

A STUDY OF SOME IODINE COMPOUNDS USED AS  
RADIOPAQUE MEDIA

By

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## INTRODUCTION

Since the discovery of X-rays by Wilhelm Conrad Röntgen in the latter part of 1895, a great deal of attention has been given to the use of these rays as a means of visualizing the various organs of the body. For this purpose various contrast media have been employed. With respect to density as compared with organ tissues, these media may be grouped as follows: those which are less dense than the surrounding tissues, such as air, carbon dioxide and oxygen, and those which are more dense than the surrounding tissues, such as barium, bismuth and certain halogen compounds. Since the halogen compounds, and especially the iodine compounds have been used extensively in the examination of tissues, a study of the latter to determine the effectiveness as radiopaque media was undertaken and is made the subject matter of this report.

A review of the literature reveals that the use of iodine as a radiopaque medium dates from 1910, when Uhle and Pfahler (1) advocated the use of silver iodide in the visualization of the bladder and the ureter. This was followed in 1913 by the work of Kelly and Lewis (2) who advocated the use of sodium iodide as an opaque medium in urography. Cameron (3), in 1918, proposed the use of 25% of sodium or potassium iodide in aqueous solution, which proposal was later modified to 13.5% of sodium iodide. In the same year, Weld (4) suggested the use of sodium bromide. Cunningham (5) who worked out a procedure for urethrography in 1910, recommended the use of a highly dilute solution of mercuric iodide to endow the radiologic sodium iodide with higher bactericidal properties.

Rowntree (6) inaugurated the use of intravenous injection for

urography in 1923, using sodium iodide as the opaque medium. Somewhat later, Roseno (7) suggested the use of a urea-iodine preparation, "Pyelognost", for this purpose. Since the clinical effect of sodium iodide was uncertain, Swick (8) and von Lichtenberg (9) suggested, in 1928, the use of a urea-iodine derivative of pyridine, a product of low toxicity and high radiopacity called "Uroselectan". Aside from its direct application as a contrast medium, this substance may also be used as a reagent for a functional test for the kidneys. Other media of this type include "Skiodan", (iodine derivative of methane sulfonic acid) and "Uroselectan B" (iodine derivative of a pyridine compound).

Greenbaum (10), in 1932, described the use of an emulsion of the halogenated esters of peanut oil for use in pyelographic work.

In the field of chest diagnosis, Sicard and Forestier (11), in 1922, described the use of Lipiodol, (iodized poppyseed oil), as a contrast medium and vehicle for roentgenology of the respiratory passages. In 1928, Glaser (12) suggested the use of iodized rapeseed oil for this purpose, stating that this product can be applied undiluted to the bronchi.

The use of iodized oils in the delineation of the maxillary sinus was carried out by Proetz and Ernst (13), in 1926, Frazier (14), in 1927, and Beeler, Smith and Collins (15), in 1930. At about the same time other observers described the use of iodized oils in studying the intracranial structures.

Iodized oils were used in the glands and gland structures of the upper respiratory tract and neck by Payne (16) and Feuz (17), in 1932, who studied the salivary structures, and by Barsky and Silberman (18), who studied the parotid gland.

In the field of gall bladder diagnosis, Graham, Cole, Copher and



Moore (19) introduced the method known as cholecystography, which procedure involves the excretion of a radiopaque substance. Of the compounds experimented with, two were clinically usable; the sodium salts of tetrabromophenolphthalein and tetraiodophthalein. Both compounds exhibited about the same toxicity, while the iodine showed the greater opacity, thereby permitting the use of smaller doses. The iodine compound had the further advantage of staining the blood serum, thereby furnishing a test for liver function.

In histerosalpingology, the work of Sicard and Forestier (11), in 1922, called attention to the use of lipiodol in the body cavities. Heuser (20) (21), in 1921, succeeded in obtaining the first rontgenograms of the uterine cavity by the use of lipiodol.

The diagnostic procedure of arteriography had its inception in 1929 in the work of Moniz (22), who used a 25% solution of sodium iodine injected into the internal carotid artery and later into the common carotid. In 1931, De Santos Lamas and Caldas (23) perfected their technic of injecting the abdominal aorta, employing iodine compounds of low toxicity.

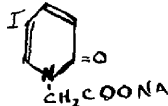
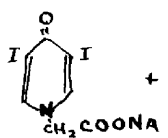
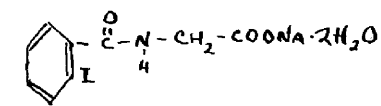
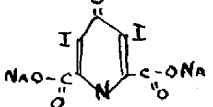
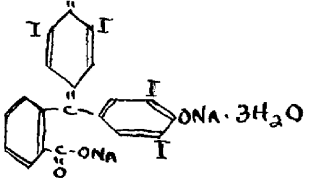
Menees, Miller and Holly (24), in 1930, reported that they were able to visualize the fetal soft parts and were occasionally able to determine the sex of the fetus, by the use of strontium iodide as the contrast medium.\*

In the table which follows, there are listed the most important iodine compounds used in rontgenology at the present time.

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\* A complete review of the development of X-ray technic may be found in "The Science of Radiology", (Charles C. Thomas, Springfield, Ill. and Baltimore, Md.), 1933.

Table I. Most Important Iodine Compounds Used in Radiography

Compound	Formula	Use	Per cent Iodine	Reference
Skiodan	$\text{ICH}_2\text{SO}_3\text{Na}$	Urography	52%	N.N.R.
Iopax (Uroselectan)		Urography	42-43.5%	N.N.R.
Diodrast	 + $\text{HN}(\text{CH}_2\text{CH}_2\text{OH})_2$	Urography	49.8%	N.N.R.
Hippuran		Urography	38.8%	N.N.R.
Neo-iopax		Urography	51.5%	N.N.R.
Iodophthalin Soluble		Cholecystography	51-53.5%	U.S.P.XI
Campiodol	Halogenated Rape-seed Oil (as emulsion)	Urography	24-26% (7-8% chlorine)	N.N.R.
Lipiodol	Iodized Poppyseed Oil	In spinal cord, bronchial diagnosis, and gynecology	40%	N.N.R.
Lipiodol Radiologique Ascendant	Iodized Poppyseed Oil	Diagnosis of intradural tumors	9.8-11.2%	N.N.R.
Lipiodine	Ethyl diiodo brassidate	Same as Lipiodol	41%	N.N.R.
Iodized Oil	Iodinated Vegetable Oil	Same as Lipiodol	38-42%	U.S.P.XI

In a survey of the elements used in radiography, among which were the elements, iodine, bromine, strontium, lead, silver and gold, Frazier and Glaser (25), in 1928, stated that the opacity of an element to X-rays is, "in general, proportional to the atomic weight and number of the element. The atomic weight is the more important factor". On the basis of opacity and toxicity they found iodine to be the "element of choice".

These authors prepared dilutions of iodized rapeseed oil and compared the opacity of these compounds. They state, "It is evident that the higher the iodine content, the more opaque is the shadow cast". Further proof of this relationship is afforded by the fact that Santiago Codino (26), in 1932, analyzed iodine in urine by the use of X-ray photographs. This relationship is confirmed by work done by the author and reported in the following pages.

## EXPERIMENTAL

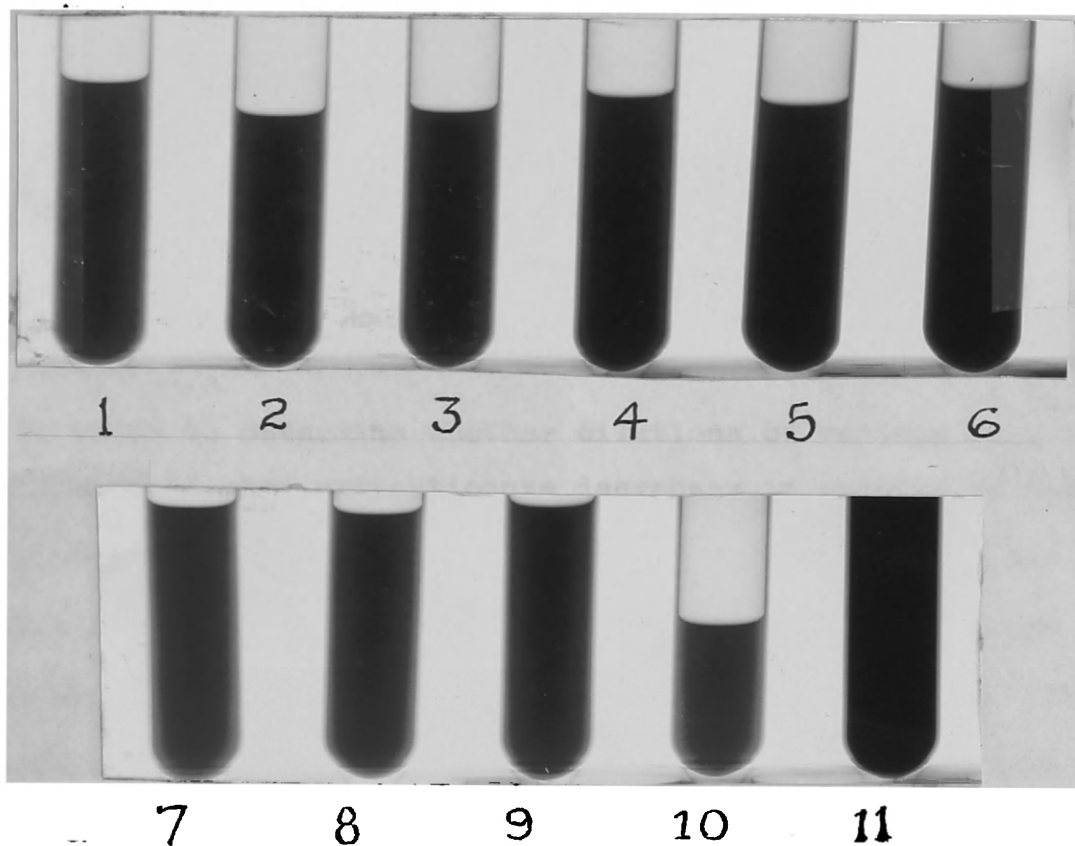
The report of experimental work carried out by the author is divided into three parts. The first deals with the opacity of several iodine compounds, in varying strengths, to X-rays; the second, with a study of the preparation, properties, and assay of iodized oils; and the third, with the preparation of two fluorinated iodine compounds, together with a toxicological study of one of these compounds.

