Effect of Histamine, Epinephrine and Anti-Histamine (Antistine) on Glucose Turnover in the Isolated Liver. (20877)

RAUL HERNANDEZ JAUREGUI* AND MARTIN G. GOLDNER.

From Departments of Medicine, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N. Y., and the State University of New York, Medical Center at New York.

The clinical observation that certain antihistaminics induce a lowering of the blood sugar level(1,2) has led us to investigate the mechanism of this action. Since, as shown previously(3), this hypoglycemic effect can be obtained in the depancreatized and the alloxanized experimental animal, it does not seem to be mediated through the insulin-producing islet cell apparatus. We have therefore considered the possibility of a direct hepatic action and have now studied the effect of an antihistaminic on the carbohydrate metabolism of the isolated surviving liver. In addition, we have carried out similar experiments with histamine, partly in order to investigate the histamine-antihistamine relationship in this experimental setting, partly because the available data on the effect of histamine on carbohydrate metabolism are contradictory (4-8).

Material and procedures. Experiments were carried out with isolated and perfused liver of the bull frog (*Rana catesbiana*). The batrachian liver, if perfused with amphibian Ringer solution (NaCl 0.66, KCl 0.015, $CaCl_2$ 0.015, $NaHCO_3$ to pH 7.8, aqu. dest. ad 100) with addition of O_2 and glucose survives for several hours without spontaneous glycogenolysis. Bullfrogs, weighing 200 to 250 g, were kept in hibernating conditions at 4°C; they were killed by pithing. Livers were removed with preservation of proximal portions of the abdominal vein and the venous sinuses. Organs were weighed before and after the experiment; a slight weight gain of about 10% due to fluid retention occurred in most instances. The perfusions were performed as described previously(9) in accordance with the original method of Froehlich and Pollack (10) and the modifications of Geiger and Loewi(11) as to rate of flow and pressure. The perfusate entered the organ through the abdominal vein and was collected from the venous sinuses. The pressure was fixed at 20 cc H_2O. Recovery rate of the perfusate at start of experiments was set at 6-7 drops per minute. The returning perfusate was collected at 10-minute intervals and its glucose concentration was determined with the Nelson modification of the Folin-Wu micro method(12). An initial period of perfusion with an O_2 saturated amphibious Ringer solution served to wash out any free blood and blood glucose. After 20-30 minutes, the fluid usually returned water-clear and was sugar-free. At 40 minutes the perfusion fluid was changed to O_2 saturated amphibious Ringer-glucose solution, with a glucose concentration of 50 mg %. Within 10 or 20 minutes glucose content of returning fluid levelled off at the concentration of the entering perfusate. At this point the substances under investigation were injected into the perfusion system near the liver. The following substances were injected: 1) Histamine 0.1 mg, using histamine diphosphate (Abbott), 2) an antihistaminic drug, Antistine (Ciba), the dose being 5 mg, 3) Epinephrine 1 cc, 1:1,000,000.

Results. In a series of control experiments, no drugs were added to the perfusate; they confirmed previous observations(9) that after an initial period glucose concentration of returning fluid equalizes to that of the entering perfusate and remains at this level for periods of 2 to 3 hours. The results of experiments with drugs are demonstrated in Fig. 1. Six different experiments, each in duplicate, are shown. Since the main experiments started only after perfusion had been stabilized to the level of 50 mg % glucose, the initial periods with their fluctuations of the glucose concentration are not shown. The dotted lines on each graph represent the control level as established in the control experiments. It can be seen that histamine (0.1 mg) as well as epinephrine 1 cc (1:1,000,000) caused a marked

* Present address: Biological Institute, Puebla University, Puebla, Mexico.

