Fate of Injected Radioiodinated 4-Iodoantipyrine in the Dog and Rat.* (29468)

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Radioiodinated 4-iodoantipyrine (RIAP) found early use as a tool in the study of body composition(1). More recently, it has been used in measurement of cerebral(2) and coronary blood flow(3). Previous studies in this laboratory indicated that in rats, RIAP was rapidly metabolized to a more diffusible form which did not distribute in the total body water as antipyrine itself does(4). These studies suggested the invalidity of using RIAP as a tool to measure the total body water compartment. It was subsequently shown that the liver was the major site of RIAP metabolism in the rat and this was used as the basis of an experimental liver function test in that species(5). This paper reports observations on the fate of injected RIAP in the dog. The diffusibility of radioactivity from plasma was studied following injection of RIAP in the dog. The effect of ablation of liver function in the dog on the diffusibility of radioactivity from plasma following RIAP injection was then studied. Finally, a chromatographic technique was used to characterize the radioactive components in plasma following RIAP injection, and to investigate the radioactive metabolic products of RIAP appearing in the bile and urine.

Materials and methods. Diffusibility of RIAP from dog plasma in vivo *and* in vitro. Normal adult dogs were anesthetized with sodium pentobarbital (30 mg/kg) intravenously. Following catheterization of the femoral artery and vein, 10 μc/kg of RIAP (Abbott) were injected intravenously. One and two hours later, 15 cc samples of arterial blood were drawn and the plasma collected. RIAP was also mixed *in vitro* for one hour with dog plasma to yield an activity of about 0.01 μc/ml. Two ml aliquots of the plasma samples were then placed inside a cellophane membrane (80 μ thick, 30 Å pore size) tube and dialyzed against 40 ml of distilled water. After a one-hour dialysis period, 1 ml samples were withdrawn from the dialysis membranes and counted in a well-type scintillation counter. The per cent radioactivity remaining inside the dialysis membrane was then calculated.

Effect of functional hepatectomy on diffusibility of RIAP from dog plasma. Normal adult dogs were anesthetized in the same manner. Following laparotomy, "functional hepatectomy" was performed by ligation of the celiac axis and portal vein, analogous to the method described by Russell(6) in rats. To prevent shock from blood pooling in the viscera, the superior and inferior mesenteric arteries were also ligated. This procedure required about one hour and was well tolerated by the animals. RIAP (10 μc/kg) was then injected intravenously. At one and two hours, arterial blood samples were drawn and treated as in the experiment described above.

Chromatographic analysis of RIAP metabolites in the dog and rat. Adult male Sprague-Dawley rats were anesthetized with sodium pentobarbital (40 mg/kg) intraperitoneally. Laparotomy was performed and RIAP (40 to 75 μc) was injected into the inferior vena cava. At 1 to 3, 15, 30, 45 and 60 minutes following injection, blood samples were collected by bleeding the tail.

An ascending paper chromatographic technique was used to separate the radioactive components in the plasma samples collected. Eight-lambda aliquots of the collected plasma samples, along with 8 lambda of RIAP and NaI^{131} were spotted on Whatman No. 2 paper and chromatographed using normal butanol-acetic acid-water (200-30-75) as the solvent. Radioautographs were then made from the dried chromatograms, and areas on the chromatogram corresponding to areas of radioactivity on the radioautograph were cut

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TABLE I. Comparison of Diffusibility of Radioactivity from Dog and Rat Plasma After *in vivo* and *in vitro* Mixing with RIAP.

Group	No. of experiments	Mixing time (hr)	% radioactivity remaining inside membrane
RIAP + dog plasma			
in vitro	12	1	39.8 ± 1.8
in vivo	5	1	10.8 ± 1.1
in vivo	5	2	8.0 ± 1.0
RIAP + rat plasma			
in vitro	6	1	34.9 ± 1.2
in vivo	6	1	6.7 ± 2.9
in vivo	6	2	4.2 ± 1.2

out and counted in a well-type scintillation counter. The per cent radioactivity in each sample represented by RIAP, free I^{131} and at the origin was then calculated.

Laparotomy was then performed on normal adult dogs and rats following induction of anesthesia with sodium pentobarbital. The common bile duct was catheterized to permit a free flow of bile for collection. RIAP was then injected intravenously. Bile was collected for a one-hour period following RIAP administration. Ten-lambda aliquots of bile, urine, plasma, RIAP and Na^{131} were then spotted on Whatman No. 2 paper and treated as described above.

Results. The data comparing the diffusibility of radioactivity from dog plasma following *in vitro* and *in vivo* mixing are listed in Table I. The observations reported earlier for rats are shown for comparison(4).

The results of the effect of "functional hepatectomy" on the diffusibility of radioactivity from dog plasma following injection of RIAP are reported in Table II. The data reported earlier for similar studies in the rat are listed for comparison(5).

TABLE II. Comparison of Diffusibility of Radioactivity from Plasma in Dogs and Rats with Intact Livers and Those with Functional Hepatectomy.

