143. EXPERIMENTS ON THE CHEMOTHERAPY OF CANCER

5. THE EFFECT OF MUSCLE EXTRACT AND ALIPHATIC BASES

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RESEARCH on the chemotherapy of cancer is still in the stage of examining substances which inhibit tumour growth rather than cure it by causing complete disappearance of the tumour. There have been many reports of substances present in tissues which inhibit both normal and malignant growth. In many cases the effect has been shown on growth in tissue culture; thus Walton [1914] found that the growth of cultures was inhibited by liver extract and many explants of parenchymatous cells were inhibited by muscle extracts. Roffo [1938] also describes the inhibition of tissue growth by muscle extract. Heaton [1926] described the inhibitory action of liver extract and obtained similar but more pronounced effects with certain fractions from yeast. Baker & Carrel [1925] obtained similar inhibition of growth with ether-soluble material from serum, brain, liver, egg and embryo tissue, and Medawar [1937] with malt extracts. Recently Brues *et al.* [1940] have attempted to fractionate the inhibitory factor of liver and showed that some of the activity is due to ethanolamine.

Analogous effects have been obtained in animals. Heaton [1929] observed inhibition of body growth and of tumour growth in animals treated with extracts of yeast or malt. This effect of yeast extracts on malignant growths has been confirmed by Lewisohn *et al.* [1940]. Tumour-inhibitory substances have also been obtained from mammary tissue and placenta [Macfadyen *et al.* 1939], spleen [e.g. Lewisohn, 1938], liver [Dobrovolskaia-Zavadskaia & Zephiroff, 1940] and heart muscle [Roffo, 1938]. There is thus a rough parallelism between the actions of these tissue extracts on tissue cultures and tumours growing in animals.

It is possible that tissues contain growth inhibitors and promoters, so that the effect of any extract will depend upon the method of preparation. It should be noticed that factors which inhibit the growth of established tumours do not necessarily inhibit the induction of tumours: thus Watson [1933] found that feeding with liver increased the carcinogenic response in tar-treated mice, while according to Maisin *et al.* [1938] yeast extracts inhibit the production of benzpyrene tumours in mice.

From the point of view of cancer chemotherapy the results obtained by Roffo [1938] with muscle extracts appeared to be most striking, and although the results described below are not so remarkable as those obtained by Roffo, the tumour growth-inhibiting action of muscle extract was confirmed and investigated.

Table 1. *The ratio of growth rate of treated to that of untreated mouse tumours*

In columns A, B, C and D the numerators in the fractions refer to the increase in size (length plus 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 an individual 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%.																	
	Toxicity L.D. 50 for mice (oral unless otherwise stated)	Daily dose	Effect on grafted sarcoma M.C.D.B.1. inhibition %	Effect on spontaneous carcinomata: (i) Growth in control period. (ii) Growth in treatment period. (iii) Ratio ii/i												Average effect	
				A			B			C			D				
				(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)	(i)	(ii)	(iii)		
Muscle extract M ₁ (pre- pared with cold 5N HCl)	2.0 ml.	0.5 ml.	20	17 14	2 11	0.15	10 24	2 14	0.34	8 8	2 14	0.14	10 17	8 14	0.97	60	0.05
Muscle extract M ₂ (pre- pared with cold N H ₂ SO ₄ and Ca(OH) ₂)	3.0 ml.	0.5 ml.	18	10 14	-4 14	-0.40	10 10	3 14	0.22	12 10	16 14	0.95	9 10	1 10	0.11	64	0.05
M ₃ intraperitoneal	1.0 ml.	0.2 ml.	18	15 14	-10 14	-0.66	11 18	-1 14	-0.11	9 13	4 14	0.41	13 12	5 14	0.33	101	0.02
Dialysed muscle extract	3.0 ml.	1.0 ml.	6	13 22	5 14	0.61	11 9	-5 14	-0.29	11 15	5 14	0.49	16 24	2 14	0.21	74	0.02
Muscle extract: pyridine- soluble	2.0 ml.	0.4 ml.	1	16 17	10 14	0.76	11 16	2 14	0.21	6 6	5 14	0.36	7 12	4 14	0.49	54	0.02
Muscle extract: EtOH- soluble	3.0 ml.	0.4 ml.	25	20 10	16 14	0.57	10 12	6 14	0.52	16 12	5 14	0.27	7 10	3 14	0.31	48	0.01
Muscle extract: CCl ₃ .COOH-soluble	>3.0 ml.	0.5 ml.	18	8 9	-2 10	-0.22	15 14	-2 14	-0.13	11 31	0 14	0	16 14	-4 14	-0.25	115	0.01
Muscle extract: insoluble in CCl ₃ .COOH	>3.0 ml.	0.5 ml.	15	14 7	14 14	0.50	10 8	11 14	0.63	13 12	8 14	0.54	11 14	8 14	0.73	40	0.2
Muscle extract: phospho- tungstic acid ppt.	>3.0 ml.	0.5 ml.	21	12 17	1 14	0.10	15 17	4 14	0.32	10 23	4 14	0.66	9 7	-3 10	-0.23	79	0.02
Muscle extract: phospho- tungstic acid-soluble	>3.0 ml.	0.5 ml.	0	8 12	8 14	0.86	10 8	10 10	1.25	8 12	6 14	0.64	10 12	11 14	0.94	8	0.6

