motor nerve plates and muscle cross striations may be arranged in a graded series and are evidently determined by the active and inactive fibers in the fractional contraction of the same muscle. These structural changes favor the chemical theory of impulse transmission.

13042

Effect of Angiotonin and Renin on Glomerular Circulation in Frog Kidney.

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Angiotonin is the substance produced by the interaction of renin and renin activator in the plasma.[1, 2, 3] Corcoran, Kohlstaedt and Page expressed the opinion that angiotonin is a highly active vasoconstrictor which raises the blood pressure without simultaneously depressing the cutaneous temperature. They reported the effect of angiotonin on the kidney to be predominantly a vasoconstriction of the efferent arterioles of the glomeruli.[4, 5, 6] Hill and Andrus[7] noted an increase in the amplitude of the heart beat, a decrease in coronary flow, but no consistent effect on the rate of the perfused heart of the cat when angiotonin was added to the perfusate. However, renin had no significant effect. Page and Helmer[8] observed tolerance to successive doses of angiotonin in normal dogs, but if the kidneys are removed, the degree of tolerance becomes slight.

The purpose of this investigation was to study by direct microscopic observation the effect of angiotonin* and of renin* on the

1 Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.

2 Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

3 Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

4 Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 244.

5 Corcoran, A. C., and Page, I. H., *J. A. M. A.*, 1941, **116**, 690.

6 Corcoran, A. C., and Page, I. H., *Am. J. Physiol.*, 1940, **130**, 335.

7 Hill, W. H. P., and Andrus, E. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 213.

8 Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 495.

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renal circulation by transilluminating the kidney in the intact frog.

Method. For the study of the effect of angiotonin and of renin on the circulation in the kidney of the frog, a modification of the method of illumination described by Knisely[9, 10] was used. The experiments were made on anesthetized male frogs *(Rana pipiens)* whose weights ranged from 25 to 45 g. Anesthesia was produced by injecting into the dorsal lymph sac 0.1 cc of a 25% solution of urethane per 10 g of body weight. The abdomen of the frog was opened by a paramedian incision, carefully avoiding the large vessels, and the edges of the incision were cauterized gently to prevent loss of blood. The animal was laid on a block of cork with frog Ringer's solution gently flowing over the exposed viscera to prevent drying. Water was kept trickling around the intact body of the frog to dilute the Ringer's solution in contact with the skin and prevent dehydration. The kidney was protected from the heating effect of the light, transmitted through the quartz tubing, by a constant stream of Ringer's solution flowing through the bore in the delivery tip introduced retroperitoneally, dorsad to the kidney. Such a preparation could be kept functioning normally for at least 9 hours. Healthy frogs were used and the normal renal circulation in several microscopic fields was studied carefully before any observations on the effects of angiotonin or of renin were made.

Amounts of angiotonin or of renin ranging from 0.01 to 0.1 cc were injected either into the lymph sacs or intravenously into the abdominal vein, or were applied directly through a very fine needle to the microscopic field under observation. A stop watch was used to record the time when the drug was administered, when any effect was observed, and when the affected parts returned to their original condition previous to the administration of the drug.

Results. Angiotonin. By direct application of one or 2 drops of undiluted angiotonin over the region under microscopic observation a transient cessation of circulatory activity of the glomeruli, with moderate blanching of the region, was produced within 10 to 15 seconds and lasted for a fraction of a minute. This was followed soon by moderate packing of blood corpuscles and slight engorgement in the glomeruli which lasted for more than 8 minutes. Thereafter the renal circulation gradually returned to the preadministration state. This was consistently true for the first 4 or 5 administrations, but any application after that produced neither blanching nor cessation of activity in the glomeruli; nevertheless, the slight

9 Knisely, M. H., *Anat. Rec.*, 1936, **64**, 499.

10 Knisely, M. H., *Anat. Rec.*, 1938, **71**, 503.

engorgement of the glomeruli and the increase in the activity of the heart were produced repeatedly.

Injection of 0.025 to 0.1 cc of undiluted angiotonin into the submaxillary lymph sac caused, within 2 minutes, very slight paling of the region of the kidney under observation. This was followed soon by moderate engorgement of the glomeruli for more than 10 minutes. Within 15 minutes the glomerular, as well as the rest of the renal, circulation under observation gradually returned to the preinjection state. The injection of angiotonin into the lymph sac did not cause cessation of glomerular circulation.

Intravenous administration of 0.025 to 0.1 cc of undiluted angiotonin within 15 seconds caused blanching of the region and cessation of circulatory activity in the glomeruli which lasted for about one minute. However, this was followed soon by increase in circulatory activity in the kidney and moderate engorgement of the glomeruli. This engorgement phase lasted for about 8 minutes, after which the renal circulation gradually returned to the preinjection state.

When angiotonin was administered repeatedly after the effect of a previous injection had worn off, it was observed that only moderate engorgement resulted, but no blanching was produced even with increased dosage, no matter what route was used for the administration. Under the influence of angiotonin it was observed that the heart rate was increased about 10 beats per minute and the force of the heart beat was pronounced. This is in agreement with the observation of increased amplitude of the beat of the perfused heart of the cat as reported by Hill and Andrus.[7]

In comparing the effect of angiotonin with that of epinephrine, it was observed that the vasoconstriction and blanching as well as the subsequent engorgement of the glomeruli produced by a 1:100,000 dilution of epinephrine were much more marked than those produced by undiluted angiotonin but the duration of the engorgement caused by epinephrine was shorter.

Renin. Whether directly applied or injected into the lymph sac or intravenously, renin produced no demonstrable change in the renal circulation of the frog *(Rana pipiens).*

Summary. Angiotonin, when applied directly or administered intravenously produced transient vasoconstriction with cessation of activity of the glomerular circulation for about one minute. This was followed by moderate engorgement of the glomeruli which lasted about 8 minutes. When angiotonin was administered subcutaneously, moderate engorgement of the glomeruli was produced

without an initial cessation of glomerular circulatory activity. With successive administrations of angiotonin, the blanching effect disappeared but the glomerular engorgement was observed consistently.

Renin had no detectable effect on the renal circulation in the frog.

13043 P

Agglutination of Encephalitis Virus-Coated-Bacterial Cells by Virus Antisera.

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Details of the technic involved in satisfactorily coating bacterial cells with serum antigen and the use of such cells in an agglutination test have been given in a previous report.[1] A particular advantage of the procedure is that very minute amounts of serum antibody specific for the adsorbed antigen can be detected, even though the amount of antibody is too small to be revealed by the conventional precipitation test. The use of this very sensitive type of an antigen-antibody reaction, for the detection of 'antibodies' in the blood of man and of animals against the virus of St. Louis type of encephalitis, is the basis of the present report.

The microörganisms *(Serratia marcesens)* were prepared for use in the agglutination reaction by incubating them with virus as contained in a saline emulsion of infected mouse brain. That virus was adsorbed by the bacteria in significant quantity was indicated by the observation that such cells, after repeated washing with saline, would subsequently reproduce the disease in mice upon intranasal implantation.* Suspensions of virus-coated bacteria were employed as antigens in making agglutination tests with the sera of (a) human beings convalescent from encephalitis, (b) normal individuals, and (c) of rabbits immunized against encephalitis virus.

With the sera of convalescent patients† agglutination reactions

[1] Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **47**, 11.

* Subsequent testing of the brains of such infected mice established the specific identity of the virus.

† For supplies of sera and for data pertaining to mouse neutralization tests we are indebted to Dr. G. O. Broun of the Department of Medicine of St. Louis University.