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A Study of the Stability and Radiochemical Purity of some Radiopharmaceuticals. 3. Labelled Diiodofluorescein.

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SUMMARY

The thermal and radiation stability of diiodofluorescein were studied. The decomposition products were separated by paper chromatography. Inorganic iodine, mono-, tri- and tetrafluorescein were found as thermal decomposition products of diiodofluorescein. In addition, fluorescein and two unidentified compounds were separated from gamma-irradiated solutions of diiodofluorescein. The yields of radiation decomposition of diiodofluorescein and formation of monoiodofluorescein and inorganic iodine were estimated as a function of the concentration of diiodofluorescein.

INTRODUCTION.

Diiodofluorescein labelled with iodine-131 or 125 used to be utilized for brain tumor localization. The commercial product was usually a mixture of mono-, di-, and triiodofluorescein. At present, the diagnostic use of labelled diiodofluorescein is very limited by the application of more efficient radiopharmaceuticals. We studied the radiation decomposition of pure diiodofluorescein as a model substance for a more complicated compound, i.e. labelled Bengal Rose.

1. EXPERIMENTAL PART.

Diiodofluorescein-^{131}I was prepared from fluorescein dissolved in phosphate buffer (pH 7.5) and elemental iodine labelled with ^{131}I dissolved in chloroform [1]. The aqueous layer was then acidified and iodinated fluoresceins

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were extracted into ethylacetate. Pure diiodofluorescein-^{131}I was separated by preparative thin-layer chromatography. The ethyl-acetate layer was applied on the Silicagel G plate (Merck, 200 × 200 × 0.5 mm) dried at 130° C. The plate with the applied mixture was then dried at room temperature for 15 min., then at 120° C for 2 min., cooled to room temperature and placed into the chromatographic chamber saturated with the vapours of the solvent. After 20 min. of saturation, the chromatogram was developed with the mixture of chloroform - formic acid - ethanol 10 : 1.5 : 0.1 [2, 3]. The plate was then dried at 70° C in darkness, the band of diiodifluorescein was scraped off into a small column and diiodofluorescein was eluted with ethanol. This solution was filtered and evaporated to dryness; the residue was dried in vacuo at room temperature for 10 hrs. Approximately 30 mg of diiodofluorescein was isolated from five plates. The specific activity of the product was about 6 mCi/g. The weighed amount of diiodofluorescein-^{131}I was dissolved in calculated volume of 0.1 N solution of sodium hydroxide to obtain 0.03 mole solution; this solution had the pH value 7.5. Other solutions were prepared by dilution either with water with 0.2 mole solutions of phosphate buffer (pH 5.8, 7.0 and 8.0) or with 1.0 per cent solution of benzylalcohol.

Other substances used in experiments (benzylalcohol, sodium phosphates, solvents for chromatography) were of Pharmacopoeia purity or reagent grade.

Water used for the preparation of solutions of diiodofluorescein-^{131}I was boiled with potassium chromate, then with diphenylpicrylhydrazone and distilled twice.

The samples were irradiated by gamma-rays of a cobalt-^{60}Co source under well defined conditions (each irradiation position was calibrated by a Fricke dosimeter). The dose rate of 2.80×10^{18} eV/ml per hour was used for most samples; the other values are mentioned in the text.

Solutions under study were irradiated mostly in 0.25-0.50 ml aliquots, sealed in normal glass ampoules. All ampoules were stored in darkness, at room temperature. To prevent the losses of inorganic iodine-131, 0.1 ml of 0.1 N solution of sodium thiosulphate was added to the irradiated ampoule immediately after opening.

Solution of phosphate buffer (0.2 mole, pH 7.2) was used for most chromatographic analyses of irradiated solutions. The developed and dried chromatograms were counted for activity and then the separation of diiodo-fluorescein and more iodinated fluorescein was achieved by additional twice repeated descending development with the same solvent.

Chromatographic paper Whatman No. 4 was used in all analyses. The preparative isolation of individual products of radiation decomposition was carried out on Whatman No. 3 MM paper.

The activity of paper chromatograms was counted under a thin-window GM tube (FHZ 15*a*, mica 1.1 mg per cm^2) by a Frieseke-Hoepfner assembly.

The optical absorption of solution was measured on a spectrophotometer (Zeiss VSU I.).

2. Results.

2.1. *Chromatography of diiodofluorescein and products of its decomposition.*

The phosphate buffer (pH 7.2) was used as solvent for the paper chromatographic separation. The R_f values of diiodofluorescein, monoiodofluorescein and iodide in this solvent are presented in Table 1. Two other broad radioactive and one nonradioactive spots have been observed in gamma-irradiated solutions. To identify these spots, a solution of nonlabelled diiodofluorescein (purified by TLC method as described for the labelled one) was irradiated with gamma rays and then chromatographed. Individual bands were eluted from the paper with borate buffer (pH 9) and the peaks of optical absorption were determined. The results are presented in Table 1.

