DISTRIBUTION OF NEW FLUORENE DISULPHONAMIDO DERIVATIVES IN RATS WITH TRANSPLANTED WALKER CARCINOSARCOMA

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The previous investigation by this author dealt with the distribution of two new fluorene disulphonamides: N,N'-bis(thiazole)-2,7-fluorene[35S]disulphonamide, I, and N,N'-bis(guanidine)-2,7-fluorene[35S]disulphonamide dihydrate, II, in rats bearing Walker carcinosarcoma and in tumour-free animals (Malejka, 1965). While the bis-thiazole derivative revealed an appreciable concentration in tumour—35 μg. per g. tissue (8 per cent of the dose given, the bis-guanidyl derivative was not found in tumour. In the case of Compound I, autoradiograms of tumour slides showed a considerable uptake of radioactivity by tumour cells and particularly by their nuclei (Kasprzak, Malejka, Gabryel, 1965). The tumour concentration for this compound appeared, however, not to be highly selective: the ratios of the radioactivity concentration in tumour to that in liver or spleen or blood plasma were lower than 0.5. These findings favour the hypothesis (Argus, 1961) that the presence of free acidic groups in the fluorene disulphonamido high molecular compounds contributes to their binding at the cellular adsorption sites by basic proteins.

The other aspect of the diagnostic studies with the fluorene disulphonamido group was to observe the reaction of the reticulo-endothelial system in the presence of tumour. The phenomenon of an impaired phagocytic function of the reticulo-endothelial system was demonstrated in the presence of various tumours in different species using N,N'-bis(naphthalene)-2,7-fluorene[35S]disulphonamide (Argus and Hewson, 1954; Argus, Hudson, Seepe, Kane and Ray, 1962). The same reaction was observed with N,N'-bis(p-sulphamoyl-phenyl)-2,7-fluorene[35S]disulphonamide (Malejka, 1962). The importance of the precise chemical structure is shown by the failure of N,N'-bis(thiazole)-2,7-fluorene[35S]disulphonamide to localize in liver and spleen of tumour-free animals to a greater extent than in tumour-bearers (Malejka, 1965).

The present investigation was aimed at studying the influence of various substituents in the fluorene disulphonamide molecule on the distribution of the compounds in tumour-bearing and tumour-free rats. Five new derivatives of 35S-labelled 2,7-disulphonamido-fluorene were synthesized in which the substituents on the nitrogens were chosen so as to permit study of the effect of different structural types on the tissue localization.

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N,N’-bis(carbamyl)-2,7-fluorene\(^{35}\text{S}\) disulphonamide, III

N,N’-bis(p-tolylsulphonyl-carbamyl-n-butyl)-2,7-fluorene\(^{35}\text{S}\) disulphonamide, IV

N,N’-bis(4-carboxy-phenyl)-2,7-fluorene\(^{35}\text{S}\) disulphonamide, V

N,N’-bis(5(6)-nitro-benzimidazolyl)-2,7-fluorene\(^{35}\text{S}\) disulphonamide, VI

N,N’-bis(6(2,4-dimethoxy-pyrimidyl))-2,7-fluorene\(^{35}\text{S}\) disulphonamide, VII
Compound III is the condensation product of 2,7-fluorene$^{35}$S disulphonylchloride with 2 molecules of urea, thus representing a simple aliphatic side chain and showing a structural resemblance to the previously described bis-guanidyl derivative (Compound II) (Malejka, 1965). The urea moiety occurs also in Compound IV in which 2 molecules of tolbutamide, (1-butyl-3(p-tolyl-sulphonyl)urea), a hypoglycaemic sulphonamide, have been used to increase the molecular weight of the new compound. Compound V is an analogue of N,N'-bis(p-carboxyphenyl)-4,4'-biphenyl$^{35}$S disulphonamide which in the earlier studies in mice revealed an appreciable concentration in tumour (Argus, Seepe, Gutierrez, Hewson, Ray, 1958).

Previous studies with the bis-thiazole derivative (Compound I) emphasized the possible meaning of heteroatoms in the thiazole rings, particularly electronegative sulphur for its ability to bind liver and serum proteins and which might influence the affinity of this compound for neoplasms (Kasprzak, Malejka, Gabryel, 1965; Malejka, Kasprzak, Radola, 1966). These studies were now extended by substituting 2,7-disulphonamido fluorene with groupings containing the somewhat more electronegative nitrogen. Two heterocyclic groupings, 5(6)-nitrobenzimidazolyl and 2,4-dimethoxy-6-amino-pyrimidyl were chosen for this purpose and the respective condensation products are Compounds VI and VII.

**MATERIALS AND METHODS**

2,7-Fluorene$^{35}$S disulphonylchloride.—

The procedure described previously (Argus and Hewson, 1954) was employed, but double batches of material were prepared. Fifty millicuries of $^{35}$S (H$_2$$^{35}$SO$_4$) were used to label each portion of chlorosulphonic acid. The yield of crude material was 25 g. (71 per cent of theory) per batch. Recrystallization from toluene gave 10-7 g. of the product (30.4 per cent of theory) melting at 219–221° C.; specific activity was not determined on this intermediate.

