In Vitro Association of Iodide and Thyroidal Lipids (34840)

ISRAEL POSNER AND LUIS ORDOÑEZ
(Introduced by Piero P. Foà)
Laboratorio de Endocrinología, Hospital Central de las Fuerzas Armadas, Caracas, Venezuela

The active thyroidal transport of iodide is assumed to be carrier-mediated (1). Vilkki (2) found that lecithin of thyroidal origin enhanced the solubility of iodide in a nonpolar organic solvent, equilibrated with an aqueous solution in which the anion was preferentially soluble. On the other hand, lecithins of sources other than the thyroid gland did not work in this system, suggesting that lecithin is a possible physiological iodide carrier. Schneider and Wolff (3) attributed the solubilization of iodide in the organic phase to a specific electrostatic binding of the anion by the lipid, and postulated that the ion was probably in phosphatide micelles formed during the shaking of the organic and aqueous layers. The reversibility of the binding was demonstrated by the ease with which the solubilized ion could be removed from the organic phase by shaking with a new aqueous solution. Dhopeshwarkar *et al.* (4) claimed to have shown that the enhancement of the solubility of iodide by lecithin in an organic solvent could be increased by the addition of ferric ion to the system, and proposed the existence of a lecithin–iron complex involving 2 moles of lecithin and 1 mole of the cation with a strong affinity for iodide. No appropriate control experiments, however, were reported to show the effect of Fe^{3+} on iodide solubility in the absence of lecithin. The purpose of the present studies was to investigate the nature of the lipid–iodide association in the *in vitro* systems described above.

Materials and Methods. Thyroidal lipids of adult male rats were extracted with chloroform:methanol (2:1) and the extracts washed according to a micromodification of the method of Folch *et al.* (5). The lower phases of various extracts were pooled, aliquots were pipetted into test tubes, and the solvent was evaporated to dryness at 45° under a stream of nitrogen.

Partition of iodide between aqueous and organic phases was studied in two manners: (A) 2 ml of chloroform:heptane (1:9) and 2 ml of 0.01 *M* acetate buffer (pH 4.5) containing 1 m*M* methimazole, 1 m*M* iodide, and 0.5 μCi of ^{131}I were added to a series of tubes, some of which contained thyroidal lipids. The tubes were shaken gently for 45 min, centrifuged, and the radioactivity distribution between the organic and aqueous phases was determined (3). (B) Five milliliters of chloroform, 0.1 ml aqueous ^{131}I-solution (0.8 μCi/mmole), and ferric chloride in methanol were added to a series of tubes with or without thyroidal lipids and propylthiouracil (PTU). One milliliter of methanol was added to form a homogeneous solution and the tubes were shaken for 1 hr. Five milliliters of water were added, the tubes shaken again, and the upper phases withdrawn. The lower chloroformic phase was washed three times with water, and the radioactivity distribution between the chloroformic and the combined aqueous phase and washings was determined (4).

Lipid-bound iodine and free iodide were fractionated on Sephadex LH-20 columns, using either chloroform:methanol (2:1) or chloroform:heptane (1:9) as solvents. Hereafter, these columns will be called "C:M-columns" or "C:Hept-columns," respectively. The same solvent system was used for dissolving the samples, swelling the gel, and eluting the columns. After swelling in the appropriate solvent for 48–72 hr, the gel was packed into 9-in. disposable pipettes to a height of 2 cm. Eluates were collected manually in fractions of 30 drops each. Thyroidal lipids were found to elute quantitatively from

C:M-columns in fractions 1–12 (Peak I), while free inorganic iodide was eluted in fractions 15–18 (Peak II). The lipids were eluted from C:Hept-columns in fractions 1–6, whereas free iodide was retained by these columns.

Two-dimensional thin-layer chromatography (TLC) was carried out on Kieselgel G. Chloroform:methanol:water (75:25:4) was used as solvent for the first dimension and *n*-butanol:2 *N* acetic acid (1:1, upper phase), for the second. The TLC plates were sprayed with Rhodamine B, and the spots were viewed under ultraviolet light. Small areas of the chromatograms were scraped off for radioactivity determinations.

