A method for the determination of free ^{131}I in radio-iodinated lipids

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SYNOPSIS Experiments are described showing that an anion exchange resin may be used as a simple means of determining the amount of free radio-activity in emulsions of triolein and oleic acid labelled with ^{131}I in the form of ^{131}ICl . In the case of radio-iodinated triolein no special modifications are necessary. With radio-iodinated oleic acid, the working solution is made up with an excess of unlabelled oleic acid emulsion to prevent uptake of bound radio-activity by the resin. Recovery experiments show the method to be approximately 90% efficient, which is adequate when the radio-lipids are to be used for clinical purposes.

In the last 10 years increasing use has been made of radio-iodinated lipids in the diagnosis of steatorrhoea and the investigation of related disorders. The materials used are radio-iodinated triolein and radio-iodinated oleic acid. A measured dose of the radio-lipid is given by mouth, and the percentage of the dose appearing in the plasma or excreted in the faeces is determined. It is clearly essential that the administered dose should not contain any significant proportion of free ^{131}I . Two methods have been reported for the determination of free ^{131}I in radio-lipids; Cheers, McCraw, Tyor, and Ruffin (1958) dialysed radio-iodinated triolein to separate free ^{131}I from bound ^{131}I ; Beres, Wenger, and Kirsner (1957) and Balint, Pendower, and Ramsey (1960) dissolved it in a fat solvent and extracted free ^{131}I into water. These methods do not lend themselves to frequent and rapid determinations.

Scott and Reilly (1954) and Slade (1956) have described methods for the determination of serum protein-bound ^{131}I in thyroid tracer studies using an anion exchange resin. The principle of their method is the measurement of plasma radio-activity before and after treatment of the sample with an ion exchange resin which selectively removes unbound ^{131}I in the anionic form. Employing the principles used in these studies, a new method for the determination of free ^{131}I in radio-iodinated lipids is described. Since the technical details were found to be of material importance, the development of the method is given in full.

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DEVELOPMENT OF THE METHOD

TECHNICAL DATA All measurements of activity were made on a well-type scintillation counter, with a thallium-activated sodium iodide crystal, linked to an automatic scaler. The polythene liquid source containers were of the annular well type; they will be referred to as 'cups' for the sake of brevity. Calculations were based on net counts per second. All cups were counted in duplicate and a minimum of 2,500 counts registered. The majority of samples gave a net count of over 50 times background.

The radio-lipids were used as emulsions prepared by shaking the labelled oil vigorously with water and Tween 80 in a stoppered tube. In this way it was possible to minimize the radiation hazards inherent in the repeated handling of radio-active materials dissolved in volatile fat solvents. The use of working solutions in aqueous form greatly facilitated the experiments and none of the results suggested that emulsification affected the conclusions. In the majority of experiments a volume of 60 ml. was used.

The resin used throughout was Amberlite IRA-400 in the chloride form¹. This is a particulate anion exchanger with a cross-linked polystyrene matrix and has strongly basic characters due to the quaternary ammonium group. It has a total exchange capacity of 1.2 mg. equivalent per ml. of resin.

LABORATORY PROCEDURES Two unexpected difficulties were encountered. First, a fully stable emulsion could not be prepared despite many attempts with the active oil and Tween 80 in differing proportions. Secondly, the cups were found to retain a high percentage of the activity of the emulsions after thorough washing.

¹ Rohm and Haas Co.

FIG. 1. Comparison of the efficiency of the resin in removing Na^{131}I from water and from a 5% Teepol solution in water. Two minutes' treatment with resin removes 99% of the radio-activity from water. Four minutes' treatment with resin removes less than 50% of the radio-activity from Teepol solution.

If the emulsions were left to stand in a test-tube, two layers were seen to develop in a few minutes and made pipetting of aliquots of equal activity difficult. The problem was partially overcome by ordering a large batch of suitable pipettes made to a standard specification and by ensuring that they were thoroughly washed. Acceptable accuracy was achieved by meticulous attention to pipetting technique combined with shaking the test emulsion by hand before pipetting each aliquot.

