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Received April 3, 1956. P.S.E.B.M., 1956, v92.

Immersion Hypothermia: Effect of Glycine. (22464)

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Factors responsible for and methods of preventing ventricular fibrillation during hypothermia have been vigorously pursued of late. The anti-arrythmic effects of many pharmacologic agents have been investigated(1,2), but little attention has been paid to the use of metabolic stimulants in hypothermia. Such substances may not directly prevent the onset of ventricular fibrillation, but they may prolong the time required to approach a lethal hypothermic level by increasing heat production within the body. Thus the survival chances of individuals accidentally exposed to hypothermic conditions would be greatly enhanced. An effective metabolic stimulant would also be of therapeutic value during the rewarming process by its ability to aid in rapid reattainment of normal body temperature.

Proteins are known to exert a definite thermogenic effect due to high specific dynamic action of certain amino acid constituents, principally phenylalanine, tyrosine, and glycine(3). The practical applicability of this phenomenon was demonstrated by Gubner, DiPalma, and Moore in patients with peripheral vascular diseases(4). These workers observed that oral ingestion of glycine induced a marked rise in oxygen consumption, heat production, and blood flow. It therefore appeared that glycine might be able to alter rate of cooling and rewarming during and following exposure to acute immersion hypothermia. This present study was executed in an effort to demonstrate a possible thermogenic effort of glycine at low body temperatures.

Methods. Twenty experiments were carried out on 10 apparently healthy mongrel dogs of both sexes, ranging from 10-26 kg. All dogs were anesthetized with 30 mg/kg of intravenous pentobarbital and then immersed in iced water bath of 8°C in the manner described by Hegnauer, *et al.*(5). Shivering was inhibited by appropriate administration of 50-100 mg of pentobarbital. Animals were cooled to rectal temperature of 28°C at which point the cold water was drained. They were exposed then to room air until such time as the rectal temperature fell to 26°C. Rewarming was instituted by immersion in warm water bath of 42-44°C. Rectal, bath, and room temperatures in °C were recorded continuously by means of a Leeds-Northrup iron-constantan potentiometer. During cooling and rewarming the following measurements were made. Oxygen consumption was determined at 35-33°C, 30-28°C, 28-26°C, and during rewarming at 30°C and 35°C. Expired air was collected in a Tissot spirometer and oxygen content measured with Beckman oxygen analyzer. Heat production in Cal./kg/hr was then calculated from oxygen consumption data by the method of Weir(6). Carbon dioxide in expired air was determined with a Haldane-Guthrie gas analyzer and blood sugar levels were measured prior to cooling and at a rectal temperature of 26°C. Continuous electrocardiograms (lead 2) were recorded throughout the experimental procedure. Each dog was rendered hypothermic on 2 separate occasions, separated by 2 to 5 days. On first exposure 5 dogs received 500 cc of 5% glycine solution intravenously, the remaining animals received 500 cc of 5% glucose solution. On the second exposure the so-

lutions were reversed. In each instance the infusion was started 15 minutes prior to immersion and continued until rectal temperature of 26°C was achieved. At beginning of the rewarming phase those animals which had received glycine while being rendered hypothermic were switched to glucose, and vice versa. During rewarming 300 cc of each solution was administered. The procedure of transferring solutions at start of the rewarming phase was carried out in an effort to evaluate the thermogenic property of glycine in non-treated subjects who already had been made hypothermic.

Results. Intravenous administration of 5% glycine solution significantly prolonged the cooling time in dogs subjected to immersion hypothermia (Fig. 1). Average time required to lower the rectal temperature of 10 control dogs from 38.3°C to 26°C was 135.2 minutes. These same animals when receiving glycine required 169.8 minutes to cool from 38.2°C to 26°C, an increase of 34.6 minutes ($p = <0.01$). The difference in cooling rates between the two groups is reflected in the significantly greater heat production (Cal./kg/hr) of dogs receiving glycine (Fig. 2). As indicated in Fig. 2 glycine exerted its most marked effect at rectal temperature of 30-28°C ($p = <0.05$). As the animals cooled further, the difference between the 2 groups became negligible. Measurements of blood sugar revealed a significantly higher concentration at 26°C than at 38°C in the glucose treated dogs, as expected, and also in the

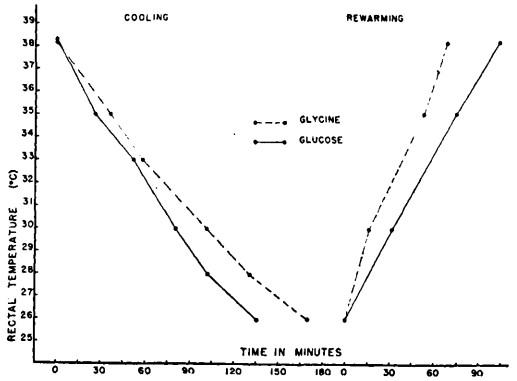

FIG. 1. Total cooling and rewarming time in glucose and glycine treated dogs.

