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CYTOTOXIC AGENTS: I, METHYLOLAMIDES WITH TUMOUR INHIBITORY ACTIVITY, AND RELATED INACTIVE COMPOUNDS

BY

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In 1945 our colleagues Templeman and Sexton reported a study of the effects of arylcarbamic esters and related compounds upon the growth and morphology of cereal seedlings. They confirmed and extended the observations of Lefèvre (1939) that ethyl-N-phenylcarbamate produces bulbous hypertrophies of the coleoptile, the mesocotyl, and the root tip of wheat seedlings with progressive retardation or even arrest of growth. They found *iso*propyl-N-phenylcarbamate to be even more active in this sense and regarded the underlying cytotoxic effect of these substances as a blocking of mitosis in a pseudo-metaphase essentially similar to that produced by colchicine. Ethyl carbamate (urethane) was found to be inactive.

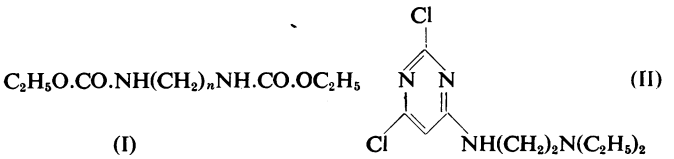
These findings prompted a study of the effects of the carbamates upon tumour growth in mammals. It was found (Haddow and Sexton, 1946) that both ethyl and *iso*propyl phenylcarbamate produce some retardation of the growth of the Walker carcinoma 256 in rats, but, somewhat unexpectedly, that urethane itself is even more active in this respect. These observations led ultimately to the clinical trial of urethane in advanced and intractable cancer in man and to the discovery that the drug causes striking though only temporary remissions in a high proportion of cases of myeloid and lymphatic leukaemia (Paterson, apThomas, Haddow, and Watkinson, 1946).

In the hope of finding a compound with an even more pronounced selective effect upon malignant tissues and at the same time of discovering some clue to the mode of action of urethane we prepared some 30 simple homologues of that substance and examined them for growth inhibitory activity against the Walker tumour. An account of this work is in preparation; suffice it here to say that of those compounds only bis- and tris-carbethoxyamine even approach urethane in activity. From the results of similar studies both Haddow (private communication; *v.* also Haddow and Sexton, 1946) and Skipper and his associates (Skipper *et al.*, 1948, 1949) have concluded that the effect of urethane upon tumours and upon leukaemic cells is shared by only a few substances very closely related to it.

In spite of the considerable amount of speculation and laboratory and clinical investigation that has been devoted to the problem, the mode of action of urethane remains obscure. The sequence of observations recorded here had as their starting point speculations upon this subject. Urethane is comparatively inert in the chemical sense but in some circumstances will react with amino groups according to the general equation:

$$NH_2CO.CC_2H_5 + NH_2R \longrightarrow NH_2CO.NHR + C_2H_5OH$$

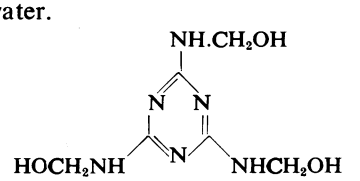
Although not facile *in vitro*, the reaction might occur *in vivo* under relatively mild conditions. We envisaged the possibility that urethane might interfere with cellular proliferation by reacting in this manner with some amino derivative within the cell essential to the process of division. In consequence we examined a range of substances formally capable of analogous interaction with amino groups to yield not only ureas and ureides as urethane does, but also other functional derivatives. These substances included organic halides, carboxylic esters, nitriles, cyanamides and dicyandiamides, cyanates, isocyanates, and isocyanate "generators," thiocyanates, ketones, aldehydes, imino-ethers, acetylenes, thiols, and alkylthiols. The majority were monofunctional in that the molecule carried only one reactive group, and all these were inactive against the Walker tumour, in daily doses near the maximum tolerated, in the sense that none produced any greater inhibition of tumour growth than could be accounted for on the basis of non-specific toxicity. A minority were polyfunctional, having two or more reactive groupings attached, for example, to an aliphatic chain or heterocyclic nucleus as in compounds of types I and II.



(I) $C_2H_5O.CO.NH(CH_2)_nNH.CO.OC_2H_5$

(II) 2-chloro-4-[bis(2-chloroethyl)amino]-6-diethylamino-1,3,5-triazine

These were included deliberately in consideration of the work of Gilman and Philips (1946) upon the biological effects of the alkyl nitrogen mustards. We had realized that the latter fell within the scope of our working hypothesis as substances capable of reacting with amino groups, and noted that in this series two alkylating groups appeared to be necessary for characteristic biological activity. It was in the polyfunctional class that tumour inhibitory activity was eventually encountered, namely, with compound (III) which was included for test in a series of polymethylolamides derived from amides by condensation with formaldehyde and characterized by their capacity to condense in turn with amines and other active hydrogen systems with the elimination of water.



(III) Trimethylolmelamine

Activity was subsequently found in several compounds related to (III).

The first polymethylolamides examined were obtained from the textile auxiliaries section of our research organization. When incorporated in fabrics derived from

natural or synthetic fibres these substances modify the mechanical properties of the fabrics in various ways, conferring, for example, increased wet strength upon paper and crease-resisting properties upon cotton or woollen textiles. Their usefulness in this direction is probably associated, as described by our colleague Mr. J. G. Evans (1949), with their poly-alkylating function and with the formation of cross-linkages between adjacent polymer fibres. The possible biological significance of the poly-alkylating and cross-linking potentialities of the sulphur and nitrogen mustards had been brought to our attention by Professor A. Haddow and his group (private communication, and Haddow, Kon, and Ross, 1948), more particularly in connexion with the aryl nitrogen mustards then under investigation by them ; this observation gave additional stimulus to the hypothetical ideas then being developed. The dependence of the characteristic toxicological properties of the nitrogen mustards upon the presence in the molecule of at least two β-chloroethyl groups emerges from early work with these agents and had been noted, for example, by Karnovsky, Burchenal, Ormsbee, Cornman, and Rhoads (1947) ; similarly Elmore, Gulland, Jordan, and Taylor (1948) found evidence for cross-linkage in one of the products isolated from the reaction between desoxyribonucleic acid and mustard gas, and suggested that their investigation provided a chemical parallel to previous cytological observations with the latter. While the methylolamides were under investigation we learnt also from Professor Haddow of the hypothesis, since elaborated and published (Goldacre, Loveless, and Ross, 1949 ; Loveless and Revell, 1949), that the specific cytotoxic action of the nitrogen mustards might be due to the formation of linkages between cell nuclear components.

At this stage it appeared that the methylolamides found active as tumour inhibitors were to be regarded essentially as " mustard " analogues rather than as acting like urethane, and this conception appeared to be substantiated by a more detailed study of the biological action of trimethylolmelamine. Moreover, it seemed probable that cross-linkage was in some way involved in the mechanism of action. Certain results, however, made it clear that the capacity to function as a simple cross-linking agent was not alone sufficient as a criterion of potential tumour inhibitory activity. Meanwhile the results obtained with trimethylolmelamine had already directed our attention to other chemical types having similar technological applications, namely, bis-epoxides and certain compounds containing two or three ethylenimine residues (Rose, Hendry, and Walpole, 1950), and with the discovery of tumour inhibitory activity among these substances a further chemical condition for biological activity became apparent. The results obtained with these alternative types, however, form the subject of a later communication in this series. The present paper is concerned with the initial group of substances capable of reacting with amino groups and with the methylolamides which immediately followed them.

Finally, in addition to the many compounds mentioned above, there were also included in these investigations several substances selected in connexion with another hypothesis relating to the action of urethane. Although it has never been demonstrated *in vitro*, the possibility exists that urethane, like the related isocyanate ion, might, *in vivo*, give a trimeric condensation product of the 1 : 3 : 5-triazine type or, more specifically, triethyl cyanurate. This compound, being tri-functional, might then produce cross-linking effects. It was therefore synthesized and examined together with the trimethyl ester and the corresponding acid halide, cyanuric chloride.

METHODS

As a preliminary to the examination of compounds for tumour inhibitory activity toxicity tests were carried out in mice and rats. The compounds were prepared for injection by dissolving them in physiological saline solution or in arachis oil, or in some instances in propylene glycol. Substances of limited solubility in these media were suspended by milling in arachis oil or in an aqueous solution of Dispersol OG (1.5 per cent) and Dispersol LN (0.05 per cent). They were then given to mice, usually by intraperitoneal injection, in single doses of 1,000, 500, 250, 125, and 50 mg. per kg., four mice being dosed at each level. The animals were inspected from time to time during the first half hour or so for the more readily observable signs of acute pharmacological action, such as hypnosis or convulsions, and deaths were recorded up to seven days. With the more toxic compounds the range of doses was extended downward until at least two of the four mice at any level survived.

On the basis of the results obtained in this acute test in mice, doses were chosen for repeated administration to rats. Rats were dosed once daily for five successive days in groups of three at each level. The animals were weighed each day before injection, and the doses given adjusted in proportion to the body weight. Daily weighing and recording of deaths was continued up to the tenth day. The doses employed in testing for inhibitory activity against the Walker carcinoma were based upon the results of these experiments in rats, the trends in body weight as well as deaths among the treated animals being taken into account; it was also borne in mind that tumour-bearing rats are rather more susceptible to the toxic action of many substances than are healthy animals. In the inhibitory tests dosing was usually started on the day after tumour implantation and continued daily, Sundays excepted, till the tenth to twelfth day. We aimed at giving the largest daily dose of each compound possible without any of the rats dying by the fourteenth day, and the toxicity tests described above usually sufficed for the estimation of this dose. Sometimes the condition of the treated animals made it desirable to reduce the level of dosage during the course of an experiment, at other times it was found possible to raise it; but a more precise determination of maximum tolerated doses would have required larger samples of the compounds than were usually available and a greater expenditure of time, labour, and animals than seemed expedient.

Our routine method of test for inhibitory activity against the Walker tumour, and some considerations involved in evaluating the results, have been dealt with in detail elsewhere (Walpole, 1951). Briefly the method was as follows: Single subcutaneous implants of the tumour were made by trochar and cannula in albino rats of a heterozygous stock, which were then separated into groups of 10 to 15 animals. One group was kept untreated as control and the others dosed as described above with the compounds under test. On the fourteenth or fifteenth day (the day of implantation being taken as day 0) all the surviving animals were again weighed; they were then killed and the tumours dissected out and weighed. The percentage increase in gross weight ($\triangle W$) of the survivors in each group and the percentage inhibition of tumour growth (I) in each of the treated groups were calculated. In each group I was calculated according to the formula:

$$I = \frac{M_{50}\ \text{controls} - M_{50}\ \text{treated}}{M_{50}\ \text{controls}} \times 100$$

where M_{50} was the mean weight of the n largest tumours in any group of $2n$.

RESULTS

In Table I are listed a number of "amine reactants" of various chemical types, most of which were tested before the discovery of activity in trimethylolmelamine.

