CVI. A NOTE ON GLUTATHIONE AND VITAMIN C IN TUMOUR TISSUE.

By ERIC BOYLAND.

From the Research Institute of the Cancer Hospital (Free), London.

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VITAMIN C and the hexuronic acid of suprarenal tissue (ascorbic acid) were found to be identical by Svirbely and Szent-Györgyi [1932], and the identity has been confirmed by many other workers. This has led to the chemical estimation of vitamin C by Birch, Harris and Ray [1933]. Birch and Dann [1933] have pointed out that determinations of glutathione in tissues by the method of iodine titration are probably too high because in such a titration iodine would be reduced by ascorbic acid as well as by sulphydryl groups. Thus in an acid extract of suprarenal cortex as much as 77 % of the total iodine required may react with ascorbic acid and only 23 % with glutathione. Almost simultaneously with the publication of this suggestion Bierich and Rosenbohm [1933] produced evidence showing that the figures normally given for the glutathione content of tissues as obtained by iodine titration were probably too high and that some other reducing substance, "x," was present. This conclusion was arrived at by comparison of results obtained by iodine titration and a colorimetric method of estimating glutathione. These authors suggest in a footnote that the reducing substance may be the hexuronic acid of Szent-Györgyi. The conclusions of Birch and Dann and of Bierich and Rosenbohm have been confirmed in this laboratory and similar estimations have been carried out upon tumours.

EXPERIMENTAL.

Tissues were removed from animals immediately after killing, rapidly weighed and ground with 3 times their weight of ice-cold 10 % trichloroacetic acid. After standing 30 minutes in the cold the tissue suspension was centrifuged and the extract filtered. The tumours examined were carcinomata (C 63) of mice and sarcomata (LM $\frac{157}{23 A}$, $\frac{4}{59 E}$, $\frac{LR 10}{35 F}$ and Baker No. 2) of mice, rats and fowls. The designation LM $\frac{157}{23 A}$ is applied to a mouse tumour produced originally by the subcutaneous injection of 5 : 6-cyclopenteno-1 : 2-benzanthracene in lard and grafted through 23 generations¹. Similarly, $\frac{LR 10}{35 F}$ refers to the 35th grafted generation of a rat tumour produced by the subcutaneous injection of 1 : 2 : 5 : 6-dibenzanthracene dissolved in lard, and $\frac{4}{59 E}$ to the 59th generation of a mouse tumour produced in a similar manner. I am indebted to Prof. McIntosh of the Middlesex Hospital for the fowl tumour (Baker No. 2).

¹ A description of this tumour will be published by Dr J. W. Cook and Miss Barry.

Ascorbic acid was estimated by titration against phenol-2 : 6-dichloroindophenol as described by Birch, Harris and Ray [1933]; Dr Harris was good enough to furnish full details of the method. In the absence of a specimen of pure ascorbic acid as standard the dye solution was titrated with filtered orange juice. Orange juice was found to contain no glutathione as estimated by the colorimetric method. The ascorbic acid content of the orange juice (and the iodine equivalent of the 2 : 6-dichloroindophenol solution) were therefore estimated by titration with $N/100$ iodine in the presence of 5 % trichloroacetic acid.

Glutathione was estimated colorimetrically by a method similar to that of Bierich and Rosenbohm except that it was carried out on the CCl_3COOH extract, and the colour produced was compared with that produced by glutathione in standard controls containing 0.5, 1.0, 1.5 and 2.0 mg. glutathione. The neutralised CCl_3COOH extract was saturated with $(\text{NH}_4)_2\text{SO}_4$, and volumes ranging from 2 to 12 cc. of the solution were transferred with a pipette into a 15 cc. graduated flask; 1.0 cc. M KCN and 1.0 cc. 1 % sodium nitroprusside were added and then saturated $(\text{NH}_4)_2\text{SO}_4$ solution to 15 cc. The reddish-purple colour produced was rapidly compared in a colorimeter with that produced at the same time by standard glutathione solution.

