be more effective after homogenization, when such contact would be enhanced.

Costa(8) reported SKF *cis* 385 to be less active than the *trans* isomer as an *in vivo* inhibitor of MAO, although no quantitative data were presented. This finding agrees with the tryptamine potentiating potency of this compound; SKF *cis* 385 being approximately one-half as potent as the corresponding *trans* isomer. Biel and co-workers(9) have indicated that the dextro-isomer of PIH is approximately 50% more potent than the racemate *dl*-PIH as an *in vivo* inhibitor of MAO. Although *d*-PIH was found to be twice as potent as the racemate as a tryptamine potentiator, comparison of the respective ED_{50}'s revealed that this difference in potency was not statistically significant ($P > .05$).

Although Tickner(16) reported that diphenhydramine and tripelennamine were effective *in vitro* inhibitors of MAO, the *in vivo* data presented here do not support this conclusion. Diphenhydramine, for example, potentiated tryptamine only at barely subtoxic doses, whereas tripelennamine failed completely to potentiate tryptamine. It is concluded that these compounds as well as the other compounds listed in Table II are not selective *in vivo* inhibitors of MAO.

Summary. The *in vivo* monoamine oxidase (MAO) inhibitory potency of a number of drugs was tested by measuring their activity as potentiators of the convulsant effects of tryptamine. Effective MAO inhibitors, arranged in descending order of potency, included harmine (s.c.) tranylcypromine, SKF *cis* 385, *d*-PIH, *dl*-PIH, harmine (oral), RO-4-1018, nialamide, phenelzine, iproniazid, and isonicotinyl-PIH. Compounds ineffective as MAO inhibitors included diphenhydramine, tripelennamine, isoniazid, dihydroergotamine, mescaline, SKF 525-A, SKF 5-A, procaine, and procaine amide.

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Received January 12, 1960. P.S.E.B.M., 1960, v103.

Action of Digoxin and Insulin on Transport of Glucose through Myocardial Cell Membrane. (25634)

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A decrease in myocardial glucose utilization has been found to be associated with failure in the dog heart-lung preparation(1). Administration of glucose or insulin results in an increase in myocardial glucose utilization and a restoration of the competency of the preparation(2). Administration of lanatoside C to the failing heart-lung preparation has also

been found to increase myocardial glucose utilization as evidenced by an increase in respiratory quotient of the cardiac glycoside-compensated preparation(3). Investigations with C^{14}-labeled substrates have demonstrated that cardiac glycosides increase rate of metabolism of glucose to carbon dioxide in the dog heart slice(4) and in the heart *in situ* (5). In view of the increased myocardial metabolism of glucose following administration of the cardiac glycosides or insulin, and the similarity of action of the cardiac glycosides and insulin on the dynamics of the failing heart-lung preparation, it appeared worthwhile to compare the action of digoxin with that of insulin on the rate-limiting reaction in utilization of glucose by the myocardium, *i.e.*, its transport across the membrane of the myocardial cell(6,7,8). This investigation deals with the effects of digoxin and insulin on rate of transport of galactose into the myocardium of the intact anesthetized dog. The criteria for the use of galactose as an indicator of glucose transport have been set forth by Levine (6).

Methods. The myocardial intracellular transport of glucose was determined by measuring rate of galactose-1-C^{14} entry and accumulation in the heart of normal dogs. Galactose-1-C^{14} used in these experiments had a specific activity of 0.88 μC/mM (Volk). Dogs weighing between 10 and 20 kg were anesthetized with pentobarbital sodium (35 mg/kg) in the post absorptive state, and allowed to respire room air supplemented with 100% oxygen *via* a nasal catheter. The external jugular vein was isolated and cannulated for administration of the drugs and isotopic substrate. Three groups of 6 animals were used in these studies. The first group received digoxin (0.065 mg/kg), the second group received insulin (1 unit/kg) and the third group served as the control. One-half hour after drug administration galactose-C^{14} was administered (0.5 μC/kg). The heart was quickly removed by a thoracotomy in the fourth intercostal space at 1, 5, and 10 minutes respectively, after administration of the isotope. The heart was freed of pericardium, fat and blood and homogenized in N perchloric acid (2 ml/g). Carbon dioxide liberated from the tissue was collected by means of a series of 4 gas washing towers, each filled with N sodium hydroxide. The carbon dioxide thus collected was precipitated as the barium salt and assayed for radioactivity. An aliquot of the total myocardial extract was assayed for radioactivity and the remainder was evaporated to dryness *in vacuo*. A sample of the dried extract, redissolved in a small amount of water was placed on Whatman #1 filter paper for chromatography in a butanol-formic acid-water(9) and a phenol-water solvent system(10). The chromatograms thus developed were placed on X-ray film for visualization of areas of radioactivity. R_f values thus obtained were compared with those obtained by chromatography of authentic galactose-1-C^{14}. Blood samples in the 3 groups were obtained from a femoral artery at time intervals of 1, 5, and 8 minutes following administration of the isotopic substrate. These samples were assayed for radioactivity as above. Statistical analyses of the data were made on the basis of a paired analysis of control and experimental groups throughout the time course of experiment.