TABLE I

Substances capable of reacting with amino groups examined for their effect upon the growth of the Walker tumour in rats, with the total dose in mg. per 100 g. given during the first 10–12 days after implantation of the tumour. With the exception of the three compounds in Table II, none of these produced a percentage inhibition of tumour growth (*I*) greater than 50. The effect on overall weight gain was variable. Dosage intraperitoneal unless otherwise indicated

Serial No.	Number	Compound		Total dose (mg./100g.)
		Name	Formula	
		Cyanates and isocyanates, etc.		
1	5648	Potassium cyanate	KCNO	500 *p.o.*
2	5648a	Sodium cyanate	NaCNO	80 *i.v.*
3	5475	Methyl isothiocyanate	CH_3NCS	15
4	5476	*Iso*propyl isothiocyanate	$(CH_3)_2CH.NCS$	80
5	5482	Phenylisocyanate bisulphite complex	$C_6H_5NH.CO.SO_3Na$	60
6	7957	Hexamethylenediisocyanate	$NCO.(CH_2)_6NCO$	10.5
7	5481	1 : 6-Hexamethylenediiso-cyanate bisulphite complex	$NaSO_3CO.NH(CH_2)_6NH.CO.SO_3Na$	75
8	5480	Nitrourea	$NO_2NH.CO.NH_2$	550 *p.o.*
		Ethynyl compounds		
9	6526	Phenylpropiolic acid	$C_6H_5C{\equiv}C.CO_2H$	120
10	6532	Ethyl *o*-chlorophenylpropiolate	$2\text{-}Cl.C_6H_4C{\equiv}C.CO_2C_2H_5$	120
11	6534	Tetrolic acid	$CH_3C{\equiv}C\ CO_2H$	60
12	6535	Ethyl tetrolate	$CH_3C{\equiv}C.CO_2C_2H_5$	275
		Nitriles, cyanamides, and dicyandiamides		
13	6005	Carbethoxy cyanamide (K salt)	$C_2H_5O.CO.NK.CN$	1,100
14	6184	Diethylcyanamide	$(C_2H_5)_2N.CN$	60
15	6146	Methylphenylcyanamide	$C_6H_5N(CH_3)CN$	164
16	5103	*p*-Chlorophenylcyanamide	$4\text{-}Cl.C_6H_4NH.CN$	110
17	6145	*p*-Bromophenylcyanamide	$4\text{-}Br.C_6H_4NH.CN$	55
18	6100	*ω-Iso*propyldicyandiamide	$(CH_3)_2CH.NH.C(:NH).NH.CN$	300
19	6336	β-Diethylaminoethyl dicyandiamide oxalate	$(C_2H_5)_2N.CH_2CH_2NH.C(:NH).NH.CN,\frac{1}{2}(COOH)_2$	275
20	5046	ω-(*p*-Chlorophenyl)-dicyandiamide	$4\text{-}Cl.C_6H_4NH.C(:NH)NH.CN$	220
21	6141	ω-(*o*-Chlorophenyl)-dicyandiamide	$2\text{-}Cl.C_6H_4NH.C(:NH)NH.CN$	275
22	6142	ω-(*p*-Chlorophenyl)-ω'-methyl-dicyandiamide	$4\text{-}Cl.C_6H_4NH.C(:NCH_3)NH.CN$	55
23	6829	ω-(*p*-Chlorophenylazo)-dicyandiamide (Na salt)	$4\text{-}Cl.C_6H_4N{=}N.NH.C(:NH)NH.CN$	162.5
24	7348	β-Diethylaminopropionitrile hydrochloride	$(C_2H_5)_2N.CH_2CH_2CN,HCl$	225

TABLE I (*continued*)

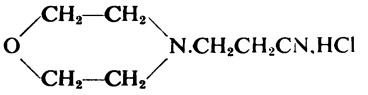
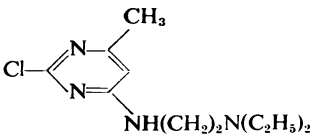
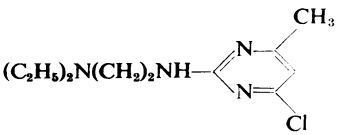
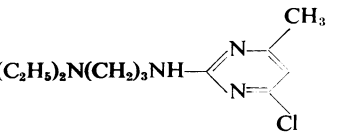
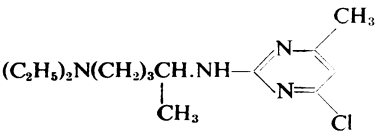
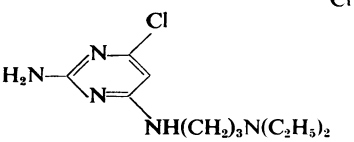
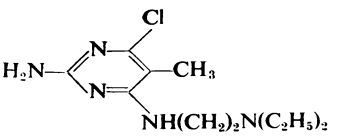
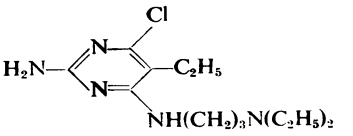
Serial No.	Number	Compound		Total dose (mg./100g.)
		Name	Formula	
		Nitriles, cyanamides and dicyandiamides (contd.)		
25	7349	β-Piperidinopropionitrile hydrochloride	$(CH_2)_5{>}N.CH_2CH_2CN,HCl$	22.5
26	7351	β-Hexamethyleniminopropionitrile hydrochloride	$(CH_2)_6{>}N.CH_2CH_2CN,HCl$	22.5
27	7350	β-Morpholinopropionitrile hydrochloride		600
		Halogenopyrimidines		
28	7744	2-Chloro-4-β-diethylamino-ethylamino-6-methyl-pyrimidine		40
29	7745	4-Chloro-2-β-diethylamino-ethylamino-6-methyl-pyrimidine		40
30	7746	4-Chloro-2-γ-diethylamino-propylamino-6-methyl-pyrimidine		40
31	7743	4-Chloro-2-δ-diethylamino-α-methylbutylamino-6-methylpyrimidine		40
32	4552	2-Amino-4-chloro-6-γ-diethylaminopropylamino-pyrimidine		67.5
33	4718	2-Amino-4-chloro-6-β-diethylaminoethylamino-5-methylpyrimidine		32
34	4442	2-Amino-4-chloro-6-γ-diethylaminopropylamino-5-ethylpyrimidine		60

TABLE I (*continued*)

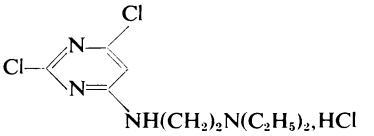
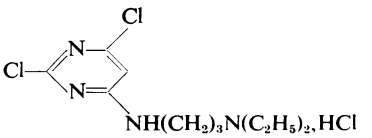
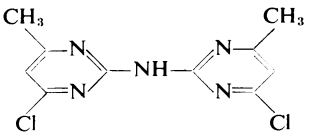
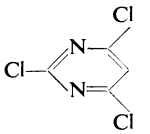
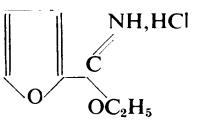
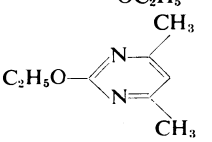
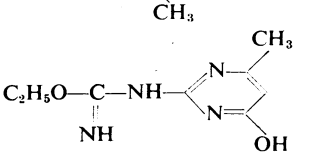
Serial No.	Number	Compound		Total dose (mg./100 g.)
		Name	Formula	
		Halogenopyrimidines (contd.)		
35	8179	2 : 4-Dichloro-6-β-diethylaminoethylamino-pyrimidine hydrochloride		40
36	8180	2 : 4-Dichloro-6-γ-diethylaminopropylamino-pyrimidine hydrochloride		45
37	8085	4 : 4′-Dichloro-6 : 6′-dimethyl-2 : 2′-dipyrimidylamine		60
38	8328	2 : 4 : 6-Trichloropyrimidine		13.5
		Esters and O-alkyl derivatives		
39	8285	N-*Iso*propyl-O-methyl*iso*urea	$(CH_3)_2CH.NH.C(:NH)OCH_3$	140
40	8284	N-*Iso*propyl-N-methyl-O-methyl*iso*urea	$(CH_3)_2CH.N(CH_3)C(:NH)OCH_3$	160
41	8283	N : N-Di-*n*-butyl-O-methyl*iso*urea	$(C_4H_9)_2N.C(:NH)OCH_3$	8
42	8282	N-*p*-Chlorphenyl-O-methyl*iso*urea	$4\text{-}Cl.C_6H_4N:C(NH_2)OCH_3$	87.5
43	6671	α-Furoiminoethyl ether hydrochloride		90
44	6065	4 : 6-Dimethyl-2-ethoxypyrimidine		200
45	5097	2-Carbiminoethoxyamino-4-methyl-6-hydroxypyrimidine		300
46	5142	Ethyl *p*-chlorophenylguanidinoformate	$4\text{-}Cl.C_6H_4HN.C(:NH)NH.CO_2C_2H_5$	4

TABLE I (*continued*)

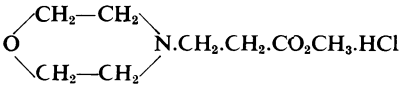
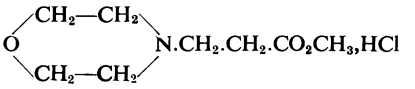
Serial No.	Number	Compound		Total dose (mg./100 g.)
		Name	Formula	
		Esters and O-alkyl derivatives (contd.)		
47	6010	Ethyl *p*-chlorophenylallophanate	4-Cl.C_6H_4NH.CO.NH.$CO_2C_2H_5$	110
48	7340	Methyl β-diethylaminopropionate hydrochloride	$(C_2H_5)_2$N.$CH_2CH_2CO_2CH_3$,HCl	700
49	7358	Methyl β-hexamethyleniminoα-methylpropionate hydrochloride	$(CH_2)_6$>N.$CH_2CH(CH_3)CO_2CH_3$,HCl	450
50	7342	Methyl β-piperidinopropionate hydrochloride	$(CH_2)_5$>N.$CH_2CH_2CO_2CH_3$,HCl	275
51	7343	Methyl β-morpholinopropionate hydrochloride		800
52	7344	Methyl β-hexamethyleniminopropionate hydrochloride	$(CH_2)_6$>N.$CH_2CH_2CO_2CH_3$,HCl	800
53	7356	Methyl α-methyl-β-piperidinopropionate hydrochloride	$(CH_2)_5$>N.$CH_2CH(CH_3)CO_2CH_3$,HCl	690
54	7357	Methyl α-methyl-β-morpholinopropionate hydrochloride		660
		Thiols and S-alkylthiols		
55	5491	Ethylthiocarbamate (thiourethane)	$NH_2CO.SC_2H_5$	75
56	5102	N-*p*-Chlorophenyl-S-methyl-*iso*thiourea sulphate	4-Cl.C_6H_4NH.C(: NH)SCH_3,$\frac{1}{2}H_2SO_4$	110
57	6349	N-Phenyl-N′-asym.dimethylguanylthiourea	C_6H_5NH.CS.NH.C(: NH)$N(CH_3)_2$	100 *s.c.*
58	6350	N-(*p*-Chlorophenylthiocarbamyl)-S-methyl*iso*thiourea	4-Cl.C_6H_4NH.CS.NH.C(: NH)SCH_3	100 *s.c.*
59	5385	N-*p*-Chlorophenyl-N′-(N″:N″-dimethylguanyl)-thiourea	4-Cl.C_6H_4NH.CS.NH.C(:NH)$N(CH_3)_2$	50
60	5403	N-*p*-Chlorophenyl-N′-(N″:N″-dimethylguanyl)-S-ethyl*iso*thiourea hydrobromide	4-Cl.C_6H_4NH.C(SC_2H_5) : N.C(:NH)$N(CH_3)_2$,HBr	50 *s.c.*
61	6143	N-*Iso*propyl-N′-cyano-S-methyl*iso*thiourea	$(CH_3)_2$CH.N:C(SCH_3)NH.CN	110
62	6414	N-Cyano-N′-phenyl-S-methyl*iso*thiourea	C_6H_5N:C(SCH_3)NH.CN	200
63	6144	N-(*p*-Chlorophenyl)-N′-cyano-S-methyl *iso*thiourea	4-Cl.C_6H_4N:C(SCH_3)NH.CN	350
		Bis- or polyfunctional esters and carbamates		
64	5606	Carbethoxyurethane	$NH(CO.OC_2H_5)_2$	550 *p.o.*
65	5609	Tricarbethoxyamine	$N(CO.OC_2H_5)_3$	1,050 *p.o.*
66	5317	Dicarbethoxyhydrazine	C_2H_5O.CO.NH.NH.$COOC_2H_5$	60 *p.o.*

TABLE I (*continued*)

