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Influence of Reduced Glutathione on Oxygen Consumption.* (29188)

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A marked reduction in basal oxygen consumption levels is associated with the obese hyperglycemic syndrome in mice(1). In addition, these animals are particularly sensitive to a variety of traumata(2,3). They are also more susceptible to decompression sickness following pressure-decompression treatment than thin littermate controls(4). Observations in our laboratory indicate occasional symptomatic improvement when reduced glutathione (GSH) is administered to obese mice exhibiting decompression sickness. The present series of investigations were carried out to explore the role of possible metabolic mechanisms in the protective effects of GSH in the obese mouse. Determination of the effects of GSH administration on total oxygen consumption levels in obese hyperglycemic mice and their thin siblings were performed. Effects of GSH on respiration of salamanders were also investigated in order to provide broader characterization of the effects of GSH on metabolic rate.

Materials and methods. Obese mice and their thin siblings of both sexes were obtained from the Roscoe B. Jackson Memorial Laboratory and housed in standard laboratory cages in a thermostatically controlled animal room (25°-27°). Purina laboratory chow and water were given *ad libitum*. Oxygen consumption measurements were carried out in the Warburg apparatus utilizing standard Machlett manometers and specially designed flasks which could accommodate a single mouse (Fig. 1). Flasks were placed in a 20°C water bath for 5 minutes with stopcocks open to the outside atmosphere, and oxygen consumptions were then determined during a 2nd 5-minute period following closing of the stopcocks. Values were expressed as μl O_2/g/hr. The gas phase was air. GSH dissolved in saline was administered subcutaneously in doses ranging from 30-180 mg.

Salamanders were injected weekly with GSH and/or various nutrient supplements (5). Body weights and O_2 consumptions were determined on groups of 3-5 animals placed in the Warburg flask. Since considerable day-to-day variations were encountered in the O_2 consumption levels of salamanders (Fig. 2), values in the experimental groups were treated as absolute deviations from control groups which were run simultaneously. The low oxygen consumption levels of the salamander necessitated group measurements. In chronic experiments with GSH in mice and in salamanders, O_2 consumptions were carried out at least biweekly.

Results and discussion. Experiments were carried out with an initial group of 43 obese mice averaging 39 g in weight and a second group of 43 thin mice averaging 19 g in weight. Fasting oxygen consumption levels

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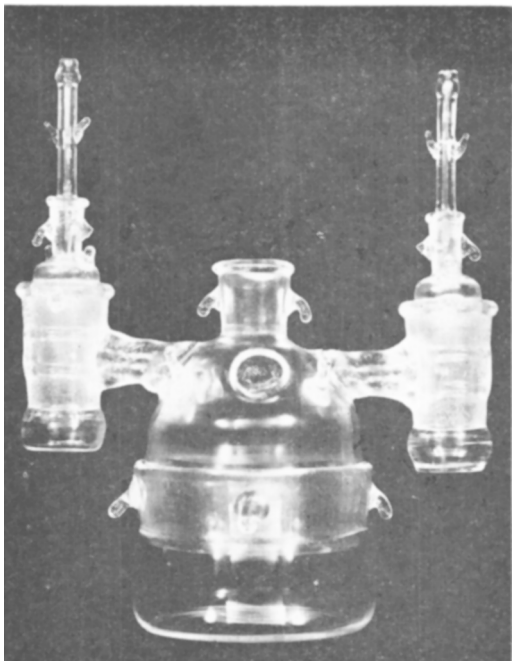

FIG. 1. Modified Warburg flask for determination of oxygen consumption in small mammals.

for the obese group averaged 1085 μl/g/hr (s.e. $= \pm$ 49.1) and for the thin group, 2087 μl/g/hr (s.e. $= \pm$ 53.7). The weight-specific oxygen consumption level is approximately half that of thin littermates. On the basis of $W^{0.73}$ as a unit of metabolic mass this becomes 63%. This is in accordance with the low BMR characteristic of this metabolic type of obesity(6).

GSH produces a significant but not completely compensatory increase in the O_2 consumption of obese mice and is without effect on non-obese littermates (Table I).

