41. STUDIES IN TISSUE METABOLISM

12. THE ACTION OF COLCHICINE ON TRANSPLANTED, INDUCED AND SPONTANEOUS MOUSE TUMOURS

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In a previous paper [Boyland & Boyland, 1937] it was shown that spontaneous mouse tumours were very much less susceptible than grafted tumours (S 37, Mal. and Crocker 180) to the action of colchicine. Intraperitoneal injection of 0.05 mg. of colchicine into mice bearing grafted tumours caused (1) haemorrhage in the tumours, (2) reduction of the ascorbic acid content and (3) lowered tissue respiration and glycolysis. The combination of these three phenomena is called the "colchicine effect". The effects of injection of colchicine on primary induced tumours and on a grafted sarcoma, M.C.D.B. 1, which has always been grown in the same pure line of Dilute Brown mice [see Boyland & Boyland, 1939], as well as further results with spontaneous mammary tumours are described in this paper.

The grafted sarcoma, M.C.D.B. 1, has been extremely valuable in experiments on chemotherapy and in studying the effects of treatment on tumour metabolism. An analogous mammary carcinoma (C.C₃H 1) has been established in the C₃H strain of mice and its reaction to colchicine has been studied.

EXPERIMENTAL

Tumours used. (a) The M.C.D.B. 1 sarcoma which, having only grown in the same line, might be expected to bear more resemblance to a primary tumour in its behaviour than the usual strains of grafted tumours which have not been grown entirely in one line of mice.

(b) C.C₃H.1 refers to transplants of a carcinoma that arose spontaneously in a mouse of the C₃H strain; the tumour has been grown only in C₃H mice. The first and second grafted generations were used.

(c) Induced tumours were obtained by subcutaneous injections of 2 doses each of 1 mg. of methylcholanthrene in lard into mixed stock mice. They were in all cases sarcomata.

(d) Spontaneous mammary tumours.

Mice were injected intraperitoneally with solutions of colchicine 18–20 hr. before examination of the tumours. Respiration and anaerobic glycolysis were measured by the Warburg technique at 37°. The ascorbic acid content was determined by grinding the healthy tumour tissue with 3 volumes of 5% trichloroacetic acid and titrating the extract with 2:6-dichlorophenol-indophenol. The degree of haemorrhage was estimated macroscopically as in the previous paper.

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Table 1. Effect of injection of colchicine on the properties of tumours

A. Dilute Brown mice bearing M.C.D.B. 1

B. C₃H mice bearing C.C₃H.1

mg. colchicine	Degree of haemorrhage in tumours	Metabolism of tumours		mg. colchicine (untreated)	Degree of haemorrhage in tumours	Metabolism of tumours		Ascorbic acid content of tumours mg./g.
		-Q _{O₂}	Q _L ^N			-Q _{O₂}	Q _L ^N	
Control (untreated)	-	9.2	27.7	Control (untreated)	-	10.4	27.6	0.41
"	-	9.4	28.8	"	-	14.0	23.2	0.30
"	-	11.8	25.2	"	-	10.2	19.0	0.62
"	-	9.1	34.2	"	-	11.0	25.5	0.30
"	-	10.0	35.7	"	-	14.7	26.0	0.38
"	-	11.4	31.0	"	-	Mean	24.3	0.40
Mean		10.1	30.4			12.1		
0.025	+	6.8	13.9	0.05	-	11.2	21.2	0.08
0.025	+	10.1	30.0	0.05	-	11.1	23.8	0.26
0.025	++	3.4	7.8	0.05	-	7.2	5.0	0.29
0.025	++	2.0	20.1	0.05	-	12.6	15.0	.
0.025	-	11.3	22.9	0.05	-	14.1	29.4	.
0.025	-	6.9	23.5	0.05	-	12.8	27.1	0.43
0.025	-	6.9	21.6	0.1	+	7.0	20.0	0.06
0.025	-	11.6	22.7	0.1	-	4.0	28.0	0.08
0.025	-	8.2	23.6	0.1	+	0.6	3.3	0.14
0.05	++	1.9	8.0	0.1	-	1.2	13.3	0.16
0.05	-	7.5	22.2	0.1	-	4.2	11.9	.
0.05	++	2.0	2.6	0.1	-	10.5	8.4	.
0.05	++	6.5	11.6	0.1	-	0	6.4	.
0.05	++	0.6	2.5	0.2	++	0.4	2.8	0.18
0.05	++	0.6	1.6	0.2	++	0	4.6	0.16
0.05	++	1.5	8.2	0.2	++	0	2.0	0.20
0.05	++			0.2	+++	0.1	4.5	0.07
0.05	++			0.2	+	1.2	4.4	0.07

