deteriorate more quickly in the higher concentrations of glucose than do fibroblasts and epithelial cells. Migration of the polymorphonuclear cells is stimulated by concentrations of 390, 650, and 1,150 mg. %. These concentrations do not injure the cells. The higher concentrations, although stimulating in the beginning, later become inhibiting and quite toxic. The monocytes appear to be stimulated by glucose in all the concentrations except the very highest. The cells are active in all, but appear coarsely granular in the three highest concentrations. The 3,200 mg. % concentration is, in fact, toxic.*

Iris epithelial cells tolerate each concentration of glucose except that of 3,200 mg. %.* In the latter, the cells become granular. However, after transfer into ordinary nutrient medium, all the cultures recover and grow normally.

8830 C

Reaction of Sensitized Dog's Kidney to Horse Serum Injected into the Renal Artery.

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The rôle of allergy in the pathogenesis of glomerulo-nephritis has been a subject of experimental investigation for many years and offers an attractive theory of the etiology of this disease. It has long been known that injections of foreign proteins into previously sensitized animals produce lesions in the kidney. Longcope[1] in 1913 showed that animals which were sensitized to horse serum or egg albumin and later given a shock dose of the same protein developed focal renal lesions among which were marked glomerular changes. Duval and Hibbard[2] found that parenteral injections of

* It is possible, of course, that this inhibiting effect is due to the reduction in salts that had to be made to keep the solution isotonic. When adding glucose, the solution will be hypertonic unless the salt concentration is lowered. From previous work, it seemed probable that injury to the cells would occur more quickly in hypertonic solutions than in those with reduced salt content. It is interesting to note that Wilmer, using hypertonic solutions and fresh tissue, found approximately the same limits of tolerance as those reported here.

1 Longcope, W. T., *J. Exp. Med.*, 1913, **18**, 678.

2 Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, **44**, 567.

streptococcus filtrates into animals produced glomerular lesions only if the animals had been previously sensitized to the filtrates. The fact that post-scarlatinal glomerulo-nephritis appears during convalescence rather than at the height of the disease speaks strongly in favor of the "allergic" theory of this disease. Hepler and Simonds[3] studied the effects of injecting proteins directly into the kidneys of sensitized animals. Rabbits were sensitized by subcutaneous injections of horse serum or egg albumin; later, the protein was introduced directly into the kidney through the skin. They observed hemorrhage and necrosis of the injected kidneys and concluded that these changes resulted from an anaphylactic inflammation caused by the direct toxic effect of the antigen on the sensitized tissues (Arthus phenomenon). The lesions thus produced, however, bore no analogy to the histological changes occurring in human glomerulo-nephritis. Masugi,[4] on the other hand, claimed to have produced the picture of a true glomerulo-nephritis by injecting proteins directly into the renal arteries of rabbits which had been previously sensitized. He emphasized the importance of having the foreign protein reach the kidney in a concentrated form, and believed that the unconvincing results of previous workers were due to a failure to bring about the necessary concentration in the glomeruli.

The work to be described in this paper is an attempt to produce renal lesions in dogs by methods similar to those that Masugi used in rabbits, and then to study the kidneys from both functional and histological aspects.

Method. Nine healthy, well-nourished dogs which were without evidences of previous nephritis were used in these experiments. Two sensitizing injections of 5.0 cc. of horse serum were given intravenously at 2-day intervals to all dogs excepting one which received 7 injections at intervals of 3 days, the doses being 4.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 cc. From 16 to 21 days later about 3.0 cc. of horse serum were injected directly into the left renal artery according to the technique described by Quinby and Fitz[5]. The kidney was approached retroperitoneally and the renal artery and vein were exposed, freed by blunt dissection, and ligatures placed about them. Simple traction on these ligatures sufficed to shut off the flow of blood in the vessels. Both renal vessels were occluded and about 3.0 cc. of horse serum were injected into the artery by means of a

[3] Hepler, O. E., and Simonds, J. P., *Am. J. Path.*, 1929, **5**, 473.

[4] Masugi, M., and Sato, Y., *Virchow's Arch. f. path. Anat.*, 1934, **293**, 615.

[5] Fitz, R., and Quinby, W. C., *Arch. Int. Med.*, 1915, **15**, 303.

syringe fitted with a fine bent needle. The artery was then released to allow the blood to drive the serum into the kidney. After 30 seconds the artery was again occluded, and 10 to 15 cc. of blood were withdrawn from the distended vein by a sharp hypodermic needle attached to a 20 cc. syringe. This blood, presumably, contained most of the injected horse serum. Normal circulation was then allowed to reestablish itself. Pressure of 2 or 3 gauze wipes on the renal vein usually controlled the hemorrhage. With complete hemostasis the wipes were removed and the wound repaired. In 5 of the 9 animals a second shock dose was administered from 12 to 34 days after the first. The method of operation was the same, but increased difficulty was encountered because of adhesions and scar tissue from the first operation. Biopsy specimens were taken from the kidney at the beginning of the experiments and before each arterial injection in 8 out of 9 cases.