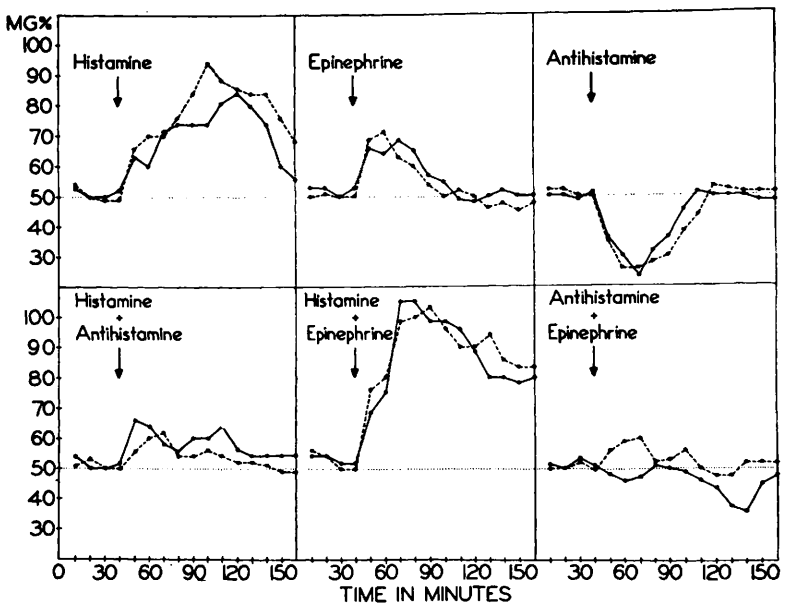

FIG. 1. Glucose concentrations of perfusates returning from isolated liver of bull frog, perfused with oxygenated amphibian Ringer solution containing 50 mg % glucose to which the following substances are added: Histamine 0.1 mg; epinephrine (1 cc—1: 1000000); antihistamine (Antistine) 5 mg; histamine + epinephrine, and Antistine + epinephrine. Two experiments shown in each instance. Initial periods of experiments during which livers were perfused without glucose are not included. Control level indicated in each graph by dotted line.

and transitory increase of the glucose concentration in the returning perfusate, while the antihistaminic caused an equally significant and transitory decrease. It must be emphasized that during these experiments, the rate of the perfusion remained grossly unaltered and that approximately the same amounts of fluid were collected during 10-minute periods before and after the addition of the test material. The second group of experiments shows the effect of simultaneous administration of Histamine + Antihistamine, Histamine + Epinephrine, and Antihistamine + Epinephrine in the above mentioned dosages. The antihistaminic is seen here to inhibit both the effect of histamine and that of epinephrine. Epinephrine and histamine, on the other hand, appear to have mutually augmenting effects; the increment of increase in the glucose content of the returning perfusate is not only greater but also more prolonged than if either substance is injected separately.

The last group of experiments (Fig. 2) was carried out to study the effect of antihistamine on spontaneous glycogenolysis of the surviving liver. The organ was perfused with a non-oxygenated sugar free amphibian Ringer solution. We have shown previously(9) that under these circumstances spontaneous glycogenolysis starts after 30 to 40 minutes of perfusion, and that this glycogenolysis is enhanced by epinephrine. This effect of epinephrine is demonstrated in the lower graph of Fig. 2. After spontaneous glycogenolysis had developed during the first 90 minutes of the experiment, epinephrine was added to the perfusate and a marked transitory rise in the glucose concentration of the returning fluid was found. The upper graph of Fig. 2 shows that the epinephrine effect was not only prevented by simultaneous injection of the antihistaminic, but that the glycogenolytic process itself was inhibited significantly. The decrease in the glucose concentration of the returning fluid indicates that glucose was retained in the organ, since the flow rate of the perfusion remained unchanged and no excessive amount of fluid was retained, as checked by the weight of the liver.

Comment. Our findings demonstrate that histamine and the antihistaminic substance used (Antistine) have direct effects upon the