N,N'-bis(carbamyl)-2,7-fluorene$^{35}$S disulphonamide, III.—

Urea, 1.8 g. (0.03 moles) was dissolved in 1.6 ml. of hot water and 10 ml. of acetone was added. The solution was stirred and maintained at 55° C. (water bath) throughout the addition (30 minutes) of the suspension of 2,7-fluorene$^{35}$S disulphonylchloride, 2 g. (0.0055 moles) in 60 ml. of acetone. After refluxing 3 hours the resulting white precipitate was collected and dried over phosphorus pentoxide. The yield of crude material was 2 g. (91.7 per cent of theory); it did not melt, but darkened at 240° C. Purification from 96 ml. of n-butanol and methanol mixture (2:1) with charcoal gave 1.3 g. (59-6 per cent of theory) of cream-coloured crystalline material, darkening at 250° C. (complete decomposition at 320° C.) and having a specific activity of 2000 disintegrations per second per mg. (0.054 μC per mg.).

Analysis.—Calculated for C$_{15}$H$_{14}$N$_3$S$_2$O$_6$: C, 43.90; H, 3.44; N, 13.65; S, 15.63. Found: C, 43.72; H, 3.51; N, 13.33.

N,N'-bis(p-tolylsulphonyl-carbamyl-n-butyl)-2,7-fluorene$^{35}$S disulphonamide, IV.—

N-p-tolylsulphonyl-N-n-butyl-urea (Tolbutamide, U. S. P.), 6.9 g. (0.025 moles)
was dissolved in 15 ml. acetone and methyl-ethyl-ketone mixture (1 : 1) at 60° C. (water bath). The solution was stirred and maintained at the same temperature throughout the addition (15 minutes) of the solution of 2,7-fluorene$^{[35]S}$disulphonylchloride, 3 g. (0-008 moles) in 75 ml. mixture of the same solvents. The reaction mixture was stirred throughout the following 12 hours at 65–70° C. (a cream-coloured precipitate started to form after 2 hours of stirring). On cooling to room temperature the precipitate was collected and dried over phosphorus pentoxide. The yield of crude material was 5-6 g. (81-2 per cent of theory), melting point 290° C. Triple recrystallization from absolute methanol (first time with charcoal) gave 1-45 g. (21 per cent of theory) of white product melting at 313–315° C. and having a specific activity of 1200 disintegrations per second per mg. (0-032 μC per mg.).

**Analysis.**—Calculated for C$_{27}$H$_{20}$N$_2$S$_2$O$_8$: C, 53·70; H, 5·07; N, 6·71; S, 13·56. Found: C, 53·42; H, 5·12; N, 6·60.

N,N'-bis(4-carboxy-phenyl)-2,7-fluorene$^{[35]S}$disulphonamide, V.—

p-Aminobenzoic acid, 3-6 g. (0-027 moles) was dissolved in a mixture of 20 ml. acetone and 15 ml. of toluene at 50–60° C. (water bath). The solution was stirred and maintained at the same temperature throughout the addition (15 minutes) of solid 2,7-fluorene$^{[35]S}$disulphonylchloride, 2 g. (0-0055 moles) and throughout the following 5 hours. The resulting pale pink precipitate was collected, washed free of chlorides with warm distilled water and then with toluene and dried over phosphorus pentoxide. The yield of crude material was 2-6 g. (86-6 per cent of theory) carbonizing at 260° C. Purification by double extraction with hot aniline (30 ml. and 20 ml.) followed by several washings with hot toluene gave 0·9 g. (30 per cent of theory of white, fine crystalline material, carbonizing within the range of 325–350° C. and having a specific activity of 1330 disintegrations per second per mg. (0·036 μC per mg.).

**Analysis.**—Calculated for C$_{27}$H$_{20}$N$_2$S$_2$O$_8$: C, 60·88; H, 3·79; N, 5·26; S, 12·01. Found: C, 5·40; S, 12·21.

N,N'-bis(5(6)-nitro-benzimidazolyl)-2,7-fluorene$^{[35]S}$disulphonamide, VI.—

Nitro-5(6)-benzimidazole, 3 g. (0-018 moles) was dissolved in 45 ml. of anhydrous pyridine at 60° C. (water bath). The solution was stirred and maintained at the same temperature throughout the addition (30 minutes) of the solution of 2,7-fluorene$^{[35]S}$disulphonylchloride, 3 g. (0-008 moles) in 60 ml. hot toluene. The reaction mixture was stirred throughout the following 3 hours at 80° C. (a yellow precipitate started to form after 30 minutes of stirring). The precipitate was allowed to cool to room temperature and then collected, washed with methanol and dried over phosphorus pentoxide. The yield of crude material was 3·5 g. (68·7 per cent of theory), melting point 232° C. Purification from 200 ml. mixture of N,N-dimethylformamide and dioxane (1 : 1) with charcoal gave 1·9 g. (37·3 per cent of theory) of white crystalline material melting at 296–298° C. and having a specific activity of 730 disintegrations per second per mg. (0·02 μC per mg.).

