in stomach weight is due to protein rather than carbohydrate or minerals. The underlying mechanism is unknown.

Summary. Vagal denervation produced a marked increase in stomach weight and a less dramatic loss of intestinal weight. Changes in fat and water content of these structures could not account for the observed changes in weight.

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Action of Protamine on Production and Activity of Heparin-induced Clearing Factor.* (25139)

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It is well established that protamine inhibits clearing factor *activity*. This inhibition can be demonstrated *in vivo*(1,2) and *in vitro*(3, 4). The effect of protamine injections on clearing factor *release* is not clearly established. Bragdon and Havel(5) showed that protamine caused hyperlipemia in fasting rats, and this has been confirmed by several other workers. Bragdon(6) subsequently found that protamine inhibits the disappearance of injected phospholipids from blood. He concluded that this was the result of anti-heparin activity of protamine. Similar conclusions could be drawn from the work of French and Morris(7) who injected C^{14}-labelled chylomicrons obtained from lymph of rats. They found that heparin caused an increase in rate of removal of chylomicron while protamine caused a decrease. According to the hypothesis of Robinson and French(8), clearing factor occurs normally on the capillary surface and may be displaced into the blood stream by injection of heparin or by adsorption on circulating chylomicra. Our recent results(9) support such an hypothesis. The question arises whether protamine may inhibit *release* of clearing factor by attaching itself to the capillary surface. Experiments were carried out to distinguish between an inhibitory effect on *release* of clearing factor and inhibition of clearing factor itself. Determinations were made first on the time protamine remained in the circulating blood after injection and, secondly how long after a protamine injection was it before a challenging dose of heparin would elicit full release of clearing factor.

Materials and methods. Protamine. Commercial product (Connaught Medical Labs) prepared as 1% solution, was used. *Clearing factor assay* was carried out by the method of Monkhouse and Mackneson(9). Lipemic substrate was prepared by adding either commercial fat emulsion, "Ediol," or chylomicrons to pooled oxalated dog plasma previously heated to 56°C for 5 minutes and stored at –20°C. Changes in turbidity were measured in Coleman Junior spectrophotometer at 500 mμ after 1 hour incubation at 37° C, and the activity expressed as changes in transmission for 0.1 ml of plasma being assayed. *Clearing factor release.* Each rat was given 50 units of heparin in 1 ml of saline solution intravenously, and the post heparin plasma taken 6 minutes thereafter. *Heparin* was kindly supplied by Connaught Medical Labs. Blood was removed from rats *via* exposed jugular vein. All samples were taken into one-tenth volume of 3.8% trisodium citrate solution. *Fat diet* contained approximately 40% protein (7% casein, 28% alcohol extract of peanut meat, 5% soya protein) 34% lard, 2% cholesterol, 10% sucrose and adequate vitamin supplements.

Results. Fig. 1 illustrates the relationship between protamine concentration and inhibition of clearing activity. Various dilutions of protamine were made with pooled citrated rat plasma. To each of 2 tubes, one containing

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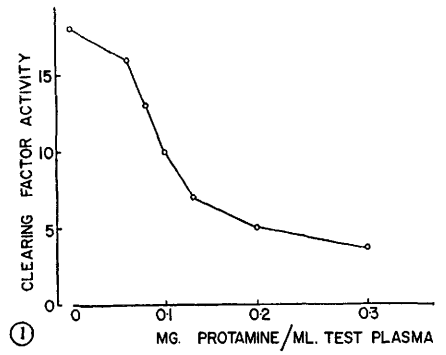
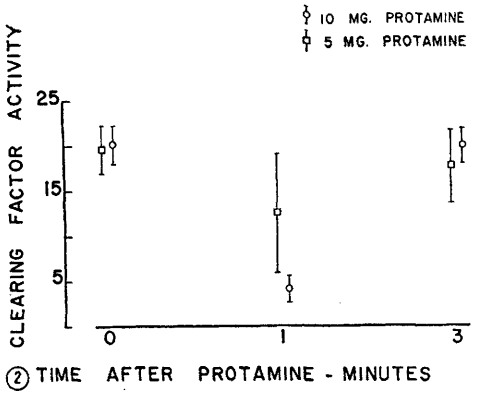
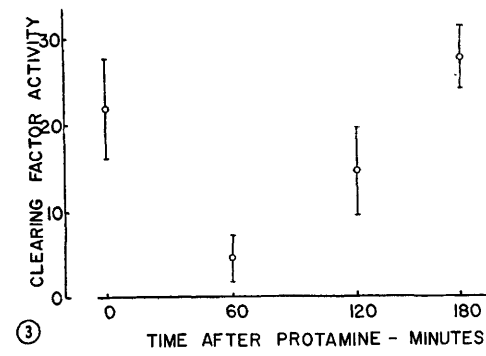


① FIG. 1. Inhibition of clearing factor activity with different concentration of protamine added *in vitro*.



