Hormones and the Metabolism of Isolated Tissues

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In diabetes there are disturbances in the utilization of glucose, in amino acid and protein metabolism and in the synthesis and breakdown of fat. The present brief review is confined to our own recent experiments on the first and second of these subjects. Experiments on the third topic, showing that fat synthesis from 2 carbon substances is deficient in diabetes, have appeared from two laboratories.[1, 3, 4]

GLUCOSE UTILIZATION BY STRIATED MUSCLE

The test object which we have employed in experiments on glucose utilization is the excised rat diaphragm. The diaphragm is carefully removed, washed and incubated for a definite time at 37°C. in a medium simulating extracellular fluid. Glucose uptake and other changes can be measured by analyses of aliquots of diphragm and medium before and after incubation.

INSULIN. Gemmill[6] showed that glucose use and glycogen formation by diaphragm are enhanced by addition of insulin to the medium in vitro, an observation later confirmed by many investigators.

Glucose use by diaphragm from severely diabetic (alloxan) rats is only 50 to 60 per cent of normal.[10, 16] Added insulin returns the rate toward normal (Figure 1).

These experiments indicate clearly that glucose utilization by a striated muscle can be increased by extra insulin and decreased in insulin deficiency. They supplement earlier experiments to the same end on whole and eviscerated animals and on perfused organs; the relation of these to the in vitro measurements has recently been reviewed,[5, 7, 8] the present experiments also provide the basis for a further study of the inhibitory factors against which insulin acts in increasing glucose uptake.

PITUITARY—ADRENAL CORTICAL FACTORS. Krahl and Park[11] observed that glucose uptake of diaphragms from hypophysectomized rats is higher than that of the normal, 4.9 mg. per gram per hour, as compared to a control rate of 3.5 (Figure 2). The maximum effect is reached about 15 to 20 days after operation. This has been repeatedly confirmed by new experiments of our own group[14] by Villee and Hastings[16] and by Bornstein and Nelson,[2] but not by Li, Kalman and Evans.[12] The reason for this discrepancy is not clear. Glucose uptake of diaphragms from adrenalectomized rats is slightly increased.[10, 16] But in no case is the effect from adrenalec-

Read at the Annual Meeting of the American Diabetes Association, Chicago, Illinois, June 8, 1952.

The experimental work described in this paper was supported in part by research grants from Eli Lilly and Company and from the National Institutes of Health.

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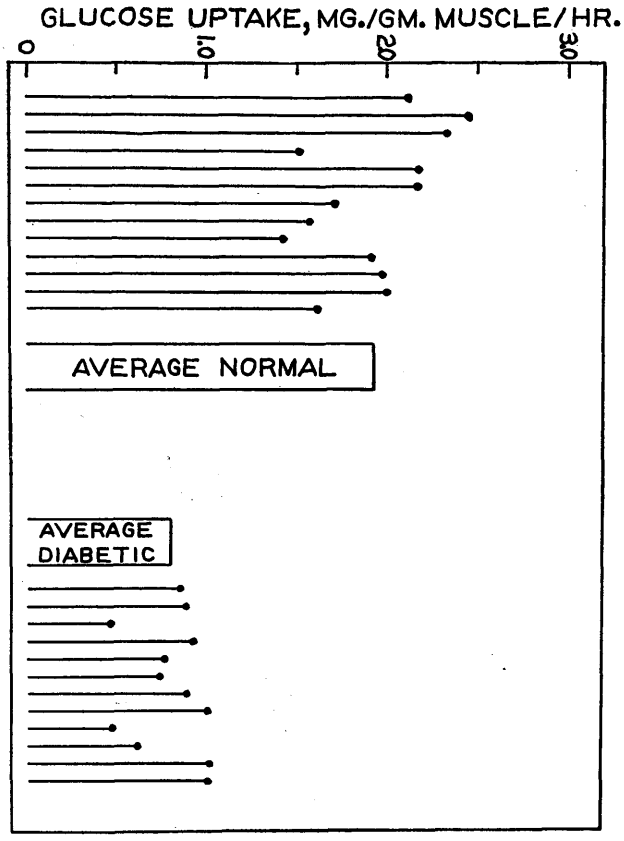

FIGURE 1 Glucose uptake by diaphragms from normal and severely diabetic (alloxan) rats. The muscle was shaken in oxygenated Krebs-bicarbonate solution and the medium analyzed for glucose before and after two hours of incubation at 37°C. The initial glucose concentration of the medium was 100 mg. per cent. From Cori (5) and Krahl (8)