**Analysis.**—Calculated for C$_{27}$H$_{16}$N$_2$S$_2$O$_8$: C, 52·60; H, 2·62; N, 13·63; S, 10·40. Found: C, 52·42; H, 2·70; N, 13·49.

The presence of nitro groups in this compound was proved by their reduction to amino groups, diazotization and coupling with β-naphtol to give a red dye.
N,N'-bis(6(2,4-dimethoxy-pyrimidyl))-2,7-fluorene\(^{35}\text{S}\)disulphonamide, VII.—

2,4-Dimethoxy-6-amino-pyrimidine, 2·8 g. (0·017 moles) was mixed thoroughly (in mortar) with 2,7-fluorene\(^{35}\text{S}\)disulphonylchloride, 2 g. (0·0055 moles). The mixture was divided into four 1·2 g. portions. Each portion was introduced into a glass tube, 10 ml. toluene was added and tubes were sealed. Four tubes were placed in an oil bath and temperature was raised up to 180° C. and maintained throughout the following 10 hours. The tubes were allowed to cool to room temperature and opened. The dark beige reaction mass which settled on the tube walls was separated from the toluene solution, and methanol was added (several portions) to help to remove residue from the tubes. Yield of 3·65 g. of crude material was obtained which after drying and powdering in the mortar was extracted with 20 ml. of toluene under refluxing. The material was dried over phosphorus pentoxide. The yield of crude material was 3 g. (91 per cent of theory), carbonizing within the range of 240–300° C. Purification from 20 ml. mixture of N,N-dimethylformamide and dioxane (1 : 1) with charcoal and precipitation with methanol gave 1·8 g. of beige product which purified again in the same way resulted in 0·8 g. (24·3 per cent of theory) of yellow precipitate, decomposing at 330° C. and having a specific activity of 1330 disintegrations per second per mg. (0·036 \(\mu\)c per mg.).

Analysis.—Calculated for C\(_{23}\)H\(_{24}\)N\(_2\)S\(_2\)O\(_8\) (molecular weight 600-55) : C, 49·98; H, 4·03; N, 13·99; S, 10·86. Found : C, 49·60; H, 3·94; N, 14·12.

**ANIMAL EXPERIMENTS**

A total of 52 young male Wistar rats was used; 24 were employed for the subcutaneous transplantation of Walker carcinosarcoma (which was received from Deutsche Akademie der Wissenschaften zu Berlin, Institute für Medizin und Biologie); 28 tumour-free rats served for the control groups. A week before transplantation, the experimental animals received subcutaneous injections of hydrocortisone-acetate suspension (15 mg. per rat in 3 doses—5 mg. each every second day). When the tumours were 11–20 days old and weighed 3·5–20 g. (in 3 rats metastases were noted after 17 days), the radioactive compounds were administered by tail vein injection. Each rat was given 1·2 ml. 0·05 N NaOH containing 12 mg. Compounds III, IV, V, VI or VII. Each animal was then placed in an individual metabolism cage and killed 6 hours following administration of the compounds. The concentration and per cent recovery of radioactive material in tissues and excreta of rats were determined by methods described previously (Argus, Kane and Ray, 1960; Malejka, 1965). Radioactivity measurements were made in a "Tracerlab" gas-flow GM counter of low background with automatic sample changer. The efficiency of the instrument at 1300 volts and 35 cc per minute GM gas flow was approximately 15 per cent.

**RESULTS AND DISCUSSION**

The complete tissue distribution of five new fluorene disulphonamides labelled with sulphur-35 (Compounds III, IV, V, VI and VII) in Wistar rats bearing a transplanted Walker carcinosarcoma and tumour-free animals was compared. This investigation was conducted with a 6 hour time interval between administration of the compounds and killing the animals in order to obtain data which
TABLE 1-A.—Distribution of Radioactivity in Tumour-Bearing and Tumour-Free Wistar Rats 6 Hours After Intravenous Injection of Fluorene Disulphonamides Labelled with Sulphur-35.*