Group	No. of experiments	RIAP mixing time (hr)	% radioactivity remaining inside membrane
Dogs with functional hepatectomy	5	1	33.7 ± 5.8
	2	2	33.8 ± 2.5
Dogs with intact liver	5	1	10.8 ± 1.1
	5	2	8.0 ± 1.9
Rats with functional hepatectomy	10	1	26.5 ± 5.8
Rats with intact liver	6	1	6.7 ± 2.9

A typical radioautograph of a chromatogram of rat plasma at various intervals following RIAP injection is shown in Fig. 1.

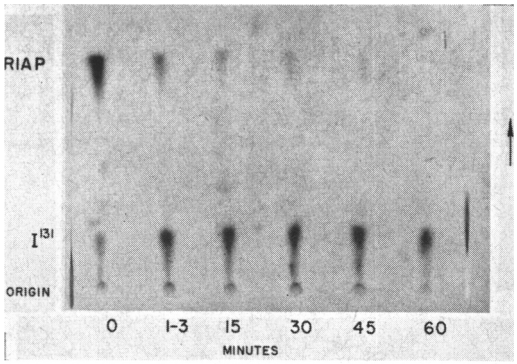

FIG. 1. Radioautograph of chromatogram of serial samples of rat plasma drawn at various times following RIAP administration.

The per cent radioactivity in each sample due to RIAP, free I^{131} and that left at the origin is listed in Table III. These results are shown graphically in Fig. 2.

A typical radioautograph illustrating the radioactive metabolites present in the bile and urine of the dog and the rat following RIAP administration is shown in Fig. 3.

TABLE III. Quantitative Analysis of Radioactive Components of Rat Plasma at Various Time Intervals After RIAP Administration.

RIAP mixing time *in vivo* (min)	% plasma radioactivity represented by RIAP	I^{131}	Origin
RIAP + plasma *in vitro*	94.6 ± 1.8	3.2 ± .8	2.2 ± .2
1 to 3	82.1 ± 1.6	13.8 ± .8	4.2 ± 2.4
15	42.1 ± 2.7	44.8 ± 2.3	10.1 ± 5.8
30	25.3 ± 3.6	57.4 ± 5.6	17.3 ± 8.1
45	19.6 ± 4.1	61.7 ± 3.3	18.6 ± 3.6
60	16.3 ± 3.7	71.4 ± 6.8	13.9 ± 6.1

The per cent of radioactivity represented by each component is listed in Table IV. The chromatograms of dog bile appeared identical to those of the rat as reported earlier from this laboratory(8). The radioactive components present in the urine of the dog and the rat seemed identical to those present in the

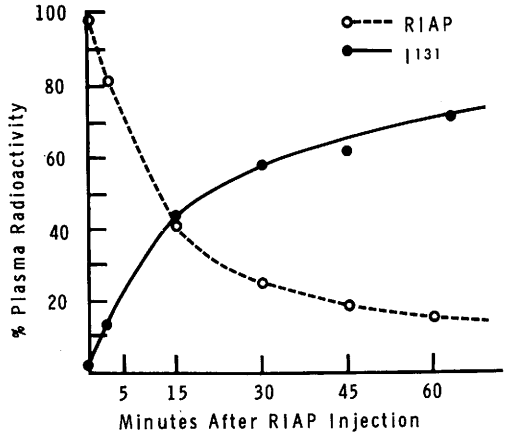

FIG. 2. Graphic illustration of % of plasma radioactivity due to RIAP and I^{131} at various times following RIAP administration in the rat.

bile; however, in the radioautograph shown in Fig. 3, there is not good resolution of the X and Y components of bile as was evident on other radioautographs.

Discussion. Earlier studies in the rat indicated that RIAP was rapidly metabolized to a more diffusible form which did not distribute in the total body water. This finding suggested that it was invalid to use RIAP as a tool to measure the total body water compartment, an area in which RIAP has found most use to date. The results of the first experiments described above indicate that the dog also rapidly metabolizes RIAP to a more diffusible form, thus adding further support to the belief that RIAP should no longer be used as a tool to measure total body water.

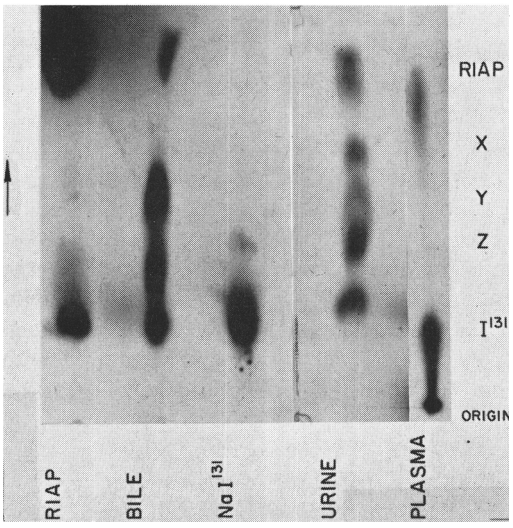

FIG. 3. Radioautograph of chromatogram of samples of dog urine, bile, and plasma collected 1 hr after RIAP administration. RIAP and NaI^{131} are shown for identification.