The tri- and tetraiodofluorescein remained on the start. To obtain a better separation from the main component, i.e. diiodofluorescein, the chromatograms were usually rechromatographed twice after the counting of activity and then counted again. In the first counting the more iodinated fluoresceins were counted together with diiodofluorescein. In the second (after two additional development) the tetraiodofluorescein remained on the start line, the triiodofluorescein formed a spot with R_f approximately 0.1 and diiodofluorescein a spot with a R_f value of approximately 0.25.

The spot of iodide interferes with the spot of unknown compound II. Fortunately the activity in the spots U I and U II was comparatively low, and distributed over broad areas, while the spot of iodide was small and the activity peak was sharp. In model experiments, the same activity of iodine-131 was diluted either with water or with solutions of iodide carrier with concentration increasing from 10^{-5} mole to 10^{-2} mole. Aliquots of these solutions were chromatographed and chromatograms counted. No broadening of the

Table 1. The R_f values and peaks of optical absorption in borate buffer of pH 9.0 of compounds found in gamma-irradiated solution of diiodofluorescein.

R_f	Activity	Max, nm		Compound
		Observed	Theor.	
0	+	508-515	517	triiodofl.
0.12	+	508	508	diiodofl.
0.33	+	497	498	monoiodofl.
0.50	0	490	490	fluoresc.
0.55-0.70	+	498-503	—	unknown I
0.75-0.92	+	488	—	unknown II
0.85	+	—	—	iodide

activity peak was observed with decreasing carrier concentration. Therefore the activity of U I and II can be easily subtracted from the peak of iodide activity.

2.2 *The thermal stability of diiodofluorescein.*

Preliminary experiments showed that during autoclaving at 122° C diiodofluorescein decomposed to inorganic iodide, monoiodofluorescein and more iodinated fluoresceins, presumably triiodofluorescein. The content of triiodofluorescein increased with increasing duration of heating and decreasing pH of solution, while content of inorganic iodide usually decreased with increasing autoclaving time. The changes are demonstrated in the Table 2. The formation of unidentified compounds U I a U II was not observed in any solution analysed.

2.3. *The radiation stability of diiodofluorescein.*

The radiation decomposition experiments were carried out with solutions of labelled diiodofluorescein having radioactive concentration of ^{131}I not

TABLE 2. The percentage of activity in individual compounds as a function of autoclaving time and composition of the solution.

Solution	Duration of heating min.	% of activity in				
		Iodide	Iodofluorescein			
			Mono-	Di-	Tri-	Tetra-
3×10^{-2} M aqueous	0	0.9 ± 0.1	—	99.1 ± 0.1	—	—
	20	3.4 ± 0.7	2.1 ± 0.2	94.5 ± 0.9	—	—
	40	3.1 ± 0.2	1.5 ± 0.1	91.2 ± 0.4	4.2 ± 0.7	—
3×10^{-3} M aqueous	0	1.7 ± 0.1	0.8 ± 0.1	97.5 ± 0.2	—	—
	20	6.2 ± 0.3	1.5 ± 0.3	92.3 ± 0.5	—	—
	40	4.8 ± 0.3	1.6 ± 0.2	89.8 ± 0.0	3.8 ± 0.5	—
3×10^{-3} in buffer pH 8	0	2.4 ± 0.3	0.6 ± 0.1	97.0 ± 0.2	—	—
	20	4.0 ± 0.8	1.2 ± 0.1	94.8 ± 0.8	—	—
	40	2.9 ± 0.2	0.8 ± 0.1	93.5 ± 0.1	2.8 ± 0.2	—
3×10^{-3} in buffer pH 6	0	4.2 ± 0.3	1.2 ± 0.1	94.6 ± 0.4	—	—
	20	4.0 ± 0.4	4.4 ± 0.1	78.9 ± 0.7	9.8 ± 0.5	2.9 ± 0.2
	40	2.8 ± 0.1	5.0 ± 0.4	78.1 ± 0.0	12.4 ± 0.4	1.7 ± 0.1

