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THE PROPERTIES OF *n*-PROPYL 3:5-DI-IODO-4-PYRIDONE-N-ACETATE (PROPYLIODONE)

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The suggestion to use organic iodine compounds for visualizing the bronchial anatomy was first made by Waters, Bayne-Jones, and Rowntree (1917). They reported experiments on dogs in which a mixture of iodoform and olive oil had shown considerable promise. Human bronchography first became practicable in 1922 when Sicard and Forestier employed iodized poppy-seed oil. Chemically similar contrast media used in bronchography have been iodized sesame or arachis oils and ethyl di-iodobrassidate. Viscous aqueous solutions of diodone were first used by Titus, Tafel, McCullen, and Messer (1938, 1939). Contrast media of this type were designed to overcome the delayed lung clearance, characteristic of iodized oils, by using the concentrated aqueous solution of an organic salt. Their use involves administration of a very hypertonic solution which causes instantaneous "flooding" and a fleeting bronchogram.

It occurred to the authors that a suspension of a contrast medium which was slightly soluble in body fluids should overcome the major disadvantages of both iodized oils and diodone solutions, viz., delayed lung clearance and hypertonicity respectively.

We have examined several contrast media for their solubility in saline and their toxic effects on the lung. Practically all were rejected for one or both reasons. Diodone was the least irritant, but its solubility was too great. Since diodone is the diethanolamine salt of 3:5-di-iodo-4-pyridone-N-acetic acid, a search was made for a suitable derivative of the present acid. Among the derivatives examined were the alkyl esters, which appeared to satisfy both initial requirements. Finally the *n*-propyl ester was chosen, and the following is an account of the pharmacological and metabolic investigations carried out with it.

MATERIALS AND METHODS

The normal propyl ester of 3:5-di-iodo-4-pyridone-N-acetic acid,

(propyliodone, Dionosil brand), is a white crystalline solid, m.p. 186–7° C. (with decomp.). It is readily sterilized by dry heat and will withstand a temperature of 150° C. for at least two hours without undergoing any detectable changes in composition. It is slightly soluble in saline and serum. The solubilities in water at 15, 35, and 95° C. are 0.014, 0.020, and 0.110 g./100 ml. respectively.

The pulverized crystals can be suspended either in aqueous media with a mixture of sodium carboxymethyl-cellulose and a suitable wetting agent, or in vegetable oils. The terms "aqueous propyliodone" and "oily propyliodone" employed throughout the text refer to 50% w/v suspensions in an aqueous medium and in arachis oil respectively.

Toxicity

Intratracheal.—The aqueous and oily suspensions were injected into the trachea (0.4 ml. per kg. body weight) at 15-minute intervals into rabbits anaesthetized with a combination of sodium pentobarbitone and chloralose. The animals tolerated four to five doses before becoming asphyxiated.

Two groups each of 18 rabbits were injected through the crico-thyroid membrane, the first receiving aqueous propyliodone and the second control group the aqueous vehicle. A third group of 18 rabbits was injected with oily propyliodone. Two more groups of 12 rabbits each were injected, one with iodized poppy-seed oil and the other with a viscous preparation of diodone (50% w/v). Each rabbit received 1 ml. of the appropriate substance. At intervals of 16 hours, 3 days, 5 days, 2 weeks, and monthly up to 5 months after the injection, two rabbits from each of the first three groups were killed, and the lungs were examined both macro-

scopically and histologically. Sections of the lungs from all 36 rabbits in the first two groups were fixed and stained for sodium carboxymethyl-cellulose by a modified Feyrter method (Vischer, 1951). The lungs from the rabbits in the third group were fixed, and frozen sections were prepared and stained with "Oil Red 4B." Macroscopic examinations were carried out at intervals on animals from the fourth and fifth groups.

Bronchography in Rabbits.—Comparative bronchographic studies were carried out in rabbits with the new compound, iodized poppy-seed oil, and a viscous preparation of diodone (50% w/v). Both aqueous and oily propyliodone were investigated lest the aqueous suspension should provoke excessive coughing.