In columns A, B, C and D the numerators in the fractions refer to the increase in size (length plus 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 an individual 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%.

β -Alanine	200 mg.	50 mg.	7	$\frac{8}{14}$	$\frac{2}{14}$	0.25	$\frac{18}{24}$	$\frac{17}{14}$	1.62	$\frac{14}{14}$	$\frac{14}{9}$	1.55	$\frac{11}{18}$	$\frac{5}{14}$	0.58	0	—
Aneurin	—	0.5 mg.	11	$\frac{11}{17}$	0	0	$\frac{24}{17}$	$\frac{6}{14}$	0.30	$\frac{25}{26}$	$\frac{8}{14}$	0.59	$\frac{8}{24}$	$\frac{2}{14}$	0.43	67	0.1
Arcaïne sulphate	50 mg.	5 mg.	11	$\frac{10}{5}$	$\frac{13}{14}$	0.47	$\frac{10}{7}$	$\frac{5}{14}$	0.25	$\frac{10}{6}$	0	0	$\frac{8}{13}$	$\frac{-2}{11}$	-0.30	89	0.01
Betaine	120 mg.	25 mg.	10	$\frac{23}{11}$	$\frac{10}{14}$	0.34	$\frac{8}{25}$	$\frac{1}{14}$	0.22	$\frac{9}{12}$	$\frac{8}{14}$	0.76	$\frac{7}{14}$	$\frac{7}{14}$	1.00	42	0.1
Carnosine nitrate	—	5 mg.	0	$\frac{13}{17}$	0	0	$\frac{15}{4}$	$\frac{15}{14}$	0.64	$\frac{13}{8}$	$\frac{8}{11}$	0.45	$\frac{16}{18}$	$\frac{-2}{12}$	-0.19	77	0.05
Choline chloride	120 mg.	25 mg.	9	$\frac{20}{30}$	$\frac{5}{14}$	0.54	$\frac{14}{12}$	$\frac{8}{14}$	0.49	$\frac{13}{11}$	$\frac{-1}{14}$	-0.06	$\frac{10}{12}$	0	0	75	0.02
Creatine	200 mg.	50 mg.	11	$\frac{14}{11}$	$\frac{-7}{14}$	-0.39	$\frac{11}{10}$	$\frac{11}{14}$	0.71	$\frac{12}{5}$	$\frac{11}{14}$	0.33	$\frac{10}{17}$	$\frac{8}{14}$	0.97	59	0.2
Creatinine	200 mg.	50 mg.	4	$\frac{9}{18}$	$\frac{-3}{14}$	-0.43	$\frac{12}{10}$	$\frac{0}{14}$	0	$\frac{10}{14}$	$\frac{6}{14}$	0.60	$\frac{10}{17}$	$\frac{13}{14}$	1.57	56	0.3
Ethanolamine	40 mg.	10 mg.	9	$\frac{11}{8}$	$\frac{-8}{11}$	-0.53	$\frac{11}{15}$	$\frac{1}{14}$	0.10	$\frac{15}{12}$	$\frac{-4}{14}$	-0.23	$\frac{7}{10}$	$\frac{3}{14}$	0.31	109	0.01
Methylguanidine	12 mg.	3 mg.	16	$\frac{13}{17}$	$\frac{5}{14}$	0.45	$\frac{10}{11}$	$\frac{11}{14}$	0.86	$\frac{12}{14}$	$\frac{4}{14}$	0.33	$\frac{17}{14}$	$\frac{8}{14}$	0.47	47	0.2
Trimethylamine oxide hydrochloride	40 mg.	10 mg.	17	$\frac{13}{7}$	$\frac{9}{14}$	0.35	$\frac{12}{11}$	$\frac{-2}{14}$	-0.13	$\frac{10}{19}$	$\frac{3}{14}$	0.41	$\frac{18}{12}$	$\frac{-2}{14}$	-0.10	87	0.01
Ethylenediamine hydrochloride	100 mg.	10 mg.	4	$\frac{9}{14}$	$\frac{6}{14}$	0.66	$\frac{8}{18}$	$\frac{7}{14}$	1.12	$\frac{7}{25}$	$\frac{2}{14}$	0.51	$\frac{13}{13}$	$\frac{-4}{14}$	-0.28	50	0.1
Putrescine	50 mg.	10 mg.	13	$\frac{13}{9}$	$\frac{7}{11}$	0.43	$\frac{10}{10}$	$\frac{6}{14}$	0.43	$\frac{8}{14}$	$\frac{1}{14}$	0.13	$\frac{10}{18}$	$\frac{8}{14}$	1.03	50	0.1
Cadaverine hydrochloride	100 mg.	25 mg.	4	$\frac{7}{24}$	$\frac{-5}{14}$	-1.22	$\frac{13}{7}$	$\frac{2}{11}$	0.10	$\frac{10}{10}$	$\frac{-3}{10}$	-0.30	$\frac{8}{7}$	$\frac{7}{12}$	0.51	123	0.05
Spermine hydrochloride	60 mg.	5 mg.	8	$\frac{8}{13}$	$\frac{3}{14}$	0.33	$\frac{9}{14}$	$\frac{4}{14}$	0.44	$\frac{12}{14}$	$\frac{-4}{14}$	-0.33	$\frac{8}{11}$	$\frac{-3}{14}$	-0.29	96	0.02
Tetradecamethylene- diamine dihydrochloride	2 mg.	1 mg.	19	$\frac{7}{20}$	$\frac{1}{14}$	0.20	$\frac{9}{21}$	$\frac{-1}{14}$	-0.17	$\frac{13}{9}$	$\frac{2}{14}$	0.10	$\frac{10}{8}$	$\frac{10}{14}$	0.57	86	0.05

