Effects of Injection of Large Molecular and Particulate Substances on Body-Temperature of Rats.* (17418)

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In an earlier paper[1] it was shown that autolyzed yeast and the magnesium salt of yeast nucleic acid produce a rise in the body-temperatures of albino rats when injected subcutaneously, but produce a fall in the body-temperatures when injected intraperitoneally. On the other hand, the purines, pyrimidines and allantoin derived from nucleic acid do not materially affect body-temperature by either route of administration. It seemed possible, therefore, that the property of altering body-temperatures may be associated with the high molecular weight or particulate nature of the substances employed rather than with chemical groups present. India ink injected subcutaneously, as well as autolyzed yeast, has been used for producing fever experimentally. India ink is also known to be phagocytized after intraperitoneal injection and taken up by the cells of the reticuloendothelial system where it is easily seen. However, we have not found any record of body-temperature measurements after intraperitoneal injections of this substance.

In this paper we are reporting experiments with India ink, with blood charcoal and with two dyes that have been used for visualizing the lymph channels and the reticuloendothelial system, carmine and trypan blue. In addition to these, a variety of substances was tested either because of their particulate nature or because they have been reported to alter body-temperature. In the latter group were skim milk, barium sulfate as prepared for gastrointestinal roentgenography, lipiodol, egg albumin, and 2, 4-dinitrophenol. Of these, only the last named showed any influence on body-temperature by either subcutaneous or intraperitoneal administration, and it produced fever by both routes. Because of the negative results no data are reported for this group of substances.

It seemed possible that the fall in body-temperature observed after intraperitoneal injection of some substances was a response to shock. To test this hypothesis blood cell volumes and plasma proteins were measured before and at intervals after treatment. These measurements are compared with similar measurements made after intraperitoneal injections of ox bile and solutions of peptone, two substances that are used for the experimental production of shock.

Materials and methods. The carmine used was a Grubler and Company preparation. The trypan blue was obtained from the Coleman and Bell Company. For most of the experiments both were prepared for injection by shaking 100 mg of the dye with 100 cc of 0.9% NaCl solution. Neither dye was completely soluble at this concentration, the carmine less so than the trypan blue, but both remained suspended long enough after vigorous shaking for injection. Except as indicated, Higgins India ink was used as purchased. Two g of Merck's blood charcoal were shaken vigorously with 100 cc of 0.9% NaCl solution just before injection. Ox bile was used as received from the packing house. The peptone was a 20% aqueous solution of J. T. Baker's powdered "Peptone, Bacteriological." Blood for cell volume and plasma protein determinations was obtained by clipping the tail and allowing the blood to drop into a small cuvette containing heparin. Cell volumes were determined with Smith tubes and plasma proteins by the copper sulfate specific gravity method.[2] The methods of

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1 Hill, R. M., and Rutledge, E. K., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 9.

2 Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Hamilton, P. B., and Archibald, R. M., Publication of the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research.

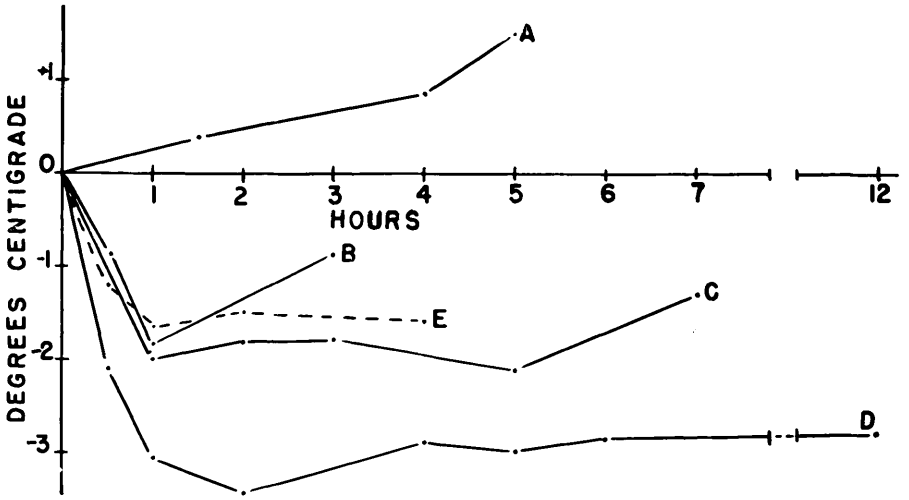

FIG. 1.

