STUDIES WITH RADIOACTIVE IODIZED FAT. II. THE TISSUE DISTRIBUTION OF EMULSIFIED FAT FOLLOWING INTRAVENOUS ADMINISTRATION [1]

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(Received for publication March 14, 1949)

The rate of absorption of emulsified fat labelled with radioactive iodine from the subcutaneous tissues and the peritoneal cavity in animals is too slow to serve as a source of current caloric requirements (1). Since parenteral administration to patients for this purpose would require that the fat be given in high concentration intravenously, it is necessary to prepare a stable, non-toxic emulsion. This has not yet been accomplished (2–7). Pending the availability of such an emulsion, it is desirable to study the metabolic fate of fat given intravenously. One of the first steps in such a study is the distribution of the injected fat. Numerous studies of fat, tagged with non-radioactive iodine, given by vein, fail to agree as to its distribution (8–19). The conflicting results are in part due to variations in particle size and to lack of reliable techniques for determining tissue localization and quantitative distribution.

In this communication, we report the quantitative tissue distribution of the radioactivity found after the intravenous injection into dogs and mice of an emulsion of radioactive iodized soya bean oil. The oil was labelled with radioactive iodine by a technique reported in a preceding paper (1). It was then prepared as an emulsion as follows:

Preparation of the emulsion

Radioactive iodized soya bean oil (1) was emulsified in water with the aid of "Demal 14." [2] Twenty-five gms. of this detergent were dissolved in 50 gms. of the oil, and the resulting solution was warmed to 80° C. and slowly added to 425 cc. of warm distilled water in a rotating Waring blender. The blending was continued for ten minutes after the oil mixture had been added. The resulting crude emulsion (10%) was then homogenized by recirculation for 60–90 minutes in a two-stage homogenizer at 3300–3500 lbs. of pressure per square inch. The temperature rose to 70–85° C. The final product appeared blue by reflected light and reddish by transmitted light, an indication of fine particle size (20). On microscopic examination the oil globules ranged from approximately 0.5 to 2.0 micra in diameter. The emulsion was stable at room temperature for one to two weeks. Since sterilization by autoclaving destroyed the emulsion and decreased its pH from 8.0 to 4.3, it was passed through a Seitz filter before use. The emulsion was found apparently non-toxic and well tolerated when given orally and intravenously to mice and dogs.

ANIMAL EXPERIMENTS

Intravenous administration in dogs

Each of eight mongrel dogs (weight range 8–12 kgs.) was given a single slow intravenous injection (1.2–4.2 cc./kg.) of the emulsion. Venous blood samples were drawn at intervals. Two cc. of blood or 1 cc. of plasma was used for radioactivity measurements. Standards consisted of highly diluted aliquots of the emulsion, to which 2 cc. of normal blood or 1 cc. of normal plasma was added. The technique of radioactivity measurement has been described elsewhere (1).

The results, listed in Table I, show that at the end of the first hour following injection about 2% of the total injected radioactivity was circulating in the blood of Dog 8, and about 1% in the plasma of Dogs 8, 20–24. There followed a slow increase, so that some 24 hours later the maximum concentration of circulating radioactivity was reached (some 3% to 7.5% of the total injected radioactivity). This maximum, with some variation, persisted for another 24–48 hours and gradually declined thereafter.

Plasma Fractionation Experiments

The distribution of the circulating radioactivity among the plasma components was examined by fractionation of plasma specimens drawn from several dogs 24–30 hours after injection of the emulsion.

[1] Aided by a grant from the Patrons of Research, Beth Israel Hospital, Boston.

[2] A blend of di-tri and higher polyglycerols, partially esterified with oleic acid. Supplied by the Emulsol Corp., Chicago, Illinois.