EXPERIMENTAL

The methods of testing were the same as those used in the previous paper [Boyland, 1940] and the results are given in Table 1. Muscle extracts were prepared by extracting minced ox heart muscle with 5 volumes of acid for 48 hr. at room temperature. Extract M_1 was prepared with 5*N* HCl and the acid after extraction was neutralized with NaHCO_3 . Extract M_5 was prepared with *N* H_2SO_4 , the acid neutralized with $\text{Ca}(\text{OH})_2$ and the precipitated CaSO_4 removed. Both these extracts produced significant inhibition of growth of spontaneous mouse tumours.

A greater effect was obtained when the extract was given by intraperitoneal injection, but as definite effects were obtained with the oral administration of the extracts, the latter method was used in subsequent experiments.

Fractionation of muscle extract. A sample (50 ml.) of the extract M_5 was dialysed against distilled water in a cellophane bag for 24 hr. at 5°; this treatment did not remove all the activity. The precipitate obtained from another sample (100 ml.) on addition of 20 ml. 25% trichloroacetic acid was suspended in 50 ml. 1% NaHCO_3 ; the trichloroacetic acid filtrate was boiled for 45 min. to destroy the acid and was made up to 50 ml. The trichloroacetic acid precipitate was not significantly active in inhibiting tumour growth, but the filtrate showed more activity than the original extract. Two preparations of the trichloroacetic acid filtrate were evaporated to dryness on the water bath; the dry residue of one was extracted with absolute alcohol and the other with pyridine. In each case the solvent was removed and the residue dissolved in water equal in volume to that of the trichloroacetic acid used. In both cases the extracts had some action, but not as much as the original trichloroacetic acid filtrate.

Another portion of the neutralized filtrate was extracted with butyl alcohol in a continuous extractor for 48 hr. The extract, which contained purines, was inactive against grafted sarcomata.