Average body-temperature changed after (1) subcutaneous injection (A) (15 rats) of 1.0 cc of Higgins India ink; (2) intraperitoneal injection of (B) 0.2 cc (8 rats), (C) 1.0 cc (8 rats), and (D) 2.0 cc (15 rats) of Higgins India ink, and (3) intraperitoneal injection of 2.0 cc of 2.0% suspension of blood charcoal (8 rats).

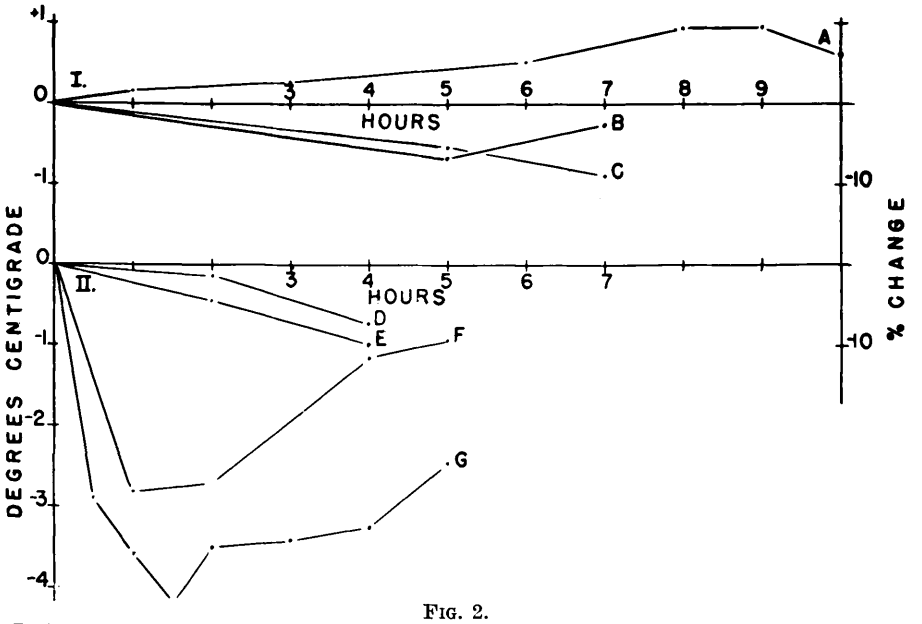

FIG. 2.

I. Average body-temperature change after subcutaneous injection of 4 cc of 1.0% carmine (A). Per cent change in cell volume (hematocrit) shown in C and plasma protein in B (15 rats).

II. Average body-temperature change after intraperitoneal injection of 4 cc (G) (8 rats) and 2 cc (F) (8 rats) of 1.0% carmine. Per cent changes in cell volume are shown in E and plasma protein in D for the rats of curve G.

injection, measurement of body-temperature and the care of the animals were the same as those already described.[1] Young adult white rats were used in all instances.

Experimental Results. Fig. 1 shows average curves of body-temperature change after intraperitoneal administration of 0.2 cc (B), 1.0 cc (C), and 2.0 cc (D), and the subcutaneous administration of 1.0 cc (A) of India ink. In this figure is included a curve (E) that

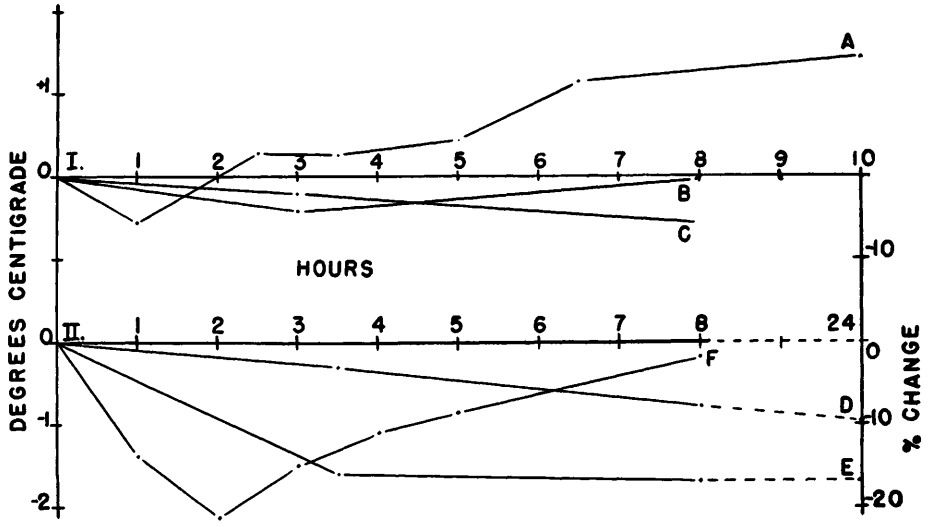

FIG. 3.

