apparent. It is not repeated later even in the first experiment (Fig. 1) in which no anaesthesia was used.

Fig. 2 shows the changes in body-temperature and in blood lactic acid following the

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1. Hill, R. M., and Rutledge, E. K., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 9.

2. Hill, R. M., and Rutledge, E. K., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 310.

3. Barker, J. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, v138, 535.

4. LePage, G. A., Method for Lactic Acid, p. 104, Manometric Techniques and Related Methods for the Study of Tissue Metabolism, by W. W. Umbreit, R. H. Burris, and J. F. Stauffer, Burgess Publishing Co., Minneapolis, 1948.

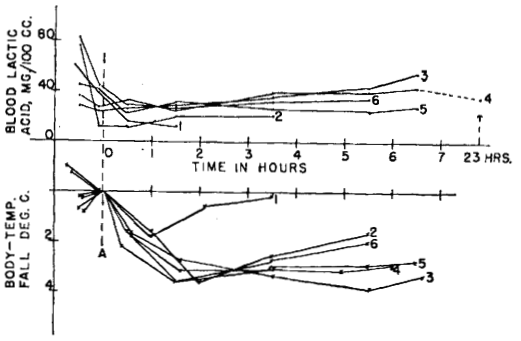

FIG. 1.
Fall in body temperature and blood lactic acid curves after intraperitoneal injection of 2 cc of India ink without anesthesia. Rat numbers given at the ends of the curves.

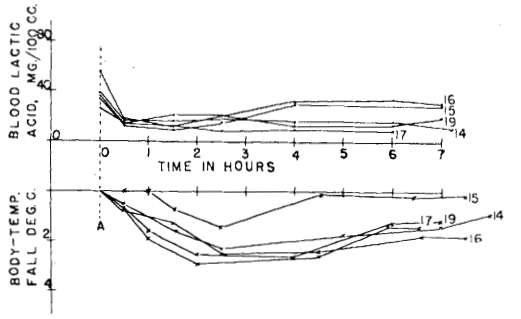

FIG. 2.
Body temperature and blood lactic acid curves following the subcutaneous injection of 0.2 cc of 2.5% pentothal sodium per 100 g body weight, without any other treatment. Rat numbers are given at the ends of the curves.

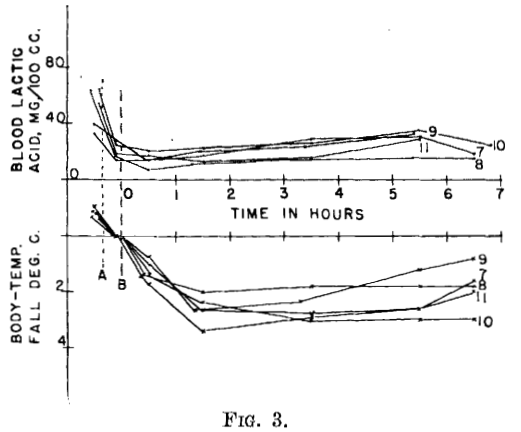

FIG. 3.
Body temperature and blood lactic acid curves after the subcutaneous injection of 0.2 cc of 2.5% pentothal sodium per 100 g body weight at A, and the intraperitoneal injection of 2 cc of India ink at B. Rat numbers are given at the ends of the curves.

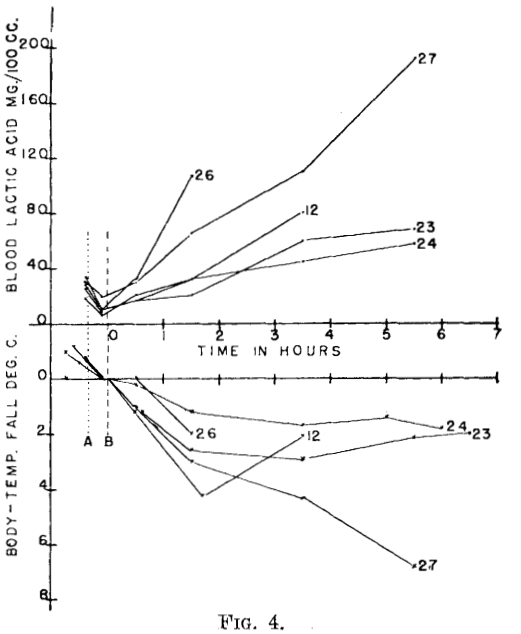

FIG. 4.
Body temperature and blood lactic acid curves following the subcutaneous injection of 0.2 cc of 2.5% pentothal sodium per 100 g body weight at A, and the intraperitoneal injection of 0.5 cc of ox bile at B. Rat numbers are given at the ends of the curves.