The appropriate contrast medium (0.6 ml.) was administered to rabbits anaesthetized with sodium pentobarbitone. The medium was injected either into the trachea through the crico-thyroid membrane or directly into the bronchial tree by means of an oral catheter. Local anaesthesia was not practised, as it would depress the cough reflex, which serves as a guide to the cough-promoting properties of the various preparations. Not unexpectedly it was found that the aqueous suspension was somewhat more irritant than the oily one, although the extra coughing induced was not excessive and in no way impaired the quality of the bronchogram. Iodized oils caused as much coughing as the oily suspension. Diodone invariably produced violent and persistent coughing. The progress in the lung of the various media was followed on a fluoroscopic screen.

Animal Metabolism Studies

Excretion Rate and Serum Levels in the Rabbit.—Aqueous propyliodone (0.8 ml.) was administered to female rabbits by the intratracheal or oral route. To facilitate excretion studies the compound was labelled with I_{131}. Hourly blood samples were taken for the first six hours from the animals placed in metabolism cages, and the urines were collected until they were no longer radioactive. At the end of the experiment the animals were killed and the faeces, alimentary tract, and major organs were examined for radioactivity.

Chromatographic Identification of Metabolite.—Urine samples were collected from rats that had previously received oral doses of labelled propyliodone. The undiluted urines were spotted on filter paper (Whatman No. 2) and developed with saturated sodium bicarbonate by the ascending technique. After drying, the paper strips were examined in ultra-violet light. The same strips were also used to develop radio-autographs.

Human Metabolism Studies

Excretion Rate after Bronchography.—Aqueous propyliodone (22 ml. of 50% w/v suspension) was injected into a patient through the crico-thyroid membrane. During the next 82 hours urine was collected. The volumes were recorded and aliquots analysed for total iodine by the method of White and Rolf (1940). Sputum samples were not collected. All urine samples were tested for free iodine (starch) and iodide ion (starch-nitrite).

Isolation and Identification of Metabolite in Human Urine.—A second patient was injected by the crico-thyroid technique with 20 ml. of oily propyliodone. It was not possible to collect his urine for more than 28 hours. The excreted metabolite was identified as 3:5-di-iodo-4-pyridone-N-acetate ion as follows (Baker and Briggs, 1943):

250 ml. of urine (3.73 mg. I/ml.) were made alkaline (*p*H 10) and filtered. The filtrate was saturated with sodium chloride and the resulting precipitate separated on a centrifuge. The mother liquors contained 8 μg. I/ml. The precipitate was washed twice with saturated brine and dissolved in distilled water. After adjusting the resultant solution to *p*H 3.5 with HCl, white crystals separated. Final purification of the material was achieved by dissolving the crystals in boiling water and cooling the solution in a refrigerator overnight. The resultant solid was separated, dried to constant weight, and analysed. The equivalent weight of the acid was determined by titrating an aliquot against standard NaOH solution. The m.p. was compared with that of the laboratory standard. Isotope-dilution experiments were carried out on a bulked sample of the urine.

Excretion Rate after Oral Administration.—Solid propyliodone (10 g.) was ingested by a volunteer and urine samples were collected during the next 10 hours. The samples were assayed for organic iodine. Here again isotope-dilution experiments were performed on a bulked sample.

RESULTS

Toxicity

Intratracheal.—After administration of aqueous propyliodone and the aqueous vehicle, macroscopic signs of lung irritation were apparent at 16 hours and reached a maximum at three days. After two weeks the lungs appeared normal. They showed the same degree of congestion after the propyliodone or its aqueous vehicle, but in no instance was the congestion greater than was observed in the animals dosed with iodized oil. Microscopic examination revealed a few scattered foci of round cell infiltration in the interstitial tissue adjoining the small bronchi of the rabbits killed 16 hours, three days, five days, and one month after instillation of propyliodone. Small deposits of carboxymethyl-cellulose could be demonstrated during the first few days in the mucosa and cartilage of small bronchi and in the alveoli. Traces were seen in the few macrophages of the inflammatory foci in the interstitial tissue. In no animal could sodium carboxymethyl-cellulose be demonstrated after five days.