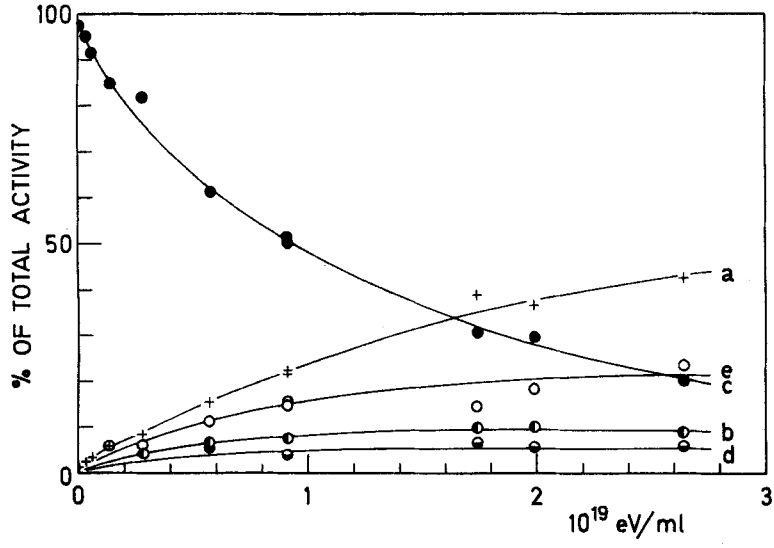

FIG. 1. Plot of radiation decomposition of diiodofluorescein and formation of decomposition products vs. absorbed energy dose. (*a*) inorganic iodine, (*b*) mono-, (*c*) di-, (*d*) tri- and tetraiodofluorescein, (*e*) unidentified products. The initial concentration of diiodofluorescein was 3×10^{-3} M.

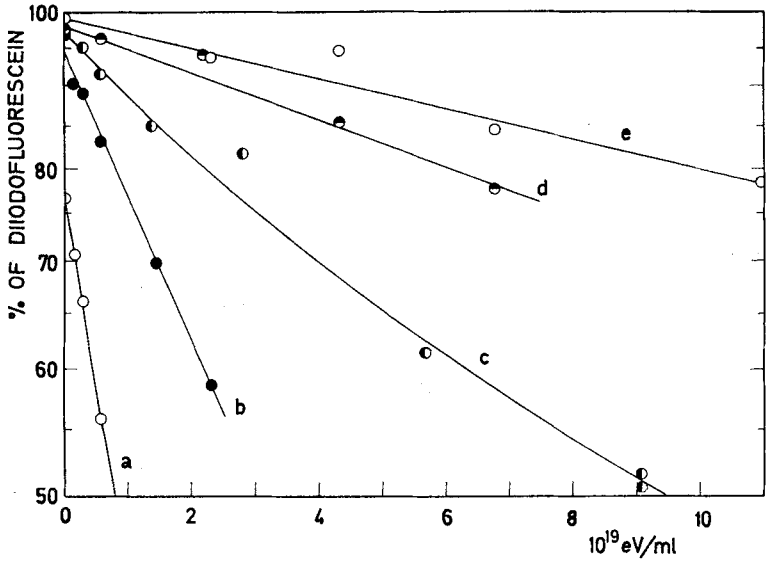

FIG. 2. The percentage of decomposition of diiodofluorescein as a function of absorbed energy dose. The initial concentrations were as follows : (*a*) 3×10^{-4} M, (*b*) 1×10^{-3} M, (*c*) 3×10^{-3} M, (*d*) 1×10^{-2} M, (*e*) 3×10^{-2} M.

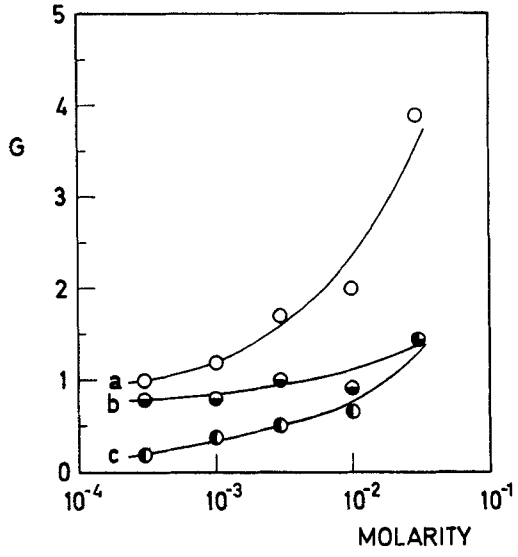

FIG. 3. The initial radiation yields of decomposition of diiodofluorescein (*a*) and formation of inorganic iodine (*b*) and monoiodofluorescein (*c*) as functions of the concentration of diiodofluorescein.