② FIG. 2. *In vitro* clearing factor inhibitory activity of plasma withdrawn from rats at different times after they had received intrav. protamine. Each point is represented by 4 animals. Mean values and stand. dev. are shown.



③ FIG. 3. Inhibition of *release* of clearing factor in rats following intrav. inj. of 5 mg of protamine. Each point represented by 4 animals. Mean values and stand. dev. are shown.

0.05 ml of post heparin plasma and the other 0.10 ml was added 0.2 ml of various protamine dilutions. Each tube contained 1.6 ml of lipemic substrate and total volume was adjusted to 2.0 ml with saline. Increasing inhibition was obtained with plasma protamine concentrations up to 0.20 mg/ml plasma.

Protamine was injected intravenously into rats and samples of blood withdrawn every minute thereafter. Fig. 2 shows that protamine leaves the circulation extremely rapidly. Even with doses as high as 10 mg/rat no significant inhibition of clearing factor was observed with plasma obtained from animals 4 minutes after they received the protamine. When injections of protamine were made intraperitoneally only slight inhibition of clearing factor was obtained with plasma from injected animals. If inhibition did occur it was usually detectable in plasma between 30 and 60 minutes after injection.

In contrast to its transient stay in the circulation, a single injection of protamine has a prolonged effect on *release* of clearing factor. Fig. 3 shows the effect of intravenous injection of protamine on release of clearing factor activity by heparin. Twelve rats each received 5 mg of protamine intravenously. At each of 3 intervals, 60, 120 and 180 minutes thereafter a group of 4 rats received 50 units of heparin intravenously and 6 minutes later a sample of blood was withdrawn for clearing activity assay. There was still a decrease in ability of rats to release clearing factor 2 hours after protamine injection. This experiment was repeated with intraperitoneal injections of protamine in doses of 10 mg/rat. Again inhibition of clearing factor release was obtained, but to a much lesser degree and the results were more variable. It is worth noting that inhibition could be obtained in some animals as early as 5 minutes after injection.

Experiments were then carried out to determine whether repeated daily injections of protamine over 2 weeks would result in a more permanent impairment of clearing factor production. In a preliminary experiment intraperitoneal injections of protamine, saline or heparin were given. One ml of one of the solutions was given daily for 2 weeks to each animal. The protamine and heparin solutions both contained 10 mg/ml. Three of the rats receiving heparin died with intraperitoneal hemorrhage. Twenty-four hours after the last injection each animal received the stand-

ard 50 units of heparin and 6 minutes later a sample of blood was removed for clearing factor assay. The protamine treated animals showed no decrease in ability to produce clearing factor as compared to saline injected animals.

The experiment was repeated and extended to include variations in diet and temperature. Two groups of 24 rats each were used. One group was kept at room temperature and in a refrigerated room at 2°C. Animals were given a chow diet and maintained in their respective environments for 6 months. Six weeks prior to measuring clearing factor production, half of the rats in each group were put on a fat diet. After 4 weeks on the diet, half of the rats on the fat diet and half of those on the chow diet were given daily intracardial injections of 10 mg of protamine for 2 weeks. Twenty-four hours after last protamine injection, all animals were tested for ability to produce clearing factor. There was no significant decrease in ability to release clearing factor in the protamine treated rats when compared with controls. However, animals in the cold appeared to release more clearing factor than those at room temperature.

The effect of cold adaptation was repeated. Clearing factor release was compared in 10 rats which had been on a chow diet in the cold for 6 months with 10 rats of same age kept at room temperature for similar time. This time, clearing activity was measured according to rate of change in transmission minute for the first 15 minutes incubation. This is considered to be a more sensitive test for enzyme activity(4). The results are shown in Table I. It can be seen that animals kept in the cold, release more clearing factor activity following standard heparin injection. Only one of 10 rats kept at room temperature had a value as high as animals in the cold.

