CXIV. A NOTE ON THE REDUCING ACTIVITY OF THE TISSUES OF NORMAL AND TUMOUR-BEARING RATS AND MICE.

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HARRIS and his colleagues have recently studied the reducing activity of acid extracts of a number of animal and vegetable tissues, and have found a correlation in most cases between this activity and the antiscorbutic potency of the tissues. Whether their technique [Birch, Harris and Ray, 1933] affords a specific test for such potency is still a matter of controversy but the authors themselves recognise the limitations of the method unless confirmed by biological assay. During the examination of the tissues Harris [1933, 1, 2] found that an extract of the Jensen rat sarcoma had a high reducing activity although he found by biological tests on guinea-pigs that this activity could not be attributed entirely to its ascorbic acid content. Similarly Borghi and Deotto [1934] were unable to demonstrate any antiscorbutic activity in extracts of the Ehrlich mouse carcinoma. Boyland [1933] confirmed the high content of reducing substances in the Jensen rat sarcoma but found a lower content for a second rat sarcoma, two mouse sarcomas, a mouse carcinoma (C 63) and a fowl sarcoma. In the case of the Jensen sarcoma but not in the other five tumours, the activity of the malignant tissues was higher than that of other tissues of the same animal. In commenting on Harris' results, Quastel [1933] cited in confirmation the greater reduction of ammonium molybdate by acid extracts of human tumours than by those of non-malignant tissues.

These observations have been extended in the present study to a number of different strains of transplantable rat and mouse tumours which have been propagated over a long period of years in the laboratories of the Imperial Cancer Research Fund. The reductions of both ammonium molybdate and 2:6-dichlorophenolindophenol by the tumours and other organs have been examined. The rats were maintained on a mixed diet of bread, meat biscuits, boiled beans, maize and kitchen scraps, and the mice on bread and oats, with cod-liver oil and yeast three times a week.

The reduction of ammonium molybdate by tissues of tumour-bearing rats and mice.

The following method was adopted for estimating the relative reducing activities of the tissues.

The tumour-bearing animal was killed and equal quantities of the different tissues ground up with dilute acetic acid and purified silver sand in hard glass centrifuge pots. 20 % ammonium molybdate was then added in equal amounts to each of the pots, which were incubated at 37° or 50° for 60 or 90 minutes.

The best results were usually obtained when 2 ml. of 3*N* acetic acid and 8 ml. of 20 % ammonium molybdate per 0.2 g. of tissue were used.

The intensity of the blue colour of the supernatant fluid after centrifuging was employed as a criterion of the degree of reduction of the molybdate.

Two rat tumours and eight mouse tumours were examined by this method and the results are shown in Table I.

Table I. *The reduction of ammonium molybdate by the tissues of rats and mice with transplantable tumours.*

No.	Animal	Tumour			Relative reducing values							Necrotic tumour tissue	Non- necrotic tumour tissue
		Strain	Type	Age in days	Liver	Spleen	Brain	Muscle	Kidney	Lung			
1	Rat	Jensen	Sarcoma	22	3	2	—	1	—	—	—	—	5
	"	"	"	19	2	—	2	—	—	—	—	—	4
2	"	Walker	Carcinoma	38	2	2	4	—	2	$\frac{1}{2}$	—	—	5
1	Mouse	Crocker	Sarcoma	15	2	$\frac{1}{2}$	—	0	—	—	—	—	4
2	"	37	"	14	2	3	—	1	—	—	—	—	4
3	"	Bonne	Tar sarcoma	21	3	4	—	1	—	—	—	—	2
4	"	173	"	28	4	3	5	—	1	—	—	—	4
5	"	2146	Tar carcinoma	20	2	4	—	1	—	—	—	—	4
	"	2146	"	15	1	2	—	$\frac{1}{2}$	—	—	—	—	3
6	"	91	Carcinoma	66	5	4	—	1	1	—	—	—	4
7	"	113	"	28	2	3	—	$\frac{1}{2}$	1	—	—	$\frac{1}{2}$	4
8	"	206	"	18	2	3	4	—	—	—	—	1	—

