

venin plays no rôle in the production of the resistant state to bacterial toxic filtrates produced by the venom.

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### Effect of the Prolonged Use of Ethyl Alcohol on Renal Function and Pathology in the Dog.\*

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Observations were previously made on the acute toxic effect for the kidney in both normal and naturally nephropathic dogs of an alcoholic distillate obtained after the fermentation by yeast of a mixture of corn meal and sugar or corn meal and molasses.<sup>1</sup> It was later demonstrated<sup>2</sup> that such distillates were able to induce a type of chronic nephropathy in the dog which was largely confined to the endothelium of the glomerular capillaries. The animals in the 2 groups of experiments were given 10 cc. per kg. of the distillate. Normal dogs were used as controls and received 10 cc. per kg. of a 40% solution of ethyl alcohol. The experiments lasted from 6 weeks to 3 months. The animals receiving the alcoholic distillate as well as the control animals were then sacrificed. Both the normal and naturally nephropathic dogs which received the alcoholic distillate showed definite evidence both functionally and anatomically of renal injury. The control dogs gave but slight evidence in so far as renal function was concerned of any departure from the normal. Similar observations of the relative nontoxicity of ethyl alcohol for pigeons have recently been made by Hanzlik.<sup>3</sup>

The present investigation is concerned with the effect of ethyl alcohol on renal function and pathology in the dog when taken over long periods in the amount of 10 cc. per kg. of a 40% solution. The experiments have lasted from 6 months to 2 years and 2 months. Twenty-three adult dogs have been used. The animals were kept in metabolism cages and allowed an unrestricted amount of water except on the days set aside for observation. They were fed on bread, scraps of meat, a small amount of butter and milk.

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<sup>1</sup> MacNider, Wm. deB., *J. Pharm. and Exp. Therap.*, 1925, **26**, 97.

<sup>2</sup> MacNider, Wm. deB., *Proc. Soc. Exp. Biol. and Med.*, 1925, **22**, 52.

<sup>3</sup> Hanzlik, P. J., *J. Pharm. and Exp. Therap.*, 1931, **43**, 339.

The solution of ethyl alcohol was given once a day before the morning meal. For 3 days prior to the day on which observations were made, the animals were given 300 cc. of water twice a day in order to insure a constant water intake. At such periods the urine was studied qualitatively for the presence of albumin, glucose, acetone, and diacetic acid. Centrifugalized portions of urine obtained by catheterization were examined for casts. The elimination of phenolsulphonephthalein over a 2 hour period was determined by the method of Rowntree and Geraghty. Blood urea and non-protein nitrogen estimations were made by the methods of Folin and Wu. The reserve alkali of the blood was determined by the method of Marriott.

The results are as follows:

1. The use of ethyl alcohol in the amount and percentage indicated, induces for a period which has varied in the respective animals, a state of semiintoxication characterized by muscular incoordination especially of the hind limbs and a staggering gait which is followed by drowsiness and usually by a period of sleep. Twelve of the dogs after having received the alcohol for periods which varied from 4 to 7 months failed to show any of the above mentioned symptoms. The remaining 11 animals gave evidence of such an intoxication until the experiments were terminated. The development of a tolerance to alcohol in so far as the effect on the central nervous system is concerned is variable and suggests that in those animals which fail to develop a tolerance some factor other than the alcohol may be concerned in this variation.

2. Nineteen of the 23 dogs showed an increase in body weight, varying from 3.75 kg. in an animal that had received alcohol for 6 months and 10 days to 6.32 kg. in an animal receiving the alcohol over a period of 7½ months. The dogs were presumably full grown at the commencement of the experiments, aged from 3½ to 7 years. The increase in body weight cannot be attributed to the natural processes of growth nor to the length of time over which the animals were subjected to the use of alcohol. Six animals that received the alcohol for 2 years and 2 months increased less in weight than did 17 of the dogs which received alcohol for a shorter length of time. Of the 19 animals which gained in weight 12 showed both acetone and diacetic acid in the urine and 8 developed a glycosuria.

