promote growth of the adenohypophysis and atrophy of the thymus. It is clear that, even when a definite oestrogenic effect was elicited, the weight of the thymus was not affected. The atrophy of the thymus after administration of oestrogen is caused through more than one mechanism[4]; experiments to determine the action of oestrone-17β-thiol on adrenal function and release of ACTH are necessary, even considering that the adrenal gland weight was not affected. The fact that even without showing any oestrogenic effect the drug was able, in small doses, to block the appearance of castration cells in the adenohypophysis, can be of some importance. We have not determined whether this histological picture corresponds to low concentrations of pituitary gonadotrophin.

This work was supported in part by a grant from the Rockefeller Foundation.

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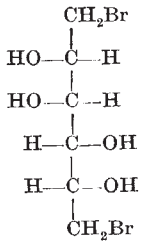
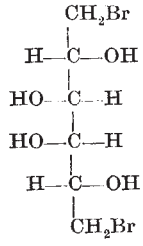
1,6-Dibromo-1,6-dideoxy-dulcitol: a New Antitumoral Agent

Among the α,ω-substituted sugar-alcohols the dibromo derivatives represent a special group because of their biological properties[1]. The first dibromo-hexitol introduced into clinical use, the dibromo-mannitol ('Myelobromol^{R}'), has myelotoxic effects[2]. Its diastereoisomer dibromo-dulcitol (DBD) revealed, in pharmacological experiments, remarkable qualities.

DBD was prepared by treating dulcitol with aqueous hydrobromic acid saturated with gaseous hydrogen bromide at a temperature less than 0° C. On dilution of the reaction mixture with water, crude DBD precipitated to give a yield of about 70 per cent. Recrystallization from methanol gave colourless plates, of melting point 186°–190° C (decomp., corr.). Analysis showed 23·8 per cent carbon, 4·1 per cent hydrogen, and 51·85 per cent bromine. The formula $C_6H_{12}O_4Br_2$ requires 23·4 per cent carbon, 3·9 per cent hydrogen, and 51·9 per cent bromine.



1,6-Dibromo-1,6-dideoxy-D-mannitol, 'Myelobromol^{R}' (DBM)



1,6-Dibromo-1,6-dideoxy-dulcitol (DBD)

In acute toxicity experiments the LD_{50} is 685 mg/kg for mice, intraperitoneal, 470 mg/kg for rats, intraperitoneal, whereas it is 1,400 mg/kg for rats and 200 mg/kg for rabbits when given perorally. (Because of its poor solubility in water, 1 : 2,500, the DBD was administered in solution prepared with 'Tween 80'.) A single dose of 10–30 mg/kg markedly inhibits growth of more sensitive tumours (Yoshida, Walker, Shay tumours). A pronounced inhibition of more resistant tumours was brought about by six to ten repeated doses of 100–200 mg/kg. The peroral equivalent of these doses was about twice as much DBD. Both the individual doses and the total quantity administrable could be raised considerably by giving the dose every second or third day; the side-effects became much milder.

The administration of 200 mg/kg to normal rats resulted in a transient increase of about 30 per cent in the leucocyte count of the peripheral blood which lasted for 12 h, together with the appearance of some immature forms. By 24 h a distinct leucopenia had set in, and reached its maximum, 50 per cent, within 72–96 h. The leucopenia ceased within a fortnight. There was no change in the red blood cells, even after larger doses, while the platelets decreased in number slightly. Optimal treatment did not cause a loss of weight; there was some gain in weight. Post-mortem examination revealed that the spleen was of normal size, the duodenum free of any microscopic lesion, and the inner organs appeared normal. The larger dose of 400 mg resulted in a severe depression of bone marrow: at the time of greatest effect the myeloid elements disappeared almost completely, and the nuclei of megakaryocytes showed lytic and pycnotic changes. Regeneration of myeloid elements commenced 120–144 h after damage. A fairly extensive destruction of white blood cells began in the spleen and lymph nodes after 3–6 h. There was also a large number of disintegrating cells, with