The instability of the emulsions caused a steady fall in the counts registered in the cups, when activity was successively measured over a short period and was far greater than might be explained by natural decay of the isotope as a simple Na^{131}I standard counted at the same time showed no such change. The difficulty was overcome by counting during relatively short periods and using emulsions of relatively high activity. Each cup was shaken before each count, using surgical finger cots as watertight seals.

The problem of heavy residual cup contamination was

considered important. Low residual contamination would permit a rapid turnover of cups by reducing the time required for natural decay. Thorough washing with water, detergents, or fat solvents such as petroleum ether, carbon tetrachloride, or chloroform made no substantial difference to contamination. Contamination was markedly reduced by adding to the emulsions a small volume of the water-soluble detergent Teepol² but unfortunately this device led to erratic results, which were explained by the finding that Teepol inhibits the uptake of Na^{131}I by the resin (Fig. 1). The cups were therefore prepared in advance with 1 ml. of Teepol from a burette, so utilizing the property of Teepol to reduce contamination and avoiding its effect on the resin uptake of free ^{131}I from solution. The residual contamination, with and without the use of Teepol, was compared (Table I). Teepol was effective in keeping contamination to a level of 1% of the original activity and also assisted in keeping the counting rate constant.

TABLE I

EFFECT OF TEEPOL ON RESIDUAL CUP CONTAMINATION IN CUPS USED FOR RADIO-IODINATED LIPIDS

Treatment of Cups	Residual Activity in Cups as % of Original Activity
Without Teepol	15.6 ¹
With Teepol	0.7

¹Values are means of four experiments

TESTS OF VALIDITY The proposed method involved the measurement of the activity of the radio-lipid under test before and after treatment with the resin. By this means, it may be anticipated that any fall in the activity after resin treatment would represent the amount of free ^{131}I in the ionic state. The procedure must meet three requirements: 1 Sufficient resin must be used to remove the amount of free ^{131}I likely to be encountered; 2 the resin must not affect the activity of the labelled lipids; 3 the resin should remove unbound ^{131}I from the labelled lipids with acceptable efficiency and a substantial proportion must therefore be in the anionic state.

Amount of resin required to remove ^{131}I Three aqueous solutions of Na^{131}I were made up. The first contained an amount of activity equal to that used in subsequent experiments (approximately 100 c.p.s./9 ml. aliquot); the second the same amount of Na^{131}I and

TABLE II
EFFICIENCY OF ^{131}I REMOVAL USING 3 g. RESIN

	Time Treated with Resin (min.)	Iodide Content of Aqueous Solutions		
		Na^{131}I (100 c.p.s.)	$\text{Na}^{131}\text{I} + 1 \text{ mg. KI}$ (100 c.p.s.)	Na^{131}I (1,000 c.p.s.)
Activity of aliquots	0	100	100	100
(activity of starting solution taken as 100%)	1	3.2	1.7	0.9
	2	0.9	0.4	0.4
	3	0.9	0.4	0.3
	4	0.7	0.4	0.3

All values are means of three experiments

²Shell Chemical Company Ltd.

1 mg. of carrier KI to increase the total iodide content several hundred fold; the third 10 times the usual amount of activity (approximately 1,000 c.p.s./9 ml. aliquot). After measuring the starting activity, each solution was shaken with 3 g. of resin and aliquots were taken at one-minute intervals for four minutes. In every test, over 99% of the original activity was removed after two minutes' treatment with the resin (Table II). Therefore 3 g. of resin was sufficient to remove much more than the amounts of ^{131}I to be used in later experiments. It was not necessary to measure the resin precisely and a simple spoon spatula was used.

Effect of the resin on activity of radio-lipids Emulsions of radio-iodinated triolein and radio-iodinated oleic acid were made up. Aliquots were first taken to measure the starting activity; resin was added and shaken with the active emulsions for six minutes, aliquots being taken for activity readings at one-minute intervals. The results (Table III) show that the activity of the triolein emulsion did not change, whereas the oleic acid emulsion showed an appreciable fall in activity over the period of the test, showing that the method required modification if it was to be applied to the measurement of free ^{131}I in radio-iodinated oleic acid. The experiments were repeated except that the solutions of radio-lipids were made up with unlabelled oleic acid emulsified with Tween 80 and an equal volume of water giving a concentration of unlabelled oleate radicals grossly in excess of the concentration of labelled radicals. Under these conditions, the activity of both radio-iodinated oleic acid and triolein remained unaltered when treated with the resin (Table IV). The method can therefore be applied to radio-iodinated oleic acid, provided the solution is made up with an excess of unlabelled oleic acid emulsion.

