

The Unexpected Incorporation of Unbound Iodide-131 Into the Non-Radioactive Hippuran Molecule^{1,2}

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When aqueous solutions of sodium iodide and a salt of an organic acid containing iodine covalently bound to non-activated aromatic sites (such as sodium o-iodohippurate [Hippuran] or sodium diatrizoate) are mixed together at room temperature and pressure and stored in the dark, no reaction would be expected. Further, it would be anticipated that a mixture of solutions of sodium iodide-131 and non-radioactive Hippuran, tested for per cent of radioactivity existing as radio-iodide, would yield a value of 100 per cent which would remain essentially constant for a reasonable period of time.

Interestingly though, it has been observed in this laboratory that the per cent of radioactivity as free radioiodide diminishes rapidly in the non-radioactive Hippuran-radioiodide system. Tested by the method of Meschan, Hosick, Schmid and Watts (1), the *free iodide* in such a system dropped from 100 per cent immediately after mixing to ca. 50 per cent in 24 hours, then to ca. 4 per cent after a seven day interval.

EXPERIMENTAL

A series of experiments were run wherein excess radioactive or non-radioactive iodine-containing organic salts (Hippuran or sodium diatrizoate) were treated with sodium iodide-131, and the per cent activity due to iodide-131 was determined at various intervals (Table I) by the I_2 - CCl_4 test method (1). Ascending chromatography was used with Whatman No. 1 paper and n-butanol saturated with N acetic acid as the developing solvent (2). Spots were located by means of autoradiographic techniques. The R_f value varied from 0.14 to 0.20 for sodium iodide-131 and from 0.78 to 0.82 for sodium o-iodo¹³¹ hippurate. Corresponding non-radioactive carriers were added to the spots at the origin prior to development whenever the radioactive material had a high specific activity. The unknown radioactive organic compound formed from non-radioactive Hippuran and sodium iodide-131 had an R_f value of 0.83.

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DISCUSSION

Initially it was observed that when a solution of commercial I^{131} -labeled Hippuran containing 14.8 per cent radioiodide contamination was diluted with 1 per cent non-radioactive Hippuran, which results in the lowering of the specific activity by a factor of about 10,000, the free radioiodide had dropped to 6.4 per cent after one week.

The determination of the per cent radioactivity due to iodide-131 in an aqueous solution of a water-soluble salt of a radioiodinated organic compound is based upon the following: when a large excess of non-radioactive elemental iodine dissolved in carbon tetrachloride is allowed to come into contact and reach equilibrium with the aqueous solution to be tested, the radioiodide essentially will have exchanged into the carbon tetrachloride-iodine solution, leaving the radioactivity due to the water-soluble organic material in the aqueous phase.

At first it appeared that the observed phenomenon might have been an exchange reaction between radioiodide and organically bound iodine. The radioactive organic compound which formed during the disappearance of free radioiodide in a non-radioactive Hippuran solution has an R_f value of 0.83, as com-

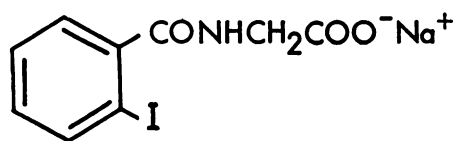
TABLE I
DISAPPEARANCE OF FREE IODIDE-131 UPON CONTACT WITH HIPPURAN, RADIO-HIPPURAN, AND SODIUM DIATRIZOATE WITH AND WITHOUT ADJUNCTIVE RADIATION

<i>Experiment</i>		<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
<i>Compound Treated with Na I^{131}</i>		<i>Hippuran</i>	<i>Hippuran</i>	<i>Hippuran</i>	<i>Hippuran</i>	<i>Radio- Hippuran</i>	<i>Sodium Diatrizoate</i>
<i>Adjunctive Radiation</i>		<i>None*</i>	<i>None</i>	<i>Co-60 Spheres</i>	<i>U.V. Light</i>	<i>None</i>	<i>None</i>
Determined % Radioactivity as Free Iodide-131 at various time intervals	Initial	102%	101%	100%	100%	14.8%	101%
	1 day	52%	46%	47%	56%		
	2 days					10.8%	
	3 days				6%	9.9%	
	4 days	8%	8.5%				
	1 week	3.5%	4%			6.4%	99%
	1 month						97%