Table 1. GROWTH-INHIBITING EFFECT OF DBD ON VARIOUS TUMOURS

Tumour	Dose, treatment schedule (mg/kg)*	Duration of observation (days)	Tumour weight(g) Controls	Percentage inhibition	No. of animals dead/total†
		Mice			
NK/Ly ascites lymphoma	6 × 150/24 h i.p.	8	4·8 / 11·0	56	0/10 / 0/10
Ehrlich ascites carcinoma	6 × 150/24 h i.p.	8	2·2 / 3·2	32	0/10 / 0/10
Crocker sarcoma	8 × 200/24 h i.p.	10	0·29 / 0·64	55	1/10 / 0/10
		Rats			
Walker carcinosarcoma	7 × 100/24 h** i.p.	8	1·19 / 31·25	96	0/11 / 0/11
	7 × 200/24 h** oral	8	0·99 / 31·25	96	1/11 / 0/11
Yoshida sarcoma	6 × 50/24 h i.p.	9	1·03 / 15·75	93	0/6
	6 × 25/24 h i.p.	9	0·85 / 15·75	94	0/6
	6 × 12·5/24 h i.p.	9	1·69 / 15·75	89	0/6
	6 × 6·25/24 h i.p.	9	11·43 / 15·75	27	0/6 / 0/6
Yoshida ascites	7 × 200/48 h i.p.	60		100‡	
Guérin carcinoma	7 × 200/48 h i.p.	14	1·54 / 13·47	98	0/10 / 0/10
	7 × 200/48 h oral	21	0·54 / 22·80	98	0/10 / 0/10
	5 × 200/72 h i.p.	16	7·24 / 22·12	67	0/10 / 0/10
Rhabdomyosarcoma	9 × 200/24 h i.p.	12	0·97 / 16·15	93	0/10 / 0/10
Shay acute leukaemia	5 × 150/24 h i.p.	60		75§	

* Treatment commenced 24 h after inoculation.
** Treatment commenced 72 h after inoculation.
† Weight loss not more than 10 per cent.
‡ Free of tumour after 60 days. Cured 10/10.
§ 75 per cent, with 25 per cent average life span of 28 days (controls 11 days).

pycnotic nuclei, in the duodenum. This effect, however, regressed within 48–72 h and after a further 1 or 2 days the tissue damage seemed to be repaired. In the parenchymatous organs histological investigation showed no damage. Data concerning the effects of DBD on tumour inhibition are given in Table 1.

With the Guérin tumour large doses of DBD resulted in visible cytological alterations by 1–3 h. The chromatin substance showed, under the electron microscope, a rough bundle-like arrangement[3]. Pycnotic derangement soon resulted in necrosis of the tumour (within 72–96 h). By this time there were multinuclear giant cells scattered around the vessels and in the connective tissue at the periphery of the tumour. Mitoses were scarce and occasionally deformed, even in the early stage. Yoshida ascites cells were enlarged in diameter to multiple size. *NK/Ly* ascitic lymphoma cells also showed less enlargement[4]. The same changes also occurred in tissue cultures of HeLa cells, especially with chronic treatment. The nuclei and nucleoli appeared puffy[5].

The biological and tumour inhibiting properties of DBD differ from those of any other drug which we have so far tested: its cytological effects are obvious very soon and last for a long time, especially in tumours, and it therefore seems advisable to have long intervals between doses. DBD has a wide therapeutic range. The effect on the lymphoid organs and the duodenum fades much sooner than that on the myeloid elements and tumours. Relatively large doses are tolerated when given perorally and the effect seems even greater than that following intraperitoneal administration.

DBD in therapeutic doses provokes leucopenia and minor organic lesions of short duration. Of the cytological findings, cellular, nuclear and nucleolar enlargement is noteworthy, together with the early and long lived reduction of mitosis and a few deformed mitoses.

