Iodinated Organic Compounds
As Contrast Media for Radiographic Diagnoses

IX. Experimental Studies on the Visualization of the Biliary Tract

GLENN E. JONES, ALPHONSUS L. GROHOWSKI, M.D., HAROLD D. ROBERTSON, GEORGE H. RAMSEY, M.D., JOHN A. SCHILLING, M.D., and WILLIAM H. STRAIN, Ph.D.

Departments of Radiology and Surgery, School of Medicine and Dentistry and Strong Memorial Hospital, The University of Rochester, Rochester, N. Y.

In the course of testing \( o-(4\text{-hydroxy-3,5-diiodobenzyl})\text{-benzoic acid} \) (I) as a cholecystographic agent in dogs, it was observed that the extrahepatic biliary ducts were visualized as well as the gallbladder. Since duct visualization would be a desirable addition to cholecystography, a comparison was made against the standard cholecystographic media to determine whether this property was unique with the dophthalein (IV), all delineated the ducts. There were marked differences, however, in the rate and degree of visualization achieved with each preparation. Delineation was most rapid with \( o-(4\text{-hydroxy-3,5-diiodobenzyl})\text{-benzoic acid} \), and slowest with the iodinated derivatives of phenolphthalein. The time interval for initial duct visualization was in the range of ten to forty minutes.

\[
\begin{align*}
\text{I. } & o-(4\text{-Hydroxy-3,5-diiodobenzyl})\text{-benzoic Acid, 52.9\% Iodine} \\
\text{II. } & \text{Iodoalphonic Acid (Priodax), 51.5\% Iodine} \\
\text{III. } & \text{Tetraiodophenolphthalein, 61.8\% Iodine} \\
\text{IV. } & \text{Phenoltetraiodophthalein, 61.8\% Iodine}
\end{align*}
\]

To eliminate the effect of the gallbladder on the secretion of the cholecystographic media, a number of examinations were made on dogs both before and after cholecystectomy. Intravenous injections of the benzoic acid derivative (I) and of iodoalphonic acid (II) gave satisfactory duct delineation.

**ANATOMY**

The dog is the most satisfactory of the laboratory animals for the study of chole-

\[1\text{ Presented before the Radiological Society of North America at the Thirty-third Annual Meeting, Boston, Mass., Nov. 30-Dec. 5, 1947. This work was aided by grants from the U. S. Public Health Service and from Eli Lilly and Co., Indianapolis, Ind.}\]
cystographic media, and is particularly suitable for the investigation of the secretion of such media into the biliary tract. The liver of the dog is divided into five chief lobes which are separated by deep fissures and drained by three main hepatic ducts. Although in situ the lobes overlap, the fresh specimen may be spread out so
twenty-four hours and then anesthetized. After a control film had been taken, the cholecystographic agent was administered by stomach tube at a dosage level of 50 mg./kg. For the first hour the medium was confined to the stomach, but the one-and-a-half-hour film showed that a part of the medium had passed into the small intestine. By four and a half hours none of the medium was left in the stomach. A small portion of the common duct was visualized on the two-hour roentgenogram. The duct shadow increased in extent and intensity on succeeding films, and reached a maximum intensity at three and a half hours. On this film the hepatic ducts also were delineated. The neck of the gallbladder first was visualized five and a half hours after drug administration, and the entire gallbladder was outlined by seven and a half hours. The gallbladder shadow increased in intensity on each successive roentgenogram until the experiment was terminated twelve and a half hours after administration of the cholecystographic agent. Some unabsorbed medium was visualized in the large bowel on the twelve-and-a-half-hour film.

*Intravenous Administration:* To eliminate the variable of intestinal absorption, the sodium salts of the cholecystographic media were given intravenously at a dosage level of 50 mg./kg, based on the acid. Individual doses of \(o\)-(4-hydroxy-3,5-diiodobenzyl)-benzoic acid and of iodoaliphonic acid were prepared by warming the weighed amount of the free acid with a 10 per cent excess of sodium carbonate (calculated to form disodium salt) in slightly less than 10 c.c. of distilled water, filtering, and finally adjusting the volume with distilled water to 10 c.c. Solutions of tetraiodophenolphthalein and phenoltetraiodophthalein sodium salts were prepared from the commercial products by dissolving in distilled water, filtering, and finally making the volume up to 10 c.c.