C. Mice bearing tumours induced with methylcholanthrene

D. Mice bearing spontaneous tumours*

Control (untreated)	-	6.6	23.2	Control (untreated)	.	12.8	25.6	0.62
"	-	9.3	14.0	"	.	8.7	11.4	0.35
"	-	10.7	16.5	"	.	5.3	17.0	0.16
"	-	8.5	17.5	"	.	6.8	10.2	0.22
"	-	10.2	18.5	"	.	.	.	.
Mean		9.1	17.9			10.9	29.0	0.17
0.1	+	4.7	13.0	0.1	.	10.1	17.5	0.27
0.1	-	13.9	21.5	0.1	.	6.5	8.9	0.11
0.1	-	7.0	9.0	0.1	.	11.5	20.0	0.23
0.1	++	6.4	13.0			4.2	7.6	0.01
0.2	++	1.1	6.0	0.2	.	4.8	5.4	0.19
0.2	++	5.6	9.5	0.2	.	6.8	9.5	0.25
0.2	++	0.5	0.6	0.2	.	7.6	19.2	0.20
0.2	++	5.7	12.7	0.2	.	4.4	7.8	0.19
0.2	-			0.2	.	10.6	11.4	0.29

* Some of these results are taken from Boyland & Boyland [1937] for comparison.

Results

(a) Table 1A shows that 0.05 mg. in most cases produces the typical colchicine effect in M.C.D.B. 1 tumours, but this dose was often fatal; 0.025 mg. sometimes has a slight effect on the tumours but is generally ineffective; in about 20 % of cases this dose was fatal; 0.1 mg. was always fatal to these mice. It was shown [Boyland & Boyland, 1937] that all mice bearing Crocker sarcoma 180 survived a dose of 0.025 mg. and 8 out of 20 survived 0.1 mg. Thus the Dilute Brown mice with tumours are more sensitive to colchicine than stock mice with the Crocker tumour. This may be due to the relatively small size of the former mice. This transplanted tumour therefore resembles other grafted tumours previously investigated, rather than induced or spontaneous tumours, in its reaction to colchicine.

(b) The untreated carcinoma C.C.H. 1 is sometimes haemorrhagic, so that the estimation of the haemorrhage due to colchicine is not so definite as with the other sarcomata which have been used; however the signs ++ and +++ are used where there was extensive haemorrhage caused by the treatment. Table 1B shows that 0.05 mg. colchicine sometimes had a slight effect, 0.1 mg. was generally effective and 0.2 mg. always so. The mortality with 0.2 mg. was low, 1 out of 6 mice dying within 24 hr. This tumour (in C₃H mice) is therefore more resistant to the action of colchicine than the Crocker sarcoma in mixed stock mice or the M.C.D.B. 1 sarcoma in Dilute Brown mice. It more nearly resembles induced sarcomas in mixed stock mice.

(c) Most of the induced tumours examined (Table 1c) were unaffected by 0.1 mg. but 0.2 mg. frequently produced the "colchicine effect".

