142. EXPERIMENTS ON THE CHEMOTHERAPY OF CANCER

4. FURTHER EXPERIMENTS WITH ALDEHYDES AND THEIR DERIVATIVES

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STRONG [1938; 1939] has shown that the growth of spontaneous mammary tumours in mice is inhibited by feeding heptaldehyde. This was confirmed by Boyland & Mawson [1938] who found, however, that citral had a greater inhibitory effect than heptaldehyde. Some other aldehydes have now been examined but none has proved to be as effective as citral. In an attempt to understand the action of these aldehydes on tumours a number of related compounds and possible metabolic products have been tested; of these malonic acid was found to have marked inhibitory action.

An attempt has been made to put the experiments on a quantitative basis so that the activities of compounds can be more accurately compared and the significance of the results assessed.

EXPERIMENTAL

Grafted tumours

The toxicity of each substance was determined and it was given daily by forced feeding to groups of five Dilute Brown mice grafted with sarcoma M.C.D.B.I. [cf. Boyland & Boyland, 1939]. Dosing was commenced on the day on which the tumour was grafted. No doses were given to the animals on Sundays. The daily dose chosen was about one-quarter of the L.D. 50 and was suspended or dissolved in 0.5 ml. water with gum. Some compounds had a cumulative toxic action and killed mice when one-quarter of the L.D. 50 was administered repeatedly; in these cases a smaller dose was given.

The tumours were measured with callipers twice weekly and their sizes compared with those of tumours grafted at the same time in similar but untreated mice. The mean rate of growth of the tumours for all the periods between measurements was calculated and compared with the mean growth rate of the tumours in control mice. The percentage inhibition of growth due to the treatment is given in Table 2. The probability (P) that the inhibition is greater than could have arisen by errors of sampling is calculated by the method of t [Fisher, 1936]. If the value of P exceeds 0.05 then the inhibition cannot be considered significant. This does not necessarily imply that the hypothesis that the inhibition is real is not true but that the experiment for which P is calculated cannot be taken by itself as proof of the hypothesis, within the limits of likelihood corresponding to $P=0.05$.

On the whole there is rather poor correspondence between the results with grafted tumours and spontaneous tumours. The effect on the grafted sarcomata is generally smaller but the variations also are smaller and, as the results depend on a larger number of measurements, the effect is often significant.

Sebacic acid, sebacic acid hydrazide, citral, furfuralamide and phloroglucin-aldehyde were less effective on grafted tumours than on spontaneous tumours; while adipamide, ethyl malonate, malonamide, acrolein, crotonaldehyde, furfuraldehyde and furfuralacrylic acid were more effective. Only heptaldehyde (50 mg. per day), malonic acid and citral produced significant inhibition of growth with both types of tumours. These last three compounds must be considered the most effective of the substances described in this paper.

Boyland & Mawson [1938] obtained no inhibition of the Crocker Sarcoma 180 by feeding 50 mg. heptaldehyde per day but a definite inhibition with 5 mg. phloroglucinaldehyde daily.

Primary tumours

As the growth rates of primary tumours vary greatly each tumour must be used as its own control. Induced primary tumours were obtained by injection of 1 mg. methylcholanthrene into stock mice. Induced tumours and spontaneous mammary tumours were measured twice weekly as soon as they were obtained. They were allowed to grow until the sum of two diameters of the tumour had increased by at least 7 mm. and this, the control rate of growth, was expressed in mm. growth per day ((i) in the tables).

The mice were then dosed for 14 days and the rate of growth per day determined ((ii) in the tables). The ratio of these two rates of growth shows the effect of the treatment and from this ratio the inhibition expressed as a percentage of the original growth can readily be obtained. Those cases in which partial retrogression occurred during the treatment are shown by the negative ratio of growth and the inhibition is taken as being over 100%.

The tables show large variations in the response of different mice treated with the same compound. The inhibition obtained in each series was examined according to the methods for treating the difference of means on a unique sample described by Fisher [1936] and the values for *t* and the probability (*P*) determined.

