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Reduced Glutathione of Tissues and Insulin Sensitivity.

R. LEVINE, O. HECHTER, A. GROSSMAN AND SAMUEL SOSKIN.

From the Department of Metabolism and Endocrinology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.*

The sensitivity of an animal to insulin is usually judged by the extent and duration of the depression of the blood sugar level after the administration of insulin. The factors which are usually considered to exert an important influence upon the insulin depression curve are: the available carbohydrate stores in the liver and the ease with which these stores may be mobilized.[1] The state of the endocrine glands is important in both these respects, and it is well known that adrenalectomy,[2] hypophysectomy,[3] and thyroidectomy[4] increase the sensitivity to insulin. Another important factor in insulin sensitivity, to which less attention has been paid, is the rate of destruction or inactivation of insulin by the tissues of the body. That this factor is significant is shown by the well-established fact that the same amount of insulin becomes more effective when administered in divided doses or by prolonged constant injection than when given in a single dose.[5]

The exact mode of insulin inactivation in the body is unknown, but ever since it was shown that insulin is a protein, the supposition has been that it is destroyed by proteolytic enzyme systems.[6] However, it is also known that the physiological action of insulin depends upon the integrity of its S-S groups,[7, 8] and that the *in vitro* reduction of these groups by sulphydryl compounds renders the insulin inert upon subsequent injection.[9-13] That a similar mode of inacti-

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vation applies to insulin in the living organism is indicated by the work of Jacobs, who has recently demonstrated that the administration of cysteine decreases the reaction to subsequently injected insulin.[14]

These experimental considerations raise the question as to what rôle the normally occurring sulphydryl compounds of the tissues play in insulin inactivation, and therefore in the sensitivity to insulin. Such compounds are glutathione, which is present in the body almost wholly in its reduced form (GSH), and the sulphydryl groups of the tissue proteins. In the absence of satisfactory methods for determining the latter we have tried to obtain a partial answer to the above question by determining the GSH content of blood, muscle and liver in normal animals, and in animals known to be hypersensitive to insulin.

Methods. The GSH of fixed tissues was determined in rats. For practical reasons the estimations of blood GSH were made on dogs and also on humans. The GSH was estimated by the iodometric titration of 4% trichloracetic acid filtrates of blood and tissues. Vitamin C was determined by titration with 2-6 dichlorphenol-indophenol and the equivalents subtracted from the iodometric titration.[15] The results obtained for normal animals agreed quite well with figures obtained by the specific manometric method of Woodward[16] and the cadmium lactate method of Binet and Weller.[17] All determinations were run in triplicate.

The completeness of the hypophysectomies as well as of the adrenalectomies was checked at the time the animals were sacrificed. As a contrast to hypophysectomy we administered to normal rats an extract of the anterior pituitary gland† which we have elsewhere shown is able to maintain the blood sugar level of fasting hypophysectomized dogs. Our rats received 1 cc of "Phyone" subcutaneously per day (divided into 3 doses), for 2 days prior to the day on which they were sacrificed for tissue analyses.

Results. The chief results, namely the GSH content of liver and muscle in rats, are summarized in Table I. The results on the GSH of blood, and of some additional exploratory experiments, appear in the text below.

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[15] Fujita, A., and Iwataka, D., *Bioch. Z.*, 1935, **277**, 284.

[16] Woodward, G. E., *J. Biol. Chem.*, 1935, **109**, 1.

[17] Binet, L., and Weller, G., *Bull. Soc. Chim. Biol.*, 1936, **18**, 358.

† "Phyone" (Wilson and Co.) for supplies of which we are indebted to Dr. David Klein.

TABLE I.

