Preparation of some mercuri-derivatives of fluorescein labelled with isotopes ^{197}Hg and ^{203}Hg

III. Recoil-labelling of derivatives

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SUMMARY

The possibility of preparation of mercuriderivatives of fluorescein labelled with ^{197}Hg by irradiation of the respective compounds in a nuclear reactor has been studied. For all investigated compounds, specific activity high enough could be obtained without any significant radiation decomposition. In cases, when the target molecule contained more than one target atom-bis (hydroxymercurifluoresceinyl) mercury and bis (hydroxymercurifluorescein)-higher enrichment factor for some products could be obtained. The recommended procedure for preparation of difluoresceinylmercury-^{197}Hg by this method has been given.

INTRODUCTION.

In the preceding papers [1,2] we have studied the possibility of preparing labelled mercuric derivatives of fluorescein by a substitution or exchange reaction. The synthesis gives relatively low yields of less mercurized components which are of great interest for medical reasons; the exchange reaction leads to the presence of impurities in the form of derivatives mercurized to a higher degree. We have tried to verify the possibility of the preparation of these substances by direct neutron irradiation.

The Szillard-Chalmers reaction taking place when organic mercuric compounds are irradiated by neutrons has been studied elsewhere [3,6]. Heitz and Adloff [7] have found when irradiating diethylmercury that the secondary reactions lead to the decrease in the yield of ^{197}Hg in the inorganic form and increase the retention in the target substance. An opposite result has been found with diphenylmercury. The retention in these both target substances

exceeds 50 %. Similarly, Wheeler and McClin [8] have found more than 90 % of the activity in target diphenylmercury.

The preparation of organomercuric compounds by direct neutron irradiation, significant from medical point of view, has been studied by Kronrád and Cífka [9]. They have found that 3-chloromercuri-2-methoxypropylurea (chlormerodrine) and 3-chlormercuri-2-methoxypropylurea succinate (meralluride) are relatively considerably decomposed when irradiated by neutrons. The maximum specific activity reached by recoil-labelling was 40 mCi/g.

The relatively short half-life makes the preparation of materials labelled with ^{197}Hg easier than the preparation of organic compounds with ^{203}Hg, where in many cases this method cannot be used at all, due to the radiation decomposition of the substances. The yield of the organic molecules labelled with ^{197}Hg can depend in general on the irradiation conditions; on the other hand, all exchange and recombination reactions that lead to the return of the hot atom into the original target molecule effect favourably the preparation of the labelled target substance.

EXPERIMENTAL.

Target substances (difluoresceinylmercury, hydroxymercurifluoresceine, *bis* (hydroxymercurifluoresceinyl) mercury, and *bis* (hydroxymercuri) fluoresceine) were synthetized from mercuric acetate and fluoresceine. Because all these substances are formed in the reaction mixture simultaneously, they were separated chromatographically on a column of aluminium oxide. The purity of singular fractions was checked by paper chromatography.

The samples placed in sealed quartz tubes were irradiated in the vertical channels of the VVR-S reactor in Řež by a neutron flux of $1.10^{13}n/cm^2sec$. The average neutron flux during each irradiation was determined from the activity of red phosphorus (0.19 barn) irradiated simultaneously with the sample. The irradiation time varied between 1 and 20 hours.

Analysis of samples.

The irradiated sample was dissolved in 10 ml of 0.1 N sodium hydroxide, the solution filtered with a Witt's needle and both the filtrate and the filter with the eventually insoluble residue were measured under equal conditions. A part of the filtrate was further put on chromatographic paper and separated in a 6 % ammonia-methanol 1 : 1 system. After measuring the chromatograms, they were cut into pieces and singular fractions eluted with 0.1 N sodium hydroxide solution and the eluates measured photometrically at 490 mμ. In order to determine the presence of mercury bound inorganically, a part of the fitrate was chromatographied in diluted acetic acid.

The activity of the samples in the form of filtrates or precipitates was measured on a 200-channel gamma spectrometer, Intertechnique Co., chromatograms were measured by β-tube Frieseke-Hoepfner counter.

Recommended procedure for the preparation of difluoresceinylmercury-^{197}Hg :

A sample of difluoresceinylmercury is irradiated in the active zone of the reactor for 20 hours at the neutron flux of 1.5×10^{13} n/cm^2 sec. The irradiated sample is dissolved in 0.1 N sodium hydroxide solution and the solution poured on a column of aluminium oxide 1.5 cm in diameter and 5 cm high. The active difluoresceinylmercury is eluted from the column with 0.1 N sodium carbonate solution and the first coloured active fraction is taken. The activity of the eluate is measured and the chemical content determined.

The production gives in this way yield three times as high as those obtained by means of a synthetic reaction, it is more simple and the number of operations with the radioactive substance is limited to a minimum.

RESULTS AND DISCUSSION.