3. The influence of ethyl alcohol on urine formation was ascertained by the following procedure. After a period of 3 days during which the dogs were given 300 cc. of water twice a day by

stomach tube, they were given on the fourth day the normal dosage of alcohol in a sufficient amount of water to bring the total volume up to 300 cc. During a 3 hour period following the use of alcohol in such a dilution there was an increase in the output of urine by all the animals over the amount formed on the 3 preceding days when the volume of fluid remained constant. The average increase in urine formation by all of the dogs varied from a minimum increase of 84 cc. by the animal of Exp. 6, to a maximum increase of 211 cc. by the animal of Exp. 21. The diuretic effect of alcohol cannot be solely explained on the basis of the associated water intake.

4. Eighteen of the 23 dogs developed an albuminuria, varying from a faint trace of albumin to 1.6 gm. per liter. Seven of the animals within 6 hours after the intake of alcohol showed albumin varying from 0.5 to 1.6 gm. per liter. With the exception of 2 of these dogs the albuminuria was transitory and disappeared within 24 hours. Any subsequent use of alcohol resulted in its recurrence. The observation is interpreted as a transitory functional disturbance not dependent upon structural changes in the kidney. The histological study of the kidneys of such animals showed no abnormality other than an increase in stainable lipid material in the cells of the ascending limb of Henle's loop. These 7 animals which reacted to the use of alcohol by the development of more than a trace of albumin, a definite albuminuria, also showed a glycosuria, the presence of acetone and diacetic acid in the urine, a high elimination of phenolsulphonophthalein which varied during a 2 hour period from 72 to 96% and a reduction in the reserve alkali of the blood from the normal of 8.0 or 8.1 to a maximum reduction of 7.85. There was no retention of urea or non-protein nitrogen.

The presence of casts in the urine of those animals showing only a trace of albumin was variable. In the group developing an albuminuria which varied from 0.5 to 1.6 gm. per liter, narrow hyaline casts were constantly present. With the disappearance of the albuminuria the number of casts was first reduced and gradually disappeared. This sequence of events would again indicate a functional rather than a structural disturbance in the kidney.

5. The elimination of phenolsulphonophthalein has been very variable when contrasted with the output of this dye before the administration of alcohol. The normal elimination of this substance over a 2 hour period has varied from a minimum output of 58% to 71%. There was no constant change in the time of appearance of the dye in the urine. The variations in the elimination of phenolsulphonophthalein by the respective animals have developed

within the first month after the use of alcohol was commenced. It must therefore be attributed to some rather acutely developing disturbance. In 8 of the 23 dogs there was a persistent reduction in the elimination which continued without much change throughout the experiments. The percentage reduction in the following 2 animals is indicative of this type of modification in the total output of the dye. The animal of Exp. 2 had a normal elimination of 58% which within a month was reduced to 41% and at the termination of the experiment one year and 8 months after the commencement of the alcohol was 36%. The animal of Exp. 19 had a normal phenolsulphonephthalein elimination of 67% which was reduced within a month to 52% and at the end of the second year when the experiment was terminated the output was 46%.

In a second group of 8 animals the elimination of the dye was practically unaffected by the use of alcohol. The normal elimination of the dye by the dogs in this group varied from 59 to 71%. The output during the course of the experiments varied from 50 to 64%. In a third group of 7 dogs which has been previously referred to, the elimination of the dye was definitely increased after the commencement of the use of alcohol. This increase in elimination above the percentage which these animals had established as their normal percentage elimination depended to some extent on the relationship between the time at which the estimations were made after the alcohol had been administered. The most marked increase in the output of the dye occurred when the determinations were made within 3 hours after the injection of alcohol. If the use of alcohol was withheld for 48 hours there developed a reduction in the elimination of the dye. This observation would indicate the occurrence in certain of the animals of either a functional disturbance in the mechanism concerned in the elimination of the dye by the organism or the development of some structural defect which could be readily repaired. The following experiments illustrate the increased elimination of the dye by the animals falling in this third group. The normal elimination of phenolsulphonephthalein by the animal of Exp. 14 was 60%. During the course of the first month of the experiment there developed an increased output to 78%. In the animal of Exp. 19 the output of the dye increased from 62% to 96%. In this group of animals that have shown an increase in the elimination of phenolsulphonephthalein there has also occurred an albuminuria with casts, a glycosuria, the appearance of both acetone and diacetic acid in the urine and a reduction in the reserve alkali of the blood. There was no retention of urea or non-protein nitrogen.

