LXII. STUDIES IN TISSUE METABOLISM

IX. THE ACTION OF COLCHICINE AND *B. TYPHOSUS* EXTRACT

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Colchicine

Dixon & Malden [1908] showed that the injection of colchicine into rabbits produced a rapid increase in the number of leucocytes of the blood. Further investigations of the action of the drug by Lits [1934], Dustin [1934] and Ludford [1936] have shown that injection of 0·025 mg. colchicine into mice arrests cell division at the metaphase in grafted tumour tissue, the thymus gland, testis and the small intestine. Amoroso [1935] claimed that injection of colchicine inhibited tumour growth in a dog and in mice but no confirmation of this has as yet been published.

Toxicity

All injections in these experiments were intraperitoneal. The survival times of mice injected with colchicine are shown in Table I. It will be noticed that the lethal dose of colchicine does not kill some animals until after 48 hours. Also a given dose is much more toxic to mice bearing tumours than to normal animals: thus 60% of normal animals survived a week with 0·1 mg., whereas

Table I. *Toxicity of colchicine to mice*

Dose mg.	Number surviving 24 hours	48 hours	72 hours	1 week
A. Normal mice. 10 mice given each dose				
0·2	2	1	0	—
0·1	10	9	8	6
0·05	10	9	8	8
0·025	10	10	10	10
B. Mice bearing Crocker 180. 10 mice given each dose				
0·2	0	—	—	—
0·1	6	0	—	—
0·1	2	0	—	—
0·05	8	5	2	1
0·025	10	10	10	10
C. Normal mice injected with colchicine and 2 doses each of 4 mg. ascorbic acid				
0·2	10	4	3	0
0·2	5	0	—	—
0·2	8	0	—	—
0·1	5	0	—	—

D. Effect of tumour size. 10 mice bearing Crocker 180 tumours injected with 0·05 mg. colchicine

Weight of tumour	8·3	5·0	3·7	2·3	1·65	1·4	0·9	0·7	0·6	0·5
Hours of survival	<12	<12	17	18	48	1 week				

Fig. 1. Normal Jensen rat sarcoma. × 120. Ascorbic acid content, 0·32 mg. per g. Metabolism, $Q_{O_2}=11{\cdot}8$; $Q_{N_2}^{M}=23{\cdot}4$.

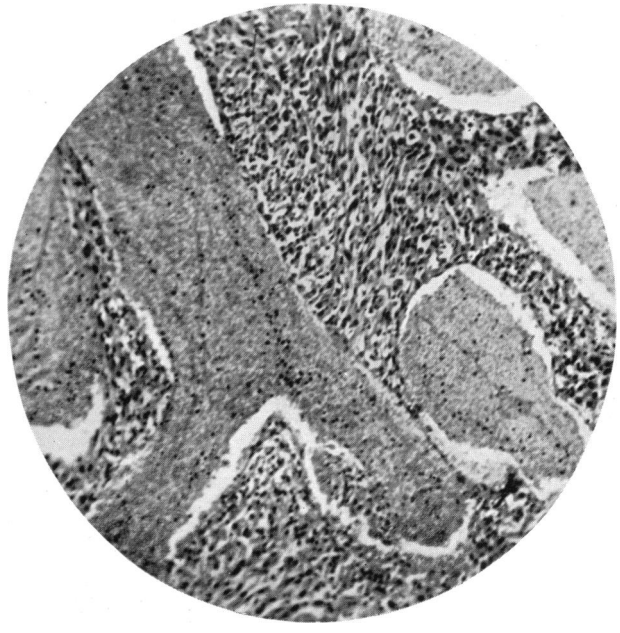

Fig. 2. Jensen sarcoma from a rat 16 hours after intraperitoneal injection of 0·2 mg. colchicine. × 120. Ascorbic acid content, 0·13 mg. per g. Metabolism, $Q_{O_2}=1{\cdot}2$; $Q_{N_2}^{M}=1{\cdot}8$.

0·05 mg. was fatal to nearly all animals with tumours. The size of the tumour affects the survival rate, the animals with large tumours being very sensitive (Table I D). Ascorbic acid had no favourable effect on the survival rate.

