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Role of Phospholipids in Iodine Binding* (33373)

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Nonspecific binding between halides and phospholipids was first reported by Phillips *et al.* (1) who observed that most of the fluoride present in egg yolk was associated with acetone-insoluble lipids, after alcohol extraction. Christensen and Corley (2) later reported that a portion of bromide administered to animals, or added *in vitro* as sodium bromide to lipid solutions, could be recovered in the phospholipid fraction, and that this was true with sodium chloride or sodium iodide. Vilkki (3) demonstrated the presence in the thyroid of lecithin-like material that could reversibly bind iodide ion *in vitro*. Schneider and Wolff (4) further studied the problem to show that the iodide-concentrating effect was not merely one of nonspecific anion binding to a positively charged phospholipid species soluble in nonpolar solvents. These authors as well as Vilkki (5) have shown that the iodide-concentrating effect is not shared by synthetic dipalmitoyl, di-oleyl, or calf brain lecithin.

Our present interest in this subject was stimulated by a chance observation of high radioactivity in serum phospholipids isolated from a thyroid cancer patient given a therapeutic dose of $Na^{131}I$. Such a phospholipid-iodide complex formed *in vivo* has not been documented and the present study was undertaken to examine both *in vivo* and *in vitro* formation of a phospholipid-iodide complex.

Materials and Methods. In vivo *studies.* Albino rats weighing 200–300 g were given an oral dose of $Na^{131}I$ (50–60 μCi $Na^{131}I$, carrier-free) and sacrificed after 48 hr. Livers were perfused with 0.9% NaCl via the portal vein to remove blood from the liver tissue. They were then excised and washed thoroughly in tap water before extracting the lipids.

To study the incorporation of radioactivity in the thyroid, liver, and blood plasma, larger animals, such as rabbits, were used. Rabbits weighing 2.5–3.5 kg were given an intraperitoneal injection of $Na^{131}I$ (4–5 μCi) and sacrificed 24 hr later. Blood was collected by heart puncture, and the thyroid and

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liver were excised carefully and washed with water. A suitable piece of liver (8–10 g) and the whole of the thyroid gland were used for extracting the lipids. Blood plasma was also obtained for determining plasma lipids.

Two dogs were given an intravenous dose of $Na^{131}I$ (100 μCi), and serial 20-ml samples of blood were collected at intervals up to six hr. Plasma was separated and lipids were extracted.

In vitro *study. Rat liver homogenate.* Freshly excised rat livers were homogenized in Ringer phosphate buffer pH 7.4 (6) and incubated with $Na^{131}I$ (0.1 μCi).

Rat liver slices. Rat liver slices (6) were substituted for the homogenate and incubated with $Na^{131}I$ with and without added glucose. In all cases lipids were extracted and purified by the Folch method (7). Fractionation of total lipid was accomplished by silicic acid chromatography as described in a previous publication (8). Further fractionation of the total phospholipids was carried out using DEAE cellulose chromatography followed by silicic acid-silicate-water column chromatography (9). Thin-layer chromatographic separations, both for identification and preparative purposes, were carried out using the method suggested by Vogel *et al.* (10). A trichloracetic acid–precipitated fraction from tissue homogenate was washed several times to determine protein-bound iodide.

In order to study the nature of the lecithin-iodide complex, pure lecithin samples were isolated from liver, thyroid, and brain tissue by the above methods. Two procedures were used:

Procedure I. An aqueous solution of $Na^{131}I$ (0.1–0.5 ml, containing 0.01–0.1 μCi of $Na^{131}I$, carrier-free) was added to 5 ml of chloroform containing 10–30 mg of TLC-pure lecithin. Sufficient methanol was added to make the solution homogenous and it was shaken on a mechanical shaker for one hr. Water was added to separate the chloroform layer, and the mixture was shaken gently and centrifuged. The aqueous layer was aspirated out into a measuring cylinder, and the chloroform layer was washed several times with water. Usually the fifth washing was almost free from radioactivity. The chloroform layer was dried with anhydrous sodium sulfate and transferred to a counting vial. The volume of the washings was noted, and an aliquot was used for counting the radioactivity.

