CII. OXIDATIVE MECHANISMS OF TUMOUR TISSUE.

I. THE ANAEROBIC HABIT OF TUMOUR TISSUE.

By BARBARA ELIZABETH HOLMES.

From the Biochemical Laboratory, Cambridge.

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WARBURG has shown in a recent series of papers [Minami, 1923; Warburg, Posener and Negelein, 1924] that the metabolism of cancer tissue may be regarded as resembling in some respects that of an anaerobic rather than an aerobic organism. It is capable of converting glucose into lactic acid with great rapidity, but the oxidative removal of the lactic acid is not so rapid. Thus, if slices of tumour tissue are placed in a Ringer's solution containing glucose, large amounts of lactic acid accumulate even when an abundant supply of oxygen is ensured. The oxidative activity of the tissue does not keep pace with the fermentative, and Warburg found that cancer tissue differed from normal tissues in this respect.

In his first paper on glutathione Hopkins [1921] stated that "actively growing cancer cells seem to show a nitro-prusside reaction of surprisingly low intensity." Sir F. G. Hopkins informs me that this statement was mainly based upon experiments with the Jensen rat sarcoma. Later observations on human material did not seem to confirm it, but this was at a time when no distinction was made between the reaction due to the "fixed SH group" of the tissue, and that due to soluble glutathione.

Callow and Robinson [1925] carried out a series of glutathione determinations on aerobic and anaerobic bacteria, and found the anaerobic to be notably deficient in their content of glutathione.

In view of this work and of the work of Warburg it seemed to be of interest to carry out determinations of the glutathione content of cancerous tissues.

The glutathione was extracted and titrated by the method of Tunncliffe [1925]. The tissue was repeatedly ground and extracted with 10 or 20 % trichloroacetic acid and filtered, and the filtrate then titrated with $N/100$ iodine, using the nitroprusside reaction as an external indicator. Necrotic tissue was most carefully avoided.

Some typical results are given in Table I. The glutathione content of cancerous tissue is very low indeed for a cellular tissue. For the liver (rabbit and rat) Tunncliffe obtained a mean value of about 200 mg. per 100 g. wet tissue. Hopkins (unpublished experiments) for the rabbit's liver has obtained values ranging from 250 mg. after 24 hours' starvation to 340 mg. in a well-fed animal. For the kidney, Tunncliffe's mean figure was 170 mg., while

Hopkins' data show a range from 130–145 mg. Tunnicliffe's figure for muscle is about 35 mg., the amount in muscle being small for a normal tissue.

It will be seen from the table that, in five cases out of seven examined, cancer tissue contained too little reduced glutathione to make estimation possible by the method used. It was in all these cases certainly less than 3.0 mg. per 100 g. tissue. The highest figure for cancer tissue, in the case of a rat sarcoma, was about 16 mg. The dry weight of tumour tissue is not much more than half that of muscle, however, and this, the highest figure and apparently exceptional, if calculated per 100 g. dry weight instead of wet weight, might give a value about equal to that of muscle. The tissues of young embryos, which have also a very high water content, and which, since they are growing tissues, give an interesting comparison, have been found to contain a large amount of glutathione [Murray, 1926]. The contrast with the quick-growing rat carcinoma and the quick-growing human mammary carcinoma is striking.

Table I.

Description of tissue	Glutathione content in mg. per 100 g. wet weight of tissue
Jensen rat sarcoma 2.4 g.	Small trace
" " 6.1 "	16.5
" " 5.0 "	Small trace
Rat carcinoma 9 2.2 "	"
" " 1.4 "	"
Quick growing and cellular human mammary carcinoma	7.5
Scirrhus human mammary carcinoma	Small trace
Rat brain 1.5 g.	50
Rabbit brain—grey matter	75
" " white matter	52
Metastatic nodules in axillary lymph glands from human mammary carcinoma	63
	75

The results given by human tumours correspond well with those given by rat tumours.

The only exception I have so far found to the low values given by tumour tissues is in the case of metastatic growths in lymph glands. In two cases of human mammary carcinoma I have obtained normally high values in infected axillary glands. It has not been possible as yet to determine whether this puzzling answer is due to an increased power of the tissue to reduce its glutathione (the iodine titration estimates, of course, only the reduced form) or whether the metastatic nodules actually pick up or form a larger amount of the substance than the parent tumours.

Similarly it is not yet certain whether the low figures obtained for tumour tissue are due to a low content of glutathione or to a decreased power to reduce the oxidised form. Analyses of the total sulphur in the tissue extracts will be carried out to settle this point.