After the animal was anesthetized with nembutal, intravenously, and placed on its right side, a scout film was taken. Once properly positioned, the dog was not moved throughout the rest of the procedure. Although the external jugular vein sometimes was employed, ordinarily the vein on the lateral aspect of the leg was used for the injections of the solution of the cholecystographic media. Since the same dogs were employed again and again, great care was taken to prevent extravasation of the medium from the veins. Following the control roentgenogram, the solution of the cholecystographic agent was administered over a period of five minutes, and serial roentgenograms were taken at 10-minute intervals from the time of completion of the injection. Most series were terminated at the end of ninety minutes, but some were continued for as long as nine hours. During the longer experiments, it was necessary to give the dogs supplementary doses of nembutal intravenously.

A total of 40 examinations with intravenous injections were carried out, 22 of which were comparative studies on the two litter mates, dogs “A” and “B.” In the comparison series the cholecystographic agents were alternated in successive experiments at the same dosage level of 50 mg./kg. The results of the comparison are given in Table II. Although the values for each compound vary somewhat, it is evident that \(o\)-(4-hydroxy-3,5-diiodobenzyl)-benzoic acid is secreted into the duct system much more rapidly than the other three compounds.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Duct Visualization</th>
<th>Gallbladder Visualization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog “A”</td>
<td>Dog “B”</td>
</tr>
<tr>
<td>(o)-(4-Hydroxy-3,5-diiodobenzyl)-benzoic acid</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Iodoaliphonic acid</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>(Pridax)</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Tetraiodophenolphthalein</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Phenoltetraiodophthalein</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

Table II: Time Interval in Minutes for Initial Visualization of Biliary Ducts and of the Gallbladder in Dogs Following Intravenous Administration of Cholecystographic Media
Fig. 3. Roentgenologic visualization of the extrahepatic biliary system of dog "A" after intravenous injection of \( \text{a-(4-hydroxy-3,5-diodobenzyl)-benzoic acid} \). The film taken ten minutes after administration of the medium (A) shows the extra hepatic and the common bile ducts outlined. The twenty-minute roentgenogram (B) shows filling of the neck of the gallbladder, and by forty minutes (C), the fundus also is delineated. The ninety-minute film (D) shows an increase in gallbladder radiopacity as well as more extensive biliary duct visualization.
Iodine Concentration for Visualization of the Extrahepatic Ducts: The concentration of iodine required for the delineation of the extrahepatic ducts was determined by a series of cholangiograms on both living and dead dogs, and by an analysis of a sample of bile obtained ninety minutes after the intravenous injection of the sodium salt of $o$-(4-hydroxy-3,5-diodobenzyl)-benzoic acid. Neither method was entirely satisfactory but both gave the same range of 1 to 2 per cent of iodine as the concentration required for visualizing the duct system by the roentgenographic method used.
For the cholangiograms, aqueous solutions of the sodium salts of cholecystographic agents were employed as well as various dilutions of 50-per cent emulsion of ethyl iodophenylundecylate (1). By the procedure adopted, a laparotomy was carried out on each dog, the duodenum was opened, and the ampulla of Vater cannulated with a blunt No. 17 lumbar puncture needle. The bile was aspirated, and the biliary tract injected with the fluid under study. After waiting five minutes the injected fluid was aspirated, and a second injection was made. The diluting effect of hepatic bile draining from the fine radicles was thus minimized. By this method the ducts were faintly defined when a 1 per cent iodine mixture was used, and definitely delineated by a 2 per cent iodine content.

Mixed bile was aspirated from a dog that died from an overdose of anesthetic ninety minutes after intravenous injection of the disodium salt of o-(4-hydroxy-3,5-diiodobenzyl)-benzoic acid at a level of 50 mg. kg. On analysis by one of the iodine methods (4) developed for diodrast, an iodine content of 1 per cent was found. In this case the duct system was as clearly defined as in Figure 3D, but it is uncertain whether the iodine content of the mixed bile was the same as that of the bile in the extrahepatic ducts.

