CLX. THE CARBOHYDRATE METABOLISM OF CERTAIN PATHOLOGICAL OVERGROWTHS.

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An important biochemical aspect of the cancer problem has been stressed by Warburg and his collaborators (1924–1927) in their study of the carbohydrate metabolism of surviving tissues, normal and pathological.

An enquiry into the problem whether the basic chemical reactions which yield energy for the maintenance and growth of cells are the same in kind and velocity for normal and abnormal tissues has resulted in establishing the fact that these tissues exhibit fundamental differences in their manner of utilising carbohydrates.

Warburg has shown that malignant tissues, like certain varieties of yeasts, possess the dual capacity to metabolise carbohydrates by oxidative and splitting processes.

Normal growing tissues, while using the oxidative process (respiration) almost exclusively under *aerobic* conditions, have the power of splitting carbohydrate to lactic acid under *anaerobic* conditions (glycolysis).

The characteristic property of the cancer cell is its power and habit, even under *aerobic* conditions, to metabolise carbohydrate by both these processes.

That the energy freed by the glycolytic process (amounting, in the case of tumour tissue to about 35–40 % of that freed by respiration) is utilised in the cell economy, cannot be definitely established, but experiments on survival of tumour cells *in vitro* by Okamoto [1925] suggest that this is the case, while Warburg's studies on the inhibiting effect of narcotics on the glycolytic functioning of tumour cells would seem to show that glycolysis is a reaction associated with cell structures, the liberated energy being at the disposal of the cell.

In this connection the following questions arise. (1) Is this peculiarity of metabolism an exclusive feature of malignant tissue? (2) Are there quantitative relationships between the magnitudes of the respiration and the *aerobic* and *anaerobic* glycolyses, which afford a means of differentiating normal from pathological tissues?

Warburg has, at various times, suggested the following generalisations. (*a*) The ratio $\frac{\text{aerobic glycolysis}}{\text{respiration}}$: this was found to be approximately three for

tumour tissue, and small or zero for normal tissues; (*b*) the value U, or excess fermentation, based upon the assumption that the Pasteur reaction is functioning at its maximum efficiency. U would then represent the minimum theoretical value for the aerobic glycolysis. For tumour tissue, this expression was found to be positive, for normal tissues, negative.

Many facts have been brought forward, which suggest that such attempts to differentiate malignant tissues, with respect to their carbohydrate metabolism, from normal resting or growing tissues are not fully justified.

Murphy and Hawkins [1925] found great variability in their results. Rat placenta, according to these authors, behaved as a malignant tissue, and many spontaneous mouse tumours fell into the category for normal resting tissues. Pentimalli [1927], regarding tumours as the result of a repeatedly disturbed regeneration process, found that regenerating muscle in the abdominal wall of young chicks behaved, with respect to its glycolytic metabolism of carbohydrates, qualitatively in the manner of tumour tissue, though quantitatively the results were considerably smaller. Fleischmann and Kubowitz [1927] found that the leucocytes of geese show a carbohydrate metabolism similar to that of tumours. Warburg [1927] records the fact that red blood corpuscles also possess a positive U value, and suggests that this anomaly may be due (as also with white blood cells) to the fact that they are free-living cells which are nearing the end of their life-cycle in the circulation. Warburg, Krebs [1927] and Tamiya [1927] have found that the retina is an exceptional tissue, since it possesses a glycolytic capacity of greater magnitude than that of any other animal tissue. In this case Warburg has introduced a new conception, showing acceleration or retardation of anaerobic glycolysis with the progressive development of the tissue, to differentiate tumour from retinal metabolism.

For normal tissues the value $\frac{dQ_{\mathrm{M}}^{\mathrm{N}_2}}{dt}$ (see p. 1291) is negative, for tumour tissues zero, and for retina tissues positive.

It appeared of interest, in the light of extrinsic hypotheses on the cause of carcinogenesis, to study the carbohydrate metabolism of overgrowths associated with recognised viruses, and accepted generally as being devoid of the essentials of malignancy.

