PHARMACOLOGICAL REVIEWS
Vol. 18, No. 1, Part 1

Printed in U.S.A.

R. EFFECTS OF GLUCOSE DEPRIVATION ON THE SYMPATHETIC OUTFLOW TO THE ADRENAL MEDULLA AND ADIPOSE TISSUE[1]

ALAN GOLDFIEN

Cardiovascular Research Institute and Departments of Obstetrics and Gynecology and Medicine, University of California School of Medicine, San Francisco

Mechanisms for the storage and retrieval of substrates for intermediary metabolism are required for the intermittent feeding pattern observed in many species. The integrative function of the central nervous system and its unique dependence on glucose for energy metabolism suggest that it might play an important role in the regulation of these mechanisms.

Studies of the activity of the sympathetic nervous system during periods of glucose deprivation have led us to conclude that it plays an important part in the regulation of the retrieval mechanisms for glucose and free fatty acids (FFA) in the dog. The experiments to be described indicate that insulin-induced hypoglycemia or the administration of 2-deoxyglucose (2-DG),[2] known to stimulate adrenal medullary secretion, or both, increase the mobilization of FFA from adipose tissue stores by activation of the thoracic sympathetic outflow. The evidence to be presented also shows that centers mediating these activities are in the spinal cord. Preliminary evidence indicates that glucose receptors regulating adrenal medullary secretion are located in the spinal cord.

The following studies were carried out with male mongrel dogs anesthetized with pentobarbital (25 to 30 mg/kg) after a 12- to 24-hr fast. The femoral arteries were cannulated bilaterally for the purpose of measuring arterial pulse and blood pressure and for obtaining arterial blood samples to be analyzed for glucose by the method of Teller (20), FFA by the method of Trout *et al.* (21) and 2-DG by the method of Blecher (2). The animals were artificially respired by a pump. Adrenal vein plasma concentrations of epinephrine (E) and norepinephrine (NE) were measured by the method of Goldfien *et al.* (11). Additional procedures were performed as noted.

Hypoglycemia resulting from an injection of insulin to a fasting dog produces a marked increase in adrenal medullary secretion (3, 10) with a large increase in the rate of secretion of E and a small increase in the release of NE (10). These effects are reversed by the administration of glucose (10), and when the fall in blood sugar is prevented by the simultaneous administration of insulin and glucose, the increase in adrenal medullary secretion is not produced (10). The secretory response to hypoglycemia is mediated through the nerves supplying the adrenal medulla (3).

In order to locate the portion of the central nervous system required for the the response to hypoglycemia, insulin was administered to dogs with lesions in

[1] Supported in part by grants H-06285, HD-00640, and TIHD7-06 from the National Institutes of Health, U. S. Public Health Service.

[2] 2-Deoxy-D-glucose (glucose free), A grade, Calbiochem.

Effect Of A Lesion In The Area Postrema On The Response Of Adrenal Venous Catecholamines To Insulin

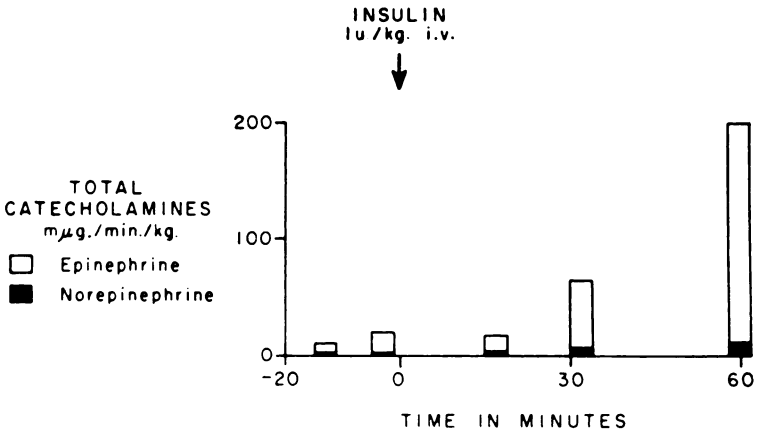

FIG. 1. The effect of insulin given to 2 dogs with chronic lesions in the area postrema. A very high rate of secretion from the right adrenal was observed.