Results and Discussion. Partition of iodide between chloroform:heptane and water. The distribution coefficient (*d*) of iodide between chloroform:heptane and aqueous acetate buffer (3) was found to be 0.01 ± 0.01, whereas in the presence of thyroidal lipids, *d* was 0.45 ± 0.09. When aliquots of the organic phase which contained the lipid-bound ^{131}I were filtered throught C:Hept-columns, the label was quantitatively eluted in the first 10 fractions indicating that the radioiodide was intimately associated with the lipids. To test whether the ion was in lipid micelles, the radioactive eluates were pooled and evaporated to dryness under a stream of nitrogen at 45° and then *in vacuo*. Any micelles present should have been disrupted by this treatment. The dried residues were redissolved in chloroform:heptane (1:9) and filtered through C:Hept-columns. In this case, only less than 20% of the label was eluted from the columns, the rest being retained as free iodide. When the dried residues were redissolved in chloroform:methanol (2:1) and loaded onto C:M-columns, less than 10% of the label could be eluted under Peak I as lipid-bound ^{131}I, and the rest was eluted under Peak II as free iodide. These experiments offer further evidence to support the assumption of Schneider and Wolff (3) that the solubilization of iodide in chloroform:heptane was due to formation of lipid micelles. Furthermore, the electrostatic nature of the lipid-iodide binding (3) is apparent from the fact that the iodide could be dissociated from the lipids by the simple expedient of evaporating the organic solvent to dryness and redissolving the residue in the same solvent.

The physiologic significance of the lipid–iodide association cannot be ascertained at the present time. The concept of a thyroidal iodide–carrier has been inferred from kinetic studies and from the fact that iodide is lipid-insoluble, and, thus, cannot traverse the lipidic double layer of the cellular membrane, unless bound to a carrier such as a phospholipid (1, 3). Nevertheless, the existence of an iodide carrier remains purely hypothetical. Whether the specific binding of iodide by lipid micelles *in vitro* is merely a test-tube phenomenon or whether it represents a model for iodide transport *in vivo* remains to be elucidated.

Partition of iodide between chloroform: methanol and water. The distribution of iodide between chloroform:methanol and water, in the presence of increasing amounts of Fe^{3+} in a lipid-free system is shown in Fig. 1. The ^{131}I in the chloroformic phase increased as a direct function of the Fe^{3+} concentrations in the system. These experiments suggest that, in the system of Dhopeshwarker *et al.* (4), ^{131}I was oxidized to iodine by Fe^{3+} and retained as iodine in the chloroformic phase due to its greater solubility in chloroform than in water.

As can be seen from the data in Table I, the distribution coefficient of ^{131}I between the chloroformic and aqueous phases was 0.01 in the absence of Fe^{3+} and 1.5–1.6 in its presence, whether thyroidal lipids were present or not. PTU prevented the Fe^{3+}-induced changes in the *d* values, although we had found that iodine, once formed, could not be reduced to iodide by the addition of PTU. We do not know why PTU prevents the oxidation of iodide. Perhaps, PTU forms complexes with Fe^{3+}, thus lowering its redox potential. This might explain the action of PTU in blocking the iodination of thyroglobulin, as there is good evidence for the participation of a ferriporphyrin with iodide-peroxidase activity in the biosynthesis of thyroid hormones (6). In the system of Schneider and Wolff (3) oxidation of iodide was

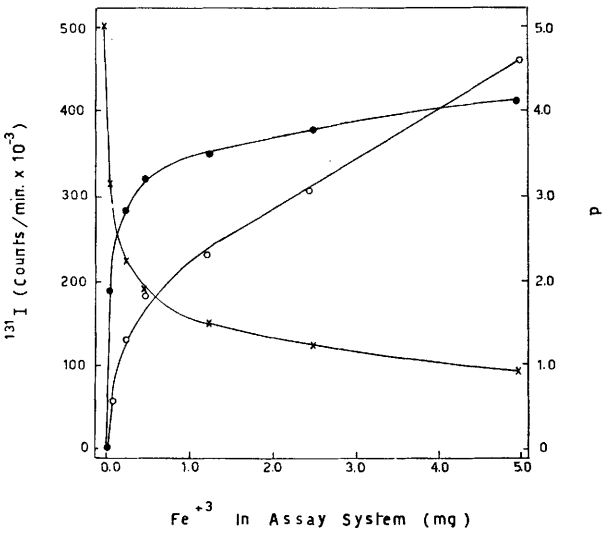

FIG. 1. The effect of increasing concentrations of Fe^{3+} on the solubilization of iodide in chloroform. ^{131}I in the aqueous phase (X) and in the organic phase (●). d = ^{131}I in the organic phase/^{131}I in the aqueous phase (○).

prevented with methimazole. It is not surprising that no ^{131}I could be found in the organic phase in the presence of thyroidal lipids, unless Fe^{3+} was added to oxidize the iodide since the results of the present experiments confirm the suggestion of Schneider and Wolff (3) that the preferential solubilization of iodide in a nonpolar solvent, in equilibrium with an aqueous phase, requires the formation of lipid micelles and these cannot be formed in the chloroform:methanol:water system.