The lesions of fowl pox in pigeons, vaccinia lesions in young chickens, vaccinia lesions in rabbits, and human warts have been examined up to the present time. A study of the metabolism of brain tissue of cavies dying of rabies has also been made, where an intracellular virus is active, but unaccompanied by any tissue proliferation. The Rous sarcoma has also been examined.

Where proliferation has taken place and a gross lesion is manifested, the carbohydrate metabolism was found to be greatly enhanced, and in a manner analogous to that of tumours. Where no hyperplasia was present, no deviation was found from the normal values for the tissue concerned.

Technique and expression of results.

The manometric technique elaborated by Warburg has been followed throughout. It is fully described in Warburg's collected papers [1926]. Any variations from the standard methods of determination of the metabolism quotients are referred to under separate headings.

The mode of expressing results is that used by Warburg. Simultaneous measurements are made of respiration, aerobic and anaerobic glycolysis, and their magnitudes expressed as the number of mm.^{3} of gas consumed or evolved per mg. of dried tissue per hour.

$$Q_{O_2}\text{ (respiration)} = \frac{\text{mm.}^3\text{ oxygen consumed}}{\text{hours} \times \text{mg. dry tissue}}.$$

$$Q_M^{O_2}\text{ (aerobic glycolysis)} = \frac{\text{mm.}^3\ CO_2\text{ evolved by lactic acid formation in } O_2}{\text{hours} \times \text{mg. dry tissue}}.$$

$$Q_M^{N_2}\text{ (anaerobic glycolysis)} = \frac{\text{mm.}^3\ CO_2\text{ evolved by lactic acid formation in } N_2}{\text{hours} \times \text{mg. dry tissue}}.$$

Fowl-pox.

In measuring the metabolism quotients of these lesions, certain inherent difficulties arise, which are not met with in the more straightforward cases of transplanted animal tumours or normal animal tissues. In order to get the maximum effects *in vitro* of dissolved gaseous and solid metabolites, perfect diffusion of these to the cells most remote from the surface must be ensured. Under the experimental conditions used, it can be calculated theoretically that the maximum thickness of the tissue slices must be no greater than 0·5 mm. Owing to the fatty nature of these lesions, the practical difficulties of obtaining such thin sections are great, with the result that the quotients obtained are probably less than the true values which would be found under perfect conditions of diffusion. These lesions moreover are not homogeneous in character, and allowances must obviously be made on this account.

The sections were prepared by cutting perpendicularly to the skin surface, and, on the average, each section had an area of approximately 0·5 cm.^{2}, and a dry weight of 15–30 mg. A histological examination showed a greatly thickened and proliferated epidermal layer, a high percentage of fat, connective tissue, and occasionally a few muscle cells.

The muscle cells, as shown by Meyerhof [1921], Pentimalli [1927], and controls quoted later for Rous tumours, show a very low carbohydrate metabolism under the experimental conditions. Figures quoted by Warburg [1926] indicate that connective tissue shows a negligible metabolism, and it seems probable that the fat makes no contribution to the results obtained.

On the basis of both histological and chemical estimations of fat, a minimum of 50 % of the total dry weight was found. It seems justifiable, therefore, to multiply the actual figures obtained by at least 2, in calculating the metabolism of the proliferating epithelium. The following table of results shows the experimentally obtained quotients for a series of lesions at their maximum phase of hyperplasia.

Table I. *Carbohydrate metabolism quotients of fowl-pox lesions.*

No. of days after transmission	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	Meyerhof quotient	U
10	−3·3	+4·7	+10·0	1·6	+3·4
9	−3·8	+5·1	+11·8	1·7	+4·2
10	−4·4	+6·5	+12·3	1·3	+3·5
9	−4·8	+5·2	+ 8·4	0·7	−1·2
9	−1·1	+3·3	+10·0	—	+7·8
7	−6·0	+5·2	+ 8·5	0·6	−3·5
5	−3·2	+5·5	+11·3	1·8	+4·9
8	−4·3	+5·9	+12·4	1·5	+3·8
13	−3·1	+2·8	+ 8·3	1·8	+2·1
11	−7·8	+8·5	+18·0	1·2	+2·4
15	−2·1	+2·9	+ 7·3	2·1	+3·1

The measurements were made in Ringer solution, containing glucose and sodium bicarbonate, at 37·8°, the gas phase being 5 % CO_2 in oxygen for aerobic, and 5 % CO_2 in nitrogen for anaerobic measurements.