the brain or spinal cord. Direct evidence suggesting a role of the hypothalamus in the response to hypoglycemia is lacking, but the suppression of adrenal medullary secretion by the injection of glucose into certain areas of the hypothalamus, demonstrated by Dunér (5), has led many to conclude that such centers are present. If they are present, it is unlikely that they coincide with the loci studied by Dunér since the injection of Ringer's solution without glucose did not increase the secretion of E.

When hypoglycemia was produced in dogs after transection of the midbrain at the midcollicular level, to exclude hypothalamic connections, we observed a normal increase in amine secretion (4). These results indicate that the hypothalamus is not required for the response but do not exclude its participation.

Evidence linking the brain stem and area postrema with the control of glucose metabolism has been noted by Wise *et al.* (22). We have studied the effect of insulin-induced hypoglycemia in dogs with chronic lesions in the area postrema. The details of the surgical procedure and the effects of insulin on blood sugar levels have been reported (22). The mean secretion rate of E and NE in 2 of these dogs is shown in figure 1. The response may have been enhanced in these dogs since each showed a higher rate of secretion than we have observed in other experiments.

Further experiments indicated that lesions in the cervical region of the spinal cord did not abolish the response. However, removal of segments of the spinal cord which included T_5– T_7 entirely prevented an increase in the secretion of E and NE (4). In order to obtain further evidence for a center in this region,

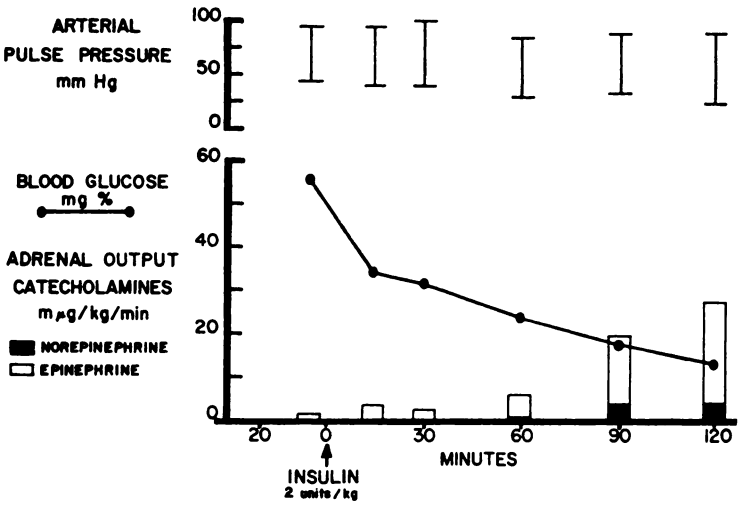

FIG. 2. The effects of insulin given to a dog in which the upper and lower parts of the spinal cord were removed, leaving the segment from C_6–T_{11} *in situ*. The dorsal roots to the segment were severed.

several attempts were made to create an island of spinal cord severed from its connections above and below and isolated from its afferent pathways by cutting the dorsal roots inside the dura. Only one such animal responded in a normal fashion (19). This experiment is illustrated in figure 2. These results indicate the presence of glucose receptors in the spinal cord. We have not pursued these experiments because of technical difficulties. The glucose receptors in the pancreas and liver that regulate insulin secretion and the output of glucose by the liver do not seem to be required for the response since pancreatectomized (18) and eviscerated (9) dogs respond to hypoglycemia with an increase in adrenal medullary secretion.

Augmentation of adrenal medullary secretion is not the only manifestation of increased sympathetic activity during periods of glucose deprivation. Havel and Goldfien (12) found evidence of tonic stimulation of fat mobilization in fasting dogs. We also found NE to be almost as effective as E in raising plasma FFA levels in man and the dog. The following experiments were designed to determine whether enhanced fat mobilization during periods of glucose deprivation might also be under the control of centers in the nervous system.