From the small number of experiments done with rats to determine the toxicity, it was shown that the toxic dose is about twice that for mice, and that tumour-bearing animals are again more sensitive and die more quickly. No normal rats have died within a few days with doses of 0·2 mg., but this amount killed 4 out of 12 animals bearing tumours within 18 hours.

Haemorrhage in tumours

In most cases the tumours of animals injected with a dose of colchicine amounting to half the lethal dose became very haemorrhagic within 6–12 hours of the injection. No effect has been noticed in less than 4 hours and the maximum effect appears in 18–24 hours. Normally the tumours used in these experiments are almost white. In albino animals injected with colchicine the haemorrhage causes the tumour to appear dark beneath the skin. The haemorrhagic tumours are generally entirely red except for the necrotic area. Microscopic examination of sections of these tumours shows numerous areas filled with red blood corpuscles: the surrounding tumour tissue contains very little blood (see Pl. III, figs. 1 and 2).

Effect on ascorbic acid content of tissues

Injection of colchicine in lethal and sublethal doses diminishes the ascorbic acid content, as measured by the indophenol-reducing power, of the tumours, intestines and livers of rats and mice within 18 hours of injection. Table II

Table II. *Average indophenol-reducing power (mg. of ascorbic acid per g.) of various rat and mouse tissues: rats injected with 0·2 mg. and mice with 0·1 mg. colchicine 18 hours previously*

The numbers in brackets refer to the number of determinations

Tissue	Treated	Untreated
Mouse:		
S 37	0·11 (4)	0·32 (2)
Mal.	0·11 (3)	0·21 (2)
Crocker	0·14 (5)	0·30 (5)
Intestine	0·24 (4)	0·30 (4)
Liver	0·24 (3)	0·43 (3)
Brain	0·56 (3)	0·50 (2)
Rat:		
Jensen sarcoma	0·18 (3)	0·33 (3)
Intestine	0·16 (3)	0·37 (3)
Liver	0·15 (4)	0·26 (4)
Brain	0·43 (2)	0·45 (2)
Testis	0·29 (2)	0·24 (2)

gives a summary of determinations on various tissues. Although this effect is generally shown it does not invariably occur and some of the treated animals apparently remain normal. For this reason individual results of any treatment cannot be taken as typical. The ascorbic acid contents of the brain and testis are not affected by colchicine. Glutathione estimations have been made on many tissues of treated animals and no significant difference from the normal values has been observed.

The minimum dose for the effect on the ascorbic acid content of tumours was found to be fairly close to the toxic dose as shown by the results with different doses in Table III. A definite effect is obtained in mice with 0·025 mg.

colchicine and in rats with doses over 0·1 mg. In mice the ratio of effective dose to toxic dose is much lower than in rats. The effective dose in mice is about the same as that used for therapeutic experiments in mice by Amoroso [1935].

Table III. *Effect of different doses of colchicine on the ascorbic acid content of mouse and rat tissues, 16 hours after injection*

Dose mg.	Ascorbic acid, mg. per g.		
	Tumour	Liver	Intestine
	Mice bearing Crocker 180 sarcoma		
0	0·16	0·27	0·20
0	0·18	0·30	0·22
0·012	0·17	0·27	0·19
0·012	0·15	0·29	0·25
0·025	0·13	0·18	0·17
0·025	0·12	0·21	0·21
0·050	0·08	0·12	0·17
0·05	0·10	0·12	—
0·10	0·07	0·13	0·12
0·20	0·09	0·14	0·14
	Rats bearing Jensen rat sarcoma		
0	0·39	0·26	0·39
0	0·31	0·27	0·33
0·03	0·24	0·27	0·29
0·06	0·37	0·26	0·28
0·10	0·21	0·27	0·34
0·12	0·24	0·24	0·28
0·2	0·09	0·10	0·13
0·2	0·13	0·24	0·23
0·25	0·14	0·09	0·14
0·4	0·13	0·17	0·16
1·0	0·17	0·18	0·20