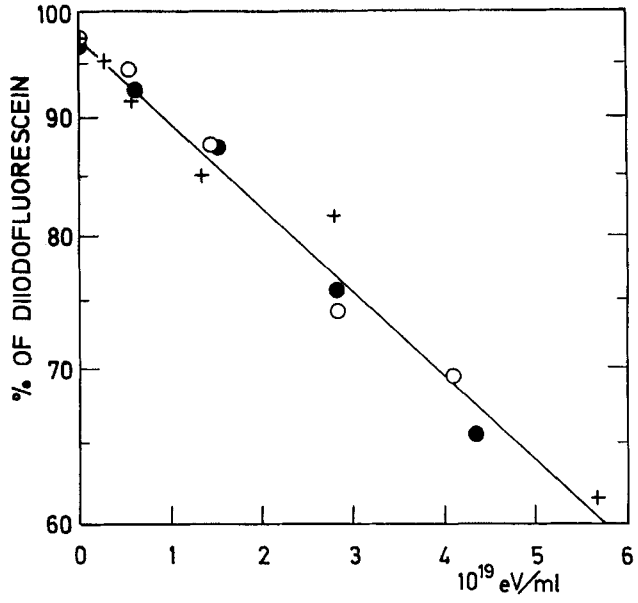

FIG. 4. The radiation decomposition of diiodofluorescein as a function of absorbed energy. Applied dose rates were : (+) 2.80×10^{18}, (o) 7.85×10^{17}, (●) 3.31×10^{17} eV/hr/ml. The initial concentration of diiodofluorescein was 3×10^{-3} M.

higher than 50 μCi/mol. Figure 1 shows the decomposition of diiodofluorescein and formation of various compounds as a function of absorbed dose of gamma radiation. In a semilogarithmic plot nearly straight lines were obtained for the decomposition of diiodofluorescein; the results obtained for various initial concentrations of diiodofluorescein are presented in Figure 2 (the results were not corrected to the zero time decomposition). The "initial" yields of radiation decomposition of diiodofluorescein as well as the "initial" yields of radiation formation of monoiodofluorescein and inorganic iodine were calculated from these results. (Owing to the nonlinearity of decomposition curves, the values were calculated for 5 per cent of decomposition.) The plot of respective G values as a function of concentration is shown in Figure 3.

The radiation decomposition of diiodofluorescein was independent of the dose rate within the range from 3.3×10^{17} to 2.8×10^{18} eV/ml/hour (Fig. 4). The change of pH value from 5.8 to 8 was also without effect on the radiation decomposition of diiodofluorescein (Fig. 5).

The presence of 0.9 per cent of benzyl-alcohol showed a protective effect (Fig. 5).

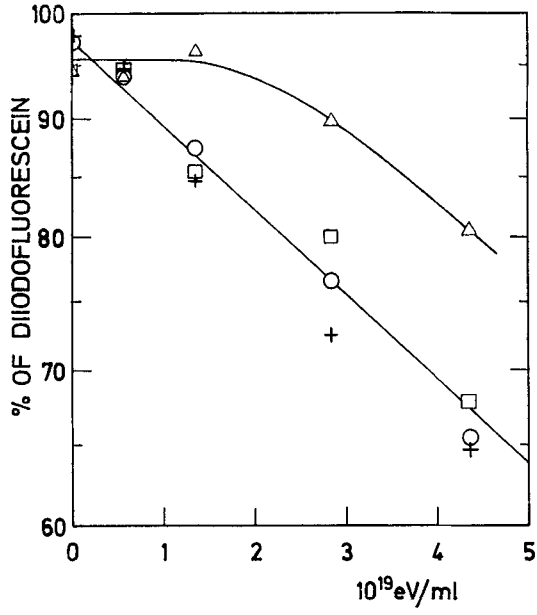

FIG. 5. The radiation decomposition of diiodofluorescein as a function of absorbed energy. (□) pH 5.8 (+) pH 7, (o) pH 8. (△) solution contained 0.9% of benzylalcohol. The initial concentration of diiodofluorescein was 3×10^{-3} M.

3. Discussion.

The heating experiments show that inorganic iodine is released during autoclaving of diiodofluorescein and that this inorganic iodine can be bound again by the diiodofluorescein molecule to give more iodinated fluoresceins, presumably triiodofluorescein. The elemental or atomic iodine is needed for further iodination of diiodofluorescein; it is therefore necessary to suppose the presence of these species in the solution. An additional study of thermal decomposition of various iodofluoresceins would be very interesting.

The accelerated radiation decomposition experiments [4] show in addition, the formation of two yet unidentified compounds. In solutions of diiodofluorescein the ionizing radiation caused the abstraction of iodine from the molecule. If iodine is replaced by hydrogen, the monoiodofluorescein arises, which is easy to identify. The replacement by hydroxy group results in hydroxyiodofluorescein, the chromatographic behaviour of which is unknown; may be that this compound can be regarded as one of the unidentified products (Table 1).