EFFECT OF HEPTALDEHYDE ON INDUCED TUMOURS

Heptaldehyde was given in 50 mg. doses daily to mice with primary induced tumours. The treatment had very slight and variable effect on the growth of the tumours, as the results in Table 1 show. Induced tumours like grafted tumours are less susceptible to the action of heptaldehyde than are spontaneous carcinomata.

Table 1. *The ratio of growth rate of induced tumours under treatment with heptaldehyde to their normal growth rate*

In columns A, B, C and D, which refer to separate mice, the numerators of fractions refer to the increase in size of the tumour (as the sum of length and breadth in mm.) while the denominators refer to the number of days required for that growth.

Mouse	A	B	C	D
(i) Growth in control period	$\frac{15}{22}$	$\frac{9}{5}$	$\frac{20}{14}$	$\frac{8}{10}$
(ii) Growth in treatment period	$\frac{12}{14}$	$\frac{6}{14}$	$\frac{18}{14}$	$\frac{18}{14}$
(iii) Ratio $\frac{(ii)}{(i)}$	0.69	0.24	0.90	1.60
Average of $\frac{\text{Growth in treatment period}}{\text{Growth in control period}}$ 0.86 or 14% inhibition.				

Table 2. *The ratio of growth rate of mouse tumours under treatment to their normal growth rate*

In columns A, B, C and D the numerators in the fractions refer to the increase in size (length and breadth in mm.) of the tumour while the denominators refer to the number of days required for that growth; each column A, B, C and D refers to a separate mouse. If any diminution in size occurred during the treatment period this is recorded by a minus sign and the inhibition is considered as over 100%.

over 100 %.

Toxicity L.D. 50 for mice mg.	Daily dose mg.	Effect on grafted sarcomata M.C.D.B.I. in groups of 5-15 mice			Effect on spontaneous carcinomata												Average effect on spontaneous carcinomata	
		Inhibition %	Probability (P) that the inhibition is significant	Inhibition of growth under treatment %	(i) Growth in control period.				(ii) Growth in treatment period.				(iii) Ratio (ii)/(i)					
					A		B		C		D		(i)		(ii)			
					(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)	(i)	(ii)		
Heptaldehyde	500	25	9	0.1	8 22	4 14	0.78 14	15 17	12 14	0.97 14	7 7	2 14	0.14 7	12 7	17 14	0.71 14	35	0.2
Heptaldehyde	—	50	20	0.02	11 14	-2 14	-0.18 14	13 16	7 14	0.61 14	10 21	2 14	0.30 14	14 7	8 14	0.29 14	75	0.02
Heptoleic acid	160	25	5	—	8 18	10 14	1.60 14	10 14	12 16	1.05 14	8 32	5 14	1.42 14	25 30	3 12	0.30 12	Nil	—
Sebacic acid	150	25	19	0.2	10 13	4 14	0.37 14	9 10	1 14	0.08 14	10 11	2 14	0.16 14	9 11	4 14	0.35 14	76	0.01
Sebacic acid dihydrazide	10	2	6	0.2	23 12	14 14	0.52 14	11 10	9 14	0.58 14	13 12	5 11	0.42 11	16 17	11 14	0.83 14	41	0.02
Pinelic acid	120	25	6	0.1	10 19	7 16	0.84 14	12 12	8 16	0.86 14	11 12	5 14	0.39 14	15 23	4 14	0.44 14	37	0.05
Adipic acid	200	25	0	—	9 9	4 14	0.29 14	13 12	8 14	0.53 14	10 10	11 14	0.79 14	12 18	9 14	0.97 14	35	0.1
Adipamide	150	25	28	0.01	18 12	3 14	0.14 14	7 11	11 14	1.22 14	12 14	11 14	0.91 14	10 11	0 14	0 14	43	0.3
Mucic acid	200	25	16	0.1	9 20	4 14	0.63 14	28 28	4 14	0.89 14	7 14	3 10	0.60 8	9 9	10 14	0.63 14	30	0.05
Glutaric acid	150	25	8	0.5	9 12	1 14	0.10 14	14 40	0 14	0 14	16 8	23 14	0.82 14	7 14	8 14	1.14 14	48	0.2
Malonic acid	100	20	32	0.05	18 38	-6 14	-0.91 14	15 14	-1 14	-0.07 14	18 34	2 14	0.27 14	13 14	-7 14	-0.54 14	131	0.02
Ethyl malonate	160	40	27	0.05	6 14	4 14	0.66 14	11 12	3 14	0.43 14	7 18	6 14	1.10 14	8 13	7 14	0.81 14	25	0.15