The technical difficulties involved in cross perfusion of the dog brain and the local action of insulin in adipose tissue led to the use of 2-DG to produce glucose deprivation in these studies (8). Some effects of 2-DG in the intact anesthetized dog are shown in figure 3. In this experiment, 2-DG (100 mg/kg) was injected intravenously over a period of 20 min using a constant infusion pump, 2 hr after the right adrenal was cannulated. These results confirm the studies of Laszlo *et al.* (14), who found an increase in FFA, and the increase in adrenal medullary section reported by Hökfelt and Bydgeman (13).

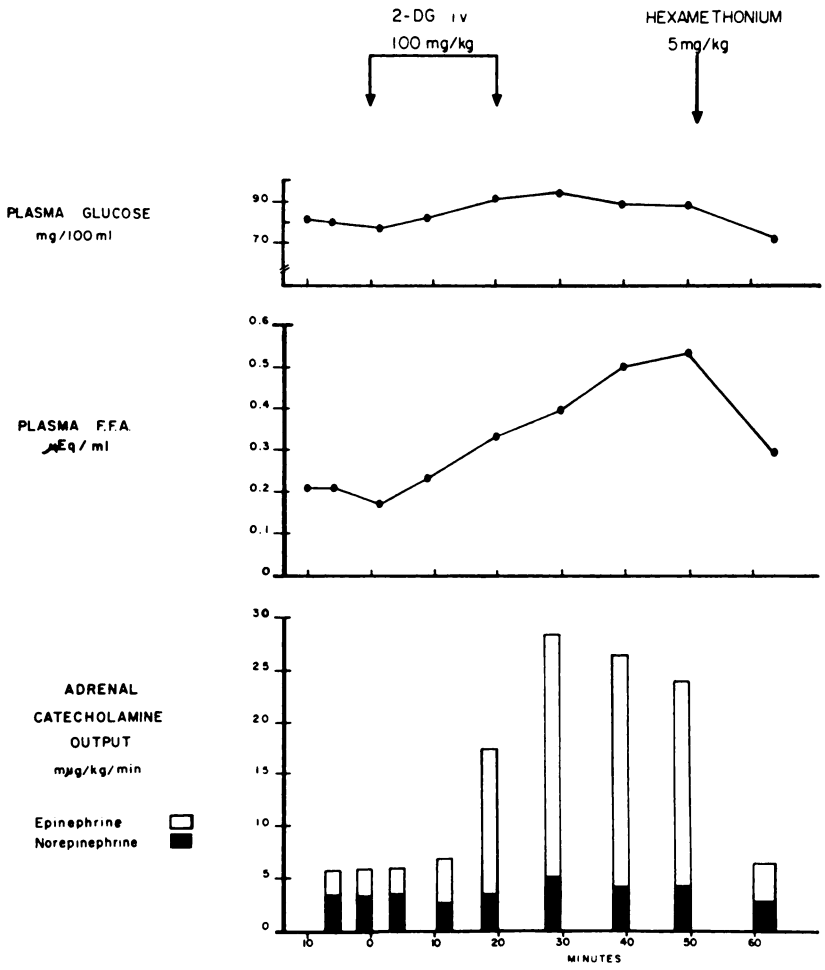

FIG. 3. The effect of 2-deoxyglucose and hexamethonium on plasma glucose and FFA and on the right adrenal secretion of E and NE.

In order to determine whether the increase in FFA was the result of the increased adrenal medullary secretion, as suggested by these investigators, similar studies were carried out in 6 dogs 2 hr after bilateral adrenalectomy. Cortisone acetate (50 mg) and hydrocortisone hemisuccinate (25 mg) were injected intramuscularly at the conclusion of the surgical procedure. The results of this study are shown in figure 4. The marked increase in FFA levels indicate that the adrenal medulla is not required for the response and the prompt fall after the injection of hexamethonium (5 mg/kg) suggests that the changes noted were due to alterations in the sympathetic outflow. The slight change in plasma glucose reflects interference of 2-DG in the assay (15) and confirms previous reports that hyperglycemia does not occur in adrenalectomized dogs (1).