The intensity of the blue colour was determined by comparison with an arbitrarily chosen standard, and the data in the right-hand columns afford an approximate comparison of the reducing activities of the tissues. The Jensen rat sarcoma and the Walker rat carcinoma showed activities of a higher order than the other tissues of the same animals. In the majority of the mouse tumours a similar increased activity of the malignant tissues was found. Exceptions were the Bonne and the 173 sarcomas and the 91 carcinoma, where the spleen, brain and liver respectively showed greater reducing activities. A slight reduction of the ammonium molybdate was effected even by the highly necrotic portions of a tumour as was shown in the case of tumours 113 and 206. Both the spleen and brain showed high reducing activities and in most cases these organs were superior to the liver in this respect.

The reduction of 2:6-dichlorophenolindophenol by acid extracts of the tissues of normal and tumour-bearing rats and mice.

A slight modification of the technique of Birch *et al.* [1933] was employed for testing the reducing activity of the extracts.

The animals were killed and the organs removed. 2 ml. of 10 % ice-cold trichloroacetic acid were added to small weighed quantities of each of the different organs which were then ground with 0.5 g. of purified silver sand in small hard glass centrifuge-pots. 2 ml. of cold distilled water were added in each case, and the material was centrifuged for 5 minutes. After centrifuging, the supernatant acid extracts were poured off into labelled beakers. 4 ml. of distilled water were added to the mixture of sand and tissue in each case and the centrifuging was continued for a further 5 minutes. This second supernatant was then added to the contents of the corresponding beaker. A third washing was found to be unnecessary. The 8 ml. of extract and washings contained therefore the acid extractives of the original weighed tissue.

These extracts were then titrated against a measured volume of the 2:6-dichlorophenolindophenol indicator. The indicator solution was made by dissolving 0.05 g. of the dried substance in a small quantity of boiling distilled water and filtering. The undissolved residue was extracted with a further quantity of boiling water and the total volume of the filtrate made up to 50 ml. The tissue extracts under examination were run from a micro-burette into 1.0 or 0.5 ml. of a 1 in 10 dilution of the indicator as prepared above, in a small beaker. Table II shows the reducing activities of the various tissues. The figures represent the ml. of the indicator solution (1 ml. of which was equivalent to 0.04 mg. of a commercial sample of ascorbic acid) reduced by the extract from 1 g. of tissue.

Table II. *The reducing activity of the acid extracts of the tissues of normal and tumour-bearing rats and mice.*

				Relative reducing activities of tissues ml. 2:6-dichlorophenolindophenol solution (1/10,000) reduced by extract from 1 g. of tissue									
No.	Animal	Tumour		Age in days									Non- necrotic tumour tissue
		Strain	Type		Liver	Spleen	Brain	Kidney	Lung	Testes	Embryo	Necrotic tumour tissue	
1	Rat	Jensen	Sarcoma	31	5.2	8.7	8.7	3.5	—	—	—	—	10.7
	"	"	"	8	8.2	8.8	7.8	5.7	—	—	—	—	11.2
	"	"	"	26	—	—	8.3	—	—	—	—	—	10.8
2	"	Walker	Carc.	39	7.6	9.4	9.7	—	8.4	—	—	6.0	14.5
	"	"	"	80	4.9	7.5	6.6	3.0	—	—	—	—	13.3
	"	"	"	21	3.8	6.9	4.9	3.1	5.2	—	—	—	10.7
	"	Normal	—	—	10.0	8.7	8.0	5.3	5.7	8.0	—	—	—
	"	"	—	—	10.0	9.7	—	5.8	8.2	7.4	—	—	—
	"	"	—	—	9.1	9.1	8.6	5.7	3.7	8.4	—	—	—
1	Mouse	Crocker	Sarcoma	21	10.5	14.5	14.3	9.0	13.3	—	—	—	14.1
2	"	37	"	13	9.5	8.3	16.3	5.0	8.0	—	—	—	7.2
	"	37	"	25	8.4	8.2	12.3	7.5	—	—	—	—	9.2
3	"	Bonne	Tar sarc.	7	7.3	—	11.6	—	—	—	—	—	10.0
4	"	173	"	15	8.5	7.6	13.1	—	8.5	—	8.0	—	6.7
	"	173	"	22	5.7	10.0	12.5	—	6.9	—	—	—	6.7
5	"	2146	Tar carc.	9	10.5	11.2	11.1	—	—	—	—	—	9.5
6	"	113	Carc.	80	8.3	13.0	14.0	—	12.0	—	—	—	14.3
7	"	4108	Sporadic	—	9.5	16.3	13.5	8.0	—	—	—	11.9	16.0
	"	Normal	—	—	12.3	16.0	15.7	9.6	15.8	—	—	—	—
	"	"	—	—	8.9	10.4	14.8	7.0	14.0	—	—	—	—
	"	"	—	—	9.0	12.7	17.0	7.6	9.3	—	—	—	—

