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SECTION MEETINGS

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Mechanism of Hypoglycemia Responsiveness in Relation to Changes in Blood Eosinophile Level.* (19500)

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In the normal individual the intravenous administration of a small dose of insulin causes an abrupt decline of the blood sugar concentration which is followed within 20 to 30 minutes by a sharp rise. This phenomenon is considered to be a result of stimulation of a homeostatic mechanism for restoration of normal blood sugar levels and not of the cessation of the activity of the injected insulin. In previous investigations we demonstrated that the moderate hypoglycemia caused by the intravenous administration of 0.1 unit of crystalline insulin per kg of body weight in normal individuals is accompanied by a significant rise of the absolute lymphocyte count with the peak at 45 to 90 minutes after the insulin administration(1). In a subsequent report it was shown that the mean percentage increase of the absolute lymphocyte count above the fasting value in diabetic patients given the same insulin dosage declined with increasing degrees of impairment of hypoglycemia responsiveness(2). Moreover, 2 diabetic patients in whom there was no evidence of counter-regulatory response to the falling blood sugar level in the Insulin Tolerance Test, failed to show a rise in the absolute lymphocyte count. This suggested that the same mechanism which is responsible for the rise of the absolute lymphocyte count is also responsible for antagonizing the action of insulin on the blood sugar level.

The present study was undertaken with the objective of elucidating the mechanism of the lymphocytosis after insulin and at the same time it was hoped to clarify the trigger mechanism for hypoglycemia responsiveness. Since

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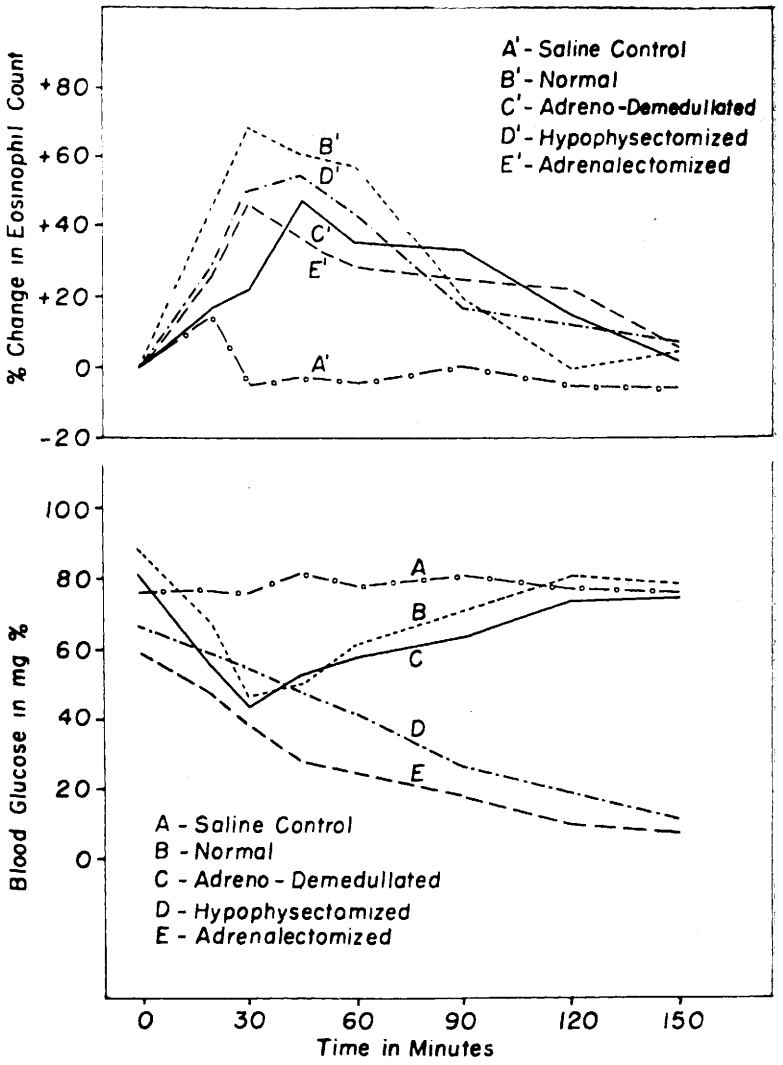

FIG. 1. Mean blood sugar levels and mean percentage change of the eosinophil count after intravenous injection of insulin in normal, adreno-demedullated, adrenalectomized, and hypophysectomized dogs compared with saline controls.

it was observed that the change in the eosinophil level paralleled the change in absolute lymphocyte count, for technical reasons this response was studied rather than the lymphocyte response(3-6).

