Experiments on the Chemotherapy of Cancer

6. THE EFFECT OF AROMATIC BASES

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The growth of tumours in mice has been shown to be inhibited by administration of large and repeated doses of certain aromatic amines containing sulphur in the molecule, such as 4:4'-diaminodiphenylsulphoxide and sulphanilyl sulphanilic acid (Boyland, 1938). Later experiments (Boyland, 1942) indicated that other amines which did not contain sulphur, such as benzidine, 4:4'-diaminodiphenylether and 4:4'-diaminodiphenylmethane also had tumour inhibiting activity. Most of the substances dealt with in the present paper can be considered in four groups: (1) sulphonamides, following the original observation that Prontosil-soluble acted as a vital stain and inhibitor of tumour growth; (2) diaminodiphenyl derivatives and possible precursors of these, in an attempt to find more potent substances than diaminodiphenylsulphoxide; (3) nitrosophenols, tried on the hypothesis that the hydroxylamino forms might inhibit catalase—an enzyme in which tumours are particularly deficient and (4) substances which might combine with metals, probably present in low concentrations in tumours and essential for cellular function (e.g. 2-mercaptopbenzthiazole and the nitrosophenols which combine with copper). The object of the experiments has been to detect tumour growth-inhibiting action in compounds which might be safe to use over prolonged periods.

EXPERIMENTAL

Methods

The experimental technique was the same as that used in earlier experiments (Boyland, 1940) each substance being tried on four mice with spontaneous mammary cancer and on groups of five Dilute Brown mice with the grafted sarcoma MCD B1. The mice were dosed orally, 12 times in 14 days with a dose of each compound equivalent to one-quarter of the L.D. 50. If mice died during the repeated administration the dose was reduced to the maximum which the mice could tolerate. The growth of tumours was followed by measuring with calipers during a preliminary control period, then during the period of treatment and again for a period following treatment. The probability, *P*, of the effect on tumour growth being due to chance was calculated by the method of *t*. (All the experiments were completed by May 1941 but preparation of the data for publication has been delayed owing to the war.)

Inactive compounds (preliminary tests)

Several compounds were found to be inactive when tested on two mice bearing spontaneous tumours and were not investigated further, on account of the shortage of tumour bearing mice. These compounds (with the daily dose in parenthesis) were *p*-aminobenzenesulphonate-ethylamine (20 mg.), tetrabromobenzidinesulphonate (5 mg.), sodium-2-amino-8-naphthol-6-sulphonate (25 mg.), sodium 1-amino-8-naphthol-4:6-disulphonate (25 mg.), *p*-phenylenediamine (1 mg.), 2-nitrodiphenylsulphide (5 mg.), *p*-nitrosophenol (5 mg.), nitroso *R*-salt (10 mg.), nitrosodimethylaminophenol (2 mg.) and phenylisopropylamine (2 mg.).

Results

The data summarized in Table 1 show that complete inhibition of growth of spontaneous tumours in four out of four mice was produced by only three compounds—methylene blue (no. 87), 4:4'-diaminodiphenylsulphoxide (no. 48) and 4:4'-diaminodiphenylether (no. 42). The difference in effect of thionine (no. 35) and methylene blue may be due to the larger dose of the latter which could be employed. In addition to these diamines mean inhibitions of 100 % were obtained with compounds nos. 11, 18, 23, 41, 43, 46, 48, 57 and 73, all of which are diaminodiphenyl compounds or precursors of such compounds, and by no. 74 which is a sulphonamide derivative. Inhibitions recorded as over 100 % mean that the tumour appeared to diminish in size. Such diminution is much more variable than growth, so that differences in the rate of retrogression or 'negative growth rate' are probably less important than differences in growth.

The diaminodiphenyl compounds were generally more effective than sulphonamide compounds. Some diaminodiphenyl derivatives had only slight activity; thus 4:4'-diaminodiphenylamine (no. 38) was almost inactive possibly because only small doses could be given repeatedly. The lengthening of the chain between the phenyl groups (as in nos. 32, 33, 36, 37 and 49) did not appear to increase the inhibitory action. The two diaminostilbenes (nos. 51 and 52) are similar to the 4-dimethylaminostil-

Table 1. *Ratios of the growth rates of mouse tumours under treatment to their normal growth rates*

(Each column A, B, C and D refers to a separate mouse. If any diminution in size occurred during the treatment period this is recorded by a minus sign in heavy type and the inhibition is considered as over 100 %.)