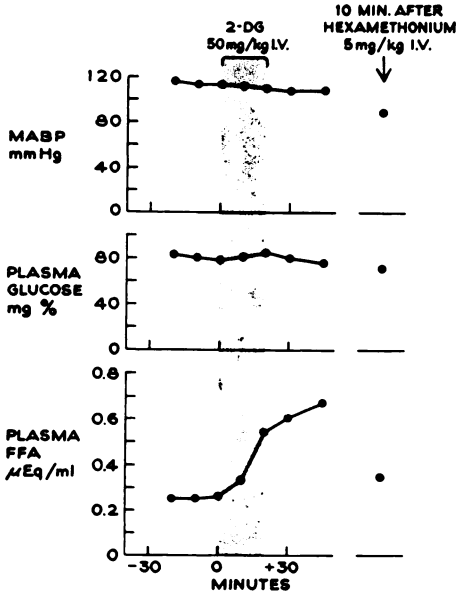

FIG. 4. The effects of 2-deoxyglucose given to 6 adrenalectomized dogs. Note the reduction of plasma FFA after hexamethonium.

The response to 2-DG (100 mg/kg) was measured in 1 dog following removal of the thoracic spinal cord and in 3 dogs following epidural spinal anesthesia produced by injecting 0.5% tetracaine (Pontocaine) into catheters placed in the epidural space. In no instance was a rise in FFA observed (8). These studies provide further evidence for the nervous origin of the increase in plasma FFA. These results agree with those recently reported by Fröberg *et al.* (6) in insulin-induced hypoglycemia but differ from the results obtained by Richardson and Hökfelt in the rat (17).

The role of pituitary hormones in this response was also studied. Growth hormone is known to increase plasma FFA concentrations (16), but even when it is given intravenously the response is too slow to account for the observed changes (23). The requirement for the release of other adipokinetic factors was studied by administering 2-DG (100 mg/kg), in a 20-min intravenous infusion, to 5 dogs 3 hr after completion of bilateral adrenalectomy and hypophysectomy. An increase in FFA was noted in each experiment and the mean FFA concentration rose from 0.6 to 1.2 μEq/ml in 30 min (8). These results indicate that augmented pituitary secretion is not responsible for the changes found, but they are compatible with studies showing that the hormones of pituitary target organs have a permissive action in regulating fat mobilization.

Having established that the response is mediated by the nervous system, we studied 14 acutely adrenalectomized dogs after transection of the spinal cord in an attempt to locate controlling centers in the nervous system (8). Figure 5 shows the effect of 2-DG (100 mg/kg) injected intravenously in 20 min in 3 dogs

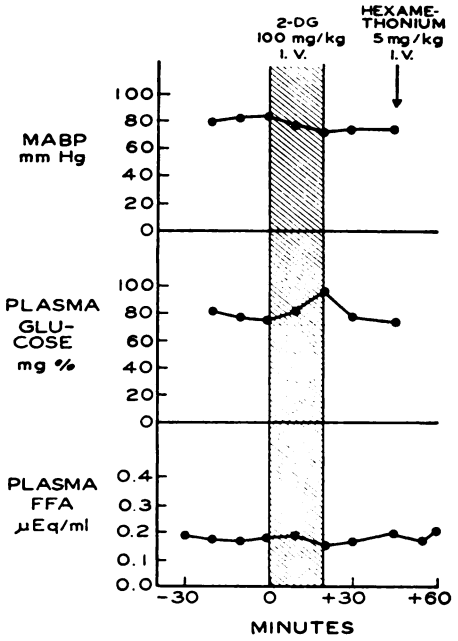

FIG. 5. The lack of increase in plasma FFA in 3 dogs with transections of the spinal cord (T_1, T_4, T_5) is shown in the above figure. The rise in glucose is unexplained.

