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Choleretic Action and Excretion of Cinchophen in Rabbit Bile.

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Since the report of Brugsch and Horsters[1] many investigators have definitely proved that cinchophen, or 2-phenylquinoline-4-carboxylic acid, increases the volume output of bile in dogs and man. The choleretic effect of cinchophen in the rabbit has been disputed in the literature. Stransky[2] reported no increase in volume output in anesthetized and unanesthetized rabbits in doses of 0.05-0.2 g. Franke[3] reported that small doses of atophan, up to 0.1 g, had no effect on bile flow in anesthetized rabbits, whereas larger doses, 0.15 to 0.6 g, increased the bile flow. Matsuoko[4] noted a choleretic effect of atophan in rabbits.

Our work was undertaken to determine the effect of cinchophen on bile flow in anesthetized rabbits, and to correlate the effect with the amount of cinchophen excreted in the bile.

Methods. Rabbits weighing from 1.0 to 2.5 kilos were anesthetized with nembutal (sodium pentobarbital). The abdomen was opened and the common duct cannulated near its entrance into the duodenum. The cystic duct was then ligated securely. The bile was allowed to flow for about one hour until the volume had reached a constant basal level. Cinchophen (Calco), as the sodium salt, was injected into the femoral vein in doses of 50, 25 or 10 mg per kilo of body weight. The bile was then collected at half-hour intervals for 2 hours, and the volumes noted and recorded. At the end of the experiment the bile secreted after the injection of cinchophen was pooled, so that the amount of cinchophen secreted could be determined. The method devised by Bradley[5] was used for the determination of cinchophen in bile.

Results. Fifty mg of cinchophen were injected slowly intravenously into 11 rabbits. The injection of cinchophen did not cause an increase in bile volume output. The average control output of bile for the 11 rabbits was 2.6 cc per half-hour, while the average output for

1 Brugsch, T., and Horsters, H., *Z. Ges. Exp. Med.*, 1923, **38**, 367.

2 Stransky, E., *Biochem. Z.*, 1925, **155**, 256.

3 Franke, K., *Arch. Exp. Path. u. Pharm.*, 1930, **151**, 219.

4 Matsuoko, Y., *Japan. J. Gastr.*, 1936, **8**, 145.

5 Bradley, W. B., Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 143.

the first half-hour after the injection of cinchophen was 2.4 cc. During the remainder of the test period, the output usually showed a gradual decrease, or showed normal fluctuations. A normal variation of ±17% in volume output was noted in 5 control rabbits which received no cinchophen. The injection of 50 mg per kilo of sodium dehydrocholate 2 hours after the cinchophen provoked a brisk choleresis.

It was thought that possibly a dose of 50 mg per kilo was too large for rabbits. Therefore, in subsequent experiments 25 and 10 mg of cinchophen per kilo were injected. In 5 rabbits, 25 mg per kilo gave no choleretic response. Similar results were obtained with 10 mg of cinchophen per kilo in 3 rabbits.

It was quite obvious that, under the conditions of our experiments, cinchophen in large or small doses manifested no choleretic property in rabbits. Bradley[5] in his studies on anesthetized and unanesthetized biliary fistula dogs found that the increase in bile volume occurring with the administration of 50 mg of cinchophen per kilo was associated with the recovery in the bile of approximately 20 to 78% of the administered cinchophen. Thinking that this fact, confirmed by us during this study, may be concerned in the mechanism of cinchophen choleresis, the bile obtained during the experiment was analyzed for cinchophen content.

The total amount of cinchophen excreted by 11 anesthetized rabbits when 50 mg per kilo was administered was 32.7 mg or an average of approximately 3 mg per rabbit during the collection period of 2 hours. In anesthetized dogs, Bradley[5] recovered 51 mg of cinchophen per dog during the same period of time. When the smaller doses were administered correspondingly smaller quantities of cinchophen were recovered in the bile. No cinchophen was found in the control bile, nor in the bile obtained after the injection of sodium dehydrocholate (Decholin-Sodium) which is an excellent choleretic in rabbits according to our observations on 12 rabbits. When small amounts of cinchophen were added to bile approximately 100% recovery was obtained. It is remotely possible that the liver of the rabbit is more sensitive to sodium pentobarbital anesthesia than the liver of the dog, even though the pentobarbitalized rabbit's liver secreted briskly in response to sodium dehydrocholate. To test this possibility 50 mg of sodium cinchophen per kilo was given slowly intravenously to 3 unanesthetized (chronic) biliary fistula rabbits. A choleresis did not occur.