I. Average body-temperature change (A) after intramuscular injection of 1 cc of 4% carmine. Average per cent change in cell volume (hematocrit) (B) and plasma protein (C) in the same animals (4 rats).

II. Average body-temperature change (F) after intraperitoneal injection of 1 cc of 4% carmine. Average per cent change in cell volume (D) and plasma protein concentration (E) in the same animals (8 rats).

represents the changes in body-temperature after intraperitoneal administration of 2.0 cc of a 2.0% suspension of blood charcoal.

The expected rise in body-temperature was observed after subcutaneous injection of India ink. After intraperitoneal injection, the body-temperature showed a fall, the degree and duration of which depended upon the amount of ink administered. Dilutions of India ink 1-100 given in 2 cc doses were ineffective; 1-10 dilutions given in the same volumes gave small responses in some animals. These data are not represented in the figure. Blood charcoal given intraperitoneally gave less intense but qualitatively the same response as India ink.

Carmine (Fig. 2) in doses of 4 cc of a 1.0% preparation produced a fever when given subcutaneously (A) but was somewhat less active than 2 cc of India ink. The same dose given intraperitoneally (G) reduced the body-temperature, both qualitatively and quantitatively, much as was done by 2.0 cc of India ink. Intraperitoneal injection (F) of 2.0 cc of 1.0% carmine gave a less prolonged response. The changes in cell volume (subcutaneous injection C; intraperitoneal injection E) and plasma protein concentration (subcutaneous injection B; intraperitoneal injection D) may have been caused by dilution with the water injected. Similar changes followed the injection of saline alone.

The results of intramuscular injection of carmine are shown in Fig. 3 (I). In order to avoid over-distention of the tissue and also to give the same dose of carmine, 1 cc of a 4% preparation was used instead of 4 cc of a 1% preparation. After a short period of hypothermia, a prolonged hyperthermia followed this treatment. The more mild decreases in cell volume and plasma protein concentration after injection of the smaller volumes are consistent with the hypothesis that these phenomena are due to dilution.

In Fig. 3 (II) are shown the results of intraperitoneal injection of 1 cc of the 4% preparation of carmine. This more concentrated preparation given in smaller volume has less effect on body-temperature (F) than 4 cc of the 1% preparation (Fig. 2). At the same time there is a greater fall in the plasma protein concentration (E) and a somewhat greater fall in the cell volume (D). The great fall in plasma protein concentration

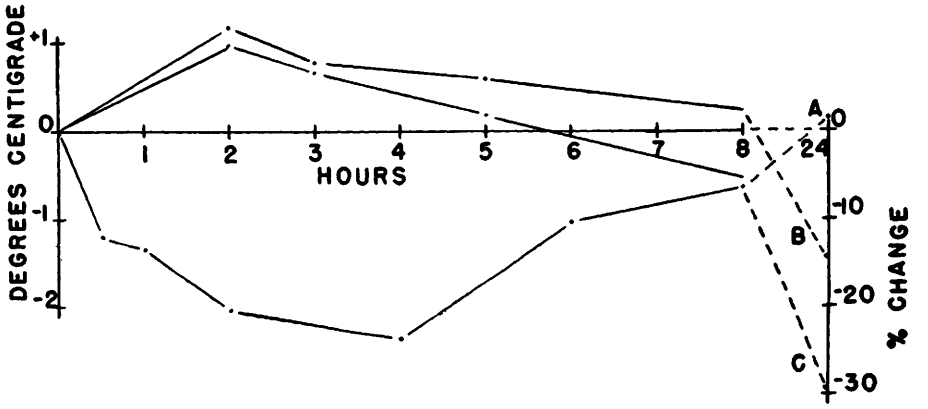

FIG. 4.
Average changes in body-temperature (A) after the intraperitoneal injection of 4 cc of 20% peptone. Per cent change in cell volume (hematocrit) (C) and plasma protein (B) in the same animals (6 rats).