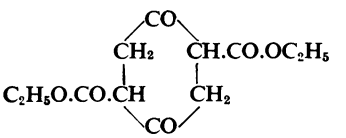
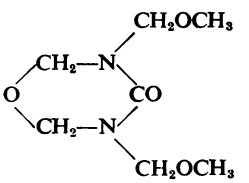
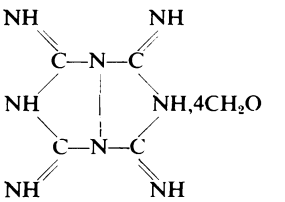
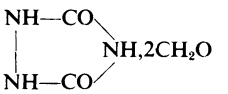
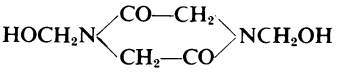
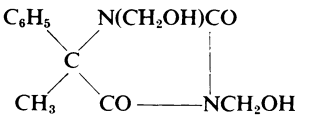
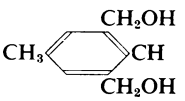
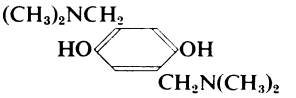
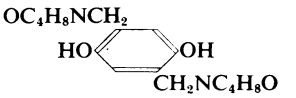
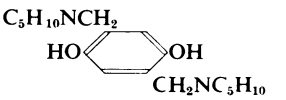
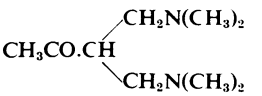
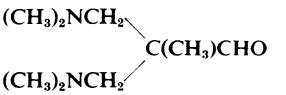
Serial No.	Number	Compound		Total dose (mg./100g.)
		Name	Formula	
		Bis- or polyfunctional esters and carbamates (contd.)		
67	5818	Dicarbethoxyethylenediamine	$C_2H_5O.CO.NH.CH_2CH_2NH.CO.OC_2H_5$	550
68	6107	Dicarbethoxypentamethylene-diamine	$C_2H_5O.CO.NH(CH_2)_5NH.CO.OC_2H_5$	200 *p.o.*
69	5819	Dicarbethoxyhexamethylene-diamine	$C_2H_5O.CO.NH(CH_2)_6NH.CO.OC_2H_5$	100
70	5993	Dicarbethoxydecamethylene-diamine	$C_2H_5O.CO.NH(CH_2)_{10}NH.CO.OC_2H_5$	550
71	5765	Ethyleneglycol bis-carbamate	$NH_2CO.OCH_2CH_2OCO.NH_2$	800
72	5766	Propyleneglycol bis-carbamate	$NH_2CO.O(CH_2)_3OCO.NH_2$	500
73	5767	Butyleneglycol bis-carbamate	$NH_2CO.O(CH_2)_4OCO.NH_2$	225
74	5814	Hexamethyleneglycol bis-carbamate	$NH_2CO.O(CH_2)_6OCO.NH_2$	350
75	5815	Decamethyleneglycol bis-carbamate	$NH_2CO.O(CH_2)_{10}OCO.NH_2$	300
76	5816	Ethyleneglycol-bis-diethyl-carbamate	$(C_2H_5)_2NCO.OCH_2CH_2OCO.N(C_2H_5)_2$	500
77	5817	Propyleneglycol bis-diethyl-carbamate	$(C_2H_5)NCO.O(CH_2)_3OCO.N(C_2H_5)_2$	200
78	7207	2:5-Dicarbethoxy*cyclo*hexane-1:4-dione		300
79	4955	N:N′-Bis-(4:6-dimethoxy-2-pyrimidyl)urea		25
		Miscellaneous methylolamides		
80	7927	Methylolurea	$NH_2.CO.NH.CH_2OH$	200
81	5429	Dimethylolurea	$HOCH_2NH.CO.NH.CH_2OH$	500
82	5430	Dimethylolurea dimethyl ether	$CH_3OCH_2NH.CO.NH.CH_2OCH_3$	300
83	9499	Dimethylolbisurea	$HOCH_2(HN.CO.NH)_2CH_2OH$	200
84	7926	Dimethylol biuret	$HOCH_2NH.CO.NH.CO.NH.CH_2OH$	250
85	3357	Bis(methoxymethyl)uron		1,050

TABLE I (*continued*)

Serial No.	Number	Compound		Total dose (mg./100g.)
		Name	Formula	
		Miscellaneous methylolamides (contd.)		
86	8376	Tetramethylolguanazo-guanazole		350
87	7925	Dimethyloladipamide	$HOCH_2NH.CO(CH_2)_4CO.NH.CH_2OH$	350
88	9402	Dimethyloldiphenylmalona-mide	$(C_6H_5)_2C(CO.NH.CH_2OH)_2$	400
89	7922	Dimethylolurazole		120
90	8103	Dimethyloldiketopiperazine		350
91	8375	Dimethylol-5-methyl-5-phenylhydantoin		200
		Miscellaneous methylol derivatives		
92	8397	2:6-Bis(hydroxymethyl)*p*-cresol		425
93	8031	2:5-Bis(dimethylaminomethyl)-hydroquinone		10
94	8032	2:5-Bis(morpholinomethyl) hydroquinone		375
95	8034	2:5-Bis(piperidinomethyl) hydroquinone		75
96	8036	1-Dimethylamino-2-dimethyl-aminomethyl-butan-3-one		6.5
97	8037	α:α-Bis(dimethylaminomethyl) propionaldehyde		20

The serial number, code number, name, and formula of each of these compounds is shown, but they will be referred to by the serial number only (or by name) in the sequel; the total dose in mg. per 100 g. rat given over the first 10 to 12 days after implantation of the tumour is also given. Unless otherwise indicated dosing was intraperitoneal. With most of these compounds any tumour inhibition produced could be accounted for on the basis of non-specific toxicity as indicated by their effect on the gross weight gain. The only substances which produced a percentage inhibition of tumour growth, *I*, of 50 or more were sodium cyanate, given intravenously, and di- and tri-carbethoxyamine (serial nos. 64 and 65), given orally. With these, some preferential effect upon the tumour was possibly in evidence, but even here activity was low. Details are shown in Table II.

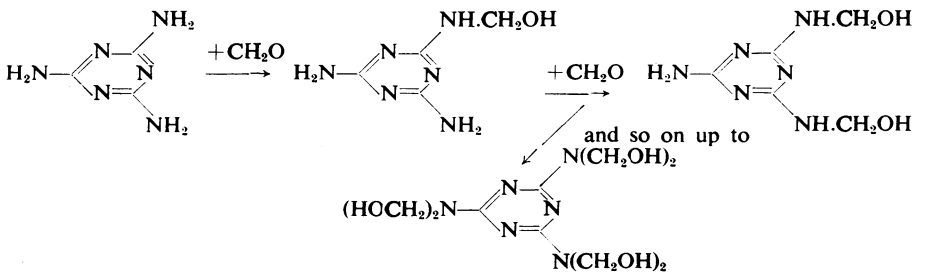
TABLE II

Substances capable of reacting with amino groups producing a percentage inhibition (*I*) of the growth of the Walker tumour in rats greater than 50 when given in the total doses shown spread over the first 10–12 days after implantation of the tumour. $\triangle W$, mean percentage increase in gross weight of the tumour-bearing rats; M_{50}, mean weight of the *n* heaviest tumours in groups of $2n$; a.s., aqueous solution; a.e., aqueous emulsion

Serial No.	Compound	Form	Total dose mg./100 g.	$\triangle W$		M_{50}		*I*
				Control	Treated	Control	Treated	
1	Sodium cyanate ..	a.s.	80 *i.v.*	30.0	−6.8	22.3	8.1	64
64	Dicarbethoxyamine (carbethoxyurethane)	a.s.	550 *p.o.*	40.2	14.8	35.9	12.4	65
65	Tricarbethoxyamine ..	a.e.	1,050 *p.o.*	37.9	6.5	33.1	14.8	55

In Table III are listed a number of " cross-linking " agents tested after the demonstration of the activity of trimethylolmelamine. None of these produced an inhibition of tumour growth greater than 45 per cent, and none appeared to have an effect which was in any sense directed preferentially towards the tumour.

Results obtained in single experiments with melamine and with the products formed by the condensation of this substance with formaldehyde in various molecular proportions are shown in Table IV, section A. These products are amorphous and difficult to characterize, and their composition is by no means established. They may be formulated provisionally as derived by the successive replacement of the hydrogen atoms in the melamine molecule by the methylol group $-CH_2OH$, thus:



In fact, however, with the exception of the hexamethylol derivative, they are probably complex mixtures of several chemical entities and very likely consist of

TABLE III

Miscellaneous " cross-linking " agents examined for their effect on the growth of the Walker tumour in rats, with the total dose in mg. per 100 g. given during the first 10–12 days after implantation of the tumour. None of these compounds produced an inhibition of tumour growth (*I*) greater than 50. The effect on gross weight gain was variable

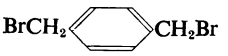
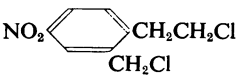
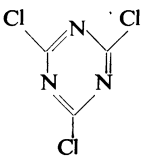
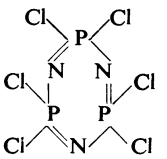
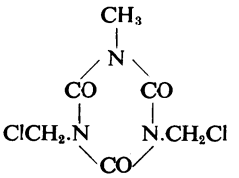
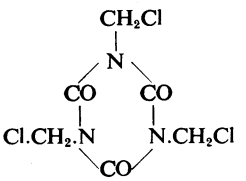
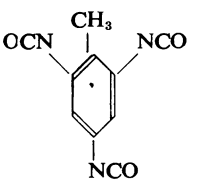
Serial No.	Number	Compound		Total dose (mg./100 g. rat i.p.)
		Name	Formula	
		Halogeno compounds		
98	9589	*p*-Xylylene dibromide		4.5
99	8361	4-*β*-Chloroethyl-3-chloromethyl-nitrobenzene		25
100	8351	Cyanuric chloride		8.0
101	9418	Phosphorus chloronitride		2.25
102	8843	N: N′-bis-chloromethyl-N″-methyl isocyanuric acid		100
103	8842	N: N′: N″-Tris-chloromethyl-isocyanuric acid		65
		Isocyanates and isocyanate generators		
104	8781	Toluene tris-isocyanate		50

TABLE III (*continued*)

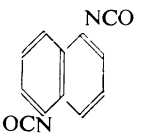
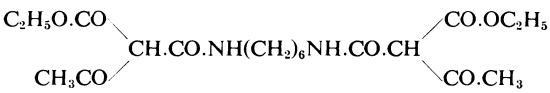
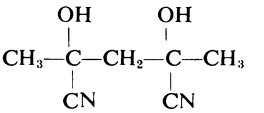
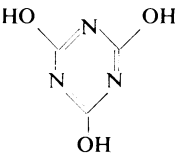
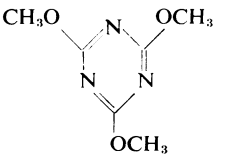
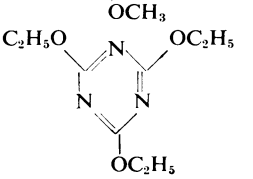
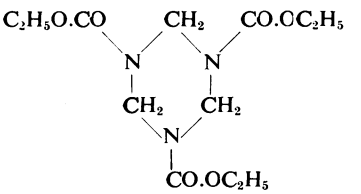
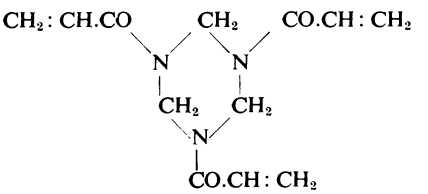
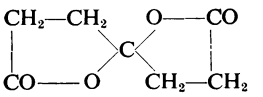
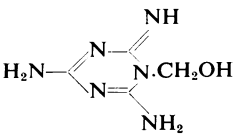
Serial No.	Number	Compound		Total dose (mg./100 g. rat i.p.)
		Name	Formula	
		Isocyanates and isocyanate generators (contd.)		
105	9861	Naphthalene-1 : 5-diisocyanate		22.5
106	9598	Hexamethylene-bis-*tert*-butylcarbamate	$(CH_3)_3CO.CO.NH(CH_2)_6NH.CO.OC(CH_3)_3$	300
107	9599	Hexamethylene diisocyanate dibenzoic anhydride	$C_6H_5CO.O.CO.NH(CH_2)_6NH.CO.O.CO.C_6H_5$	210
108	9600	N : N′-bis-(α.α-dicarbethoxy-acetyl) hexamethylene diamine	$(C_2H_5O.CO)_2CH.CO.NH(CH_2)_6NH.CO.CH(CO.OC_2H_5)_2$	275
109	9601	N : N′-bis-(α-carbethoxyaceto-acetyl)-hexamethylene diamine		325
		Aldehyde and keto derivatives		
110	9602	Glyoxal-bis-sodium bisulphite	$SO_3Na.CHOH.CHOH.SO_3Na$	325
111	9594	Acetyl acetone dicyanhydrin		14
		Acids, esters, nitriles, etc.		
112	8533	Cyanuric acid		250
113	8541	Trimethylcyanurate		100
114	8532	Triethyl cyanurate		87.5