### Part I.-- Opacity of Several Iodine Compounds to X-Rays

To determine whether or not the type of compound to which iodine is attached is a factor in the opacity of the compound, X-ray photographs were made of several iodine compounds in ether and alcohol solutions. The results are shown in Figures 1 and 2.

All of the tubes in Figure 1, except the last two, contain 12.7% of iodine. All of the tubes, except the last two, exhibit about the same opacity.

FIGURE 1. X-ray Photographs showing the Comparison of the Opacity of Various Organic Iodine Compounds.



- The tubes contain:
1. n-Propyl iodide in 1 molar concentration.
  2. n-Butyl iodide " " "
  3. Iso-butyl iodide " " "
  4. sec-butyl iodide " " "
  5. n-Amyl iodide " " "
  6. n-Hexyl iodide " " "
  7. n-Heptyl iodide " " "
  8. Iodobenzene " " "
  9. o-Iodotoluene " " "
  10. Iodized Oil containing 10% of iodine.
  11. Iodized Oil containing 26% iodine.

The last two tubes contain, respectively, a commercial iodized oil reported to contain 10% iodine, and a commercial iodized oil reported to contain 25% of iodine. It is apparent from the photographs that the type of compound to which the iodine is attached has no bearing on the opacity of the compound to X-rays, whether the compound is a straight chain, a branched chain, a secondary iodine compound or an aromatic compound. Tube 10 (10% iodine) is somewhat lighter in color, and tube 11 (26% iodine) is darker than tubes 1 to 9, inclusive (12.7% iodine).

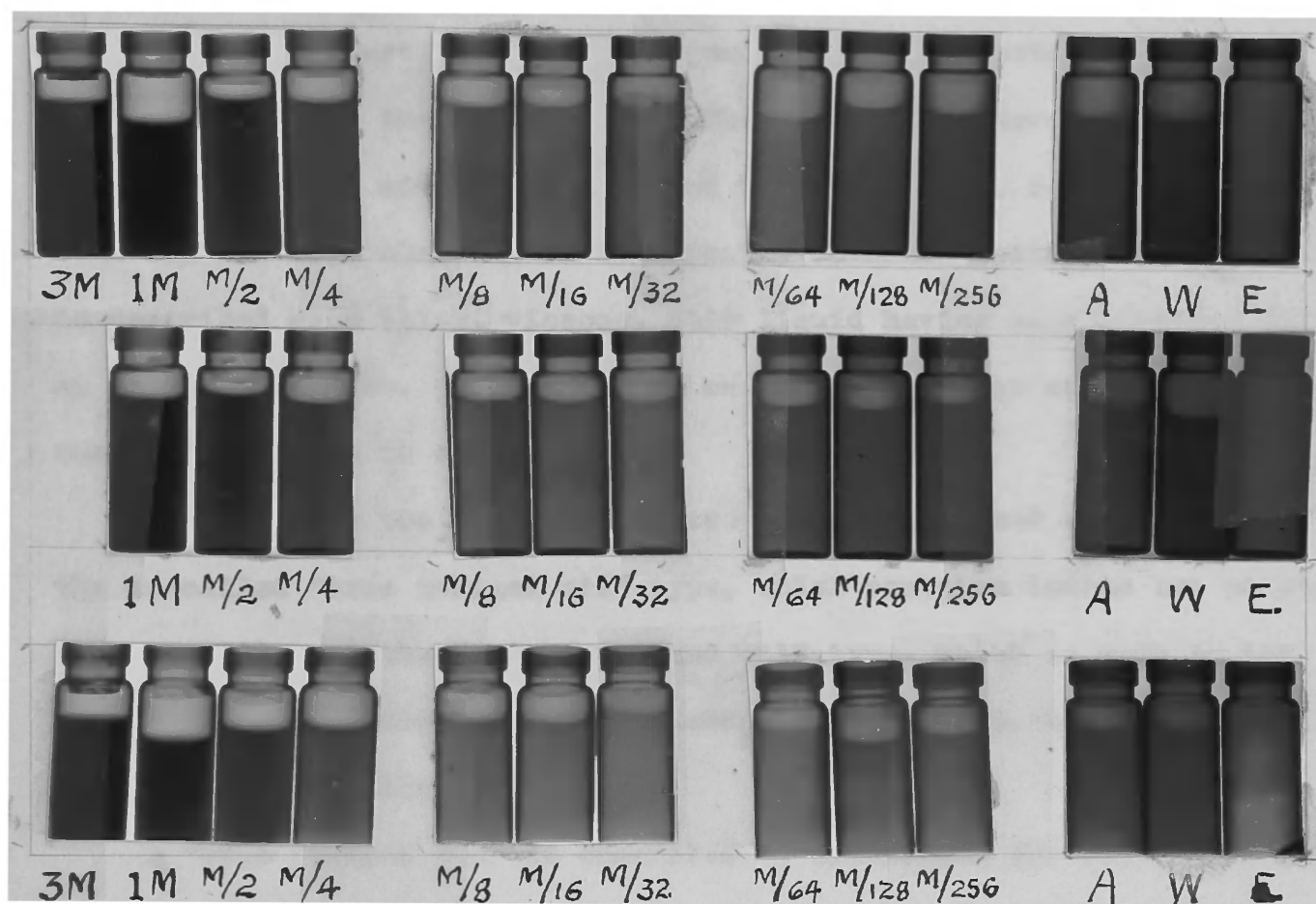
In order to determine whether dilutions of various type iodine compounds would show proportionate decreases in opacity to X-rays, photographs were prepared of sec-butyl iodide, n-heptyl iodide and iodobenzene in dilutions of 3M, M, M/2, M/4, M/8, M/16, M/32, M/64, M/128 and M/256 in 95% alcohol.\* These photographs are shown in Figure 2. From these photographs, it is apparent that equal dilutions of the three type compounds show equal decreases in their opacity to X-rays.

In general, the foregoing results substantiate those found by Frazier and Glaser (25) for dilutions of iodized rapeseed oil.

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\*n-Heptyl iodide was not soluble in sufficient amount to permit of a 3 Molar solution.

FIGURE 2. X-ray Photographs of Dilutions of Three Organic Iodine Compounds



The tubes in the top row contain dilutions of sec-butyl iodide; those in the middle row, n-heptyl iodide; and those in the bottom row, iodobenzene, in the strengths shown. The last three tubes in each row contain alcohol (A), water (W) and an empty tube (E).

## Part II.--Iodized Oils

Iodized oils are vegetable oils which have been treated with iodine or iodine and chlorine to form halogen addition products.

According to the United States Pharmacopeia (Eleventh Revision) Iodized Oil is an addition product of vegetable oils, containing not less than 39% nor more than 41% of organically combined iodine. The product is described as a thick, viscous, oily liquid having an alliaceous odor and an oleaginous taste. It decomposes on exposure to air and sunlight, becoming dark brown in color.

There are at the present time two types of iodized oil on the market; the so-called "true iodized oil" type, which contains iodine but no other halogen (10), and the "chloro-iodized oil" type, which is made by the action of iodine chloride upon a fixed oil, and which therefore, contains both iodine and chlorine.

A "true iodized oil" is described by Sicard and Forestier (11), who first suggested the use of iodized oil as a radiopaque medium in röntgenology, as "an amber-coloured oily substance with a slight aromatic odor and with no taste whatever. It is viscid at room temperature, but heating to 35 - 45° C. (body temperature) causes it to become more fluid, a property which greatly facilitates injection".

Merck (27) describes the chloro-iodized oil type as follows: "At the present time (1899) Iodipin is marketed in the form of two products, one containing 10% of iodine and the other 25% of iodine. The 10% product is similar in taste and appearance to pure sesame oil, is very stable, is insoluble in water and alcohol, and is comparatively soluble in ether, chloroform, benzol, etc. Its specific gravity is 1.025. The 25% product is a thick, sticky, more or less reddish or violet colored,



oily liquid, having a honey-like consistence in the cold, and must be warmed before use. Its specific gravity is 1.22, and its solubilities are similar to the 10% product."

Iodized oil is used at the present time principally as a diagnostic and therapeutic agent in bronchial affections (28, 29, 30, 31, 32, 33, 34, 35), and in diagnosis of spinal block (36, 37, 38). It has also been used in diagnosis of disease in the genito-urinary system, and in various other parts of the body (39, 40, 41).

The relative merits of the two types of iodized oil are a matter of controversy. Lafay (42) states that lipiodol (true iodized oil) is superior to chloro-iodized oils since it does not contain chlorine, and is lighter in color than chloro-iodized oil. The fact that decomposition can be detected by the darkening of the product is considered an asset. On the other hand, Balyeat and associates (35) say that the presence of chlorine is in no way objectional clinically, in fact, it lends increased stability to the product.

These products have been reported to have an antiseptic action (29), but the concensus of opinion is that the antiseptic action, if manifested at all, is very weak (34, 35).

### A. - Methods of Preparation

To prepare these two types of oils, it is necessary to follow slightly different procedures.