TABLE I

Circulating radioactivity at various intervals after injection of radioactive fat emulsion in dogs*

Dog no.	8	8	20	21	23	24	22
Specimen	Blood	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Volume emulsion injected *cc./kg.*	4.2	4.2	1.2	2.5	3.1	3.2	3.8
Hours after injection	Circulating radioactivity in per cent of total activity injected						
0.25				0.8			1.2
0.50			1.8	1.4			0.9
0.75	2.0	0.7	0.9				
1			1.1	1.1			1.2
2	1.9	1.1	1.6				
3	2.3	1.3	2.0	1.1			1.7
5	2.2	1.4	2.1	1.6		1.3	2.6
7	2.0	1.2					
8			3.6	3.8			2.4
12	2.2	1.5	4.2	2.4			3.0
20	4.5	2.7				3.5	
24–26	5.5	3.2	7.4	7.0	6.5		6.1
28–30	4.6	2.3	7.5			3.0	
45–54	4.4	1.9	6.3	10.5	7.8	2.9	
63	3.6	2.0					
68–72	3.1	1.7	4.3	12.4	7.0		
84–88		1.7	2.6				
102		1.5					
112			0.7				
120				9.0	6.7		
170					5.2		
216					4.2		
243					1.2		
252					0.2		

* Assuming blood volume to be 10% and plasma volume to be 5% of body weight.

The isolated fractions or their aliquots were transferred to aluminum cups (2 cm. in diameter), evaporated to dryness and the radioactivity measured (1). The results were calculated in per cent of the radioactivity of the plasma which was fractionated.

Four types of fractionation experiments were performed as follows:

Experiment 1.—Analysis of lipoid fractions of plasma by alcohol-ether extraction

Total lipoids and phospholipids were isolated from the plasma according to the method of Bloor (21). The plasma proteins precipitated by the alcohol-ether mixture were also collected. The total protein and phospholipid fractions contained only traces of radioactivity, while the total lipoids contained 50–65% of the radioactivity.

Experiment 2.—Analysis of the alcohol-ether extract of plasma

An alcohol-ether (lipoid) extract of plasma was evaporated to dryness and the lipoids in the residue redissolved in hot petroleum ether. The petroleum ether extract contained 60% of the total radioactivity of plasma. The residue, after lipoid extraction, was redissolved in water. An aliquot of this solution showed that it contained 20% of the total radioactivity of whole plasma.

The aqueous solution remaining after extraction with alcohol-ether was acidified with nitric acid. Carrier sodium iodide and a solution of silver nitrate were added in that order. The yellow silver halide precipitate was collected, washed thoroughly with water, acetone and ether. The radioactivity of this precipitate (*i.e.*, ionic iodine) was 9–12% of the total radioactivity (standard prepared with the same quantity of silver halide).

Experiment 3.—Further analysis of the alcohol-ether extract of plasma

A sample of plasma was extracted with alcohol-ether. The extract was divided into two portions. These were treated as follows:

(*a*) *Isolation of ionic iodine from alcohol-ether extract:*

To one portion nitric acid, carrier sodium iodide, and silver nitrate in aqueous solution were added in that order. The yellow precipitate was collected, dried, and washed thoroughly with ether and petroleum ether. The halide fraction contained 25–30% of the radioactivity of the alcohol-ether extract.

(*b*) *Isolation of ionic iodine from lipoid-free alcohol-ether extract:*

Another portion was evaporated to dryness and extracted with water. Silver halides precipitated from the extract contained approximately 10% of the total activity.

Experiment 4.—Analysis of the protein and lipoid-free filtrate of plasma

Protein was precipitated with sodium tungstate from an aliquot of plasma by the method of Folin and Wu (21). The filtrate was evaporated to dryness, extracted with ether and the residue dissolved in water and filtered. Ionic iodine was precipitated with silver nitrate as above. The precipitate of silver chloride and silver iodide was collected, washed thoroughly with water, acetone, and ether, and its radioactivity determined. The protein precipitate had no measurable radioactivity. The silver precipitate (*i.e.*, presumably the ionic iodine fraction) contained 15–20% of the activity in plasma.