Results. Fig. 1 illustrates myocardial galactose concentration (cpm/g) in control, insulin and digoxin-treated groups, as a function of time after intravenous administration of galactose-C^{14}. There was a significant increase in rate of galactose-C^{14} entry and accumulation in the myocardia of the digoxin-treated group ($P = 0.01$) and of the insulin-treated group ($P = 0.001$). There was no significant difference between myocardial galactose concentration in the digoxin and the insulin-treated animals. Integration of these curves revealed that administration of insulin or digoxin increased the extent to which galactose accumulated in the myocardium by 125 and 90%, respectively.

The myocardium did not metabolize galactose during the short duration of this experiment. There was no isotopic activity in carbon dioxide liberated from the heart tissue. Paper chromatography of the extract of the myocardium revealed, upon autoradiography, that all radioactivity on the developed chromatograms coincided with the R_f values of authentic galactose-C^{14}.

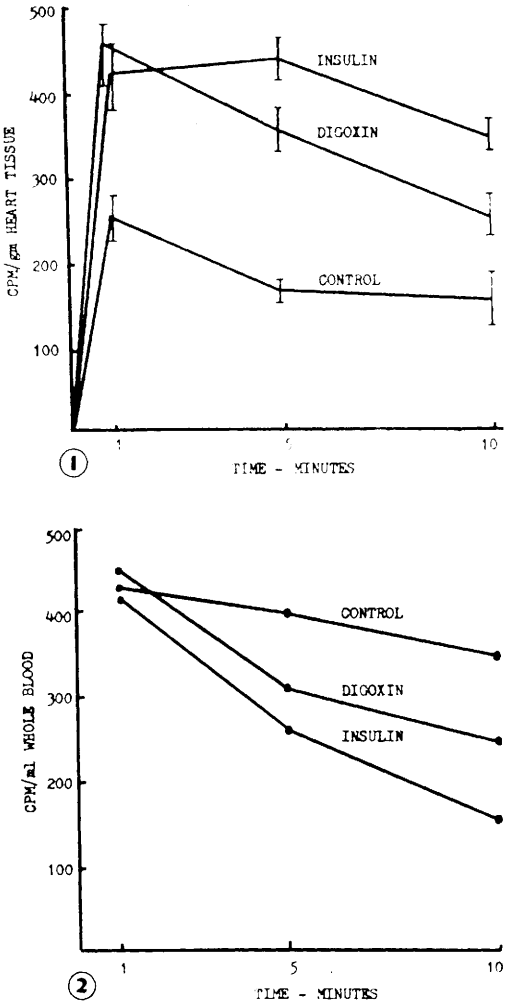

FIG. 1. Rate of accumulation of galactose-l-C^{14} in myocardia of control group, insulin-treated and digoxin-treated group, as a function of time following intrav. administration of labeled substrate. Each curve represents response in 6 dogs, 2 at each time interval. Perpendicular lines at each time interval represent range of deviation of the duplicate experiments.

FIG. 2. Rate of decrease of galactose-l-C^{14} in arterial blood as a function of time after intrav. administration of labeled substrate. Control, insulin-treated and digoxin-treated groups are represented by the mean responses in 3 dogs.