Macroscopic examination of the lungs after administration of oily propyliodone showed that congestion was greatest at three days and absent after two weeks. In none of the rabbits was the degree of congestion greater than was seen in animals treated with iodized oil. Droplets of non-opaque oil were present in the bronchi and alveoli up to five days after injection, but later there was no evidence of residual oil.

In those rabbits which received iodized poppy-seed oil marked congestion of the bronchial mucosa had developed after 16 hours and was most apparent in the small bronchioli. After one week the degree of congestion was undiminished and the lungs still contained appreciable amounts of iodized oil visible to the naked eye. Presumably the lung changes continued for varying periods depending on the persistence of the oil. Compared with iodized oils, vegetable oils are cleared rapidly from the healthy rabbit lung; iodine is slowly liberated by the former. There may be some connexion between these two phenomena.

Diodone preparations caused immediate flooding of the lung tissue with serous fluid, and by the time this had been resorbed (2 hours) erythema and swelling of the mucosa were pronounced. Slight interstitial oedema persisted for five days.

Oral.—Propyliodone was administered as a single oral dose of 18 g./kg. to mice. No immediate toxic symptoms were observed and thereafter growth rates were normal. Fluoroscopic examination revealed a radio-opaque bladder and no contrast medium in the colon.

Intravenous.—Aqueous propyliodone was injected intravenously into forty mice in a dose of 120 mg./kg. The mice were disorientated at first, but recovered within an hour. Their subsequent behaviour was normal. The LD50 for mice by this route was 300 mg./kg. Death was almost certainly due to occlusion of blood vessels.

Subcutaneous, Intramuscular, and Intraperitoneal.—Injection of mice and rabbits by these routes with large doses of aqueous or oily propyliodone (1 ml.) produced no signs of systemic toxicity. Invariably unabsorbed contrast medium was found at the injection site. Absorption by these routes must be extremely slow.

Bronchography in Rabbits

Figs. 1, 2, and 3 are of bronchograms taken immediately, two hours, and three days respectively after administration of propyliodone. It will be seen that no alveolar filling had occurred after two hours and that all contrast in the lungs had disappeared within three days. The bronchograms shown are typical of both the aqueous and oily media. Although many experiments have been performed in rabbits with these two preparations, alveolar filling has not yet been encountered. On many animals a further bronchographic examination was made after three days (i.e., when all traces of the first dose had disappeared), and invariably the second bronchogram was identical with the first. Presumably serial bronchography should present no difficulties in the human patient.

Figs. 4, 5, and 6 were obtained with iodized poppy-seed oil. They show all the characteristics of the immediate, fifteen-minute, and three-day bronchograms respectively. When coughing occurred during administration, it was immediately succeeded by alveolar filling.

All efforts to obtain a satisfactory bronchogram with a viscous solution of diodone (50% w/v) were unsuccessful.

Animal Metabolism Studies

Excretion Rate and Serum Levels in the Rabbit.—Figs. 7 and 8 show the urinary excretion rates of I_{131} in the animals dosed by the intratracheal and oral routes respectively. In both a minimum of 95% was recovered within 72 hours. In no instance during the first six hours was the total iodine concentration in the serum greater than 0.01% (calculated as propyliodone). At the end of the experiment the faeces, alimentary tract, and major organs were not radioactive. Apart from traces in the lung and urinary tract, no radioactive iodine remained in the animals that had been injected by the intratracheal route.

Chromatographic Identification of Metabolite.—On examining the paper strip in ultra-violet light, a single fluorescent purple spot (Rf 0.68) was observed, corresponding with sodium 3:5-di-iodo-4-pyridone-N-acetate. Radio-autographs developed from these paper strips revealed the presence of a single radioactive spot coinciding with the fluorescent spot. When chromatographed under these conditions, propyliodone remained at the site of application.

Human Metabolism Studies

Excretion Rate after Bronchography.—It will be seen in Fig. 9 that a steady elimination of iodine-containing metabolite, totalling 50%, occurred in the first 72 hours. The absence of any lag in excretion was shown by the appearance of organic iodine in the first voiding, suggesting that no secondary accumulation of the compound or its metabolite occurs in the body. All tests for iodine or iodide ion in the urine gave negative results.

