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# A Chromatographic Study of the Stability of Iodine-131 Labeled Sodium o-Iodohippurate

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A series of experiments have been carried out in order to study the stability of sodium *o*-iodohippurate (Hippuran) labeled with iodine. Solutions of sodium *o*-iodohippurate I-131 in pharmaceutical form are widely used as a radioisotopic diagnostic agent in kidney function studies.

The purpose of the experimental work was to determine the stability of the radioactive material under various conditions of pH, salt concentration, and time.

Assays were carried out using sodium *o*-iodohippurate labeled with I-131 by exchange (1). Preparations possessing different specific activities were tested in order to determine the effect of specific activity on the rate of decomposition of the product.

## EXPERIMENTAL

A chromatographic procedure was used to determine the amount of iodide liberated by break down of the initially iodide-free material. Descending chromatography, using a N-butanol-acetic acid-water (4:1:1 v/v), was employed. Under the conditions employed, the $R_f$ found for sodium *o*-iodohippurate was 0.82-0.86 and for iodide 0.09-0.14.

The extent of decomposition was determined 10, 20, and 30 days after the date of preparation using sodium *o*-iodohippurate preparations initially possessing specific activities of 0.05, 0.20, and 2.0 mc/mg.

The effect of solution pH on stability was also studied. Original solutions of sodium *o*-iodohippurate containing 0.9 per cent of benzyl alcohol and 0.2 per cent of sodium citrate possessed a pH of 8.0. Solution pH was altered to 10.0, 2.0, and 7.0 by the addition of 0.04 ml of 0.1 N NaOH, 0.1 N HCl, or phosphate buffer (pH 7.0) to 0.1 ml of the original solution stored in a plastic container. The effect of solution pH on the liberation of free iodide is shown in Table I.

The same technique was employed to study the effect of salt concentrations on the stability of high specific activity (2 mc/mg) sodium *o*-iodohippurate. The

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TABLE I

FREE IODIDE RELEASED AFTER STORAGE OF SODIUM O-IODOHIPPURATE AT DIFFERENT PH VALUES

| | *Per Cent Activity in the Iodide Chromatographic Spot* | | | |
|---|---|---|---|---|
| *mc/mg* | *pH* | *10 Days* | *20 Days* | *30 Days* |
| 0.05 | 2.0 | 4.0 | 4.1 | 6.2 |
| | 7.0 | 4.8 | 4.8 | 6.2 |
| | 8.0 | 2.4 | 2.8 | 3.4 |
| | 10.0 | 4.1 | 4.7 | 5.8 |
| 0.2 | 2.0 | 4.8 | 6.4 | 10.1 |
| | 7.0 | 5.1 | 7.2 | 11.3 |
| | 8.0 | 4.1 | 6.2 | 10.3 |
| | 10.0 | 5.2 | 8.2 | 10.3 |
| 2.0 | 2.0 | 18.0 | 18.3 | 18.3 |
| | 7.0 | 16.4 | 22.7 | 31.0 |
| | 8.0 | 13.2 | 20.6 | 20.8 |
| | 10.0 | 18.1 | 23.4 | 28.7 |

effects of sodium chloride concentrations of 0.33, 0.82, 1.65, 3.33, and 8.25 per cent on the stability of sodium *o*-iodohippurate solutions are summarized in Table II.

Table III indicates the effect of light on the stability of high specific activity sodium *o*-iodohippurate. A "stabilized solution" of 2 mc/mg sodium *o*-iodohippurate in a sealed serum vial was exposed continuously for 10-, 20-, and 30-day intervals to the light from a "cool white" 40 watt fluorescent lamp at a distance of 10 centimeters.

In order to achieve better separation of the possible compounds formed in the break down of sodium *o*-iodohippurate, a two-dimensional chromatograph was prepared employing two separate solvent systems. The test material was applied as a small (4 mm) diameter spot at one corner of a chromatographic filter paper sheet. The spot was initially developed in the ascending manner using N-butanol-acetone-water (5:5:1 v/v). After drying, the sheet was rerun at 90° from the initial solvent direction using N-butanol-acetic acid-water (4:1:1

TABLE II

FREE IODIDE RELEASED AFTER STORAGE OF SODIUM O-IODOHIPPURATE WITH DIFFERENT SALT CONCENTRATIONS

| | *Per Cent Activity in the Iodide Chromatographic Spot* | | |
|---|---|---|---|
| *NaCl %* | *10 Days* | *20 Days* | *30 Days* |
| 0.33 | 14.1 | 17.8 | 19.4 |
| 0.82 | 5.9 | 6.0 | 10.3 |
| 1.65 | 4.9 | 8.0 | 15.6 |
| 3.33 | 3.0 | 8.0 | 14.3 |
| 8.25 | 3.5 | 5.6 | 13.8 |

TABLE III

FREE IODIDE RELEASED AFTER STORAGE OF SODIUM O-IODOHIPPURATE WITH AND WITHOUT LIGHT EXPOSURE

| | *Per Cent Activity in the Iodide Chromatographic Spot* | |
|---|---|---|
| | *With Exposure* | *Without Exposure* |
| 10 Days | 17.6 | 7.0 |
| 20 Days | 22.7 | 20.6 |
| 30 Days | 37.1 | 20.8 |

v/v). After the removal of the second solvent, a radioautograph was obtained by placing the chromatographic sheet in contact with Gevaert No. D 4 industrial x-ray film, obtaining the radioautograph shown in Figure 1. The identity of the