Warburg [Warburg *et al.*, 1924] showed that the grey matter of the brain and the retina were the only normal adult tissues which could convert glucose into lactic acid as actively as cancer tissue. On the other hand, the grey matter of the brain is also capable of oxidising the lactic acid rapidly. It

seemed, therefore, of particular interest to compare the oxidative mechanisms of nervous tissue and cancerous tissue.

The amount of glutathione in the grey matter of rabbit's brain, even when this is only very roughly divided from the white, seems to be about 75 mg. per 100 g. wet weight of tissue. Values obtained are given in Table I.

Experiments have been carried out on three different tumours to determine to what extent cancer tissue is capable of converting added oxidised glutathione into the reduced form under strictly anaerobic conditions. All normal tissues examined by Hopkins have shown this capacity to a high degree.

About 3–5 g. of tissue were placed in a vacuum tube with 20 cc. of Ringer's solution containing 50–100 mg. of oxidised glutathione. The tube was very carefully exhausted, washed out with nitrogen and re-exhausted. A control tube containing the same amount of tissue and of fluid, and at the same p_H (7.4), but containing no glutathione, was treated exactly similarly. The tubes were then incubated for 3 or 4 hours at 37°.

Under these circumstances a considerable reduction of the oxidised glutathione would normally be expected. The tumour tissue proved to be extraordinarily inactive in this respect. In one case definite reducing power was displayed, but very small compared with normal tissues. The results are shown in Table II with data from normal tissues for comparison.

Table II.

Tissue	mg. of GSH per 100 g. tissue after incubation anaerobically	
	Without added G_2S_2	With added G_2S_2
Rabbit's liver	297	780
„ kidney	138	1020
Rat sarcoma (a)	± 1.5	± 4.5
„ (b)	Trace	± 1.5
„ (c)	Trace	91

The fact that the tumour tissue can reduce a further amount of oxidised glutathione suggests that, under the conditions of the original estimation, the glutathione already present is all reduced and therefore all estimated. On the other hand, the tissue is quite remarkable in that it only reduces such a very small amount of the added glutathione. In this respect tumour tissue seems to differ from anaerobic bacteria, as these can reduce added G_2S_2 . Quantitative comparison of the two is, however, very difficult. A further investigation on these lines should yield very interesting results.

In addition to glutathione, the tissues of rat sarcoma and carcinoma have been examined for the respiratory pigment *cytochrome*. Dr Keilin, who first described this pigment [1924], has very kindly examined the tissues. He has so far always found that aerobic tissues and organisms, and particularly those which might be expected to show great metabolic activity, contain considerable amounts of this pigment, whereas it is lacking in anaerobic organisms.

In order to remove as much haemoglobin as possible from the tumour tissue, so that the cytochrome spectrum might not be obscured, the rat bearing

the tumour was perfused for about 1 hour with Ringer's solution. The animal was killed by chloroform anaesthesia, the heart exposed, and a cannula tied into the left ventricle. Warmed Ringer's solution was allowed to flow through this and eventually to escape through a cut in the right ventricle, after passing through the systemic circulation.

After perfusion, small portions of the tumour and of the grey matter of the brain were removed for examination. The pieces of tumour tissue were taken from the most quickly growing portion of the tumour and necrotic tissue was carefully avoided.

The results of Dr Keilin's spectroscopic examination of these tissues were of very great interest.

The grey matter of the brain was found to contain a high concentration of cytochrome. The tumour tissue, on the other hand, contained very little of the pigment, and the position of the absorption bands resembled that shown by brewer's yeast.

The tissue of the Jensen rat sarcoma was examined twice, that of rat carcinoma 9 once, and the results were exactly similar on all three occasions. The grey matter of the rat brain was twice examined.

These results undoubtedly support very strongly the idea that the cancerous cell may live in some respects like an anaerobic organism. They also emphasise the difference, which was first pointed out by Warburg, between the activities of the cells of the grey matter of the brain and those of cancerous cells.

SUMMARY.

1. The tissues of rat sarcoma, rat carcinoma and human mammary carcinoma have all been found to contain abnormally small amounts of reduced glutathione.

2. Rat sarcoma and rat carcinoma tissue, unlike normal cellular tissue, appear also to show only very slight activity in reducing added oxidised glutathione.

3. Spectroscopic examinations of rat sarcoma and rat carcinoma tissue indicate deficiency in the respiratory pigment, cytochrome.

4. It is thought that these observations support the contention of Warburg, that the cancer cell resembles in some respects an anaerobic rather than an aerobic organism.

I have much pleasure in thanking Professor Gask, Dr Piney, Mr Perrin, Professor Ellis and Dr Byrom for their kindness in providing me with the human cancer material.

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