The results for fractions extracted by organic solvents are somewhat high (error approximately 15%) and those for the silver halide precipitates somewhat low (error approximately 10–20%), because of differences in the dry weights of these samples as compared with standard plasma specimens. Corrections were not made for such variations in weight except to determine the per cent of error given above by suitable control experiments.

These experiments (Table II) show that 24 hours after the injection of the emulsion approximately 60% of the circulating plasma radioactivity was in the total lipoid fraction and approximately

TABLE II

Radioactivity circulating in fractions of plasma of dogs 24 hours after injection of radioactive fat emulsion, expressed in per cent of the activity in the circulating plasma

Experiment no.*	Per cent of radioactivity in circulating plasma				
	Lipoid fraction	Inorganic fraction		Protein	Phospholipid
		Precipitated with silver	Insoluble in organic solvents but soluble in H_2O		
	%	%	%	%	%
1	50–65			0	0
2	60	9–12†	20		
3a		25–30‡			
3b		10			
4		15–20†		0	

* The results listed for each type of experiment were obtained from two to four experiments.

† These samples contained silver chloride in addition to silver iodide.

‡ These samples may have contained the silver salt of radioactive fatty acid.

20% was in the water soluble residue, free of lipoid and protein (Experiments 2 and 4). The value, 9–12%, is a low estimate of what was present as ionic iodine (Experiment 3b). In a pilot experiment this discrepancy of 11–8% was accounted for by the self-absorption of β-rays by the heavy mass of the silver precipitate. Part of the 25–30% radioactivity in the silver halides precipitated from an alcohol-ether extract (Experiment 3a) may be due to radioactive fatty acids carried down with the halides.

Tissue Distribution of Radioactivity After Injection of Fat Emulsion

A. In Dogs

Tissue analyses for radioactivity by methods previously described (1) were performed in five dogs sacrificed by exsanguination at various intervals after injection of the emulsion.

The results, listed in Table III, are expressed in (*1*) activity per organ in per cent of the total activity injected, and (*2*) activity of 1 gm. of tissue in per cent of activity circulating at zero time in 1 cc. of plasma, assuming the plasma volume of each dog to be 5% of the body weight.

Samples (1 gm.) of various tissues when wet did not yield dry tissues of uniform weight. Error was thus introduced into the radioactivity measurements by difference in self-absorption of β-rays. However, the dry weight of any given tissue from one animal to another was remarkably uniform and changes in the radioactivity of any given tissue with time could be reliably compared (*22*).

Of the tissues of Dog 22, sacrificed one day after

TABLE III

Total and specific† radioactivity in tissues of dogs at various intervals after injection of radioactive fat emulsion*

Dog no.	22		24		21		23		20	
Interval from injection to death (*days*)	1		2		5		10		25	
Volume of emulsion injected (*cc./kg.*)	3.8		3.2		2.5		3.1		1.2	
Tissue	Total*	Specific†	Total*	Specific†	Total*	Specific†	Total*	Specific†	Total*	Specific†
Plasma	6.1	6.1	2.8	2.8	9.0	9.0	0.2	0.2		
Lung	9.9	73.8	2.9	19.2	0.5	3.0	0.06	0.3		
Liver	15.6	35.2	4.7	6.2	1.3	2.2	0.3	0.4	0.2	0.3
Spleen	4.0	93.4	1.5	28.4	0.5	11.0	0.05	1.1	0.02	0.6
Kidneys	0.3	3.4	0.2	2.5	0.5	4.6	0.03	0.2		0
Intestines	0.7	1.4	0.9	0.8	1.0	1.5	0.2	0.2		
Mesenteric fat	0.1	0.5	0.2	0.5	0.05	0.6	0.04	0.3		0.3
Muscle	4.7	0.7	4.1	0.7	3.9	0.7	0.9	0.1		
Thyroid	0.02	9.6	0.1	60.7	0.3	123.5	0.1	87.7	0.3	89.2
Bile	0.04	1.5	0.04	2.2	0.02	0.8	0.04	0.1		
Total	41.46		17.44		17.07		1.92		0.52	

* Expressed in per cent of total radioactivity injected. Muscle assumed to be 30% of body weight (23). All other organs weighed wet.