Lafay (42), who was the first to prepare a "true" iodized oil, stated that a product of this type containing 40% of iodine could be prepared by the interaction of hydriodic acid and poppyseed oil. Unfortunately the publication containing the reference to his work is not available in this country; hence, the procedure which he followed cannot be described here. However, G. Kasuya (43) gives the following procedure for the application of this method: Introduce hydriodic acid into an ethereal solution of purified vegetable or animal oil under ultra violet light with sodium iodide as a catalyst. When the reaction is completed, remove free iodine by treating with sulfur dioxide. Add a small amount of urea to the finished product as a stabilizer.

An entirely different procedure is described by Takagi (44): Dissolve 15 to 20 parts of iodine in 75 parts of fatty oil (cod-liver, olive, peanut, etc.) with agitation at 50 deg. C. Heat the mixture at 15° with 25 parts of cineol in a closed vessel for 1 hour or more. After cooling, add 100 parts of a 5% potassium iodide solution, shake the mixture and set aside at 0°. Separate the wax and wash the product with water. The product is not viscous, and is reported to be suitable as a food, having no taste of iodine.

The methods which have been used for the preparation of chloriodized oils are based on the fact that iodine chloride reacts with the unsaturated portions of fatty oils to form halogen addition products. The iodine chloride may be prepared by the decomposition of iodine trichloride or by the chlorination of iodine in solution using chlorine gas, aqua regia, or

mercuric chloride.

Lindblom's Method (45) is as follows: Dissolve the iodine in glacial acetic acid and pass in chlorine gas until the iodine color disappears. Mix the solution with the fatty acid dissolved in chloroform. After stirring and letting stand until the next day, tap off the separated oil, wash with water several times to remove the acetic acid, and finally remove the chloroform by passing a current of air through the oil for 10 - 20 minutes, at 60 - 70°. Schou and Jacobsen, commenting on this method, say that the oil still smells of acetic acid, and after a few days, the light brown-yellow oil darkens in color and has a distinct sour odor. As an improvement over this method, Schou and Jacobsen (45) recommend the following modification: Dissolve sixty five grams of iodine in five liters of glacial acetic acid by warming on a water bath. After cooling, lead chlorine into the solution. When the iodine color begins to disappear, continue the flow of chlorine cautiously and stop the chlorination as soon as the mixture assumes the light red-yellow color characteristic of iodine chloride solutions. Dissolve 300 Gm. of fatty oil in 500 Gm. of chloroform and add slowly to the iodine chloride solution. Stir the mixture well and add 500 Gm. of water. When the oil has collected at the bottom of the container, separate it from the supernatant liquid and wash by shaking with water. Separate the water as completely as possible and place the oil in a flask on a water bath at 60 - 70°. Lead a strong current of air through the oil for twenty four hours. The product is reported to be light in color and stable even when exposed to air. This procedure gives an iodized oil containing approximately 17% of iodine, and which also has a fairly constant chlorine content (about one third equivalent of chlorine for each equivalent of iodine).

Bauman and Woodford (46) used the above method up to the point where the oil is separated from the mixture. They proceeded then as follows: After separating the oil from the acetic acid mixture, dissolve it in chloroform, filter the product, and remove the chloroform by evaporation over a water bath. Remove the acid adhering to the oil by placing the product in an evacuated desiccator over concentrated sodium hydroxide. Remove the last traces of water by drying in vacuum over phosphorous pentoxide. This procedure requires from four to seven days for completion.

Schwank (47) used the following method: Mix a solution of 2 Gm. of iodine in ether with 2 Gm. of rape oil, and heat the mixture until the ether is evaporated. Pass chlorine through the mixture, drive off the excess chlorine by heat, and wash the product with water until the washings cease to give a precipitate with silver nitrate.

Conradt (48) recommended the method of Winternitz which is as follows: Mix the calculated quantity of iodine chloride (in absolute alcohol solution) with almond or sesame oil. Heat the mixture to 40° with frequent shaking. Remove the alcohol by washing with water, and drive off the last portions in an evacuated vessel at 40°. The product is said to be colorless and to contain no free iodine. The author stated that a 10 - 20% iodized sesame oil preparation remained unchanged in color when heated to 100-110°, while a 25% iodized oil was not changed at 100° but assumed color at 110°.

Lami (49) employed the following procedure: Mix 354 parts by weight (or 300 by volume) of hydrochloric acid (Sp. G. 1.18) with 84 parts by weight (or 60 by volume) of nitric acid (Sp. G. 1.40), dissolve therein 250 parts of iodine, and add the mixture with shaking to 750 parts of sesame oil in a separatory funnel. When the reaction is completed and the aqueous layer is colorless, add 350 parts of ether. When the layers sep-

arate, remove the acid, wash the oil once with water, and then treat with 50 parts of calcium chloride. The product obtained is said to contain 25% of iodine and must be protected from heat and moisture.

In no instance is it claimed that an oil containing 40% of iodine can be prepared by any of the foregoing methods. However, Schou and Jacobsen (45) state that by increasing the initial quantity of iodine, products with a high iodine content may be prepared. For this reason the Schou and Jacobsen method was investigated in some detail.

B. - Preparation of Iodized Oil:

The iodized oils cited in the medical literature are made with sesame, poppyseed, or rapeseed oils. Since pure rapeseed oil was not obtainable for these experiments, the products were made with sesame and poppyseed oils. The oils used gave the following constants:

Table II.- Constants Sesame and Poppy Seed Oils

	Sesame Oil	Given <sup>b</sup>
	Observed	
Iodine Value	109-111	106-114
Saponification Value	190	187.6-194.6
Specific Gravity	0.921 at 25°	0.917-0.921 at 20°
Acid Value <sup>a</sup>	1.30	
	Poppyseed Oil	Given <sup>c</sup>
	Observed	
Iodine Value	133-135	132.6-136
Saponification Value	190	189-197
Specific Gravity	0.919 at 22.6°	0.924-0.927 at 15°
Acid Value <sup>a</sup>	0.50	

a. The acid value is expressed as the number of cc. of tenth normal sodium hydroxide required to neutralize the free acid in 10 Gm. of substance (U. S. P. XI, page 444).

b. Lewkowitsch, M., Chemical Technology and Analysis of Oils, Fats, and Waxes (London, Macmillon and Co., Ltd., 1909). Edit. 4, Vo. 2., 173.

c. *ibid.*, 95.

For the first portion of the work, it was decided to determine how much iodine would be absorbed by a fatty oil upon irradiation with ultra-violet light. Eleven grams of poppyseed oil were placed in an open crucible and powdered iodine was added in two portions as shown in the table which follows and the mixture was placed under a mercury arc lamp. The results are as follows:

Table III. Amount of Absorption of Iodine by Poppyseed Oil (11 Gm.) upon Irradiation with Ultra-violet Light

Amt. of Iodine	Time Exposed	Remarks
0.55 Gm.	10 minutes	The dark reddish solution changed to a light brown color, similar to the color of poppyseed oil.
0.10 Gm.	30 minutes	A dark reddish-brown solution was obtained, which did not change color.
" "	7 hours	The product became black and tarry.

Upon standing the liquid became tarry in appearance and consistence which was evidently due to oxidation of a portion of the oil.

It is evident that ultraviolet light irradiation of an iodine-oil solution is impracticable, since less than 10% of iodine was absorbed, even after seven hours.

A second attempt to iodize the oil was made, using, in this case, a suitable solvent. A solution of 1 Gm. of iodine in 25 cc. of chloroform was added to 10 Gm. of poppyseed oil dissolved in 50 cc. of chloroform. The resulting solution was allowed to stand for a week in diffused sunlight. At the end of this period, the solution was still dark colored and contained free iodine. Repeating the experiment with sesame oil gave no better results.

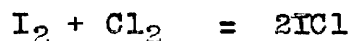
Attempts to prepare a "true iodized oil" by the patented methods of Kasuya (43) and Takagi (44), from the information available, were unsuc-

cessful. Attempts were also made to prepare a "true iodized oil" by the use of dry hydrogen iodide gas. The gas was passed into the unsaturated oil with and without the aid of ultraviolet light for varying periods of time (15 minutes to 2 hours). The resulting products contained only very small amounts of iodine.

A further experiment was tried in which 10 Gm. of sesame oil and 20 cc. of concentrated hydriodic acid solution (48% HI) were shaken mechanically for six hours. Upon separation of the oil, it was found to contain only a small amount of iodine.

Since the patent literature on the preparation of this type of oil is very limited and all methods tried were unsuccessful, attention was turned to the preparation of a chloro-iodized oil.

The procedure of Schou and Jacobsen (45) was chosen as the one which appeared to offer the greatest possibility for success from a practical standpoint. These authors state that they prepared and used an iodized oil containing 17% of iodine, but that the amount of iodine in the finished product could be increased by increasing the original quantity of iodine. They made, for example, a product containing 30% of iodine. The method used was as follows: Dissolve 37 Gm. of iodine in 2,500 cc. of glacial acetic acid with the aid of gentle heat, and determine its iodine content with N/10 sodium thiosulfate solution. Pass washed and dried chlorine gas through the solution until the iodine titre has doubled. The solution assumes a light red-yellow color at this point. The doubling of the titre shows that the following reaction has taken place:



Dissolve 50 Gm. of poppyseed oil in 50 cc. of chloroform and add to the iodine-chloride solution slowly. After stirring well, add 100 cc. of water and allow to stand over night. Separate the oil which has collected



at the bottom of the container and wash by shaking with water. Separate the water as completely as possible and place the oil in a flask on a water bath at 60-70°. Pass a strong current of air through the oil for twenty-four hours. At the end of this period the oil has a light color and is quite stable even when exposed to air. The product obtained by this method contained 30.0% of iodine.