A third group of experiments was performed to show

that increased amounts of resin did not alter the activity of the radio-lipids. After starting activity had been measured, the emulsion was shaken with the standard amount of resin (3 g.) and an aliquot taken after two minutes. A further 3 g. of resin was then added and aliquots taken again after shaking for two and four minutes. Only small changes in the activity of these aliquots were detected, except where radio-iodinated oleic acid was tested in the absence of excess unlabelled oleic acid emulsion (Table V).

RECOVERY TESTS In three groups of solutions, recovery of ^{131}I in two different chemical forms was measured. The first was ^{131}I in the ionic form as an aqueous solution of Na^{131}I . The second was ^{131}I prepared as iodine monochloride (^{131}ICl), which is the standard label for radio-iodinated lipids. The method of preparation of ^{131}ICl was the same as described by Lubran and Pearson (1958) for the radio-iodination of triolein, except that it was performed in aqueous solution. No more than 0.5 ml. of Na^{131}I or ^{131}ICl was used. Since this was added to a volume of 60 to 70 ml., the dilution effect has been ignored in the calculations.

1 Recovery from unlabelled solutions Na^{131}I or ^{131}ICl were added to water, emulsified triolein, and emulsified oleic acid. Where the recovery of ^{131}I from the emulsified fats was being measured, the unsaturated double bonds were previously blocked with carrier ICl to prevent uptake of active ^{131}ICl during the test. An aliquot was taken to measure the starting activity in each case. The solution was then shaken with the resin and aliquots taken at one-minute intervals for four minutes. In all instances, the resin removed over 98% of the original activity (Table VI) and the results show that no significant amounts of activity are removed after two

TABLE III
EFFECT OF RESIN ON ACTIVITY OF LABELLED TRIOLEIN AND OLEIC ACID
EMULSIONS WITHOUT CARRIER OLEIC ACID

	Activity of Aliquots as % of Starting Activity						
	Time Treated with Resin (min.)						
	0	1	2	3	4	5	6
Emulsion of labelled triolein	100.0	100.7	102.1	102.1	100.3	102.0	102.3
Emulsion of labelled oleic acid	100.0	94.3	90.0	85.7	82.0	79.7	77.0

All values are means of three experiments

TABLE IV
EFFECT OF RESIN ON ACTIVITY OF LABELLED TRIOLEIN AND OLEIC ACID
EMULSIONS WITH CARRIER OLEIC ACID

	Activity of Aliquots as % of Starting Activity						
	Time Treated with Resin (min.)						
	0	1	2	3	4	5	6
Emulsion of labelled triolein with emulsion of unlabelled oleic acid	100.0	99.1	99.7	98.5	99.5	100.1	102.3
Emulsion of labelled oleic acid with emulsion of unlabelled oleic acid	100.0	101.0	99.8	99.8	100.2	100.8	99.7

All values are means of three experiments

TABLE V

EFFECT OF EXTRA RESIN ON ACTIVITY OF LABELLED TRIOLEIN AND OLEIC ACID

Activity of Aliquots as % of Starting Activity

	Starting Activity	Treated with 3 g. Resin	Treated with 3 g. Extra Resin	
		2 Min.	2 Min.	4 Min.
Labelled triolein emulsion	100	96.6	93.8	96.4
Labelled triolein + emulsion of unlabelled oleic acid	100	98.9	98.4	102.5
Labelled oleic acid emulsion	100	93.7	84.0	77.4
Labelled oleic acid + emulsion of unlabelled oleic acid	100	97.7	96.6	96.3