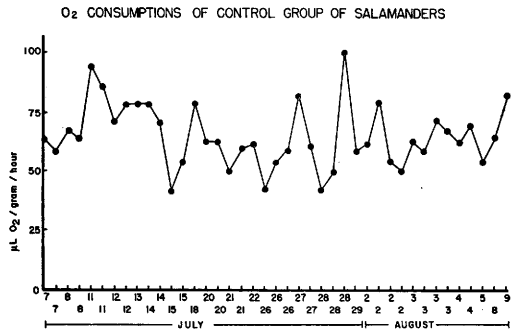

FIG. 2. Variations in oxygen consumption of a group of salamanders over a 2-month period.

In obese animals doses of 33 mg of GSH produced an average increment in O_2 consumption of 36.4% in 7 animals; 66 mg resulted in 26.8% increment in 25 animals, while 180 mg GSH produced an average increment of 24.6% in 7 animals. In the first dosage group 6 of 7 showed a rise, in the second group 18 of 25 went up, while in the third group 6 of 7 went up. O_2 consumption in mice treated chronically with neutralized GSH (66 mg dose), injected thrice weekly, did not show marked differences from untreated controls over a 6-week period (Fig. 3). Repeated O_2 consumption measurements

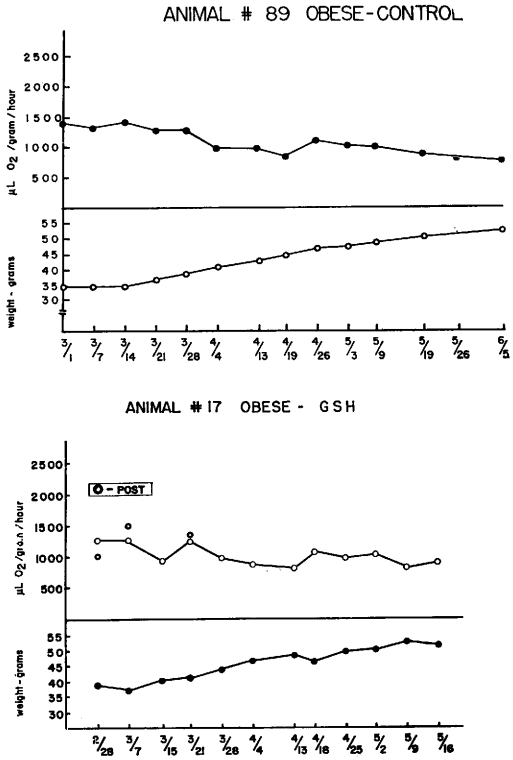

FIG. 3. Comparison of saline-treated (control) and GSH-treated (experimental) obese mice over an approximate 3-month period. In 3 instances, single post-injection values with GSH are indicated.

were carried out at intervals in selected instances following a single dose of GSH and no sustained elevations were apparent. Effects on the pattern of weight increase in thin and obese mice with GSH were not noted.

TABLE I. Effect of GSH on Obese Hyperglycemic Mice and Thin Littermates.

No. of animals	Type	Treatment	% O_2 consumption change (mean ± s.e.)	"t" test
39	Obese	GSH	+28.0 ± 5.2	<.01
17	"	Saline	+ 5.0 ± 5.7	
19	Thin	GSH	+ 2.50 ± 4.3	Not significant
14	"	Saline	+ 8.2 ± 1.9	
39	Obese	GSH	+28.0 ± 5.2	<.001
19	Thin	GSH	+ 2.50 ± 4.3	

In salamanders, GSH injection not only produced immediate increments ranging from 20-40 μl/g/hr above controls in 16 animals (4 groups) but also an overall average increment of 24 μl/g/hr for the 5-week period following initial injection. In the case of simultaneous administration of GSH and a fat supplement, Lipomul (Upjohn), striking increases in O_2 consumption were noted, both as an overall average (38 μl/g/hr) and immediately following each injection (Fig. 4). This increase in weight-specific oxygen consumption is all the more remarkable in view of the higher weights encountered in the Lipomul-GSH-treated animals(5). Controls demonstrated an approximate 30% weight loss during the 5 week experimental period, compared to a mere 8% loss in the GSH-Lipomul group.