The method is very crude as the colour produced by glutathione fades rapidly and is not exactly proportional to the amount of this compound. By taking an amount of standard solution such that the control and the experiment contain about the same amount of glutathione, duplicate results are obtained which do not differ by more than 10 %.

The iodine titration which presumably gives a measure of ascorbic acid *plus* glutathione was carried out by the method of Tunncliffe [1925].

Table I shows the result of ascorbic acid determinations on some rat and mouse tissues. The figures obtained by Birch and Harris and by Bierich and

Table I. *Ascorbic acid content of normal and malignant tissues.*

Tissue	Ascorbic acid			
	Estimated with indophenol mg. per g.	Calculated equivalent $N/100$ I per g. Average cc. per g. tissue	From Birch and Dann. Equivalent $N/100$ I cc. per g. tissue	From Bierich and Rosenbohm. Substance "x," equivalent $N/100$ I cc. per g. tissue
<i>Mouse</i>				
Muscle	0.04, 0.03, 0.05	0.048	—	—
Liver	0.25, 0.31, 0.26	0.31	—	—
Sarcoma				
LM $\frac{157}{23 A}$	0.16, 0.11, 0.15	0.16	—	—
$\frac{4}{59 E}$	0.20, 0.16	0.20	—	—
<i>Rat</i>				
Blood	0.05, 0.03	0.04	0.023	—
Muscle	0.04, 0.04, 0.05	0.05	0.068	0.03-0.04
Liver	0.32, 0.34, 0.36	0.39	0.188	0.07-0.56
Kidney	0.26, 0.27	0.30	0.25	0.60-1.2
Spleen	0.22	0.25	—	0.22-0.47
Brain	0.26	0.30	—	0.33-0.48
Jensen rat sarcoma	0.50, 0.48, 0.47, 0.45	0.54	—	—
Sarcoma LR 10 $\frac{35 F}{35 F}$	0.22, 0.27	0.28	—	—

Rosenbohm are given for comparison. The ascorbic acid values are of the same order as those of Bierich and Rosenbohm for the unknown reducing substance "x." It seems highly probable that the reducing substance "x" is identical with ascorbic acid.

The estimations on muscle and blood are less accurate than the others because of the small amounts of both ascorbic acid and glutathione present in these. Results obtained with kidney tissue are also open to suspicion on account of the possible presence of cysteine.

Table II. *Ascorbic acid and glutathione.*

		1	2	3	4	5	6	7
		Ascorbic acid from phenol-2 : 6-dichloro-indophenol titration		Glutathione determined colorimetrically		Total I required Tunnicliffe method cc. N/100 I	I equivalent of ascorbic acid + glutathione (col. 2 + col. 4)	Percentage of total I (col. 5) due to GSH (col. 4)
		mg. per g.	Equivalent cc. N/100 I	mg. per g.	Equivalent cc. N/100 I			
<i>Mouse</i>								
Liver	(1)	0.28	0.32	3.50	1.14	1.35	1.46	84
	(2)	0.26	0.30	3.30	1.08	1.15	1.38	93
Muscle	(1)	0.045	0.05	0.31	0.11	0.23	0.16	48
	(2)	0.052	0.06	0.25	0.08	0.11	0.14	73
Carcinoma C 63	(1)	0.105	0.12	0.21	0.07	0.19	0.19	37
	(2)	0.115	0.13	0.18	0.06	0.21	0.19	29
	(3)	0.13	0.15	0.25	0.08	0.19	0.23	42
<i>Rat</i>								
Liver	(1)	0.26	0.29	1.45	0.47	0.72	0.76	65
	(2)	0.25	0.28	1.54	0.50	0.73	0.78	68
	(3)	0.23	0.26	1.54	0.50	0.70	0.76	71
Kidney	(1)	0.35	0.40	0.86	0.28	0.63	0.68	45
	(2)	0.38	0.43	0.92	0.30	0.72	0.73	42
Spleen	(1)	0.18	0.20	0.89	0.29	0.45	0.49	64
	(2)	0.19	0.21	0.80	0.26	0.53	0.47	49
Muscle	(1)	0.03	0.035	0.14	0.045	0.07	0.08	64
	(2)	0.04	0.05	0.12	0.04	0.06	0.09	66
Brain	(1)	0.22	0.25	0.40	0.13	0.40	0.38	33
	(2)	0.28	0.32	0.68	0.22	0.53	0.54	42
<i>Jensen rat sarcoma</i>								
	(1)	0.38	0.43	0.55	0.18	0.67	0.61	27
	(2)	0.27	0.31	0.52	0.17	0.47	0.48	36
	(3)	0.42	0.47	0.65	0.21	0.69	0.68	30
<i>Sarcoma LR 10 35 F</i>								
	(1)	0.23	0.26	0.37	0.12	0.34	0.38	35
	(2)	0.17 (necrotic)	0.18	—	Nil	0.20	0.18	—
<i>Fowl</i>								
Liver		0.27	0.31	1.35	0.44	0.70	0.71	63
Kidney		0.16	0.18	1.20	0.39	0.63	0.57	62
Muscle		0.04	0.04	0.29	0.10	0.16	0.14	62
Sarcoma (Baker No. 2)		0.11	0.13	0.38	0.12	0.28	0.25	43