TABLE III (*continued*)

Serial No.	Number	Compound: Name	Compound: Formula	Total dose (mg./100 g. rat i.p.)
		Acids, esters, nitriles, etc. (contd.)		
115	9141	N: N′: N″-Tris-carbethoxy-trimethylene triamine		145
116	9140	N: N′: N″:-Tris-acryloyl-trimethylene triamine		3.75
117	9165	Hexamethylene bis-dicyandiamide	$CN.NH.C(:NH)NH(CH_2)_6NH.C(:NH)NH.CN$	475
118	8353	Dimethyl-α: α′-azodiisobutyrate	$CH_3O.CO.C(CH_3)_2N:N.C(CH_3)_2CO.OCH_3$	50
119	8355	α: α′-Azodiisobutyronitrile	$CN.C(CH_3)_2N:N.C(CH_3)_2CN$	80
120	8354	1: 1′-Azo*cyclo*hexane carbonitrile	$(CH_2)_5>C(CN)N:N.C(CN)<(CH_2)_5$	275
121	9505	*Spiro*-anhydroketopimelic acid		175

mixtures of several of the simple substitution products formulated above. In addition, they may well contain a proportion of dimeric and/or polymeric forms containing linkages such as -NH.CH_2NH-, while the presence of a proportion of isomeric compounds of the type (IV),



(IV)

though unlikely, is not entirely excluded by the available evidence. Thus it is difficult to draw any firm conclusions as to the relationship between constitution and activity in this series. It will be seen from Table IV, however, that melamine itself proved inactive, and indeed in the experiment cited the tumour grew better in the melamine treated animals than in the controls. Condensates containing 2, 3, 4, 5, and 6 molecular proportions of formaldehyde inhibited the growth of the tumour to an extent which was clearly greater than could be accounted for by non-specific

TABLE IV

Action of some methylolmelamines and related compounds upon the growth of the Walker tumour in rats. $\triangle W$, mean percentage increase in gross weight of the tumour-bearing rats; M_{50}, mean weight of the n heaviest tumours in groups of $2n$; I, percentage inhibition of tumour growth; a.d., aqueous dispersion (suspension); a.s., aqueous solution; L, supply of compound limited, maximal tolerated dose not given

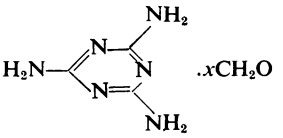
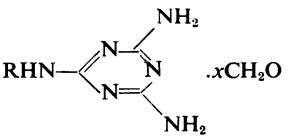
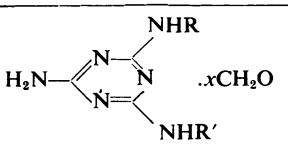
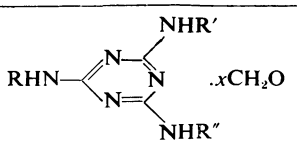
Serial No.	Number	Formula	Form	Total dose i.p. (mg./100 g.)	$\triangle W$ Control	$\triangle W$ Treated	M_{50} Control	M_{50} Treated	I	Remarks
A	Type									
122	8455	$x = 0$	a.d.	175	29.4	21.7	19.3	23.6	−22	
123	8372	1	,,	275	44.2	20.0	30.7	17.8	42	
124	8396	2	,,	250	25.3	14.2	30.2	4.8	84	
125	7924	3	,,	450	41.8	1.2	31.9	0	100	
126	8373	4	,,	225	50.4	12.9	31.3	1.0	97	
127	8713	5	,,	237	39.1	26.4	21.8	5.6	74	
128	7923	6	,,	225	36.3	9.7	29.4	11.5	61	
B	Type									
129	8599	R = CH_3 $x = 3$	,,	265	45.6	30.6*	24.6	16.5	33	*Ascites
130	9416	C_6H_5 3	,,	250	40.0	7.8	30.4	4.4	86	
131	9205	4-Cl.C_6H_4 2	,,	112.5	33.1	−1.0	35.7	18.5	48	
132	9204	4-Cl.C_6H_4 3	,,	137.5	33.1	3.9	35.7	17.4	51	
133	9203	4-Cl.C_6H_4 4	,,	137.5	33.1	22.5	35.7	34.4	4	
134	9415	β-naphthyl 2	,,	90	32.8	24.4	40.4	35.4	8	
135	9414	β-naphthyl 3	,,	—	—	—	—	—	—	Untested *v.* Table VI
C	Type									
136	8711	R = CH_3 R′ = CH_3 $x = 3$	,,	187.5	39.1	−2.0	21.8	0.28	99	
137	8712	CH_3 CH_3 4	,,	175	39.1	15.4	21.8	1.9	91	
D	Type									
138	8575	R=CH_3 R′=CH_3 R″=CH_3 $x=3$	,,	87.5	31.3	3.0	30.9	4.9	84	
139	8100	4-Cl.C_6H_4 CH_3 CH_3 2	,,	25	32.4	27.5	20.6	27.5	−33	L
140	8973	β-naphthyl CH_3 CH_3 2	,,	112.5	50.8	8.8	41.1	26.1	37	

TABLE IV (*continued*)

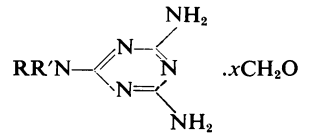
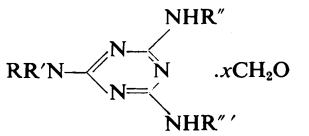
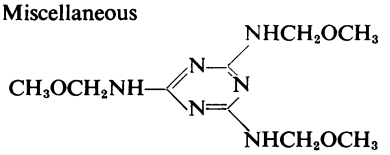
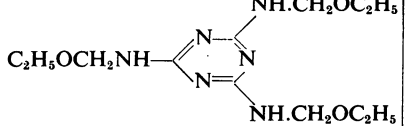
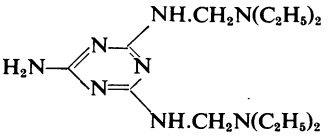
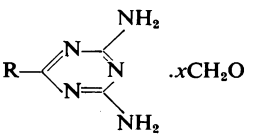
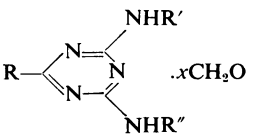
Serial No.	Number	Formula	Form	Total dose. i.p. (mg./ 100 g.)	$\triangle W$		M_{50}		I	Remarks
					Control	Treated	Control	Treated		
E	Type									
141	8578	R=CH_3 R′=CH_3 x=2	a.d.	106.3	43.4	16.5	12.6	9.4	25	
142	8656	CH_3 CH_3 3	,,	125	43.4	39.0	12.6	1.3	90	
143	8576	C_2H_5 C_2H_5 2	,,	192.5	31.3	26.4	30.9	20.5	34	
144	9207	CH_3 C_6H_5 2	,,	75	45.6	40.5	38.5	35.9	2	L
145	9206	CH_3 C_6H_5 4	,,	100	49.0	35.3	42.0	29.4	30	L
F	Type									
		R R′ R″ R‴ x								
146	8972	CH_3 C_6H_5 CH_3 CH_3 1	,,	125	50.8	22.2	41.1	30.8	25	L
147	9094	CH_3 C_6H_5 CH_3 CH_3 2	,,	237.5	51.0	19.4	44.3	27.8	37	
G	Miscellaneous									
148	8454		o.s.	250	40.5	19.8	25.9	5.2	80	
149	8696		a.d.	125	23.5	18.6	24.0	16.0	33	
150	8393		,,	185	39.4	23.0	30.7	24.1	22	
H	Type									
151	8456	R=H x=2	,,	175	41.7	32.4	40.3	30.3	25	
152	8542	CH_3 0	,,	225	28.5	19.5	23.9	15.9	33	
153	8394	CH_3 2	,,	300	(25.3)	23.7	30.2	29.3	3	
154	8809	C_6H_{13} 2	,,	205	16.2	7.5	30.2	17.7	41	
155	9095	$C_6H_5CH_2$ 4	,,	140	49.6	36.4	37.4	31.7	15	L

TABLE IV (*continued*)

Serial No.	Number	Formula	Form	Total dose.i.p. (mg./100 g.)	$\triangle W$ Control	$\triangle W$ Treated	M_{50} Control	M_{50} Treated	*I*	Remarks
H *contd.*										
156	8102	R = C_6H_5—CH=CH— x = 0	a.d.	175	46.2	32.0	35.8	37.3	−4	L
157	9087	C_6H_5—CH=CH— 2	,,	230	37.8	23.8	44.7	36.3	19	
158	9088	C_6H_5—CH=CH— 3	,,	275	49.6	28.0	37.4	23.9	36	
159	9089	C_6H_5—CH=CH— 4	,,	275	49.6	24.2	37.4	29.3	22	
160	8808	α-furyl 2	,,	145	16.2	20.2	30.2	29.3	3	
161	9208	α-furyl 3	,,	125	50.0	52.5	39.9	37.8	5	L
162	8543	C_6H_5 0	,,	80	25.0	13.0	24.3	18.3	25	
163	8395	C_6H_5 2	,,	275	26.6	10.1	38.7	11.2	71	
164	8657	C_6H_5 3	,,	325	12.5	4.2	27.8	0.2	99	
165	8780	C_6H_5 4	,,	170	16.2	26.4	30.2	31.9	−6	
166	9090	4-$CH_3C_6H_4$ 2	,,	260	37.8	25.8	44.7	29.9	33	
167	9091	4-$CH_3C_6H_4$ 3	,,	275	37.8	23.5	44.7	24.0	46	
168	8974	4-$CH_3C_6H_4$ 4	,,	225	50.7	33.0	41.1	34.9	15	
169	9417	4-$CH_3O.C_6H_4$ 2	,,	270	40.0	8.4	30.4	0.8	97	
170	9092	4-$CH_3O.C_6H_4$ 3	,,	275	49.6	19.6	37.4	8.6	77	
171	9093	4-$CH_3O.C_6H_4$ 4	,,	230	49.6	25.8	37.4	11.6	69	
172	9340	4-$Cl.C_6H_4$ 2	,,	35	29.1	12.8	39.4	23.5	40	
173	9339	4-$Cl.C_6H_4$ 3	,,	39	29.1	4.9	39.4	17.7	55	
174	9338	4-$Cl.C_6H_4$ 4	,,	30	29.1	8.8	39.4	23.1	41	
175	9343	3-$Cl.C_6H_4$ 2	,,	140	42.9	22.2	34.7	21.1	39	
176	9342	3-$Cl.C_6H_4$ 3	,,	60	33.8	9.2	35.7	27.2	24	
177	9341	3-$Cl.C_6H_4$ 4	,,	60	33.8	19.3	35.7	23.2	35	
178	9346	2-$Cl.C_6H_4$ 2	,,	220	29.1	26.4	39.4	33.7	14	L
179	9345	2-$Cl.C_6H_4$ 3	,,	220	33.8	11.0	35.7	27.2	24	
180	9344	2-$Cl.C_6H_4$ 4	,,	160	33.8	18.2	35.7	31.9	10	L
181	9139	β-naphthyl 2	,,	162.5	34.6	0.9	35.4	21.2	40	
182	9086	β-naphthyl 3	,,	162.5	37.8	11.4	44.7	18.9	58	
183	9101	β-naphthyl 4	,,	275	32.4	18.5	20.6	19.3	6	
I	Type									
184	8658	R=CH_3O R′=CH_3 R″=CH_3 x=2	,,	135	12.5	12.9	27.8	20.0	28	
J		Formaldehyde and Formaldehyde Generators:								
		HCHO	a.s.	20 *i.v.*	36.4	31.2	45.1	38.6	14	
		,,	,,	32 *i.v.*	17.0	15.4	39.6	30.9	22	
185	8548	$(HCHO)_n$ Paraformaldehyde	a.d.	25	36.2	27.9	29.4	27.5	6	
186	7508	$(CH_2)_6N_4$ Hexamethylene tetramine	a.s.	900	39.6	29.0	33.0	30.7	7	
187	9610	$HOCH_2SO_3Na.H_2O$ Formaldehyde sodium bisulphite monohydrate	,,	875 *i.v.*	31.6	27.8	40.4	33.6	17	L

toxicity. With monomethylolmelamine (serial no. 123) it is not clear from the figures obtained whether or not the inhibition produced (42 per cent) was due to non-specific factors.