Table IV. *Effect of 0·2 mg. colchicine injected into rats with J.R.S. tumours*

Time after injection hours	Degree of haemorrhage in tumour	Ascorbic acid mg. per g.			Metabolism of tumour		
		J.R.S. tumour	Liver	Small intestine	Q_{O_2}	$Q^{M}_{N_2}$	Q_{O_2} (with glucose)
Control	–	0·20	0·30	0·25	—	—	—
,,	–	0·32	0·23	0·19	12·2	26·3	—
,,	–	0·26	0·25	0·24	11·1	19·2	—
,,	–	0·35	0·25	0·25	10·0	28·5	—
4	+ +	0·22	0·15	0·15	2·0	16·0	7·6
4	+	0·23	0·25	0·35	8·3	8·4	13·0
8	+ + +	0·21	0·12	0·15	—	—	—
12	+ + +	0·12	0·10	0·14	0·6	0·0	0·6
12	+ + + +	0·11	0·15	0·13	—	—	—
16	+ + +	0·13	0·14	0·19	1·2	1·8	0·2
16	+ + +	0·19	0·12	0·105	1·2	0·0	5·5
18	–	0·24	0·20	0·18	11·2	20·4	—
18	+ + +	0·06	0·13	0·20	0·5	2·1	—
20	+ + +	0·09	0·10	0·11	—	—	—
24	+ + + +	0·11	0·08	0·13	0·2	1·1	—
24	+	0·35	0·25	0·15	8·0	27·7	6·6
24	+ +	0·15	0·33	0·25	5·8	17·0	7·7
48	+ +	0·18	0·32	0·24	—	—	—
48	+ + +	0·04	0·23	0·32	0·6	0·0	0·4
72	+	0·21	0·18	0·14	—	—	—
72	–	0·31	0·25	0·24	—	—	—
92	–	0·23	0·27	0·23	—	—	—

Degree of haemorrhage: – =normal white tumour; + =slight haemorrhage; + + =fair haemorrhage; + + + =bright red tumour; + + + + =dark red tumour.

Table V. *Effect of 0·05 mg. colchicine injected into mice with Crocker 180 tumours*

Time after injection hours	Degree of haemorrhage of tumours	Ascorbic acid, mg. per g.		Metabolism of tumour	
		Tumour	Liver	Q_{O_2}	$Q^{M}_{N_2}$
Control	–	0·20	0·35	8·5	22·0
,,	–	0·26	0·38	7·6	20·1
4	–	0·24	0·41	8·2	20·2
4	–	0·18	0·22	—	—
18	+ +	0·24	0·31	2·2	1·1
21	+	0·18	0·30	2·0	0·9
24	+	0·17	0·20	—	—
24	–	—	0·10	6·0	22·7
28	+ +	0·15	0·32	—	
28	–	—	0·42	4·4	24·0
42	+	—	0·24	1·7	2·1
42	+	—	0·32	1·7	1·1
46	–	0·21	0·43	—	—
70	–	0·25	0·42	10·0	16·0
70	–	0·18	0·32	12·0	29·2
72	–	0·44	0·32	—	—
13 days	–	0·16	0·44	12·0	34·1

The duration of the effects of colchicine on various properties of animal tissues is shown in Tables IV and V.

Effect on tissue metabolism

Using the Warburg technique, measurements of the respiration and glycolysis of tumours taken from animals treated with colchicine were made. Although the similar doses of colchicine did not always produce similar effects it was invariably the case that when the tumours were very haemorrhagic the metabolism was low and was sometimes completely inhibited. After a few days (see Tables IV and V) the effect appeared to pass off and the tumour to become normal if the animal survived.

The liver respiration was measured in several cases, but was (with the exception of one fatty liver) never below normal, even in such animals as were almost moribund.

Effect on metabolism of colchicine added in vitro

The effect of colchicine added to liver and tumour slices *in vitro* was also determined. A concentration of 0·03 % reduced the respiration without added

Table VI. *Effect on metabolism of compounds added* in vitro *to J.R.S. slices*

Concentration	Ratio of Q_{O_2} to normal Q_{O_2}	
	1st hour	2nd hour
Control	0·82	0·66
Control (with glucose)	0·90	0·85
A. Colchicine:		
1 : 1,000	0·64	0·30
1 : 1,000 (with glucose)	0·92	0·79
1 : 3,300	0·73	0·46
1 : 10,000	0·81	0·57
B. Colchiceine:		
1 : 1,000	0·28	0·04
1 : 3,300	0·40	0·10
1 : 10,000	0·65	0·22

glucose after 1–3 hours (see Table VI). Colchiceine, which is far less effective when injected into the animals (unpublished observations), is much more active than colchicine when tested *in vitro*: a concentration of 1 in 10,000 reduces the respiration to one-quarter of the normal value in 1 hour.