The irradiation in a reactor does not lead to any visible change in the mercuric derivatives of fluoresceine. After dissolving the samples in 0.1 N sodium hydroxide solution, practically no insoluble residue remains (amounts of activity higher than 0.5 % were not found on the filter irrespective of the time of sample irradiation. On the other hand, increasing amount of activity

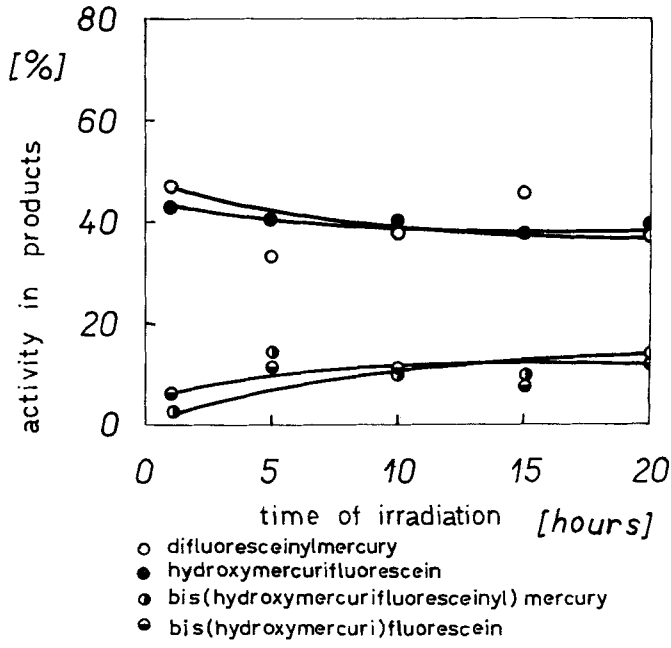

FIG. 1. Dependence of activity distribution of ^{197}Hg among singular products on time of irradiation.

in the precipitate was found with increasing irradiation time with samples of chlormerodrine and meralluride [9].

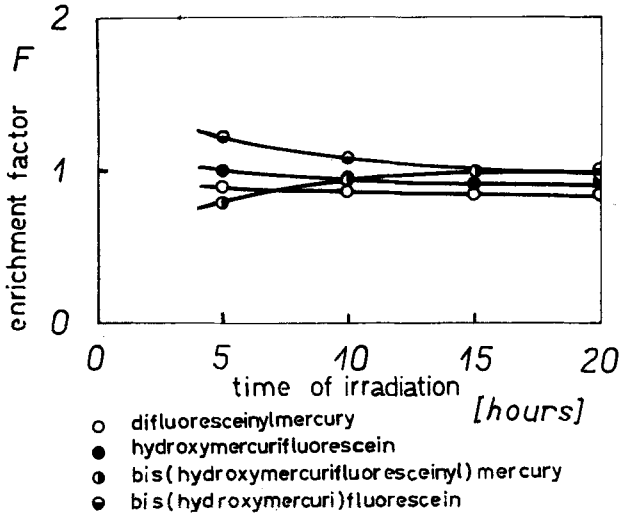

FIG. 2. Dependence of enrichment factor on time of irradiation for target difluoresceinylmercury.

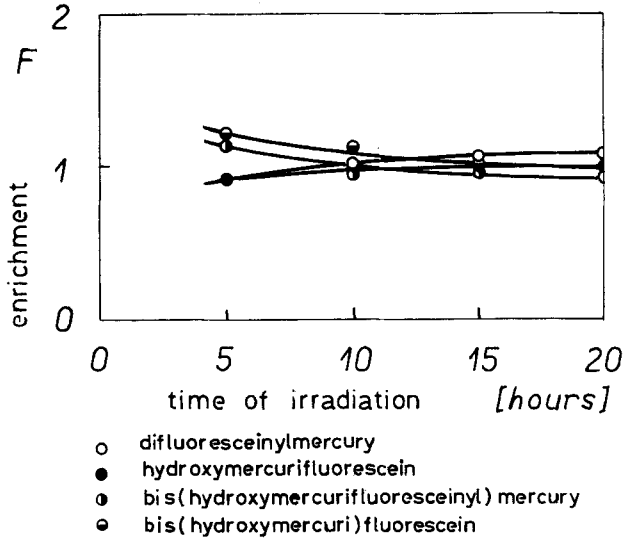

FIG. 3. Dependence of enrichment factor on time of irradiation for target hydroxymercurifluoresceine.

Chromatographic analysis of the filtrate has shown that practically all activity is concentrated in the organic phase. When difluoresceinylmercury was irradiated, the predominant part of the activity was found in the form of the target compound and in the form of hydroxymercurifluoresceine. The distribution of the activity among the particular mercuric derivatives of fluoresceine as the function of the irradiation time is presented for target difluoresceinyl mercury in Figure 1. Chemical distribution of mercury among the particular derivatives showed that these compounds are present after dissolving the irradiated difluoresceinylmercury in the ratio roughly equal to their activities. This fact is especially evident in Figure 2, where the dependence of the enrichment factor (the ratio of the specific activity in the given fraction to the total specific activity) on irradiation time is presented. Figure 2 shows that the enrichment factor for all four mercuric derivatives of fluoresceine does not practically differ from 1. Due to the fact that practically no exchange reaction between singular mercuric derivatives of fluoresceine takes place at room temperature at which all samples were dissolved [1], it may be supposed that during the irradiation, mercury atoms are predominantly liberated from the target substance and when the sample is dissolved, the active atom of