In view of the experimental evidence summarized above, it may be concluded that the effect of Fe^{3+} on the solubility of ^{131}I in the chloroformic phase was due to the oxidation of iodide to iodine and not to the formation of a lecithin–iron complex with a strong affinity for iodide, as postulated by Dhopeshwarker *et al.*, (4).

From criteria similar to those employed elsewhere (7), we concluded further that the lipid-bound ^{131}I which was retained in the chloroformic phase in the presence of Fe^{3+},

TABLE I. The Effect of Fe^{3+} on the Solubilization of Iodide in Chloroform in the Presence and Absence of Thyroidal Lipids and Propylthiouracil (PTU).

Assay system	Mean ^{131}I in: Organic phase (cpm)	Aqueous phase (cpm)	d[a]
$Na^{131}I$	1060	106,000	0.01 ± 0.01
$Na^{131}I$ + thyroidal lipids	1070	107,000	0.01 ± 0.01
$Na^{131}I$ + Fe^{3+}[b]	62,000	39,000	1.60 ± 0.08
$Na^{131}I$ + Fe^{3+} + thyroidal lipids	61,000	40,500	1.51 ± 0.10
$Na^{131}I$ + PTU[c]	820	100,200	0.01 ± 0.01
$Na^{131}I$ + Fe^{3+} + PTU	1340	87,000	0.01 ± 0.01
$Na^{131}I$ + Fe^{3+} + PTU + thyroidal lipids	1275	95,000	0.01 ± 0.01

[a] d = ^{131}I in the organic phase/^{131}I in the aqueous phase. Values are means ± SD of three experiments.

[b] 250 μg of ferric chloride added to the system.

[c] 1 mg of PTU added to the system.

was covalent in nature. Indeed, the label could not be washed out of the chloroformic phase with 1% NaI, with 0.1 *M* EDTA, or with buffers having a pH of 3.0 or 10.0 and the lipid-bound iodine could be removed by alkali, but not by acid hydrolysis. Furthermore, after evaporation of the chloroform to dryness, solubilization of the residue in chloroform:methanol and filtration through C:M-columns, the radioactivity of Peak I accounted for all the label, none being eluted as free iodide. On TLC the radioactivity was distributed as follows: 8.5% bound to lecithin, 54.5% bound to cephalins and 34.5% bound to nonpolar lipids. It should be mentioned that recently we have demonstrated the presence of iodine bound covalently to thyroidal lipids *in vivo* (7).

Summary. Evidence is presented to show that enhancement of iodide solubilization in a nonpolar organic solvent in equilibrium with water, is due to micellar formation and that the lipid–iodide binding is ionic in nature. The enhancement of ^{131}I solubility in chloroform by the addition of Fe^{3+} was shown to be due to oxidation of iodide to iodine, a reaction which could be prevented by addition of PTU to the *in vitro* system. In the presence of thyroidal lipids, the iodine formed was subsequently bound to the lipids covalently.

1. Wolff, J., Physiol. Rev. **44**, 45 (1964).

2. Vilkki, P., Arch. Biochem. Biophys. **97**, 425 (1962).

3. Schneider, P. B., and Wolff, J., Biochim. Biophys. Acta **94**, 114 (1965).

4. Dhopeshwarkar, G. A., Mandlik, M. Y., Atmaram, R. H., and Mead, J. P., Proc. Soc. Exp. Biol. Med. **129**, 751 (1968).

5. Folch, J., Lees, M., and Sloane-Stanley, G. H., J. Biol. Chem. **226**, 497 (1957).

6. De Groot, L. J., N. Engl. J. Med. **272**, 243 (1965).

7. Posner, E., and Ordoñez, L., Biochim. Biophys. Acta **187**, 588 (1969).

Received Feb. 26, 1970. P.S.E.B.M., 1970, Vol. 134.