Beeston and Channon[3] have observed an enhancing effect of cystine on fatty liver production and Tucker and Eckstein[4] have shown that methionine has the opposite effect. Furthermore, Newburgh and Curtis[5] and Cox, Smythe and Fishback[6] have reported the occurrence of hemorrhagic kidneys on a diet containing casein and added cystine and Hartwell[7] noted a similar effect on diets containing edestin. Our data suggest that these renal lesions were in reality due to choline deficiency and experiments to answer this question are now in progress.

The remarkable effect of small amounts of choline in preventing a severe pathological state associated with hemorrhagic degeneration of the kidneys demonstrated its important rôle in the maintenance of normal kidney structure in young rats. The choline requirement is dependent upon certain other factors, among which the methionine-cystine ratio of the protein may be particularly important. It is provisionally suggested that the choline requirement is increased by dietary protein relatively richer in cystine than in methionine.

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## Observations Indicating Absence of Glomerular Intermittence in Normal Dogs.*

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Khanolkar[1] and Hayman and Starr[2] have reported experiments on rabbits which they interpreted as showing that usually only a fraction of the renal glomeruli are open to circulating blood. The principle in both sets of experiments was essentially the same, the injection of hemoglobin or dye into the blood stream with subsequent examination of the kidneys to determine what fraction of the capsules or glomeruli

3 Beeston, A. W., and Channon, H. J., *Biochem. J.*, 1936, **30**, 280.

4 Tucker, H. F., and Eckstein, H. C., *J. Biol. Chem.*, 1937, **121**, 479.

5 Newburgh, L. H., and Curtis, A. C., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 963.

6 Cox, G. J., Smythe, C. V., and Fishback, C. F., *J. Biol. Chem.*, 1929, **82**, 95.

7 Hartwell, G. A., *Biochem. J.*, 1928, **22**, 1212.

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1 Khanolkar, V. R., *J. Path. and Bact.*, 1922, **25**, 414.

2 Hayman, J. M., Jr., and Starr, Isaac, Jr., *J. Exp. Med.*, 1925, **42**, 641.

contained the injected material. The percentage of glomeruli receiving the injected material varied in different experiments from 11 to 100.

The present paper reports findings on 12 kidneys of 8 normal dogs which are interpreted as evidence that all the glomeruli are normally open all the time. The renal arteries were exposed usually by flank incision, sometimes by midline laparotomy, under nembutal, 32 mg per kilo, intravenously. Injection of 0.4 to 0.5 cc filtered Higgins Eternal Black ink into renal artery was completed in 5 seconds, renal vessels were clamped 5 seconds after end of injection. The injection was through a fine hypodermic needle, sometimes with and sometimes against the stream. The kidneys were removed soon after; 3 to 6 blocks taken from different parts of the kidney were put into 10% formalin over night and frozen sections of 100 micra cut. From 7 to 12 sections of each block were counterstained with eosin and permanently mounted. With this technic the ink-free as well as the ink-containing glomeruli are easily seen and the proportion of injected to uninjected readily determined in a very large sample. Since each section contained 200 to 800 glomeruli, with 30 to 50 sections from each kidney, there were 10,000 to 30,000 glomeruli inspected with each kidney.

In every case it was found either (a) that every glomerulus was injected or (b) that the distribution of injected and uninjected was such as to indicate that failure of injection was due to the manner of distribution of ink in the larger preglomerular vessels rather than to closure of the glomeruli themselves or of the afferent arterioles. Of 12 kidneys examined the injection was into the main renal artery in 10 cases; 2 of these showed every glomerulus injected. In 2 cases the injection was into 1 of the 2 main branches of the renal artery just outside the kidney; in these 2 all the glomeruli of one half were injected, none of the other. In 1 case the boundary was very sharp. in the other there was a boundary zone 3 or 4 mm wide within which there were both injected and uninjected, the proportion of injected in the zone increasing as one passed from the uninjected to the injected side. On the injected side the vessels other than glomeruli were also injected, but not on the uninjected side.

The remaining 8 cases are of particular interest; in all of these the injection was into the main renal artery. In 3 of these, one-half of the kidney was completely injected, the other half completely uninjected; the picture resembled the 2 cases where the injection was into one branch of the artery. In one of these 3 cases the boundary was very sharp, in the other 2 there was an intermediate zone a few

millimeters wide. In one case the plane separating injected from uninjected side passed sagittally through the pelvis, in the other 2 it differed somewhat from this plane. The only acceptable interpretation of these cases is that all of the ink by chance was swept into one of the 2 main branches of the artery; the only alternatives are that all the glomeruli in one-half of the kidney are closed while all those in the other half are open, or that one of the 2 main branches of the renal artery is showing intermittence.

In the other 5 cases there were various degrees of transition between the bilaterally symmetrical hemi-injection of the above 3 cases and a complete injection; in one case the injection was complete except for a wedge of about 1/40 the kidney volume. As would be expected, isolated fields within the various boundary zones showed a picture which, if considered alone, was quite compatible with the view of glomerular intermittence. The complete picture, however, in my opinion, gives undoubted evidence that incompleteness of injection is in all cases due to a distribution of ink in the preglomerular vessels which is determined by the characteristics of stream flow; if the picture is to be ascribed to intermittence, such intermittence must be assigned to some relatively large artery. In any area where the glomeruli are injected the vessels other than glomeruli are also injected and *vice versa*. Ink injected into the main renal artery, even though in some cases the injection was made against the stream, may by chance completely escape one of the 2 main branches or any subsequent smaller branch. It is obvious that if one depended for his conclusions upon the total count of injected glomeruli, as did Hayman and Starr, varying degrees of completeness of injection would be obtained. The procedure employed in the present paper of counterstaining the sections has made it possible to determine directly the proportion of injected to uninjected glomeruli and, more important, details of their distribution.

A few normal rabbits have also been observed, some with injection into the renal artery and some into the aorta; the conclusions to date are the same as reached above with dogs.