However, it has been shown by Friend and Robinson,[5] using this technic, that sodium thiocyanate exerts a depressant action on liver metabolism both when added to serum *in vitro* and when present in blood serum as drawn. A hitherto unpublished experiment, furthermore, showed that serum from a patient to whom a large amount of thiocyanate had been administered depressed the liver metabolism 30%. The addition of methylene blue to this serum restored the liver metabolism to its normal $Q_{O_2}$ of 13.0.

*Summary.* The effect on liver metabolism of adding methylene blue, chloral hydrate and sodium barbital to serum has been studied. Methylene blue has been found to increase the oxygen consumption of rat liver respiring in human serum. Chloral hydrate and sodium barbital both depress the metabolism. Following depression of the oxygen consumption by chloral hydrate, methylene blue increases the metabolism. Following sodium barbital, the dye further depresses the metabolism. This is interpreted as indicating a difference in the site of action of the drugs on the oxidative system of the tissue cells.

## 11609

## Excretion and Determination of Cinchophen in Bile.

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It has been established that cinchophen has a choleretic action in man and dog.[1-7] It does not apparently stimulate bile volume output in the rabbit.[8] The effect of cinchophen on the output of the various constituents of bile is disputed in the literature,[1-7] and good quantitative data on the excretion of cinchophen in the bile could not be found by us. However, cinchophen is known to be excreted in bile;[2]

5 Friend, D. G., and Robinson, R. W., *J. Lab. Clin. Med.*, 1939, **24**, 832.

1 Brugsch and Horsters, *Z. ges. exp. Med.*, 1923, **38**, 367.

2 Taubmann, *Arch. exp. Path. u. Pharm.*, 1927, **121**, 204.

3 Chabrol and Maximin, *Press. Med.*, 1929, **37**, 666.

4 Taschenberg and Hofmann, *Deut. med. Wochn.*, 1925, **51**, 1611.

5 Speerling and Hartman, *J. Lab. and Clin. Med.*, 1928, **13**, 854.

6 Horsters, *Arch. exp. Path. u. Pharm.*, 1925, **105**, xi.

7 Goffin, *Compt. rend. Soc. de Biol.*, 1936, **123**, 97.

8 Strausky, *Biochem. Z.*, 1926, **155**, 256.

in fact, diiodoatophen (2-o-iodophenyl-6 iodoquinoline-4 carboxylic acid, or biloptin) has been used for cholecystography.[9, 10]

Our work was primarily undertaken to determine the rate and extent of the excretion of cinchophen in bile. At the same time, however, the choleretic action and effect of cinchophen on the output of various bile constituents was compared with sodium dehydrocholate.

*Methods.* The effect of cinchophen on bile flow and the excretion of cinchophen in the bile was studied by using 5 anesthetized dogs and 4 "chronic" bile fistula dogs prepared by the Rous-McMaster method. In the "acute" experiments the dogs were anesthetized with pentobarbital. The common bile duct was cannulated and the cystic duct tied. Bile was collected for periods of one hour each. After the secretion had become constant, cinchophen (as the sodium salt) was injected intravenously in doses of 50 mg/K of body weight. The volume of bile and the amount of cinchophen in the bile were determined for hourly intervals for a period of 5 hours after the injection of cinchophen. Five other anesthetized dogs were also used to study the effect of sodium dehydrocholate.

Bile was collected from the "chronic" bile fistula dogs for 24-hour periods. After the volume output of bile had become constant, one gram of cinchophen was fed daily for 15 days. The volume of bile and the amount of cinchophen in the bile were determined for 24-hour periods.

The method devised for the determination of cinchophen in bile follows: A 1 ml sample of bile is pipetted into a 6-inch test tube. Eight drops of concentrated NaOH are added together with a few drops of caprylic alcohol to prevent foaming and a few beads to prevent bumping. The mixture is refluxed for 2 hours after which 1 ml of saturated $NaNO_2$ is added through the condenser followed by 3 ml of concentrated HCl. The mixture is again refluxed for 15 minutes and then evaporated down to half its volume in an oil bath at 115°C. The contents of the tube are then cooled and filtered quantitatively with suction through filter paper pulp into a 15 ml conical centrifuge tube, the test tube and filter being washed repeatedly with small quantities of 1% $HNO_3$. At this point the volume of the filtrate is about 13 ml. One ml of 10% phosphomolybdic acid is added to precipitate the cinchophen. If there is no precipitate there is no cinchophen in the sample.

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9 Pribram, *Deut. med. Wochn.*, 1926, **52**, 1291.

10 Einhorn and Stewart, *Med. J. and Rec.*, 1926, **125**, 457; *Ibid.*, 1927, **126**, 430.

If there is a precipitate, it is centrifuged down and washed twice by centrifugalization with a few ml of 1% $HNO_3$. The washed precipitate is dissolved in 5 ml of N/10 NaOH and is transferred quantitatively into a 100 ml volumetric flask. Enough wash water should be used to make the volume about 60 ml. Twenty ml of 20% potassium ferrocyanide and 10 ml of concentrated HCl are then added and the contents of the flask made up to volume with distilled water.