Conclusions. (1) Samples of plasma taken from rats one to 3 minutes after they received an intravenous injection of protamine will inhibit clearing factor. Degree of inhibition varies with dose given, but even with doses as high as 10 mg no inhibition can be detected in the plasma 4 minutes after injection. An intravenous dose of 5 mg of protamine inhibited *release* of clearing factor for a period of more than 2 hours. It would seem that protamine which has been so quickly removed from the circulation, has been deposited at sites of clearing factor release. The simplest explanation, but by no means the only one, would be to assume that the site of clearing factor release is the endothelial lining of the vessels. This explanation would be in support of the hypothesis proposed by Robinson and French (8). (2) With intraperitoneal injections of protamine, inhibition of clearing factor release was detected in some animals within 5 minutes, whereas inhibitory activity was not detected in plasma until 30 to 60 minutes after injection, if at all. This was probably due to the fact that protamine was removed from the circulation almost as rapidly as it entered, and its removal blocked release of clearing factor. (3) Experiments in which protamine was injected daily for periods of 2 weeks indicated that the reported increase in atheromatous plagues(10) was probably not attributable to any prolonged impairment in clearing factor production. There would however be daily periods of from 2 to 4 hours immediately after each injection when there would be an impairment and this could be a contributing factor. (4) The increased ability to release clearing factor in animals adapted to cold environment may be associated with the general increase in metabolic rate. This increase in level of enzyme activity brought on by a physiological change encourages the thought that it has indeed a physiological function.

TABLE I. Clearing Factor Activity in Rats. Expressed as rate of change of transmission/min./ml of post heparin plasma. Figures are avg values and their stand. dev. based on estimates from 10 animals in each group. The differences are significant ($p = 0.005$).

Exposed to cold	At room temp.
14.7 ± 1.4	11.1 ± 2.2

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Differential Survival to Leukemia as a Function of Infantile Stimulation in DBA/2 Mice.* (25140)

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The influence of environmental factors on physiological activity has been well documented. The effects of emotional stress(1) and periodicity(2) are examples of environmental influences on physiological functioning. Recently there has appeared evidence to indicate that the nature of environment during organism's infancy exerts profound influence on physiological activity later in life. For example, rats which received extra stimulation during infancy show less mortality(3) following 120 hours of food and water deprivation and less adrenal hypertrophy 24 hours following an injection of hypertonic glucose(4) than do nonstimulated controls. This communication presents findings concerning the effects of infantile stimulation on length of survival following implantation of leukemia 1210 in DBA/2 mice.

Materials and methods. Ninety mice were used. Litters were assigned at birth alternately to either the stimulated or non-stimulated condition. Experimental treatment consisted of removing the pup from the nest, placing it in a 2 x 3 x 6″ compartment for 3 minutes, then returning the infant to the nest. This procedure was initiated on day following birth and was continued once daily until weaning on day 24. There were 42 mice in this treatment group. The non-treated subjects (N = 48) remained in the nest and were not treated in any manner until weaning. On day 24 all mice were weaned and placed in group cages. No animals were manipulated from weaning until time of implantation. All animals were implanted interperitoneally with 2 x 10^6 leukemic cells in 0.1 ml volume of Locke's solution when they reached a weight of at least 18 g, *i.e.,* between ages 45 to 55 days. The tumor used, L-1210, is a transplantable lymphoid leukemia induced in DBA/2 mice(5), maintained in the tumor bank at Battelle Memorial Inst. by weekly implantation of 2 x 10^6 tumor cells into the peritoneal cavity. This tumor has been very consistent in causing death in 100% of implanted animals with mean survival time over the past year between 8 to 9 days, and with a latent period of 2 to 3 days after implantation.

Results. (Table I) shows that animals stimulated by manipulation during postnatal period have lower survival times than nonstimulated mice. In the first and second replications there was no overlap between groups, thus obviating the need for statistical analysis. A median test was used for the third replication and yielded an X^2 of 20.16 significant beyond the .001 level of probability. Although there were some differences between the replications, these may in part be attributable to the fact that a transplantable tumor shows changes during the course of repeated transplantation. These changes may manifest themselves as slight variations in mean survival time of animals implanted. Furthermore, the experimental subjects, although highly inbred, may show fluctuation in re-

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