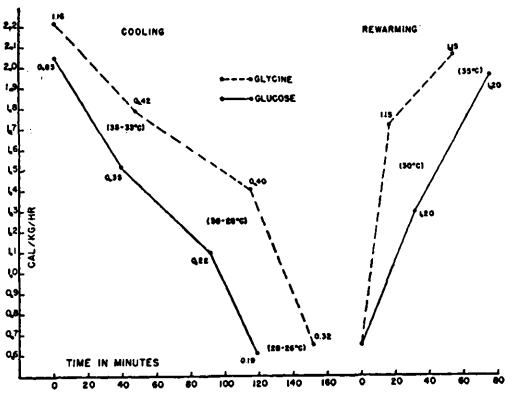

FIG. 2. Heat production in cal./kg/hr in glucose and glycine treated dogs during cooling and rewarming. Figures above and below points indicate stand. dev. Rectal temperatures in °C are presented in the parentheses.

group receiving glycine ($p = <0.01$). The latter is due perhaps to a depletion of liver glycogen as demonstrated by Peterson, *et al.* (7).

During the rewarming phase the marked thermogenic ability of intravenous glycine was illustrated. Those dogs in which the glycine infusion was started at 26°C reattained normal body temperature in average time of 69.7 minutes. These same dogs not treated with glycine during the rewarming phase required 104 minutes to achieve a rectal temperature of 38°C. The difference in rewarming time of 34.3 minutes was significant at the 0.01 level. The heat production calculated from the oxygen consumption again was greater in glycine treated group during rewarming. However, the scatter of values was considerably greater and so the difference between the 2 groups did not attain statistical significance ($p = >0.05<0.1$). The failure of the latter to achieve significance most likely was due to the residual effect of glycine in those dogs which were switched from glycine to glucose at the start of the rewarming phase.

An effort was made to quantitate the increase in heat production due to the administration of glycine and to compare this with the increase anticipated on the basis of the specific dynamic action of glycine alone. Two methods were used to calculate the heat pro-

duction which can be directly attributed to glycine thermogenesis: 1) The difference in heat content between each group after 135 minutes of immersion (average time required for controls to cool to 26°C). The heat content of each group was determined according to the formula: Caloric content = Mass X Specific heat X Mean body temperature(8). As calculated by this method the caloric content of the glycine group was 14.16 calories greater than controls. 2) The difference between the total caloric production of each group during 135 minutes of hypothermic exposure. The total caloric content for each group was determined by measuring the area under the respective curves in Fig. 2. Utilizing this technic the glycine treated dogs showed a total caloric production of 40.9 calories. The glucose group produced an average of 28.4 calories. Thus the caloric difference as determined by this method was 12.5 calories. Therefore, there is good agreement in 2 methods used to determine the caloric difference between groups.

During the 135 minute exposure period approximately 20 g of glycine had been infused. Assuming that glycine has a caloric content of 4 calories/g and a specific dynamic action of 30%, the maximum increase in heat production that can be attributed to the specific dynamic action of glycine would be 24 calories. Therefore, the increased caloric content of the glycine treated dogs can be explained solely on the basis of the specific dynamic action of glycine.

Discussion. The results presented herein indicate that agents which increase thermogenesis may be of practical importance in hypothermia. Glycine appears capable of delaying the rapidity with which a lethal hypothermic state is attained and of augmenting the rate of rewarming in dogs already rendered hypothermic. It is interesting in this regard to note that the relatively small caloric increase of 12-14 calories was sufficient to cause a significant difference in the cooling time of the two groups. Possibly the administration of larger quantities will exert an even greater effect. This is true, of course, only if the heat loss does not increase concomitant with the increase in heat production. Gubner, *et al.*(4) reported that 20 g of glycine per orum induced in human patients a rise in oxygen consumption and also a marked peripheral vasodilatation. The latter indicates a rapid dissipation of the extra heat produced by glycine. Under hypothermic conditions this avenue of heat loss is minimal, and so thermogenic agents should provide an effective adjunctive means of therapy in accidental and induced hypothermia.