**DISCUSSION**

That cholecystographic media are secreted into the biliary tract of dogs at a concentration sufficiently great to visualize the extrahepatic ducts on roentgenograms is the significant finding of this study. The retrograde injections into the biliary tract show that an iodine concentration of at least 1 per cent is necessary before the ducts are seen on the roentgenograms, and that the probable iodine content is of the order of 2 per cent. Since the media were administered in the intravenous experiments at a level of 50 mg./kg., and all had an iodine content of 51.5 to 61.8 per cent, the level of the iodine administered was of the order of 25 mg./kg. If the plasma volume of dogs is taken to be 50 c.c./kg., the iodine content of the plasma after injection of each medium is of the order of 0.05 per cent. It follows that a concentration of twenty- to forty-fold takes place as the cholecystographic media enter the biliary ducts. Both the extent and the rapidity with which this concentration takes place are somewhat surprising.

It is difficult by roentgenologic methods to obtain more precise information on the concentration changes that occur in the biliary tract. It is significant, however, that there are definite differences in the type of shadow produced by the several preparations. With the iodinated phenolphthalein, the delineation of the ducts is least satisfactory and only thin lines of opacity are evident. Somewhat more definite is the delineation obtained with iodoalphonic acid, but the best results are given by o-(4-hydroxy-3,5-diiodobenzyl)-benzoic acid. Not only does the iodinated benzoic acid derivative give more complete filling of the duct system, but also the rate of filling is definitely more rapid.

The studies made after oral administration bring out a number of differences in the behavior of the three preparations: o-(4-hydroxy-3,5-diiodobenzyl)-benzoic acid (I), iodoalphonic acid (II) and tetraiodophenolphthalein (III). That tetraiodophenolphthalein was not absorbed is explicable on the basis of the high molecular weight (822) and the probable persistence of an anhydride structure during the passage of the medium through the gastro-intestinal tract. The absorption of the other two compounds, I and II, seemed to be about equal; that is compatible with their molecular weights (480 and 496, respectively), and the fact that both compounds contain the same number and kinds of acid groups.

The rate of elimination of the absorbable cholecystographic agents into the biliary tract is probably more rapid than their absorption from the gastro-intestinal tract. This is shown clearly by the experiment in which the benzoic acid derivative was administered orally, and the characteristics of the duct and gallbladder shadows were
followed for a period of twelve hours. After one and a half hours the medium passed through the stomach into the small intestine. At two hours a small portion of the common duct was visualized, and this shadow grew in intensity and extent with the cystic duct and the neck of the gallbladder showing at the end of five and a half hours, and the entire gallbladder at the end of seven and a half hours. During this interval of time not all of the administered medium had been absorbed, and even at the end of twelve hours there was still some of the medium in the large intestine.

The elaboration and concentration of cholecystographic media constitute special problems of gallbladder physiology. Apart from studies on phentetaiiodophthalein, relatively little work has been done on the rate of disappearance from the blood stream, and still less on the rate of reappearance in the bile. It appears to be generally accepted that a four- to ten-fold concentration of bile takes place in the gallbladder itself (5, 6), but Kirklin, Caylor, and Bollmann (7) did not find a good correlation between bile pigment concentration and the intensity of the gallbladder shadow obtained clinically with tetraiodophenolphthalein. This suggests that the secretion of bilirubin and of the cholecystographic agents is governed by different factors. The studies with the cholecystectomized dogs support this view. After the gallbladders of these animals had been removed, the amount of cholecystographic medium elaborated into the biliary ducts was sufficient for visualization ten minutes after injection. A considerable degree of concentration was necessary to achieve this, and it is improbable that bile pigment concentration took place at the same time. As time passed, however, the intensity of the duct shadow did not increase, and this suggests that the medium was being secreted at a uniform level while bile was escaping through the ampulla. The observations are not good enough to permit more than speculation on this issue, and a great deal is still to be learned concerning the mechanisms and chemistry governing the secretion of cholecystographic media.

With respect to recelylization of cholecystographic media, there are inadequate data. The results of Delario (8) with dogs indicate that tetraiodophenolphthalein after intravenous injection leaves the blood stream in two to four hours; subsequently only a trace of the medium is found in the blood as a result of intestinal absorption. Eitel (9), on the other hand, found that after intravenous injection of tetraiodophenolphthalein there was a gradual decrease of the blood level in normal dogs for ten hours, and that after tying off the common duct the blood level fell off very slowly after an initial drop of 50 per cent during the first hour. In view of the extensive use that tetraiodophenolphthalein formerly enjoyed, it is rather surprising that more work has not been done on the factors involved in the elimination of this medium from the body. In part, this has been due to the fact that the analytical procedures that must be employed are somewhat cumbersome.

