CLXII. EXPERIMENTS ON THE CHEMOTHERAPY OF CANCER

I. THE EFFECT OF CERTAIN ANTIBACTERIAL SUBSTANCES AND RELATED COMPOUNDS

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MANY dyes containing aminonaphtholsulphonic acid residues have affinity for some cells contained in grafted tumours and lymph glands. Such dyes are isamine blue [Roosen, 1930], trypan blue [Opitz *et al.* 1926] and an azo dye formed from H-acid [cf. Kottman, 1935]. According to Ludford [1929] these dyes stain the macrophages of tumours rather than the malignant cells proper. Prontosil soluble, an antibacterial agent, contains an acetaminonaphtholsulphonic acid residue and on injection into animals with tumours it stains tumour tissue apparently in the same way as do the other dyes of this class. Prontosil soluble was found to have some slight inhibitory action on the growth of tumours in mice and many drugs related to this have been tested for their actions on the growth of tumours. The Schiff's base of 4:4'-diaminodiphenylsulphone and heptaldehyde was prepared and tested because heptaldehyde has been described as causing retrogression of spontaneous tumours [Strong, 1938].

The study of the effect of substances on tumour growth is much easier with grafted tumours as an unlimited number of comparable tumours can be obtained at any one time. With spontaneous tumours, however, each tumour must act as its own control and each has its own properties. It is also at present difficult to obtain sufficient spontaneous tumours.

Whereas grafted tumours become haemorrhagic on treatment with bacterial filtrates [Andervont, 1936] or colchicine [Boyland & Boyland, 1937] spontaneous tumours do not react in the same way. This difference in behaviour makes it appear possible that some substance might be effective against spontaneous cancers and not against grafted tumours. All substances mentioned in this paper have therefore been tested on both spontaneous tumours and grafted tumours but it is thought that more weight should be placed on the results with the spontaneous tumours.

EXPERIMENTAL

Benzidinesulphone was prepared as described by Culinane & Davies [1936]. Tolidinesulphone was prepared in the same way. Heptylideneaminobenzene-sulphonamide was prepared by the general method of Gray *et al.* [1937]. It was crystallized from alcohol and had m.p. 171–172°. (Analysis (Schoeller) found: C, 57·3; H, 7·55; N, 10·15 %. $C_{13}H_{20}SO_2N_2$ requires C, 58·0; H, 7·5; N, 10·20 %.)

Female mice with spontaneous tumours which were either detected among the old breeding mice of the Strong A or Dilute Brown strains or supplied by dealers were examined and the tumours measured with calipers three times per week. The length and breadth of the tumour was recorded and the sum plotted. Normally the growth of tumours plotted in this way is linear [Boyland & McClean, 1935]. After the rate of growth of a tumour had been recorded for such a time

that the sum of length and breadth had increased by about 10 mm. the animal was treated as shown in the figures with the compound under investigation. All the substances were administered orally using a syringe with a long blunt needle. The toxicity of each substance was determined and the daily dose given was less than 1/4 of the toxic dose. Each substance was ground with water and gum tragacanth to give a suspension 0.5 ml. of which contained the daily dose. The results of the treatment with each compound were observed with four mice with spontaneous tumours as shown in the tumour growth curves. On the death of each mouse the tumour or tumours were removed for histological examination and in all cases were shown to be mammary carcinomata. The results are shown in Figs. 1-23 and summarized in Table I. The figures marked (*G*) are taken from Gray *et al.* [1937]. If the size of the tumour was less at the end of the period of treatment than at the beginning the tumour is described as having retrogressed.

Each substance was also tested in a similar way on the growth of the Crocker Sarcoma 180 in mice of the Strong A or Dilute Brown strains. In this case five mice of the same strain were treated with the compound while five mice grafted with the same tumour at the same time served as controls. If the substance produced definite inhibition of growth this is indicated by + in Table I.

Table I. *Action of substances on tumour growth in mice*

Substance	Toxic dose mg.	Dose given mg.	Effect on spontaneous tumours. Each substance tested on 4 mice		Maximum survival under treatment days	Inhibitory action on grafted tumours (Crocker 180)
			No. of tumours showing			
			Inhibition of growth	Retro- gression		
Prontosil soluble	> 50	25	3	2	16	—
Prontosil rubrum	2 (<i>G</i>)	3	3	0	50	—
<i>p</i> -Aminobenzenesulphonamide	100 (<i>G</i>)	25	4	2	58	+
<i>p</i> -Heptylideneaminobenzenesulphonamide	200	25	3	2	28	—
<i>p</i> -Aminobenzenesulphonyl- <i>p'</i> -sulphon- amidophenylamide hydrochloride	100 (<i>G</i>)	25	2	1	46	+
Sodium <i>o</i> -aminobenzenesulphonate	> 200	50	2	2	21	+
Sodium <i>m</i> -aminobenzenesulphonate	> 200	50	0	0	19	—
Sodium <i>p</i> -aminobenzenesulphonate	> 200	50	4	1	43	—
Sodium sulphanilylsulphanilate	> 200	20	4	3	33	+
Sodium <i>p</i> -hydrazinobenzenesulphonate	200	25	4	1	33	—
4:4'-Dinitrodiphenylsulphide	25	5	1	0	15	—
4:4'-Dinitrodiphenylsulphoxide	20	5	4	2	38	—
4:4'-Dinitrodiphenylsulphone	25	5	3	1	25	+
4:4'-Diaminodiphenylsulphide	30	5	3	1	35	—
4:4'-Diaminodiphenylsulphoxide	20	2	4	3	60	+
4:4'-Diaminodiphenylsulphone	10	2	4	2	16	+
4- <i>p</i> -Methoxybenzylideneamino-4'-amino- diphenylsulphone	200	4	3	3	15	+
4:4'-Diaminodiphenylsulphone glucose anil	100	10	4	2	37	+
Benzidinesulphone	20	4	3	0	44	+
Tolidinesulphone	10	4	3	2	15	—
Tetradecamethylene-1:14-diaminedihydro- chloride	2	1	3	2	25	+
Synthalin A (decamethylene-1:10-di- guanidine hydrochloride)	0.2	0.05	4	1	37	+
Synthalin B (dodecamethylene-1:12-di- guanidine hydrochloride)	0.2	0.05	2	1	32	—