Attempts to increase the amount of iodine in the finished product to 40% by increasing the original amount of iodine used proved unsuccessful. Increasing the original amount of iodine to 50 Gm. produced a product containing no more than 34% of iodine.

Since none of the aforementioned procedures is used to prepare an iodized oil containing 40% of iodine, it was decided that a procedure following the Wijs method for the determination of the iodine number be developed. Several modifications were tried, the following being the most satisfactory: Dissolve 37 Gm. of iodine in 1 liter of chloroform, by the aid of gentle heat, and determine its iodine content with standard sodium thiosulfate solution. If the concentration of iodine in chloroform is greater than 37 Gm. per liter, the finished product tends to have a dark color. Pass washed and dried chlorine gas through the solution until the titre of the solution has just doubled. This point is indicated when the dark red solution assumes a reddish-orange color. Add 50 Gm. of oil (sesame or poppyseed) dissolved in 100 cc. of chloroform to the solution and let stand over night. Add 100 cc. of water, which forms a layer on top of the chloroform layer, and add, in small portions, a 10% solution of sodium thiosulfate, shaking after each addition. Continue adding the thiosulfate solution until the chloroform layer assumes a light yellow color and the aqueous layer becomes colorless. Wash the chloroform

layer with water until the wash water gives no precipitate with silver nitrate T.S. Separate the chloroform layer, add 5 Gm. of anhydrous sodium sulfate, shake and allow to stand for one half hour, and filter. The filtrate is a clear light yellow liquid. Drive off the chloroform as completely as possible by distillation under reduced pressure at a temperature not exceeding 40° (45). At this point the product still has a sweet odor, due in part to a trace of chloroform. All of the chloroform may be removed by passing a current of air through the product for one hour.

The iodine content and the constants of the products prepared by this method are given in the following table.

Iodized oil with a 40% iodine content could not be obtained by this method. The highest percentages of iodine obtained using this procedure was 34%. It is apparent that manufacturers who obtain a product containing 40% of iodine must employ some procedure which has not been published. It would seem advisable, therefore, to consider lowering the official requirement for iodine content (39% to 44%) to some more readily obtainable standard. Schou and Jacobsen (45), for example, have found an iodized oil containing 30% of iodine to be entirely satisfactory as a radiopaque medium.

C. - Properties of Iodized Oils Prepared in the Laboratory

Iodized sesame oil prepared by the foregoing process was of a light brown color, and had a viscosity about the same as the iodized sesame oil. The following properties were obtained for the two products:

Table IV. Properties of Iodized Oils Prepared in the Laboratory

	Iodized Sesame Oil		Iodized Poppyseed Oil	
	No. 1	No. 2	No. 1	No. 2
Iodine Content*	30.5%	29.6%	34.0%	33.7%
Specific gravity	1.348	1.341	1.419	1.413
Refractive index	1.5192	1.5159	1.5280	1.5227
Ash	0.05%	0.05%	0.05%	0.07%

Solubility Both products were freely soluble in chloroform, ether, carbon tetrachloride, carbon disulfide, and benzene. Neither was soluble in water or alcohol.

Limit of Acidity (U. S. P. XI, page 255) -- 1 cc. of the oil dissolved in 10 cc. of chloroform in a glass stoppered bottle, and shaken with three drops of phenolphthalein T.S. and 0.3 cc. of tenth normal sodium hydroxide solution produced a red color.

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\* Assayed by the method outlined on page 33.

#### D. - Assay of Iodized Oils for Halogens

Since the assay of these oils for iodine by the U. S. P. XI method gave inconsistent results, other published methods for the determination of halogens were investigated and the results reported herein. The method which produced the best results and which was finally adopted is given in detail on page 33.

Hok (50) recommends the following procedure: Weigh about 1 Gm. of oil into an Erlenmeyer flask, and add 25 cc. of alcohol and 2-3 Gm. of potassium hydroxide. Reflux the mixture for 30 minutes. Dilute the material with water, transfer to a 250 cc. volumetric flask, and fill to the mark. Take a 25 cc. Aliquot and weakly acidify with sulfuric acid. Add 1 cc. of a 0.001 N. solution of iodine and 1-2 cc. of starch indicator solution, and titrate with standard silver nitrate solution to the end point, (olive green to pale yellow). Chlorine does not interfere.

With this method it was found that the end point was obscured to this investigator, and therefore the results were not reliable.

The second method tried was a modification of the above method, as follows: Reflux the oil with alcoholic-potassium hydroxide. Transfer the mixture to an iodine titrating flask and neutralize the alkali with concentrated hydrochloric acid. Add concentrated hydrochloric acid to double the volume, and titrate with M/20 potassium iodate, shaking vigorously. When the dark brown color which develops becomes light brown, add 5 cc. of chloroform and continue the titration until the chloroform becomes colorless and the supernatant liquid is clear yellow. 1 cc. of M/20 potassium iodate is equivalent to 0.01269 Gm. of iodine.

In using this method, it was found that the end point was indistinct,

since the chloroform did not completely decolorize at the end point, but had a brown color. It was noted that when the mixture was made slightly acid, oily drops appeared on the surface of the liquid. When all of the acid is added a light brown, transparent, but not entirely homogenous mixture was obtained. Apparently not all of the oil was saponified. Increasing the quantity of alkali to 5 Gm. and continuing the refluxing for one hour did not produce any better results.

A third method tried consisted of fusing the product with potassium hydroxide and titrating with M/20 potassium iodate: Weigh about 7 Gm. of dry powdered potassium hydroxide in a nickel crucible. Weigh accurately about 1 Gm. of iodized oil into the crucible, and heat gently over a bunsen burner until a dark homogenous mass is obtained. Heat strongly until smoking ceases, and allow the crucible and mass to cool. Dissolve the mass in 100 cc. of hot water in a beaker, and filter the hot solution into a 500 cc. flask. Wash the beaker, crucible and filter with three 10 cc. portions of hot distilled water. Neutralize the alkali with concentrated hydrochloric acid to double the volume, and titrate with M/20 potassium iodate as in the preceding method.

Upon the addition of concentrated acid, iodine was liberated, so that the potassium iodate titration gave very low results. Middleton (51) says of this method, "Methods in which a substance is evaporated with caustic alkali require much attention to prevent creeping, and it is impossible to prevent some loss as spray. If the ignition is carried out with dry alkali, creeping still occurs, while there is some doubt whether the whole of the iodine is recovered". Middleton (51) then proposed the method of ignition with sodium carbonate in a double crucible, which is essentially the U. S. P. XI method.

In the U. S. P. XI the investigator is referred to the assay of iodine under Soluble Iodophthalein (U. S. P. XI, p. 196). This method is as follows: "Mix about 0.35 Gm., accurately weighed, of the iodized oil, with 2 Gm. of anhydrous sodium carbonate; place the mixture in a small porcelain crucible, and completely fill the crucible with anhydrous sodium carbonate well pressed down; invert the crucible and contents in a larger porcelain crucible, and add sufficient anhydrous sodium carbonate to seal the junction of the two crucibles. Heat rapidly and strongly over the Bunsen burner, and continue the heating for twenty minutes. Allow the crucibles and contents to cool, and dissolve the residue in 100 cc. of hot distilled water in a beaker. Filter the hot solution into a 500 cc. flask and wash the beaker, crucibles and filter with three 10 cc. portions of hot distilled water. Allow the filtrate and washings to cool, and add hydrochloric acid cautiously until effervescence ceases, then add an equal volume of hydrochloric acid, and titrate with twentieth-molar potassium iodate, shaking vigorously, until the dark brown solution which is formed becomes light brown; add 5 cc. of chloroform and continue the titration until the chloroform becomes colorless and the supernatant liquid is clear yellow. One cc. of twentieth-molar potassium iodate is equivalent to 0.01269 Gm. of I."

This method gave inconsistent results, which depended on the intensity of heat and the length of time of heating. Low heat resulted in incomplete combustion, giving low results. High heat or prolonged heating caused some loss of iodine, thereby giving low results. Even under the most carefully controlled conditions of heating, results which fell within the bounds of experimental error could not be obtained.

Butler & Burdett (52), in 1939, who studied this method of assay say

"The official method for sodium iodophenolphthalein is not a rapid method, and furthermore it is subject to several inherent errors which combine to give a low value for iodine. Free iodine is lost by volatilization and the sodium tetra-Iodophenolphthalein is not completely decomposed by the sodium carbonate fusion. The iodine lost was found to amount to as much as 1% of the weight of the sample. The iodine left in the mass in the form of undecomposed sample varied in quantity, probably depending on the temperature and length of time of the fusion. This iodine was found to amount to as much as 3%. The results are not particularly concordant."

These authors then developed the method which was adopted as the official method of assay for sodium iodophenolphthalein in the Second Supplement of the U. S. P. XI. This assay follows: "Place about 0.2 Gm., accurately weighed, of the tetraiodophenolphthalein obtained in the assay described above in a 500 cc. Erlenmeyer flask and add 15 cc. of an aqueous solution of sodium hydroxide (1 in 20). Place the flask on a steam bath and, when the substance has dissolved completely, add 25 cc. of an aqueous solution of potassium permanganate (1 in 15). Rinse the inside of the flask with a small amount of distilled water and heat the mixture on a steam bath for forty-five minutes, rotating the contents of the flask at intervals of five minutes. Remove the flask from the steam bath, allow the contents to cool to room temperature, and add 75 cc. of distilled water followed by 15 cc. of diluted sulfuric acid. Add, all at once, 15 cc. of an aqueous solution of sodium bisulfite (1 in 5) and, when the solution has become colorless, add the solution of potassium permanganate, drop by drop, until a faint yellow color appears, then add at once additional solution of sodium bisulfite, drop by drop, to dis-

charge the color. Add 2 cc. of glacial acetic acid, 1 Gm. of freshly powdered ammonium carbonate, and 1 cc. of diiodofluorescein T. S. Titrate the solution in a diffuse light with tenth-normal silver nitrate until the color just changes from brownish-red to bluish-red. Each cc. of tenth normal silver nitrate is equivalent to 0.01269 Gm. of iodine.\*\*

Attempts were made to apply this method to iodized oil with the following results:

Exp. 1 -- About one Gm. of iodized oil was weighed off and treated as in the above method. It was noted that the oil did not completely saponify and that the end point was obscured. It was apparent that complete saponification is necessary for the determination. Repeated experiments with smaller quantities of iodized oil ranging down to 0.2 Gm. were tried with no better results.