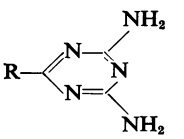
As already mentioned the doses given in these experiments were near the maximum tolerated, so that the total doses shown are inversely proportional to the toxicity of the products. It will be seen that as one proceeds from melamine to the hexamethylol derivative, increasing formaldehyde content is accompanied at first by an increase and then by a decline in tumour growth inhibitory activity. Activity reaches its maximum at or near the product analysing as trimethylolmelamine (serial no. 125). At or near this point in the series toxicity on the other hand is minimal.

The condensation between melamine and formaldehyde is reversible and the products fairly readily dissociate, liberating free formaldehyde. The question naturally arose whether the observed tumour inhibition was in fact due to the latter substance liberated *in vivo*. The total doses given of the condensates containing one to six molecular proportions of formaldehyde, expressed in terms of their formaldehyde content, were 53, 81, 187, 110, 129, and 132 mg. respectively per 100 g. rat. As far up the series as tetramethylolmelamine there is some parallelism between these figures and the observed inhibition of tumour growth, but beyond this point no such parallelism obtains. Other compounds will be found in the Tables which, in doses high in terms of readily dissociated formaldehyde, have produced no significant inhibition of tumour growth. This applies, for example, to dimethylolurea (no. 81), tetramethylolguanazoguanazole (no. 86), and dimethylolacetoguanamine (no. 153). These results all militate against the suggestion that formaldehyde liberated *in vivo* is the active agent. The results obtained with formaldehyde itself and with the simple formaldehyde generators paraformaldehyde, hexamethylene tetramine, and sodium formaldehyde bisulphite do not provide unequivocal evidence on this point. It will be seen (Table IV, J) that whereas formaldehyde given intravenously in aqueous solution and paraformaldehyde given intraperitoneally produce comparatively little inhibition of the tumour the tolerated doses are very low. While very large doses of hexamethylene tetramine and sodium formaldehyde bisulphite could be given with no greater effect on the tumour, these complexes are comparatively stable.

Results obtained with substituted methylolmelamines and with closely related derivatives of various types are shown in sections B to I inclusive of Table IV. They may be summarized in the following terms.

Activity is high in the trimethylol derivatives of alkyl and aryl substituted melamines and lower or absent in the corresponding dimethylolamines. Activity tends to be lower also in the corresponding tetramethylol derivatives.

Acyl and aroyl guanamines of the type (V)



(V)

are virtually inactive. The activity of the corresponding polymethylol derivatives depends on the nature of R as well as upon the formaldehyde content of the con-

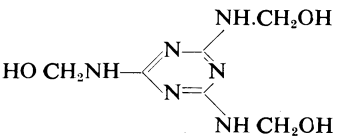
densate. Where R is hydrogen or a methyl, *n*-hexyl, benzyl, or styryl group, activity is negligible irrespective of the formaldehyde content. The same is true where R is an α-furyl or an *o*- or *m*-chlorophenyl group. Where R is *p*-chlorophenyl, *p*-tolyl, or β-naphthyl, activity is negligible in the di- and tetra-methylol derivatives, but the corresponding trimethylolamides inhibit the tumour to a rather greater extent than can be attributed to non-specific toxicity alone. Where R is phenyl or *p*-anisyl, the di- and tri-methylolamides are highly active, as is the tetramethylolamide from *p*-methoxybenzguanamine.

It was found that the water soluble trimethyl ether of trimethylolmelamine (serial no. 148) was quite active, whereas the activity of the corresponding triethyl ether (serial no. 149) was negligible.

These results will be considered further when the properties of trimethylolmelamine, one of the most active of the series, have been described in greater detail.

Trimethylolmelamine (serial no. 125)

This substance, which we formulate provisionally as (VI),



(VI)

is a white amorphous solid, sparingly soluble in water. Throughout our experiments it has been used in the form of an aqueous suspension. At first Dispersol OG (1.5 per cent) and Dispersol LN (0.05 per cent) were added to assist dispersion, but it was then found that the compound formed a fine suspension quite readily when milled with distilled water alone, and for all subsequent experiments it was made up in this way.

Tumour inhibition.—In preliminary toxicity tests in which the compound was given to mice in single intraperitoneal doses up to 1 g. per kg. none of the treated mice died up to seven days after injection. In a further test the median lethal dose in this species was found to be of the order of 1.35 g. per kg. Rats were found to tolerate five daily doses of up to *ca.* 75 mg. per 100 g. i.p. In the first test of this compound for inhibitory action on the Walker tumour, the dosage schedule per 100 g. body weight was 100 mg. on days 3, 4, and 5, 50 mg. on day 6, and 25 mg. on days 7, 10, 11, and 12, totalling 450 mg. per 100 g. i.p. Single rats died on days 12 and 14. By the fourteenth day the gross weight of the control animals had increased by 42 per cent, and the weights of the tumours in this group were 39, 34, 33, 31, 28.5, 26, 4, 0, 0, 0, 0, and 0 g. In the treated group the increase in gross weight of the survivors was only 1.2 per cent; no tumours were found although fragments of tissue of about the same size as the original implants were present in some animals.

The effect of varying the dosage and route of administration of the compound is shown in Table V. It will be seen that the degree of tumour inhibition produced depends not only on the total dosage but also upon its distribution and timing in relation to the time of implantation of the tumour. Thus a total dose of 125 mg. per 100 g. given in single doses of 25 mg. between days 1 and 6 or 1 and 7 is very

TABLE V

Effect of variations in dosage schedule and route of administration upon the inhibitory effect of trimethylolmelamine on the growth of the Walker tumour in rats. Conventions as in Table IV

Expt. No.	Route	Total dose mg./ 100 g.	Schedule of dosage	M_{50}		I	Remarks
				Control	Treated		
48/46	i.p.	450	100 on days **3**, 4, and 5; 50 on day 6; 25 on days 7, 10, 11, and 12	31.9	0	100	2/12 rats dead (on days 13 and 14)
48/39	,,	250	50 on days **2**, 3, and 5; 25 on days 6, 7, 8, and 9	28.0	<1	>96	2/11 rats dead (at 20 days)
48/46	,,	225	50 on days **3** and 4; 25 on days 5, 6, 7, 8, and 10	30.7	2.5	92	
	,,	120	20 on days **3** and 4; 10 on days 5, 6, 7, 8, 10, 11, 12, and 13		24.5	20	
	,,	100	100 on day **3**		17.0	45	1/12 rats dead (on day 8)
48/65	,,	125	25 on days **1**, 2, 5, 6, and 7	23.9	1.7	92	
48/71	,,	125	25 on days **1**, 3, 4, 5, and 6	26.6	3.5	87	
48/70	,,	87.5	50 on day **1**; 25 on day 2; 12.5 on day 3	25.3	2.2	91	
48/45	,,	200	50 on days **8** and 9; 25 on days 10, 12, 13, and 14	30.7	8.1		Dissected on day 15
48/65	i.v.	125	25 on day **1**, 2, 5, 6, and 7	23.9	3.7	85	
48/46	p.o.	550	100 on days **3** and 4; 50 on days 5, 6, 7, 8, 10, 11, and 12	30.7	29.8	3	
48/48	,,	1,800	200 on day **3**, 4, 5, 6, 7, 8, 10, 11, and 12	30.4	17.0	44	$\triangle W$ control = 40.4 $\triangle W$ treated = 47.2

much more effective than a total of 120 mg. given between days 3 and 13. In general the longer the time that elapses between the implantation of the tumour and the start of treatment with this compound the less marked is the effect upon tumour growth. This appears to be true also with compounds of the bis-epoxide and polyethylenimine class and will be discussed in a later paper in relation to the mode of action of compounds of all three types. Trimethylolmelamine is as active by the intravenous route as when given intraperitoneally. By mouth the compound produces much less effect, but with the enormous dose of 1.8 g. per 100 g. a 44 per cent inhibition of tumour growth was observed with no reduction in the gain in gross weight of the treated animals as compared with controls.

The compound was examined by our colleague Dr. G. T. Stewart for activity against a transmissible lymphoid leukaemia in Afb mice. Mice were inoculated with leukaemic cells and five days later dosing with the compound was begun. Three intravenous doses of 2.5 mg. per 20 g. followed by 10 of 5 mg. per 20 g., intraperitoneally, were given between the fifth and twenty-second days. This treatment caused a slight but definite retardation of the proliferation of leukaemic cells and restriction of leukaemic infiltration in the liver.

Dr. Edith Paterson, of the Christie Hospital and Holt Radium Institute, Manchester, gave trimethylolmelamine intraperitoneally to Strong A mice bearing established grafts of a spontaneous mammary tumour of that strain. The total doses given in different

groups of mice were 35, 75, 100, and 112.5 mg. per mouse in 7, 10, 10, and 9 days respectively. Tumour growth was restrained in the three latter groups, and, in the last, two permanent and three temporary regressions were seen in the seven mice treated. A dog with enlargement of the lymph nodes, liver, and spleen diagnosed as malignant lymphoma ("leukaemia") was treated with the compound by Mr. H. B. Parry, of the Animal Health Trust. Two courses, one of five and the other of eight intravenous doses of 50 mg. per kg., were given over seven and fourteen days respectively. There was a rapid reduction in the size of the glands, the liver and spleen became no longer palpable, and the general malaise of the animal disappeared. It remained in apparent health until some 18 days later, when it contracted an acute infection (undiagnosed) and died.

Clinical trial of the compound in human malignant disease is in progress at the Christie Hospital. The results will be reported in due course.

Toxicology.—In the second experiment (48/39) recorded in Table V, the tumour weights in the treated group were estimated by palpation on the fourteenth day (mean, less than 1 g.) and the survivors killed and the tumours actually weighed on the twenty-first. By that time the weights were 15, 14.3, and 2.4 g. respectively, while in six animals only tiny nodules of tissue, necrotic in whole or part, could be found. Two rats died on day 20, one with a tumour weighing 1.7 g. and the other with no tumour. Both these animals looked anaemic shortly before death, and when the survivors in this group were killed the organs of one other were seen to be very pale. Blood taken from the heart contained 9,650 leucocytes and 1,400,000 erythrocytes per c.mm., and haemoglobin *ca.* 35 per cent. Tissues were taken *post mortem* from the treated animals in this experiment and examined histologically. Renal damage was found in all, varying in severity up to destruction of parts of the convoluted tubules. "Dead cellular" casts were often present in the lumina, and in some kidneys odd tubules were calcified. Damage was also seen around the central veins in the liver and ranged from partial destruction to full acute necrosis of the liver parenchyma in this region. These findings were later amplified by study of the effects of trimethylolmelamine in normal animals.

Experiments in rats.—No attempt will be made to detail the many experiments which have been carried out; the essential findings are illustrated by the following. In a group of six young male rats, weighing about 100 g. at the start of experiment and dosed intraperitoneally with 50 mg. trimethylolmelamine per 100 g. on days 0–3 and 5–9 inclusive, there was marked reduction in mean body weight gain as compared with untreated controls (Fig. 1). Food intake was not recorded, and it is not known to what extent, if any, loss of appetite was responsible for this result. Individual rats died on days 16 and 17 and the remainder were killed at intervals up to the twenty-seventh day. Tissues were taken from all these animals *post mortem* for histological study. The effects of treatment upon the leucocyte and erythrocyte counts of one rat, typical of the group, are shown in Fig. 2.

