and terramycin(11-13), than to streptomycin (14), in that it occurs gradually in a stepwise fashion, and in that there has been so far no demonstration of an amethopterin-dependent strain of this organism.

This resistance is presumed to be due to a random mutation selected out by the presence of the drug as previously demonstrated for *Staphylococcus aureus* with both penicillin and streptomycin(7,14).

The fact that the resistant bacterial cell is able to utilize 4-amino PGA and 4-aminopteroyl aspartic acid, as well as amethopterin, to supply its PGA requirement is of considerable interest. The mechanisms by which this resistance and this ability to utilize amethopterin are achieved, are under study at the present time.

Summary. (1) By growing *Streptococcus faecalis* (ATCC No. 8043) in a medium containing successively higher concentrations of amethopterin, more than a thousand-fold increase in resistance has been achieved. (2) This amethopterin-fast variant has a slightly lower requirement for folic acid in the absence of amethopterin than the parent sensitive strain. In the absence of folic acid, however, amethopterin and certain other 4-amino derivatives of PGA can replace this folic acid requirement in the resistant strain.

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Evaluation of Some Iodine-Containing Organic Compounds as X-Ray Contrast Media. (19059)

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Organic compounds containing iodine have been useful aids in radiographic diagnoses. We have previously studied the physiological behavior of one of these, Urokon(1), which has proven of value in the visualization of the pelves and ureters of the kidneys of man(2). Five analogues of Urokon and a number of other iodine-containing compounds have now been investigated. Some of these which are of experimental and potential clinical interest are listed in Table I. Two approaches to the determination of their value have been made, radiographic study of the compounds after intravenous or oral administration (dogs) and analyses of the iodine content of kidney, liver, and blood after intravenous administration (rats).

Roentgenographic studies. Dogs 5 to 9 kg in weight were maintained in the laboratory over several months, so that the same animals might be used in a comparative series of investigations with various potential cholecystographic agents. A portable Picker x-ray unit was employed with instrumental settings at 50 kilovolts and 84 milliamperes and exposures of 1 to 1.75 sec, depending on the size of the animal. Roentgenograms were taken at intervals as shown in Table II. Under these conditions, it was possible to obtain clear visualization of the gall bladder and biliary ducts. For intravenous injection, 10% solutions (pH 7-9) of the sodium salts (sodium carbonate as neutralizing agent) of the compounds studied were used. After a 20 hr fast, the dog was fed the yolk of a hard boiled egg, 4 hr preceding the injection of

1. Neuhaus, D. R., Christman, A. A. and Lewis, H. B., *J. Lab. Clin. Med.*, 1950, v35, 43.
2. Nesbit, R. M., and Lapides, J., *J. Urol.*, 1950, v63, 1109.

TABLE I. Compounds studied.

Chemical name	Identification No.	LD_{50} in mice mg/kg	Iodine %
3-Formylamino-2,4,6-triiodobenzoic acid	1	8000	70.0
3-Acetylamino-2,4,6-triiodobenzoic acid*	2	8000+	68.4
3-Propionylamino-2,4,6-triiodobenzoic acid	3	8100	66.7
3-Butyrylamino-2,4,6-triiodobenzoic acid	4	—	65.1
3-Caproylamino-2,4,6-triiodobenzoic acid	5	1450	62.1
N-(3-amino-2,4,6-triiodobenzoyl)-ε-amino-n-caproic acid	6	410	61.7
2-(4-hydroxy-3,5-diiodobenzyl) benzoic acid	S	—	52.9
α-Phenyl-β-(4-hydroxy-3,5-diiodophenyl) propionic acid†	P	—	51.5

* This compound, as the sodium salt, is known as Urokon Sodium.
† Priodax.

the compound. This preliminary treatment is necessary to obtain clear visualization of both the gall bladder and the biliary ducts after injection of the compounds. After the dog was anesthetized (35 mg of sodium pentobarbital per kg) and placed on its right side on the table, a scout film was made to establish the proper length of exposure and then the experimental solution was slowly injected intravenously over a period of 3-5 min. After repeated anesthesia and exposure to x-ray, the anesthetic was frequently effective for as long as 8 hr rather than 3 hr as in the initial experiments. Dogs D and E, which had been injected and x-rayed approximately 60 times over a period of 3 months, were sacrificed. Histological examination showed normal liver, sternum and ribs; the hematocrit values were slightly low however. Since these animals had been used more frequently than any of the others of the entire group, it is believed that the experiments, details of which are reported in Table II, were carried out on essentially normal animals.