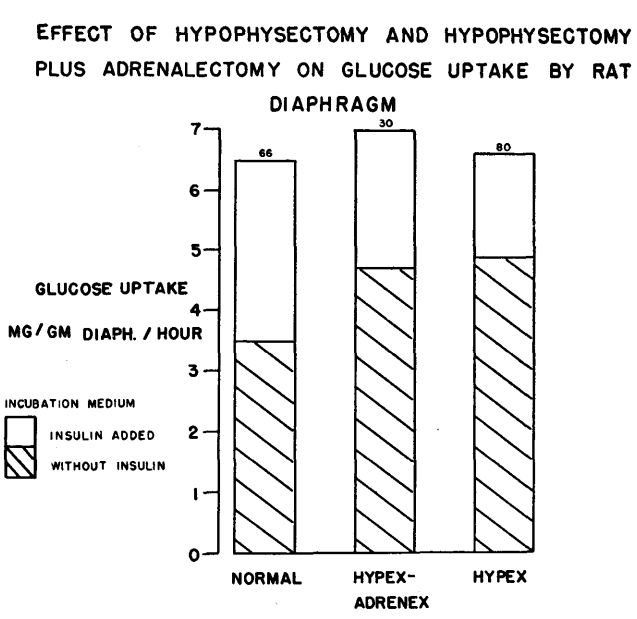

FIGURE 2 Effect of hypophysectomy and hypophysectomy plus adrenalectomy on glucose uptoke by rat diaphragm. The height of the cross-hatched area represents the rate without added insulin; the total height represents the rate with 0.1 unit per ml. of incubation fluid. The number of rats used is given at the top of each bar. From Krahl (8)

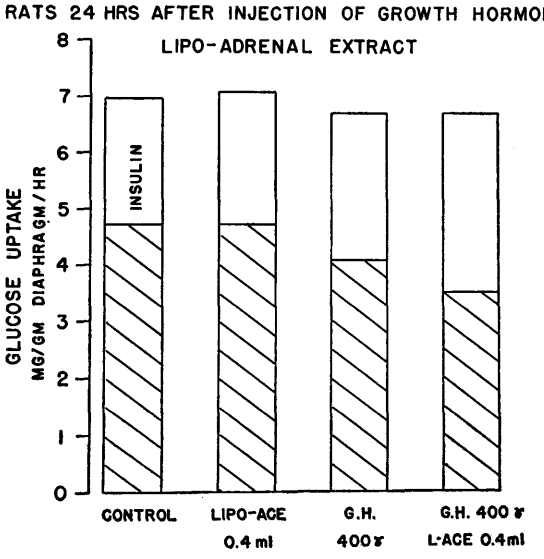

FIGURE 3 Glucose uptake of diaphragms from hypophysectomized-adrenalectomized rats. The growth hormone was given intraperitoneally 24 hours, the lipo-adrenal extract in divided doses at 24 and 6 hours, prior to removal of diaphragms. For significance of cross-hatched and white areas, see Figure 2. From Krahl (8)

tomy large enough to account for the increase in glucose uptake after hypophysectomy.

The isolated muscle of the hypophysectomized-adrenalectomized rat is still responsive to insulin, the glucose uptake being raised to a common level of about 7 mg. per gram per hour for the normal, for hypophysectomized, or for hypophysectomized-adrenalectomized cases. Hence the glucose uptake of muscle is in part under an insulin-reversible inhibitory influence from the pituitary and adrenals and in part under an inhibition which persists even when both these glands are removed.[11, 14, 15]

The nature of the inhibitory factor from the pituitary has now been extensively investigated with the diaphragm as test object.[13, 14] Partially purified anterior pituitary fractions, and even some samples of once crystallized growth hormone, produced significant depression of glucose uptake when injected into hypo-

physectomized rats 3 hours before excision of the diaphragm. Four times crystallized growth hormone, on the other hand, was inactive at 3 hours, but active if given 24 hours to take effect; these highly purified samples were inactive at 3 hours even when large doses of adrenocorticotropic hormone were given concurrently.[8] This shows conclusively that growth hormone, as now prepared, does not account for all of the inhibitory activity of cruder pituitary extracts toward glucose uptake by muscle. The suggestion has been made that the inhibitor can be derived from growth hormone in the body.[8, 14]

Both growth hormone and adrenal cortical factors are involved in inhibition of glucose uptake by muscle.[13, 14] For example, in one series of experiments 400 micrograms of growth hormone lowered the rate of glucose uptake by diaphragms from hypophysectomized-adrenalectomized rats from a control value of 4.7 to 4.1 mg. per gram per hour; concurrent injection of lipo-adrenal extract (Upjohn) equivalent to 800 micrograms of compound E, a dose which was inactive alone, lowered the rate still further to 3.4 (Figure 3).