Exp. 2 -- About 0.2 Gm. of iodized oil were weighed off and 30 cc. of a solution of sodium hydroxide and 20 cc. of alcohol were added.

Two runs were made as follows:

- a. The mixture was heated on a steam bath for two hours. At the end of this time, the mixture was not saponified.
- b. The mixture was heated over a direct flame under a reflux condenser for two hours. At the end of this time, the oil was not saponified.

It was then decided to attempt to saponify the oil by following the U.S.P.XI method for determining saponification numbers.

Exp. 3 -- About 0.5 Gm. of iodized oil was placed in a 250 cc. flask,

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\*Since no mention of Iodized Oil is made in the Second Supplement of the U.S.P.XI, a question arose as to whether Iodized Oil is to be assayed by the original Soluble Iodophthalein assay or by the new method for Soluble Iodophthalein official in the Second Supplement.



25 cc. of half-normal alcoholic potassium hydroxide were added and the mixture was heated over a direct flame. The mixture became homogenous in about thirty minutes. It was then cooled to room temperature and a solution of potassium permanganate (1:15) was added. The mixture became dark brown immediately. Upon addition of more permanganate, the mixture continued to produce brown manganese dioxide. It is evident that the use of alcohol must be avoided in this method.

Exp. 4 - In still another attempt, the oil was dissolved in about 20 cc. of chloroform instead of alcohol in order to avoid the formation of manganese dioxide when the saponified oil is oxidized. The mixture was heated on a water bath with intermittent shaking for two hours. This method was found to be unsatisfactory, however, as some of the oil remained unsaponified.

Exp. 5 - The above (Exp. 4) modification was tried again, using a mixture of 1 Gm. oil, 20 cc. of chloroform, 15 cc. of 5% sodium hydroxide solution and 25 cc. of a solution of potassium permanganate (1:15). The mixture was heated under a reflux condenser for one hour and allowed to stand overnight. The following day the mixture was acidified and titration attempted but the dark brown color which developed was so intense that the end-point was obscured. From these trials it was concluded that this method is not suitable for the assay of iodized oil.\*

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\* In a private communication, Butler, one of the co-authors of this method of analysis stated that he had not had occasion to assay iodized oil by this method.

Another method which showed promise was that of Cocking and Middleton (53). This method is as follows:

"About one gram of the oil, accurately weighed, is boiled for one hour under a reflux condenser with 10 cc. of glacial acid and 1 Gm. of zinc filings. Thirty cc. of hot water are then added down the condenser tube, and the liquid is filtered through a plug of wet cotton wool, and the flask and filter are washed with two portions of 20 cc. of water. (Note, it is not necessary that the filtrate should be clear). The combined filtrates and washings are cooled, 100 cc. of concentrated hydrochloric acid are added, and the liquid treated with M/20 potassium iodate. When the deep brown colour, which at first develops, changes to a light brown, a few cc. of chloroform are added and the titration is continued, with vigorous shaking, until the chloroform is colourless (the aqueous layer being clear yellow). Each cc. of M/20 potassium iodate is equivalent to 0.01269 Gm. of iodine."

With some samples the chloroform layer assumed a brownish color and obscured the exact end-point, which is a change from violet to colorless.

A modification of the method of titrating, produced better results. The complete method, including this modification is as follows: Weigh accurately about 0.5 Gm. of iodized oil into a 250 cc. Erlenmeyer flask, add 1 Gm. of zinc filings and 10 cc. of glacial acetic acid. Boil the mixture under a reflux condenser for one hour. Add 30 cc. of hot distilled water down the condenser tube. Filter the mixture into a 250 cc. flask, and wash the flask and filter with three 10 cc. portions of hot distilled water. Add 1 cube of ammonium carbonate. When effervescence has ceased, add 1 cc. of diiodofluorescein indicator and titrate with N/10 silver

nitrate solution until the color changes from a brownish-red to a bluish-red.

The end-point using diiodofluorescein is distinct when titrating for iodine alone, but is not very clear in the presence of chlorine. In order to make the end-point more distinct when chlorine is present, a modification of the above procedure was tried, making use of the Lang iodine cyanide titration method (54).

The above procedure was followed until the mixture was filtered, and then as follows: To the combined filtrate and washings, add 30 cc. of 25% concentrated hydrochloric acid and 5 cc. of a 10% potassium cyanide solution, and titrate with M/20 potassium iodate solution. At the start the mixture becomes dark brown and toward the end a light yellow. Now add 1-2 cc. of a freshly prepared starch solution, and titrate to the disappearance of blue color. This method gave a very distinct end-point, and the assay may be run in two or three hours. The results of these methods are shown in Table V.

Table V. Determination of Iodine by Cocking and Middleton Method (33)  
Using Silver Nitrate with Iodofluorescein Indicator, and  
Potassium Iodate with Starch Indicator.

	Silver nitrate titration with iodofluorescein indicator	Potassium iodate titration with starch indicator
Commercial Sample reported to contain 10% of iodine	9.3% 9.2 9.4 9.2 9.4 9.2	
Commercial Sample reported to contain about 26% of iodine	25.6% 24.9 27.0 27.7 26.5	26.6% 26.2 26.2 26.4
Iodinated Sesame Oil No. 1		30.4% 30.3 30.5 30.7
Iodinated Sesame Oil No. 2		29.5% 29.7
Iodinated Poppyseed Oil No. 1		33.9% 33.9 34.2
Iodinated Poppyseed Oil No. 2		33.6% 33.8 34.0

In order to check the accuracy of the titration procedure, a commercial sample which is reported to contain iodine but no other halogen, was refluxed with zinc and glacial acetic acid (page 21, and titrated by the iodate method and also by the Volhard method. The results are shown in Table VI.

Table VI. Comparison of the Iodate and Volhard Titration Methods

Volhard Method (Total halogen calculated as iodine)	Iodate Method (Iodine alone)
38.0%	38.1%
38.1	38.2
38.1	38.2

This table indicates that the sample used contained iodine but no other halogen, and that the iodate titration procedure checks well with the Volhard method.

Since there are no pure iodine compounds of the iodized oil type, the method employed for determining whether or not the iodine is quantitatively extracted from the oil was as follows (52): A solution of iodine in glacial acetic acid was made up to be about tenth normal in iodine. The iodine titre was taken and an equivalent amount of bromine was added to prepare U.S.P. Iodobromide T.S. The iodine content of 10 cc. of this solution was determined by the method outlined on page 33, 10 cc. of the solution was then permitted to stand for one hour with 0.3 Gm. of linseed oil for absorption to take place, and the iodine content was then determined by the same method. The results are given in Table VII.

Table VII. Analysis of Iodobromide Solution by Boiling with Zinc and Glacial Acetic Acid. Titration with  $KIO_3$ .

Iodobromide Solution	Iodine found (Gm./cc. of solution)
Without oil	0.0145
	0.0145
With 0.3 Gm. of linseed oil	0.0145
	0.0146
	0.0146
	0.0147

The iodine content of the iodine-acetic acid solution was found to be 0.0144 Gm. of iodine per cc. of solution. It is apparent that the procedure of boiling with zinc and acid quantitatively extracts the iodine from the oil.

It was thought advisable to compare this method with the official method of assay (fusion with sodium carbonate). A commercial sample was used, four analyses being run by each method. The results are given in Table VIII.

Table VIII. Comparison of U.S.P. XI Method with the  
Cocking and Middleton Method (53) (Page 33)

U.S.P. XI Method	Cocking and Middleton Method (see page 33)
36.8%	38.9%
37.2	39.0
37.9	39.1
38.1	39.2

The U. S. P. method gives results which are not concordant, and which are lower than the official requirement for iodine (39% to 41%). The organic matter is not completely decomposed in the fusion process, and the filtration requires some length of time if a clear, colorless filtrate is to be obtained. Besides this, there is the tediousness and danger of vigorous shaking of about 300 cc. of a solution which must contain at least 12% of hydrogen chloride after the addition of each drop of iodate solution. The method of Cocking and Middleton with the modifications as described below is, on the other hand, rapid, simple, and accurate, and is recommended as being superior to the present official procedure.

Since many of the commercial samples contain both iodine and chlorine, it was thought advisable to include a method of assay for chlorine as well as iodine. The method is given in detail below:

#### ASSAY

##### Determination of Iodine:

Weigh, accurately, about 1 Gm. of the oil into a flask, add 1 Gm. of zinc filings and 15 cc. of glacial acetic acid, and boil for 1 hour under a reflux condenser fitted with standard glass con-

nections. Add 30 cc. of warm distilled water down the condenser tube. Filter the mixture through a pledget of wet cotton into a glass stoppered 250 cc. flask and wash the flask and filter with three portions of distilled water. To the filtrate add 30 cc. of concentrated hydrochloric acid and 5 cc. of a 10% potassium cyanide solution. Titrate with M/20 potassium iodate solution, and when the color becomes a light yellow, add 1-2 cc. of a freshly prepared starch solution, and continue the titration to the disappearance of blue color. Each cc. of potassium iodate solution is equivalent to 0.01269 Gm. of iodine.

