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Enhancing Effect of Polyamines on Yield of Film Sarcoma*

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Abstract—*The salts of the natural polyamines, protamine sulphate, clupeine sulphate, spermidine phosphate, putrescine dihydrochloride and spermine diphosphate, and of the synthetic poly-L-lysine hydrobromide (mol. wt 85 k) and poly-L-arginine hydrochloride (mol. wt 50 k) were tested for their effect on film sarcoma by application in gel to nitrocellulose filters that were implanted in groups of 20–45 BALB/c mice. Five experiments were carried out using filters of decreasing pore size and increasing carcinogenicity. Control groups had filters treated with saline. The tumours that arose were fibrosarcomas, some with vascular elements. Tumour yield was expressed as the number of mouse-weeks of exposure within each group divided by the number of tumours arising. The yield was up to three times higher in the test groups, spermidine and poly-L-arginine being weakest and not reaching significance. The substances may act by producing a basic pH in the environment, by an effect on nucleic acid, by facilitating a virus or by stimulating production of a natural mitogen.*

INTRODUCTION

FILM SARCOMA arises on smooth, unadsorbable surfaces embedded under the skin of rats [1]. The system has the advantage of being devoid of carcinogenic chemicals. It is conveniently used in the mouse, and it responds to substances applied to the film [6].

When tested in this model, crude histone augmented tumour growth, as did some of its fractions [3]. Histones are basic proteins concerned in the activity of nucleic acids. Polyamines are smaller units thought to have a similar function.

It was of interest to see if their effects on film sarcoma were similar and how they compared with synthetic polyamines. Five experiments were run comparing respectively protamine sulphate, poly-L-arginine (mol. wt 50 k), clupeine and spermidine, putrescine, and spermine, protamine and poly-L-lysine (mol. wt 85 k), with controls.

MATERIALS AND METHODS

Protamine sulphate (histone-free), clupeine sulphate (histone-free), spermidine phosphate, putrescine dihydrochloride, spermine diphosphate and poly-L-lysine hydrobromide (85 k) were obtained from Sigma London Chemical Co., and poly-L-arginine hydrochloride (50 k) from Pilot Chemicals, Watertown, MA. The poly-L-amines were dissolved at 0.5 g/dl in physiological saline, the others at 1 g/dl and the latter protamine sulphate at 2 g/dl, and were sterilised by passage through a 0.22-μm sterilising filter (Millex or Swinnex).

Nitrocellulose filters (Millipore) of 25 mm diameter were autoclaved. The pore size ranged from 0.22 to 0.05 μm in separate experiments. Smaller pores are known to be more carcinogenic [4, 5]. Test substance was applied by beading onto each filter 0.1–0.12 ml until saturated and part-drying at 37°C for 10 min. Uptake was approximately 0.05 ml per filter. Gelatine 16 g/dl in saline was then similarly applied. In the fifth experiment the filters were instead dipped in the test substance, part-dried at room temperature and similarly treated with gelatine.

Filters for control groups were treated as were those of the test groups, with the test solution being replaced by saline.

BALB/c strain mice about 5 months of age were used. For testing poly-L-arginine, clupeine and spermidine they were of mixed sex, for the rest female only. Similar sex and numbers were used in test-substance and control groups. Under ether anaesthesia, an incision was made across the nape

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Table 1. *Survival in weeks of test and control animals with and without tumour*

	Week of tumour onset (death)	Incidental deaths
Group 1		
Protamine	38, 53, 56, 69, 76, 80	24, 35, 38, 53, 66, 73, 89, 89, 92, 96, 102, 102, 106, 106
Controls	59, 75	20, 33, 35, 42, 42, 51, 71, 75, 88, 105, 107, 109, 109, 112, 114, 114, 124, 128
Group 2		
Poly-L-arginine	29, 50, 72	29, 31, 39, 54, 57, 58, 62, 76, 79, 87, 90, 93, 100, 100, 109, 111, 123
Controls	63, 87	30, 41, 42, 48, 49, 57, 62, 73, 74, 79, 87, 88, 90, 90, 90, 105, 114, 128
Group 3		
Clupeine	40, 41, 53, 53, 61, 65, 67	61, 62, 62, 68, 77, 82, 83, 85, 86, 86, 87, 98, 101
Spermidine	48, 59, 59	68, 69, 69, 70, 71, 71, 71, 72, 74, 74, 74, 74, 74, 91, 101, 105, 117
Controls	42, 72	50, 50, 59, 66, 67, 69, 72, 75, 77, 77, 78, 81, 82, 84, 86, 89, 92, 104
Group 4		
Putrescine	26 , 38, 39, 41, 48, 53, 53, 59, 90	21, 31, 35, 36, 39, 41, 41, 42, 42, 45, 48, 55, 60, 61, 61, 61, 63, 63, 64, 64, 66, 68, 70, 70, 72, 78
Controls	50, 67, 86	26, 28, 34, 35, 42, 43, 45, 45, 47, 47, 48, 50, 50, 50, 54, 56, 57, 58, 60, 63, 63, 70, 70, 70, 75, 76, 76 82, 82, 87, 89, 110
Group 5		
Poly-L-lysine	30, 32, 38, 41, 41, 45, 47, 47, 47, 49, 49, 49, 49, 49, 51, 51, 54, 55, 57, 63, 63, 75, 79, 81	18, 21, 26, 30, 30, 36, 45, 49, 52, 57, 57, 57, 60, 60, 75
Spermine	21, 25, 29, 29, 35, 39, 39, 39, 41, 41, 45, 47, 49, 49, 49, 51, 54, 54, 56, 56, 58, 64, 73, 73, 88	19, 24, 27, 32, 36, 39, 39, 42, 50, 50, 51, 61, 63, 64, 65, 66, 78, 89
Protamine	31, 42, 42, 42, 45, 49, 49, 52, 55, 56, 58, 68, 68, 68, 68, 69, 73, 76, 88	19, 22, 25, 27, 31, 34, 37, 42, 43, 45, 47, 50, 56, 57, 57, 58, 66, 66, 68, 76, 100
Controls	25, 38, 41, 43, 44, 44, 46, 56, 56, 56, 59, 60, 68	24, 24, 35, 35, 37, 37, 40, 47, 48, 51, 51, 52, 54, 54, 55, 60, 60, 60, 62, 66, 66, 66, 68, 69, 72, 76, 76, 77