Compound	Toxicity L.D. 50 for mice (mg. per mouse)	Daily dose (mg. per mouse)	Effect on grafted sarcoma MCDB 1 in groups of 5-15 mice Inhibition (%)	Effect on spontaneous carcinomata Ratio of growth in treatment period to control period				Average effect on spontaneous carcinomata	
				A	B	C	D	Inhibition of growth under treatment (%)	Probability (P) that the inhibition is significant
(1) <i>p</i> -Aminobenzene-sulphonamide	30	5	7	-0.75	0.57	-0.14	0.91	85	0.1
<i>p</i> -Aminobenzene-sulphonamide	100	25	18	0.40	0.75	0.48	-0.37	69	0.05
(2) 2-(<i>p</i> -Aminobenzene-sulphonamido)-pyridine	100	5	7	0.06	0.10	0.14	0.80	75	0.05
(3) 6-(<i>p</i> -Aminobenzene-sulphonamido)-quinoline	20	3	17	0.85	0.20	0.28	0.17	63	0.05
(4) <i>p</i> -Aminobenzene-sulphone-ethyl-piperidine	10	2	7	0.78	1.03	0.96	0.93	8	0.3
(5) <i>p</i> -Aminobenzene-sulphonyl- <i>p</i> -sulphonamido-phenyl-amide hydrochloride	100	25	20	0.27	0.07	0.22	0.27	83	0.01
(6) 2-(<i>p</i> -Aminobenzene-sulphonamido)-thiazole	50	10	2	-0.31	-0.14	0.00	0.66	95	0.02
(7) 4-Amino-4'-dimethylamino-diphenylether	25	5	10	-0.85	0.16	1.19	0.00	88	0.1
(8) 4-Amino-diphenylether-4'-trimethylammonium iodide	40	5	7	0.27	0.00	0.45	0.28	75	0.01
(9) 2-Amino-6-ethoxy-benzthiazole hydrochloride	10	2	10	-0.33	-0.28	0.86	-1.21	64	0.2
(10) 1-Amino-8-naphthol-3-disulphonic acid	200	25	12	0.39	0.45	0.87	0.78	55	0.2
(11) 2-Amino-2'-nitro-diphenyl-sulphide	20	5	12	-1.62	-0.06	0.10	0.39	105	0.02
(12) <i>p</i> -Aminophenol	30	5	12	0.30	0.83	0.36	0.45	51	0.1
(13) 2-Aminopyridine-5-sulphonamide	20	5	14	0.40	0.59	0.95	0.00	51	0.1
(14) <i>o</i> -Aniline-sulphonic acid	>200	40	13	1.60	0.77	0.33	0.12	28	0.5
(15) <i>m</i> -Aniline-sulphonic acid	>200	50	—	1.09	0.39	1.21	0.43	22	0.4
(16) <i>p</i> -Aniline-sulphonic acid (Sulphanilic acid)	>200	50	—	0.71	-0.54	0.20	0.17	86	0.05
(17) 1,2'-Azonaphthalene	>200	50	—	-0.05	0.57	0.25	0.45	70	0.02
(18) Benzidine	20	3	5	0.44	-0.48	0.23	-0.31	102	0.02
(19) Benzidine-sulphone	20	5	5	0.19	1.03	0.08	0.22	62	0.1
(20) Benzylamine hydrochloride	100	15	4	0.63	-0.39	0.29	0.35	78	0.05
(21) <i>p</i> -Cresol-sulphoxide	30	5	8	0.45	0.69	1.12	1.02	18	0.3
(22) 4,4'-Diacetamido-diphenyl-amine	20	5	—	1.50	-0.31	0.57	0.64	40	0.4
(23) 4,4'-Diacetamido-diphenyl-ether	200	10	30	-0.11	-0.72	0.05	0.33	111	0.01
(24) 4,4'-Diamidino-diphenylether dihydrochloride	12	1.5	21	0.09	0.37	0.34	0.68	63	0.02
(25) 4,4'-Diamidino-diphenyl-methane dihydrochloride	45	5	18	0.43	0.43	0.89	-0.27	62	0.1
(26) Diamino-acridinium sulphate (Proflavine)	15	2	19	0.52	0.56	0.08	0.48	59	0.01
(27) 4,4'-Diamino-azobenzene	25	5	14	0.29	0.00	0.71	0.62	60	0.05
(28) 4,3'-Diamino-benzenesulphonamide	100	25	10	2.20	0.91	1.66	1.22	-50	—