#### Determination of Chlorine:

Proceed as above until after the mixture is filtered. Then add 30 cc. of diluted sulfuric acid (10%) and 1-2 cc. of ferric ammonium alum indicator solution (40%). Run in 25 cc. of N/10 silver nitrate solution and back titrate with N/10 potassium thiocyanate solution. Continue the titration until the appearance of the first pink color which is not discharged by strong shaking.

#### Calculation for Chlorine Content:

$$(A - B) \times \left( \frac{35.457}{126.92} \right) = \% \text{ chlorine.}$$

Where A represents the % of total halogen (Volhard) calculated as iodine.

B represents the % of iodine (Iodate Method).

Analyses of a commercial chloro-iodized oil reported to contain about 26% of iodine were run. The results are shown in Table IX.



Table IX. Results of analyses of a Commercial Sample of Chloro-iodized Oil by the Assay Method Given on Page 33.

	Iodine (Iodate Titration)		Total Halogen as Iodine (Volhard Method)		Chlorine (calculated)
Sample 1.	26.2%	26.2%	52.1%	52.7%	7.4%
	26.3		53.7		
	26.1		52.3		
Sample 2.	26.1%	26.2%	52.5%	51.9%	7.2%
	26.3		51.6		
	26.2		51.7		

The results of these analyses indicate that the product contains approximately an equivalent amount of iodine and chlorine.

### Part III. Fluorinated Iodine Compounds.

Since the opacity to X-ray of any iodine compound is directly proportional to the amount of iodine contained therein (see page 8 of this thesis), it was decided to prepare some organic iodine compounds of high iodine content for possible use as X-ray contrast media.

Vaeder Leonard (55) in his work on resorcinols stated that, "the secretion of bactericidal urine depends on the small quantities of unchanged substance (hexylresorcinol) appearing in the urine." On the basis of this statement it was deemed advisable to prepare some iodoresorcinols for possible use in X-ray diagnosis of the urinary tract, since some of the compound might be excreted unchanged.

4-Propyl resorcinol was prepared by the method of Dohme, Cox and Miller (56). Attempts to iodinate this compound by the method of Stenhouse (57) produced a brown insoluble compound which could not be crystallized. Attempts to prepare 4-allyl resorcinol, for iodination of the alkyl chain, from allyl alcohol and resorcinol resulted in the formation of a plastic mass from which no pure compound could be isolated.

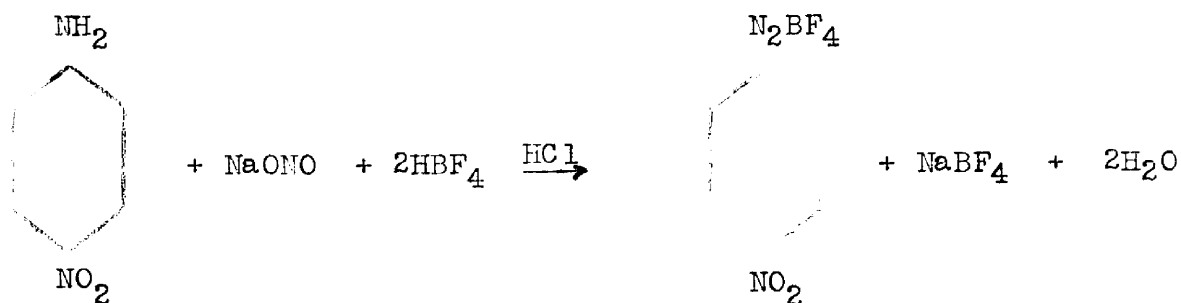
Since these results were disappointing, it was decided to discontinue further attempts to prepare a radiopaque medium from this type of compound. In view of the findings of Midgely and Henne (58) and Henne (59, 60) on the low toxicity of dichloro-difluoromethane (Freon 12) and fluorofom, further research along this line was directed toward the preparation of some fluorinated aromatic iodine compounds to determine whether or not the addition of fluorine would stabilize the compounds and thus make them less toxic than the iodine compounds themselves.

Lang (61), in 1930, investigated the pharmacologic activity of fluoroacetanilid, fluorobenzene, and p-fluorotoluene on rabbits. He found that these compounds did not lead to storage in the organism, except in the heart. He states "They have no specific pharmacologic effect on the heart. p-Fluorotoluene is excreted as p-fluorobenzoic acid. The fate of fluorobenzene is as yet unknown."

Accordingly, it was decided to prepare o-, m-, p- iodofluorobenzene for investigation as to possible use as X-ray contrast media in the urinary tract.

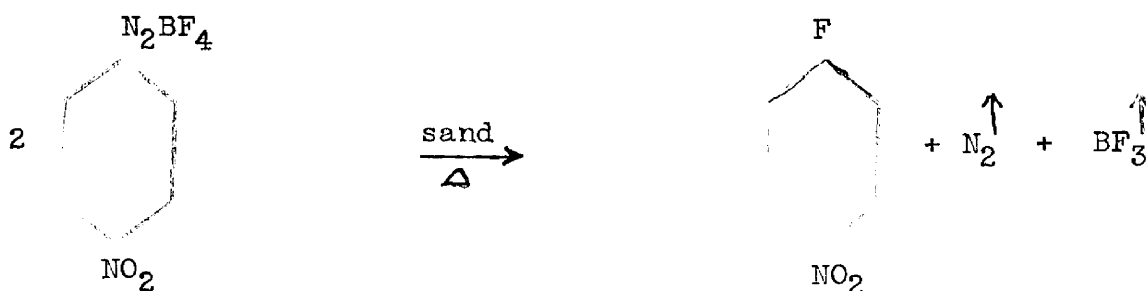
#### A. Chemical Preparation.

Preparation of p-nitrobenzene diazonium borofluoride. The method used was that of Balz and Schiemann (62).



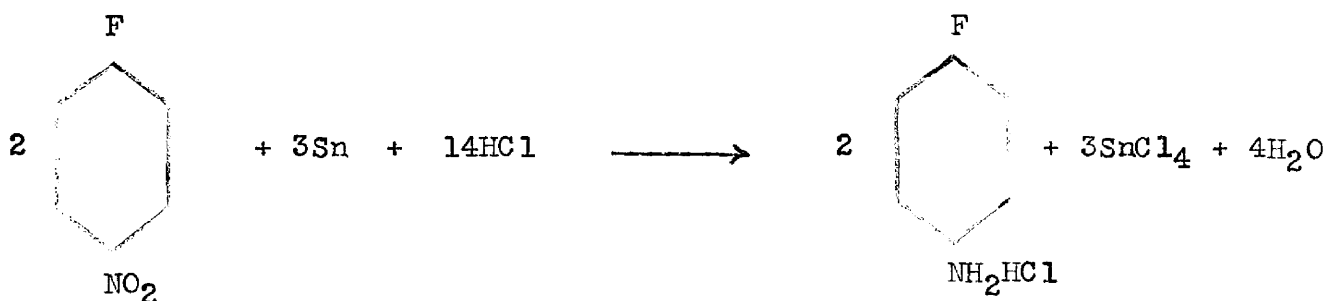
Dissolve 138 Gm. (1 mol) of p-nitraniline in 206 cc. of concentrated hydrochloric acid (36%) in a 1 L. beaker with the aid of mechanical stirring. Place the beaker in an ice bath, and, while stirring vigorously, add slowly from a dropping funnel a solution of 60 Gm. of sodium nitrite in 140 cc. of water. Maintain the temperature of the mixture at about 5°. When the addition is complete, add, all at once, 200 cc. of fluoroboric acid (40% Harshaw). Stir the mixture for a few minutes and filter through a fritted glass funnel with the aid of suction. When the liquid has been removed, wash the solid with about 200 cc. of alcohol. Repeat the washing with about 100 cc. of alcohol, and then with ether until the solid is a light yellow and the wash-ether is almost colorless. The solid should be thoroughly mixed with each portion of alcohol and ether. Dry the solid as completely as possible by passing air through it, and place in a desiccator over phosphorous pentoxide overnight. The product is a light powder, and the yield is practically quantitative (230 Gm.).

Preparation of p-nitrofluorobenzene. The method followed was that of Schiemann and Pillarsky (63).



Mix 111 Gm. of p-nitrobenzene diazonium borofluoride with about three times its volume of sand. Place about one-fifth of this mixture in a 500 cc. distilling flask having the delivery tube bent so that the flask may be clamped in an almost horizontal position. The delivery tube is connected with a condenser and the distillate is collected in a cooled 500 cc. suction flask. The suction flask is connected with tubing of large diameter extending into a hood. Heat the flask slowly, beginning at the surface and working down slowly as the compound is decomposed. When the decomposition is complete, heat strongly to drive over the last traces of p-nitrofluorobenzene. The sand is shaken out after cooling for use in subsequent decompositions. Shake the distillate with two portions of sodium carbonate solution to remove dissolved boron trifluoride, and steam distill the oily layer. 37 Gm. of p-nitrofluorobenzene were obtained (56% yield).