This initial study of the secretion of cholecystographic media into the biliary ducts is largely of theoretical interest and does not lead to any immediate recommendations for refinement of clinical roentgenography. The accuracy of clinical cholecystography is high — Scott and Moore (10) report 92.7 per cent and Sosman (11) 95 per cent — yet the failures are frequently in patients where added information is sorely needed. If it becomes possible to make oral or intravenous cholangiograms, the radiological evaluation and surgical attack of biliary tract diseases would be greatly expedited. This would be particularly true in the post-cholecystectomy patient where partial or complete occlusion of the common duct is present from a calculus or inadvertent damage to the duct itself. As Ivy (12) has emphasized, improved media and a better understanding of the physiological factors involved in the filling and emptying of the gallbladder will contribute to this goal.
SUMMARY

Using dogs as experimental subjects, comparisons have been made of the secretion into the biliary tract of the following cholecystographic agents: \( o-(4\text{-hydroxy-3,5-diiodobenzyl})\)-benzoic acid, iodoalphoninic acid, tetraiododophthalamide, and phenoltetraiodophthalein.

Administered orally, only the benzoic acid derivative consistently gave good visualization of the common duct. Given intravenously in the form of the sodium salts, all four compounds delineated certain of the hepatic ducts, the common duct, and finally the gallbladder. The most rapid and complete delineation was obtained with the benzoic acid derivative, the ducts being visible ten minutes and the gallbladder twenty minutes after injection. Similarly, the duct system was outlined after cholecystectomy.

REFERENCES


DISCUSSION

Aubrey O. Hampton, M.D. (Washington, D. C.):
This paper is so complete that a discussion of the content is not necessary. Its implications, of course, are tremendous. All of us are being asked over and over again, "Why is this patient jaundiced? Is the common duct obstructed or not?" I don't know whether this will solve that problem, but it is at least worth a whirl; a definite trial is certainly in order.

An old and accepted view—I started to call it a theory but I guess it has been proved—is that the reason we see the gallbladder is that the dye is concentrated in it. Why couldn't it be that the gallbladder merely fills with concentrated dye from the liver and thus becomes visible?

The dog, of course, is different from a human being. It seems to be in more than one respect an ideal animal for this study. The relation of soft-tissue density to the size of the common duct looks to be ideal when you think of a big fat patient.

I'd like all of you to know, as I'm sure you do know, that Dr. Strain has already produced one of our best contrast substances, which is being used everywhere, "Pantopaque," and I am certain he is going to produce other contrast substances. As you know, progress in radiology is practically dependent upon these things, so we are very grateful that he has come here to speak to us.

I think when the radiologist is able to control the funds of the x-ray department, he might be able to assist in some of this research by aiding other excellent chemists. I hope some day to be able to offer Dr. Strain a place in my laboratory if he is unhappy elsewhere.

Chairman Rigler: Does anyone else wish to discuss this paper or ask any questions? I am sure Dr. Strain will try to answer any that appear.

Question: Has this medium been tried on any human beings yet?

Dr. Strain (closing): As to the clinical test, there is only one patient so far. I have taken half a gram and I planned to take more so that we could answer this question, which I knew would be raised. Unfortunately, I had a streptococcus infection which involved my children and myself, and I felt that the circumstances were unfavorable to further experimentation. We shall have clinical tests in the very near future.
Los Compuestos Orgánicos de Yodo como Medios de Contraste para el Diagnóstico Radiográfico. Estudios Experimentales sobre la Visualización del Aparato Biliar

Con perros como sujetos de experimentación, se hicieron comparaciones de la secreción al aparato biliar de las siguientes sustancias colecistográficas: ácido o-(4-hidroxi-3,5-biyodobencílico)-benzoico, ácido yodoalifónico (Priodax), tetrayodofenolftaleína y fenol(tetrayodof)taeleína.

Con la administración oral, sólo el derivado del ácido benzoico facilitó constantemente buena visualización del colédoco. Administrados endovenosamente en forma de las sales sódicas, los cuatro compuestos delinearon algunos de los conductos hepáticos, el colédoco, y por fin la vesícula biliar. La delineación más rápida y completa se obtuvo con el derivado del ácido benzoico, quedando los conductos visibles a los diez minutos y la vesícula biliar a los veinte minutos de la inyección. En forma semejante, quedó demarcado el grupo de las vías después de la colecistectomía.