Malonamide	150	25	36	0.05	$\frac{9}{13}$	$\frac{7}{14}$	$\frac{8}{10}$	$\frac{11}{14}$	0.98	$\frac{8}{14}$	$\frac{3}{14}$	0.38	$\frac{10}{7}$	$\frac{3}{14}$	0.15	44	0.3
Geranio	60	15	18	0.2	$\frac{10}{11}$	$\frac{6}{14}$	$\frac{16}{26}$	$\frac{8}{14}$	0.93	$\frac{9}{15}$	$\frac{6}{14}$	0.71	$\frac{8}{14}$	$\frac{10}{14}$	1.25	16	0.2
Citral	150	25	13	0.05	$\frac{10}{11}$	$\frac{1}{14}$	$\frac{9}{18}$	$\frac{-6}{17}$	-0.70	$\frac{20}{9}$	$\frac{5}{14}$	0.16	$\frac{16}{28}$	$\frac{0}{14}$	0	111	0.01
Geranic acid	100	20	0	—	$\frac{9}{14}$	$\frac{4}{14}$	$\frac{8}{14}$	$\frac{8}{14}$	1.00	$\frac{23}{6}$	$\frac{9}{14}$	0.17	$\frac{15}{6}$	$\frac{15}{14}$	0.43	49	0.2
Hildebrandt acid	100	10	3	—	$\frac{8}{12}$	$\frac{-6}{15}$	$\frac{10}{7}$	$\frac{11}{14}$	0.55	$\frac{10}{14}$	$\frac{13}{14}$	1.30	$\frac{10}{22}$	$\frac{9}{14}$	1.41	33	0.4
Hildebrandt acid	—	25	10	0.3	$\frac{14}{7}$	$\frac{20}{14}$	$\frac{9}{11}$	$\frac{11}{14}$	0.96	$\frac{7}{11}$	$\frac{-5}{14}$	-0.56	$\frac{17}{32}$	$\frac{2}{14}$	0.27	66	0.2
Acrolein	1	0.25	25	0.05	$\frac{19}{21}$	$\frac{-3}{14}$	$\frac{10}{28}$	$\frac{4}{14}$	0.80	$\frac{9}{11}$	$\frac{11}{14}$	0.96	$\frac{12}{21}$	$\frac{-1}{14}$	-0.23	67	0.1
Acrylic acid	60	15	19	0.1	$\frac{7}{17}$	$\frac{2}{14}$	$\frac{9}{14}$	$\frac{12}{14}$	1.33	$\frac{8}{7}$	$\frac{9}{14}$	0.56	$\frac{8}{6}$	$\frac{12}{14}$	0.64	28	0.2
Crotonaldehyde	6	0.5	19	0.01	$\frac{13}{19}$	$\frac{2}{14}$	$\frac{14}{14}$	$\frac{0}{14}$	0	$\frac{7}{18}$	$\frac{7}{14}$	1.28	$\frac{9}{23}$	$\frac{3}{14}$	0.54	49	0.2
Crotonic acid	120	25	5	—	$\frac{7}{18}$	$\frac{6}{14}$	$\frac{9}{14}$	$\frac{9}{10}$	1.40	$\frac{12}{10}$	$\frac{10}{14}$	0.59	$\frac{10}{14}$	$\frac{6}{14}$	0.80	8	0.5
Maleic acid	60	15	3	0.8	$\frac{9}{24}$	$\frac{4}{14}$	$\frac{7}{18}$	$\frac{9}{14}$	1.47	$\frac{16}{10}$	$\frac{9}{14}$	0.40	$\frac{10}{10}$	$\frac{9}{14}$	0.64	18	0.2
Furfuryl alcohol	40	2.5	3	—	$\frac{12}{20}$	$\frac{6}{14}$	$\frac{9}{11}$	$\frac{12}{14}$	1.05	$\frac{7}{9}$	$\frac{14}{14}$	1.28	$\frac{24}{12}$	$\frac{15}{14}$	0.54	10	0.5
Furfuraldehyde	10	2.5	15	0.01	$\frac{10}{21}$	$\frac{-3}{14}$	$\frac{7}{10}$	$\frac{11}{14}$	1.12	$\frac{8}{10}$	$\frac{9}{14}$	0.80	$\frac{10}{7}$	$\frac{9}{14}$	0.45	52	0.2
Furfuramide	30	7.5	14	0.2*	$\frac{14}{26}$	$\frac{3}{14}$	$\frac{29}{20}$	$\frac{6}{14}$	0.30	$\frac{18}{21}$	$\frac{7}{14}$	0.58	$\frac{9}{9}$	$\frac{11}{14}$	0.71	50	0.02
Furoic acid	25	5	0	—	$\frac{10}{24}$	$\frac{7}{14}$	$\frac{15}{12}$	$\frac{13}{14}$	0.74	$\frac{15}{21}$	$\frac{8}{14}$	0.80	$\frac{13}{17}$	$\frac{6}{14}$	0.63	16	0.2
Furfuracrylic acid	20	5	44	0.01	$\frac{14}{24}$	$\frac{4}{14}$	$\frac{9}{10}$	$\frac{7}{14}$	0.55	$\frac{9}{14}$	$\frac{9}{14}$	1.00	$\frac{9}{10}$	$\frac{5}{14}$	0.40	39	0.5
Cuminal	60	5	0	—	$\frac{10}{16}$	$\frac{10}{14}$	$\frac{11}{32}$	$\frac{0}{14}$	0	$\frac{10}{19}$	$\frac{6}{14}$	0.82	$\frac{14}{24}$	$\frac{2}{14}$	0.24	45	0.2
Phloroglucin-aldehyde	80	5	0	—	$\frac{11}{24}$	$\frac{0}{14}$	$\frac{9}{10}$	$\frac{11}{14}$	0.87	$\frac{12}{10}$	$\frac{0}{14}$	0	$\frac{11}{14}$	$\frac{6}{14}$	0.55	65	0.05

