Protein labelling by 2-imino-1, 2-fluorenoquinone, a metabolite *in vitro* of the carcinogen *N*-(2-fluorenyl)acetamide

A mechanism for the interaction of the carcinogen *N*-(2-fluorenyl)acetamide with protein, which involves *o*-quinone imines, has recently been proposed. According to this mechanism, 2-imino-1,2-fluorenoquinone or 2-imino-2,3-fluorenoquinone, which are enzymic oxidation products *in vitro* of 2-amino-1-fluorenol or of 2-amino-3-fluorenol, respectively, combine with the appropriate functional group of the protein[1-3]. This tentative conclusion was based on indirect spectrophotometric evidence[2]. Recently, CRAMER, MILLER AND MILLER[4] have discovered *N*-hydroxy-2-fluorenylacetamide as an important metabolite of *N*-(2-fluorenyl)acetamide[4], and MILLER, MILLER AND HARTMANN consider that this metabolite may be the "proximate" carcinogen[5]. MILLER *et al.* have also advanced the view that a quinolimide, which may arise through a spontaneous rearrangement of *N*-hydroxy-2-fluorenylacetamide, may be the bound compound[6]. We have therefore examined the labelling of bovine serum albumin by *N*-hydroxy-2-[9-^{14}C]fluorenylacetamide as well as by the enzymic oxidation product of 2-amino-[1-^{14}C]fluorenol-1. The results of this work indicate that only the latter is bound to the protein, while *N*-hydroxy-2-fluorenylacetamide, 2-aminofluorenol-1 and *N*-(1-hydroxy-2-fluorenyl)acetamide[7] *per se* are not. The failure of *N*-hydroxy-2-fluorenylacetamide to combine with albumin also contradicts its spontaneous rearrangement to a quinolimide. Moreover, such a rearrangement was excluded by the absence of any phenolic compounds after *N*-hydroxy-2-fluorenylacetamide was incubated in acid or neutral media. Phenolic derivatives would be the end products of the spontaneous rearrangement of *N*-hydroxy-2-fluorenylacetamide to a quinolimide[6]. The data support the view, previously held[2,3], that the enzymic oxidation products of *o*-aminofluorenols play

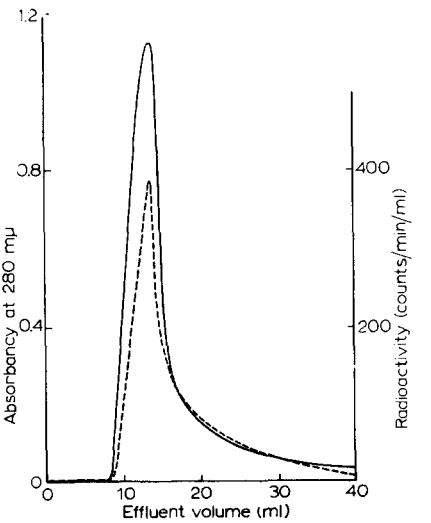

Fig. 1. Elution of the radioactivity and of the protein from a DEAE-cellulose column after incubating bovine serum albumin (0.15 μmole) with 2-amino-[1-^{14}C]fluorenol-1 (12.6 μmoles, 16900 counts/min/μmole) and cytochrome *c* – cytochrome oxidase. ———, absorbancy at 280 mμ; – – – –, radioactivity.

a dominant role in the binding of the carcinogen *N*-(2-fluorenyl)acetamide to tissue proteins.

2-Amino-[1-^{14}C]fluorenol-1 (ref. 8) was oxidized by cytochrome *c* – cytochrome oxidase in the presence of bovine serum albumin. Alternatively, oxidation was accomplished by adding the aminofluorenol to a solution of $K_3Fe(CN)_6$ and bovine serum albumin in phosphate buffer. The protein was then isolated by chromatography on *N*,*N*-diethyl aminoethyl cellulose columns, essentially as previously described[3]. Fig. 1 shows coincidence of radioactivity measurements and spectrophotometric protein determinations in the elution profile; this coincidence and the impossibility of extracting the radioactivity prove binding of the oxidized metabolite to albumin.

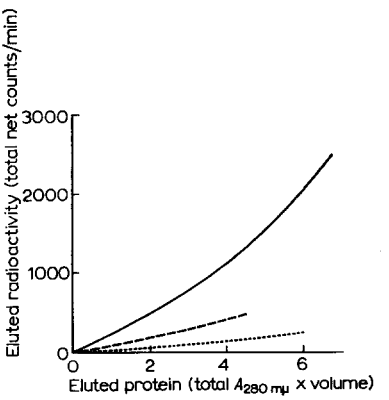

Fig. 2. Elution of the protein-bound radioactivity from DEAE-cellulose columns. The cumulative net radioactivity of the effluent fractions is plotted as the ordinate. The sum of the products of the absorbancy at 280 mμ and the volume of each fraction is plotted as the abscissa. ———, after incubating bovine serum albumin (0.15 μmole) with cytochrome *c* – cytochrome oxidase and 2-amino-[1-^{14}C]fluorenol-1 (12.6 μmoles, 16900 counts/min/μmole); – – – –, after incubating bovine serum albumin in the above system but omitting cytochrome *c*; ·····, after incubating bovine serum albumin (0.15 μmole) with *N*-hydroxy-2-[9-^{14}C]fluorenylacetamide (1.87 μmole, 16500 counts/min/μmole).

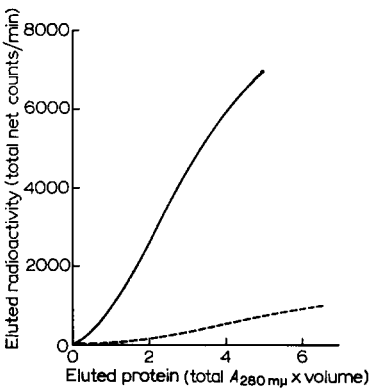

Fig. 3. Elution of the protein-bound radioactivity from DEAE-cellulose columns. The cumulative net radioactivity of the effluent fractions is plotted as the ordinate. The sum of the products of the absorbancy at 280 mμ and the volume of each fraction is plotted as the abscissa. ———, after the oxidation of 2-amino-[1-^{14}C]fluorenol-1 (11.8 μmoles, 21200 counts/min/μmole) by $K_3Fe(CN)_6$ in the presence of bovine serum albumin (0.15 μmole); – – – –, after incubating bovine serum albumin in the above system but omitting $K_3Fe(CN)_6$.

N-hydroxy-2-[9-^{14}C]fluorenylacetamide, prepared essentially by the published procedure[4], was incubated in a phosphate buffer containing the bovine serum albumin and the protein was isolated as above. The results of this experiment and the binding of the *o*-quinoneimine are shown in Fig. 2. In separate experiments, *N*-hydroxy-2-fluorenylacetamide was incubated in buffers of pH 1 and 7.4. Ether extracts of these solutions were chromatographed[4], and the chromatograms treated with *p*-dimethylaminobenzaldehyde and diazotized 7-nitro-2-fluorenamine[4]. The details of the foregoing experiments will be published at a later date.

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