The monoiodofluorescein balanced only from one fifth to one third of the inorganic iodine amount; the remaining part is bound mainly in unidentified products and in tri- and/or tetraiodofluorescein. The presence of fluorescein among the radiation decomposition products can be explained by successive abstraction of iodine from the molecule; simultaneous abstraction seems to be less probable. The iodination of diiodofluorescein can be realized by molecular as well as atomic iodine; both species are present during radiolysis of iodide solutions [5, 6]. The equilibrium concentration of elemental iodine can cause the loss of inorganic iodine from the analysed solutions, the addition of reducing agent before analyses of iodinated radiopharmaceuticals can be therefore recommended.

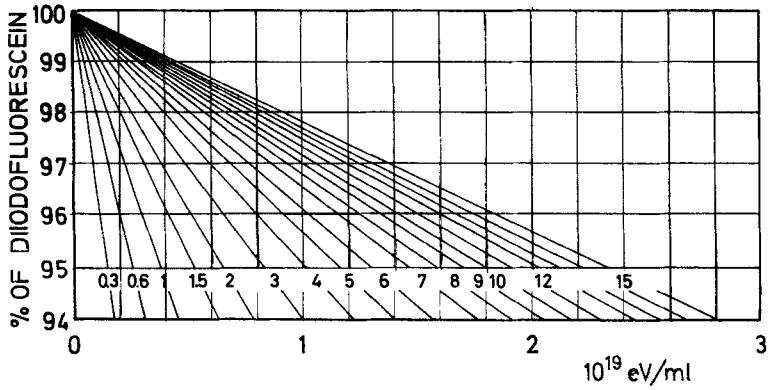

FIG. 6. The calculated radiation decomposition of diiodofluorescein as a function of absorbed energy. Individual initial concentrations of diiodofluorescein are expressed in mg/ml.

The independence of radiation decomposition of diiodofluorescein on the dose rate was found. Results obtained in accelerated experiments can be therefore extrapolated to dose rates which are usual in autoradiolysis.

The independence of radiation decomposition of diiodofluorescein on the pH value of solution shows that the decomposition of slightly acid solutions during storage is caused mainly by normal chemical processes (hydrolysis).

The obtained values for radiation yields were used for the calculation of the radiation decomposition of diiodofluorescein and formation of inorganic iodine as a function of the absorbed energy for various initial concentrations of diiodofluorescein. The results presented in Figures 6 and 7 can be used for the rough evaluating of the radiation decomposition of diiodofluorescein (or formation of inorganic iodine); the following procedure is to be recommended.

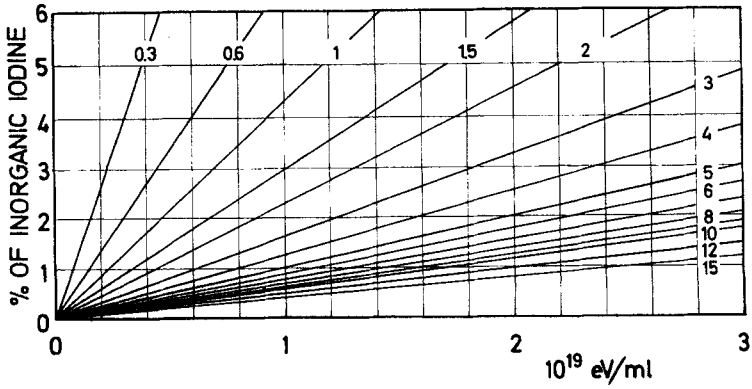

FIG. 7. The calculated radiation formation of inorganic iodine as a function of absorbed energy. Individual initial concentrations of diiodofluorescein are expressed in mg/ml.

(1) From the initial value of radioactive concentration of iodine-^{125}I or ^{131}I, and the storage time the respective value of total absorbed energy (in eV/ml) is calculated using the Tables 4 and 5 (and corrections for geometrical shape, if necessary) of the previous paper [4].

(2) The radiation decomposition or formation for this value of absorbed energy and the given concentration of diiodofluorescein is found using the Figure 6.

The value found must be corrected with respect to the initial radiochemical impurities. The calculated values are valid only for the pure solutions and can be taken as maximum values. Most of additives lower the radiation decomposition. The protective effect of benzylalcohol was studied as an example.

Our results show that the decomposition of diiodofluorescein is not balanced by the formation of inorganic iodine. The estimation of unbound iodine, which is usually the only one criterion for the radiochemical purity of iodinated radiopharmaceuticals, seems to be therefore insufficient.

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