† Radioactivity of 1 gm. tissue, expressed in per cent of the radioactivity circulating at zero time in 1 cc. plasma, assuming plasma volume to be 5% of total body weight.

injection, the liver, lungs, plasma, muscle and spleen, in decreasing order, contained the largest fractions of the total radioactivity. Mesenteric fat and thyroid showed the lowest radioactivity content of all tissues examined. Some 41% of the total radioactivity injected was recovered in the tissues examined.

In Dog 24, sacrificed two days after injection, only 17% of the total radioactivity injected was recovered in these same tissues. The same amount was recovered from the same tissues in Dog 21, sacrificed five days after injection. In Dog 23, sacrificed ten days after injection, only some 2% of the amount injected was found in these tissues.

The spleen, lungs and liver, in decreasing order, showed the highest specific radioactivity one day after injection (Dog 22). The same was true after two days (Dog 24) although a substantial decline was obvious in all these organs, while the thyroid showed a very large increase in specific activity. This was all the more striking after five days (Dog 21). The spleen exhibited the slowest decline in specific activity. The continuously very low total and specific radioactivity of mesenteric fat is noteworthy. This is all the more so, in view of the fact that every other tissue studied showed a higher specific activity, at least during the first five days after injection. Ten days after injection very little activity was found in any of the tissues except the thyroid.

Specimens of urine were collected at various intervals after injection. The total urine collected in one of the dogs (Dog 20) during the first eight hours following injection contained 2½% of the total activity injected. By the third day, the specific [3] activity of the urine averaged 0.5% in all dogs. It remained at that level for the duration of the experiment in Dogs 20, 21 and 23.

B. In Mice

White stock mice, injected with 0.25 cc. of emulsion via a tail vein, were killed by decapitation at specified intervals. Blood collected in heparin during decapitation, and various tissue specimens taken immediately thereafter, were examined for radioactivity. The specific activity of each tissue,

[3] Radioactivity in 1 cc. of urine, expressed in per cent of the activity circulating at zero time in 1 cc. of plasma.