(29) 4,4'-Diamino-benzene-sulphanilide	100	10	9	0.56	0.40	-0.44	-0.22	92	0.05
(30) 4,4'-Diamino-benzene-sulphonyl-phenol	50	8	5	0.53	0.05	1.43	0.31	42	0.3
(31) 4,4'-Diamino-3-carboxy-benzene-sulphonamide	100	25	0	0.10	0.36	0.47	0.40	67	0.01
(32) 4,4'-Diaminodibenzyl	15	5	16	0.87	-0.12	0.61	0.37	57	0.1
(33) 4,4'-Diamino-dibenzyl ether	20	5	7	0.41	0.88	0.31	0.47	48	0.1
(34) 4,4'-Diamino-2,2'-dinitro-diphenyl-methane	—	5	22	0.20	0.90	0.37	0.66	47	0.05
(35) Diamino-diphenaz-thionium chloride (Thionine)	100	10	2	0.32	0.48	-0.21	0.60	65	0.05
(36) 4,4'-Diamino- α : β -diphenoxymethane	80	20	—	-0.49	1.0	-0.11	-0.10	92	0.07
(37) 4,4'-Diamino-diphenoxypentane	10	3	—	0.05	0.70	-0.55	0.37	86	0.05
(38) 4,4'-Diamino-diphenylamine	50	0.2	—	0.86	0.39	1.56	0.72	12	0.5
(39) 4,4'-Diamino- α : β -diphenyl-amino-methane	8	2	—	0.30	0.14	0.51	0.56	62	0.1
(40) 2,2'-Diamino-diphenyl disulphide	12	2	18	0.07	-0.05	-0.47	-0.75	92	0.05
(41) 2,4'-Diamino-diphenyl ether	30	5	6	-1.19	1.10	-1.28	1.16	105	0.25
(42) 4,4'-Diamino-diphenyl ether	20	5	16	-1.25	-0.50	0.00	-0.17	148	0.02
(43) 4,4'-Diamino-diphenylmethane	15	3	19	0.21	-0.95	-0.75	0.20	132	0.03
(44) 4,4'-Diamino-diphenylthiourea	2	0.5	—	0.00	0.21	-0.22	0.26	94	0.02
(45) 4,4'-Diamino-diphenyl sulphide	30	5	6	1.36	0.00	-0.18	0.07	69	0.2
(46) 4,4'-Diamino-diphenyl sulphone	10	2	15	-0.18	0.00	-0.63	0.79	100	0.05
(47) 4,4'-Diamino-diphenyl-sulphone-glucose anil.	—	10	23	0.73	0.19	-0.49	0.36	80	0.05
(48) 4,4'-Diamino-diphenyl sulphoxide	10	2	—	-0.46	-0.32	0.00	0.00	119	0.01
(49) 4,4'-Diamino-phenylbenzyl ether	20	5	14	1.06	-0.06	0.86	-0.11	56	0.2
(50) Di-(<i>p</i> -amino-phenyl-sulphone-ethyl)-amine	100	10	0	0.87	0.26	0.56	-0.20	63	0.1
(51) 2,2-Diaminostilbene hydrochloride	100	25	13	0.63	0.63	1.00	1.07	17	0.2
(52) 4,4'-Diaminostilbene	20	5	8	-1.03	0.49	0.12	0.62	95	0.05
(53) 4,4'-Diamino-triphenylmethane	50	5	6	0.82	-0.20	0.24	-0.43	89	0.05
(54) Diansidine	70	10	3	0.66	-0.28	0.36	0.61	66	0.1
(55) 4,4'-Dibenzamido-diphenyl ether	80	20	—	0.80	0.14	0.25	0.94	46	0.1
(56) Diguano-diphenyl ether	20	5	21	0.62	-0.56	1.01	0.00	73	0.1
(57) 2,4-Dinitro-2'-amino-diphenyl sulphide	25	5	15	0.62	0.00	-1.22	-0.05	116	0.05
(58) 2,6-Dinitro-diphenylamine sulphoxide	200	5	2	0.18	0.86	0.57	0.47	48	0.05
(59) 4,4'-Dinitro-diphenyl sulphide	25	5	—	0.72	-0.44	0.44	0.77	63	0.05
(60) 4,4'-Dinitro-diphenyl sulphone	20	5	—	-0.31	0.28	0.12	0.79	78	0.07
(61) 4,4'-Dinitro-diphenyl sulphoxide	25	5	—	0.0	-0.18	-0.14	0.22	102	0.05
(62) Dinitrosoresorcinol	25	5	9	-1.30	0.97	1.15	0.35	71	0.3
(63) 4,4'-Dipropionaminodiphenyl ether	60	15	—	0.94	0.55	0.07	0.21	56	0.1
(64) γ : γ -Dipyridyl	25	3	22	0.24	-0.07	-0.08	0.28	91	0.02