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Effect of Histones, Other Basic Proteins and Some Antibiotics on the Transplantability of Mouse Mammary Tumours

Unfractionated preparations of histones from normal mammalian tissues have been reported to inhibit the growth of transplantable tumours in rats[1]. Incubation of mucous cancer cells of liver with rat liver histone resulted in the inhibition of the growth of tumour by as much as 95·5 per cent. No cell or species specificity was found and there was a similar inhibition with rat thymus or calf thymus histones. Histones have been reported to play an important part in the regulation of RNA synthesis through the inhibition of the DNA-dependent RNA polymerase[2,3]. The ability to suppress DNA-primed RNA synthesis varies with different fractions of histones. Interest, therefore, now centres on histone fractions rather than on whole histone as regulators of gene action. We have examined the effects of whole histone, its two chief fractions and other chemicals on the growth of transplantable mouse mammary tumours.

Histone was prepared from calf thymus according to the method of Laurence *et al.*[4]. The fractions rich in lysine and arginine were isolated according to the methods of Johns *et al.*[5] and Johns and Butler[6]. The lysine-rich fraction was extracted in 5 per cent perchloric acid.

Mouse mammary tumour was induced in Swiss albino mice and maintained in our laboratory. Solid tumours were passed through a tissue press. The material was then suspended in Hanks solution and forced several times through a 20 gauge needle in a 20 ml. syringe into a sterile serum bottle. It was thus possible to obtain a satisfactory homogeneous cell suspension. The chemicals were dissolved in Hanks solution and the *p*H was adjusted to 7. They were mixed with the cell suspension, in an appropriate concentration and incubated for 2 h at 37° C. A sample (0·1 ml.) of this suspension containing approximately 2×10^5 cells was injected into the left hind leg of a mouse. Tumours of 10–12 days growth were collected and weighed. A total of more than 400 Swiss albino mice of 5–7 weeks of age were used in these experiments.

The results of the effects of the basic proteins and other chemicals on the growth of the transplantable tumours are shown in Table 1. It was noticed that the lysine-rich histone, when used in a concentration greater than 5 mg/ml., inhibited tumour growth significantly. For example, at a concentration of 10 mg/ml. it showed 69 per cent inhibition. Whole histone or the arginine-rich fraction produced little or no inhibition. Unfractionated histone at a concentration of 7 mg/ml. showed 34–35 per cent stimulation in tumour growth. Spermine in a concentration of 10 mg/ml. resulted in 35–36 per cent inhibition while protamine and spermidine were ineffective. Polylysine when used in concentrations greater than 7 mg/ml. completely blocked the growth of transplanting tumours. Actinomycin had some effect, as 25 *u*g/ml. resulted in 59 per cent inhibition, but puromycin showed only 20–21 per cent inhibition at a concentration of 100 μg/ml.

In contrast with earlier observations[1] we found that unfractionated histone had no inhibitory effect on the growth of tumour. On the other hand, some stimulatory effect was observed with total histone (Table 1). Only the lysine-rich fraction reduced growth significantly. It

Table 1. EFFECT OF HISTONES, OTHER BASIC PROTEINS AND ANTIBIOTICS ON GROWTH OF TRANSPLANTING TUMOURS

S. No.	Compound	Concentration (mg/ml.)	Total weight, average of tumours in five mice (g)	Percentage inhibition or stimulation
(1)	Control Hanks solution	—	32·58	0
(2)	Unfractionated histone	3	28·86	−11·39
		5	36·88	+13·22
		7	43·95	+34·92
(3)	Arginine-rich histone	3	26·97	−17·19
		5	29·63	− 8·89
		10	28·38	−12·89
(4)	Lysine-rich histone	3	26·66	−18·14
		5	24·79	−23·90
		10	10·09	−69·00
(5)	Protamine sulphate	3	27·85	−14·49
		5	26·49	−18·67
		10	32·60	+00·07
		20	35·54	+09·09
(6)	Polylysine	3	13·94	−57·19
		4	7·18	−77·96
		5	3·32	−89·78
		7	0·75	−97·68
		10	0·00	100·00
(7)	Spermine	3	25·48	−21·77
		5	21·54	−33·88
		10	20·87	−35·93
(8)	Spermidine	3	36·73	+12·76
		5	32·60	00·00
		10	30·91	−5·12
		(μg/ml.)		
(9)	Actinomycin-*D*	10	24·13	−25·93
		25	13·35	−59·00
(10)	Puromycin	25	31·50	− 3·31
		100	25·84	−20·67