The composition of the saline medium used was:

Salt	Moles per litre
NaCl	0·121
KCl	0·0025
$CaCl_2$	0·0018
$NaHCO_3$	0·0025
Glucose	0·2

In dealing with sections composed of different types of tissue, it is clear that the aerobic values obtained (dependent upon a calculation from the observed gas-exchange in two vessels containing different amounts of nutrient media) would only be valid if the sections used were entirely similar in composition. This condition is difficult to obtain, and two methods have been used to minimise errors arising from this:

(*a*) measurements were made with adjacent sections;

(*b*) measurements were made with the same section, making consecutive observations in different volumes of Ringer solution.

The results obtained have been similar by both methods. These results, making a minimum allowance of 50 % for the fat included in this dry weight, may be summarised as follows:

Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	M.q.	U
−6·6 to −15·6	+9·4 to +17·0	+20 to +36	1·2 to −2·1	+4·8 to +15·6

A comparison of these values with those obtained by Warburg for animal, human and avian tumours, shows them to be of the same order of magnitude, and to possess the same relationships to one another. In particular, the excess fermentation, U, is positive in nearly every case, making these lesions fall into the category of malignant tumours, if the most recent classification of Warburg be adopted.

Certain results obtained with embryonic tissue and liver show different values for the aerobic glycolysis when measured in serum from those obtained

by measurements in Ringer. Warburg attributes this to the extreme sensitiveness of the Pasteur reaction, the respiratory activity failing to function effectively owing to rapid damaging of tissue placed in Ringer solution. Hence the metabolism of fowl-pox lesions was measured in serum, both foreign (inactivated horse-serum) and autologous serum (pigeon).

Table II gives the results of a series of experiments, and indicates that the change of medium has little effect on the metabolism quotients.

Table II.

Serum	No. of days after transmission	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	M.q.	U
Pigeon	4	−1·9	+ 4·8	+ 7·0	1·2	+ 3·2
	10	−4·6	+12·9	+20·5	1·65	+11·3
	11	−2·1	+ 9·1	+16·3	3·0	+12·1
	10	−4·2	+13·0	+20·5	1·8	+12·1
	13 (regressing)	−1·0	+ 2·7	+ 7·5	—	+ 5·5
Inactivated horse	7	−4·0	+ 3·3	+ 8·2	1·2	+ 0·2
	6	−3·4	+ 2·8	+ 7·3	1·3	+ 0·5
	9	−3·1	+ 5·8	+ 9·6	1·2	+ 3·4
	10	−4·2	+ 5·9	+11·3	1·3	+ 2·9

In order to see if there was a correlation between the metabolism and the progressive epithelial hyperplasia, a series of measurements was made from day to day of the developing lesions. The results are represented graphically in Fig. 1.

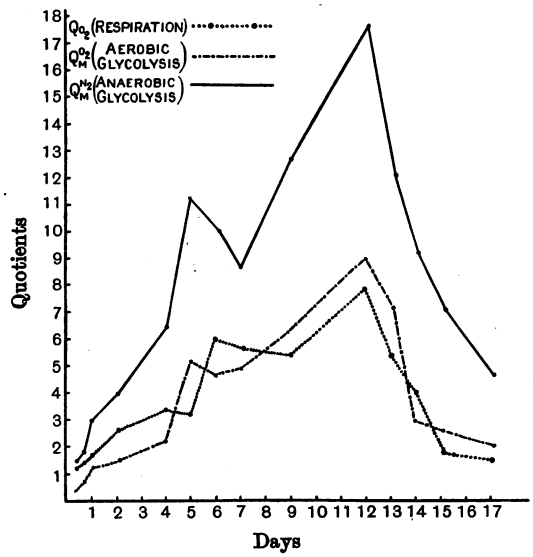

Fig. 1. Curves showing the changes in the carbohydrate metabolism of fowl-pox lesions, during development and regression.