Procedure II. A dialyzing tube was suspended in a large beaker containing 2000 ml of an aqueous solution of 3 μCi of $Na^{131}I$. Pure lecithin (5–7 mg) dissolved in chloroform was quantitatively transferred to the dialyzing tube and sufficient $NaHSO_3$ was added to the water to make a concentration of 0.1%. Dialysis was continued for 16–18 hr with constant stirring and the dialyzate was emptied into a counting vial and washed twice with water before determining the radioactivity.

In order to test the involvement of metal ions in the formation of the lecithin-iodide complex, ferric chloride ($FeCl_3 \cdot 6H_2O$), manganese chloride ($MnCl_2 \cdot 4H_20$), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2 \cdot 6H_20$) were used in both the procedures. Usually 0.1 ml of a 5% methanolic solution of salts was used. Chelating agents, such as EDTA and sodium citrate, were also used (1 ml of 1% solution).

All counting of radioactivity was done with a Nuclear Chicago automatic scintillation counter equipped with a 3 $\times$ 3-in. thalium-activated sodium iodide well-type crystal, connected to a scaler and a printer.

Results. Figure 1 shows the distribution of radioactivity in liver lipids of rats given an oral dose of $Na^{131}I$. Neutral lipids had negligible radioactivity and lecithin had the major portion of the phospholipid activity. For this reason much of the *in vitro* work was done with pure lecithin.

Table I shows the distribution of radioactivity in the rabbit thyroid, liver, and blood plasma. It was noted that liver lipids had higher radioactivity than those of thyroid or blood plasma.

In the dog, after an intravenous injection of $Na^{131}I$, the blood plasma lipids, neither in the early period of 15 min nor as late as 6 hr, exhibited any appreciable radioactivity, indicating that plasma lecithin was not involved as a carrier of iodide in the blood.

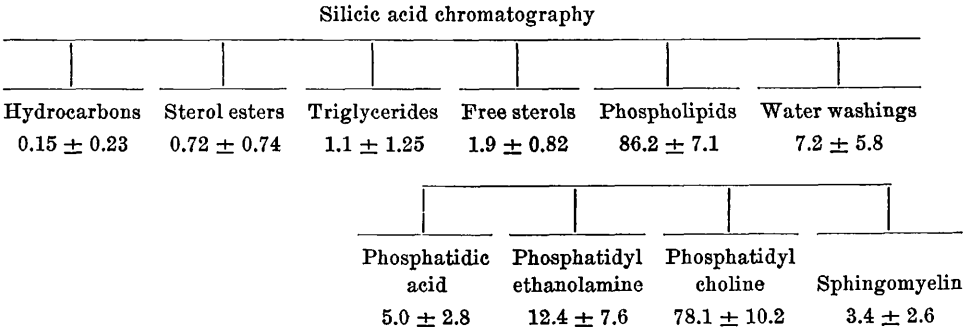

FIG. 1. Distribution of radioactivity in liver lipids. Liver total lipids, 157,300 ± 10,500 cpm/100 mg; after washing, 93,862 ± 4,097 cpm/100 mg. Figures represent percentage of the total radioactivity. Each experiment involved pooled livers of two rats. Values represent average of five experiments.

The homogenate, as well as intact liver slices, failed to show any enzymatic uptake of radioiodide associated with lipids even when supplemented with glucose or a hydrogen peroxide generating system. The boiled preparations gave results identical to those with the active experimental samples.

The effects of mild alkaline hydrolysis (11) or enzymatic cleavage of lecithin iodide complex formed *in vitro* by phospholipase C or D (12, 13), washing by $AgNO_3$ solution, and hydrogenation of lecithin prior to formation of the iodide complex, are shown in Table II. It was noted that hydrolyses by any method led to loss of radioactivity from the lecithin-iodide complex, indicating that the entire phosphatidyl choline structure was essential for the formation of the complex. Washing with silver nitrate had very little effect, and hydrogenation of lecithin before making the complex reduced the ability to form iodide complex by about 50%.

Table III shows the effect of $FeCl_3$ on the ability of the lecithin isolated from various sources to form an iodide complex. In all cases addition of $FeCl_3$ markedly increased the ability of the lecithin to form the complex.