Rabbit's bronchograms after administration of propyliodone

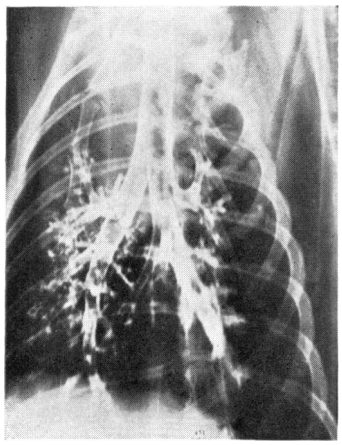

FIG. 1.—Immediately.

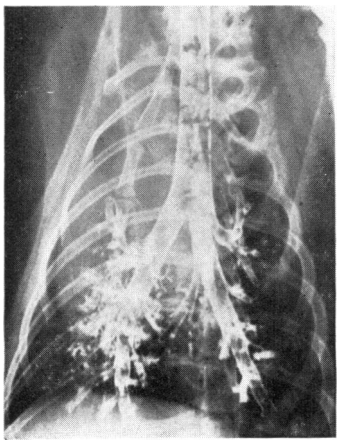

FIG. 2.—2 hours.

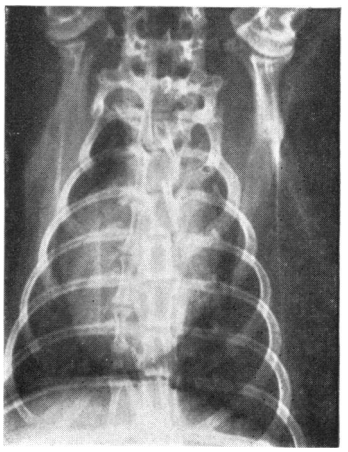

FIG. 3.—3 days.

Rabbit's bronchograms after administration of iodized poppy-seed oil

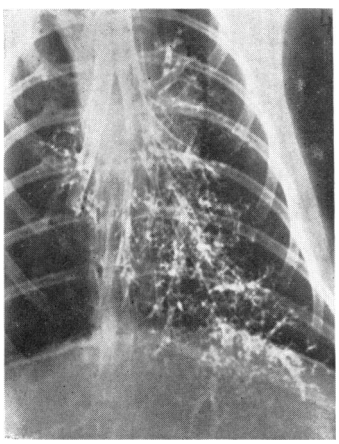

FIG. 4.—Immediately.

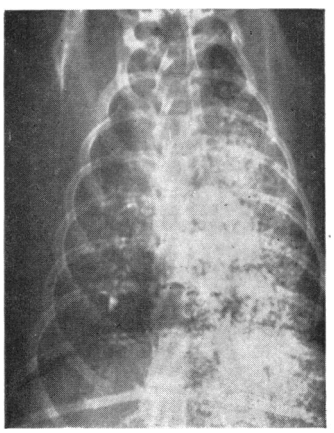

FIG. 5.—15 minutes.

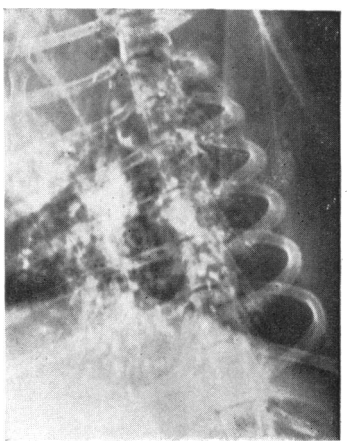

FIG. 6.—3 days.

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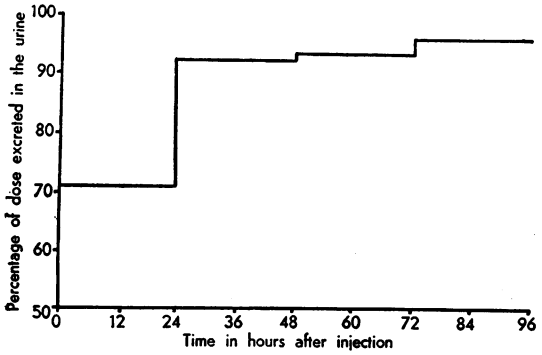

FIG. 7.—Urinary excretion in rabbits of 3:5-di-iodo-4-pyridone-N-acetate ion after intratracheal administration of propyliodone.