The exact mechanism responsible for the extra heat production attributed to glycine is difficult to evaluate. As stated previously the increased caloric production can be adequately explained on the basis of the specific dynamic action of glycine if we assume that the SDA of amino acids is the same at low body temperatures. No evidence is available at present on this particular subject. Amino acids such as glycine are reported to possess a definite hyperglycemic effect. The significant rise in blood sugar observed in the glycine group supports this thesis. Petersen, *et al.*(7) have shown that glycine has a metabolic adrenergic effect, depletes liver glycogen and so produces hyperglycemia. This breakdown of glycogen to glucose which is an exothermic reaction may be responsible in part for the extra heat production observed in the glycine group. It is not certain, however, whether the latter should be considered a part of the specific dynamic action of amino acids or an entirely separate phenomenon.

Summary. The intravenous administration of a 5% glycine solution caused a significant increase of 34.6 minutes in the time required to lower the rectal temperature of dogs from 38°C to 26°C. Total rewarming time was decreased by 34.3 minutes in the glycine treated group. The differences in cooling and rewarming rates between the treated and non-treated animals was due to the increased heat production observed in the dogs receiving glycine. The possible applicability of thermogenic agents in accidental hypothermia is discussed.

The authors wish to thank Dr. D. W. Rennie for his advice during the course of this study and K. Behymer for his technical assistance.

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Received April 9, 1956. P.S.E.B.M., 1956, v92.

Action of Butyltolylsulfonylurea on Liver Glycogenolysis.* (22465)

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The hypoglycemic action of a sulfonamide derivative, sulfonamido-isopropylthiadiazole, was first discovered by Janbon and coworkers in 1941(1). Loubatieres(2) suggested that this compound caused hypoglycemia by stimulating insulin secretion. A similar conclusion was reached by Chen and coworkers(3) who investigated the hypoglycemic action of sulfanilamido-cyclopropyl-thiazole. It is possible that one mechanism by which the sulfonamide produces hypoglycemia is by inhibiting the rate of degradation of insulin, as has been reported by Mirsky(4) and Williams(5) to occur with certain other sulfonamides. Holt and coworkers(6) reported that sulfonamido-cyclopropylthiadiazole produces morphologic changes in the alpha cells of the pancreas in rabbits and they suggested that the compound caused hypoglycemia by inhibiting glucagon secretion. Franke and Fuchs(7), Bendfeldt and Otto(8), and Achelis and Hardebeck(9) attributed the hypoglycemic action of butylaminobenzenesulfonylurea to decreased production of glucagon. However, Tyberghein and Williams(10) found that extracts of serum of rabbits treated with butyltolylsulfonylurea (BTSU), which also produces hypoglycemia, had the same glycogenolytic stimulating activity in liver slices as extracts of serum of normal rabbits. Since there was good indication that this glycogenolytic effect was due to glucagon, these observations weaken the hypothesis of a decreased glucagon secretion. Therefore another mechanism was sought to explain the hypoglycemic effect of the sulfonamide preparations. An action on liver glycogen has been discussed by Miller and Dulin(11) and by Loubatieres(12). In the present study we investigated whether or not the hypoglycemic action of BTSU† could be related to an inhibition of glucose release by the liver and whether enzymes involved in glycogenolysis were affected by BTSU.

Methods. Some studies were conducted with rats and others with rabbits.

Rat Experiments. The effect of BTSU on blood sugar and liver glycogen was studied in Sprague-Dawley rats, each weighing approximately 300 g, prepared in one of the 2 following ways: (a) fasted for 24 hours, or (b) fed a standard amount of glucose for 24 hours. One group was fasted since it was believed that in this manner an inhibitory effect of BTSU on glycogenolysis might be more apparent, since normally the glycogen stores are considerably depleted after 24 hours. In all experiments with rats 80 mg of the sodium salt of BTSU, dissolved in 2 ml of water, was given every 12 hours by stomach tube. The first dose coincided with the beginning of the

* This research was supported by funds granted by the Atomic Energy Commission, the U.S.P.H.S. and the Upjohn Co.

† We are indebted to Dr. C. J. O'Donovan of the Upjohn Co. for generous supplies of 1-3-butyl-p-tolylsulfonylurea ("Orinase").