Fig. 2 illustrates rate of decrease of isotope activity in arterial blood of the 3 groups of animals. Digoxin increased rate of removal of galactose from the circulating blood volume. This has been observed in experiments with insulin(11). There was no significant difference between arterial concentrations of galactose-C^{14} in the 3 groups of animals at the one minute time interval. At the 5 and 10 minute time interval, however, there was significantly less galactose in the blood of the treated groups than the control ($P = 0.1$). There was no significant difference in galactose level between the digoxin and the insulin-treated group at any time interval.

Discussion. On the basis of conclusions drawn from experiments on galactose permeability with reference to the kinetics of glucose transport processes(6,7,8,11), these data indicate that digoxin and insulin augment transport of glucose into the myocardial cell. This effect of insulin is generally accepted as the mechanism whereby it increases the metabolism of glucose(12). The increase in myocardial galactose content following administration of digoxin may explain the earlier findings that the cardiac glycosides increase rate of metabolism of glucose by the myocardium(4,5).

Earlier experiments in this laboratory have shown that administration of therapeutic dose levels of digoxin increased rate of metabolism of glucose by the myocardium of the intact anesthetized dog and increased the flux of isotope through the cellular intermediates of the glycolytic pathway, the tricarboxylic acid cycle and the amino acids associated with these (5). These changes occurred in absence of changes in dynamics of the heart and hence without changes in metabolic requirements of the myocardium. Wollenberger(4) has demonstrated similar metabolic changes following administration of ouabain in the dog heart slice preparation. From these studies(5) it was concluded that the action of the cardiac glycosides appeared to augment one or more of the rate-limiting reactions of the glycolytic pathway of the myocardium, and in this manner, increased the contribution of cellular intermediates of the glycolytic pathway (*i.e.*, acetate, or oxaloacetate by carbon dioxide fixation) to the tricarboxylic acid cycle. This in turn, resulted in an increased rate of myocardial respiratory activity.

Burdette(13) has shown that administration of lanatoside C to human heart muscle slices resulted in an increase in oxygen consumption of the preparation and an increase in glucose uptake. Wollenberger(14) dem-

onstrated that this respiratory stimulating effect of the cardiac glycosides in heart slices occurred only if lactic acid or glucose was present in the incubation media. Furthermore, this effect of cardiac glycosides could not be elicited if the heart tissue was homogenized. Thus, the presence of a functional cell membrane was necessary for the actions of cardiac glycosides.

Our experiments indicate that the increase in rate of metabolism of glucose to carbon dioxide (as observed in our earlier experiments) by the myocardium, subsequent to administration of the cardiac glycoside, is a function of an increase in transport of glucose (galactose) across the membrane of the myocardial cell.

Summary. Galactose transport into the myocardium of the anesthetized intact dog was measured as function of time following administration of either digoxin (0.065 mg/kg) or insulin (1 unit/kg). Administration of these drugs resulted in an increase in galactose entry and accumulation in the myocardium, thus indicating an increase in rate of glucose transport into this tissue. The data support the conclusion that digoxin increases myocardial glucose metabolism by facilitating entry of glucose into the myocardial cell.

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Received January 13, 1960. P.S.E.B.M., 1960, v103.

Effect of Neurohypophyseal Principles on Adenohypophyseal Activity in Toads. (25635)

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It was shown that lysine-vasopressin injected into the common toad *Bufo bufo* (L.) was able to release hormone(s) from the pars distalis of the hypophysis(1), supporting the theory that vasopressin is chemically related to natural pars distalis stimulating factors(2). The present paper reports further investigation of the effect of neurohypophyseal principles on pars distalis function in toads. We were especially interested in determining minimal effective dose of vasopressin and in seeing whether the oxytocic principle was also able to stimulate the pars distalis.

Methods. In the toad, extirpation or inactivation of the pars distalis inhibits shedding of the slough, but not keratinization of the epidermis. Eventually, therefore, the skin becomes thickly cornified. The pars distalis stimulating activity of the preparations used could be evaluated from their effect on the skin. A preparation was judged capable of stimulating the pars distalis if it caused total or partial shedding of the slough within 24 hours after injection into toads with inactivated pars distalis, but not after injection into toads with extirpated pars distalis. Inacti-