| Tissue            | N,N'-bis(carbamyl)-2,7-fluorene [35S]disulphonamide, III | N,N'-bis(p-tolysulphonyl-carbamyl-n-butyl)-2,7-fluorene[35S] disulphonamide, IV |
|-------------------|-----------------------------------------------------------|---------------------------------------------------------------------------------|
|                   | Experimental Group† | Control Group† | Per cent | Per cent | Per cent | Per cent | Per cent |
| Blood Cells§      | 0  0-00            | 0  0-00         | 0  0-00   | 0  0-00   | 1‡  0-04 | 0-00  0-04 |
| Blood Plasma§     | 2  0-05            | 0**  0-00       | 5  0-15   | 25  0-73  |
| Brain             | 0  0-00            | 0  0-00         | 0  0-00   | 0  0-00   |
| Liver             | 7  0-33            | 0‡  0-00        | 3  0-13   | 8  0-28   |
| Lungs             | 0  0-00            | 0  0-00         | 4  0-02   | 11  0-15  |
| Spleen            | 8  0-04            | 0‡  0-00        | 7  0-03   | 5  0-02   |
| Kidneys           | 15  0-15           | 9  0-10         | 7  0-05   | 30  0-43  |
| Skin              | 32‡  4-61          | 8  1-80         | 24  4-84  | 65  10-98 |
| Leg muscle        | 5  0-15            | 0  0-00         | 0  0-00   | 2  0-02   |
| Stomach + contents| 7  0-07            | 11  0-15        | 10  0-06  | 8  0-11   |
| Small intestine+ contents | 33  2-81   | 26  1-37        | 38  2-33  | 35  1-40  |
| Large intestine + contents | 174 7-23 | 158 6-88        | 349 8-39  | 212 5-40  |
| Carcass           | 0  0-00            | 0  0-00         | 8  3-41   | 8  3-73   |
| Urine§            | 864 80-61          | 1017 91-83      | 8600 71-68| 8080 67-36|
| Faece§            | 862 77 0-16        | 0  0-00         | no 137** 0-21 |
| Tumour            | 0  0-00            | —               | 10 0-44   |
| Total             | 96-90              | 102-29          | 91-33     | 90-86     |

* Dose of Compound III, IV, V, VI or VII—12 mg. per rat.
† Average value from 6 rats.
‡ Average value from 4 rats.
§ Concentration in µg. compound per ml.; other data are in µg. compound per g. tissue.
| Average value from 5 rats.
| Average value from 2 rats.
| Average value from 3 rats.

could be compared to those for Compounds I and II (Malejka, 1965), and for other fluorene disulphonamides tested previously (Argus, Kane and Ray, 1960; Malejka, Argus and Ray, 1961; Malejka, 1962). The details of present studies for Compounds III–VII are shown in Tables I-A and I-B; concentrations are expressed in µg. of compound per g. tissue or ml. blood or urine, and in percentages of administered dose recovered.

Substituents in the 2,7-fluorene-disulphonamido molecule influence the physico-chemical properties and biological behaviour of the resulting compounds. The chemical structure of N,N'-bis(carbamyl)-2,7-fluorene[35S]disulphonamide, III, with the lowest molecular weight (410-3) of Compounds I–VII, and its easy solubility in water relate Compound III to the bis-guanidyl derivative, II, tested previously (Malejka, 1965). Six hours after administration, Compound III is almost completely excreted (trace amounts of radioactivity remain in the kidney) into urine in which in experimental group—80-61 per cent, and in controls—91-83 per cent of the given dose is recovered: the difference between the two groups is statistically significant at P = 0.05 (Table II). This finding together with the
### Table I-B. — Distribution of Radioactivity in Tumour-Bearing and Tumour-Free Wistar Rats 6 Hours After Intravenous Injection of Fluorene Disulphonamides Labelled with Sulphur-35.*

| Tissue                        | Control Group† | Experimental Group† | Control Group † | Experimental Group † | Control Group † | Control Group † |
|-------------------------------|----------------|--------------------|-----------------|--------------------|-----------------|-----------------|
| Blood cells§                  | 0 0.00         | 0 0.00             | 1 0.02          | 1 0.03             | 7 0.24          | 11 0.33         |
| Blood plasma§                 | 2 0.07         | 6 0.15             | 8 0.19          | 9 0.28             | 40** 1.24       | 33 1.02         |
| Brain                         | 0 0.00         | 5 0.03             | 0 0.00          | 7 0.08             | 0 0.00          | 0 0.00          |
| Liver                         | 13 0.52        | 33 1.17            | 0 0.00          | 6 0.27             | 258** 9.11      | 249 9.04        |
| Lungs                         | 8 0.04         | 5 0.03             | 5 0.02          | 11 0.06            | 57** 0.31       | 57 0.28         |
| Spleen                        | 0 0.00         | 4 0.03             | 13 0.11         | 14 0.10            | 81** 0.26       | 121 0.36        |
| Kidneys                       | 0 0.00         | 5 0.04             | 18 0.12         | 19 0.16            | 275** 3.09      | 197 1.71        |
| Skin                          | 67 9.81        | 34 5.31            | 7 0.87          | 30 5.08            | 177 27.09       | 85 13.15        |
| Leg muscle                    | 26 0.20        | 20 0.17            | 5 0.03          | 2 0.01             | 8 0.08          | 0 0.00          |
| Stomach + contents            | 32 0.39        | 42 0.35            | 17 0.09         | 30 0.25            | 37 0.40         | 50 0.60         |
| Small intestine + contents    | 354 13.57      | 274 10.47          | 40 1.25         | 39 1.76            | 787 28.31       | 190 8.41        |
| Large intestine + contents    | 1451 65.52     | 2372 60.33         | 361 8.07        | 91 3.41            | 31 0.94         | 1081 35.61      |
| Carcass                       | 31 10.83       | 22 10.56           | 11 3.06         | 13 7.08            | 35|| 16.08       | 25 11.76        |
| Urine§                        | 0 0.75         | 250** 2.08         | 10910 86.04     | 8170 68.06         | 850 7.41        | 1862 15.52      |
| Faeces                        | 0 0.00         | 4833 6.08          | 110 0.14        | 24## 0.03          | 450†† 1.51      | 450†† 1.51      |

* Dose of Compound III, IV, V, VI or VII—12 mg. per rat.
† Average value from 6 rats.
‡ Average value from 4 rats.
§ Concentration in μg. compound per ml.; other data are in μg. compound per g. tissue.
∥ Average value from 5 rats.
¶ Average value from 2 rats.
*** Average value from 3 rats.
†† Value from 1 rat.