Normal pigeon skin gave low values for all the quotients,

$$e.g.\ Q_{O_2} = -0{\cdot}9;\ Q_M^{O_2} = +0{\cdot}5;\ Q_M^{N_2} = +1{\cdot}7.$$

As the lesion attained its maximum growth (12 or 13 days), the respiration, aerobic and anaerobic glycolysis increased, and with its slow regression all these quotients decreased to the level typical of normal skin.

To account for the aerobic glycolysis found in red and white blood cells, Warburg suggests that they are in a degenerating condition. The progressive character of the metabolism with proliferation of the epithelium in fowl-pox lesions, where histological examination shows numerous mitotic figures, suggests that the concept of degeneration does not account for such increases as are observed. Moreover, when regression commences, and it would be expected that degeneration would follow, the glycolytic metabolism tends to disappear.

Vaccinia.

(*a*) *Rabbit.* The vaccinia lesion consists of dense infiltration of the dermis with polymorph leucocytes. The adventitial or reticulo-endothelial cells are numerous and in active mitosis. There is no marked change in the Malpighian layer, except early chromatolysis and swelling. Mitotic figures are occasionally seen.

Table III shows the quotients obtained in two experiments with these lesions, and with normal rabbit skin.

Table III.

Tissue	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$
Vaccinia lesion (rabbit)	−3·3	+2·3	+2·8
	−3·0	+2·0	+2·7
Normal rabbit skin	−1·0	+1·3	+1·9

Sections perpendicular to the skin surface were used and the measurements made in Ringer solution containing glucose and bicarbonate. The figures refer to the whole lesion, with no allowances for material of very small, or no, measurable metabolism.

The increase in metabolic activity is small, and might be attributed to the presence of leucocytes.

(*b*) *Young chickens.* The lesions produced in one to seven days old chickens by the vaccinia virus are identical histologically with those produced by the fowl-pox virus, as shown by Findlay [1928] and Ludford [1928].

Table IV.

Tissue	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	M.q.	U
Vaccinia lesion	−5·0	+4·2	+11·1	1·4	+1·1
	−3·2	+3·6	+ 7·8	1·3	+1·4
	−4·8	+4·0	+10·6	1·4	+1·0
Normal chicken skin	−1·0	+0·4	+ 1·5	—	—

Corresponding to the epithelial hyperplasia, a great increase in the carbohydrate metabolism is noticed (Table IV).

The measurements were made in Ringer solution, and the figures represent the metabolism of the whole lesion. The same difficulties of obtaining sections of the necessary thickness for perfect diffusion of metabolites and the presence of non-reacting tissues make the values quoted minimal.

Human warts.

The metabolism quotients found in the three cases examined are much smaller than those found for fowl-pox lesions (Table V).

The gas exchanges observed during the experiments were comparatively large, and suggest an active metabolism for the relatively small percentage of physiologically active epithelial cells present. Considerably more than half of the section was composed of fully keratinised epithelium and connective tissue, giving the dry weight a value many times greater than that of the reacting portion.

Table V.

	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	M.q.	U
A	−1·7	+2·2	+4·1	1·1	+0·7
B	−1·2	+1·7	+3·9	1·8	+1·5
C	−1·2	+1·6	+3·8	1·8	+1·4

Rabies.

The strain of virus used was a "virus fixe" which is highly pathogenic for guinea-pigs, and on intracerebral inoculation produces a fatal result in a week. The animals were killed when moribund and the brain removed for examination.

Sections were cut tangentially from the cerebral cortex and hippocampal region.

The average quotients obtained for the outer layer of normal guinea-pig cerebral cortex were:

$$Q_{O_2} = -10{\cdot}3;\ Q_M = +2{\cdot}5;\ Q_M^{N_2} = +20{\cdot}3.$$

Table VI shows the figures obtained for the brain of guinea-pigs dying of rabies.

Table VI.

Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$
− 9·2	+2·9	+25·6
− 8·8	+2·7	+18·8
− 7·0	+1·0	+20·4
−10·8	+1·9	+20·2

Here no hyperplasia is found and the activities of an intracellular virus seem to cause no alteration in the carbohydrate metabolism of the cells.

ROUS CHICKEN SARCOMA.