Material and methods. The study was carried out on normal, adreno-demedullated, bilaterally adrenalectomized and hypophysectomized mongrel dogs weighing from 15 to 20 kg. A 125 mg Percorten tablet (Ciba) was inserted subcutaneously immediately postoperatively in all adrenalectomized animals. Both the adrenalectomized and hypophysectomized animals were maintained on normal saline in addition to the regular diet of canned dog food and table scraps. The experimental animals were divided into 4 groups: 1. 10 normal controls; 2. 5-adreno-demedullated dogs; 3. 3 hypophysectomized dogs; 4. 5 adrenalectomized dogs.

The experiments in the operated animals were carried out after the 14th post-operative day and were repeated on the same dog at 2-week intervals. After a 24-hour fast and

2 hours after the induction of intraperitoneal nembutal anesthesia, 0.1 unit of crystalline insulin (Lilly) per kg of body weight was injected intravenously. When necessary, as indicated by a returning corneal reflex, the animals were given additional small doses of anesthesia. Furthermore, 6 control experiments using saline instead of insulin were carried out. Duplicate venous blood samples were collected before and at 20, 30, 45, 60, 90, 120, and 150 minutes after the injection of insulin. The blood sugar determinations were done on serum by the Nelson modification of the Folin-Wu micro method(7) and the eosinophil counts were done according to the procedure described by Randolph(8). The change of the eosinophil level was expressed in terms of the percentage change from the fasting value which was taken as 0%. The experiments were started 2 hours after the induction of anesthesia since it was found that in the untreated normal dog this time interval allowed for recovery from the initial stress incident to anesthetizing the animal.

Results. Both the blood sugar and the eosinophil level of the saline control animals showed a non-significant variation from the fasting level (Fig. 1, Curve A, A^1). In the intact animal the lowest blood sugar after insulin was reached within 20, 30, or 45 minutes whence it rose gradually. This was accompanied in all instances by a significant rise in the eosinophil level which reached its peak at 30 to 60 minutes and was then followed by a gradual decline. The mean values are shown in Fig. 1 (Curve B, B^1). The response of the blood sugar level and of the eosinophil level of the adreno-demedullated animal after insulin administration was in all respects similar to that of the intact dog (Fig. 1, Curve C, C^1). In the hypophysectomized and the adrenalectomized animals the fasting blood sugar level was depressed and after injection of insulin it declined progressively with no evidence of hypoglycemia responsiveness (Fig. 1, Curve D, E). However, the eosinophil level in both groups, on the other hand, showed a significant rise which was similar to that of the normal (Fig. 1, Curve D^1, E^1).

Discussion. Cannon *et al.*(9) showed that hypoglycemia causes a release of epinephrine. In view of the glycogenolytic action of the medullary secretion it has been assumed that hypoglycemia responsiveness is based on a stimulation of the adrenal medulla(10,11). However, Zucker and Berg(12) demonstrated that the adreno-demedullated animal has a normal response to insulin. This has been confirmed by our experiments. Furthermore, the rise of the eosinophil level in the adreno-demedullated animal is similar in all respects to that of the normal, indicating that this response is not mediated by activation of the adrenal medulla.

However, it is generally agreed that in the absence of the hypophysis or the adrenal there is a failure of hypoglycemia responsiveness (13,14). This has led to the hypothesis that an immediate response of the pituitary-adrenal axis is the mechanism for hypoglycemia responsiveness. This viewpoint is supported by the finding that there is a decline of adrenal cholesterol and ascorbic acid as a response to a lowered blood sugar level, indicating a release of glucocorticoids(15).