with lesions at T_1, T_4 and T_5. There was no increase in plasma FFA. The cause of the rise in plasma glucose is unclear. Increased FFA levels were not observed in dogs with lesions above T_8 or below C_4. However, similar experiments done in dogs with cord transections between C_2 and C_3 indicated that the FFA response to glucose deprivation remained intact. In 5 animals with lesions at this level and a mean plasma glucose concentration of 68 mg%, a significant increase in plasma FFA was noted (8). The results of one of these experiments is shown in figure 6. The increase in this dog was the same as the mean for the group, and a sharp decrease was noted in each experiment after the injection of hexamethonium. Three of the animals with lesions in this region developed hypoglycemia during the control period (8). It was of interest to find that the control FFA concentrations were in the range of 0.8 μEq/ml; this fact indicates that this stimulus was as effective as 2-DG and perhaps suggests the presence of a regulatory center in this area.

In 3 experiments the surgical procedure was delayed 45 min after the induction of anesthesia. The FFA concentrations in samples obtained immediately before and 3 hr after adrenalectomy and transection of the spinal cord between C_2 and C_3 fell from 0.55 to 0.3 (8). This finding is consistent with the presence of higher centers but the experiment is too complicated to permit any conclusion of its nature. Additional experiments were performed in which a metal coil cooled by hexane at −15°C was applied to the surface of the cord at C_2 (7). In 2 experiments it was possible to produce a fall in plasma FFA levels by cooling the cord without

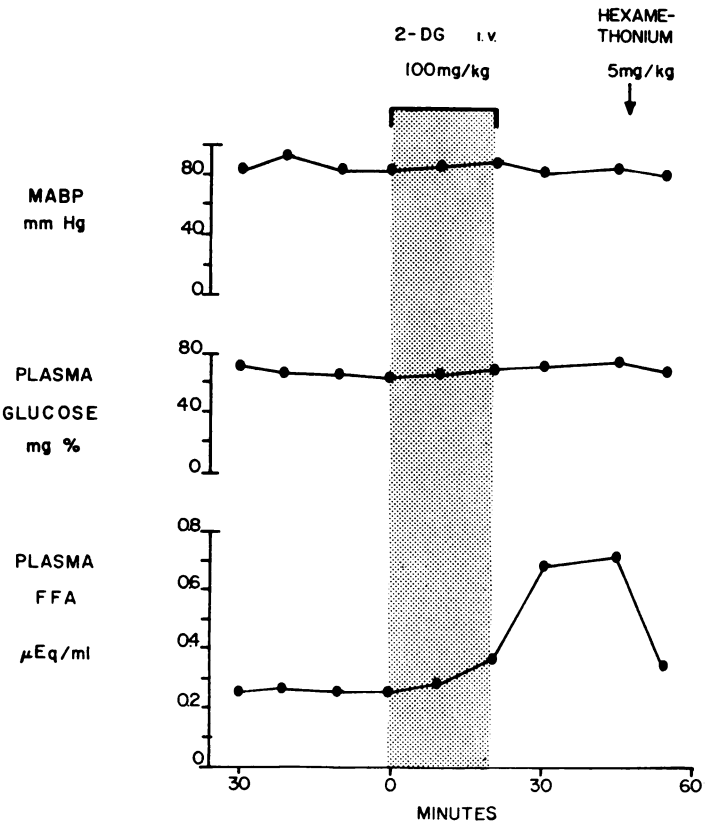

FIG. 6. The effects of 2-DG in a dog with a lesion in the spinal cord at the C_{2-3} level

eliminating the response to 2-DG. In other experiments the fall occurred but the response to 2-DG was prevented, possibly because the requirement of the center for glucose had been reduced by the cooling. Figure 7 illustrates the findings in 1 of the 2 dogs in which the response remained intact.