The experimental procedure for the study of compounds after oral administration was similar to that already described for intravenous injection and to that used by Jones and coworkers(3). The compound to be studied was mixed with boiled egg yolk, crumbled and moistened to a suitable consistency with water. This mixture was fed 8 hr after food had been removed from the cage. After a further period of 16 hr, the animals were anesthetized and roentgenograms were taken as described. Occasionally an animal required additional pentobarbital to maintain satisfactory anesthesia for the 6 hr experimental period (*i.e.*, 16th to 22nd hr after feeding the egg yolk-compound mixture); in these cases, one fourth of the amount given intravenously was injected intramuscularly. No symptoms of toxicity (nausea, vomiting, diarrhea, etc.) were observed with any of the compounds listed in Table I. In the investigation of Jones and coworkers(3), the time required for the initial visualization of the biliary ducts and gall bladders after administration of their new compound, 2-(4-hydroxy-3,5-diiodobenzyl) benzoic acid (Compound S of Table I), was compared with that for Priodax, Isoiodeikon, and Iodeikon. Compound S showed a distinct advantage over the others. In Table II, Compound S and Priodax (P), given under our conditions, are compared with Urokon and its analogues (Compounds 1-5) and Compound 6. After intravenous injection differences are small; Compound S how-

3. Jones, G. E., Grohowski, A. S., Robertson, H. D., Ramsey, G. H., Schilling, J. A., and Strain, W. H., *Radiology*, 1948, v51, 225.

TABLE II. Initial Visualization of Biliary Ducts and Gall Bladders of Dogs after Oral and Intravenous Administration of Iodine-Containing Compounds.

Tissue visualized	Dosage	Dog No.	Time interval of initial visualization compound administered S	P	6	5	4	3	2	1
			Intravenous							
	mg/kg		min	min	min	min	min	min	min	min
Gall bladder	53	B			20					
	60	B	40							
	75	B	20	20	40	20	40	—	—	60*
	75	E	20	60	40					
			100		40					
	75	F	20	—	20	20	20	20	—	
Biliary ducts	53	B			20					
	60	B	20							
	75	B	20	40	40	20	40	—	—	60*
	75	E	20	40	40					
			20		40					
	75	F	20	—	20	20	20	20	—	
				Oral						
			hr	hr	hr	hr	hr	hr	hr	hr
Gall bladder	50	B	19		19		—		—	
			—		16					
	100	D	19		22					
	44	F			19					
	50	F	19		16	—		—		
Biliary ducts	50	B	16		16		16		22	
			16		16					
	100	D	16		16					
	44	F			16					
	50	F	16		16	16		19		

* Appeared only on one film.

ever is somewhat better than Compound 6 in visualization of the biliary ducts and inferior with respect to the gall bladder. After oral administration, they are of approximately equal value in both respects. On the other hand, visualization of the gall bladder was not obtained after oral administration of Urokon or any of its analogues (Compounds 1-5). Perhaps the reason for this can be found in the fact that this group included the only compounds tested which made possible the visualization of the kidneys within 3 hr after injection. Epstein and his associates(4) have noted that the more water-soluble compounds appeared rapidly in the urinary tract and were less likely to give clear biliary contrast on x-rays. Urokon (Compound 2) has been shown to be an excellent pyelographic medium (1).

In view of these results it is interesting to note from Table I that compounds 5 and 6 are very similar in structure. The prime difference lies in the placement of the caproyl group (Compound 5) and the polarity of Compound 6. The lack of similarity in their LD_{50} values in mice (Table I) and in their biological behavior in dogs (Table II) may be related to this difference in chemical structure.