Since in both the hypophysectomized and adrenalectomized animals the rise of the absolute eosinophil count after insulin was similar to that in the normal, it can be assumed that this response is not effected by a change in activity of the anterior pituitary or the adrenal cortex. As previously stated, the mechanism for the rise of the eosinophil level is probably identical with that of hypoglycemia responsiveness. Therefore, it can also be inferred that an immediate response of the adrenal cortex and the anterior lobe of the pituitary are not a necessary component of the homeostatic mechanism even though the hypophysectomized and adrenalectomized animals are hypoglycemia unresponsive. This is supported by a previous hypothesis(16) that the failure of hypoglycemia responsiveness which is observed after removal of the pituitary or adrenal glands may be primarily the result of depletion of the glycogen stores of the liver rather than the destruction of the mediating mechanism.

Summary. 1. The intravenous administration of 0.1 unit of crystalline insulin/kilo body weight to the intact anesthetized dog caused

an abrupt decline of the blood sugar level which is followed within 20 to 45 minutes by a sharp rise. This is accompanied by a significant rise of the eosinophil level which is thought to be mediated by the same mechanisms as is responsible for the rise of the blood sugar level. 2. The adreno-demedullated animal shows normal hypoglycemia responsiveness in the insulin tolerance test and an accompanying rise of the eosinophil level similar to that of the intact animal. This indicates that the medullary secretion is unnecessary for either of these phenomena. 3. In the adrenalectomized and hypophysectomized animals a rise of the eosinophil level similar to that of the normal was demonstrated after insulin, even though the blood sugar changes showed no evidence of hypoglycemia responsiveness. This is interpreted to signify that the mechanism for hypoglycemia responsiveness in the hypophysectomized and adrenalectomized animal is intact but is not apparent, due to depletion of liver glycogen in the absence of the anterior-pituitary and adreno-cortical secretions.

1. Lazarus, S. S., Volk, B. W., Jacobi, M., and Zymaris, M., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 432.

2. Lazarus, S. S., Volk, B. W., Jacobi, M., Slade, W. R., and Zymaris, M., *Am. J. Clin. Path.*, 1951, v21, 436.

3. Elmadjian, F., Freeman, H., and Pincus, G., *Endocrin.*, 1946, v39, 293.

4. Freeman, H., and Elmadjian, F., *J. Clin. Endocrin.*, 1946, v6, 668.

5. Jordan, P. H., Last, J. H., Pitesby, I., and Bond, E., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 243.

6. Gellhorn, E., and Frank, S., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 112.

7. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

8. Randolph, T. G., *J. Lab. Clin. Med.*, 1949, v34, 1696.

9. Cannon, W. B., McIver, M. A., and Bliss, S. W., *Am. J. Phys.*, 1924, v69, 46.

10. Somogyi, M., *J. Biol. Chem.*, 1949, v179, 217.

11. Bouckaert, J. P., and deDuve, C., *Physiol. Rev.*, 1947, v27, 39.

12. Zucker, T. F., and Berg, B. N., *Am. J. Phys.*, 1937, v119, 539.

13. Fraser, R. W., Albright, F., and Smith, P. H., *J. Clin. Endocrinol.*, 1941, v1, 297.

14. Houssay, B. A., *Endocrinology*, 1942, v30, 884.

15. Steeples, G. L., Jr., and Jensen, H., *Am. J. Phys.*, 1949, v157, 418.

16. Engel, F. L., and Scott, J. L., *J. Clin. Invest.*, 1950, v29, 151.

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Autonomous Transplantable Pituitary Tumors Arising in Growths Dependent on Absence of the Thyroid Gland.* (19501)

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"Invasive," "autonomous," "unrestrained" are adjectives commonly used to characterize cancerous growth. The study now to be described indicates the uncertainty of these terms in relation to neoplastic growth. Autonomy may be only apparent or partial, can never be certified to be absolute, and metastases may occur with conditioned neoplasms. Destruction of the thyroid by I^{131} (250-300 μc) is followed by the development of pituitary growths 10 to 14 months later(1). These growths can be grafted readily in the muscle of mice similarly radio-thyroidectomized but not in normal mice(2). They grow progressively in the former until the death of the host and almost invariably metastasize to the draining lymph nodes. In over 100 such tumor-bearing mice not a single spontaneous regression was noted.

The procedures of inducing and transplanting the pituitary tumors have been described (2). All studies have been made in C-57 black mice. Seven transplantable strains were established thus far in radio-thyroidectomized

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