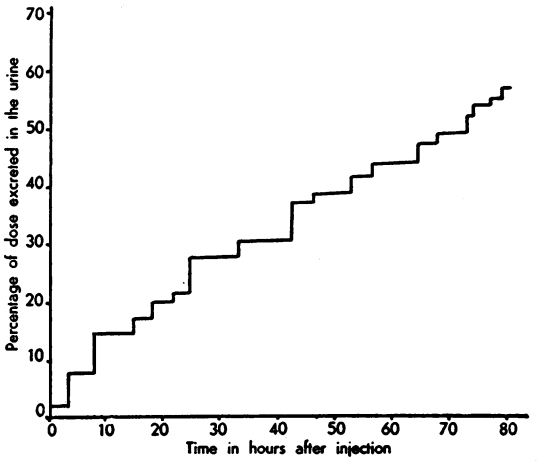

FIG. 9.—Urinary excretion in man of 3:5-di-iodo-4-pyridone-N-acetate ion after intratracheal administration of propyliodone.

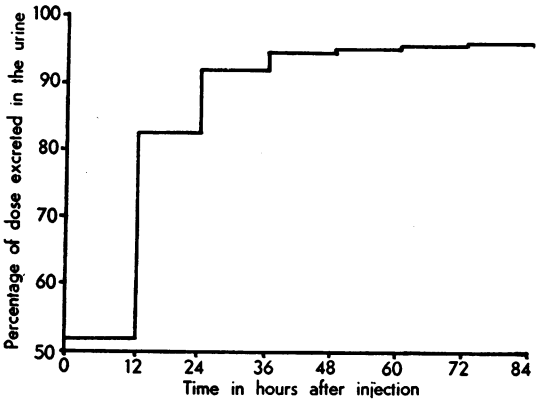

FIG. 8.—Urinary excretion in rabbits of 3:5-di-iodo-4-pyridone-N-acetate ion after oral administration of propyliodone.

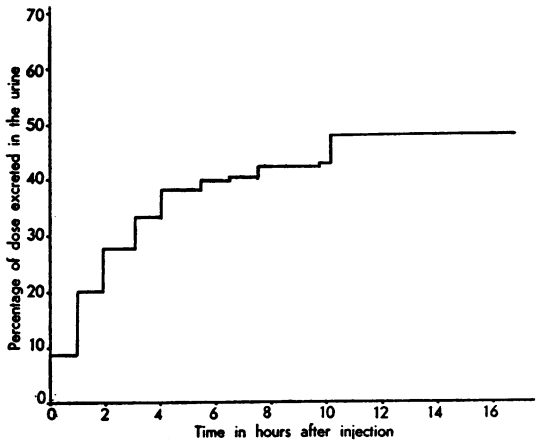

FIG. 10.—Urinary excretion in man of 3:5-di-iodo-4-pyridone-N-acetate ion after oral administration of propyliodone.

Isolation and Identification of Metabolite in Human Urine.—The weight of pure acid finally obtained was 754 mg. (I=62.73%) which represented a recovery from the urine of 50%. Analysis gave C, 20.40; H, 1.06; N, 3.25; I, 62.73% (calculated for $C_7H_5O_3NI_2$: C, 20.76; H, 1.24; N, 3.46; I, 62.68%). The equivalent weight of the acid was 403 (calculated for $C_7H_5O_3NI_2$, 405). The crystals melted at 242° C. (decomp.), whilst the laboratory standard melted at 243° C. No depression of the mixed melting point was observed. The isotope dilution experiments confirmed the presence in the urine of only one metabolite containing iodine.

Excretion Rate after Oral Administration.—After the ingestion of 10 g. of propyliodone, no nausea or other untoward reactions were observed. The rate of organic iodine excretion by the kidney is shown in Fig. 10. Here again the isotope dilution experiments proved that only one iodine-containing metabolite (the anion of the parent acid) was present in the urine.