The effect of sex hormones

Mosonyi [1936] found that the injection of male hormone into male guinea-pigs and of female hormone into female guinea-pigs caused a reduction in the ascorbic acid contents of the adrenal glands and liver. The effects of androsterone benzoate and oestrone benzoate on the ascorbic acid content and metabolism of tumour and liver tissue of mice are shown in Table VII. It will be seen that no significant change was produced.

Table VII. *The effect of sex hormones on ascorbic acid content and metabolism of tissues of mice with Crocker 180 tumours*

Sex of mouse	Hormone injected	Dose (in mg. of benzoate)	Time after injection days	Ascorbic acid, mg. per g.			Q_{O_2} of tumour
				Tumour	Liver	Intestine	
♀	Oestrone	0·02	1	0·18	0·38	0·23	9·2
		0·02	2	0·24	0·42	0·39	7·8
		0·02	3	0·24	—	0·29	6·2
		0·1	1	0·30	0·36	0·38	8·6
		0·2	2	0·26	0·33	0·36	6·8
♂	Oestrone	0·02	1	0·25	0·35	0·24	7·1
		0·02	2	0·23	0·23	0·23	6·4
		0·02	3	0·22	0·27	0·25	9·7
		0·1	1	0·23	0·26	0·29	7·5
		0·2	2	0·23	0·27	0·21	9·9
♀	Androsterone	0·5	1	0·22	0·27	0·38	5·7
		0·5	2	0·22	0·32	0·36	7·1
		0·5	3	0·22	0·31	0·33	7·7
		2·5	1	0·20	0·34	0·32	7·2
		5·0	2	0·21	0·27	0·24	7·3
♂	Androsterone	0·5	1	0·15	0·23	—	5·7
		0·5	2	0·19	0·20	0·19	7·4
		0·5	3	0·26	0·28	0·25	8·1
		2·5	1	0·20	0·29	0·29	11·6
		5·0	2	0·21	0·38	0·33	6·8
Average normal (5 mice)		—	—	0·21	0·35	0·35	8·5

Effect of *B. typhosus* washings

The effect of colchicine in producing haemorrhage in tumours appeared to be similar to that of such bacterial filtrates and washings as elicit the Shwartzman reaction in rabbits described by Gratia & Linz [1931] and Shwartzman & Michailovsky [1932]. Agar washings from a culture of *B. typhosus* (Watson) containing 0·2% formalin were injected into tumour-bearing animals in the same way as colchicine was injected. Haemorrhage was produced in the tumour and also reduction in the ascorbic acid content and metabolism as shown in Table VIII. The effect of the bacterial extract in reducing the ascorbic acid content is in agreement with results obtained by Harde & Kobozieff [1936] using a killed culture of *Salmonella typhimurium*.

Table VIII. *Effect of* B. typhosus *filtrate 24 hours after injection*

Mice bearing Crocker 180

Dose ml.	Degree of haemorrhage of tumour	Ascorbic acid, mg. per g.			Metabolism of tumour	
		Tumour	Liver	Intestine	Q_{O_2}	$Q^{M}_{N_2}$
Control	–	0·18	—	—	8·2	20
,,	–	0·18	0·35	0·23	7·6	18
0·05	–	0·16	0·26	—	2·8	0
0·05		—	—	—	6·0	22
0·1	+	0·10	0·33	0·31	0	0
0·1	–	0·22	—	—	6·2	13·5
0·2	+	0·10	0·35	0·28	0	0
0·2	+ +	0·13	—	—	1·8	2·6