Table 1 (cont.)

Compound	Toxicity L.D. 50 for mice (mg. per mouse)	Daily dose (mg. per mouse)	Effect on grafted sarcomata MCDB 1 in groups of 5-15 mice Inhibition (%)	Effect on spontaneous carcinomata Ratio of growth in treatment period to control period				Average effect on spontaneous carcinomata	
								Inhibition of growth under treatment (%)	Probability (P) that the inhibition is significant
				A	B	C	D		
(65) 4:4'-Disuccinylaminodiphenyl ether	100	20	—	0.63	0.98	0.00	0.10	57	0.1
(66) Disulphanilamide	60	15	—	0.25	-0.11	0.93	0.44	62	0.1
(67) Di- <i>o</i> -tolylthiourea	—	0.5	—	0.00	-0.14	1.03	1.46	41	0.3
(68) Di- <i>p</i> -tolylthiourea	200	0.5	—	0.33	1.00	-0.00	0.23	61	0.07
(69) Di- <i>p</i> -tolylthiourea	—	5	20	0.71	0.28	0.45	0.57	50	0.01
(70) <i>p</i> -Heptylidene-amino-benzene-sulphonamide	100	25	4	-0.07	0.81	0.25	1.68	33	0.6
(71) 2-Meraptobenzthiazole	80	20	15	0.62	0.55	0.21	0.96	41	0.1
(72) 2-Meraptothiazoline	25	5	12	-0.37	0.65	0.79	0.40	63	0.1
(73) Methoxybenzylideneamino-4'-amino-diphenyl sulphone	100	4	17	—	-0.22	0.40	-0.70	113	0.1
(74) 4-Methyl-2-(<i>p</i> -sulphonamido-phenyl-amino)- thiazole	200	50	0	-1.02	-0.14	-0.20	0.33	126	0.02
(75) 2-Nitro-2'-carboxy-diphenyl sulphide	—	5	—	0.0	0.86	0.64	0.50	49	0.05
(76) Nitroso- <i>o</i> -cresol	20	5	20	0.21	0.70	0.71	0.72	42	0.05
(77) α -Nitroso- β -naphthol	25	5	36	0.28	0.54	-0.18	-0.08	86	0.01
(78) Nitrosoresorcinol	40	5	32	0.25	-0.10	0.78	0.40	67	0.05
(79) <i>o</i> -Phenylenediamine	20	5	20	0.71	-0.43	1.00	0.18	64	0.1
(80) Phenylhydrazinesulphonic acid	100	25	28	0.58	0.44	0.71	0.23	51	0.02
(81) <i>p</i> -(γ -Phenyl-propylamino)benzene-sulphonamide- α,γ -disulphonic acid	200	50	8	0.09	0.00	0.05	0.46	85	0.01
(82) β -Pyridinesulphonic acid	200	50	0	-0.57	0.23	0.36	0.40	89	0.02
(83) 4-Sulphamido-2':4'-diamino-azo-benzene (Prontosil rubrum)	30	4	8	0.23	0.00	0.80	-0.22	80	0.05
(84) 4'-Sulphonamido-phenyl-azo-1-hydroxy-7- acetamido-naphthalene-3:6-disulphonic acid (Prontosil soluble)	80	20	19	0.44	0.48	0.57	0.73	45	0.01
(85) Sulphanilyl-sulphanilic acid	100	10	6	0.25	-0.64	0.37	0.10	98	0.01
(86) Tetramethyl-4:4'-diamino-diphenylmethane	25	2	2	0.00	0.00	0.26	0.75	75	0.05
(87) Tetramethyl-diamino-diphenaz-thionium chloride (methylene blue)	40	8	1	0.0	-0.03	0.0	0.0	102	0.01
(88) 2:4:2':4'-Tetranitro-diphenyl sulphide	30	5	19	0.57	-0.21	-0.29	-1.00	98	0.01
(89) Tolidine sulphone	20	5	3	0.54	0.31	0.00	0.50	61	0.1
(90) 2:4:6-Trinitro-4'-acetamido-diphenyl sulphone	30	5	5	1.46	0.63	1.20	0.71	0	—

bene reported to have inhibitory action by Haddow, Harris & Kon (1945). The 4:4'-diaminostilbene was active while the isomeric 2:2'-diaminostilbene was ineffective.