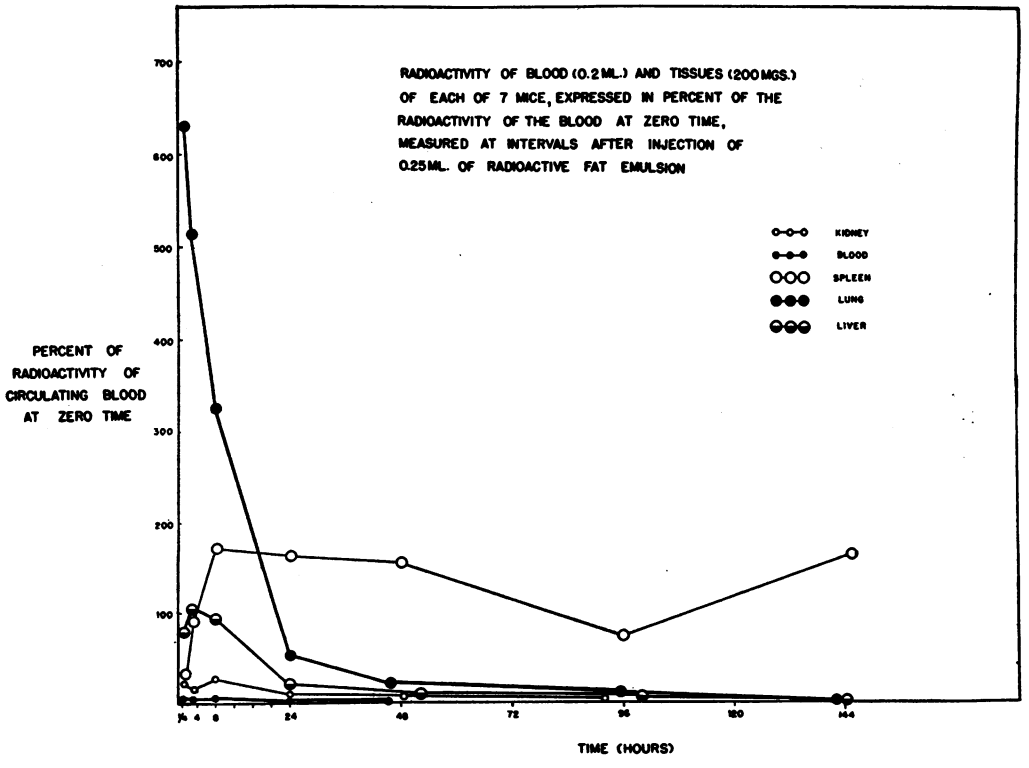

FIG. 1

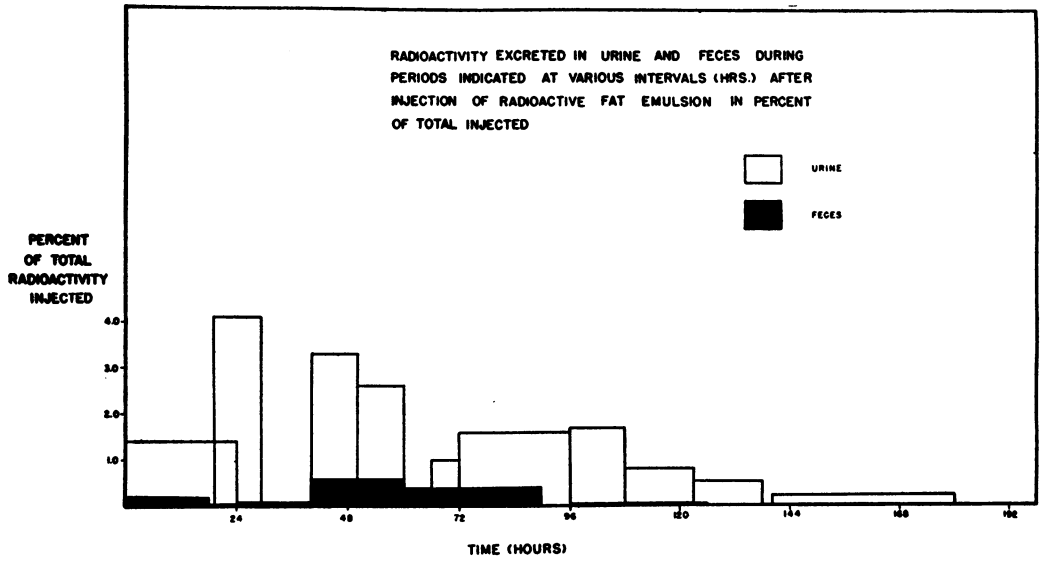

FIG. 2

in per cent of the activity circulating at zero time, was determined by a technique previously described (1, 22, 24). This experiment was performed in three groups of eight to 12 mice each.

The results of a representative distribution study (Figure 1), calculated according to methods reported elsewhere (22) are expressed in per cent of total activity injected. Errors in radioactivity determinations due to differences in dry weights of different tissues were not corrected, but the dry weight of the same tissue in different animals was remarkably uniform (22).

Following injection, the radioactivity disappeared rapidly from the blood. Three hours after injection, lung, liver, and spleen showed high levels of specific radioactivity, that of lung being extraordinarily high.

Twenty-four hours after injection, however, the level of radioactivity was highest in spleen, next highest in lung, and next in liver. The level in spleen increased thereafter, and continued at a high level for six to eight days, but that in liver and lung slowly declined. The specific activity in thyroid was high, but the cumulative increase with time, observed in the dog, did not occur. Intestine and fat showed a very low level of activity. Brain, muscle and skin showed no measurable activity.