INSULIN AND PEPTIDE SYNTHESIS

Many experiments are available to show that insulin raises the rate of amino acid removal from plasma in whole or eviscerated animals. The experiments summarized here represent an attempt to follow the amino acids one step further and to determine their fate and the controlling factors in their disappearance.

Liver slices or diaphragms were incubated in a physiological medium containing radioactive glycine-1-C^{14} or phenylalanine-3-C^{14}; the radioactivity incorporated into the peptide glutathione and the total proteins of the tissue was measured.[9]

The results are expressed as counts per minute per mg. of the substance isolated. The higher the count incorporated under a given set of conditions, the greater the degree of synthesis, and vice versa.

Glutathione, containing the amino acids glutamic acid, cysteine, and glycine, was chosen for this purpose largely because it is the only typical simple peptide which can be readily isolated in crystalline form with high purity. Tissues from normal and from severely diabetic (alloxan) rats were used.

Diabetes reduces radioactive glycine incorporation into cuprous glutathione from a level of 3200 c.p.m. per mg. for normal liver to a level of 497 for diabetic liver. The values for protein fractions from normal and diabetic were: liver, 138 for normal, 19 for diabetic; diaphragm, 53 for normal, 21 for diabetic (Table 1).

Fasting alone (24 hours) results in rates of glycine incorporation which are intermediate between the normal non-fasting and the diabetic levels.

TABLE 1 Glycine-1-C^{14} incorporation into glutathione and protein fractions of liver and diaphragm from normal and diabetic rats. No glucose was added to the incubation medium.

Fraction Isolated	Insulin Added to Medium units per ml.	Radioactivity incorporated C.P.M. Per Mg. Normal, Non-Fasting	Normal, Fasting	Diabetic, Fasting
Liver Cuprous Glutathione	0	3208	1068	497
	0.1	3525	1097	—
Liver Protein	0	138	43	19
	0.1	127	41	—
Diaphragm Protein	0	53	33	21
	0.1	70	43	—

Similar experiments with phenylalanine-3-C^{14} instead of glycine-1-C^{14} gave: normal liver protein 160 c.p.m. per mg., diabetic 45; normal diaphragm protein 130, diabetic 20. Thus, the deficiency in uptake of radioactive amino acids is not confined to glycine.

This net decrease in labeled amino acid uptake in diabetes could be due to decreased peptide synthesis or to dilution, in the diabetic, of the glycine-1-C^{14} by a larger glycine pool; the larger pool might arise from increased protein breakdown. In an attempt to decide in a preliminary way between these two alternatives, new experiments with normal and diabetic liver were performed. Liver slices were incubated in medium containing forty times as much labeled glycine as in the initial experiments. At this higher glycine concentration, dilution of the radioactivity by unlabeled glycine from liver protein is much smaller than before. Therefore, incorporation of radioactivity into glutathione by normal and diabetic liver should be at rates much closer together than before if the difference between normal and diabetic which was initially observed were due solely to a larger pool of unlabeled glycine in the diabetic. Actually, it turns out that the relative reduction in glycine-1-C^{14} incorporation for the diabetic is 77 per cent at the higher glycine concentration as compared to 84 per cent at the lower. Since the reduction is nearly the same in the two cases, it must be concluded that the net decrease in amino acid uptake is attributable principally to decreased peptide synthesis in the diabetic tissues. A limited concurrent increase in protein breakdown in the diabetic is not excluded.

The question of the specific relation of insulin to rates of amino acid uptake by normal and diabetic tissues has been explored and found to be related to the availability in the medium of glucose as a substrate.

In all the experiments on amino acid uptake de-

scribed above, no glucose or other substrate was added to the incubation medium, the only glucose present being that carried over with the tissue. Under these circumstances, addition of insulin in vitro at a concentration of 0.1 unit per ml. produced no consistent increase in amino acid uptake by normal or diabetic tissues.

In another series of experiments carried out with 140 mg. per cent glucose in the medium, the cuprous glutathione isolated after incubation of diabetic liver was found to incorporate glycine-1-C^{14} to the extent of 1480 c.p.m. per mg.; with insulin present in vitro, the value was 2940 c.p.m. per mg. Hence, glucose alone raises the uptake in the diabetic from the value of 497 c.p.m. which was obtained without substrate. Insulin in vitro raises it still further, to a value very close to the mean normal level of 3200. A similar relationship to the availability of glucose and insulin was observed for the protein fractions of liver and diaphragm (Table 2).