Some of the compounds were tested for any action which they might have upon the increase in body weight of young mice. Groups of four mice weighing 10 g. each were weighed daily and while one group remained as control other

groups were given *p*-aminobenzenesulphonamide, 4:4'-diaminodiphenylsulphone, 4:4'-diaminodiphenylsulphoxide and 4-*p*-methoxybenzylideneamino-4'-aminodiphenylsulphone. None of these compounds produced continued inhibition of body growth (see Fig. 24). Inhibition occurred with all of the compounds for periods of 3-9 days after the beginning of treatment but subsequently growth at the normal rate occurred, although the compounds were administered at the same level. The loss of weight which occurred during the first few days of administration of the drugs may be due to increase in the excretion of urine, such as occurs when *p*-aminobenzenesulphonamide is given to rats [Rimington, 1938].

DISCUSSION

Although treatment with prontosil soluble (Fig. 1) produced inhibition of tumour growth, the mice did not survive the treatment very long; red prontosil (Fig. 2) and *p*-aminobenzenesulphonamide (Fig. 3) which are equally effective as antibacterial compounds appeared to be more effective in inhibiting tumour growth and animals so treated survived much longer. Heptylideneaminobenzene-sulphonamide (Fig. 4) and *p*-aminobenzenesulphonyl-*p*-sulphonamidophenylamide hydrochloride (Fig. 5) also have some action of this kind.

Buttle *et al.* [1936] found that sulphanilic acid has only about 1/20 of the therapeutical action of *p*-aminobenzenesulphonamide and that *m*-aminobenzene-sulphonic acid and *p*-hydrazinobenzene-sulphonic acid had only slight actions against streptococcal infections in mice. Tested on tumours *m*-aminobenzene-sulphonic acid had no action (Fig. 7) while *p*-aminobenzene-sulphonic acid (Fig. 8) had definite action and *o*-aminobenzene-sulphonic acid (Fig. 6) and *p*-hydrazinobenzene-sulphonic acid (Fig. 10) some slight action. The marked difference between the *m*- and *p*-aminobenzenesulphonates indicates that some specificity in tumour-inhibiting action may be found. Sodium sulphanilyl-sulphanilate (Fig. 9) which has been recommended for treatment of canine distemper [Dochez & Slanetz, 1938] was more effective than sulphanilic acid or *p*-aminobenzenesulphonamide.

The 4:4'-diaminodiphenyl- and 4:4'-dinitrodiphenyl-sulphides, -sulphoxides and -sulphones (see Figs. 11-16) all showed some action but the most effective compounds were the sulphoxides, particularly 4:4'-diaminodiphenylsulphoxide. Levaditi *et al.* [1937] claim that 4-nitro-4'-aminodiphenylsulphoxide is particularly efficacious against gonococcal infections. In the case of one mouse (Fig. 14, no. 426) treatment with 4:4'-diaminodiphenylsulphide appeared to increase the rate of growth.

The Schiff's bases 4-*p*-methoxybenzylideneamino-4'-aminodiphenylsulphone (Fig. 17) and 4:4'-diaminodiphenylsulphone-glucose anil (Fig. 18) did not appear to be as effective as the original sulphones. Two diphenylene sulphones, benzidine-sulphone (Fig. 19) and tolidinesulphone (Fig. 20), produced definite inhibition, although in these compounds the amino groups are situated in the *m*-position to the sulphone group. It is hoped to be able to test other substances related to benzidinesulphone.

Tetradecamethylene-1:14-diaminedihydrochloride, which King *et al.* [1937] have shown to be effective against some trypanosome infections, had some inhibitory action (Fig. 21) as also had synthalin A and synthalin B (Figs. 22 and 23) which Jancsó & Jancsó [1935] found to have trypanocidal action in mice.

In those cases in which the mice survived and measurements were made after treatment had been stopped, the rate of growth of the tumours returned to normal indicating that the compounds did not produce a permanent inhibition

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

For explanation of Figs. 1-12 see p. 1213.