Dose ml.	Mice bearing S 37. Ascorbic acid, mg. per g.		Rats bearing J.R.S. Ascorbic acid, mg. per g.			Metabolism		
						Tumour		Liver
	Tumour	Liver	Tumour	Liver	Intestine	Q_{O_2}	Q^{M}	Q_{O_2}
0	0·26	0·35	0·30	0·41	0·44	—	—	—
0·05	0·10	0·38	0·07	0·15	0·15	2·4	9·2	10·0
0·10	0·12	0·38	0·04	0·07	0·10	2·1	0·3	13·1
0·20	0·14	0·36	0·04	0·13	0·12	2·4	0·0	10·7
0·25	—	—	0·10	0·42	0·40	—	—	—

Injection of colchicine and B. typhosus *filtrate into mice bearing spontaneous tumours*

All the grafted tumours that have been used (S 37, Mal. and Crocker 180 in mice and J.R.S. in rats) have, with a few individual exceptions, given the typical responses with colchicine and *B. typhosus* washings.

Duran-Reynals [1933] had found that slowly growing spontaneous tumours were practically non-susceptible to *B. coli* filtrates which produced haemorrhage in rapidly growing transplantable tumours. The effect of colchicine on spontaneous mouse tumours was therefore examined and the observations are recorded in Table IX. (Sections of the tumours were examined and all were

Table IX. *Mice, bearing spontaneous tumours, injected with colchicine 24 hours previously*

Dose mg.	Degree of haemorrhage	Ascorbic acid, mg. per g.			Metabolism tumour	
		Tumour	Liver	Intestine	Q_{O_2}	$Q^{M}_{N_2}$
Control	+ +	0·16	0·26	0·30	—	—
,,	–	0·16	0·21	0·33	—	—
0·1	+	0·17	0·27	0·19	10·9	29·0
0·1	+ + +	0·27	0·23	0·30	10·1	17·5
0·1	+ + +	—	—	—	11·2	11·8
0·1	+ +	—	—	—	12·0	11·2
0·1	+ +	0·11	0·30	0·40	6·5	8·9
0·1	–	0·23	0·24	0·25	11·5	20·0
0·2	+ +	0·19	0·25	0·33	4·4	7·8
0·2	–	0·29	0·21	0·23	10·6	11·4

found to be carcinomata.) It is difficult to assess the results obtained on the spontaneous tumours on account of the wide variation in extent of haemorrhage and in metabolism of the untreated tumours, but there is no doubt that the spontaneous tumours used did not respond to treatment with colchicine or with *B. typhosus* filtrate to the same extent as did transplantable tumours.

The effect of colchicine on rabbit skin

As colchicine resembles bacterial filtrates in its effect on grafted tumours the effect of colchicine in producing the Shwartzman phenomenon was tested. The shaved skin of albino rabbits was injected with 0·025, 0·05, 0·1 and 0·2 mg. of colchicine. Intravenous injections of 1·0 mg. of colchicine were given 24 hours later and the skin examined frequently during the next few days. No reaction was obtained in any of the rabbits.

Discussion

The results show that the effects of colchicine injected into rats and mice bearing grafted tumours (in producing haemorrhage, reducing ascorbic acid content and metabolism of the tumour) are similar to those of a "filtrate" of *B. typhosus*. The nature of the factor in bacterial filtrates which produces these effects is not known but it appears to be identical with the factor responsible for the Shwartzman phenomenon (in the skin of the rabbit). In order to obtain these effects doses of colchicine approaching the toxic dose and ten times as great as those necessary to produce inhibition of mitosis are required. Colchicine resembles the bacterial filtrates in that, in order to produce injury to the tumour, doses are required which almost kill the animals.

The fate of the ascorbic acid which disappears from the tissues is not known. Some of the decrease of this compound in tumours must be due to increase in the amount of blood in the tissue, but that increase does not appear to be large enough to account for all the difference. Colchicine also causes a diminution in the ascorbic acid of the intestine, without the appearance of haemorrhage. Experiments to determine whether the tissues of colchicine-treated animals contained dehydroascorbic acid showed that none was present. There also appeared to be no increase in the urinary excretion of ascorbic acid on treating rats with colchicine.

Summary

Colchicine in doses approaching the toxic dose produces haemorrhage in grafted tumours accompanied by a reduction in the ascorbic acid content and metabolism of the tumours. In these effects colchicine behaves like a *B. typhosus* filtrate.

Colchicine also causes a reduction in the ascorbic acid contents of the liver and intestine of rats and mice, but does not inhibit the metabolism of the liver.

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