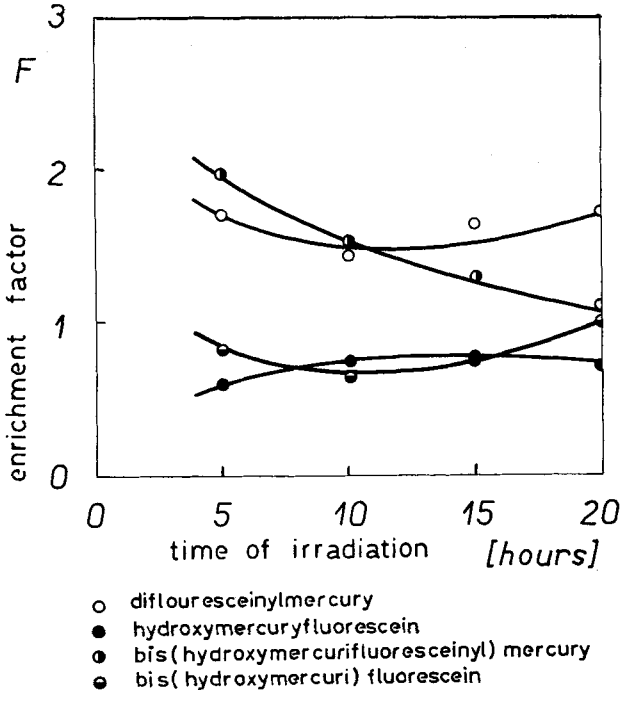

FIG. 4. Dependence of enrichment factor on time of irradiation for target *bis* (hydroxymercurifluoresceinyl) mercury.

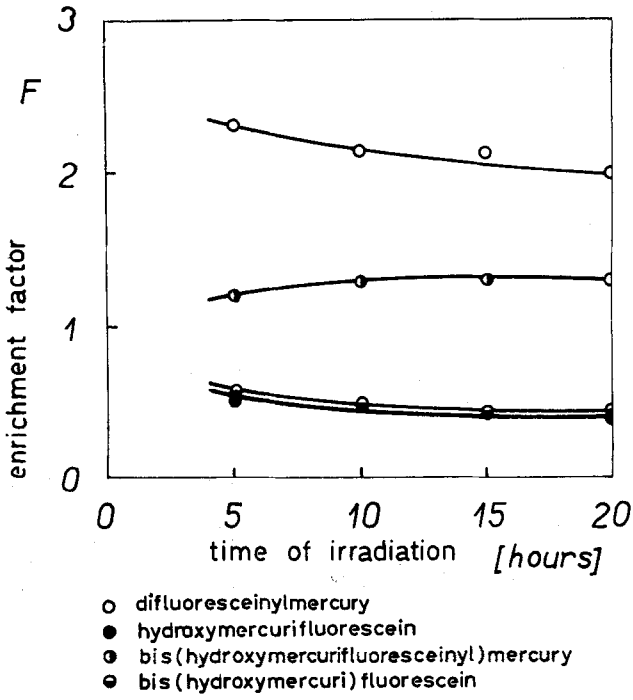

FIG. 5. Dependence of enrichment factor on time of irradiation for target *bis* (hydroxymercuri) fluoresceinyl.

mercury is introduced by a secondary reaction by means of exchange to the singular compounds resulting from the radiolysis of the target substance.

Similar effects are likely to appear also with hydroxymercurifluoresceine. The enrichment factor of singular mercuric derivatives of fluoresceine in the dependence on the irradiation time is presented for this target substance in Figure 3.

With *bis* (hydroxymercurifluoresceinyl) mercury and *bis* (hydroxymercury) fluoresceine on the contrary, i.e. if the target molecule contains more than one target atom, higher enrichment factor may be found for some derivatives as demonstrated in Figures 4 and 5. Figure 4 represents the dependence of the enrichment factor F on the irradiation time for *bis* (hydroxymercurifluoresceinyl) mercury; Figure 5 shows the same dependence for *bis* (hydroxymercuri) fluoresceine. The bond of the newly formed atom with the residue of the molecule seems to remain to a certain extent unchanged in these compounds. The subsequent reactions during the dissolution lead then to the predominant formation of two basic structures : to difluoresceinylmercuri and *bis* (hydroxymercuri) fluoresceine. This fact supports in general the opinion that the energy of the newly formed atom may be distributed to the molecule as a whole and

the deexcitation of the excited molecule can happen by the rupture of its bonds.

It may be concluded that in contradistinction to meralluride and chlormerodrine, when the radiation decomposition predominantly occurs and the liberated atoms of mercury do not produce the target material by means of secondary reactions, which results in the fact that only low specific activities can be obtained with these substances by recoil-labelling, mercuric derivatives of fluoresceine make the preparation by direct irradiation quite possible.

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