** Transplanted Walker Carcinosarcoma
### TABLE II.—Comparison of the Concentration of Radioactivity in the Tissues of Tumour-Free Wistar Rats and in the Tissues of Tumour-Bearing Wistar Rats 6 Hours After Intravenous Injection of Fluorene Disulphonamides Labelled with Sulphur-35.*

| Control Grp. (tumour free) | Range | Average† | Probability | Mean diff.±std. dev. | Ratio (av. cont. / av. exp.) |
|---------------------------|-------|----------|-------------|----------------------|-----------------------------|
| Blood plasma Compound    | III   | IV       | V           | VI                   | VII                         |
| Control Grp. (tumour free) | 10-77 | 3-9      | 6-14        | 25-47                | 4-18                        | 10-50                      | 3-12                       | 230-265 \*               |
| Average†                 | 0**   | 25||     | 6||        | 9†                    | 33†                         | 0†                         | 8                          | 33||                      | 6                          | 240†                      |
| Range                    | 0-4   | 0-12     | 0-6         | 1-21                 | 38-46                      | 0-20                       | 0-6                        | 0-50                       | —                          | 245-285 \*               |
| Average†                 | 1-6   | 5†       | 2†          | 8†                   | 40**                       | 6-7                        | 13                         | 13†                        | 258**                     |
| Probability              | 0.2< p< 0.3 | 0.2 | 0.05        | 0.3< p< 0.4          | 0.1< p< 0.2                | 0.1< p< 0.2                | 0-1                        | 0.02< p< 0.05              | 0-3                       |
| Mean diff.±std. dev.     | 1-6± 1-3 | 20±15   | 4±1.8       | 1±1.9                | 7±7.2                      | 6-7±4.2                    | 5±3                        | 20±11.5                    | 6±2.2                     | 18±15.1 \*               |
| Ratio (av. cont. / av. exp.) | ∞      | 5-00     | 3-00        | 1-12                 | 0.82                       | ∞                          | 2.67                       | 2-53                       | ∞                          | 0-93                     |
| Experimental Grp. (tumour-bearing) |       |          |             |                      |                             |                             |                             |                             |                             |
| Range                    | 9100-11850 | 7500-8900 | 150-350 | 5850-9500 | 1000-2750               | —                          | 0-8                        | 0-15                       | 0-51                       | 95-145 \*               |
| Average†                 | 10717 | 8080     | 250†       | 8170                | 1882†                      | 0†                         | 5                          | 4                          | 14                        | 121†                     |
| Control Grp. (tumour-free) |       |          |             |                      |                             |                             |                             |                             |                             |
| Range                    | 5600-11000 | 7900-8930 | 0-450   | 8300-12700 | 600-1150                | —                          | 0-25                       | 0-15                       | —                          | 6-25                      | 65-95 \*               |
| Average†                 | 8642  | 8600†    | 75         | 10310†             | 850†                       | 8                         | 7†                        | 0                          | 13†                       | 81**                     |
| Probability              | 0-6< p< 0.6 | 0.6 | 0.1         | 0.3< p< 0.4          | 0.5< p< 0.6                | 0.1< p< 0.2                | 0.02< p< 0.05              | 0.9                        | 0.05< p< 0.1              |
| Mean diff.±std. dev.     | 2075±925 | 520±833 | 175±318   | 2140±1153           | 1012±386                   | 8±6.6                      | 2±3.1                     | 4±2.6                     | 1±10.8                    | 40±15.3 \*               |
| Ratio (av. cont. / av. exp.) | 1.24   | 0.94     | 3.33       | 0.79                | 2.19                       | ∞                          | 0.71                      | ∞                          | 1-07                      | 1-50                     |

* Dose of **S-labelled compound—12 mg. per rat. Concentration in μg. compound per ml. or g. tissue.

† Average value from 6 rats.

‡ Average value from 4 rats.

Average value from 3 rats.

** Average value from 3 rats.
fact that some radioactivity remained in organs of tumour-bearing rats indicate
an impaired elimination of the $^{35}$S-labelled compound in the presence of tumour.
In general, the distribution of Compound III occurs very similarly to the bi-
guanidyl derivative (Compound II). Among other factors, no radioactivity of
Compound III is found in tumour which suggests a lack of affinity of the bi-
carbamyl derivative for protein binding.