Peptide synthesis in isolated tissues is thus correlated with glucose uptake in a general way. This finding is consistent with the requirement for energy-yielding metabolites of glucose in glutathione synthesis by a cell-free system.[17] Whether insulin influences peptide synthesis by a mechanism other than by stimulation of glucose uptake remains to be determined.

TABLE 2 Glycine-1-C^{14} incorporation into glutathione by liver slices from severely diabetic rats.

Concentration Glucose Added to Medium	Radioactivity incorporated C.P.M. Per Mg.	
	No Insulin Added to Medium	Insulin Added to Medium
mg. per cent		
0	632	598
140	1480	2940

SUMMARY

Glucose utilization by muscle is under at least two types of insulin-reversible inhibition.

One type of inhibititon is produced by a factor from the anterior pituitary gland which requires the concurrent presence of adrenal cortical products for full activity. This pituitary factor is closely related to growth hormone as now prepared, but apparently is not identical with it.

A second type of insulin-reversible inhibition is present even when both the pituitary and the adrenal glands have been removed. The chemical nature of this inhibition remains to be defined.

Incorporation of radioactive glycine into tissue glutathione and proteins is reduced in severe diabetes and enhanced by insulin in vitro in presence of glucose. The effect of insulin is apparently principally upon rate of peptide formation rather than upon rate of breakdown of proteins to their constituent amino acids.

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DISCUSSION

DR. F. D. W. LUKENS (*Philadelphia*). With the exception of Dr. Best's lecture, Dr. Krahl's paper is the only presentation of current work on the action of insulin, a topic of primary interest to this Association. He reminded us that insulin accelerates the utilization of carbohydrate by all three of the major pathways, namely, glycogen formation, fat formation and oxidation. If time permitted, he would have made a summary of other work in this field. Therefore, he may forgive me if I indicate in an oversimplified way three approaches to the study of the action of insulin which I find particularly thought-provoking. The first is the work of Drs. Krahl, Cori and their associates who have attempted to learn the biochemical site of action and to study the role of insulin inhibitors. The second is Dr. Levine's examination of the altered permeability of the cell to glucose under the influence of insulin. The third is the work of Dr. Stadie who has observed the binding of insulin to tissue and recently, with radioactive insulin, has begun to quantitate the relations between the amount of insulin bound to tissue, the glycogenic function of insulin so bound, and the effect of hormones and other inhibitors on both the binding and the glycogenic function.

As a result of this comment, one question occurs to me. Does the effect of insulin in the hypophysectomized-adrenalectomized rat mean that there is an inhibitor which persists in the absence of these glands? Might this not be an action of the increased dose of insulin on the permeabiliity to glucose or on the binding of insulin independent of any hypothetical inhibitor? Some of Stadie's results suggest that this may be more than a matter of semantics. Thus, with labeled insulin Stadie has found that the *binding* of insulin by the diaphram is the same in normal and hypophysectomized rats, although the insulin effect is greater in the hypophysectomized tissue. In other words, the insulin sensitivity of the hypophysectomized animal is here measured as a greater effect per unit of insulin bound. On the other hand, in examining the serum of insulin-resistant patients, this group finds a striking prevention of the binding of radio-insulin. In summary, I suppose that the assumption of inhibitors is made with due regard for possible future developments.

Another point which prompts an attempt at correlation is this. We showed that in Houssay animals, in the absence of insulin, growth hormone had no effect on nitrogen retention. Dr. Krahl's experiment showing that amino acids are not incorporated by tissue in the absence of glucose even when insulin is present is a thought-provoking companion experiment. It looks as if protein anabolism by muscle were dependent on the system insulin plus glucose much as the metabolism of the brain is dependent on the system oxygen plus glucose.

DR. CHARLES H. BEST: (*Toronto*) I wonder if Dr. Krahl would review again the data I heard him give elsewhere on how long he thinks insulin may persist after cessation of dosage or removal of the pancreas.

DR. M. E. KRAHL (*closing*): With respect to Dr. Lukens' question about inhibitors, what I should have said, perhaps, was that there is a limitation in glucose uptake which persists after the hypophysis and adrenals are removed. I shall be very much interested in seeing how the experiments which Dr. Levine and others are conducting finally turn out in this respect.

With respect to Dr. Best's question, it has been our experience, that if one uses rats which have received alloxan, it is very difficult to demonstrate a reduced glucose uptake or a reduced amino acid uptake unless, first, the rats have been given very large doses of alloxan so that they have become maximally diabetic and, second, these animals have been kept for at least five to seven days after the alloxan injections, or after insulin treatment has been withdrawn.