A standard is prepared by pipetting into a 15 ml conical centrifuge tube 1 or 2 ml of a standard solution of recrystallized cinchophen (as the sodium salt) per ml. Ten ml of 1% $HNO_3$ are added followed by 1 ml of 10% phosphomolybdic acid. The resulting precipitate is treated in the same manner as the unknown.

After standing for 30 minutes the unknown is matched against the standard in a colorimeter. The quantity of cinchophen in the unknown is calculated by dividirg the reading of the standard by the reading of the unknown and multiplying by the number of mg of cinchophen in the standard.

Normal bile yielded no cinchophen by this method. Cinchophen added to normal bile could be recovered quantitatively with an error of not more than 5%. The cinchophen used in this work was a white, odorless powder, soluble in alkaline solution (Calco Chemical Co.).

TABLE I.

Effect of Cinchophen and Dehydrocholic Acid on Bile Formation.
The data represents the averaged results obtained on 5 dogs.

| | | Bile pigment | | Cholic acid | | Cholesterol | | Cinchophen | |
|---|---|---|---|---|---|---|---|---|---|
| Hr | Vol. cc | Mg/ 100 cc | Total Mg | Mg/ 100 cc | Total Mg | Mg/ 100 cc | Total Mg | Mg/ 100 cc | Total Mg |
| Control | 1.98 | 263.0 | 4.66 | 2120.0 | 37.2 | 19.9 | .314 | | |
| | | | | 50 g/kilo cinchophen intravenously. | | | | | |
| | | | | Avg 5 dogs. | | | | | |
| 1st | 12.4 | 109.7 | 14.0 | 264.0 | 32.6 | 4.66 | .589 | 169 | 22.3 |
| 2nd | 11.7 | 70.7 | 8.21 | 85.5 | 9.0 | 3.80 | .430 | 237 | 28.6 |
| 3rd | 10.2 | 70.7 | 7.16 | 89.0 | 7.9 | 3.64 | .356 | 195 | 20.0 |
| 4th | 9.02 | 78.0 | 6.95 | 79.7 | 6.6 | 3.81 | .329 | 164 | 15.0 |
| 5th | 7.9 | 94.5 | 6.61 | 132.0 | 9.03 | 8.64 | .578 | 146 | 11.9 |
| | | | | | | | | | 97.8 mg total |
| | | | | Avg wt—10.1 K. | | | | | |
| Control | 2.19 | 489.0 | 11.6 | 2230.0 | 33.2 | 17.2 | .261 | | |
| | | | | 50 mg/kilo sodium dehydrocholate intravenously. | | | | | |
| | | | | Avg 5 dogs. | | | | | |
| 1st | 16.4 | 130.0 | 22.1 | 451.0 | 56.2 | 4.29 | .532 | | |
| 2nd | 5.31 | 129.0 | 7.19 | 563.0 | 24.6 | 6.41 | .289 | | |
| 3rd | 3.03 | 202.0 | 7.24 | 755.0 | 17.7 | 9.07 | .264 | | |
| | | | | Avg wt—10.7 K. | | | | | |

The bile was analyzed for total pigment by the method of Schmidt and Jones,[11] for cholic acid by the method of Reinhold and Wilson,[12] and for cholesterol by the method of Bloor.[13]

*Results. Anesthetized dogs.* The averaged results of the "acute" experiments are shown in Table I. It is to be noted that by weight cinchophen given intravenously is a better choleretic than sodium dehydrocholate (Decholin). It is well known that sodium dehydrocholate is superior in regard to choleretic action than sodium cholate and the conjugated cholates. During the first hour after the injection of cinchophen, the total output of pigment, cholic acid and cholesterol was definitely increased. The same occurred after sodium dehydrocholate. During the remainder of the period of observation, the chief difference between the 2 choleretics pertained to volume output and cholic acid output. Cinchophen caused a more prolonged choleretic action and depressed cholic acid output.

Cinchophen was excreted in relatively large amounts. The average dose given was 505 mg, and the average amount recovered during the 5-hour period following its injection was 97.8 mg, or almost 20% The maximum excretion occurred during the second hour, but significant amounts were still being excreted during the fifth hour.

*"Chronic" biliary fistula dogs.* In these experiments we were particularly interested in obtaining data regarding the amount of cinchophen excreted daily in the bile when 100 mg per kilo body weight of cinchophen were given orally with the meal. This is definitely a toxic dose of the drug. Such a dose is used to produce experimental gastric or duodenal ulcer and causes gastritis with anorrhexia and frequently vomiting after 2 to 4 days of administration. For that reason, valid data on the effect of cinchophen in the doses used, on cholic acid, pigment and cholesterol output could not be obtained.[14] Regardless of anorrhexia and the consequent failure to consume all the food, the animals showed a choleresis of from 50 to 150% above the control level.