Compound III forms a basic structure for Compound IV—N,N'-bis(p-tolyl-
sulphonyl-carbamyl-n-butyl)-2,7-fluorene$^{35}$S disulphonamide—in which hydro-
gens of both urea nitrogens are symmetrically substituted with n-butyl- and
p-tolylsulphonyl-groups. A "double" bis-disulphonamide has thus been obtained
with the highest molecular weight (835) of all fluorene disulphonamide derivatives
tested. Despite the high molecular weight of Compound IV, polar nature of the
substituents added contributes to its solubility in water. Hence, only differences
which might be due to its high molecular weight are noted in the distribution of
Compound IV compared to the bis-carbamyl derivative, III. Compound IV is
excreted into urine at a slower rate than Compound III: 71-68 per cent of the
given dose is obtained in the experimental group, and 67-36 per cent in the con-
trols; this is not a significant difference. As the result of the lower values for
urine, more Compound IV than III is found in the other tissues and organs of
rats. The differences, however, in the distribution of Compound IV between
tumour-free and tumour-bearing rats are not statistically significant (Table II).
In tumour, 10 $\mu$g. of compound per g. tissue is found; this will be discussed
later.

The behaviour of N,N'-bis(4-carboxy-phenyl)-2,7-fluorene$^{35}$S disulphonamide,
V, may be compared with the disulphonamides known from the earlier studies.
In going from the biphenyl to the fluorene series, the activities of the compounds
are not parallel. In the biphenyl series, compound with free carboxylic groups
examined in CAF$_1$/Jax mice with a transplanted stomach carcinomata (2 and 8
hours after its administration) showed more selective localization in tumour than
the derivative with primary sulphonamido groups (Argus, Seepe, Gutierrez,
Hewson, and Ray, 1958). In the fluorene series, the effect was the opposite:
the comparison of Compound V with N,N'-bis(p-sulphamoyl-phenyl)-2,7-fluorene
$^{35}$S disulphonamide (Malejka, 1962) in Wistar rats bearing Walker carci-
osarcoma has not proved a specific affinity of the free-carboxylic groups occurring
in Compound V to tumour cells. Six hours after administration, N,N'-bis(4-
carboxy-phenyl)-2,7-fluorene$^{35}$S disulphonamide, V, is excreted mainly through
the gastrointestinal tract. Considerable amounts of radioactivity are found in
small and large intestines—13-57 and 65-52 per cent of the given dose in the experi-
mental group and 10-47 and 60-33 per cent of the dose in the controls,
respectively (Table I-B). The lower values for the control group are mostly
compensated by the activity found in faeces—6-08 per cent of the dose given.
In the tumour-bearers radioactivity is not found in faeces, and about $\frac{1}{3}$ less
compound is found in urine of this group than in tumour-free animals (Table II),
thus showing similarity to the distribution of Compound III.

N,N'-bis(5(6)-nitro-benzimidazolyl)-2,7-fluorene$^{35}$S disulphonamide, VI, was
obtained in the reaction of 2,7-fluorene$^{35}$S disульphonylchloride with imino-
nitrogen of 5(6)-nitro-benzimidazole. The nitro groups at C5 or C6 of benzene
ring as electronegative substituents were expected to increase tumour localization
of the compound. Data obtained for the distribution of Compound VI in tumour-
bearing and tumour-free Wistar rats show its very similar behaviour to Compound IV (Tables I-A, I-B and Table II). The excretion of Compound VI into urine remains on a similar level to Compound IV; the concentration of radioactivity in tumour was very much the same.

A portion of the sulphadimethoxine molecule, a sulphonamide known to remain at elevated blood levels for prolonged periods of time, appears in N,N'-bis (6(2,4-dimethoxy-pyrimidyl))-2,7-fluorene[35S]disulphonamide, VII, which similarly to the bis-thiazole derivative, I, (Malejka, 1965) is insoluble in water and requires an alkaline pH to dissolve. Compound VII has a higher molecular weight (600·5) than Compound I (490·6). These physico-chemical properties might account for the slow elimination of Compound VII in the rat, and this is slower in the experimental animals than in the controls. In the skin of tumour bearing animals twice as much, and in the small intestines (+ contents) 3 times more, of Compound VII is found as in the controls. (The difference between the two groups of animals for small intestines is at P < 0·001 (t = 7·70). On the other hand, in the large intestines (+ contents) of tumour-free animals 35 times more and in the urine 2 times more of Compound VII is present than in the tumour-bearing group. The differences in the concentration of Compound VII between the two groups found in the large intestines and in the urine are statistically significant (t = 8·82, P < 0·001 and P ≥ 0·05 in Table II, respectively).

The response of the reticulo-endothelial system towards the uptake of fluorene disulphonamides is different for every compound presented in this paper. Compound III deviates from the behaviour of four other disulphonamides in that some radioactivity is found in the liver and spleen of tumour-bearers but not in these organs of the control group. This may indicate that only liver impaired by the presence of a tumour in the animal body can take up the compound, although the effect is not statistically significant. Compounds IV, V, and VI localize to a greater extent in the liver of tumour-free animals than in the liver of the tumour-bearers (ratios 2·67, 2·57 and ∞, respectively, in Table II). The difference in the uptake of the compounds by the control and experimental liver is statistically significant only for Compound VI (0·02 < P < 0·05). The content of Compound VII in the liver of both groups of animals is similar (ratio 0·93). In the spleen of tumour-free rats 1·5 times more Compound VII is found than in the presence of tumour (0·05 < P < 0·1), and also larger amounts of Compounds V and VI are found in this organ of the control group than in the tumour-bearers (the differences between the two groups for Compounds V and VI are not, however, significant). Thus, in general, the four disulphonamides (Compounds IV, V, VI and VII) tend to follow the behaviour of the pattern compound: N,N'-bis(naphthalene)-2,7-fluorene[35S]disulphonamide with which an impaired function of the reticulo-endothelial system in the presence of tumour has been shown (Argus, 1961; Argus, Hudson, Seepe, Kane and Ray, 1962).