In general the inhibition of tumour growth produced by the compounds under discussion occurred only during the period of dosing. When dosing stopped the normal growth was restored. This was the case even with 1:2'-azonaphthalene, a compound which produced prolonged growth inhibition when injected intraperitoneally into rats or mice (Badger, Elson, Haddow, Hewett & Robinson, 1942). Exceptionally prolonged inhibition lasting for 2-3 weeks after cessation of dosing was observed in the present experiments with 4:4'-diaminodiphenyl-ether and 6(*p*-aminobenzenesulphonamido)-quinoline (no. 3). This prolonged action may have been due to an accumulation of the drug persisting in the mice for some time after dosing was stopped, although this appears to be unlikely.

DISCUSSION

The inhibitory effect observed with some compounds may be a secondary consequence of a general toxic action on the animals, as Stamer (1943) has

suggested for the inhibitory action of 9:10-dimethyl-1:2-benzanthracene on the growth of grafted sarcomata in mice and rats. In the present experiments the mice were given a daily dose of the compounds which was a constant ratio (1/4) of the median lethal dose, the aim being to measure inhibitory action, while keeping the 'toxic action' constant. Toxic effects, however, may possibly vary apart from lethal action. The dosing with an amount of drug which might be expected to kill a certain small proportion of mice might be a better method of administering more constant equivalent toxic doses of different compounds. This would involve preliminary determination of the slope of the dose-mortality curve in addition to the L.D. 50.

By measuring therapeutic effects at a fixed fraction of the lethal doses, comparative figures varying roughly with the therapeutic index should be obtained. The dosing level chosen of 1/4 of the L.D. 50, now appears to have been rather high for the following reasons: (1) a number of compounds—e.g. Prontosil soluble (no. 84), 4:4'-diaminodiphenylamine (no. 38), the diamidino compounds (nos. 24 and 25), thionine, dianisidine (no. 54), 4:4'-dinitrodiphenylaminesulphoxide (no. 58)—could

not be given repeatedly at such a high level, because they had a cumulative toxic action. (2) If the dose-mortality curve is not very steep an appreciable number of mice are killed by doses equal to 1/4 of the L.D. 50. (3) The dose is too high to differentiate between effects of the more potent compounds. The dose used was chosen at the beginning of the work when it was thought that any effect on tumour growth would be difficult to produce. The results obtained, however, do suggest that there is some correlation between chemical structure and tumour inhibiting power.

Sulphonamides have been shown to reduce the effective vitamin intake of animals by suppressing the intestinal flora, and the diminution of riboflavin, biotin or pantothenic acid in the diet of tumour-bearing mice is known to reduce the growth rate of tumours. It is therefore possible that the inhibitory effects obtained with sulphonamides may be a secondary effect of the impaired nutritional state of the mice.

4:4'-Diaminodiphenylsulphone which was shown to inhibit tumour growth (Boyland, 1938) has since been found to inhibit the growth of tubercle bacilli *in vitro*, and the glucose anil (compound no. 47) has also some therapeutic effect on tuberculosis in guinea-pigs (Feldman, Hinshaw & Moses, 1942). The growth of tubercle bacilli is also inhibited by methylene blue, thionine (Freedlander, 1944) and 4:4'-diaminodiphenylmethane (author's unpublished observations). Tuberculostatic action has also been found with 1:2-benzophenothiazine (Freedlander, 1944) which Badger *et al.* (1942) found to inhibit growth of sarcomata in rats. There may, therefore, be some relation between the structure of compounds which inhibit the growth of tubercle bacilli and the growth of some tumours.

Even the large doses used in these experiments have not produced much effect on the grafted sarcomata, and the effects on the two types of tumours did not always go together. Both types of tumour were inhibited by 4:4'-diacetamidodiphenylether but the nitrosophenols (nos. 76, 77 and 79) were relatively less active against spontaneous carcinomata than against grafted sarcomata. This is further evidence indicating that substances should be tested on more than one type of tumour. The specific effect of oestrogens in treatment of cancer of the prostate (cf. Huggins & Hodges, 1941) and of 9:10-dimethyl-1:2-benzanthracene in treatment of leukaemia in mice (Stamer, 1943) show that the use of different therapeutic agents may be found necessary to be efficacious in different types of cancer.

SUMMARY

1. Of a number of cyclic nitrogenous compounds which have been tested for tumour growth inhibition on carcinomata and sarcomata in mice the most effective of the substances used against carcinomata appear to be diaminodiphenyl derivatives such as benzidine, 4:4'-diaminodiphenylether and 4:4'-diaminodiphenylsulphoxide.

2. The inhibitory action was generally only present during the period of dosing, but in the case of 4:4'-diaminodiphenylether and 6-(*p*-aminobenzene-sulphonamido)-quinoline a more prolonged effect was obtained.

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