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

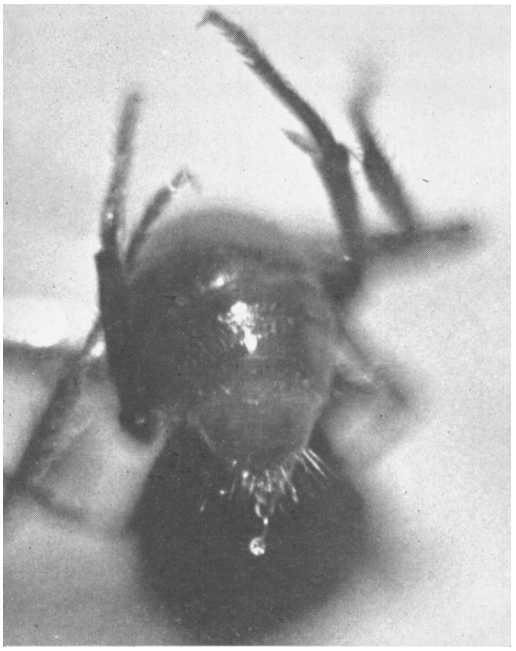

FIG. 1

seen to be like a hpyodermic syringe, a pointed plunger being repeatedly drawn up, then driven back through the external hollow needle or barrel, each time carrying a charge or droplet of venom to the tip of the stinger (Fig. 1).

A second method for collection of venom was to dismember the animals, removing the abdomens which were placed in the distilled water as previously described. As many as 20 or 30 were stimulated almost simultaneously by moving about the platinum stimulating electrodes in their proximity. The physiological phenomenon involved in secretion of venom in the intact animal was virtually duplicated in the isolated abdomen, the stinger-plunger action being identical. This technique enabled collection of larger volumes of slightly less pure material in shorter periods. With regard to purity of material, it is apparent that some loss of body fluids into the collecting reservoir will occur. Insofar as pharmacological effects that were observed, however, no distinction could be made between the venoms collected by one or the other techniques.

A third method for collection, while perhaps the least desirable from a purity standpoint, was more readily amenable to quantitation and consisted of grinding the entire abdomen of the ant in distilled water by means of mortar and pestle, centrifuging, and drawing off the supernatant fluid. By this technique, a pharmacologically potent solution was obtained containing body fluids as well as venom. This mixture was readily concentrated or diluted and has been kept frozen for long periods.

Body fluids and venom collected by the mortar and pestle technique showed no grossly apparent pharmacological difference from venom collected by electrical stimulation; thus, a solution containing 1 ant unit (AU) per milliliter (*i.e.*, venom abdominal fluids from one ant, prepared as indicated above) was utilized to determine the intraperitoneal LD_{50} in the mouse. Four groups of 10 female mice each were given 13.3, 20, 30 and 45 AU/kg intraperitoneally and the mortality was observed for 2 weeks.

Samples containing 3 ant units per milliliter were placed in tared weighing flasks over concentrated sulphuric acid and water withdrawn, the resultant solids were then weighed.

Results and Discussion. All quantitative data included in this study involved the use of the third collection technique, namely, that of homogenization of the abdomen, centrifugation of the resultant brei and removal of supernatant fluid. Intraperitoneal dosage in female weanling, Swiss Webster white mice, at levels of 13.3, 20, 30 and 45 ant units per kilogram of body weight resulted in the following mortality; at the high level *i.e.*, 45 (AU) per kilogram, all deaths which occurred were within 3 hours (5/10), the earliest deaths recorded were within one hour while at the next highest level *i.e.*, 30 AU/kilo, all deaths occurred within 24 hours (4/10). At the 20 AU/kilo level, 2 died within 24 hours, one in 48, and another in 72 hours (4/10), while at the low level 13.3 AU/kilo, one only died in 48 hours (1/10). The deaths were preceded by dragging and twisting of abdomen, respiratory distress, ataxia and extreme lethargy, with final action being a burst of excitation followed by clonic convulsion collapse and death. These data treated by the method of Litchfield and Wilcoxon(4)

gave an LD_{50} of 45 AU/kilo with upper and lower 95% confidence limits of 81 and 25 AU/kilogram of body weight respectively.

Dehydration of the homogenate in a desiccator at room temperature over concentrated sulfuric acid, when made up to 3 ant units per milliliter of solution, indicated one ant unit to contain 0.53 mg of total solids. It is interesting to note that this impure material, while a conglomerate of total abdominal fluid and contents, contained only roughly one-half milligram of solids per ant unit. Thus, an LD_{50} of 45 AU/kilo represents approximately only 24 mg/kg of solid agent. This amount places the toxicity of this material in the "highly toxic" category of Hodge's(5).

An active ant was placed on the inner aspect of the mid-left forearm of the human test subject. A moderate downward pressure with a small spatula upon the animal resulted in a sting. The phenomenon observed involved the use of the large horizontally moving mandibles on the head of the animal. A small fold of skin was taken by the insect between these mandibles for leverage, the abdomen was curved and the stinger driven deep into the skin. The stinging process was allowed to continue for 15 seconds during which waves of visible activity passed over the abdomen and charges of venom were pumped into the skin. The immediate sensation felt was that of extreme stinging pain at the site of the injection. Within 3 to 4 minutes a large flare had developed about 2 inches in diameter, while a small whitened 3 mm edematous wheal had arisen at the site of injection. Deep aching pain was felt within 10 minutes which extended from elbow to wrist. Sweating over the flare area was observed at the same time as piloerection about 12 minutes after the sting. Within 20 minutes the axillary pain developed and the deep aching pain was extended into the hand, until within 30 minutes the entire arm was in rather severe deep pain which extended into the pectoral muscles on the left side. Sweating, piloerection and pain continued over the flare area for 10 hours, while a deep itching sensation appeared after 1 hour and 25 minutes, which continued for almost 48 hours. Within 18 hours the flare area had decreased in intensity but was still visible and mild local edema coinciding with the flare was present for 48 hours. Feverishness over the site of the sting continued without sweating or piloerection and was still present 96 hours later, the area being sensitive to pressure, while mild superficial itching was present.

Summary. Venom from the desert ant, *Pogonomyrmex barbatus,* collected by 3 different techniques has been described. The observed effects of ant venom, when delivered by the intact viable animal into human skin, indicated a material of cholinergic properties which resulted in piloerection and sweating, and the capacity of eliciting an extensive and prolonged triple response. Absorption of the microgram quantities of venom are postulated to occur very slowly as a result of the time required for dissipation of the triple response and the absorption of the material through the lymphatics seems likely in view of the time course and distribution of the deep pain observed. Extracted venom by any one of the means described, was very toxic to female weanling mice and when given intraperitoneally the LD_{50} was 45 (AU) per kilogram of body weight.

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