Discussion. From our results it can readily be concluded that different doses of cinchophen had no appreciable effect on the volume output of bile in rabbits. Our next problem was to find the reason

for this non-choleretic action of cinchophen, especially since it was at variance with the results obtained in dogs. It seemed to us that cinchophen in the doses employed did not acutely damage the liver, because sodium dehydrocholate injected after the administration of cinchophen always caused a marked increase in volume output. A study of the literature revealed that, generally, rabbits are less susceptible to cinchophen poisoning than other mammals. Myers and Goodman[6] reported that 300 mg of cinchophen administered to rabbits for 45 days caused only minor cellular changes in the liver. Hanzlik and Lehman[7] found no change in the rose bengal liver function test after feeding 18 rabbits from 0.3 to 1.6 g of cinchophen per kilo over a period of 7 to 89 days. They also found that cinchophen did not increase the sensitivity of the liver to chloroform or phosphorus poisoning. Schwartz and Simonds[8] were unable to obtain peptic ulcers in rabbits fed 220 to 550 mg of cinchophen per kilo per day for 76 to 99 days.

From our results on the recovery of cinchophen in the bile, it appears that the absence of choleresis in rabbits may be associated with the observation that only very small amounts of cinchophen are excreted in the bile. We have found, in previous investigations[9, 10] that the choleresis obtained from the administration of various bile salt preparations was associated with the presence of appreciable amounts of the bile acid in the excreted bile. In the rabbit the liver is not concerned with the excretion of cinchophen as in the dog. Since cinchophen is not excreted in significant amounts in the bile of the rabbit, it would exert no osmotic effect on the bile and extra water would not be excreted in the bile. The failure of the rabbit's liver to excrete cinchophen is the only available explanation of the fact that the drug fails to cause choleresis in the rabbit.

Summary and Conclusions. The chloretic action of various doses of cinchophen was studied in a relatively large number of anesthetized rabbits. Cinchophen in doses ranging from 10 to 50 mg per kilo had no appreciable effect on the volume output of bile. It was also found that, on the average, only 3 mg of cinchophen per rabbit was recovered in the bile over a period of 2 hours. Since it

[6] Myers, H. B., and Goodman, L., *Arch. Int. Med.*, 1932, **49**, 946.

[7] Hanzlik, P. J., and Lehman, A. J., *Arch. Int. Med.*, 1933, **52**, 471.

[8] Schwartz, S. O., and Simonds, J. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1133.

[9] Berman, A. L., Snapp, E., Ivy, A. C., Atkinson, A. J., and Hough, V. H., *Am. J. Dig. Dis.*, 1940, **7**, 333.

[10] Berman, A. L., Snapp, E., Ivy, A. C., Hough, V. H., and Atkinson, A. J., *Am. J. Physiol.*, in press.

was found that the absence of cinchophen choleresis in the rabbit was not due to an acute intoxication of the liver, because after cinchophen sodium dehydrocholate caused a brisk choleresis, it is tentatively concluded that cinchophen does not cause choleresis in the rabbit because it is not excreted in the bile.

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Radioactive Ion Distribution in Protoplasmic Granules.

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The *Nitella* cell has an extensively granulated protoplasm, measurements on frozen sections giving up to 15% of the total volume of the protoplasm as being made up of nucleii, plastids, mitochondria and non-ergastic bodies. It seems important to inquire whether these granules play a significant rôle in ion transfers, and indeed, whether these bodies might not be the main seat of ion accumulation.

To determine to what extent the granuloplasm took up ions, single internodal coenocytes 5-10 cm in length of *Nitella coronata* were prepared by destroying alternate cells in the chain. Care was taken to preserve a short extension of the cellulose wall (about 5 mm long) from the adjacent cell. Surgical thread could then be tied around this cellulose projection and the cell left for 24 hours in pond water in the cold room (15°C) under conditions of intermittent light which simulated sunlight. After this treatment the cell was immersed in radioactive solutions for the desired time and then placed in a centrifuge tube, the thread tied about a cork in the tube and its length adjusted so that the cell was held clear of the sides and bottom of the tube. Centrifugal force sufficient to displace the granules in the cell was applied without any other observable damage. Control centrifuged cells kept under the previous conditions would show a gradual redistribution, reaching substantial completion in about 60 hours, of the stratified granules. These cells lived as long as untreated cells. After stratification had been obtained the experimental cells were bisected into centripetal and centrifugal halves, or fractions, and the activity of each half of the protoplasm measured under a Geiger-Muller counting tube. The only apparent error due to mixing was in the sap, which almost certainly became mixed during the sampling.