

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.

5988

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

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In the preceding publication¹ 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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¹ MacNider, Wm. deB., and Donnelly, G. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 581.

brum of the blood. There was no retention of urea or non-protein nitrogen. Histological studies of the kidneys of these animals afforded no anatomical basis for the apparent contradiction of an albuminuria with casts, no retention of urea and non-protein nitrogen and an increase in the elimination of phenolsulphonephtalein.

Rowntree and Geraghty² while studying the elimination of phenolsulphonephtalein in various pathological states of the kidney observed that in some of the intoxications occurring during pregnancy, the output of the dye was definitely increased. Later, Whipple, Pieghtal and Clark³ and very recently Hanner and Whipple⁴ have shown a relationship to exist between a liver injury and an increase in the output by the kidneys of phenolsulphonephtalein.

As a result of the observation of the increase in the elimination of this dye in certain of the animals intoxicated by alcohol, histological studies were made of the livers of such animals and of those animals in which there was either no appreciable change in the output of the dye or in which the elimination of it was reduced. On this basis the animals used in the previous study¹ may be divided into three groups. The animals falling in Groups 1 and 2 in which the elimination of the dye was either not affected by the use of alcohol or only slightly reduced are used as control animals for Group 3, represented by 7 dogs in which the elimination of the dye was definitely increased. In Group 3 the increase in the elimination of phenolsulphonephtalein developed within the first month after the commencement of the use of alcohol and was most marked when the determinations were made within 3 hours after its injection. If the use of alcohol was withheld for 48 to 72 hours the initial increase in the output of the dye underwent a reduction. In 2 of the dogs its elimination returned to the normal percentage elimination established before the commencement of the experiments. The following experiments are characteristic of the alterations in the elimination of the dye by this third group of animals. The dog of Experiment 2 had a normal output of the dye of 64%. During the first month of the alcoholic intoxication the percentage elimination increased to 82%. In the animal of Experiment 19, the normal output was 62%, which increased to 96%. In the dog of Experiment 4, the normal elimination was 58%, which following

² Rowntree, L. G., and Geraghty, J. T., *J. Pharm. and Exp. Therap.*, 1909, **1**, 579.

³ Whipple, G. H., Pieghtal, T. C., and Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1909, **20**, 278.

⁴ Hanner, J. P., and Whipple, G. H., *Arch. Int. Med.*, 1931, **48**, 598.

the use of alcohol increased to 72% and with the withdrawal of the alcohol decreased to 61%. In the dog of Experiment 14, with a normal elimination of 49%, the use of alcohol caused an increase to 60%. With discontinuance of alcohol the output was reduced to 48%, 2% less than the animal's normal elimination.

Liver tissue from the 23 dogs was fixed by Zenker's fluid and in a solution of corrosive-acetic, embedded in paraffine and sections stained with eosin and haematoxylin and with eosin and methylene blue. Tissue for frozen sections was fixed in 10% formaline. The histological study of such tissue from the animals of Group 3 which had developed a definite increase in the elimination of phenolsulphonephthalein showed a marked liver injury which first involves the periphery of the liver lobule and extends inward towards the

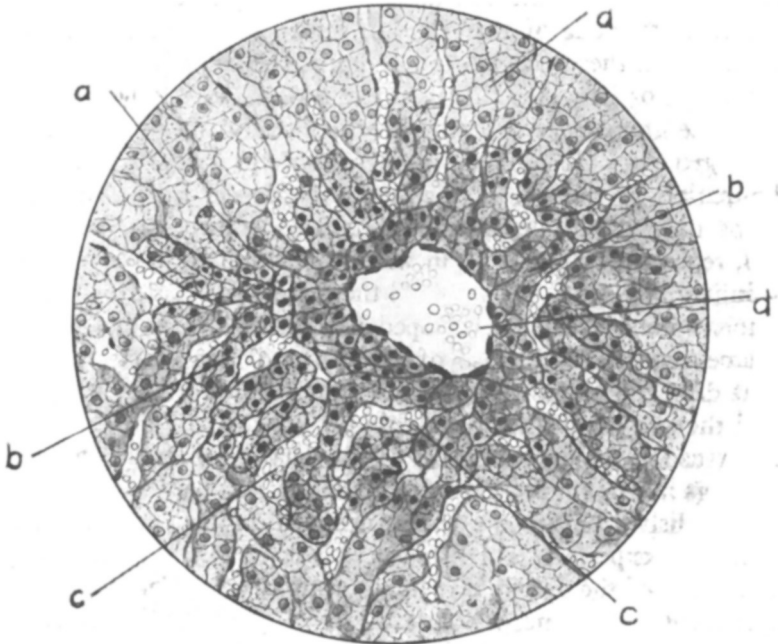


FIG. 1.