A dramatic fall in the erythrocyte count some 15 to 20 days after the commencement of dosing is characteristic of the response to the compound in this species. It occurred in five of the six animals in the present experiment and was occasionally seen in rats receiving lower doses. It has not been observed in the dog even with lethal doses.

Intraperitoneal injection of the compound was followed in this experiment by a cellular reaction on the surface of the viscera—presumably a type of irritant perisplenitis, etc. The cells were large and contained abundant protoplasm. The kidneys all showed

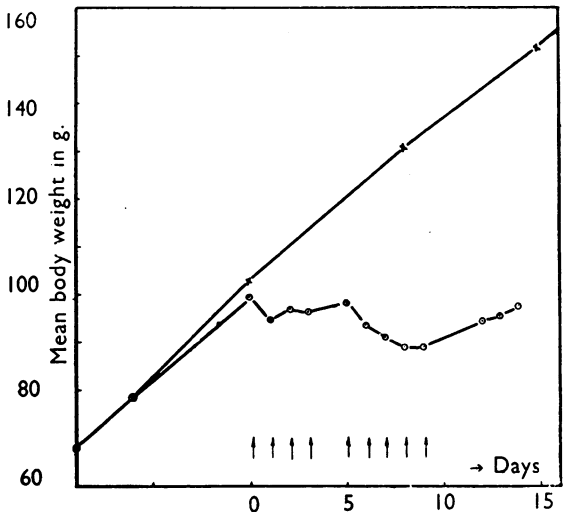


FIG. 1.—Effect of trimethylolmelamine, given intraperitoneally in aqueous suspension, upon the body weight of young male rats. ×—×: untreated controls. ⊙—⊙: rats injected at arrows with 50 mg. trimethylolmelamine per 100 g. body weight.

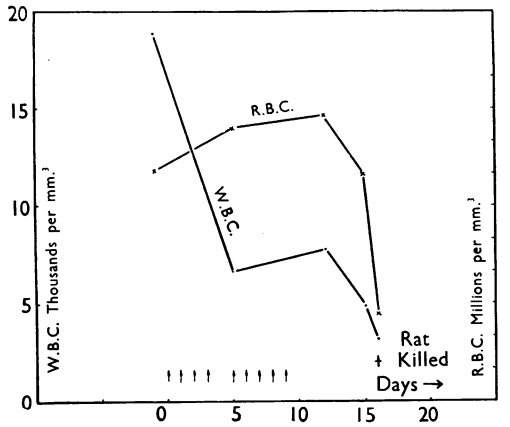


FIG. 2.—Effect of trimethylolmelamine, given intraperitoneally in aqueous suspension, upon the red and white blood cell counts of a male rat. Rat injected at arrows with 50 mg. trimethylolmelamine per 100 g. body weight.

spontaneous disease and in addition other changes certainly due to treatment. These were scarcely detectable in the rat which was killed on day 27 and were most marked in that which died on day 16. In the latter the glomeruli and first convoluted tubules were fairly normal, but the tubules of Henle and those beyond showed pathological changes. The Henle cells were highly degenerate and near necrosis. In the ducts of Bellini the tubular cells were reactive and proliferating and the lumina blocked with these and with extraneous matter which may have been the compound or a metabolite. In the rest of these rats the damage was mainly confined to the ducts of Bellini. In several animals there were minor changes in the liver (vacuolation and altered staining of the cells) in the central regions of the lobules. Throughout the series the seminiferous tubules showed advanced disorganization and spermatozoa were absent. In several instances large round cells with eosinophilic bodies in the cytoplasm were seen in the tubules, and in addition several large cells with multiple nuclei were found there. The latter were seen more consistently in the testes of rats which had received rather smaller doses of the compound and might contain anything up to 100 nuclei. Occasional cells

of this type were seen in which several of the nuclei were in division ; it is suggested that they arose from spermatocytes by repeated nuclear division without division of the cytoplasm.

Experiments in dogs.—For these studies normal dogs and bitches of the beagle breed, six to fifteen months old, were used. Trimethylolmelamine was given intravenously as a 5 per cent (w/v) aqueous suspension.

Eight doses each of 50 mg. of the compound per kg. were given over 10 days to each of 3 animals. Apart from slight depression after being dosed they appeared quite normal throughout the experiment. Treatment caused a rapid fall in the total white cell count, which reached a minimum some 20 days after the first dose and then slowly returned to normal levels. Cells of the granulocyte series were affected sooner and more markedly than the lymphocytes. There was a variable but comparatively slight downward trend in the erythrocyte count. One bitch, killed 21 days after the second of two such courses of treatment, separated by a period of 14 days, showed *post mortem* a few patches of congestion and petechial haemorrhage in the lungs and slight swelling of the glomerular endothelium in the kidney. No other pathological changes were seen in this animal.

The changes in the blood cell counts in one other of these animals (♂ 239) are shown in Fig. 3. On the 43rd day the right testicle of this animal was removed under nembutal

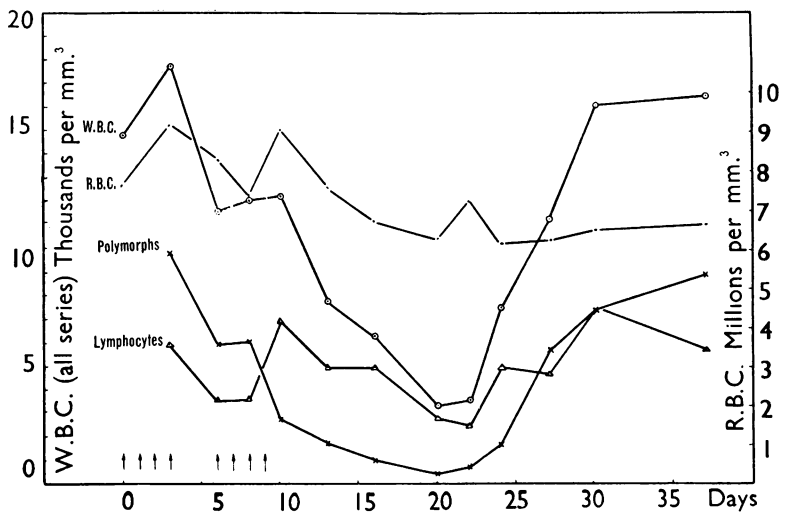


FIG. 3.—Effect of trimethylolmelamine, given intravenously in aqueous suspension, upon the blood cell counts of dog ♂ 239. Dog injected at arrows with 50 mg. trimethylolmelamine per kg.

anaesthesia. Histological examination revealed normal spermatogenesis in many of the tubules, while in others large round cells with several small dark nuclei were seen. This dog was given a second course of eight doses each of 75 mg. of the compound per kg. (days 62–65 and 68–71 inclusive). The immediate effects were slight, but a few days after the last injection the dog became listless, salivated excessively, lost appetite, and finally became comatose. It died overnight 80 days from receiving the

first (50 mg. per kg.) injection. Changes in the peripheral blood cell counts are shown in Fig. 4. The histological findings in tissues taken *post mortem* were as follows:

Lymph nodes.—There was marked central haemorrhage with local obliteration of the original structure. The peripheral ring of germinal follicles survived, but showed slight nuclear pyknosis and some depletion of lymphocytes.

Spleen.—A few lymphocytes in the Malpighian bodies showed nuclear pyknosis. The pulp was congested, and there were many haemosiderin-containing macrophages.

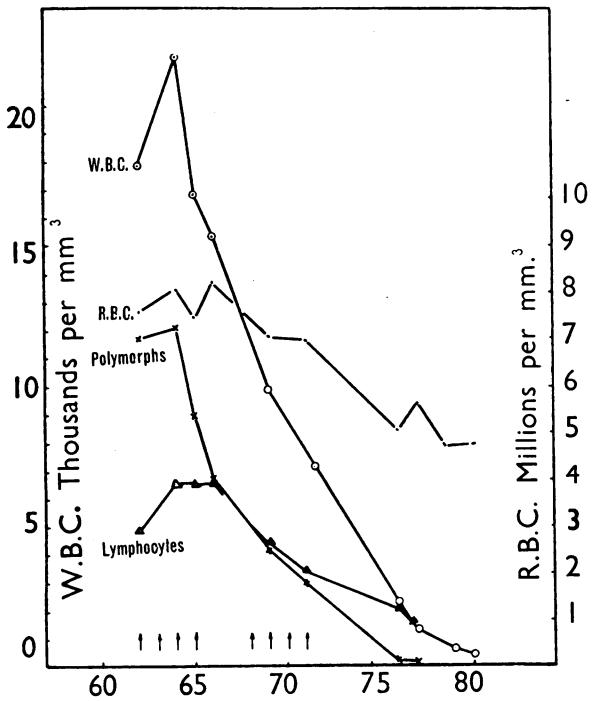


FIG. 4.—Effect of trimethylolmelamine on the blood cell counts of dog ♂ 239 continued. Dog injected at arrows with 75 mg. trimethylolmelamine per kg.

Testis.—There was no normal spermatogenesis. The lumina of the seminiferous tubules contained some large cells with numerous small nuclei, mixed with other normal cells.

Lung.—Most of the structure was lost. The outlines of some of the alveoli could still be seen, but apart from the bronchiolar cartilage almost the whole tissue was necrotic. Scattered through the section were numerous bacterial colonies, many of which had probably multiplied after the death of the animal. In considering these lung changes it should be borne in mind that the animal had been comatose some hours before death.

Liver.—There was congestion and some dilatation of the sinuses.

One other dog survived seven doses of 75 mg. of the compound per kg. and one bitch (♀ 228) succumbed to seven doses of 100 mg. per kg. (days 0–3 and 6–9 inclusive). The injection of this latter large amount was at first well tolerated although the animal vomited, defaecated, and/or urinated on several occasions shortly after being dosed and appeared somewhat depressed for an hour or two. Apart from this she appeared normal

until about four days after the last dose, when she became listless and lost appetite. By the seventeenth day she refused to eat and had become very weak ; profuse salivation was again in evidence. The blood coagulated upon dilution with Hayem's fluid and 150 mg. heparin dissolved in a little saline was given intravenously. The following day she was found moribund and was killed. White, red, and differential blood cell counts were made throughout and are plotted in Fig. 5.

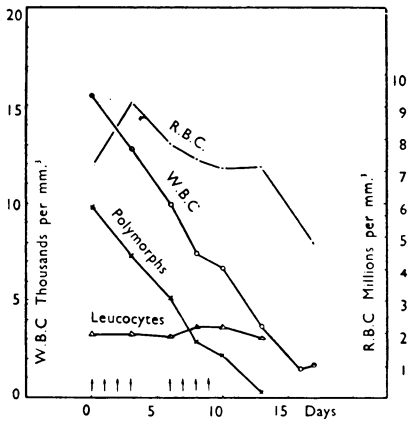


FIG. 5.—Effect of trimethylolmelamine, given intravenously in aqueous suspension, upon the blood cell counts of dog ♀ 228. Dog injected at arrows with 100 mg. trimethylolmelamine per kg.

Tissues were taken *post mortem* for histological study. In the lymph-nodes there were changes similar to those seen in dog 239, but much more severe. There was massive nuclear pyknosis and chromatorrhexis in the germinal follicles and only a few peripheral cells survived. There was depletion of lymphocytes in all areas with marked central haemorrhage. In the spleen also the damage was similar to but more severe than that seen in dog 239. Nuclear pyknosis and chromatorrhexis were very marked in the Malpighian bodies. The lungs were virtually normal. The liver sinuses in a few areas were slightly congested and some contained a granular basophilic material, possibly nuclear remains. The bone marrow taken *post mortem* from the sternum was highly abnormal. The concentration of cells was well below normal and normal mature granulocytes were absent. Such cells as were present were mainly lymphocytes, but there were some larger cells with vacuolated cytoplasm and degenerate nuclei ; these were presumably primitive cells which had undergone toxic change. There were also a few degenerate erythroblasts and normoblasts.