GSH has been implicated as a regulatory agent in cellular metabolism although an exact mechanism of action has not been demonstrated. In common with other sulfhydryl compounds, it has been assigned a role in maintenance of essential sulfhydryl groups on enzyme molecules, but its effects apparently extend beyond its ability to act solely as an H+ donor(7). A number of studies have indicated that GSH may exert a protective effect in such stress situations as drug toxicities(8), radiation(9,10), and high oxygen tension(11). Whether these phenomena are the result of some single fundamental action of GSH or whether they reflect a variety of unrelated effects cannot be evaluated at this time.

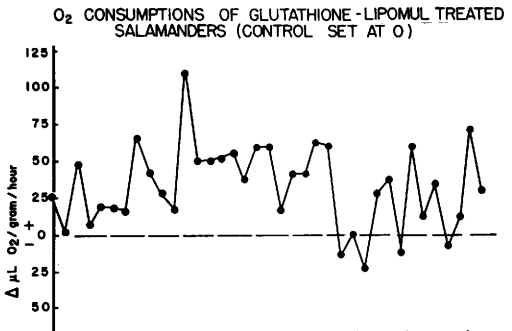

FIG. 4. Oxygen consumption response of salamanders to simultaneous treatment with GSH and Lipomul.

The fact that significant increments in O_2 consumption occur in the poikilothermic salamander, with a relatively low rate of metabolic activity, and in the obese mouse, a homeotherm whose voluntary activity and basal oxygen consumption levels are inordinately low, may be of interest in the assignment of an underlying specific biochemical mechanism to the GSH effect. Thus, a rate limiting step in the aerobic metabolic sequence may be shunted by GSH, or the enzymic component may be increased in activity by the SH moiety of the compound. The general recognition of antitoxic effects of GSH, coupled with observation of a restorative action of the compound in some comatose mice following decompression, argues for additional far-ranging systemic effects in which GSH alters the redox environment in a generalized fashion, perhaps in terms of the endocrine system(12). Additionally, differential metabolic response of obese and thin

mice to GSH suggests further underlying differences in the involvement of specific tissues (13).

Summary. The effects of reduced glutathione (GSH) on oxygen consumption of obese hyperglycemic mice and their thin (non-obese) littermates were determined manometrically. In 39 obese mice given GSH subcutaneously, elevations in oxygen consumption averaged 28.0% compared to 5.7% in 17 saline-injected obese controls. No significant effects were noted in 19 thin siblings treated with GSH (+ 2.5%) as compared to 14 saline-injected controls (+ 8.2%). Differences between thin and obese groups treated with GSH were significant at the 0.001 level. Elevations in O_2 consumption were not sustained and long-term treatment with GSH was without effect. GSH also elicited increments in the O_2 consumption levels of salamanders, and these increases were sustained.

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Collection and Toxicity Studies of Ant Venom. (29189)

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Venom from the fire ant *Solenopsis soevissima,* has been described by Caro *et al*(1), who also described one human fatality resulting from the material, while Blum *et al* (2) have written concerning a nonproteinaceous, nitrogen containing water insoluble strongly alkaline venom from this species which may be collected in microgram quantities from the animal. Blum and Ambrose (3) have described the venom of the army ant, *Eciton burchelli,* as being a water soluble cholinergic histamine-like material, which apparently is similar to the material to be described which was collected from *Pogonomyrmex barbatus.*

Because of its prevalence in the southwest, and the extremely common and painful sting of this insect, the following study was initiated to: 1) determine techniques for venom collection; 2) study the toxicity of the material.

Materials and methods. Large numbers of worker ants, *Pogonomyrmex barbatus,* were collected in silicone treated jars which were sunken to ground level in or near ant hills.

Venom was collected in 3 ways. The first method consisted of stimulation of the intact animal in small volumes of distilled water (*i.e.,* 5 to 10 ml) by means of platinum electrodes in the secondary circuit of a fully coupled Harvard inductorium with 4 to 6 volt input. Stimulation of the animal through the liquid medium produced refraction waves emanating from the stinger tip, and continuous stimulation for up to 20 minutes continued to elicit venom observable by this means.

During this process the stinger itself was