Table IV shows the effect of various metal salts, increasing amounts of ferric chloride, and chelating agents on the ability of lecithin to bind iodide. It was noted that Mn^{2+}, Mg^{2+} or Ca^{2+} could not substitute for Fe^{3+} ions. Increasing amounts of $FeCl_3$ resulted in greater association of the radioiodide with lecithin. The effect of metal ions can be prevented by use of such chelating agents as EDTA and sodium citrate.

Of the various lipid components of thyroid tissue studied, lecithin seems to have the highest capacity to form an iodide complex in the presence of $FeCl_3$ (Table V).

Finally, samples of pure lecithin isolated from three different sources were used for

TABLE I. Distribution of Radioactivity in the Blood Plasma, Thyroid, and Liver of Rabbits Given an Intraperitoneal Dose of $Na^{131}I$ and Sacrificed 24 Hr Later.

Percentage of total radioactivity recovered								
Thyroid			Liver			Plasma		
Protein-bound ^{131}I	Free iodide ^{131}I	Lipid-bound ^{131}I	Protein-bound ^{131}I	Free iodide ^{131}I	Lipid-bound ^{131}I	Protein-bound ^{131}I	Free iodide ^{131}I	Lipid-bound ^{131}I
93 ± 6	6 ± 5	0.2 ± 0.3	48 ± 29	43 ± 33	9 ± 6	36 ± 35	62 ± 36	1.6 ± 1.5

TABLE II. Effect of Hydrolysis and Other Procedures on the Lecithin-Iodide Complex.

Experimental procedure	Results	Remarks
Mild alkaline hydrolysis	85–90% radioactivity is located in the water-soluble fraction	Radioiodine not associated with fatty acids or the whole intact lecithin structure is needed for the formation of the iodide complex
Hydrolysis by phospholipase C	74–97% radioactivity is located in the water-soluble fraction	Radioiodine is associated with choline phosphate or the whole intact lecithin structure is needed for the formation of the iodide complex
Hydrolysis by phospholipase D	86–92% radioactivity is located in the water-soluble fraction	Same as above
Washing with 1% $AgNO_3$ solution	Approximately 10% of activity is removed in the wash	Evidence against loose association of lecithin and iodide
Hydrogenation of lecithin before making complex	Reduces effective iodide association by 50–60%	Unsaturated fatty acids seem to be important for the formation of the complex
Saturation of lecithin with carrier NaI followed by shaking with $Na^{131}I$	Negligible radioactivity associated with lecithin	Nonreversible association with iodide
Sphingomyelin, phosphatidyl serine, triglycerides, free fatty acids + $Na^{131}I$	Negligible radioactivity retained by any component	Complex must depend on entire phosphatidyl choline structure

TABLE III. Effect of $FeCl_3$ on Retention of ^{131}I by Lecithin.[a]

	Procedure I (see text)		Procedure II (see text)	
	Lecithin after washings (cpm)	Total radioactivity recovered (cpm)	Radioactivity of dialyzate after washing (cpm)	Radioactivity in dialyzing fluid (cpm/10 ml)
Synthetic dipalmitoyl lecithin + $Na^{131}I$	1868	331,159	121	7673
Same + $FeCl_3$ + $Na^{131}I$	14,014	304,052	11,899	7413
Egg lecithin + $Na^{131}I$	17,569	320,128	387	7115
Same + $FeCl_3$ + $Na^{131}I$	156,145	341,258	2948	6762
Dog-thyroid lecithin + $Na^{131}I$	197,333	324,042	98	7673
Same + $FeCl_3$ + $Na^{131}I$	261,797	335,703	16,216	7413
Rat-liver lecithin + $Na^{131}I$	8519	273,422	141	7673
Same + $FeCl_3$ + $Na^{131}I$	49,550	286,464	8064	7413
Vegetable lecithin[b] + $Na^{131}I$	147	273,729	—	—
Same + $FeCl_3$ + $Na^{131}I$	31,984	399,038	—	—
Beef-brain lecithin + $Na^{131}I$	—	—	131	7673
Same + $FeCl_3$ + $Na^{131}I$	—	—	22,036	7413
Beef-liver lecithin + $Na^{131}I$	—	—	74	6841
Same + $FeCl_3$ + $Na^{131}I$	—	—	26,480	6043

[a] Figures represent mean of three experiments in duplicate samples.

[b] Purified by chromatography from crude obtained from Mann Research Laboratories, New York.