Condition	No. of rats	Liver GSH mg %	% change	Muscle GSH mg %	% change
Normal	18	217 ± 26	0	32 ± 5	0
Hypophysectomized	12	134 ± 29	—38	38 ± 5	+18
Adrenalectomized	8	137 ± 24	—37	37 ± 3	+16
Normals given Phyone	8	243 ± 21	+12	27 ± 3	—16

GSH in Liver and Muscles. It is apparent from Table I that the GSH of the liver of hypophysectomized and adrenalectomized rats is significantly lower than that of normal rats. A smaller number of determinations on livers of normal and hypophysectomized dogs yielded similar results. The average liver GSH for 3 hypophysectomized dogs was 153 mg % as compared with an average of 204 mg % for the livers of 4 normal dogs. Normal rats given "Phyone" showed an increase in the liver GSH. The increase is small as compared to the decrease after hypophysectomy, but the factors of dosage and of the preëxisting level of anterior pituitary hormone in the tissues must be considered in evaluating the effects of the administered hormone.

Table I shows variations of doubtful significance between the GSH of the skeletal muscles, as compared to the definite changes in liver GSH. However, the changes in the muscle, if significant, are consistently opposite to those in the liver. We are unable to explain this difference between liver and muscle at the present time. One possibility is suggested in our discussion below.

GSH in Blood. The essential finding as regards blood was that it did not parallel the GSH content of the fixed tissues either in normal animals and humans, or in hypophysectomized dogs, or in humans suffering from diabetes mellitus, acromegaly, hepatitis and other clinical conditions. In both man and dog under all the above conditions the GSH of blood ranged from 25-40 mg %, depending upon the red blood cell volume, as determined by hematocrit readings, in the particular individual. The latter relationship may explain the variation in blood GSH observed by others[18, 19] but not found by us.

We attempted to lower tissue GSH artificially by the administration of iodoacetic acid and of alloxan, both of which substances have been previously shown to oxidize GSH *in vitro*.[20, 21] It was

[18] Zunz, E., and Vesselovsky, O., *Ann. de physiol.*, 1937, **13**, 1064.

[19] Houssay, B. A., *New Eng. J. Med.*, 1936, **214**, 1002.

[20] Goddard, E., and Schubert, M. P., *Bioch. J.*, 1935, **29**, 1009.

[21] Lieben, F., and Edel, E., *Bioch. Z.*, 1933, **259**, 8.

our purpose to determine whether such lowering of the tissue GSH would cause an increased sensitivity to insulin. Our attempts were not successful. The iodoacetic acid which lowered the tissue GSH markedly (from 217 to 113 mg %), also caused coincident hyperglycemia due to liver glycogenolysis, which prevented determinations of insulin sensitivity. The alloxan failed to lower tissue GSH *in vivo*.

Discussion and Summary. Our results show that the GSH of liver is significantly lower than normal in animals known to be hypersensitive to insulin. This relationship was not observed for skeletal muscle or blood. This may indicate that the liver is more important than other tissues for insulin inactivation. However, as noted in our introductory remarks, the GSH is only one of the factors which may be involved in this type of inactivation. It is possible that the –SH groups associated with the tissue proteins may play an important rôle in determining the sensitivity to insulin, and that this factor may be relatively more important in muscle than in liver. This possibility is indicated by the preliminary note of Lehmann[22] which appeared while our work was in progress. He stated that insulin is inactivated *in vitro* by extracts of rabbit muscle and that two substances in the extract are responsible: (1) a dialyzable, thermostable factor, probably GSH, and (2) a non-dialyzable, thermolabile factor, probably proteins containing –SH groups.

It is apparent that final conclusions as to the relationship between the sulphydryl compounds normally present in the tissues, and the inactivation of insulin, and hence insulin sensitivity, must await further work in which all the –SH compounds are quantitatively determined and compared with insulin sensitivity. However, this relationship may be tentatively assumed on the basis of the following summary:

(1) Insulin is inactivated *in vitro* by sulphydryl compounds.[9-13]

(2) Muscle extracts inactivate insulin *in vitro* by virtue of two factors which are most probably GSH, and proteins with sulphydryl groups.[22]

(3) Cysteine injected *in vivo* results in a decreased sensitivity to insulin.[14]

(4) The GSH content of the livers of animals known to be hypersensitive to insulin is significantly lower than that of normal animals.

[22] Lehmann, H., and Schlossmann, H., *J. Physiol.*, 1938, **94**, 15P.