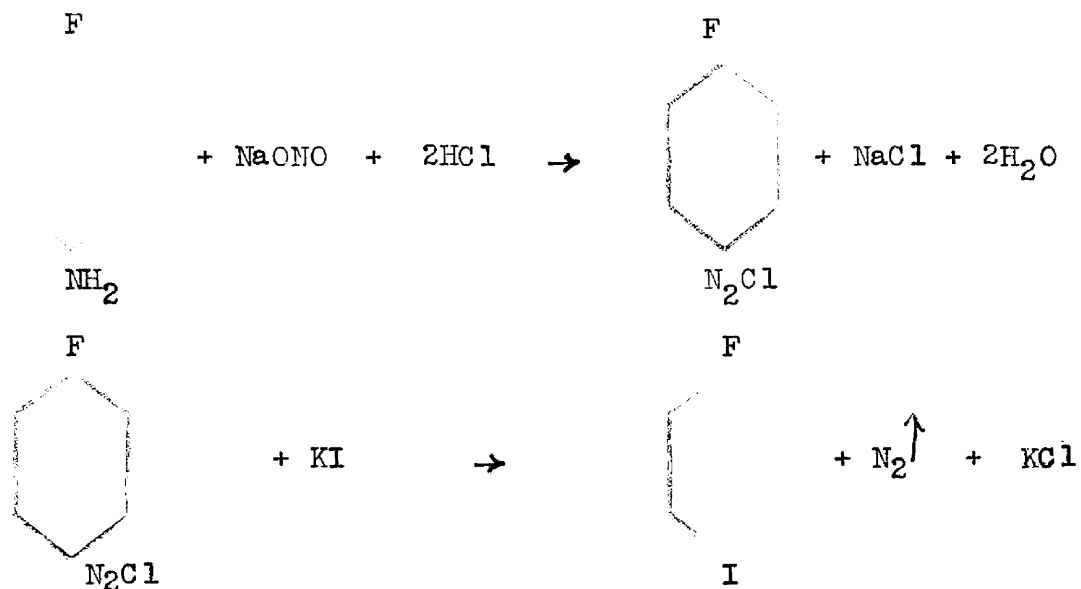
Preparation of p-fluoroaniline. Reduce the p-nitrofluorobenzene by



means of tin and hydrochloric acid (64). Add 75 Gm. of tin to 37 Gm. of p-nitrofluorobenzene in a three-necked flask fitted with a reflux condenser, mechanical stirrer and a dropping funnel. Add slowly, from the funnel, 150 cc. of hydrochloric acid with constant stirring, cooling the flask when the mixture becomes too warm. After complete addition of the acid (about one hour), reflux the mixture for 30 minutes and allow to

cool. Add 150 cc. of water and a solution of 135 Gm. of sodium hydroxide in 225 cc. of water, and steam distill the liquid. Separate the amine. The yield was 24 Gm. or 83% of the theoretical.

Preparation of p-iodofluorobenzene. Add 26 cc. of concentrated sulfuric



acid to 100 cc. of water in a 400 cc. beaker and add 24 Gm. of p-fluoroaniline while still warm. Place the beaker in an ice-salt bath (maintain the temperature at about 0°), and add slowly, with constant stirring, 20 Gm. of sodium nitrite dissolved in 50 cc. of water. Continue the addition of the nitrite solution until one drop of the reaction mixture gives an immediate blue color with moistened starch-iodide paper. Now add slowly 40 Gm. of potassium iodide solution in 50 cc. of water. After the addition is complete, continue the stirring for about one hour maintaining the reaction mixture at about 0°. Place the beaker in a water bath a room temperature, and slowly raise the temperature to 70° to completely drive off the nitrogen gas. Make the mixture strongly alkaline with sodium hydroxide and steam distill to obtain the p-iodofluorobenzene. The yield of the oil was 24 Gm. or 50% of the theoretical. Add anhydrous sodium sulfate to the iodofluorobenzene, let stand for one-half hour, filter and

distill under reduced pressure. The p-iodofluorobenzene distilled between 75 and 85° at 12 mm. pressure. The product obtained was almost colorless with a very slight yellow tinge. The boiling point was 184°. <sup>1</sup>

Preparation of m-iodofluorobenzene. The procedure outlined above was followed throughout. 138 Gm. of m-nitraniline were diazotized. The yield of m-nitrobenzene diazonium borofluoride was 90% of the theoretical. Decomposition of 138 Gm. of the diazonium compound gave a yield of 55 Gm. of m-nitrofluorobenzene, corresponding to 38% of the theoretical. Reduction of 50 Gm. of the nitre compound produced 24 Gm. of m-fluoroaniline, a yield of 70%. This compound was diazotized and treated with potassium iodide to yield 41 Gm. of m-iodofluorobenzene or 85% of the theory. The liquid distilled at 82-84° at 30 mm. pressure.

The boiling point of the redistilled compound was 182°. <sup>2</sup>

Analysis of the compound for iodine by the Stepnow method gave the following:

$C_6H_4FI$ : Calc. for iodine, 57.18%; found, 57.24%.

Preparation of o-fluoronitrobenzene. The above procedure was followed in preparing o-fluoronitrobenzene. The yield of c-nitrobenzene diazonium borofluoride was almost quantitative, but the yield of o-nitrofluorobenzene by decomposition with sand was only 12%. <sup>3</sup>

Since the decomposition resulted in a great amount of tar forma-

- 
1. Reported 182-184° Wallach and Hensler, Ann., 243 (1889). 228.
  2. m-Iodofluorobenzene could not be found in the literature.
  3. A yield of 12.5% was reported by M. F. W. Dunker, Ph. D. thesis, University of Maryland, 1939.

tion, it was decided that the diazonium compound be dropped on an inert solvent, the temperature of which could be maintained at about  $10^{\circ}$  above the decomposition point of the compound.

Tetralin (tetrahydroanaphthalein) was the solvent used. 200 cc. of tetralin were placed in a 2 L. flask and the solvent brought to a temperature of  $175^{\circ}$ , (the decomposition point of m-nitrobenzene diazonium borofluoride is  $160-165^{\circ}$ ). A strong current of air was passed through the solvent during the decomposition to facilitate the removal of boron trifluoride. The diazonium compound was added in small portions (about 5 Gm. each) into the hot solvent. After the addition of 25 Gm. of the diazonium compound to the hot solvent, the temperature was maintained at  $175^{\circ}$  for one hour. No compound could be obtained from this mixture by either direct distillation or steam distillation.

On the next run, 120 Gm. of m-nitrobenzene diazonium borofluoride were added to 300 cc. of the hot solvent as outlined above. After cooling, 30 Gm. of tin were added to the mixture, and 60 cc. of concentrated hydrochloric acid were dropped in slowly while the mixture was vigorously stirred. After addition of the acid, the mixture was stirred for 30 minutes. The mixture was allowed to cool, and the amine hydrochloride was extracted with several portions of water. The combined aqueous extractions were made alkaline with sodium hydroxide pellets, and the free amine was extracted with several portions of benzene. The benzene layer was dried and the hydrochloride salt of the amine was precipitated by passing dry hydrogen chloride through the benzene solution. A yield of 13.5 Gm. of white crystals was obtained (18% of the theoretical).

The acetanilid of this compound was formed, which melted at 83-84°. <sup>1</sup>

The poor yields obtained by this procedure of decomposition made the method impractical.

Before continuing work on the synthesis of these fluorinated compounds, it was thought advisable to determine by toxicological experiments, whether or not the presence of fluorine in the compound would show an advantage by lowering the toxicity of the iodine compound.

### B. Toxicity Studies

m-Iodofluorobenzene was chosen for the toxicity experiments because it was obtained in fairly good yields and appeared to be stable, i. e. did not develop any discoloration on standing. This compound was compared with iodobenzene. <sup>2</sup>

The toxicity experiments were run on white rats, all doses being given orally in solution in pure cottonseed oil. Seven rats were used on each dose. The doses were given at equal log. intervals for the purpose of computing the ID 50 by the Karber method. (65).

The experimental data are listed in tables X and XI.

- 
1. Reported 88° Braun, Rudolf, Ber., 64 (1931), 2470.  
84.6° Ingold, Vass, J. Chem. Soc., (1928), 421.  
83° Schiemann, Z. physik. Chem., 156A (1931), 418.
  2. Soluble Iodophthalein was used in the first experiments, but since no deaths were observed even on a dose of 552 mgm. per 100 Gm. rat, its use was discontinued.



Table X. Toxicity Data on m-Iodofluorobenzene

Dose (mgm./100 Gm. Rat)	Rat #	Weight	Death Occurred	Mortality
71	1-7	180 Aver.	All animals survived	0/7
100	8	180	S	1/7
	9	150	S	
	10	140	28 days	
	11	185	S	
	12	260	S	
	13	210	S	
	14	260	S	
141	15	170	S	5/7
	16	180	3 days	
	17	135	3 days	
	18	230	11 days	
	19	170	S	
	20	180	3 days	
	21	150	1 day	
199	22	150	3 days	7/7
	23	100	2 days	
	24	135	1 day	
	25	195	12 days	
	26	170	1 day	
	27	205	1 day	
	28	150	3 days	

S indicates survival of the animal for at least 60 days after injection.

Table XI. Toxicity Data on Iodobenzene

DOSE (mgm./100 Gm. rat)	Rat #	Weight	Death Occurred	Mortality
70.5	29-35	190 (Aver.)	All animals survived	0/0
140	36	170	S	1/7
	37	190	S	
	38	140	19 days	
	39	180	S	
	40	185	S	
	41	190	S	
	42	190	S	
278	43	225	2 days	6/7
	44	250	1 day	
	45	185	9 days	
	46	180	6 days	
	47	290	1 day	
	48	190	3 days	
	49	190	S	
552	50	220	1 day	7/7
	51	170	1 day	
	52	180	2 days	
	53	160	1 day	
	54	205	1 day	
	55	190	1 day	
	56	190	1 day	

S indicates survival of the animal for at least 60 days after injection.