(d) It is harder to determine exactly the reaction of spontaneous tumours, as there is great variation between untreated tumours, but there is no doubt

Table 2. *The effect of 2-methylnaphthoquinone and ascorbic acid with colchicine on Crocker sarcoma 180*

	Degree of haemorrhage in tumours	Metabolism of tumour		Ascorbic acid content of tumours mg./g.
		-Q _{O₂}	Q _L ^N	
A. Controls (untreated)	-	7.8	22.5	0.22
	-	8.2	20.6	0.26
	-	.	.	0.22
	-	.	.	0.14
B. 0.1 mg. colchicine	+++	0.3	0.7	0.11
	++	1.0	5.3	0.22
	++	.	.	0.13
	+	.	.	0.08
	+	.	.	0.20
C. 50 mg. ascorbic acid	-	.	.	0.24
	-	.	.	0.21
D. 50 mg. ascorbic acid followed by 0.1 mg. colchicine	++	.	.	0.05
	++	.	.	0.17
	+	.	.	0.10
E. 0.1 mg. colchicine preceded by 3 doses of 0.01 mg. 2-methyl- naphthoquinone + 5 mg. ascorbic acid	-	6.2	20.5	0.18
	+	0.2	1.5	0.10
	++	0.4	0.5	0.06
	+++	.	.	0.09
	++	.	.	0.08
0.1 mg. colchicine preceded by 2 doses of 0.01 mg. 2-methyl- naphthoquinone + 40 mg. ascorbic acid	+	.	.	0.07
	++	0.1	1.7	0.10
	++	.	.	0.09

that they are much less affected than grafted tumours, and somewhat less than induced tumours, which occupy an intermediate position.

Ascorbic acid. Andervont & Shimkin [1939] showed that ascorbic acid neutralized the effect of bacterial filtrates on tumours, but we have found no such action with ascorbic acid and colchicine. Mice bearing tumours were injected with 20–50 mg. of ascorbic acid (approximately neutralized with NaHCO_3) a few hours before injecting colchicine. The typical colchicine effect was always observed. Some results are given in Table 2. It was shown [Boyland & Boyland, 1937] that colchicine and colchicine reduced the respiration of normal tumour slices when added *in vitro*. This effect was not reversed if ascorbic acid (0.5 mg./ml.) was added to the incubation medium.

Vitamin K. As the mechanism of the haemorrhage production by colchicine is unknown, the effects of ascorbic acid and vitamin K were tested. Mice bearing the Crocker sarcoma were dosed daily with a suspension of 5–40 mg. ascorbic acid suspended in a cod-liver oil solution containing 0.01 mg. 2-methylnaphthoquinone which has been shown [Almquist & Klose, 1939] to have a high vitamin K activity. After 2 or 3 doses the mice were injected with 0.1 mg. colchicine and the tumours examined after 18 hr. The results are given in Table 2. It will be seen that the methylnaphthoquinone and ascorbic acid had no effect in reducing the "colchicine effect". In one mouse treated with the vitamins colchicine did not produce the typical results, but occasionally mice treated with colchicine alone escape the effects, so this cannot be considered significant.

DISCUSSION

The effect of injecting large doses of colchicine into mice with grafted tumours is similar to the effect of bacterial filtrates. The bacterial preparations which produce haemorrhage in grafted tumours also elicit the Schwarzman phenomenon in the rabbit skin. It seemed possible that the haemorrhage produced in grafted tumours was similar to that of the Schwarzman reaction and that the tumour was already sensitized because it was a foreign or heterologous tissue. Spontaneous tumours, which are not foreign tissues, do not react to bacterial filtrates or to colchicine. Andervont [1936] found that *Bact. coli* filtrates produced haemorrhage in induced tumours and we show that colchicine has similar effects. The fact that induced tumours which must be homologous tissue do react suggests that the mechanism of the haemorrhage is different from the Schwarzman phenomenon. The strong reactions given by the M.C.D.B. 1 tumour which must also be homologous support this suggestion. Andervont & Shimkin [1939] have shown that ascorbic acid reduces the reaction of tumours to bacterial filtrates, and we find that it has no such effect with colchicine, which would suggest that the effects produced are not identical.

SUMMARY

A spontaneous mammary carcinoma has been grafted into mice of the strain (C_3H) in which the tumour appeared, in order to obtain a grafted carcinoma of the same genetic constitution as the host.

Mice bearing (1) spontaneous mammary tumours, (2) primary tumours induced by injection of methylcholanthrene and (3) two strains of grafted tumours grown in pure lines of mice were treated with colchicine. The reactivity to colchicine increased from (1) to (3). Treatment with 2-methylnaphthoquinone and ascorbic acid does not alter the effect produced by colchicine.

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