of the neck, a pouch was formed under the skin of the back by opening a scissors, a single filter was inserted flat, the wound was closed with clips and the animal was ear-marked. The mice were housed five to a box and inspected weekly for tumours. These arise directly on the filter and are readily seen and felt, since the filter itself is visible through the skin dampened with alcohol.

The tumour was assigned to the week of discovery. The animal was killed with chloroform. The tumour was removed, sliced to show its relation to the filter and retained in fixative. All animals were autopsied. Intercurrent deaths were assigned to the week of occurrence. Observations were continued for 100 weeks. By that time 96% of the 378 mice were dead. The weeks lived by all the animals in the group were summed. When divided by the number of tumours that occurred in the group, the figure gave a measure of the exposure needed by each substance to produce a tumour as compared to its control group, despite intercurrent deaths. The tumours are fibrosarcomas, some with vascular elements, as have been described [5].

The probability of the result occurring by chance was tested with the method b of Peto [6], which uses a chi square design to compare the cumulative incidence of tumour, week by week, in the surviving mice with that which would be expected from the incidence finally observed.

RESULTS

The survival data of the animals are given in Table 1. The 0.22-μm filters are a mild carcinogenic stimulus requiring 735 weeks' mean exposure to produce tumour in 10% of controls (Table 2). Protamine and clupeine trebled the carcinogenicity by these measures. Poly-L-arginine and spermidine increased it by one half, although the number of tumours are deficient for statistical validation. The putrescine group, using the more carcinogenic 0.1-μm filters, also showed a three-fold augmentation of tumour incidence.

The 0.05-μm filters required only 173 mean weeks of exposure to generate tumour in 25% of controls. Poly-L-lysine and spermine doubled the carcinogenicity, while protamine at twice its previous concentration made less of an impact on this model with about 50% increase.

DISCUSSION

The results with polyamines correspond to those obtained with the histones previously [3]. The cells in contact with the implanted films become morphologically neoplastic in many animals after about 12 weeks and in most animals by 28 weeks [7]. In some cases they generate tumour, usually from 26 weeks on. It is not clear in which of these periods the substances exert their carcinotrophic effect, nor by what means they do so.

It is possible that they act by an effect on nucleic acids. We have no evidence for this possibility. However, both histones and polyamines are basic substances, and film sarcoma incidence is doubled in an alkaline environment around the film [8]. This is an unlikely explanation of the effect of the

Table 2. Relative incidence of film sarcoma on nitrocellulose filters treated with polyamines

Experiment	Substance	Concentration (g/dl)	Film pore size	No.	Sex	Total implant weeks (IW)	No. of tumours (T)	Implant weeks per tumour (IW/T)	$P<$
1	Protamine	1	0.22 mm	20	F	1482	7	211.7	0.1
	Controls	-		20		1491	2	745.5	
2	P-L-arginine	0.5	0.22	20	M,F	1406	3	468.7	N.S.
	Controls	-		20		1450	2	725.0	
3	Clupeine	1	0.22	20	M,F	1417	7	202.4	0.05
	Spermidine			20		1488	3	496.0	N.S.
	Controls	-		20		1468	2	734.0	
4	Putrescine	1	0.1	38	F	2092	9	232.4	0.05
	Controls	-		35		2081	3	693.7	
5	P-L-lysine	0.5	0.05*	39	F	1916	24	79.8	0.01
	Spermine	1		43		2099	25	84.0	0.02
	Protamine	2		40		2122	19	111.7	N.S.
	Controls	-		43		2252	13	173.2	

*Smaller pore sizes are more carcinogenic.

NS = not significant (method of Peto); IW = total weeks of life accumulated by the mice from implantation to death in each group; IW/T = total implant-weeks divided by the number of tumours (T) arising in the group.

substances since a gross change of pH is necessary, and further, separate fractions of histones have differing promotional effects. A third possibility is facilitation of an oncogenic virus. Evidence is not convincing, although viral particles have been found in a proportion of transmitted film sarcomas. A further possibility is that the film stimulates production of a natural mitogen, as has been postulated for mesenchymal tumours [9].

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