TABLE IV. Effect Various Metal Salts, Increasing Amounts of Iron, and Chelating Agents on the Formation of Lecithin Iodide Complex.[a]

	Procedure I (see text)		Procedure II (see text)	
	Lecithin after washing (cpm)	Total radioactivity recovered (cpm)	Radioactivity of dialyzate after washing (cpm)	Radioactivity in dialyzing fluid (cpm/10 ml)
Lecithin + $Na^{131}I$ only	22	137,803	285	7024
Idem + $FeCl_3$	88,756	146,270	4893	6906
+ $MnCl_2$	—	—	265	6766
+ $CaCl_2$	164	139,935	98	9280
+ $MgCl_2$	61	139,383	74	11171
+ $FeCl_3$ + EDTA	—	—	110	6137
+ $FeCl_3$ + Na citrate	—	—	2933	7056
+ 50 μg $FeCl_3$	21,455	277,033	—	—
+ 100 μg $FeCl_3$	40,423	276,699	94	7020
+ 200 μg $FeCl_3$	72,598	268,343	111	7016
+ 400 μg $FeCl_3$	—	—	146	7356

[a] Figures represent mean of two experiments done in triplicate.

preparing lecithin iodide complexes. Nonradioactive NaI, $NaHSO_3$, and $FeCl_3$ were used in experimental samples whereas $FeCl_3$ was omitted in the controls. The complexes were then analyzed for Fe and I by X-ray fluorescence spectrometry. It can be seen from Table VI that in the absence of $FeCl_3$ there was negligible iodide associated with lecithins and that the ratio of Fe to I was fairly constant in the three samples analyzed. The complex also contained fairly constant amounts of sulfur, presumably from the sodium sulfite used in the process.

TABLE V. Radioiodine Uptake by Various Lipids after Dialyzing in Water Containing $Na^{131}I$.

	Radioactivity in dialyzing fluid (cpm/10 ml)	Radioactivity of dialyzate after washing (cpm/mg)
Thyroid lecithin	7673	48
+ $FeCl_3$	7413	7949
Thyroid sphingomyelin	9538	32
+ $FeCl_3$	9471	3435
Thyroid phosphotidyl ethanolamine	9538	18
+ $FeCl_3$	9471	2200
Thyroid triglycerides	9538	11
+ $FeCl_3$	9471	140

In order to check whether sulfur was part of the complex, TLC-pure lecithin was mixed with $Na^{131}I$ in the presence of $FeCl_3$ with and without $NaHSO_3$. It was found that lecithin retained more radioactivity in the absence of $NaHSO_3$ after repeated washings (Procedure I). Use of ascorbate instead of $NaHSO_3$ as a reducing agent resulted in further decrease in the radioactivity retained by lecithin. Thus it seems that sulfur does not contribute directly to the formation of the complex and that Fe^{3+} seem to form a better complex than Fe^{2+}.

Discussion. Figure 1 clearly shows that the major portion of radioiodide is associated with the liver lecithin fraction. For this reason most of the later experiments involved this lipid. It may be noted here that this is the first time association of radioiodide with lecithin has been shown in the whole animal since most of the previous work was done *in vitro* (3–5). Although the total radioactivity in the rabbit thyroid gland was in great excess to that of liver or serum, the radioactivity associated with lipids of the thyroid or the serum, was considerably lower than that associated with liver lipids (Table I). As expected, most of the radioiodide in the thyroid was protein bound. It is quite possible that the affinity of thyroid proteins for iodide is

TABLE VI. X-Ray Fluorescence Analysis of Lecithin-Iron-Iodide Complex.

	Iron (ppm)	Iodine (ppm)	Phosphorus (ppm)	Atom ratio P/Fe	Atom ratio Fe/I
Beef-liver lecithin + NaI	46	8	36,200	—	—
+ $FeCl_3$	12,200	11,950	32,600	2.67	2.32
Egg lecithin + NaI	73	0	38,100	—	—
+ $FeCl_3$	13,550	17,425	29,100	2.15	1.76
Brain lecithin + NaI	73	114	33,600	—	—
+ $FeCl_3$	13,880	16,080	36,400	2.62	1.96
Av				2.48	2.01

so great that the lipids of this tissue cannot compete. Since lecithin is a major phospholipid of blood plasma, there is a possibility that it acted as a carrier of iodide. However, it was found that even during the initial periods, after an intravenous dose of $Na^{131}I$, plasma lecithin never exhibited significant activity. The major portion of the activity was either free or protein bound.