Phosphotungstic acid precipitation. Phosphotungstic acid (20% in 5% H_2SO_4) was added to 500 ml. of trichloroacetic acid filtrate of extract M_5 containing 5% H_2SO_4 until no more precipitate was formed. After 24 hr. at 5° the precipitate was removed by centrifuging and washed with a small amount of 5% H_2SO_4 . The centrifugate and washings were combined and treated with a slight excess of $\text{Ba}(\text{OH})_2$. The excess $\text{Ba}(\text{OH})_2$ was removed with CO_2 and the neutral filtrate concentrated on the water bath to 250 ml. This phosphotungstic acid-soluble material had no tumour growth-inhibiting action. The precipitate was suspended in 50% aqueous acetone and shaken with excess $\text{Ba}(\text{OH})_2$ solution (saturated at 50°). The barium sulphate and phosphotungstate were filtered off and washed twice with water at 50°. The excess of $\text{Ba}(\text{OH})_2$ was removed by CO_2 from combined filtrate and washings. The solution of bases was then concentrated to 250 ml. This material precipitated by phosphotungstic acid reduced the growth rate of tumours. This relatively clear-cut separation of the activity present in a trichloroacetic acid extract with phosphotungstic acid indicates that bases either present in muscle or liberated during the prolonged extraction with acid are responsible for the action of the extract.

Aliphatic bases. The extract M_1 was shown to contain choline and probably contained ethanolamine which would arise from the phosphatides during the prolonged extraction. Several bases which might be expected to occur in muscle extract, and related compounds, were tested; of these arcaine sulphate (tetramethylenediguanidine sulphate), carnosine nitrate, choline chloride, ethanolamine, trimethylamine oxide hydrochloride, cadaverine hydrochloride, spermine

hydrochloride and tetradecamethylenediamine dihydrochloride, all had marked inhibitory actions on spontaneous tumours. No experiments on the effects of summation of action with these compounds were carried out, but it is possible that the effects of different compounds administered in a mixture would be summed. It therefore seems probable that the tumour-inhibiting action of material obtained by prolonged acid extraction of heart muscle is due to several bases present rather than to one particular compound. The most effective substances were ethanolamine and cadaverine. Heart muscle contains lecithin and cephalin and the prolonged acid extraction would liberate choline and ethanolamine [cf. Kay, 1931].

Cadaverine is a simple diamine like tetradecamethylenediamine which had previously been tested [Boyland, 1938]. These compounds are similar to the aromatic diamines which have been examined and it is possible that their actions are related. The relatively high activity of ethanolamine and choline suggests that other hydroxy-amines should be examined. *p*-Aminophenol has been tested and is not very effective, but some nitrosophenols such as α -nitroso- β -naphthol are quite effective [Boyland & Boyland, 1939; and unpublished observations]. Other ethanolamine derivatives, diethanolamine and triethanolamine, were similar to ethanolamine in their action.

Hypoxanthine (5 mg. per day), agmatine (25 mg. per day) and inositol (10 mg. per day) were all inactive against the grafted sarcomata M.C.D.B.I. Other substances which were only tested against the grafted sarcoma included an extract of Pecten muscle, which produced 15 % inhibition in growth, glutamine (10 mg. per day, 10 % inhibition), nicotinamide (30 mg. per day, 11 % inhibition) and trigonelline (25 mg. per day, 4 % inhibition).

The aliphatic bases which have been found to inhibit tumour growth are all diamines or hydroxy-amines. The active compounds can therefore be expressed as $P-X-P^1$, where P and P^1 are polar groups. Similar activity has been found [Boyland, 1940] with aliphatic dicarboxylic acids such as malonic acid, and these compounds can be represented by the same general formula where P and P^1 are carboxyl groups. In the case of β -alanine, where P is NH_2 and P^1 is a COOH group, there is no activity. A large number of aromatic bases have been tested, and in this series also maximum activity was obtained with compounds containing two terminal amino groups, which can be represented by the same general formula.

SUMMARY

By prolonged cold acid extraction of heart muscle a preparation is obtained which inhibits the growth of grafted sarcomata and spontaneous carcinomata in mice.

The active material is heat-stable, is not precipitated by trichloroacetic acid, but is precipitated by phosphotungstic acid. The activity appears to be due to many of the bases present rather than to any one compound.

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