In another group of some 20 male mice, similarly injected, specimens of urine and feces were collected at various intervals after injection and measured for radioactivity. The urine specimens were obtained by aspiration of the exposed bladder of these mice, sacrificed ten to 24 hours following ligature obstruction of the penile urethra (22). This was done at varying intervals from zero to 140 hours after injection. The urine and feces were transferred to aluminum cups for evaporation to dryness and radioactivity determination (Figure 2). The radioactivity in urine, which was still considerable four to five days after injection (Figure 2), as already stated above for dogs, was presumably in the form of ionic iodine cleaved from the fat. The low activity in the feces may have been due to a small amount of labelled fat excreted into the intestine.

DISCUSSION

Other investigators (8–19) have studied the immediate distribution of intravenously injected, non-radioactive, iodized fat emulsion in dogs and rabbits by determining the total iodine content of various organs. The injected fat left the blood stream rapidly (10, 13) and was retained in part by the lungs (9, 18, 19) or by the liver (8, 11, 12, 14) depending on particle size. Nearly all of an emulsion of greater particle size was deposited in the lungs (14) while most of an emulsion of smaller particle size was deposited in the liver (8, 11, 12, 14) or in the spleen (10, 11). Blockade of the reticulo-endothelial system by colloidal

silver, according to one investigator, resulted in diversion of the fat to the lungs and the liver parenchyma (10), while in similar experiments by others (11) such blockade was said to have had no effect on the distribution of the injected fat, which was reported to be retained by the liver (11).

More reliable indication of the initial distribution of intravenously injected fat is provided by determining the radioactivity of tissues soon after injection of radioactive iodized fat. This is so because the liberation of iodine from the fat molecule is relatively slow, as indicated (*1*) by the fact that iodine, which appears in the urine as soon as it is liberated, is not found in the urine in any quantity until an hour after the injection of iodized fat, and (*2*) because the urine shows a continuously high level of radioactivity for five to six days after injection of the emulsion. The metabolic cleavage of iodine from the fat molecule therefore appears to take place gradually and continuously.

From our data, it is not possible to determine the rate of metabolism of the iodinated or deiodinated fat. But the data on specific activity of various tissues are interesting in respect to this question (Table III). The very high specific activity in liver, lung and spleen 24 hours after injection probably reflects the storage function of the reticulo-endothelial system for particulate matter. Comparison of these data from Dog 24 with those of Dog 21 suggest that the spleen and lungs continue to act as depots, steadily, though slowly, giving up their stores, while the liver loses its stores much more rapidly, either by catabolism or by delivery to fat depots. The latter destination of the fat before deiodination has occurred seems unlikely in view of the continuously negligible lack of specific activity of mesenteric fat in five successive experiments. One may hazard the inference that fat depots cannot store any but structurally normal fat or that fat for which there is current need does not participate in the metabolic activity of fat depots. The latter possibility is unlikely in view of Schoenheimer and Rittenberg's study of fat labelled with deuterium (25).

The excretion of radioactivity into the urine, beginning within one hour after injection, represents ionic iodine liberated continuously over a period of several days.

CONCLUSIONS

An emulsion of radioactive iodinated fat injected intravenously in dogs and mice leaves the circulation rapidly.

Approximately 60% of the radioactivity still present in the circulation 24 hours after injection is contained in the lipoid fraction of the plasma and 20% in the ionic iodine fraction.

Immediately after injection, the highest concentration of radioactivity in the tissues of dogs is found in the spleen, lung and liver, in that order, and decreases slowly. In tissues of mice the concentration of radioactivity immediately after injection is highest in the lungs. There it drops rapidly, while the concentration of radioactivity in the spleen increases rapidly and remains high for six to eight days after injection.

Iodine is gradually and slowly liberated from the iodinated fat and excreted. Iodinated fat is not stored in mesenteric fat depots.

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