In this case the inhibition is insignificant. The probability (P) that the reduction of growth during treatment might have arisen by chance is about 0.6 ($t=0.50$).

HEPTALDEHYDE AND OXIDATION PRODUCTS

The effect of daily administration of 50 mg. heptaldehyde on primary carcinomata is significant, but dosage at this level causes the mice to become ill and frequently they die with one week's treatment. Heptonic acid, the simplest oxidation product of heptaldehyde, was ineffective against both grafted and spontaneous tumours. Although heptaldehyde would probably be largely converted into heptyl alcohol and heptonic acid in the body [Parnas, 1910] a certain proportion might be expected to combine with free amino groups and suffer ω -oxidation and subsequent further oxidation to pimelic acid. (This would be analogous to the metabolism of citral, cf. below.) In order to test this, the urine of rats and rabbits which were fed with heptaldehyde was examined for pimelic and other acids but none was isolated. It is, therefore, probable that heptaldehyde is completely oxidized in the body. Pimelic acid and its possible β -oxidation products, glutaric acid and malonic acid, are probable intermediates. Of these dicarboxylic acids pimelic acid had a slight effect, while malonic acid and sebamic acid had definite inhibitory actions, which suggests that the effect of heptaldehyde may not be entirely due to the toxic action of the aldehyde group. Of this group of compounds malonic acid was much more effective than heptaldehyde. Ethyl malonate and malonamide were less active. Malonic acid is known to inhibit tissue respiration *in vitro* but the respiration of slow-growing tumours in mice treated with malonic acid was not lower than that of normal tumours.