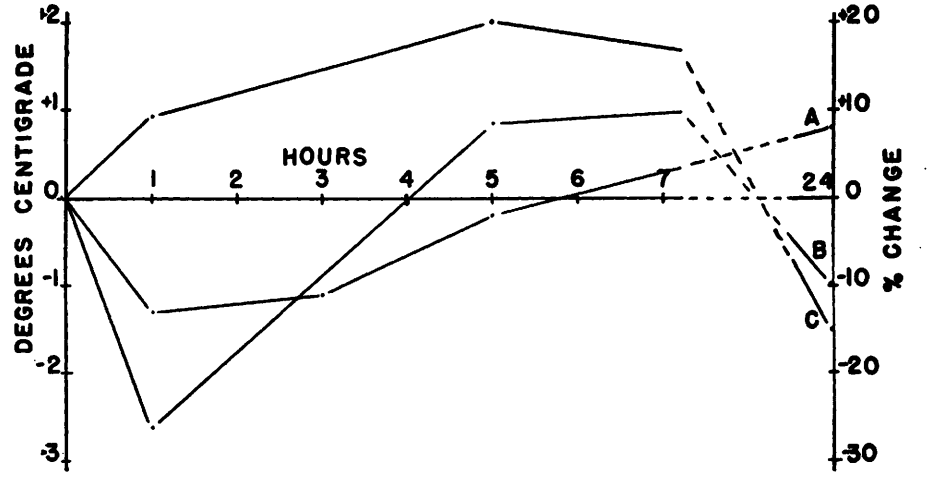

FIG. 5.
Average change in body-temperature (A) after the intraperitoneal injection of 0.5 cc of ox bile. Per cent change in cell volume (hematocrit) (C) and plasma protein (B) in the same animals (6 rats).

(16%) cannot be explained entirely on the basis of dilution. We suggest that the more concentrated carmine preparation contained enough insoluble material that local irritation resulted in mild shock. This, if it is shock, is accompanied, however, not by a more intense but less intense hypothermia.

To save space, no curves are given for the trypan blue experiments. In all cases they were qualitatively like those for carmine; quantitatively, the response was less intense.

Fig. 4 shows curves for body-temperature response and for changes in blood cell volume and plasma protein concentration after intraperitoneal injection of 4 cc of 20% peptone. The decrease in body-temperature (A) is similar to that after 1 cc of India ink or 1 cc of 1% carmine, but is not as great as that after 4 cc of either of these preparations. In spite of the large amount of water administered, there was an early concentration of the blood, as measured by blood cell volume (C) and plasma protein concentration (B), which was followed later by dilution. These changes may be due to an early loss of water into the peritoneal cavity because of hypertonicity of the peptone solution. The fact that the protein and cell volume changes were

closely parallel argues against loss of protein into the tissue through the capillary wall. If shock was present in these animals during hypothermia, it was not severe. We cannot explain the dilution of the blood the following day. The animals appeared to be in good condition.

In Fig. 5 are presented the body-temperature changes (A) and changes in the blood cell volume (C) and plasma protein concentration (B) after intraperitoneal injections of 0.5 cc of ox bile. It was found impossible to administer more than this quantity of bile and keep the animals alive as long as was desired. Following bile administration there was a decrease in plasma protein concentration at the same time that the blood was being concentrated as measured by the cell volume. This can be explained as an early local loss of protein through the damaged capillary walls. At the site of protein loss there was presumably a parallel loss of water. In other areas the water was partly restored but the protein could not be supplied as quickly. The net result of loss of water and of protein in one area and partial replacement of water alone in another area is dilution of the circulating plasma protein and a simultaneous concentration of cells. However, later, the plasma protein concentration was increased, and, at the 5th hour was 8% above the initial value. Finally, there was a roughly parallel decrease in both the cell volume and the plasma protein concentration indicating a general dilution of the blood. At autopsy it was observed that engorgement was intense in the splanchnic veins. We believe that the development of shock after intraperitoneal injection of ox bile was unquestionable. However, in spite of the shock, the intensity and duration of the hypothermia was less than was found after injection of the other hypothermia-provoking agents. Gross examination of the peritoneal surfaces after injection of the substances other than bile showed at most only mild reactions.