Camera lucida drawing, Zeiss, oc. 20X, obj. 40. The figure is from the liver of the animal of Exp. 19. The animal had a normal elimination of phenolsulphonephthalein of 62%. Following the use of alcohol and associated with the development of the liver injury the output of the dye increased to 96%. The figure represents a liver lobule which at a, shows in its periphery and extending inward towards the dilated central vein d, liver cells in an advanced stage of cloudy swelling. The nuclei are small and stain faintly. Such edematous cells contain a large amount of stainable lipoid material. The liver cells at b, immediately around the central vein and extending for a distance towards the inner third of the lobule show less swelling and the nuclei stain intensely. In such cells the amount of stainable lipoid material is greatly reduced. At C, intralobular capillaries are shown distended with blood.

central vein. By such an extension more than two-thirds of the liver lobule may participate in the pathological process. The changes in such areas of degeneration consist in a very marked cloudy swelling of the liver cells which is usually not accompanied by vacuolation or necrosis. The nuclei of such cells are small in proportion to the amount of surrounding cytoplasm and stain faintly. Their outline is usually well preserved. In such edematous cells there has occurred a marked accumulation of stainable lipoid material. As the center of the liver lobules is approached these changes diminish in their severity until immediately around the central vein the liver tissue appears normal. The intertubular capillaries are distended with blood. Hemorrhage into the liver substance has not been observed. There has been no increase in connective tissue. Fig. 1.

The study of liver tissue from the control groups of animals, Groups 1 and 2, in which the elimination of phenolsulphonephthalein was not increased has failed to show this characteristic change in the liver lobule. The livers of such animals have shown an increase in the amount of stainable lipoid material over the amount which can be demonstrated in normal animals that have not been subjected to the use of alcohol. Its distribution is of a diffuse character throughout the lobule and is not especially marked at the periphery and central portions of the lobule. Such cells may show an increase in size from an early cloudy swelling. This change has also failed to show the characteristic localization in its severity, to first, the peripheral part of the lobule with an extension towards the central vein.

Conclusions. 1. The foregoing observations of the changes which develop in the liver in those animals which show an increase in the elimination of phenolsulphonephthalein in the urine from the use of alcohol when contrasted with animals which do not show these changes, would indicate that the liver injury is responsible for the increased output of the dye by the kidney either by decreasing the excretion of the dye by the liver into the intestine with its reabsorption and elimination by the kidney (Rowntree and Geraghty²), or by decreasing the ability of the liver to destroy it (Kendall⁵). These results obtained from the use of alcohol in 7 of 23 dogs, coincide with the observation of Hanner and Whipple⁴ on the relationship which exists between a liver necrosis induced by chloroform and an increase in the elimination of phenolsulphonephthalein.

2. In this series of animals which developed a liver injury from

⁵ Kendall, E. C., *J. Am. Med. Assn.*, 1917, **68**, 343.

alcohol with an increase in phenolsulphonephthalein elimination the observation was made, that if the use of alcohol was withheld from 48 to 72 hours the elimination of the dye underwent a reduction. An investigation of the liver changes which may develop in connection with the decrease in the output of this dye is now in progress.

5989

Evidence of Physiological Mutations in the Cladoceran, *Moina macrocopa*.

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Extensive breeding experiments with *Daphnia longispina* (Banta and Wood,¹ and unpublished data) give rather conclusive evidence that recessive mutations having physiological effects accumulate during parthenogenesis in this species. In order to learn whether recessive physiological mutations accumulate similarly in *Moina macrocopa*, 2 strains of this species were inbred (sexual reproduction). One strain, Banta's Line 1012, had a laboratory history of 1090 parthenogenetic generations before inbreeding, whereas the other line, Banta's Line 1705, had only a short history of 42 parthenogenetic generations before inbreeding. Of 151 ephippial (sexual) eggs obtained from Line 1012, 118 or 78.1% hatched. Of 321 ephippial eggs obtained from Line 1705, 282 or 87.9% hatched. Eggs in both of these groups include several series which fairly consistently showed the same differential rate of hatching as is represented by the totals given above. This is suggestive of the accumulation during long-continued parthenogenesis of semi-lethal mutations in Line 1012.

Fifty-five females which hatched from the sexual eggs obtained by inbreeding Line 1012 were compared with 55 parthenogenetically-produced females of Line 1012 as to their viability, reproductive capacity, and other characteristics.* Comparisons were made be-

¹ Banta, A. M., and Wood, T. R., *Z. ind. Abs. Vererb.*, Supplementband I, 1928, 391.

* Inasmuch as there is no reduction division in the maturation of the parthenogenetic egg of Cladocera, all parthenogenetically produced descendants of an individual should be genetically identical (except as a mutation may occur). In sexual reproduction, on the other hand, segregation and recombination of genetic factors are, naturally, to be expected.