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Role of the Liver in Tolerance to Morphine in the Rat.

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Gross and Thompson[1] and Oberst[2] discovered that dogs and also man excrete morphine partly in a combined form. Since then, attempts have been made to study morphine metabolism in tolerant and nontolerant animals with a view of finding whether habituation was accompanied by a difference in metabolism.[1,3,4] Oberst[5] noticed that the glucuronic acid concentration of human urine increases with increasing dosages of morphine. Gross, Plant and Thompson[6] and Gross[4] found that liver damage causes an increase in the excretion of free morphine in both tolerant and nontolerant dogs, and that liver damage inhibits conjugation of morphine to a certain extent. Bernheim and Bernheim[7,8,9] discovered that liver slices conjugate morphine. Kidney and brain tissues were inactive.

It seemed of interest to investigate whether liver slices from tolerant rats are capable of binding more morphine than slices from normal rats.

Experimental Methods and Results. Essentially the technique of Bernheim and Bernheim[7] was followed except that double the amount of morphine was added to the slices. We have not used the silicomolybdic acid reagent because the depth of color was far from proportional to the concentration of morphine. We have utilized instead the Folin-Denis reagent according to Oberst.[10] Blank experiments were run which consisted of slices in Krebs-Henseleit buffer without added morphine. Morphine added to such suspension followed after 2 minutes by precipitation with trichloroacetic acid can be recovered quantitatively.

Rats were made tolerant by repeated daily injections of morphine hydrochloride, starting with 2 mg/100 g and reaching a dose of 20 mg/100 g in a period of 20 days. Preliminary results obtained with 9 normal rats and 4 tolerant rats showed a binding of 2.16 mg morphine per g wet slices per 3 hours for normal rats as compared with 1.86 mg for tolerant rats. With a different strain of rats the average binding by 4 normal rats was 1.39, as compared with 1.13 for 4 tolerant rats. The addition of glucuronic acid or more morphine to the suspension of slices resulted in no increase in the binding of morphine. The values given by Bernheim and Bernheim for normal rats in three different publications were: 2.31, 3.0, 3.5 mg morphine/g/3 hours.[7,8,9]

These preliminary results seemed to indicate that tolerant rats do not bind more morphine than normal rats. More direct experiments involving partially and completely hepatectomized rats were then undertaken. Tolerant rats were partially hepatectomized by removing under ether anesthesia the median and left lateral lobes which amount to 65% of the weight of the liver. These rats still tolerate the maximum dose of morphine (20 mg/100 g) injected half an hour after

1 Gross and Thompson, *J. Pharm. and Exp. Therap.*, 1940, **68**, 413.

2 Oberst, *J. Pharm. and Exp. Therap.*, 1940, **69**, 240.

3 Thompson and Gross, *J. Pharm. and Exp. Therap.*, 1941, **72**, 138.

4 Gross, PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 61.

5 Oberst, *J. Pharm. and Exp. Therap.*, 1941, **73**, 401.

6 Gross, Plant, and Thompson, *J. Pharm. and Exp. Therap.*, 1938, **63**, 13.

7 Bernheim and Bernheim, *J. Pharm. and Exp. Therap.*, 1944, **81**, 374.

8 Bernheim and Bernheim, *J. Pharm. and Exp. Therap.*, 1945, **83**, 85.

9 Bernheim and Bernheim, *Arch. Biochem.*, 1946, **11**, 119.

10 Oberst, *J. Lab. and Clin. Med.*, 1938, **24**, 318.

the operation and behave like normal rats that had been subjected to the same operation of partial hepatectomy and injected with saline.

Tolerant rats were then subjected to "functional" hepatectomy by evisceration according to Russel.[11] The operation is performed under ether anesthesia and lasts about 10 minutes. Half an hour after the operation the rats walk around and appear quite "normal." These operated rats also tolerate the maximum dose of morphine and behave like normal operated rats injected with saline. That such asphyxiated livers are effectively cut off from circulation is shown by the fact that the blood sugar falls. However, the rate of fall of blood sugar varies greatly from one animal to the other. The survival time of such animals treated with saline injections varies from 3 to 20 hours. In accordance with the observation of Ingle and Sheppard[12] we observed no clear correlation between the time of survival and the level of blood glucose at death.

It is difficult to interpret these results in any other way than to mean that tolerance is not due to metabolism of the morphine in the liver, spleen or gastrointestinal tract.

[11] Russel, *Am. J. Physiol.*, 1942, **138**, 95.

[12] Ingle and Sheppard, *Endocrinology*, 1945, **37**, 377.

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Organ Antigen in the Early Chick Embryo.

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It was previously shown[1] that early embryonic stages of the chick contain one or more antigens occurring in the adult serum. In the present report evidence is presented for the presence in the early chick of a second type of antigenic substance(s) which is common to several adult organs and is serologically distinguishable from the antigens of blood and yolk.

Our previous work on the chick[1] indicated that the serum-antigen(s) in the early stages was exclusively of vitelloid nature, that is, serologically similar to certain constituents of the yolk. Non-vitelloid constituents of the serum appear in later stages of development. In attempting to demonstrate a new organ-antigen in the early embryo, it is therefore necessary to show that it can be distinguished from both blood and yolk antigens, since one group of antigens might be common to a given organ and blood, whereas another group might be common to blood and yolk.

Preparation of antisera. Brains were obtained from chicks near the time of hatching (19-20 days of incubation). The chicks were perfused with 0.85% NaCl solution injected into the left ventricle and allowed to flow from the cut jugular veins until clear of blood. The roof of the cranium was cut away, the brain removed to sterile Petri dishes and frozen over dry ice. When properly perfused the brain is of a chalky-white color. Accumulated brains were crushed while frozen and dried *in vacuo* at −10°C. The dried tissue, very porous and friable, was broken up with a sterile pestle and mortar and stored in tightly-stoppered bottles at −25°C.

The brain powder was stirred into ice-cold saline in proportion of 100 mg of dry powder for every 3 ml of saline. After mechanical stirring for about an hour at 0° to 3°C, the suspension was left overnight at 3° to 5°C, and then centrifuged at 3000 R.P.M.

[1] Schechtman, A. M., *J. Exp. Zool.*, 1947, **105**, 329.