Warburg only quotes one experiment on the metabolism of this tissue, showing a behaviour similar to that of the rat tumours, studied so extensively.

Many measurements, in Ringer solution, have been made and Table VII shows the great variability in the results obtained. The respiration was, in some cases, found to be abnormally high, which had the effect of making U negative, and the Meyerhof quotient very low. In all cases a histological control showed no areas of necrosis, only the outer shell of the growth being used for the experiments. It has not been possible to correlate these wide variations with the age of the bird or the general necrotic or haemorrhagic conditions of the tumour.

Table VII.

No. of days after transmission with cell-free filtrate	Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$	M.q.	U
10	− 8·7	+20·3	+27	0·8	+ 9·6
11	− 4·0	+13·8	+21·8	2·0	+13·8
13	−22·0	+23·9	+36·6	0·6	− 7·4
14	−26·9	+21·3	+30·1	0·3	−23·7
14	−10·1	+18·1	+28·1	1·0	+ 7·9
10	−20·0	+21·2	+33·3	0·6	− 6·7
10	−10·4	+16·3	+31·8	1·5	+11·0
11	− 7·2	+16·2	+29·2	1·8	+14·8
12	− 4·8	+22·6	+33·8	2·3	+24·2
13	− 7·2	+21·8	+35·5	1·9	+21·1

In order to study any possible immediate effect of the agent responsible for the initiation of Rous sarcoma on the metabolism of the muscle cells near the site of injection, a series of measurements was made at daily intervals from the time of transmission.

Cut muscle under the experimental conditions shows a low metabolism and a control experiment was made simultaneously with muscle from the opposite breast of the bird. A typical value obtained for cut muscle is:

$$Q_{O_2} = -2{\cdot}5;\ Q_M^{O_2} = +1{\cdot}2;\ Q_M^{N_2} = +2{\cdot}5.$$

A slight increase in metabolic activity of the muscle was noticed during the first two days after injection of cell-free filtrate (possibly due to leucocytic reaction) followed by a return to the normal low values of the undisturbed muscle. Only when a histological examination showed foci of tumour cells scattered through the muscle was any real increase found in the metabolism quotients. The figures obtained showed a rough correspondence in their magnitude to what might be theoretically calculated from the proportion of tumour tissue seen microscopically. It would seem correct to conclude that the tumour cells possess their characteristically high metabolic rate from the time of their appearance rather than that a progressive development of this metabolism takes place over a transitional period.

Summary and Discussion.

1. Using the technique developed by Warburg, the carbohydrate metabolism of a series of lesions associated with intracellular viruses has been examined.

2. The activities of the virus in fowl-pox lesions, vaccinia lesions in young chickens, and in human warts, are accompanied by epithelial hyperplasia and in these cases an active metabolism has been found, corresponding in type to that characteristic of malignant tissue.

Making allowances for non-reacting tissue in the sections examined, the magnitude of the respiration and aerobic and anaerobic glycolysis approximates to that found for tumours.

3. In the case of vaccinia lesions in rabbits, where little or no epithelial hyperplasia is evident, the lower quotients obtained are probably due to leucocytic invasion.

4. The brain of guinea-pigs dying of rabies provides an example of a tissue where a virus is active without stimulating the cells to abnormal division, and no deviation is found from the normal metabolism.

5. The metabolic activity of fowl-pox lesions shown graphically exhibits a rough parallelism in its magnitude to the state of development or regression of the lesion.

6. Great variability in the values for respiration in the Rous chicken sarcoma are recorded.

7. After the injection of Rous sarcoma cell-free filtrate in the muscle of fowls a slight rise in the metabolism was noticed during $4\frac{1}{2}$ hours, followed by a return to the normal values obtained for resting muscle.

During the subsequent development of the tumour in its early stages, the figures obtained indicate that Rous sarcoma cells, on their first appearance, assume the high metabolic activity characteristic of the fully grown tumour.

The general conclusion which may be drawn from these results would seem to be that the magnitude and relationships of the respiratory and glycolytic processes, found by Warburg to be characteristic of malignant tissues, are not specific for malignant tissues but are a common feature of pathological overgrowths.

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