DISCUSSION

Unlike all other contrast media known at the time of its introduction, iodized poppy-seed oil was non-irritant in the lung and could be easily introduced into the trachea. It has found universal acceptance without serious rival for nearly thirty years. Nevertheless, it has serious disadvantages which detract from its value and restrict its use. Alveolar filling, commonly encountered, especially if coughing occurs during administration, results in obscured bronchograms. This would not be so serious if repeat bronchography were possible. Depending as it does on a combination of postural drainage, expectoration, and phagocytosis, complete lung clearance is often long and unpredictably delayed, making serial bronchograms difficult to obtain.

Water-soluble contrast media were introduced into bronchography in an attempt to ensure rapid lung clearance. The actual duration of such media in the bronchial tree is a matter of a few minutes. This does not permit an unhurried fluoroscopic examination, which is generally regarded as essential for successful bronchography. Since these solutions are grossly hypertonic, the lungs rapidly become flooded: coughing ensues, and the contrast medium is diluted with bronchial secretions.

There is, therefore, a need for a bronchographic agent without these disadvantages. It was argued that a suspension of a slightly soluble contrast medium should be completely eliminated from the lungs within a few days, and furthermore it should not cause osmotic disturbances.

It would appear from the experiments outlined in the text that propyliodone may fulfil both these requirements. Furthermore, it does not cause alveolar filling in the healthy rabbit lung, even after coughing. It remains to be seen whether the same lack of alveolar filling is observed in the diseased human lung. Our experience with aqueous and oily propyliodone in anaesthetized rabbits suggests that the aqueous suspension is more irritant (i.e., cough promoting) than iodized poppy-seed oil. Although oily propyliodone is a suspension, it does not appear to be more irritant than iodized oil. Presumably the oil acts as a lubricant for the crystals. Undoubtedly aqueous propyliodone will necessitate careful premedication if undue coughing is to be avoided in man.

The human excretion rate experiments suggest that the use of propyliodone should enable serial bronchographic examinations to be made at intervals of a week or so. Lung clearance rates will, without doubt, vary from one patient to another, but complete clearance should be obtained within a week or ten days.

The experimental evidence suggests that propyliodone is hydrolysed in the bronchial tree and the resulting organic anion is then rapidly removed by the kidney. Since neither iodine nor iodide ion could be detected in the urine after intratracheal or oral administration of propyliodone, it follows that it should be possible to administer the material to iodine-sensitive patients. Iodized oils should not be administered to such patients (Robertson and Morle, 1951; Lichter, 1951; Theodos, 1952). Because of the breakdown in the lung, it has been suggested that iodized oils be precluded in cases complicated by tuberculosis (Amberson and Riggins, 1933). This argument should not apply to propyliodone.

Summary

1. Aqueous and oily suspensions of normal propyl 3:5-di-iodo-4-pyridone-N-acetate (propyliodone) have been compared in rabbits with iodized oil and diodone. Propyliodone did not enter the alveoli, and clearance was complete within a few days.

2. No toxic symptoms were observed after intratracheal or oral administration of propyliodone. Elimination after subcutaneous, intraperitoneal, and intramuscular injection was extremely slow.

3. The congestion produced in rabbit lung after injection of aqueous or oily propyliodone was similar in degree to that observed with iodized oil, but of a shorter duration. Sodium carboxymethyl-cellulose could not be demonstrated for longer than five days after injection. Similarly non-opaque oil was absent after intervals of more than five days.

4. Animal experiments with propyliodone labelled with I_{131} and human metabolic studies have shown that, after intratracheal or oral administration, the compound is completely hydrolysed and eliminated via the kidney. Iodine and iodide ion are absent in the urine.

We wish gratefully to acknowledge the help we have received from Sir Harold Graham Hodson, Dr. J. N. Pattinson, Dr. J. K. Ross, Dr. C. G. Whiteside, and Sister Stone, of the Middlesex Hospital, where the first clinical trials were undertaken, and to Dr. F. Pygott and Dr. M. G. Scott, of the Central Middlesex Hospital; without the collaboration of nurses and patients at both hospitals the human metabolic studies could not have been conducted. In particular we wish to thank Drs. Pattinson and Whiteside for their stimulating advice throughout our work. We also wish to thank Miss H. M. Sharpe, B.Sc., for valuable assistance in the laboratory.

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