For explanation of Figs. 13-24 see p. 1213.

of growth. This effect is to be seen in Fig. 4 (mouse 541) treated with *p*-heptylideneaminobenzenesulphonamide, Fig. 15 (mouse 315) with *pp'*-diaminodiphenylsulphoxide and Fig. 22 (mouse 574) treated with synthalin A.

The effect of these substances on growth in body weight (Fig. 24) and the fact that the growth of tumours is inhibited only while the drugs are administered indicate that the inhibition is somewhat different from that produced by carcinogenic compounds [Haddow *et al.* 1937]. Carcinogenic hydrocarbons generally exert a prolonged inhibitory action both on tumour growth and on growth of body weight.

Many of the treated tumours in which growth was inhibited became much softer. The degree of hardness of a tumour is difficult to assess but the softened tumours vary considerably in size from day to day. As a result of this, marked variations in tumour size from day to day are recorded as shown in Fig. 2, mice 507 and 531; Fig. 4, mouse 552; Fig. 5, mouse 434; Fig. 9, mice 525 and 534; Fig. 10, mouse 446; Fig. 12, mouse 413; Fig. 15, mouse 370; Fig. 21, mouse 281; Fig. 17, mouse 318; Fig. 23, mouse 342 and Fig. 19, mice 362 and 390.

From Table I it can be seen that of the compounds tested only 4:4'-diaminodiphenylsulphoxide and sodium sulphanilylsulphanilate inhibited the growth of the Crocker sarcoma, inhibited the growth of all spontaneous tumours used and caused retrogression in the case of three out of four of the mice with spontaneous tumours. Other compounds related to these are being investigated.

SUMMARY

Some aromatic sulphur compounds, have been found to retard the growth of spontaneous mammary cancer in mice. The most effective of the compounds tested appeared to be *pp'*-diaminodiphenylsulphoxide and sodium sulphanilylsulphanilate.

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EXPLANATION OF FIGS. 1-24.

Abscissae: Figs. 1-24: Days.

Ordinates: Figs. 1-23: Tumour size as sum of length and breadth in mm.

Fig. 24: Average wt. of mice in g.

- Fig. 1. Growth of spontaneous mammary tumours in mice treated with 7-acetamino-2:4'-sulphonamidobenzene-azo-1-naphthol-3:6-disulphonate (prontosil soluble).
 In this and other figures each arrow represents the administration of the dose shown in Table I.
 Fig. 2. Growth of mammary tumours in mice treated with 4-sulphonamidobenzene-azo-2':4'-diaminobenzene (red prontosil).
 Fig. 3. Growth of mammary tumours in mice treated with *p*-aminobenzenesulphonamide.
 Fig. 4. Growth of mammary tumours in mice treated with *p*-heptylideneaminobenzenesulphonamide.
 Fig. 5. Growth of mammary tumours in mice treated with *p*-aminobenzenesulphonyl-*p*'-sulphonamidophenylamide hydrochloride.
 Fig. 6. Growth of mammary tumours in mice treated with sodium *o*-aminobenzenesulphonate.
 Fig. 7. Growth of mammary tumours in mice treated with sodium *m*-aminobenzenesulphonate.
 Fig. 8. Growth of mammary tumours in mice treated with sodium *p*-aminobenzenesulphonate.
 Fig. 9. Growth of mammary tumours in mice treated with sodium sulphanilylsulphanilate.
 Fig. 10. Growth of mammary tumours in mice treated with sodium *p*-hydrazinobenzenesulphonate.
 Fig. 11. Growth of mammary tumours in mice treated with 4:4'-dinitrodiphenylsulphide.
 Fig. 12. Growth of mammary tumours in mice treated with 4:4'-dinitrodiphenylsulphoxide.
 Fig. 13. Growth of mammary tumours in mice treated with 4:4'-dinitrodiphenylsulphone.
 Fig. 14. Growth of mammary tumours in mice treated with 4:4'-diaminodiphenylsulphide.
 Fig. 15. Growth of mammary tumours in mice treated with 4:4'-diaminodiphenylsulphoxide.
 Fig. 16. Growth of mammary tumours in mice treated with 4:4'-diaminodiphenylsulphone.
 Fig. 17. Growth of mammary tumours in mice treated with 4-*p*-methoxybenzylideneamino-4'-aminodiphenylsulphone.
 Fig. 18. Growth of mammary tumours in mice treated with 4:4'-diaminodiphenylsulphone glucose anil.
 Fig. 19. Growth of mammary tumours in mice treated with benzidinesulphone.
 Fig. 20. Growth of mammary tumours in mice treated with tolidinesulphone.
 Fig. 21. Growth of mammary tumours in mice treated with tetradecamethylene-1:14-diamine-dihydrochloride.
 Fig. 22. Growth of mammary tumours in mice treated with decamethylenediguanidine hydrochloride (synthalin A).
 Fig. 23. Growth of mammary tumours in mice treated with dodecamethylenediguanidine hydrochloride (synthalin B).
 Fig. 24. The growth in body weight of mice treated with certain sulphur compounds.