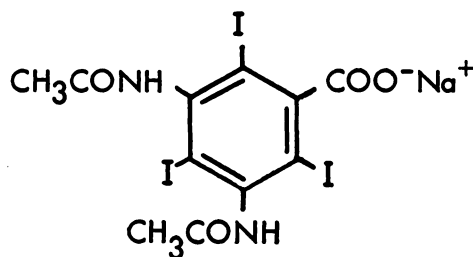
*Solutions stored in dark except during time of manipulation

pared to the 0.78 to 0.82 range for Hippuran. Such results do not conclusively show the unknown material to be other than radio-Hippuran. The non-radioiodide activity is accountable in this substance. I¹³¹-labeled Hippuran spontaneously liberates iodide-131 upon storage, apparently due to self-radiation effects (1, 2, 3). Hence, the possibility of a radiation-induced reverse reaction was considered as explaining the incorporation of radioiodide by Hippuran, with the resultant formation of radio-Hippuran. Such specificity would suggest an energy-level dependant system. Experiments using other energy levels or radiation involving mercury-vapor produced ultra-violet radiation and Cobalt-60 radiation resulted in no obvious change in the rate of radio-iodide disappearance (Table I).

If the reverse-reaction hypothesis were correct, the similar disappearance of radioiodide from a non-radioactive sodium diatrizoate solution would be anticipated. However, in such an experiment, involving sodium diatrizoate, no significant change in per cent radioiodide was observed. Unlike Hippuran, the aromatic portion of sodium diatrizoate is completely substituted.



Sodium o-iodohippurate



Sodium Diatrizoate

Experimental evidence, then, suggests that the radioiodine may be entering an unsubstituted position on the aromatic ring of Hippuran to form a radioactive diiodo- or poly-iodohippurate ion rather than the suspected exchange reaction having taken place. Further chromatographic studies are needed for the separation of the unknown radioactive organic compound(s) and to distinguish the compound(s) from radio-Hippuran. Since a di- or poly-iodo-substituted hippurate would be a much heavier ion than the mono-iodohippurate, a separation might be effected by electrophoresis. Subsequent identification of the radioactive organic product originating in the radioiodide-Hippuran system will suggest the correctness of the latter hypothesis.

Anghileri (3) found a radioactive organic contaminant of a radio-Hippuran solution which also was contaminated by radioiodide. The material was chromatographically very similar to Hippuran. In an attempt at identification, he ran functional group tests for free phenols, keto acids, and amino acids, the results being negative. It is possible that his unidentified compound is identical to our own.

The observations reported herein may have very significant ramifications, especially in regard to unexpected contamination of certain types of radioiodinated organic materials which spontaneously liberate radioiodide upon storage.

SUMMARY

Observations have been made that if the specific activity of a commercial preparation of iodine-131-labeled Hippuran containing contaminating free radioiodide is lowered by a factor of about 10,000 by dilution with a non-radioactive Hippuran solution, the per cent radioiodide contamination decreases upon standing over a period of time at room temperature in the dark. Furthermore, if sodium iodide-131 and non-radioactive Hippuran solutions are mixed, the per cent radioactivity due to iodide-131 rapidly decreases from the initial 100 per cent. An unknown radioactive compound is formed, presumably organic, which behaves chromatographically very similar to radio-Hippuran.

The addition of a solution of sodium diatrizoate to a sodium iodide-131 solution does not result in iodide-131 decrease. Cobalt-60 radiation and ultraviolet light do not appear to change the rate of iodide-131 decrease in solutions containing non-radioactive Hippuran and sodium iodide-131.

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