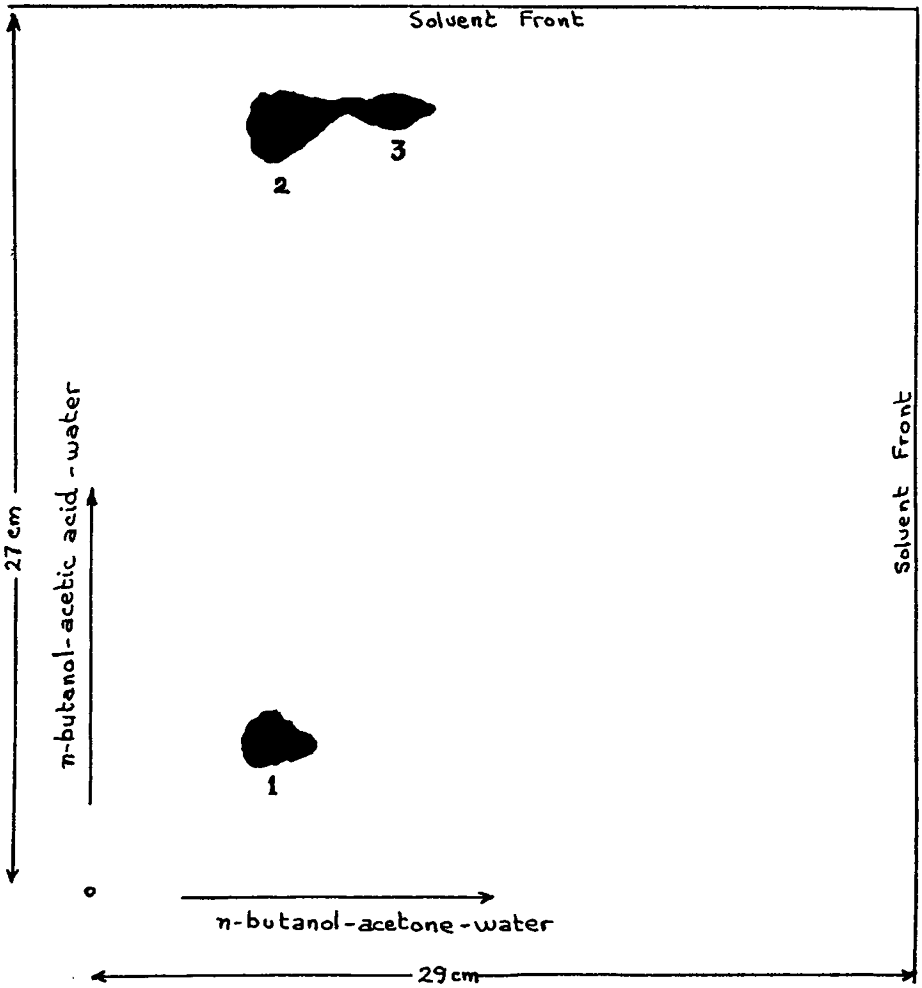

**Fig. 1.—Two-dimensional chromatograph, sodium o-iodohippurate.**

radioactive spots was determined by comparison of their measured $R_f$'s to those obtained using known materials under the same experimental conditions. After locating the areas possessing radioactivity, these were cut out of paper chromatogram, and their respective activities determined using a scintillation counter.

The ratio of the radioactivity of each area to the radioactivity initially applied is as follows: Spot 1 (iodide), 21.8 per cent; Spot 2 (sodium *o*-iodohippurate), 66.5 per cent; and Spot 3 (an unknown compound, possibly related to sodium *o*-iodohippurate), 11.7 per cent. The value for radioiodide released after 30 days corresponds well with the value obtained by one-dimensional chromatography for free iodide released from high specific activity sodium *o*-iodohippurate solution stored for 30 days. (See Table I.)

Attempts were made to identify the unknown compound constituting Spot 3. Results of tests using ferric chloride, diazotized *p*-nitroaniline, and phenolphthalein to possibly identify a free phenolic group were negative. Tests using *o*-phenylenediamine and semicarbazide, for keto acids, were also negative. The ninhydrin reaction for identification of amino acids similarly was negative, both for the material isolated in this spot and for the original mixture.

## DISCUSSION

The results of these experiments seem to indicate that the stability of iodine-131 labeled sodium *o*-iodohippurate solutions decreases as the initial specific activity of the product is increased.

The stability of sodium *o*-iodohippurate solutions seems greatest at pH 8.0. The presence of appreciable concentrations of sodium chloride in sodium *o*-iodohippurate solutions appears to partially protect them from decomposition. A possible explanation is that the sodium and chloride ions present in the solutions minimize the effect of beta particles emitted by the iodine-131 bonded to the aromatic ring, which may be the primary agent in effecting the breakdown.

The effect of exposure to light (the photoelectric effect) is not of greater magnitude than the effect of altered pH, at least with high specific activity sodium *o*-iodohippurate.

## REFERENCE

1. TUBIS, M., POSNICK, E., AND NORDYKE, A.: *Proc. Soc. Exp. Biol. Med.*, 103:497, 1960.