After an interval of 1 to 21 days after the final shock dose of horse serum, the dogs were anesthetized with an intravenous injection of 35 mg. of nembutal per kg. body weight, and the ureters exposed through a suprapubic midline incision. Small catheters were inserted into each ureter through a small incision and tied into place. Separate specimens of urine from each kidney were collected into graduated cylinders and the half-hourly output carefully recorded. A satisfactory flow of clear urine from each catheter having been established, 3.0 mg. of phenolsulphonephthalein was injected intravenously, and the excretion from each kidney during the following 2 hours recorded. According to the weight of the dogs, 0.5 to 1.0 cc. of salyrgan was then injected intravenously, and the diuretic response of each kidney was observed at half hour periods. At the end of diuresis the dogs were killed by intravenous chloroform. Throughout the procedure a continuous intravenous flow of physiological saline was maintained. The urine from each kidney was examined for albumin and microscopic sediment, and chloride and creatinine determinations were made on each specimen. After the animals were killed three blocks were taken from each kidney, and together with the biopsy specimens were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Results. All dogs behaved normally after the injections of the sensitizing doses of horse serum. At no time throughout the course of the experiments were there any urinary abnormalities with the exception of occasional slight traces of albumin following the injection of horse serum into the renal artery. There was no constant

difference between the secretion of urine from the 2 kidneys following administration of salyrgan, nor were there any differences in the chloride and creatinine excretion. The phenolsulphonephthalein excretion was the same from the 2 kidneys of all dogs excepting 2. The kidneys of these 2, however, showed gross and microscopic abnormalities which could not possibly be explained on an allergic basis. The left kidney of one presented the appearance of multiple infarction due in all likelihood to the presence of particulate matter contained in the serum. This particulate matter was noted at the time of injection into the renal artery. That of the other contained numerous microscopic abscesses caused, no doubt, by infection introduced at the time of injection. The kidneys of 5 of the 9 dogs were entirely normal both grossly and microscopically. All biopsies showed normal kidney tissue. The kidneys of 2 of the dogs showed changes which will be described and discussed below.

Dog No. 1, a male weighing 10 kilograms, was given two intravenous sensitizing injections of 5.0 cc. of horse serum, and 21 days later 5.0 cc. were injected into the left renal artery. On the following day functional studies revealed no impairment of either kidney and the animal was killed for autopsy. With the exception of the left kidney all the organs were normal. The kidneys were of equal size and of normal reddish-brown color, but scattered over the surface of the left kidney were numerous small pin-point whitish depressed areas surrounded by rings of hyperemia. The cut surface was not abnormal. The microscopic appearance was that of exudative rather than proliferative lesions. The glomerular tufts were swollen and somewhat more cellular than normal and tended to fill the capsular spaces. There was some increase in the sub-endothelial ground substance and slight proliferation of the endothelial cells. There was no increase in interstitial tissue. There was an albuminous exudate in the capsular spaces, but no inflammatory or red blood cells. The tubules showed slight cloudy swelling of the epithelium and an albuminous exudate in the lumina. The vessels were normal. The right kidney showed similar but less marked lesions.

Dog No. 2 was a male weighing 9.6 kilograms. Horse serum was injected intravenously as in the case of Dog No. 1. Twenty-one days later, horse serum was injected into the renal artery. After 6 days, routine functional studies were made, and the dog was killed. The microscopic appearance of the biopsy specimens and the functional behavior and gross appearances of both kidneys were entirely normal. Microscopically, the glomerular tufts showed little

or no proliferative change, but the epithelium of the Bowman's capsule was often seen to be thickened; the capsular spaces contained much albuminous material and red blood cells. The tubular lumina were filled with an albuminous exudate, but the tubules themselves, as well as the interstitial tissues and blood vessels, were normal. The right kidney showed similar but somewhat less marked changes.

Summary. In a series of 9 dogs, an attempt was made to produce an experimental glomerulo-nephritis by the injection of horse serum into the left renal artery of previously sensitized animals. Functional studies failed to reveal any impairment as a result of the procedure, and pathologically no animal showed a real glomerulo-nephritis. In the kidneys of 2 dogs lesions were found which were the result of traumatization and infection respectively; in 2, minor changes were noted, as just described, whose relation to the procedure of the experiment is uncertain; in 5 no pathological changes were observed. It is concluded that the procedure described by Masugi as productive of glomerulo-nephritis in rabbits failed to produce the disease in dogs.

8831 P

Effects of Increased Metabolism on the Ketone Body Excretion of Depancreatized Dogs.*

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Since fat is a source of ketone bodies, increasing the amounts of this foodstuff oxidized by completely diabetic organisms should produce an increase in the ketone body excretion. According to Shaffer's quantitative expression, every mol of fatty acid oxidized without the simultaneous combustion of antiketogenic material yields one mol of acetoacetic acid. The evidence from a variety of sources indicates that the depancreatized animal depends largely upon fat to furnish its bodily energy, both at rest and during activity,[1] and, furthermore, that it is not able to oxidize significant amounts of

* Abstract of a thesis presented to the faculty of the Graduate School of Cornell University for the degree of Doctor of Philosophy, June, 1936.

[1] Chambers, W. H., Kennard, M. A., Pollack, H., and Dann, M., *J. Biol. Chem.*, 1932, **97**, 525.