## Karber's Method of Calculating the L D 50

m-Iodofluorobenzene

Dose (mgm./100 Gm. rat)	71	100	141	199
Log. of doses	1.8513	2.0000	2.1492	2.2989
Log differences ①	0.1487	0.1492	0.1487	
Mortality	0/7	1/7	5/7	7/7
Diff. in Mort. ②	0.5	3.0	6.0	
Prod. ① * ②	0.07435	0.4476	0.8922	
Sum of Prod.		1.41415		
<u>Sum of Prod.</u> No. of animals per dose		③ = $\frac{1.41415}{7}$	= 0.20202	

$$\text{Log LD 50} = \text{log largest dose} - \text{③} = 2.2989 - 0.20202 = 2.0969$$

$$\text{Log LD 50} = 2.0969$$

$$\text{LD 50} = 125 \text{ mg./100 Gm. rat.}$$

Iodobenzene

Dose (mgm./100 Gm. rat)	70.5	140	278	552
Log of doses	1.8482	2.1461	2.4440	2.7419
Log differences ①	0.2979	0.2979	0.2979	
Mortality	0/7	1/7	6/7	7/7
Diff. in Mort. ②	0.5	3.5	6.5	
Prod. ① * ②	0.1490	1.0426	1.9364	
Sum of Prod.		3.1280		
<u>Sum of Products</u> No. of animals per dose		③ = $\frac{3.1280}{7}$	= 0.4469	

$$\text{Log LD 50} = \text{log largest dose} - \text{③} = 2.7419 - 0.4469 = 2.2950$$

$$\text{Log LD 50} = 2.2950$$

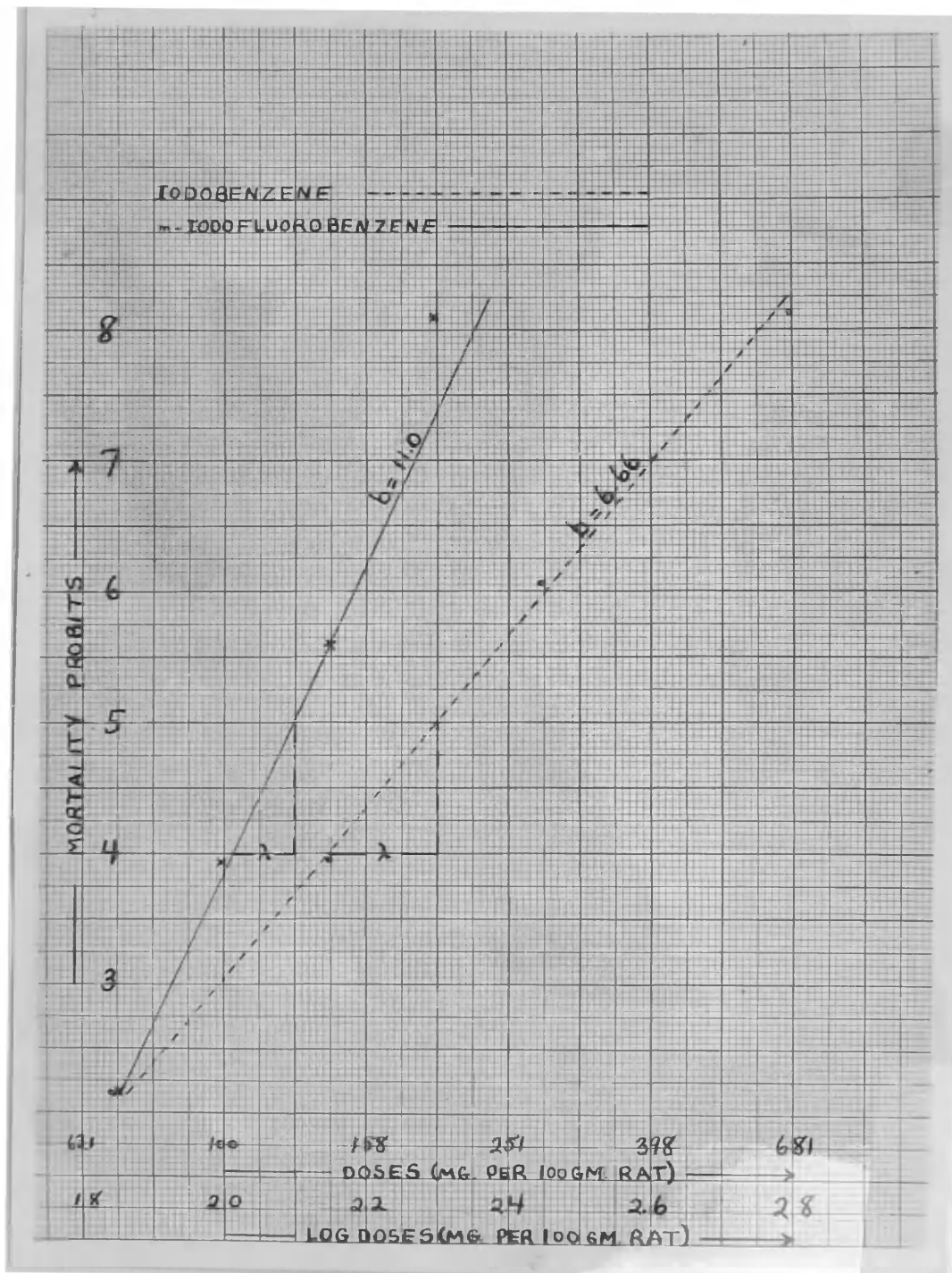
$$\text{LD 50} = 197.2 \text{ mg./100 Gm. rat.}$$

From a comparison of the LD 50 doses, it is evident that it required 36.6% less of the m-iodofluorobenzene than of the iodobenzene to produce a 50% mortality. Apparently the fluorinated compound is more toxic than the non-fluorinated compound.

To determine whether or not there is a significant difference in the LD 50 doses, the dose-mortality curves for the two compounds were plotted. The ordinates are mortality probits and the abscissae are log doses. The graph is shown on page 47.

Fig. 3 - Dose-Mortality Curves for m-Iodofluorobenzene and Iodobenzene

<u>m-Iodofluorobenzene</u>				<u>Iodobenzene</u>			
<u>Dose</u>	<u>Log dose</u>	<u>Mortality</u>	<u>Probit</u>	<u>Dose</u>	<u>Log dose</u>	<u>Mortality</u>	<u>Probit</u>
71	1.8513	0/7	2.146	70.5	1.8482	0/7	2.146
100	2.0000	1/7	3.933	141	2.1461	1/7	3.933
141	2.1492	5/7	5.566	278	2.4440	6/7	6.067
199	2.2989	7/7	8.123	552	2.7419	7/7	8.123



From the graph the following figures are calculated:

m-Iodofluorobenzene

Log LD = 2.098 ; ID 50 = 126 (125 by Kärber's Method)

Difference Probits 4 and 5 = 126 -- 103 = 23 = (Std. Deviation)

$$m \pm \delta = 126 \pm 23$$

$$\epsilon = \frac{23}{\sqrt{7}} = 8.70 \text{ (Standard error)}$$

Iodobenzene

Log ID 50 = 2.3000 ; ID 50 = 200 (197 by Kärber's Method)

Difference Probits 4 and 5 = 200 -- 141 = 59 = (Std.Deviation)

$$m \pm \delta = 200 \pm 59$$

$$\epsilon = \frac{59}{\sqrt{7}} = 30.3 \text{ (Standard error)}$$

The difference is calculated by the formula (65):

$$\frac{m_1 - m_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}} = \frac{75}{23.8} = 3.008$$

Where  $m_1$  = LD 50 for m-Iodofluorobenzene

$m_2$  = " " " Iodobenzene

$\epsilon_1$  = Std. error for m-Iodofluorobenzene

$\epsilon_2$  = " " " Iodobenzene

The figure 3.008 shows that (for P = 0.05) the difference in the ID 50 doses is probably significant, i.e., m-Iodofluorobenzene may be more toxic than Iodobenzene.

The b value is calculated by the following formula: (65)

$$b = \frac{1}{\lambda}$$

Where  $\lambda$  represents the log differences between probits 4 and 5.

(see chart).

m-Iodofluorobenzene

$$\frac{1}{\lambda} = \frac{1}{.0914} = \underline{11.0}$$

Iodobenzene

$$\frac{1}{\lambda} = \frac{1}{0.15} = \underline{6.66}$$

The difference in the b values for these two compounds probably indicates that the manner of death caused by the two compounds is different. Further work on the pathology of these compounds is required to confirm this statement.

## SUMMARY AND CONCLUSIONS

1. A short history of the use of iodine compounds as X-ray contrast media is given.
2. The opacity to X-rays of different type iodine compounds was determined. It is concluded that the opacity is directly proportional to the iodine content and is independent of the structure of the compound.
3. Several methods of preparation of iodized oils were tried. A modification of the method of Schou and Jacobsen was found to be the most practical. Iodized sesame oil and iodized poppyseed oil were prepared by this method. The products assayed approximately 30% for the sesame oil compound and approximately 34% for the poppyseed oil compound. A higher iodine content could not be obtained by any modifications of this method, or by any other method tried. It is concluded that it is not practical to make a product with the iodine content specified by the U. S. P. XI (38%-42%) by any published method available. It would therefore seem that this standard should be lowered.
4. The U. S. P. XI assay for iodized oil was found to give inconsistent results. Other assay methods were therefore investigated. The procedure which was found to give the most accurate and consistent results is essentially a combination of the Cocking and Middleton method with the Lang iodine cyanide titration procedure.
5. Meta- and para- iodofluorobenzene were prepared for possible use as contrast media in the urinary tract. The toxicity of m-iodofluoro-



benzene for rats was compared with that of iodobenzene with the following results:

	<u>L D 50</u>	<u>b value</u>
Iodobenzene	200 ± 59	6.66
m-Iodofluorobenzene	126 ± 23	11.00

It required 36.6% less of the fluorinated compound than the non-fluorinated compound to produce a 50% mortality. The difference, 3.008, which is probably significant for  $P=0.05$ , shows that the fluorinated compound may be more toxic than the non-fluorinated compound.

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