subcutaneous injection of 0.2 cc of 2.5% pentothal sodium per 100 g body-weight, without any other treatment. Pentothal sodium used in this way causes a hypothermia comparable to that following the intraperitoneal injection of India ink, and like India ink produces no significant change in the blood lactic acid.

Fig. 3 shows the changes in body-temperature and in blood lactic acid subsequent to subcutaneous injection of pentothal sodium followed in 20 minutes by the intraperitoneal injection of 2 cc of India ink. The changes produced in body-temperature and in blood lactic acid are not significantly different from those after injection of pentothal sodium or India ink alone (Fig. 1 and 2). It is noticeable that there is no additive effect of the two substances on body-temperatures when they are both injected.

Fig. 4 shows the changes in body-temperature and blood lactic acid after subcutaneous injection of pentothal sodium followed by intraperitoneal injection of 0.5 cc of ox bile. With the exception of Rat 27, the falls in

body-temperature were comparable to those in the other experiments. Ox bile injected intraperitoneally is extremely irritating. Rat 27 was in a moribund condition when the last body-temperature was taken and died shortly thereafter. Blood lactic-acid values for all the rats receiving ox bile rose sharply and continued to rise for the duration of the experiment.

Discussion. The body-temperature and blood lactic-acid curves following the intraperitoneal injection of India ink (Fig. 1), of pentothal sodium (Fgi. 2) and of India ink plus pentothal sodium (Fig. 3) are much alike. There is no evidence that the treatment caused increases in blood lactic acid. The initial high values for lactic acid found in most of the rats were surprising, particularly in view of the fact that the excitement, which was the probable cause of these high values, was not otherwise apparent in the behavior of the animals. The second value taken 25 minutes later was in some cases less than half of the initial value and must be taken as the "normal" value for the individual rat. There is little evidence from the lactic-acid curves that the subsequent hourly handling and bleeding produced comparable excitement. All the rats had been handled frequently and were gentle. This experience emphasizes the care with which "initial" values in experiments on rats must be judged.

Following the intraperitoneal injection of 0.5 cc of ox bile plus pentothal sodium, the response is different. There is a greater variation in the degree of hypothermia, a variation that seemed to reflect the physical condition of the animal. Rat 26 died at the end of 2 hours. In all instances there was a pronounced increase in the blood lactic acid.

We believe that these experiments give further support to our belief that the fall in body-temperature following the intraperitoneal injection of particulate substances is not a reflection of shock.

Summary. Adult white rats were treated in 4 ways. In 3 of these, (a) intraperitoneal injection of 2 cc of India ink, (b) subcutaneous injection of 0.2 cc pentothal sodium per 100 g, and (c) treatment (b) followed in 20 minutes by treatment (a), blood lactic acid remained normal but body-temperature fell. In the fourth treatment, subcutaneous injection of pentothal sodium followed in 20 minutes by intraperitoneal injection of 0.5 cc ox bile, a similar fall in body-temperature occurred which was accompanied by a marked rise in blood lactic acid. The rise in blood lactic acid is taken as an indication of shock.

These experiments indicate that the hypothermia produced by the intraperitoneal injection of India ink is not secondary to shock.

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Observation of a Spreading Factor Present in Mammary Gland Extracts.*† (18206)

J. R. ELLIOTT AND C. W. TURNER.

From the Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

It is well known that at the onset of pregnancy a phase of rapid growth of the lobule-alveolar system of the mammary gland is initiated. At this time there is not only an extension of the major ducts but also a rapid proliferation of side buds which invades the adjacent subcutaneous fatty connective tissue (fatty pad). In order to facilitate the forward growth of the end buds, it seemed possible that a mucolytic enzyme or collagenase might be elaborated by the growing mammary gland cells which, through liquefication

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