Certain dicarboxylic acid derivatives had inhibitory action. Sebamic acid dihydrazide (for which I am indebted to Major G. A. H. Buttle) was less active than the parent acid but still gave a significant effect. Adipamide was a little better than adipic acid but of this group only mucic acid (tetrahydroxyadipic acid) had a significant effect.

It is of interest that certain polymethylene diamines such as cadaverine and tetradecamethylene diamine also have an inhibitory action of the same order as the active dicarboxylic acid. Thus certain polymethylene compounds with either acidic or basic end groups are able to inhibit the growth of tumours.

UNSATURATED ALDEHYDES AND ACIDS

Citral with two double bonds and two methyl groups has more inhibitory action on tumour growth than heptaldehyde. No compounds related to citral have been found as effective as citral itself. When citral is fed to dogs or rabbits it suffers ω -oxidation to give "Hildebrandt acid" [Hildebrandt, 1901] or 2:6-dimethyl- Δ -2:6-octadiene-2:8-dicarboxylic acid. This acid was prepared, therefore, from the urine of rabbits which had been fed citral, and was found to be much less active than citral as was geranic acid (the simple monocarboxylic acid corresponding to citral). The alcohol geraniol was devoid of inhibitory action. Both geraniol and geranic acid are converted into Hildebrandt acid [Kuhn & Livada, 1933] in the body. Comparison of the effects of these terpenoid compounds suggests that the aldehyde group is necessary for maximal activity, and that it is not the known metabolic product which is responsible for the inhibitory effect.

Acrolein, crotonaldehyde and furfuraldehyde inhibit the growth of chick fibroblasts in tissue culture [Willmer & Wallersteiner, 1939]. The two simple unsaturated aldehydes acrolein and crotonaldehyde were only effective against grafted tumours. Acrylic acid and crotonic acid were almost without action,

indicating that the toxic aldehyde group is responsible for the activity of the corresponding aldehydes. Maleic acid, the simplest unsaturated dicarboxylic acid which could be expected to have any effect, had no significant inhibitory action.

CYCLIC COMPOUNDS

Furfuraldehyde, like acrolein and crotonaldehyde, had little effect on spontaneous tumours but had a definite action on grafted sarcomata. Furfuramide, the ammonia derivative of furfuraldehyde, was only active on carcinomata and furfuryl alcohol and furoic acid were ineffective against both types of tumour. Furfuraldehyde differs from other aldehydes in its metabolism, being excreted as furfuracrylic acid. Furfuracrylic acid (synthesized by Perkin's reaction on furfuraldehyde) had about the same effect as furfuraldehyde so that it is possible that the action of the aldehyde might be due to the metabolic product.

Cuminal, an aromatic aldehyde related to the terpenes, had no significant inhibitory action on either carcinomata or grafted sarcomata. Phloroglucin-aldehyde, which was the most promising aromatic aldehyde previously tested [Boyland & Mawson, 1938], caused significant inhibition of carcinoma growth.

DISCUSSION

Consideration of the possible fate of heptaldehyde in the body suggested that ω -oxidation might occur and give pimelic acid which might in its turn be oxidized to glutaric and malonic acids. All these acids have some inhibitory action and that of malonic acid seems to be greater than that of heptaldehyde itself. This indicates that certain dicarboxylic acids, like some diamines, have inhibitory action and opens up a new field for investigation.

Citral is much more effective than heptaldehyde but none of the citral derivatives which have been tested has the activity of citral; other unsaturated aldehydes which were examined were very toxic and not very active.

SUMMARY

1. An attempt has been made to express quantitatively and statistically the effect of substances which inhibit tumour growth.

2. Heptaldehyde given to mice has some inhibitory action on the growth of spontaneous carcinomata and grafted sarcomata. A similar effect is produced by dicarboxylic acids (particularly malonic acid) which are possible metabolic products of heptaldehyde.

3. Citral has more inhibitory action on tumour growth than heptaldehyde but the monocarboxylic and dicarboxylic acids derived from citral have only slight effect.

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