All values are means of three experiments

TABLE VI

RECOVERY OF ^{131}I FROM DIFFERENT SOLUTIONS TREATED FOR TWO MINUTES WITH 3 g. RESIN

<i>Solutions Tested</i>	<i>% Recovery of ¹³¹I as Na ¹³¹I</i>	<i>% Recovery of ¹³¹I as ¹³¹ICl</i>	
<i>Unlabelled Solutions</i>			
Water	99.6	99.6	
Emulsion of triolein	99.8	98.9	
Emulsion of oleic acid	99.2	98.8	
<i>Emulsions of Labelled Lipids</i>			
Labelled triolein	96.2	88.7	
Labelled oleic acid	109.3	105.2	
Labelled oleic acid + unlabelled oleic acid	99.0	91.9	
<i>Labelled Triolein with Intestinal Secretions</i>			
Emulsion of labelled triolein with unlabelled oleic acid	+ { 1 ml. gastric juice	100.6	91.3
	1 ml. duodenal juice	101.6	93.9
	1 ml. faeces	99.3	94.9

All values are means of at least three experiments

minutes' contact with the resin. These experiments show that the resin removes ^{131}I satisfactorily from emulsified lipids and that two minutes' treatment with the resin is sufficient.

2 Recovery from labelled lipids Emulsions of radio-iodinated triolein, radio-iodinated oleic acid, and the labelled oleic acid in an excess of unlabelled oleic acid emulsion were prepared. The emulsion of unlabelled oleic acid was made with oleic acid and an equal volume of water, using Tween 80 as the emulsifying agent. Aliquots were taken for measurement of the starting activity. Na^{131}I or ^{131}ICl was added and the activity in aliquots again recorded, the increase in net counts per unit volume representing the amount of added activity. The emulsions were then shaken for two minutes with resin and the activity in the remaining solution measured.

The fall in net counts registered per unit volume represents the amount of activity removed by the resin and was expressed as a percentage of the added activity (Table VI). In the absence of unlabelled oleic acid emulsion, the recovery from radio-iodinated oleic acid exceeded 100%, reflecting the previous finding that the resin lowers the activity of a radio-iodinated oleic acid emulsion under these conditions. In the other emulsions, the recovery of Na^{131}I was very close to 100% and of ^{131}ICl approximately 90%.

Recovery from radio-iodinated triolein in intestinal secretions These experiments were performed to assess the validity of the method when applied to the study of the stability of the radio-iodinated triolein bond in the

alimentary tract. Emulsions of radio-iodinated triolein were made containing unlabelled oleic acid emulsion and 1 ml. of gastric juice, duodenal juice, or homogenized faeces. The unlabelled oleic acid emulsion was used because of the possibility of converting radio-iodinated triolein to oleic acid, especially in the presence of the digestive enzymes of duodenal juice. The recovery experiments were carried out exactly as described in the previous section. The results showed that recovery of Na^{131}I was near to 100% and of ^{131}ICl approximately 90% (Table VI).

METHOD ADOPTED

1 One millilitre of Teepol is placed in each cup from a burette.

2 A sample of suitable activity of the radio-lipid, usually 2 to 4 drops measured with a Pasteur pipette, is placed in a stoppered tube; approximately half its volume of Tween 80 is added, again using a Pasteur pipette. With radio-iodinated triolein this is made into an emulsion of about 60 ml. by shaking vigorously with water. In the case of radio-iodinated oleic acid, the mixture of oil and Tween 80 is first emulsified with a few millilitres of water and then made up to about 60 ml. using an emulsion of unlabelled oleic acid. This is prepared in a mechanical emulsifier with equal volumes of water and oleic acid, and one quarter of this volume of Tween 80.

3 Two 9 ml. aliquots of the solution are pipetted into cups.

4 Approximately 3 g. of resin is added to the remaining solution and the mixture shaken for two minutes.

5 The resin is allowed to settle and the supernatant poured into a fresh stoppered tube, taking care to avoid transfer of resin.

6 Two 9 ml. aliquots of the poured solution are pipetted into cups.

7 Surgical finger cots are stretched over the cups to act as watertight seals.

8 The cups are counted in a scintillation counter and the percentage of free ^{131}I calculated as follows:

$$\% \text{ free } ^{131}\text{I} = \frac{\text{activity of pre-resin aliquots} - \text{activity of post-resin aliquots}}{\text{activity of pre-resin aliquots}} \times 100$$

(where the activities are expressed as net counts per unit time).