Reliable data for the output of cinchophen in the bile could be obtained usually during the first day or two after the initiation of the medication, following which reliable data could be obtained only for the days during which vomiting was known not to occur. The average daily recovery of cinchophen in the bile of the different dogs was: dog 1, 28%; dog 2, 78%; dog 3, 74%; and dog 4, 42%. These

11 Schmidt, Jones and Ivy, PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 17.

12 Reinhold and Wilson, *J. Biol. Chem.*, 1932, **96**, 637.

13 Bloor, *J. Biol. Chem.*, 1928, **77**, 53.

14 Schmidt, Beazell, Berman and Ivy, *Am. J. Physiol.*, 1939, **126**, 120.

averages represent the data for the days during which vomiting did not occur. The average of all the results yields a recovery of 55%. Twenty-four hours after cessation of the administration of the drug, it could not be detected in the bile.

*Discussion.* We were primarily interested in ascertaining the extent to which cinchophen is excreted in the bile when given in doses sufficient to cause gastritis and gastro-duodenal ulcer. It is clear from the results that cinchophen is excreted in the bile soon after it enters the blood stream. About 55% of the drug, when given orally, is eliminated daily in the bile. In the biliary fistula dog, cinchophen is excreted in the bile 24 hours after the last dose. This indicates that a considerable quantity of cinchophen may normally undergo an enterohepatic circulation.

Our results on the cholic acid output after the intravenous injection of cinchophen, show that it produces a choleresis without an increase in cholic acid synthesis. In fact, cholic acid synthesis is definitely depressed by the doses used. The same result obtained in the "chronic" bile fistula animals. In 3 of the 4 dogs, the output of cholic acid was definitely decreased. These data are not tabulated because they are equivocal. They are equivocal because the decrease in cholic acid occurred with an anorrhexia or refusal or vomiting of food. These factors are well known to decrease cholic acid output.[14] However, in some instances the cholic acid output was decreased to 0.3 g per day, and in our experience the normal fasting bile fistula dog never excretes less than 0.8 g per day. Yet, cinchophen in the doses used causes a gastritis, and gastritis *per se* may reduce cholic acid synthesis. Although we believe, on the basis of the results of the "acute" experiments, that cinchophen in the doses used directly depresses cholic acid synthesis, we doubt that our evidence on the "chronic" biliary fistula dogs warrants a definite conclusion to that effect. If a dose of cinchophen can be found which will depress cholic acid synthesis without altering the intake and digestion of food, then it will, we believe, be possible to conclude that cinchophen directly interferes with cholic acid synthesis.

*Summary and Conclusions.* A method for the quantitative determination of cinchophen in bile is described. In 5 anesthetized dogs weighing about 10 kilos, it was found that 20% of 1 g of intravenously injected cinchophen is excreted in the bile in 5 hours. In 4 "chronic" bile fistula dogs an average of 55%, or from 28 to 78%, of orally administered cinchophen (100 mg per kilo body weight) was daily excreted in the bile. This shows that the liver is significantly concerned in the excretion of cinchophen and that an enterohepatic

circulation of cinchophen may occur. By weight sodium cinchophen increases the volume output of bile more than sodium dehydrocholate, which is an excellent hydrocholeretic. Cinchophen in large doses orally or intravenously decreases cholic acid output. But, it cannot be concluded from our results on "chronic" bile fistula dogs that cinchophen specifically interferes with cholic acid synthesis.

## 11610

## Roentgen-Pigmentation in the Gold Fish.*

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One of the outstanding biologic effects of roentgen rays is the pigmentation of the skin. Roentgen pigmentation has been observed not only in human beings but in many mammals and in fish. Most characteristic is its persistence for weeks, months and years. The investigations of Miescher[1] and Peck[2] revealed the importance of chromatophores in this phenomenon. With respect to the rôle which chorial chromatophores play in the roentgen pigmentation of mammals, the eruption of chorial melanophores in the goldfish *(Carassius auratus)* after exposure to roentgen rays as described by Smith,[3, 4] is of interest.

Smith used a radiation of 100 kV, 5 MA, no filter and exposed in some instances only a portion, in other instances, the entire goldfish. Depending upon the dose, after a latent period of 5-6 days eruption of melanophores on the exposed side was observed, a general cutaneous melanosis was brought about in some cases, and some fish finally died. The eruption of melanophores usually however is a transient affair according to Smith. By a process of degeneration these melanophores disappear after 2-4 weeks and the cutaneous regions once more assume a normal color.

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1 Miescher, G., *Arch. f. Derm.*, 1922, **39**, 313.

2 Peck, S. M., *Arch. Derm.*, 1930, **21**, 916.

3 Smith, G. M., *Am. J. Cancer*, 1932, **16**, 863.

4 Smith, G. M., *Biol. Bull.*, 1932, **43**, 484.