The aspect of a possible diagnostic value of recently obtained disulphonamides can be discussed only in relation to Compounds IV, VI and VII for which radioactivity is found in tumour 6 hours after their intravenous injections into experimental rats. In Table III the ratios of the concentration of radioactivity in tumour to the concentration in other tissues for the three disulphonamides and also for Compound I (Malejka, 1965) are presented.

As has been pointed out, the distributions of Compounds IV and VI appear to be very similar as are also the values of their concentrations in tumour. For
TABLE III.—Ratios of Concentration of Radioactivity in Tumour (Walker Carcinosarcoma in Wistar Rats) to the Concentration in Other Tissues 6 Hours After Intravenous Injection of Fluorene Disulphonamides Labelled with Sulphur-35.

| Tissue         | Compound I |                | Compound IV |                | Compound VI |                | Compound VII |                |
|----------------|------------|----------------|-------------|----------------|-------------|----------------|---------------|----------------|
|                | Ratio‡ t P |                | Ratio‡ t P  |                | Ratio‡ t P  |                | Ratio* t P    |                |
| Red blood cells| 1·50 2·52 <0·05 | 20·50 18·81 <0·001 | 16·21 3·91 <0·02 | 8·50 51·00 <0·001 |
| Blood plasma   | 0·47       |                | 6·00 1·61 <0·02 | 2·00 1·09 <0·03 | 1·89 3·47 <0·05 |
| Liver          | 0·06       |                | 5·40 2·95 <0·05 | 19·00 15·31 <0·001 | 0·30       |                |
| Lungs          | 0·48       |                | 2·71 2·78 <0·05 | 13·04 2·46 <0·05 <0·1 | 1·57 1·96 <0·1 <0·2 |
| Spleen         | 0·27       |                | 4·43 1·47 <0·02 | 0·75       |                | 0·95       |                |
| Kidneys        | 0·36       |                | 4·40 1·40 <0·02 | 0·57       |                | 0·29       |                |
| Leg muscle     | 3·18 6·89 <0·01 | 20·50 18·61 <0·001 | 12·82 1·30 <0·3 | 10·90 4·40 <0·02 |
| Carcass        | 0·43 4·30 <0·01 | 1·63 0·58 <0·06 | 6·60 0·56 <0·06 | 2·65 17·54 <0·001 |

Compounds: I: N,N'-bis(thiazole)-2,7-fluorene[2,3]disulphonamide (Malejka, 1965).
II: N,N'-bis(p-tolyl)sulphonyl-carbamyl-n-butyl)-2,7-fluorene[2,3]disulphonamide.
III: N,N'-bis(5(0)-nitro-benzimidazolyl)-2,7-fluorene[2,3]disulphonamide.
IV: N,N'-bis(2,4-dimethoxy-pyrimidyl)-2,7-fluorene[2,3]disulphonamide.

‡ Average value from 4 rats.
* Average value from 3 rats.

Ratio of concentration (in μg. compound per g. tissue or ml. blood) in the tumour (x) to the concentration in the respective tissue (y) is subject of transformation $y = \log\left(\frac{x + \frac{1}{2}}{y + \frac{1}{2}}\right)$ which 1) stabilizes variance and 2) eliminates possibility of infinite ratio (Ref. : G. W. Snedecor, Statistical Methods, The Iowa State University Press, Ames, Iowa, U.S.A., 1964).

$ t $ is a " $ t $ test " on the transformed values.

$ P $ is the probability of finding a value of $ t $ to the observed value of $ t $ under the hypotheses that the ratio $ x/y $ is equal to 1·00.
Compound IV all ratios of the concentration in tumour to the concentrations in blood cells and plasma, liver, lungs, spleen, kidneys, leg muscle and carcass were greater than 1·00. The ratios obtained for blood cells and leg muscle (20·50) are of a great statistical significance: (P < 0·001), also ratios for liver (5·40) and lungs (2·71) are significant (P \( \leq 0·05 \)). The most favourable ratios for Compound VI from the statistical point of view are those obtained for liver (19·00 at \( P < 0·001 \)), red blood cells (16·21 at \( P \approx 0·02 \)) and lungs (13·04 at \( 0·05 < P < 0·1 \)). The favourable ratios obtained for the other tissues for Compound IV, and for leg muscle and carcass for Compound VI are not of statistical significance; however, this does not exclude that a significant difference may be found, using larger numbers of animals.