6. The alkali reserve determinations of the blood have been normal and have varied from 8.0 to 8.1. These determinations have remained within the normal during the course of the experiments with the exception of the 7 dogs which have shown an increase in the elimination of phenolsulphonephthalein and certain abnormalities in the composition of the urine.

At the termination of the experiments both liver and kidney tissue were immediately fixed in Zenker's solution and in corrosive-acetic, embedded in paraffine and stained with eosin and haemotoxylin and with eosin and methylene blue. Tissue for frozen sections was fixed in 10% formalin and stained for lipid material by Herxheimer's method with Scharlach R. The present study is concerned only with the changes developing in the kidneys. The kidneys of these animals regardless of the duration of the experiments have failed to show any gross surface changes. The capsules have not been adherent. The cortical substance has been smooth without scars. On section the kidneys have cut without a feeling of resistance and there has been no change in the volume relationship between the cortex and the medulla. In 9 of the dogs the kidneys showed a definite, apparently fatty zone at the junction of the cortex and medulla. The microscopic study has very largely substantiated the gross findings. The glomeruli have in general appeared normal without any very definite capsular or intracapillary changes. In 11 of the animals these structures appeared to show an increase in the number of endothelial nuclei which stained intensely and in such glomeruli the basement membrane of the capillaries was thickened. Stainable lipid material was present as fine dust-like granules in the endothelium of the capillaries in 4 of the animals. The epithelium of the tubules has shown no change of an advanced degenerative character. Edema, vacuolation and necrosis of such cells have not been observed. The most constant change in the epithelial tissue of the kidney has been an increase in the amount of stainable lipid material in the cells of the ascending limb of Henle's loop. In such a location this material may accumulate to an extent as to obliterate the outline of the cells and obscure their nuclei. Those animals which have shown such a marked accumulation of lipid material in this part of the tubule have also shown smaller droplets and dust-like particles of the same material in the cells of other segments of the tubule. There has been no definite increase in intertubular connective tissue.

*Conclusions.* 1. Ethyl alcohol when given daily to dogs over long periods in an amount sufficient to induce symptoms of alco-

holic intoxication is but slightly nephrotoxic in a structural sense. The evidence of structural changes in the kidneys has consisted in certain early changes in the glomeruli which have not been of constant occurrence and by an accumulation of stainable lipid material in the epithelium of the tubules. 2. The experiments indicate that a functional disturbance may be induced in the kidney from the use of alcohol which is not dependent upon microscopically demonstrable structural changes. Such a disturbance has been characterized by an increase in urine formation and in a certain number of the animals by an albuminuria with casts. The elimination of phenol-sulphonephthalein has been variable. Such alterations in renal function other than a persistent decrease in the elimination of phenol-sulphonephthalein tend to disappear with the discontinuance in the use of alcohol.

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**Relationship Between Liver Injury Induced by Alcohol and Elimination of Phenolsulphonephthalein.\***

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In the preceding publication<sup>1</sup> concerning the nephrotoxic action of ethyl alcohol when administered over long periods to dogs, the observation was made that the elimination of phenolsulphonephthalein was variable. On the basis of this variation the 23 animals studied could be divided into 3 fairly well defined groups. The first group showed a moderate reduction in the output of the dye persisting throughout the experiments. In a second group, the elimination was but slightly affected, while in a third group of 7 dogs the elimination of the dye was definitely increased following the commencement of the use of alcohol. The most marked example of such increase occurred in Experiment 19, in which the percentage output of the dye rose from 62 to 96%. The dogs which showed such a change in the elimination of phenolsulphonephthalein also developed an albuminuria with casts, glycosuria, appearance of both acetone and diacetic acid in the urine and reduction in the acid-base equi-

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<sup>1</sup> MacNider, Wm. deB., and Donnelly, G. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 581.