Discussion. The substances, India ink, blood charcoal, carmine, and trypan blue, whose dual action on body-temperature is described in this paper, have little in common chemically but, as administered, all are particulate. When given intraperitoneally, they are all rapidly taken up by the lymph channels and can be seen in the reticuloendothelial system. However, the fact that a substance is injected in particulate form does not assure that it will affect body-temperature. This is shown particularly well with injections of suspensions of $BaSO_4$ which are entirely inactive. It may be that the particles of $BaSO_4$ are not phagocytized and do not reach the reticuloendothelial system and that this is the reason for its inactivity. On the other hand, no particulate material can be seen in the clear yeast "autolysates" which have a dual effect on body-temperature in the rat as previously described.[1] The very large molecules of yeast nucleic acid, which are presumed to be the active constituent of yeast autolysate, may be handled similarly to particulate material, though we have, as yet, no evidence of this beyond the similarity in thermal reaction after injection.

At present, no explanation can be given for the fall in body-temperature after the intraperitoneal injection of these substances. There is little evidence of shock, and when severe shock is produced by intraperitoneal injection of ox bile, the fall in body-temperature is less than when particulate substances are injected. The cause of hypothermia would seem to lie rather in some interference with the normal functioning of the reticuloendothelial system. Intramuscular injection of carmine produces a rise in body-temperature similar to that following subcutaneous injection. This would seem to rule out differences in blood supply to the area of injection, and consequent differences in the rate of absorption, as the cause of the dual effect of carmine on body-temperature.

Summary. India ink, suspensions of blood charcoal, carmine, and trypan blue were injected subcutaneously and intraperitoneally. Carmine was injected also intramuscularly. After subcutaneous and intramuscular injection a rise in body-temperature was observed. After intraperitoneal injection a fall in body-temperature was observed. As judged by relative engorgement of capillary beds, and changes in blood cell volume (hematocrit) and plasma protein concentration, these

animals were not in shock during hypothermia. Intraperitoneal injections of ox bile, and solutions of peptone produced a less intense hypothermia although ox bile caused severe shock.

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A Highly Active Material that Promotes Conversion of Prothrombokinase Complex.* (17419)

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A procedure has been described[1] for analyzing blood coagulation in three experimental steps: 1. activation of prothrombokinase; 2. activation of prothrombin; 3. coagulation of fibrinogen. Crude thrombokinase, besides activating prothrombin, also accelerated conversion of prothrombokinase. Both activities were lost upon adsorption with barium sulfate.

It is now reported that a material, prepared by elution from barium sulfate, promotes the activation of prothrombin, and also the conversion of crude prothrombokinase. Beginning with a solution of bovine plasma globulins, fraction "A" was adsorbed on Hyflo (Johns-Manville) and eluted with phosphate-potassium chloride solution. The unadsorbed globulins were exposed to adsorption by barium sulfate. From the latter, two successive fractions were eluted, with 0.2-0.3M phosphate, pH 6.6, and with 0.6M phosphate, pH 7.9. The pH 6.6 eluate had more prothrombin. From the pH 7.9 eluate, proteins soluble in 0.35, but precipitable by 0.45 saturated ammonium sulfate were selected as the "converter fraction." This involved 3 extractions and 20 precipitations. Proteins soluble in 0.45, but precipitable by 0.60 saturated ammonium sulfate, constituted the "thrombin fraction" (3 extractions, 7 precipitations). Fifty-five liters of plasma yielded 10 ml of each fraction. Both were dialyzed simultaneously in the same beaker.

Activations were studied with calcium present, giving results summarized in Table I. At high dilution, the converter hastened both the activation of prothrombin and the conversion of crude prothrombokinase. In comparing different preparations, converter activities estimated by the 2-stage and 3-stage procedures were roughly parallel. This is compatible with the view that the tests measured two different effects of the same substance.

In contrast, converter activity did not parallel thrombin activity. The ratio of converter to thrombin activity was 100 times as great in the converter fraction as in the thrombin fraction. Conceivably, varieties of thrombin can exist with different converter activities, or some factor modifies the behavior of thrombin in one of the fractions. If, as must be contemplated, the converter is distinct from thrombin, the results suggest how difficult would be its elimination from thrombin by salting-out. Moreover, the converter is manifest in sufficiently minute amount to cause confusion, even as a small contamination in a highly purified thrombin.

The relation of "A" and converter to thrombokinase activity resembles that reported for platelets plus "globulin".[2] Their effects are complementary, but not simply additive. Clear evidence has not been obtained that their mutual reinforcement is due chiefly to progressive activation of one by the other. An additional possibility must be considered, that one is thrombokinase and the other contains a co-factor or anti-inhibitor.

* Aided by a grant from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

1 Milstone, J. H., *J. Gen. Physiol.*, 1948, **31**, 301.

2 Milstone, J. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 225.