The behaviour of Compound VII in Wistar rats can be compared to the bis-thiazole derivative (Compound I) (Malejka, 1965). It has been shown by means of radioelectrophoresis that the latter compound is strongly attached to rat and human serum albumins (Malejka, Kasprzak and Radola, 1966). This protein-binding ability might account for a high concentration of Compound I in the liver and spleen and also in tumour. The ratios of the concentration of Compound I in tumour to its concentration in blood cells, leg muscle and carcass exceeded 1·00 (1·50 at \( P \approx 0·05 \); 3·18 at \( 0·001 < P < 0·01 \); 9·43 at \( P \approx 0·01 \), respectively); the ratios for plasma, liver, lung, spleen and kidneys are smaller than 1·00.

The blood level of Compound VII (Table I-B) 6 hours after administration is not as high as Compound I (Malejka, 1965). Despite the less persistent plasma protein-binding ability of Compound VII, a more selective concentration in tumour is achieved with this derivative than with Compound I, as shown by the ratios in Table III for red blood cells (8·50 at \( P < 0·001 \)), blood plasma (1·89 at \( P \approx 0·05 \)), lungs (1·57 at \( 0·1 < P < 0·2 \)), leg muscle (10·9 at \( P \approx 0·02 \)) and carcass (2·65 at \( P < 0·001 \)). The ratios of the concentration of Compound VII in tumour to the concentration in liver, spleen and kidneys, however, remain smaller than 1·00. Compound VII, nevertheless, presents a most promising structure in searching for favourable localization in tumour, and encourages a further investigation of sulphonamido derivatives with heterocyclic rings.

CONCLUSIONS

1. The fluorene disulphonamides with aliphatic side chains symmetrically attached to 2,7-fluorene molecule, N,N'-bis(guanidyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, II, and N,N'-bis(carbamyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, III, result in rapid elimination from rat and show no tendency to localize in tumour.

2. Two high molecular weight disulphonamides: N,N'-bis(p-tolylsulphonyl-carbamyl-n-butyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, IV, and N,N'-bis(5(6)-nitrobenzimidazolyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, VI, with electronegative substituents in their molecules (sulphono-groups in IV and nitro-groups in VI) give similar distribution data and very similar concentration in tumour. The ratios of the concentration in tumour to the concentration in red blood cells, liver, lungs and also in plasma, leg muscle and carcass are favourable.

3. N,N'-bis(4-carboxy-phenyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, V, despite its free carboxylic groups is not found in tumour.
4. The compounds with heterocyclic rings: N,N'-bis(thiazole)-2,7-fluorene \(^{[35S]}\)disulphonamide, I, and N,N'-bis(6(2,4-dimethoxy-pyrimidyl))-2,7-fluorene \(^{[35S]}\)disulphonamide, VII, reveal appreciable concentrations in tumour (35 \(\mu g\) and 77 \(\mu g\) per g. tissue, respectively). It seems, however, that a strong protein-binding ability of Compound I passed its maximum for a diagnostic effect (ratios of the concentration of Compound I in tumour to the concentrations in plasma, liver, lungs, spleen and kidneys are not favourable) while a protein-binding level reached with Compound VII exhibits its more selective concentration in tumour (ratios for red blood cells, plasma, lungs, leg muscle and carcass are favourable).

**SUMMARY**

Five new radioactive compounds: N,N'-bis(carbamyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, III, N,N'-bis(p-tolylsulphonyl-carbamyl-n-butyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, IV, N,N'-bis(4-carboxy-phenyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, V, N,N'-bis(5(6)-nitro-benzimidazolyl)-2,7-fluorene\(^{[35S]}\)disulphonamide, VI, and N,N'-bis(6(2,4-dimethoxy-pyrimidyl))-2,7-fluorene\(^{[35S]}\)disulphonamide, VII, were synthesized, and their tissue distribution 6 hours after a single intravenous injection to tumour-bearing (Walker carcinosarcoma) and tumour-free Wistar rats was studied. The data obtained are discussed in relation to the bis-thiazole (Compound I) and bis-guanidyl (Compound II) derivatives examined previously (Malejka, 1965). This similarity of chemical structure of the substituents at 2,7-disulphonamido-fluorene draws a line of common factors in the behaviour of Compounds II and III. Compounds IV and VI, and Compounds I and VII. The distribution data for these paired compounds are similar. Compounds II and III are not present in tumour. The concentration in tumour of Compounds IV and VI is very similar and reveals favourable ratios for red blood cells, liver, lungs (statistically significant), and for plasma, leg muscle and carcass for both compounds, and also for spleen and kidneys for Compound IV. The highest concentration in tumour (77 \(\mu g\) per g. tissue) is achieved with Compound VII and this value gives favourable ratios for red blood cells, plasma, lungs, leg muscle and carcass (all statistically significant).

The present studies have shown an impaired elimination of fluorene disulphonamides in presence of tumour with Compounds III, V and VII. Four of the examined compounds (IV, V, VI and VII) tend to localize in the liver and spleen of tumour-free rats to a greater extent than in tumour-bearers, thus showing a weakened phagocytic function of the reticulo-endothelial system in the presence of tumour.

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