In the *in vitro* experiments no enzymatic uptake of radioiodide either by liver slices or homogenates could be seen even in the presence of a hydrogen peroxide generating system (14). Since no enzymatic process seemed to be involved in the formation of lecithin-iodide complex, experiments were undertaken to study the nonenzymatic iodide binding *in vitro*.

Pure lecithin, dissolved in chloroform and shaken with $Na^{131}I$ in the presence of $NaHSO_3$ (to prevent oxidation of iodide), formed a complex. The radioactivity could not be washed out even with repeated water washing. This complex, when subjected to mild alkaline or enzymatic hydrolyses lost all the radioactivity to the water-soluble portion, which suggests that the hydrocarbon chains of the fatty acids are probably not directly involved. However, hydrogenation of lecithin did affect its ability to form the iodide complex, probably by alteration of the structural configuration of the lecithin molecule that seems to be involved in the complex formation.

Vilkki and Jaakonmaki (5) have reported requirement of α-acyl-β-nervonic-L-α-lecithin structure (synthetic or reconstituted from natural thyrolecithin) for the *in vitro* iodide-binding effect. However, the present study shows that synthetic dipalmitoyl lecithin will bind iodide in the presence of iron; also, liver lecithin containing no detectable nervonic acid (as judged by GLC) formed an iodide complex in the presence of iron.

Although the suggestion by the same authors (5) that once iodide is concentrated in the lecithin of the cell membrane, iodide could be more readily available for further reaction, is an attractive hypothesis, we could not show any iodide uptake by intact liver cells *in vitro* even in the presence of iron. It is possible that this initial concentrating effect is extremely fast and the reaction proceeds ultimately toward the formation of protein-bound iodide resulting in failure to detect radioactivity associated with the lecithin of the cell membrane. Alternately, this could also be due to repeated binding and release cycles as suggested by Wolff (15).

Kimizuka and Koketsu (16) and others (17) have reported the interaction of Ca^{2+} ions with lecithin and sphingomyelin to give 2:1 phospholipid-calcium complexes. Ca, Mg, or Mn lecithin complexes proved to be inactive in their ability to form the iodide complex however, Fe^{3+} ion was consistently found to promote complex formation. Shah and Shulman (17) have proposed a metal complex involving two moles of lecithin bridged by an atom of calcium. We propose a similar complex involving Fe^{3+} ions that has a high affinity for iodide ion. Further, Shah and Schulman (17) pointed out that the free hydroxyl group of sphingomyelin re-

sults in a weaker metal ion complex, which may explain the weaker iodide-concentrating ability of sphingomyelin observed in the present study.

Recently Kiyasu and Duffy (18) suggested the formation of phospholipid micelles in organic solvents, which, they propose, are capable of transporting hydrophilic substances through hydrophobic matrices that are analogous to more specialized carriers in membrane transport. It is possible that lecithin-iron-iodide complex is involved in some such physiological process.

Summary. After an oral dose of $Na^{131}I$ to rats, sacrificed after 48 hr, liver lecithin had maximum radioactivity compared to other phospholipid components; neutral lipids were devoid of any activity. Liver lipids had more activity than thyroid or plasma lipids. Plasma lipids had negligible activity after intravenous injection of $Na^{131}I$ after periods ranging from 15 min to 6 hr. Plasma lecithin does not seem to be a carrier of iodide in the blood. Liver slice or homogenate experiments failed to show any enzymatic process connected with association of lecithin with iodide.

The lecithin iodide complex formed *in vitro*, when degraded by mild alkaline or enzymatic hydrolysis, loses most of the activity to the water-soluble portion rather than to the fatty acids.

Lecithin isolated from different sources will form a strong association with iodide in the presence of Fe^{3+}. Mn^{2+}, Ca^{2+} or Mg^{2+} was ineffective. Further confirmation of this metal ion involvement can be shown by preventing this effect by such chelating agents as EDTA or sodium citrate.

A lecithin iodide complex involving two moles of lecithin and one metal ion, and exhibiting a strong affinity towards iodide is proposed.

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