DISCUSSION

The purchase of commercially labelled radio-lipids is expensive, and Lubran and Pearson (1958) have published a tagging procedure which can be performed quickly and at negligible cost. The purpose of the study reported here was to establish a simple and practical means of ensuring that batches of radio-iodinated triolein and oleic acid labelled locally do not contain significant amounts of unbound radio-activity, which would invalidate their use. Chromatography, dialysis, and the separation of bound activity by ether extraction are relatively cumbersome procedures, which do not recommend themselves for routine use. The properties of an anion exchange resin suggested the possibility of a rapid and effective procedure.

In seeking to establish the validity of the method, the effect of the resin on radio-iodinated triolein and oleic acid was studied. Whereas the resin did not affect the activity of radio-iodinated triolein emulsions, treatment with resin caused a steady fall in the activity of radio-iodinated oleic acid emulsions. This may be attributed either to uptake of the anionic radio-iodinated oleate radicals or to disruption of the radio-iodinated oleic acid bond. The finding that the activity of the radio-iodinated oleic acid emulsion remained unchanged in the presence of excess unlabelled oleic acid emulsion suggests that the former is the more likely explanation but does not provide conclusive evidence. Nevertheless it provides a simple and effective means whereby the method may be applied to the measurement of free ^{131}I in radio-iodinated oleic acid.

The next stage in the study was a series of experiments designed to measure the efficiency of recovery of free ^{131}I . With ^{131}I as Na^{131}I the recoveries were uniformly satisfactory and of the order of 100%. This is scarcely surprising, since Na^{131}I is an electrovalent salt with the halogen indisputably in the anionic form and therefore suitable for uptake by an anion exchanger.

However, in the labelling of lipids, ^{131}I is custo-

marily used in the form of iodine monochloride (^{131}ICl) which belongs to the interhalogen group of substances. In related studies by other workers, no account has been taken of the properties of these compounds whose behaviour remains to be fully elucidated. Of particular relevance to the use of an anion exchanger is the fact that the interhalogens are generally covalent compounds, although ICl possesses greater ionic character than most other members of the group (Moeller, 1952).

While a detailed discussion of the chemistry of ICl would be inappropriate in this brief communication, a summary of relevant knowledge is called for. Particular attention should be paid to the behaviour of ICl in water, since the procedure is performed in aqueous solution. In electrolysis of an aqueous solution of ICl , no iodine appears at the cathode, showing that it is certainly not in the cationic form, which would invalidate the method (Sidgwick, 1950). As a result of its instability in water, ICl dissociates and gives rise to a complex series of ions, which may include, H^+ , Cl^- , OI^- , IO_3^- , and possibly I_3^- and ICl_2^- , as well as undissociated iodine and iodine-containing compounds. The presence of the latter indicates that at least part of the unbound ^{131}I is unlikely to be available for removal by the resin, although the precise amount is uncertain. The ^{131}I is otherwise present in the anionic form and will presumably be fixed by the resin, which is capable of removing all anions from solutions in a pH range of 1 to 10.

The aqueous solution of ^{131}ICl used in our recovery experiments cannot be accepted as a pure solution of the interhalogen, partly because of its dissociation in water and partly because the preparation would favour the formation of iodate. However, it may be taken as a reasonable approximation to the state of unbound ^{131}I in radio-lipids emulsified in water, since it was made in the same way as the radio-iodinating agent used in the labelling of lipids. With this preparation the recovery experiments gave slightly less satisfactory results than the solution of Na^{131}I . In both radio-iodinated triolein and radio-iodinated oleic acid with an excess of unlabelled oleic acid the recoveries were of the order of 90%.

These studies indicate that the method provides a simple means of assessing the amount of unbound ^{131}I in radio-lipids. While it is possible that the method fails to measure the precise amount, there is no doubt that only unbound ^{131}I is removed by the resin. Since any appreciable quantity of free ^{131}I in a batch of a radio-lipid renders it unsuitable for use, the exact amount is unimportant. In these circumstances our practice is to dissolve the labelled oil in ether and wash it with a solution of sodium thiosulphate and potassium iodide to remove free ^{131}I .

The recovery experiments, in which small amounts of intestinal secretions were included in the solutions, show that the method is equally valid under these conditions. The procedure lends itself to frequent repetition and therefore provides a simple means of studying the behaviour of the radio-iodinated lipid bond in intestinal secretions. This is the subject of a separate report (Cox and Hinchliffe, 1961).

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