Chemical analysis of tissues. The x-ray studies already described have demonstrated that the various compounds under discussion are concentrated in and excreted by the liver and kidneys at varying rates. Further important information should be obtained if the tissues were examined for their content of the radiographic dyes at varying intervals after administration. Rats of 100 to 150 g weight were fasted overnight and received intravenously through the tail vein the compound under investigation at a level of 50 mg per kg body weight; the sodium salts were prepared as in the previously described studies. The animals were chloroformed, blood samples

4. Epstein, B. S., Cohen, H. L., and Naletson, S., *Radiology*, 1950, v54, 87.

were taken from the left ventricles immediately after death, and the liver and kidneys were removed for analysis. The analytical procedure was essentially that of Matthews, Curtis and Brode(5) for total iodine as modified in this laboratory(1). The minimal number of animals in each group was 3; if the results were widely variant, additional animals were used to establish a more accurate value. Results presented in Table III are the average of values obtained from at least 3 animals.

From an examination of Table III, it can be seen that Urokon and its analogues (Compounds 1 to 4), having from one to 4 carbons in the acyl group, were present in the kidneys in large amounts 10 min after injection; by 180 min, however, the concentration had fallen to a relatively low level. Although Compound 5 did not reach as high an initial level as the other analogues, its concentration in the kidney during the final intervals was comparable with those of Compounds 1 to 4. By way of contrast, the amounts of Compounds 6 and S during the first 20 min were lower than those of the Urokon series, but 60 to 180 min after injection the values were considerably higher. Concurrently, the levels of all of the Urokon analogues per ml of blood fell rapidly and to a relatively low concentration by the final analytical period whereas the corresponding values for 6 and 8 remained somewhat higher.

When the concentration of radiographic dye in the kidney tissue is plotted against that in blood for each interval, the resulting curves demonstrate the rate of excretion of the dyes with increasing amounts present in the blood. In Fig. 1 the results for 3 of the analogues are plotted; the curve for Compound 2 is very similar to those for 1 and 3, and was selected to represent the curves for all 3 compounds. From the figure it can be noted that with the decreasing length of the acyl group, the slopes of the curves are steeper. Thus the rate of excretion of Compound 5, is slower than that of either Compound 2 or 4. In the cases of Compounds

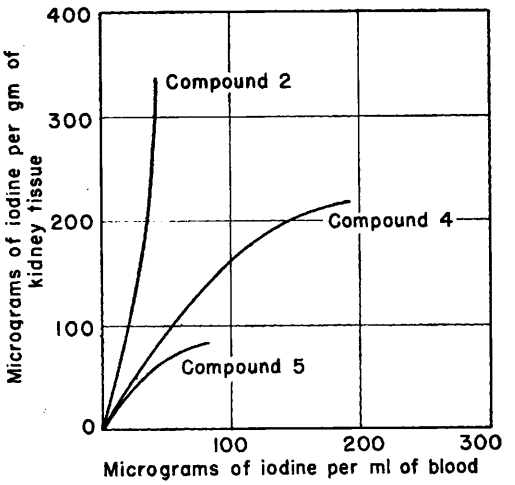

FIG. 1. Rates of Excretion of Compounds 2, 4 and 5.

4 and 5, the rates of excretion appear to reach a maximum as the amounts of the respective compounds in the blood are increased, but for Compound 2, no plateau was obtained in the range studied.

The results of the analyses of the liver after intravenous injection of these compounds (Table III) indicate that those which are excreted slowly by the kidneys are found in comparatively greater amounts in the liver. After 10 min the concentrations of Compounds 1 to 5 and S are not widely different, but after 60 min, the concentrations of the group 1, 2 and 3 are significantly lower than those of the other compounds. In the final analytical period, the concentrations of Compounds 1 to 3 are at a relatively low level. Of all of the compounds tested, it is only Compound 6 that is consistently in greater concentration throughout the three hour period. This evidence corroborates the roentgenographic findings described above; after intravenous injection the four compounds, 4, 5, 6 and S, which were maintained at a higher concentration in the livers of rats than Compounds 1 to 3, were those which were effective cholecystographic agents in dogs.