The results shown in Table II indicate that the method of iodine titration of acid extracts of tissues using starch as indicator gives a value which includes both ascorbic acid and glutathione, and that in some tissues the values that have been assumed for glutathione content are in reality too high. The amount of iodine required by the tissue extracts (column 5) agrees fairly closely with

the theoretical value of the sum of iodine equivalents of ascorbic acid and glutathione present (column 6).

It was first stated that tumours contained no glutathione [Holmes, 1926], but Bierich and Kalle [1926], Thomson and Voegtlin [1926] and Kennaway and Hieger [1927] all found glutathione in malignant tissues when the iodine titration method was employed; Yaoi and Nakahara [1926] and Bierich and Rosenbohm [1933] have found it by colorimetric (nitroprusside) methods. The results shown in Table II indicate that the values which have previously been given by the iodine titration method are possibly as much as three times too high. The determination of ascorbic acid indicates that the large discrepancy between colorimetric and iodimetric estimations is mainly due to the occurrence of this substance in malignant tissues. Brain tissue resembles tumour tissue and the suprarenal gland in having a high ascorbic acid content compared with the glutathione content.

SUMMARY.

The estimation of glutathione in tumour tissue (sarcoma of rat, mouse, and fowl, carcinoma of mouse) by a colorimetric method gives values only one-third as great as those obtained by iodine titration. Estimations of ascorbic acid show that the high iodine titre is due to the occurrence of this substance in tumour tissue.

REFERENCES.

- Bierich and Kalle (1926). *Z. physiol. Chem.* **215**, 151.
 — and Rosenbohm (1933). *Z. physiol. Chem.* **158**, 1.
 Birch and Dann (1933). *Nature*, **131**, 469.
 — Harris and Ray (1933). *Nature*, **131**, 273.
 Holmes (1926). *Biochem. J.* **20**, 812.
 Kennaway and Hieger (1927). *Biochem. J.* **21**, 751.
 Svírbely and Szent-Györgyi (1932). *Nature*, **129**, 576.
 Thomson and Voegtlin (1926). *J. Biol. Chem.* **70**, 793, 801.
 Tunncliffe (1925). *Biochem. J.* **19**, 194.
 Yaoi and Nakahara (1926). *Gann*, **20**, 51.

Note added June 19th, 1933. Biological tests on Jensen rat sarcomata carried out by Dr L. J. Harris indicate that the substance in tumours which reduces 2:6-dichlorophenolindophenol, and is designated in this paper as ascorbic acid, is not ascorbic acid. Malignant tissue must therefore contain considerable quantities of some unidentified reducing substance.