Cytotoxic effects (with J. M. Gates)

As it became evident that the toxic effects of trimethylolmelamine were most pronounced in proliferating tissues—tumours, bone marrow, lymphoid tissue, and the germinal epithelium—special attention was directed towards its action on dividing cells.

Mice were injected subcutaneously with the compound in aqueous suspension in single doses of 10, 20, and 40 mg. respectively per 20 g. and killed in groups of 2 to 5 animals at intervals from 2 to 72 hours later. Portions of duodenum and thymus and a splenic lymph node were taken from each animal, fixed in Bouin's fluid, imbedded in paraffin, cut at 5 μ, and stained with Heidenhain's haematoxylin.

The following nuclear abnormalities were seen in these tissues:

1. Pyknosis and fragmentation of the chromosomes, which appeared as small, intensely stained bodies scattered through the affected cells.

2. Pyknosis of whole nuclei ; these were the most frequent changes.

3. Stickiness of the chromosomes, which tended to form a central clump at metaphase and " bridges " during the anaphase separation.

4. Failure of odd chromosomes to attach themselves to the metaphase plate.

5. Very occasional tripolar mitoses.

The intensity of the damage varied with the dose and according to how long after dosing the tissues were taken. At two hours the changes were very slight and affected only a few of the dividing cells. There was a progressive increase in damage up to 24 hours when about half the dividing nuclei were involved and the damage was quite severe. In some animals the extent of the damage declined somewhat after 24 hours. It was observed that the changes in the gut (dividing cells of the crypts of Lieberkühn) were less marked than in either the thymus or lymph gland.

Nucleotoxic effects in the Walker tumour were first sought in sections of tissue taken from tumour-bearing rats killed at intervals after a single subcutaneous dose of 100 mg. of the compound per 100 g. The tissue was fixed, cut, and stained as above. Nuclear abnormalities similar to those described above were seen in these preparations.

At this stage we were greatly helped with this aspect of our investigations by Dr. P. C. Koller, of the Chester Beatty Research Institute, London, with whom we had made contact through Professor A. Haddow. Koller and his associates had made a detailed study of the changes in dividing cells exposed to *x* rays and to various chemicals and as a result had been able to define certain features of the cytotoxic action of *x* rays and of the sulphur and nitrogen mustards which seemed peculiar to those agents. These he called " specific chromosome effects " (Koller, 1949).

X rays and true " radiomimetic " agents act upon dividing cells to produce early and, if not fatal, transient changes which are not dissimilar from those obtained with cytotoxic agents of other types. Chromosome " stickiness " and its sundry manifestations, for example, fall within this category. The distinguishing feature of their action, however, is the production of permanent changes in chromosome structure. In this the primary attack upon the chromosomes seems to occur during the mitotic interphase, causing damage which becomes apparent only when the cells proceed to divide. Chromosome breakage, accompanied by the reunion of chromosome fragments to a variable extent and in various ways, leads to the appearance at anaphase, as visible manifestations of a " specific chromosome effect," of, e.g., dicentric chromosome bridges and acentric fragments. These true bridges superficially resemble those formed by the mutual adhesion of " sticky " sister chromatids but can be distinguished from them. Acentrics are small chromosome fragments lacking centromeres. Being unable for that reason to orientate themselves on the spindle they lie scattered in the equatorial region at the anaphase separation. If cell division proceeds to completion those chromosomes which are linked in a bridge may become unequally distributed between the daughter cells while some of the fragments will form accessory micronuclei. These daughter cells will have an unbalanced or deficient genetical complement and many will degenerate (v. Darlington and Koller, 1947 ; Loveless and Revell, 1949).

The sections of Walker tumour tissue from rats treated with trimethylolmelamine were examined by Dr. Koller, who confirmed the presence therein of specific chromosome changes. Twenty-four, forty-eight, and seventy-two hours after the subcutaneous injection of 100 mg. of the compound per 100 g. 84, 85, and 62 per cent respectively of the anaphases were abnormal, showing true chromosome bridges and/or numerous fragments. In addition at forty-eight and seventy-two hours many degenerating cells and cells with grossly abnormal nuclei and micronuclei were seen. At all times mitosis was rather less frequent than in control tissues, which contained some 10 per cent of abnormal anaphases, mostly showing only "sticky" bridges. One rat only was used for each of these counts. Both qualitatively and quantitatively these changes were almost indistinguishable from those produced in a parallel experiment by a dose of 0.02 mg. bis-chloroethylmethylamine per 100 g.

For the precise characterization of mitotic abnormalities squash preparations are to be preferred to sections, and in the subsequent evaluation of the changes induced in tumour tissue by our compounds we have used a technique demonstrated to us by Dr. Koller.

Animals with tumour implants of a few days' growth are treated with the compound at various dose levels. Tumour tissue taken at various times thereafter from these animals and from untreated controls is fixed in 70 per cent aqueous methanol, hydrolysed in N-HCl, and stained with Feulgen's reagent. Squash preparations are then made in 45 per cent acetic acid and restained with acetic lacmoid solution, which sharpens and intensifies the staining. After dehydration the preparations are mounted in Euparal. We examine 100 anaphases in each preparation and score the numbers showing (*a*) anaphases with true chromosome bridges, with or without fragments, (*b*) anaphases with fragments as the only visible abnormality, and (*c*) anaphases with sticky bridges. Any other cytological changes observed are recorded. Similar squash preparations are made of the bone marrow of the animals and examined both for specific chromosome changes and for general toxic effects.

Results obtained with some of the compounds mentioned above are shown in Table VI. The Walker rat carcinoma was used as the test object, and tumour tissue was sampled 24 hours after the injection of the single doses of the compounds. The corresponding figure in column A is the increase over controls in the percentage of anaphases showing abnormalities of any type produced. These figures were obtained in experiments made before the different types were separately scored, but wherever numerous the abnormalities were mainly of the specific types (*a*) and (*b*) above. The figure in column B is the increase over controls in the percentage of anaphases showing a "specific chromosome effect," i.e., abnormalities of types (*a*) and (*b*). Results of tumour growth inhibitory tests with the compounds using the Walker tumour are also shown in this Table. Within this limited selection of compounds there is evidence of a correlation between the capacity, on the one hand, to induce specific chromosome changes of the radiomimetic type, and, on the other, to inhibit the growth of the tumour. This possibility has been investigated further, more especially in connexion with the ethylenimino derivatives which form the subject of a later paper in this series, and will be dealt with in greater detail there.

Carcinogenic action

Carcinogenic activity has been demonstrated for several nitrogen mustards both of the original alkyl type (Boyland and Horning, 1949) and of the newer aryl type

TABLE VI

Comparison of cytotoxic and tumour inhibitory activity. A, percentage anaphases in excess of controls showing abnormalities of any type in tumour tissue from rats bearing the Walker tumour, 24 hr. after the single doses shown; B, percentage anaphases in excess of controls showing abnormalities of the " specific " type in tumour tissue from rats bearing the Walker tumour, 24 hr. after the doses shown; *I*, percentage inhibition of the growth of the Walker tumour in rats

Serial No.	Compound	Cytotoxic effect			Tumour inhibition	
		Dose mg./100 g. i.p.	A	B	Total dose mg./100 g. i.p.	*I*
122	Melamine	25	7		175	22
125	Trimethylolmelamine	50		47	450	100
126	Tetramethylolmelamine	20	26		225	97
174	Tetramethylol 2-p-chlorophenyl-4: 6-diaminotriazine	10	18		30	41
135	Trimethylol N-β-naphthyl melamine	100		3–9	—	—
111	Acetylacetone dicyanhydrin	4		1	14	0
107	Hexamethylene diisocyanate dibenzoic anhydride	20		0	210	34
105	Naphthalene 1: 5-diisocyanate	10		0	22.5	27

(Haddow, Horning, and Koller, 1951), and there is growing evidence of a close association of this property with tumour inhibitory action and cytotoxic activity of the nitrogen mustard (or radiomimetic) class.

Trimethylolmelamine has been on test as a carcinogen in mice and rats for periods up to two years. Intraperitoneal injection of the compound in aqueous suspension is followed by signs of local irritation and by a cellular reaction on the surface of the viscera. Subcutaneous injection produces a severe local reaction. The resulting lesion consists of a central area of coagulation necrosis surrounded by zones of oedema and inflammation. Ulceration through the skin may occur. When dosing is discontinued these lesions gradually heal.

Stock male and female albino mice of mixed ancestry were dosed subcutaneously with 1.25 mg. of the compound each per day (Monday to Friday) up to a total of 56 doses in one experiment and 70 in another. Nine months from the first injection one of the twenty-three surviving mice was found to have developed lymphoid leukaemia. No other sign of malignant disease has appeared in any other treated mouse, and this case may well have been spontaneous.

Of a group of 10 stock albino rats given a single intraperitoneal injection of 100 mg. of the compound per 100 g. one died with extensive malignant disease 17 months later. A large tumour mass was found in the lower part of the abdomen, a smaller mass adherent to the diaphragm, and numerous small nodules throughout the mesentery and lining the peritoneal cavity. Nodules were also found on the anterior wall of the thoracic cavity. This tumour was classified histologically as a highly malignant adenocarcinoma. The spleen of the animal presented a most unusual appearance. It consisted almost entirely of a mass of cells with large pale nuclei, many of which were abnormal. The remnants of the Malpighian bodies could still be recognized as shrunken atrophied structures. The cells forming the bulk of the organ bore no resemblance to those of the carcinoma.

In one rat out of a group of ten given a single subcutaneous dose of 200 mg. of the compound per 100 g. a subcutaneous spindle-celled sarcoma arose at the site of injection, some 19 to 20 months later. It is noteworthy that in this animal, killed 20 months after treatment, there was still a total absence of spermatogenesis, with cell desquamation and giant cell formation in the tubules. The persistence of testicular changes many months after the end of treatment has been observed in several animals in these experiments.

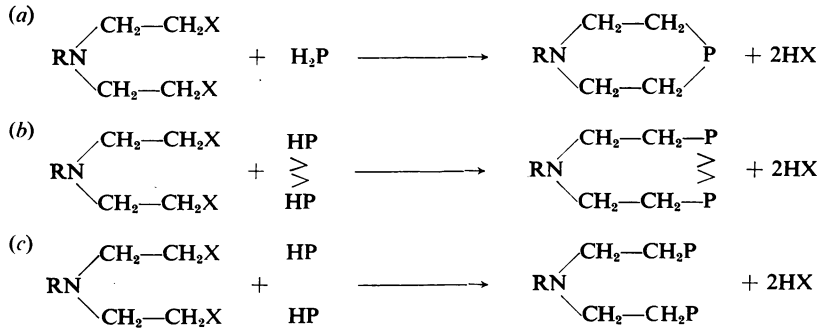
Ten rats in a third group were given two short courses, each of four daily subcutaneous doses of 50 mg. of the compound per 100 g., separated by an interval of three days. One animal in this group developed lymphoid leukaemia some 17 months after the last dose.

Eight other rats have died without tumours in these three groups. The experiment is now in the twenty-second month (December, 1950), and none of the survivors have tumours.

The commonest spontaneous tumour in our stock rats is a mammary fibroadenoma which in the later stages of its development may become malignant. Spontaneous sarcomata and carcinomata and leukaemia have also been seen from time to time so that the results described above do not constitute unequivocal evidence for carcinogenic activity. It is perhaps significant, however, that the two solid tumours in the rat experiments arose at the site of injection of the compound.

