ously(3,4,6). Sarcoma 37 in contrast to the leucocyte does not behave in the same manner toward the material from Serratia since there is no demonstrable effect in tissue culture (7,8).

The inhibition by the material from Gram negative organisms would appear to be more complex than "negative chemotaxis" since the material is distributed throughout the medium of red cells, white cells and plasma. The zone of stimulation noted with decreasing concentrations of the material would be more in favor of a direct effect on the leucocyte.

Summary. The complex carbohydrate from *S. typhosa, P. aeruginosa,* and Serratia as well as a filtrate from *N. intercellularis* inhibited the migration of human leucocytes. The same effect was noted with whole bacteria of *S. typhosa* and *P. aeruginosa.* No inhibition was seen with *M. aureus, D. pneumoniae* Type III or with the polysaccharide of Type III.

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Role of Pancreas in Hypoglycemia Responsiveness and Associated Rise of Circulating Eosinophiles.* (19852)

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In the normal organism the intravenous administration of a small dose of crystalline insulin (0.1 unit/kilo) causes an abrupt decline of the blood sugar with the nadir at 20 to 30 minutes followed by a sharp secondary rise reaching the fasting level by 150 minutes (1). This secondary rise is probably not due to a termination of the insulin effect, since in experiments in which the duration of action of insulin is measured by the time during which glucose must be injected in order to prevent the blood sugar level from declining, it has been found that 5 units of insulin have a duration of action of 6 hours(2). This evidence, together with the fact that hypophysectomized and adrenalectomized animals in which the apparent duration of insulin action is much greater than in the normal, has led to the hypothesis that the secondary rise of the blood sugar level in the insulin tolerance test is a result of stimulation of a body homeostatic mechanism which tends to restore the original blood sugar level(3,4). This phenomenon is properly termed hypoglycemia responsiveness and in order for it to make its appearance two conditions must be met: (1) There must be a sufficient reserve of hepatic glycogen to allow for the observed rise in the blood sugar concentration and (2) a mechanism for glycogenolysis must be activated by the falling blood sugar level(5). It has been hypothecated that the mechanisms stimulated by hypoglycemia are located in the hypophysis, the adrenal cortex, or the sympathetic nervous system(3,4). In a previous study it was observed that insulin administered to normal individuals is accompanied by a marked rise of the absolute lymphocyte count(6). In diabetic patients, however, the extent of the

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rise was proportional to the degree of hypoglycemia responsiveness evidenced by the insulin tolerance test(7). This led to the hypothesis that the trigger mechanism for hypoglycemia responsiveness is identical with that causing an increase in the absolute lymphocyte count after insulin administration. Similarly, it was also found that the eosinophile level parallels the change in absolute lymphocyte count(8,9,10,11). The result of experimental studies undertaken for the purpose of localizing the site of this trigger mechanism may be summarized as follows: 1) In previous studies it was shown that the eosinophile(11) and the blood sugar response(12) of the adreno-demedullated dog to insulin are similar to those of the intact animal. 2) Both hypophysectomized and adrenalectomized animals demonstrate an impairment of hypoglycemia responsiveness but a rise of the eosinophile level after insulin(11). This impairment of hypoglycemia responsiveness, however, is probably attributable to the depletion of liver glycogen in these animals rather than the ablation of the trigger mechanism. 3) In growth hormone treated adrenalectomized animals the response to insulin hypoglycemia is restored to normal(13). These data would tend to rule out the hypophysis, the adrenal cortex or the adrenal medulla as the seat of this mechanism.

The present study was undertaken to determine whether the pancreatic hyperglycemic factor may be implicated in the dual mechanism of hypoglycemia responsiveness and the associated rise of the eosinophile level.