Both the Jensen and Walker tumours showed greater activity than the other tissues of the same animals. The variation in the reducing values of the rat tumours was small (minimum 10.7; maximum 14.5). There was a greater variation in the reducing values of the different strains of mouse tumours (minimum 6.7; maximum 16.0) and in most cases the values were of a lower order than those for the spleen or brain. The high reducing activity of the brain tissue is of interest in connection with some experiments which one of us (M. M.) has carried out in collaboration with Dr F. G. Young in the Biochemistry Department at University College, London [Young and Mitolo, 1934]. The general properties of the reducing substance prepared from ox brain clearly differentiate it from ascorbic acid and in this respect it shows a similarity to the reducing substance in tumour tissue [Harris, 1933, 2; Borghi and Deotto, 1934].

Tumour 4108 was the 6-weeks old autoplast of a spontaneously occurring mammary carcinoma. Both the non-necrotic and the necrotic portions had high reducing values. The values for the tissue extracts of normal tumour-free rats and mice were of a similar order to those of the tumour-bearing animals.

Although there is a considerable variation in the reducing values of the transplantable tumours of different strains, the values of tumours of the same strain show less variation even under different conditions. This is shown by the similarity of the values for (a) tumours of the same or different ages, (b) multiple transplantable tumours of the same animal, and (c) progressing and spontaneously regressing transplantable rat tumours.

Tumours of the same and different ages. In a series of nine 2146 mouse carcinomas the reducing values were 10.0, 9.0, 8.7, 8.5, 9.5, 11.0, 10.2, 9.4 and 9.5 after the same period of growth and before the tumours showed any necrosis. The values for the Jensen and Walker rat tumours and the mouse tumours 37 and 173 of different ages were very similar (Table II).

Multiple transplantable tumours. In cases where two transplants were made in the same animal the reducing values of the subsequent tumours were approximately the same, e.g.:

	Relative reducing activities	
	Left tumour	Right tumour
2146 (mouse tar carcinoma)	1. 9.5	9.2
	2. 10.2	10.0

Progressing and spontaneously regressing tumours. In two cases Jensen rat tumours which were showing rapid spontaneous regression were found to have reducing values of 10.9 and 12.5. These values were very similar to those found for the normal progressing tumours (10.7, 11.2, 10.8, Table II).

SUMMARY.

1. Transplantable rat and mouse tumours of different strains readily reduce ammonium molybdate in an acid medium. In most cases the tumours show a higher content of reducing substances than the liver, spleen or brain.

2. The reducing substances in these tumours are soluble in dilute trichloroacetic acid, and extracts obtained in this way reduce 2:6-dichlorophenol-indophenol. In the case of the rat strains the tumour extracts were more active than those of the liver, spleen or brain. They were somewhat less active than these extracts however in the case of the mouse strains.

3. Although there is a considerable variation in the reducing activities of the tumours of different strains, the values of the tumours of the same strain are comparatively constant. This is shown by the similarity of the reducing values for (a) tumours of the same and different ages, (b) multiple transplantable tumours in the same animal, and (c) progressing and spontaneously regressing tumours.

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