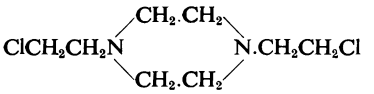
Discussion

In the preceding paragraphs we have shown that in its essential features the biological action of certain methylolmelamines closely resembles that of the nitrogen mustards. The only obvious chemical property common to these two classes is their capacity to act as alkylating agents by interaction with groupings containing active hydrogen atoms. In addition, as mentioned earlier, the characteristic biological activity of the nitrogen mustards appears to be conditional upon the presence in the molecule of at least two alkylating groups; and from our results the same appears to be true of the methylolmelamines. The interaction of a bis-functional nitrogen mustard of the type $RN(CH_2CH_2X)_2$ with cell constituents could occur in the following ways,



involving ring closure (*a*), or "cross-linkage" between two centres in the same (*b*) or separate (*c*) cellular units (P = protein).

If the alkylation of cellular constituents plays any part in the production of these biological effects it is probable that cross-linkage is involved, since with dihalogenoethylamino compounds of the type (VII)

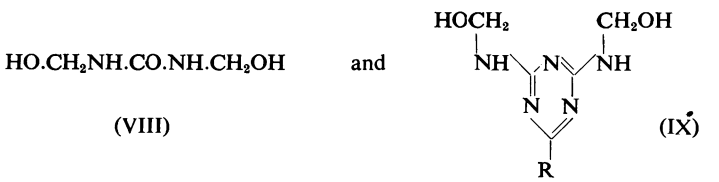


(VII)

ring closure according to scheme (*a*) above is excluded on stereochemical grounds and, as is known from the work of Burchenal and Riley (1949), compounds of this type are highly active. Assuming a symmetrical distribution of the reactive methylol groups in trimethylolmelamine our demonstration that this substance is active pointed to the same conclusion. But the main interest in this result undoubtedly lay in the fact that here for the first time cytotoxic activity of the nitrogen mustard type had been found in a structurally related but halogen-free substance which, moreover, was already in industrial use as a cross-linking agent.

At about this time, Elmore, Gulland, Jordan, and Taylor (1948), from their study of the reaction between nucleic acids, guanylic acid, and mustard gas, proposed cross-linkage by the latter substance of groups on the same or different polynucleotide chains as the possible chemical basis of its cytotoxic action, while somewhat later Goldacre, Loveless, and Ross (1949) suggested that cross-linkage might be involved in the production of chromosome abnormalities by the nitrogen mustards. Our results with trimethylolmelamine seemed to lend support to these suggestions.

On the other hand we found numerous methylolamides, which carried two prosthetic methylol groups and could therefore act as cross-linking agents, to be quite devoid of tumour inhibitory and, where tested, cytotoxic activity. These included compounds of the types (VIII) and (IX),

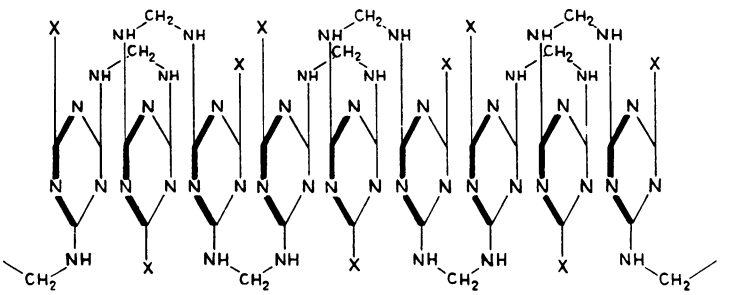


(VIII) (IX)

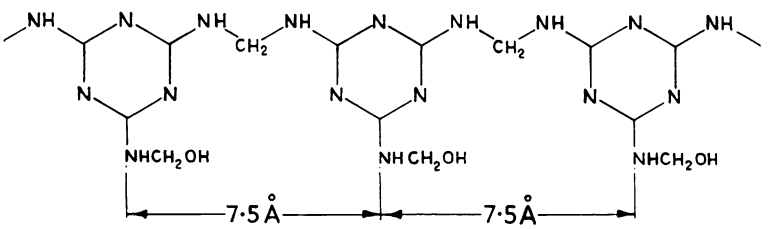
where R = H, CH_3, etc., in which the spacing of the reactive groups approximates to that of the halogen atoms in active nitrogen mustards. Certain compounds of other chemical types, and which were already known or might reasonably be expected to function as cross-linking agents, were also examined against the Walker tumour (Table III). The finding that these too were inactive further emphasized the deficiency of the hypothesis of simple cross-linkage as the basis of biological activity.

At about this time we began to examine certain epoxides and ethylenimine derivatives, and, with the discovery of nitrogen mustard-like activity in polyfunctional compounds of these two series also, a further chemical common denominator became apparent which in retrospect seemed to resolve some of the difficulties mentioned above. We were struck by the fact that the groupings in question—methylolamide, epoxide, and ethylenimine—are characterized as much by the ease

with which they polymerize as by their reactivity as alkylating agents. It seemed unlikely that this was purely fortuitous, and we began to speculate upon the possibility that the active agent in each case was not the monomeric material but a small polymer formed therefrom within the cell. Two general considerations appeared to be in harmony with this conception. The technical usage of these substances as a group is generally regarded as dependent upon the combination of the two properties mentioned above, leading to the formation of polymeric units with residual groupings capable of forming linkages between adjacent polymer chains—be they polypeptide as in wool or poly-cellobiose as in cotton fibres—with resultant modifications of structure and physico-chemical properties in the amorphous region of these fibres. It is probable, moreover, that the cellular constituents which play the fundamental role in mitosis are proteins and nucleoproteins, macromolecular structures similar in pattern to the polypeptides, and poly-cellobioses which make up the inanimate textile fibres. Unfortunately the precise nature of the polymer unit derived from trimethylolmelamine is not yet known with certainty (epoxide and ethylenimine polymers will be discussed in subsequent papers). It has been suggested (Dixon, Christopher, and Salley, 1948) that the triazine nuclei



(X)



(XI)

are arranged in it in lamellar form as represented in (X) ($X = NH.CH_2OH$); alternatively it may have an extended form in which case the most likely structure would be (XI). In either case two of the methylol groups present in each molecule of the monomer are required for the formation of the polymer chain, leaving one reactive side chain appended to each nucleus; and in either case these reactive appendages will be spaced in line at distances apart which are approximate multiples of 3.7 Å. This distance corresponds very closely to the spacing of the purine and pyrimidine residues in the nucleic acids and of the amino-acids in extended polypeptides. Such polymers would be capable of forming a comparatively stable multi-

point attachment along either protein or nucleic acid chains—such, for example, as those associated with the chromosomes and chromatids—or, in the polymers of type (X) at least, between parallel chains, to give a much more stable type of cross-linkage than that provided by unassociated molecules. If the cell components affected were indeed the chromosomes and chromatids this might well lead to the aberrations produced by the compound in dividing cells.

Assuming then that biological activity of the type under consideration depends upon the intervention of some such polymer unit it is clear that no activity would be expected from linear dimethylol compounds such as dimethylol-urea (VIII) since the derived polymers would have no residual reactive group. This would apply also to the linear dimethylolamides 82, 83, 84, 87, and 88, and further to the heterocyclic dimethylol derivatives 85, 89, 90, and 91 (Table I).

With dimethyloltriazines of the type (IX) above, in which both methylol groups would be involved in the formation of the polymer chain, the possibility of biological activity is seen to depend upon the third substituent, R, in the triazine ring. If this is inert no activity should be found ; if, on the other hand, R is a group with affinity for cellular macromolecules, becoming attached thereto either by covalent linkage or by residual valency forces or is even a group which is metabolized to a substituent having such affinity, interference with the mitotic process by the mechanism suggested above becomes possible.

In fact, no activity was found where R=H (151), Me (153), *p*-tolyl (166), *o*-chlorophenyl (178), *m*-chlorophenyl (175), *p*-chlorophenyl (172), β-naphthyl (181), α-furyl (160), styryl (157), *p*-chloroanilino (131), β-naphthylamino (134), dimethylamino (141), diethylamino (143), and methylanilino (146). Where R was phenyl (163) or *p*-anisyl (169) activity was present.

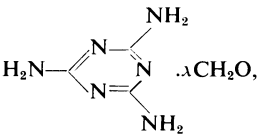
Thus the hypothesis appears to remove many of the difficulties mentioned but does not account for some few results without further assuming a metabolic change. However, the inactivity found for compounds of this type where R is a phenyl radical blocked in the *para* position by a methyl or chloro group does in some measure support the contention that metabolism of the ring is a factor concerned in the activity of the phenyl and *p*-anisyl derivatives.

The results presented and the hypothesis outlined above suggest several lines of speculation and research. The question of affinity for fibres is germane to our inquiries, and in this connexion dyestuffs and dyestuff substantivity come to mind. Substantivity in dyestuffs is usually achieved by a pattern of repeat groupings capable of binding the linear dye molecule to the fibre by residual valency forces. It would perhaps be possible to interfere with the mitotic process with such a unit or polymer chain not linked covalently with, but merely adhering to, a vital cellular component by such binding forces. Again, there is the idea, implicit in our hypothesis, that the orientation of reactive groups is a factor of importance in the process of interfering with macromolecular function. This is attained we believe with the active methylolamides by the formation of a polymer "backbone" carrying such groupings at regularly spaced intervals, but we have further suggested (Hendry, Rose, and Walpole, 1950) that a similar orientation of prosthetic groups might be brought about solely by micelle formation, perhaps, for instance, in the carcinogenic polycyclic hydrocarbons. Work in this connexion is in process and will be reported in due course.

SUMMARY

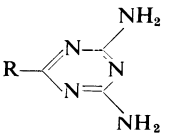
A description is given of the sequence of events leading to the discovery of tumour inhibitory activity, against the transplanted Walker carcinoma 256 in rats, in polymethylolamides prepared by condensing formaldehyde with melamine and with certain derivatives of that substance.

Compounds of the general formula



containing two or more molecular proportions of formaldehyde ($x=2$–6) have been found active. Activity is high in the trimethylol derivatives of alkyl and aryl substituted melamines and lower or absent in the corresponding dimethylolamides.

Acyl and aroyl guanamines of the type



are inactive. When R is hydrogen, methyl, *n*-hexyl, benzyl, styryl, α-furyl, or *o*- or *m*-chlorophenyl, the corresponding polymethylol derivatives are of negligible activity irrespective of formaldehyde content. When R is *p*-chlorophenyl, *p*-tolyl, or β-naphthyl the same is true of the di- or tetra-methylol derivatives, but the corresponding trimethylolamides have some activity. When R is phenyl or *p*-anisyl the di- and tri-methylolamides are highly active, as is the tetramethylolamide from *p*-methoxybenzguanamine.

Certain polymethylolamides are employed industrially for modifying the mechanical and physico-chemical properties of textile fabrics. Their usefulness in this direction is probably associated with their poly-alkylating function and with the formation of cross-linkages between adjacent polymer fibres. Our attention had been drawn to the poly-alkylating and cross-linking potentialities of the sulphur and nitrogen mustards, and it had been suggested that the specific cytotoxic effects and tumour inhibitory action of the latter might be due to the formation of cross-linkages between cell nuclear components. It appeared that the biological properties of the mustards and of the active polymethylolamides might have a common foundation. Support for this view has been obtained from a more detailed study of the biological effects of trimethylolmelamine, taken as representative of active compounds of the latter type. The toxic action of this substance in the animal body is directed mainly against those tissues where cell division is most active—the haematopoietic and lymphoid systems, the intestinal epithelium, and the germinal epithelium of the testis —with the production of minor pathological changes in epithelia and endothelia in other sites. In addition it produces in dividing cells of the Walker tumour specific chromosome effects—chromosome fragmentation and " bridge " formation—characteristic of the action of true radiomimetic agents. Tests for carcinogenic activity have given equivocal results.

The hypothesis of simple cross-linkage as the basis of biological activity is insufficient, however, to account for all the facts. In particular many bis-functional compounds which are known, or might reasonably be expected, to act as cross-linking agents have been found to be quite inactive against the Walker tumour. We postulate as an essential step in the production of their biological effects the intermolecular condensation of the methylolamides into polymeric units, in which two methylolamide groups in each molecule are involved in the formation of the polymer chain. A third active centre is then required, which may be a methylolamide group or one with similar functional capacities, which serves to attach the unit at several points along or between protein or nucleic acid chains—such as those associated with the chromosomes—in the cell.

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