Previous reports from this laboratory showed that various forms of liver damage greatly reduced the conversion of RIAP to a more diffusible form in the rat. The above results of "functional hepatectomy" experiments show the liver to be the primary site of RIAP metabolism in the dog also. This study also suggests that the dialysis technique utilized may be useful as a liver function test in the dog, and possibly higher species, as well as in the rat as previously described(5).

TABLE IV. Per Cent Radioactivity Represented by Various Components of Bile and Urine of Dog and Rat.

Chromatogram of	% radioactivity represented by RIAP	X	Y	Z	I^{131}	Origin
Bile—Dog	1.7	8.1	42.5	44.7	1.6	1.0
" —Rat	5.4	6.7	48.0	30.6	9.0	.1
Urine—Dog	4.0	30.9	—	65.0	—	.1
" —Rat	23.1	14.6	—6.9—		53.9	1.5

In the rat, increasing percentages of plasma radioactivity following RIAP administration were due to free I^{131}, while the percentage of plasma radioactivity due to RIAP fell sharply. Even as early as 15 minutes after RIAP administration, there was as much or more free I^{131} present in plasma than RIAP itself. Since one to two hours is commonly used as the equilibration time in measuring total body water with RIAP, it is apparent that by this time most of the plasma radioactivity is due to free I^{131} which does not distribute in the total body water compartment. (In the measurement of cerebral and coronary blood flow with RIAP, plasma samples are withdrawn within 4 minutes of RIAP infusion. The degree of loss of I^{131} tag from the RIAP molecule within this short time may be insignificant, and species differences in the rate of RIAP metabolism would also have to be considered.)

The chromatographic analysis of bile in

the dog and the rat following RIAP administration indicates that the liver metabolizes RIAP to at least four I^{131}-containing compounds, one of which is free I^{131} itself. It is of interest that approximately 95% of the radioactivity excreted in the bile is in the form of 3 as yet unknown compounds. Chaikoff(7) has shown that rat liver is rich in a "nonspecific deiodinase," capable of splitting the I^{131} tag from various thyroid hormone intermediates. It is possible that such a deiodinase is also responsible for splitting the I^{131} tracer from RIAP.

The major radioactive metabolic products found in the urine of the dog and the rat appear to be identical to those found in the bile following RIAP administration. Whether or not these represent metabolic products of the liver which are simply excreted by the kidney, or whether they are metabolites of the kidney itself is not known. The unknown radioactive metabolites (X, Y and Z in Fig. 3) of RIAP have not been demonstrated in the plasma.

Summary and conclusions. These studies show that the dog, like the rat, rapidly converts RIAP to a more diffusible form and that the liver is the primary site of this conversion. Chromatographic analysis of serial plasma samples following RIAP injection in the rat indicated that as early as 15 minutes most of the plasma radioactivity was due to free I^{131} and not RIAP, thus invalidating the use of this compound in total body water studies. Finally, chromatographic analyses of dog and rat bile following RIAP administration showed that in addition to RIAP and free I^{131}, the majority of radioactivity excreted was in the form of 3 as yet unknown compounds.

1. Talso, P. J., Lahr, T. N., Spafford, N., Ferenzi, G., Jackson, H. R. O., *J. Lab. and Clin. Med.*, 1955, v46, 619.

2. Sapirstein, L. A., Hanusek, G. E., *Am. J. Physiol.*, 1958, v193, 272.

3. Krasnow, N., Levine, H. J., Wagman, R. J., Gorlin, R., *Circ. Res.*, 1963, v12, 58.

4. Sullivan, J. M., Rose, J. C., *J. Lab. and Clin. Med.*, 1961, v57, 955.

5. Sullivan, J. M., Straub, W. H., Rose, J. C., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 686.

6. Russell, J. A., *Am. J. Physiol.*, 1942, v136, 95.

7. Tong, W., Taurog, A., Chaikoff, I. L., *J. Biol. Chem.*, 1954, v207, 59.

8. Straub, W. H., Sullivan, J. M., Rose, J. C., *Georgetown Med. Bull.*, 1962, v16, 20.

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Decrease in Incidence of Blood Spots in Chicken Eggs by Injection Derivatives of Pyrrole-2-Aldehyde Chalcones into Hens.* (29469)

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The cause of preovulatory, intrafollicular hemorrhage, resulting in blood spots in chicken eggs has not been determined; however, it is thought to be related to capillary fragility(1). Flavone glucosides and their water-soluble derivatives, the chalcones, have been used experimentally in the treatment of disease of man in which capillary fragility is a prominent finding(2,3). Therefore, the effect of 4 newly synthesized pyrrole-2-aldehyde chalcones on the incidence of blood spots in eggs was tested and compared with that of commercial hesperidin and hesperidin methyl chalcone.

Methods. Laying Leghorn chickens of a strain which produces a high percentage (50 to 100%) of eggs with blood spots,‡ were injected with the test materials. The pyrrole-

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