The studies shown illustrate the mechanisms by which the sympathetic nervous system responds to glucose deprivation. These studies also support the hypothesis that glucose receptors, some of which are to be found in the spinal cord, regulate the activity of centers in the cord which control the secretion of the adrenal medulla and the discharge of the sympathetic nerves to adipose tissue when the supply of glucose is limited. During hypoglycemia the increase in circulating FFA and catecholamines is accompanied by augmented secretion of glucagon, hydrocortisone, growth hormone, and other pituitary adipokinetic factors and a decrease in release of insulin by the pancreas. These alterations and their secondary effects on the hydrolysis and esterification of triglycerides and glucose uptake by muscle act to minimize the glucose uptake of peripheral tissues, to supply FFA to meet their energy needs, and, with the aid of gluconeogenesis and glycolysis to preserve and provide glucose for the central nerv-

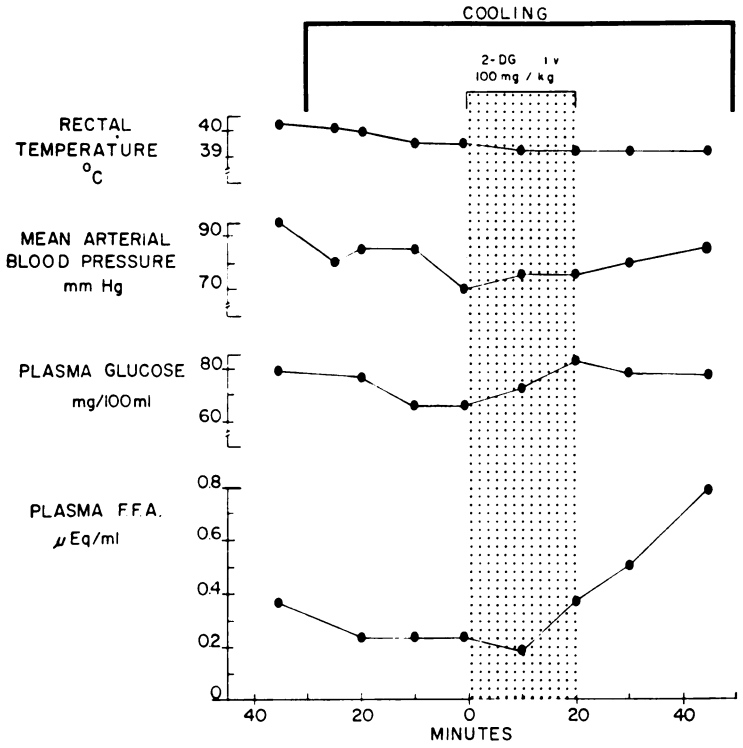

FIG. 7. In this experiment the resting level of FFA was reduced by circulating heptane at −15°C through a coil placed in position 3 hr earlier. The effect of 2-DG on plasma FFA is similar in magnitude to that shown in figures 4 and 6.

ous system. In addition to meeting the needs of the animal in the fasting state, these mechanisms must be of vital importance to the newborn of many species.

Acknowledgments. The studies of the effects of 2-DG on plasma FFA were carried out in collaboration with K. S. Gullixson, G. Hargrove and J. E. Doherty. The technical assistance of M. L. Aycock and J. Richardson is gratefully acknowledged.

REFERENCES

1. ALTSZULER, N., DUNN, A., STEELE, R., BISHOP, J. S. AND DE BODO, R. C.: Effect of 2-deoxyglucose on glucose turnover in normal and adrenalectomized dogs. Amer. J. Physiol. **204:** 1008–1012, 1963.
2. BLECHER, M.: A fluorometric method for the determination of 2-deoxy-*d*-glucose. Ann. Biochem. **2:** 30–38, 1961.
3. CANNON, W. B., MCIVER, M. A. AND BLISS, S. W.: A sympathetic and adrenal mechanism for mobilizing sugar in hypoglycemia. Amer. J. Physiol. **69:** 46–66, 1924.
4. CANTU, R. C., WISE, B. L., GOLDFIEN, A., GULLIXSON, K. S., FISCHER, N. AND GANONG, W. F.: Neural pathways mediating the increase in adrenal medullary secretion produced by hypoglycemia. Proc. Soc. exp. Biol., N. Y. **114:** 10–13, 1963.
5. DUNÉR, H.: The influence of the blood glucose level on the secretion of adrenaline and noradrenaline from the suprarenal. Acta Physiol. Scand., Suppl. **102:** 7–77, 1953.
6. FRÖBERG, S., LILJEDAHL, S. AND ORÖ, L.: Free fatty acids of plasma during insulin-induced hypoglycemia in dog. Acta Med. Scand. **176:** 685–692, 1964.
7. GOLDFIEN, A., GULLIXSON, K. S. AND DOHERTY, J. E.: Unpublished observations.
8. GOLDFIEN A., GULLIXSON, K. S. AND HARGROVE, G.: Central nervous system centers regulating fat metabolism. Submitted for publication.