Material and methods. The study was carried out on four groups of dogs: 1) 10 intact control animals who received 0.1 unit of crystalline insulin (Lilly) per kilo of body weight intravenously. 2) 5 intact animals who received insulin free of hyperglycemic factor (Lilly Lot No. T-2536) instead of crystalline insulin. 3) 5 intact animals who received 1 cc of normal saline solution intravenously instead of insulin. 4) 5 pancreatectomized dogs who received 0.1 unit of crystalline insulin per kilo of body weight intravenously. Duplicate experiments were done in this group of animals with insulin devoid of hyperglycemic factor.

All animals had been maintained on a diet of canned dog food and table scraps. In addition, the pancreatectomized animals were given 5 g of lecithin and 12 units of regular insulin twice daily. No insulin, however, was administered on the day preceding the experiment. Furthermore, the experiments in operated animals were not carried out until the 14th postoperative day and were repeated in the same dog at 2 week intervals. The test material was injected after a 24 hour fast and 2 hours after the induction of intraperitoneal nembutal anaesthesia. When necessary as indicated by a returning corneal reflex, the animals were given additional small doses of anaesthetic. Duplicate venous blood samples for sugar and direct eosinophile counts were collected at 0, 20, 30, 45, 60, 90, 120, and 150 minutes after the injection. The blood sugar determinations were done by the Nelson modification of the Folin-Wu micro method(14) and the eosinophile counts were done according to the procedure of Randolph (15). In conformity with the method of reporting results used previously(16,17,18) the change of the eosinophile level was expressed in terms of the percentage change from the fasting value which was taken as zero percent.

Results. In the 80 determinations of the absolute eosinophile level in these 10 controls, the values ranged from $+22\%$ to -16% of the initial value with a standard deviation of $\pm 8\%$. Therefore, in evaluating the experimental series, any change of the absolute eosinophile level of $\pm 24\%$ (3 times the standard deviation) of the fasting value or less was considered to be non-significant.

The experiments were started two hours after the induction of anaesthesia since it was found that in the untreated normal dog this time interval allowed for recovery from the initial stress incident to anaesthetizing the animal.

Results. The mean variation of the blood sugar and the eosinophile level of the saline control animals are represented in Fig. 1, Curve A and A'.

After the administration of commercial crystalline insulin to the intact animal the lowest blood sugar level was reached at 20,

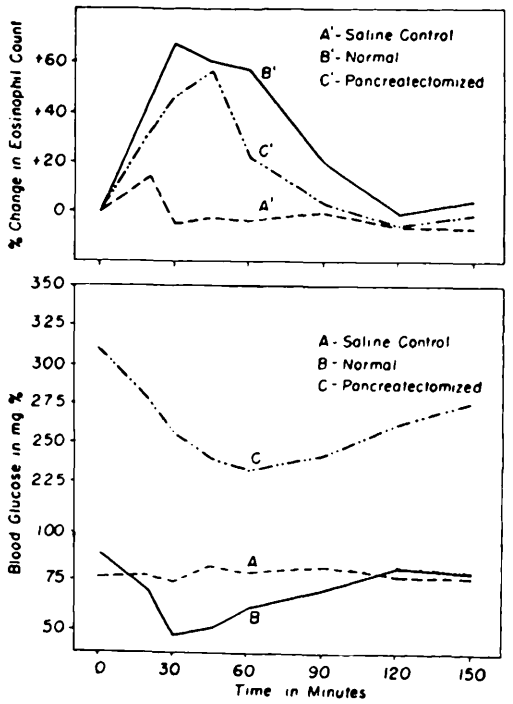

FIG. 1. Mean blood sugar levels and mean percentage change of the eosinophile count after the intrav. inj. of insulin in normal and pancreatectomized dogs compared with saline controls.

30, or 45 minutes whence it rose gradually again approaching the fasting level by 150 minutes. This was accompanied in all instances by a significant rise in the eosinophile level varying from +45% to +120% of the fasting value which reached its peak at 30 to 60 minutes and was then followed by a gradual decline. The change in the blood sugar and eosinophile level after insulin free of hyperglycemic factor was in all respects similar to that observed after crystalline insulin. The mean values both of blood sugar and eosinophiles are shown in Fig. 1, Curve B and B').