From the results of the experiments on rats, it can be concluded that Compounds 1 to 3 are too rapidly excreted by the kidneys to be of probable value as cholecystographic agents after either oral or intravenous administration. This is borne out by the roent-

5. Mathews, N. L., Curtis, G. M., and Brode, W. R., *Indust. Eng. Chem.* (Anal. Ed.)., 1938, v10, 612.

TABLE III. Concentration of Iodine in Blood, Kidneys, and Liver of Rats after Intravenous Injection of Cholecystographic Media.

Tissue analyzed	Interval after inj.	All values are expressed μg of iodine per g or ml — Compound 1	2*	3	4	5	6	S
Kidney	10	263.	363.	278.	217.	80.3	66.5	56.5
	20	125.	199.	105.	128.	111.7	78.	76
	30	120.	120.	136.	86.4	73.8	94.9	64.9
	60	31.	48.	27.5	54.	38.8	82.5	45.9
	120	10.4	6.6	20.9	22.7	22.	79.5	35.4
	180	8.6	9.2	3.7	12.7	9.4	55.1	23.7
Blood	10	50.1	40.5	54.	194.	83.5	22.7	76.9
	20	33.1	31.2	28.	55.9	45.4	57.3	66.6
	30	24.2	26.5	18.8	45.	38.9	45.6	62.4
	60	6.	6.6	8.	31.1	26.1	50.5	44.4
	120	1.	3.6	3.6	16.6	10.2	26.7	26.5
	180	0.	0.7	1.3	9.8	6.	14.8	23.4
Liver	10	45.6	48.8	73.6	73.7	68.	125.	59.7
	20	22.8	42.8	48.1	47.3	64.5	137.	60.2
	30	14.1	38.2	30.7	42.3	64.8	129.	48.3
	60	6.4	11.4	19.9	31.8	52.9	130.	28.2
	120	.6	1.6	4.8	22.	40.4	108.	17.6
	180	.0	.0	5.3	13.3	20.9	70.8	15.

* Urokon.

genographic studies on dogs already discussed (Table II). On the other hand, the Compounds 4 and 5 produce satisfactory cholecystograms after intravenous injection. Compounds S and 6 produce clear visualization of the biliary ducts and gall bladders of dogs after both oral and intravenous administration. These results were corroborated by the fact that there were present in the livers of rats, at any period after their administration, larger amounts of Compound 6 than of the other compounds, including Compound S. This latter compound has been shown by Jones and coworkers(3) and in our own experiments to be an effective cholecystographic medium in dogs after either oral or intravenous administration.

Summary. 1. After intravenous injection into dogs, the following compounds were found to be good cholecystographic dyes: N-(3-amino-2,4,6-triiodobenzoyl) ε-amino-n-caproic acid (Compound 6); 2-(4-hydroxy-3,5-diiodobenzyl) benzoic acid (Compound S); 3-caproylamino-2,4,6-triiodobenzoic acid (Compound 5) and 3-butyrylamino-2,4,6-triiodobenzoic acid (Compound 4). After oral administration, only Compounds S and 6 produced satisfactory visualization of the gall bladder and biliary tract 16 hr after feeding. 2. Analyses of the tissues of rats after intravenous administration of the compounds (1-6 inclusive and S) gave the following results: In the liver, Compound 6 was present in greater concentration than any of the others tested; in the kidney, Compounds 3-formylamino-2,4,6-triiodobenzoic acid, 3-acetylamino-2,4,6-triiodobenzoic acid, 3-propionylamino-2,4,6-triiodobenzoic acid, and 3-butyrylamino-2,4,6-triiodobenzoic acid were present in high concentration during the initial intervals, but fell to a relatively low level at the end of 3 hr. It was found that the rates of excretion of these compounds by the kidney decreased with the increase in the length of the acyl group.

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