9. GOLDFIEN, A., GULLIXSON, K. S. AND JENSEN, P.: Unpublished observations.
10. GOLDFIEN, A., ZILELI, S., DESPOINTES, R. H. AND BETHUNE, J. E.: The effect of hypoglycemia on the adrenal secretion of epinephrine and norepinephrine in the dog. Endocrinology **62**: 749–757, 1958.
11. GOLDFIEN, A., ZILELI, S., GOODMAN, D. AND THORN, G. W.: The estimation of epinephrine and norepinephrine in human plasma. J. clin. Endocrin. **21**: 281–295, 1961.
12. HAVEL, R. J. AND GOLDFIEN, A.: The role of the sympathetic nervous system in the metabolism of free fatty acids. J. Lipid Res. **1**: 102–108, 1959.
13. HÖKFELT, B. AND BYDGEMAN, S.: Increased adrenaline production following administration of 2-deoxy-D-glucose in the rat. Proc. Soc. exp. Biol., N. Y. **106**: 537–539, 1961.
14. LASZLO, J., HARLAN, W. R., KLEIN, R. F., KIRSHNER, N., ESTES, E. H., JR. AND BOGDONOFF, M. D.: The effect of 2-deoxy-D-glucose infusions on lipid and carbohydrate metabolism in man. J. clin. Invest. **40**: 171–176, 1961.
15. MCCOMB, R. B., YUSHOK, W. D. AND BATT, W. G.: 2-Deoxy-D-glucose, a new substrate for glucose oxidase. J. Franklin Inst. **263**: 161–165, 1957.
16. RABEN, M. S. AND HOLLENBERG, C. H.: Effect of growth hormone on plasma fatty acids. J. clin. Invest. **38**: 484–488, 1959.
17. RICHARDSON, L. AND HÖKFELT, B.: Plasma free fatty acids after 2-deoxy-D-glucose in intact, adrenalectomized, spinal and hypophysectomized rat. Proc. Soc. exp. Biol., N. Y. **117**: 83–86, 1964.
18. STRAND, L. J., GOLDFIEN, A. AND GANONG, W. F.: Effect of pancreatectomy on the adrenal medullary response to hypoglycemia in the dog. Endocrinology **74**: 656–657, 1964.
19. STRAND, L. J., GOLDFIEN, A. AND GANONG, W. F.: Unpublished observations.
20. TELLER, J. D.: Direct, quantitative, colorimetric determination of serum or plasma glucose. Abst. of Papers, 130th Meeting, Am. Chem. Soc., Sept., 1956, 69C.
21. TROUT, D. L., ESTES, E. H., JR. AND FRIEDBERG, S. J.: Titration of free fatty acids of plasma: A study of current methods and a new modification. J. Lipid Res. **1**: 199–202, 1960.
22. WISE, B. L., GOLDFIEN, A. AND GANONG, W. F.: Endocrine function in dogs after ablation of the area postrema. Acta Neuroveg. **22**: 1–13, 1960.
23. ZAHND, G. R., STEINKE, J. AND RENOLD, A. E.: Early metabolic effects of human growth hormone. Proc. Soc. exp. Biol., N. Y. **105**: 455–459, 1960.