In the pancreatectomized animal the mean fasting blood sugar level was 315 mg %. After the administration of insulin the lowest mean blood sugar of 230 mg % was reached at 60 minutes and it then increased gradually so that the mean 150 minute blood sugar value was 45 mg % above nadir (Fig. 1, Curve C). The accompanying change in the eosinophile level was almost the same as in the intact animal except that the peak response was moderately delayed and somewhat lower (Curve C'), the value varying from +48% to +87% of the fasting level.

Comment. These results indicate that the presence of the pancreas is not necessary for the hypoglycemia responsiveness after the injection of insulin nor for the associated rise of the eosinophiles.

That this rise of eosinophile level is probably not due to a foreign protein reaction is shown by the fact that inactivated insulin when similarly injected is not accompanied by this phenomenon.

The principal evidence for the role of hyperglycemic factor as a physiologic insulin antagonist has been the finding of an impurity in ordinary crystalline insulin which raises the blood sugar level and which can be isolated from the pancreas even in alloxan diabetic animals(19). This substance has been shown to act through hepatic glycogenolysis(20). The relatively more severe diabetes of the alloxan diabetic as compared to that of the pancreatectomized dog has also been attributed to this substance(21).

It is possible that hypoglycemia responsiveness is effected by a direct action of the glycogenolytic fibers of the sympathetic system on the liver and that the rise of the eosinophile level after insulin may also be due to sympathetic stimulation. This possibility is supported by the fact that stimulation of the nerves of the hepatic plexus will produce hyperglycemia in the fed adrenalectomized animal by breakdown of liver glycogen, and that the sympathectomized animal has an increased insulin sensitivity(22). Studies to test this hypothesis are in progress.

Summary. In the pancreatectomized animal the intravenous administration of both commercial crystalline insulin and insulin free of hyperglycemic factor is followed by a rise of the absolute eosinophile count and associated decline of the blood sugar level with a subsequent rise, similar to that observed in the normal. This is interpreted to signify that the hyperglycemic factor of the pancreas is not part of the mechanism for hypoglycemia responsiveness.

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Effects of Shock and Cold on Mouse Liver Sulfhydryl.* (19853)

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While testing the effects of *S. marcescens* tumor damaging bacterial polysaccharide(2) on concentration of iodine-reducing substances in mouse tissues, one of us noted(1) that toxic doses of this substance induced in mouse liver an appreciable decrease in concentration of non-protein sulfhydryl, measured as the difference between total iodine-reducing substances and iodine reduction due to ascorbic acid. Toxic doses of the polysaccharide markedly depress body temperature, circulation and reactivity of mice(3,4,5), as do also burns(6) and ligation trauma(7). This suggested the desirability of testing for effects of burn shock and traumatic shock on concentration of sulfhydryl compounds in mouse liver. The effects of a non-shock stress situation (exposure to cold) on liver sulfhydryl were also studied.

Materials and methods. Distilled water used: Ordinary distilled water was passed through a LaMotte Filter-ion exchange column, to remove traces of heavy metal ions reactive with sulfhydryl groupings. *Preparation of liver extracts.* A. *Solutions.* M/10 potassium phosphate buffer of pH = 6.8 (KH_2PO_4, K_2HPO_4 1:1); 22% aqueous sulfosalicylic acid. B. *Procedure.* The test mouse was decapitated, the liver promptly excised, blotted to remove excess blood, weighed to within 10 mg, placed in the micro-cup of the Waring blender, and homogenized with 10 cc of ice-cold phosphate buffer for 30 seconds. Additional phosphate buffer was then added, to a total of 19 cc of the buffer for each gram of liver tissue. Homogenization was continued for another 30 seconds. The homogenate was centrifuged at about 500 g for 5 minutes to

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