

reserpine, a supposition which deserves comparative experiments with myocardial fiber preparations at rest.

Summary. Analyzing the mechanism of thermal reaction, we found that exogenous K^+ potentiates cold contraction in rat intestine (or vas deferens) but has no similar effect on guinea pig intestine. A dibenzazepine, Protriptyline, which slightly relaxes the vas deferens of rat, induces a high potentiation of the contractions by thermal stimulus, K^+ , and norepinephrine. Norepinephrine contraction is not modified by thermal stimulation or K^+ . Reserpine is species specific on the isolated intestine: it abolishes cold contraction in guinea pig, while in rats only the spontaneous movements are decreased. However, the thermal response of the intestine in both species is inhibited by *in vivo* reserpinization. Cooling of guinea pig intestine in presence of reserpine increases K^+ release, a mechanism which may be responsible for some of its toxic action on contracting organs, such as the cardiac insufficiency in rats accompanied by K^+ re-

lease (9) and in patients treated with cardiac glycosides and reserpine (10).

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Differential Accumulation of Glycine by Avian (Chicken) and Mammalian (Human) Erythrocytes: Lack of Insulin Effect* (32691)

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Significant differences have been found in the accumulation of glycine by avian erythrocytes and mammalian erythrocytes. In the former the glycine concentration is 3–5 times higher than that of the plasma (1), while in the latter it is approximately equal to that of the plasma (1,2,3). While amino acid incorporation into avian erythrocytes is diminished by the presence of metabolic respiratory inhibitors such as cyanide, arsenate, and dinitrophenol, these agents do not affect the accumulation of glycine in mammalian erythrocytes (1).

In the course of experiments on the relationship of amino acid transport and protein synthesis, we have studied the effect of insulin on the uptake of glycine by nucleated erythrocytes, which are capable of protein synthesis (4)—as compared with nonnucleated erythrocytes, which are not capable of protein synthesis (2,3). We found that the nonnucleated cells accumulated glycine at a more rapid rate than did the nucleated cells, but the transport of this amino acid proved to be insensitive to insulin in both cell types.

Freshly heparinized whole human or chicken blood was centrifuged and the plasma decanted. The cells were suspended five times in 50–100 volumes of 0.9% NaCl at 4°C. and

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subsequently centrifuged at 4°C. for 10 min at 465g.

The incubation medium was prepared by mixing 2.15 ml of Raker's solution at pH 7.4 (5), 0.35 ml of Raker's solution containing 10 μ c of glycine- 14 C and 10 mmoles of carrier glycine, and 0.35 ml of 10 U/ml insulin in a mixture of 3.3 mM hydrochloric acid in 0.9% NaCl. The temperature of the incubation medium was raised to 37°C. and 0.65 ml of packed erythrocytes were added. At zero time and at stated intervals thereafter, 0.5 ml aliquots were removed and added to 0.5 ml of ice-cold Raker's medium. All further experiments were carried out in the cold. The cells were collected by centrifuging for 10 minutes at \sim 1050g. From the supernatant fluid a 0.2 ml aliquot was drawn and analyzed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer with a toluene-ethanol scintillating solution (6). The remaining supernatant was removed by aspiration with a Pasteur pipet and the erythrocytes were then washed three times with 1 ml 0.9% NaCl. After removal of the last saline wash, pointed filter paper wicks were used to absorb additional extracellular liquid. The packed cells were weighed and then hemolyzed by adding 0.2 ml of distilled water with stirring. The hemolyzate was stirred thoroughly with 0.4 ml of 20% trichloroacetic acid. After centrifugation 0.2-ml portions of the clear supernatant solutions were analyzed for radioactivity according to the method described above.

The constant K , representing the volume of trapped fluid in the packed cell mass (1), was determined with glycine- 14 C and with inulin- 14 C in aliquots of the experimental and control cell suspensions. An average value of 8% and 6%, respectively, of noncellular water of packed cells was obtained and no significant differences between cells exposed to insulin and control cells were observed. For the cellular water (K_2) 68% of cell weight—a value determined by Winter and Christensen (7)—was used.

It can be seen from Fig. 1 that (i) glycine is accumulated more slowly by chicken erythrocytes than by human erythrocytes during a 180-min period of observation and (ii) the presence of insulin does not affect the rate or

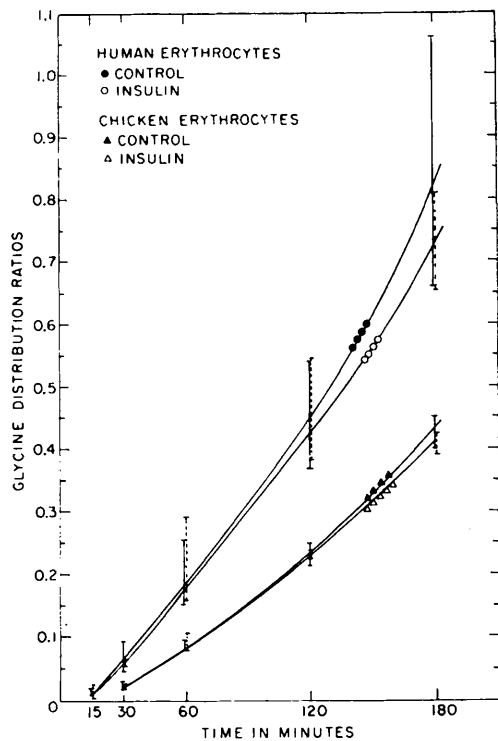


FIG. 1. Glycine accumulation by chicken and human erythrocytes. Values for human erythrocytes (● ○) represent the average of six experiments. Values for chicken erythrocytes (▲ △) represent the average of three experiments.

degree of glycine accumulation into the erythrocytes of either species.

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