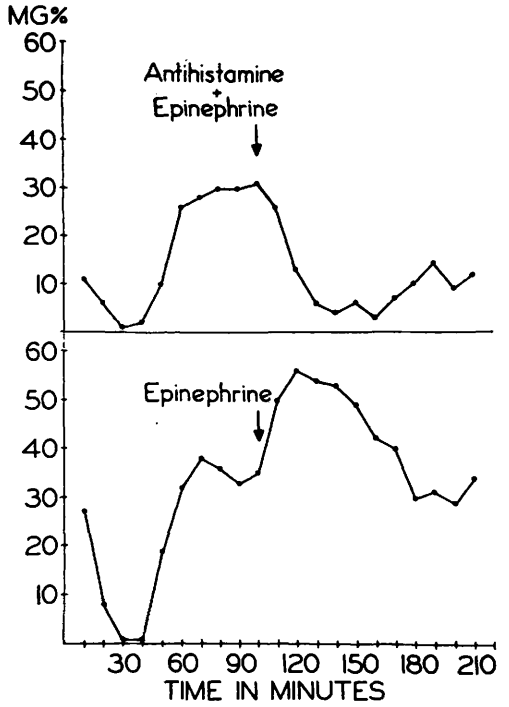

FIG. 2. Effects of epinephrine and of antihistamine (Antistine) plus epinephrine on glucose concentration of perfusate returning from isolated liver of bull frog perfused with amphibian Ringer solution. Epinephrine increases the spontaneous glycogenolysis; Antistine inhibits effect of epinephrine and diminishes spontaneous glycogenolysis.

carbohydrate metabolism of the surviving isolated batrachian liver. These effects are antagonistic inasmuch as histamine releases glucose from the hepatic tissue and the antihistaminic causes the hepatic tissue to retain glucose from the perfusate. With regard to the glycogenolytic action of epinephrine, it appears that histamine acts as a synergistic, while the antihistaminic acts as an antagonist. The observed effects seem to be the result of metabolic changes in the liver cells proper and not of changes in the vascular bed. Brauer and co-workers(13) have recently called attention to the vasomotor responsiveness of the isolated and perfused liver. In our experiments, however, pressure and rate of flow remained grossly unaltered, almost identical quantities of perfusate were collected during each fixed interval of time. The change in the glucose concentration of the perfusate would therefore seem to be caused by alterations in the cellular metabolism, *i.e.*, changes in intra-cellular glucose turn-over. Our findings tend to corroborate the observation of Chambers and Thompson(4), that histamine causes a decrease in hepatic glycogen. They are also in agreement with those investigators who reported a hyperglycemic phase after histamine administration(7,8). Furthermore, the demonstrated inhibition of hepatic glycogenolysis by the anti-histaminic used, suggests a possible mechanism for the clinically observed hypoglycemia induced by such substance.

Summary. The isolated liver of the bull frog is perfused with oxygenated amphibian Ringer glucose solution. The glucose concentration of the perfusate returning from the liver is determined in control experiments and under the influence of histamine, anti-histamine (Antistine) and epinephrine. Histamine and epinephrine cause an increase of the glucose concentration, probably due to enhanced glycogenolysis. Antistine causes a retention of glucose in the hepatic tissue. Antistine inhibits the spontaneous hepatic glycogenolysis as well as the glycogenolysis induced by histamine and epinephrine.

1. Jauregui, Raul Hernandez, and Bautista, Jr., G., *Rev. Med. y Ciencias Afines* (Mexico), 1950, v8, 116.

2. Goldner, M. G., and Jauregui, R. H., *Am. J. Dig. Dis.*, in print.

3. Jauregui, Raul Hernandez, *Rev. Med. y Ciencias Afines* (Mexico), 1950, v9, 111; 1951, v10, 127.

4. Chambers, E. K., and Thompson, K. W., *J. Infect. Dis.*, 1925, v37, 229.

5. Graf, W., and Nilzen, A., *Act. Derm. Venereal.*, 1948, v28, 7.

6. Schenk, P., *Arch. Exp. Path. Pharm.*, 1921, v89, 332.

7. La Barre, J., *Compt. Rend. Soc. Biol.*, 1926, v94, 777.

8. Takita, S., *Tohoku J. Exp. Med.* (Japan), 1950, v53, 145.

9. Goldner, M. G., and Jauregui, R. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 116.

10. Froehlich, A., and Pollak, L., *Arch. f. Exp. Path. Pharmac.*, 1914, v77, 265.

11. Geiger, E., and Loewi, O., *Pflueger's Arch. f. Physiol.*, 1923, v198, 633.

12. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

13. Brauer, R. W., Leong, G. F., and Persotti, R. L., *Am. J. Physiol.*, 1953, v174, 304.

Received January 21, 1954. P.S.E.B.M., 1954, v85.