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Effect of Cooling on the Mechanism of Insulin Action.

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The experiments reported here are concerned with the effects of lethal doses of insulin on the survival period of cooled and non-cooled rabbits. Some observations on the effect of temperature on insulin action are also reported.

1. *The Body Temperature Lowering Action of Insulin.* Forty-eight-hour fasted rabbits were used. Blood sugars were determined by the Folin-Wu method. In order to produce a lowering of body temperature without using drugs or keeping the animal in an ice bath, the rabbits were wetted with 95% alcohol and placed in front of a fan. Using this method, the rate of heat loss was increased and the body temperature usually fell 2-7° C., reaching a maximum in 90-150 minutes. In the normal animal this increase in heat loss was compensated by an increase in heat production so that when the animal was not re-wetted, the temperature returned to normal in 1-3 hours. If, however, large doses of insulin were given at the time of the initial wetting, the mechanism of heat production was interfered with and the temperature drop was not only greater in the first 2 hours but continued to fall until it reached a point a few degrees above that of the room, and remained at that level from 6-18 hours without the need of any further wetting. Prevention of the blood sugar fall by the administration of glucose with the insulin prevented this hypothermic action of the insulin. Table I summarizes the results of these 3 groups.

TABLE I.
Effect of Insulin and Insulin and Glucose on Body Temperature of Alcohol Wetted Rabbits.

	Hours						
	0	1	2	3	4	5	6
No insulin, Avg 5 animals	39.1	36.0	35.9	36.9	38.5	39.1	39.0
3 u./kg., Avg 6 animals	38.9	34.3	32.3	27.7	29.4	28.6	29.3
3 u./kg + 5 g glucose Avg 4 animals	39.3	38.4	38.1	38.7	39.1	39.2	39.2

2. *Duration of Insulin Action in Cooled Rabbits.* Twelve rabbits were cooled by repeated wettings with alcohol and when the temperature reached 25-28°, 0.5 units of insulin per kg were given intraven-

ously. Eight rabbits, not previously cooled but given the same dose of insulin, served as controls. The duration of insulin action (as evidenced from the blood sugar) was greatly prolonged in the cooled animals, returning to normal after 6-10 hours, as compared with 3-5 hours for the non-cooled. Nevertheless, the rate of lowering the blood sugar was about the same in the 2 groups.

3. *Survival Period of Cooled Hypoglycemic Rabbits.* Eight fasted rabbits were given massive doses of insulin and their temperatures were kept at normal levels. Of these animals, 6 died from 3-6 hours after the onset of convulsions, while 2 recovered spontaneously from the hypoglycemia at the end of 7½ hours.

Thirteen rabbits were cooled to temperatures between 25-30°C and given 2 injections of insulin (6 units/kg each injection) 8 hours apart. Seven rabbits lived 14 hours, 3 lived 18 hours, 1 lived 20 hours, 1 lived 26 hours and 1 rabbit was maintained for 148 hours, with blood sugars ranging from 25-45 mg %. The insulin in the latter rabbit was reduced after the second day to 3 units twice daily. The respiration and heart rate of the cooled animals were irregular and reduced.

Discussion and Conclusions. 1. The results on the hypothermic action of insulin are in line with the observations of Dworkin and Finney,[1] who found that when a woodchuck was given enough insulin to produce hypoglycemia, it lost its temperature control and could readily be put into a state of "artificial hibernation." We also confirm their observation that the administration of glucose to cooled hypoglycemic animals resulted in the rise of their body temperature.

The results reported here give support to a thesis that available glucose is essential for the maintenance of normal body temperature.

2. Insulin hypoglycemia is prolonged by cooling the animal. Whether this is due to a reduction in the rate of gluconeogenesis (as a result of cooling), or due to a prolongation of insulin action cannot be definitely established at this time.

3. The survival period of rabbits receiving lethal doses of insulin is prolonged by cooling to temperatures below 30° C. It has been established that cooling to temperatures below 30°C greatly reduces the rate of metabolism,[2] as well as inhibiting or stopping insulin convulsions.[3]

The hypothesis is offered that both the increase in the survival period and the inhibition of convulsions in cooled animals receiving

1 Dworkin, S., and Finney, W. H., *Am. J. Physiol.*, 1927, **80**, 75.

2 Finney, W. H., Dworkin, S., and Cassidy, G. J., *Am. J. Physiol.*, 1927, **80**, 301.

3 Cassidy, G. J., Dworkin, S., and Finney, W. H., *Am. J. Physiol.*, 1925, **73**, 417.

massive doses of insulin are a result of the decreased metabolic rate and the subsequent decreased demand for foodstuffs by the vital centers of the brain.

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Choline Esterase Activity in Various Portions of the Rabbit Heart.

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The concentration of acetylcholine in the auricle is approximately 10 times that in the ventricle.[1-3] Gaddum has pointed out that tissues rich in acetylcholine contain greater quantities of choline esterase.[4] It was considered of interest to determine whether the latter relationship also holds for various portions of the heart, in which case credence would be lent to the possibility that this enzyme plays the rôle of a defense mechanism in the vagus regulation of the heart.

Twelve normal rabbits weighing between 1500 and 2200 g were utilized for these experiments. The animals were sacrificed by injecting air into the marginal ear vein; the chest was then opened, and the heart immediately extirpated. The individual auricles and ventricles and the interventricular septum were dissected out. The interauricular septum was included with the right auricle as was also the mouth of the superior vena cava. The valves and their attachments were discarded. The membranous septum was included with the interventricular septum. The tissue was freed from superficial blood, weighed, and then macerated in a mortar with purified and ignited sand. Bicarbonate-Ringer solution was added in the proportion of 10 cc per gram of tissue. The mixture was centrifuged and choline esterase measurements were made with ½ cc of the supernatant fluid and 1½ cc of substrate solution (acetylcholine chloride dissolved in bicarbonate-Ringer, 5 mg/cc). The enzyme activity was determined by the manometric method using the War-

1 Witanowski, W. R., *Arch. ges. Physiol.*, 1925, **208**, 694.

2 Plattner, F., *Ibid.*, 1926, **214**, 112.

3 Engelhart, E., *Ibid.*, 1930, **225**, 721.

4 Gaddum, J. H., *Gefässerweiternde Stoffe der Gewebe*, 1936, 75, George Thieme, Leipzig.