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THE METABOLISM OF TUMORS IN THE BODY.

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In this contribution we discuss the question of whether tumor cells in living animals can be killed off through lack of energy, and the related question of how the tumors are supplied with oxygen and glucose in the body.

We assume it is understood that tumor cells obtain the energy required for their existence in two ways: by respiration and by fermentation. In respiration they burn organic materials to carbon dioxide and water; in fermentation they split glucose to lactic acid. All tumors so far tested behave fundamentally alike. There is no essential difference between the cancer cells of transplanted rat tumors and spontaneous tumors, sarcoma and carcinoma cells, and the tar carcinoma, and Rous sarcoma produced by filtrate.

The fermentation of tumors was first found with cut pieces of tumor *in vitro*.[1] C. and G. Cori[2] demonstrated it in living animals as well. They determined the glucose and lactic acid in the axillary veins of hens having in one wing a Rous sarcoma, and found in 100 cc. of blood 23 mg. less glucose and 16 mg. more lactic acid on the tumor side than on the normal side. A corresponding experiment with a human fore-arm tumor showed in 100 cc. of blood 12 mg. less glucose and 9 mg. more lactic acid on the tumor side.

In experiments on the nourishment of tumors through the blood stream, we, like Cori, determined the glucose and lactic acid in tumor veins. Our procedure differed from Cori's in that we compared tumor veins and arteries, *not* tumor veins and corresponding normal veins. Our differences were greater than Cori's because we went closer to

[1] Warburg, O., *Biochem. Z.*, 1923, cxlii, 317. Warburg, O., Posener, K., and Negelein, E., *Biochem. Z.*, 1924, clii, 309. Negelein, E., *Biochem. Z.*, 1925, clviii, 121; 1925, clx, 307.

[2] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1925, lxiv, 11; 1925, lxv, 397.

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the tumor and so obtained tumor blood less diluted with blood from normal veins.

For experimental material we used transplanted tumors of either Flexner-Jobling's rat carcinoma or Jensen's rat sarcoma. The Jensen sarcoma is the better of the two because it more rarely becomes necrotic.

Properly inoculated Flexner and Jensen tumors are practically pure cultures of tumor cells. This is their advantage over spontaneous tumors, and we believe it is their chief difference from the latter, which are mixed cultures of tumor cells and normal cells.

I. Killing-Off of Tumor Cells in Vitro.

As Y. Okamoto[3] found, tumor cells can live without oxygen in serum containing glucose. Pieces of Flexner carcinoma after 24 hours deprivation of oxygen, Jensen sarcoma after 72 hours, could be transplanted with normal results. Hence tumor cells are able to exist a certain time exclusively at the expense of fermentation.

On the other hand, respiration alone is sufficient, without fermentation, to maintain the life of the tumor cell. We dialyzed rat serum in Ringer solution until it was free from glucose, and then kept pieces of tumor in this serum, passing oxygen through. Such pieces kept for some time in glucose-free serum and then transferred to serum containing glucose, showed normal respiration and fermentation, proving that most of the tumor cells were uninjured.

Interfering with one of the two reactions furnishing energy to tumor cells is not enough to kill them. It is necessary to stop both respiration and fermentation, if the cells are to be killed for want of energy. We achieved this by keeping pieces of tumor at body temperature in serum free from glucose and oxygen for various lengths of time. After a period of hours, the normal living conditions were restored by adding glucose and oxygen, and metabolism was measured. It was found that 4 hours interference with respiration and fermentation sufficed to kill off most of the cells, and to stop metabolism.

We obtained similar results when we caused energy-want by killing tumor animals and allowing the tumors to remain in the bodies

[3] Okamoto, Y., *Biochem. Z.*, 1925, clx, 52.

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at 37° for different lengths of time. Here again, most of the tumor cells were killed after about 4 hours lack of energy.

II. Concerning Want of Energy in Living Animals.

If we consider whether the principle of killing tumor cells through want of energy is transferable to living tumor animals, the prospect is at first poor. Not only tumor cells, but all cells, require energy. Furthermore, the tumor cell is more versatile than the normal cell as far as the obtaining of energy is concerned. It can choose between fermentation and respiration, while the normal cell is confined to oxygen respiration. Imposing on the body only one of the conditions required for the killing of tumor cells would suffice to kill normal cells. But although this is true, there is one circumstance of decisive importance relating to the supplying of the tissue with glucose and oxygen through the blood stream. If the provision for tumors were less complete than that of normal cells, then depression of arterial oxygen and glucose content would harm the tumor cells without affecting the better supplied normal organs. The resistance of single tumor cells is not to be compared with that of single normal cells, but rather the tumor as a whole with the organism as a whole. An overpopulated city is more sensitive to stoppage of food supply than a normally populated city, even when the inhabitants can all endure hunger alike.

III. The Supplying of Tissue with Glucose.

How well a tissue is provided for depends on its requirements, on the number of capillaries traversing it, and on the speed of blood-flow in the capillaries. To measure the provision of one substance, glucose for example, we determined the glucose content in in-going and out-going blood-vessels. The percentage decrease in glucose concentration was thus a measure of the provision of glucose. If this percentage was small, the provision was plentiful, and *vice versa*.[4]

[4] If C_0 is the glucose concentration in the artery and C that in the vein, the percentage decrease in glucose is $\frac{C_0-C}{C_0}$, and the provision is equal to the reciprocal, or $\frac{C_0}{C_0-C}$.

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The experimental animals were narcotized with ethyl urethane (1 cc. of 20 per cent urethane solution per 100 gm. of body weight subcutaneously) and the blood-vessels were dissected out during narcosis. If the veins were large enough, like the jugular, iliac, renal, and portal veins, 1 cc. of blood was removed with a syringe. Tumor veins were cut out with scissors. The out-flowing blood was taken up with filter paper and weighed in closed balance-vials. The removal of arterial blood followed, by puncturing the abdominal aorta. The glucose estimation was done after the method of Hagedorn-Jensen.

In tumor experiments the following precautions are to be observed:

1. The tumors should be abdominal, and not too small, 10 gm. being a good average. Every time, after the removal of the blood, necrosis should be looked for. Only blood from non-necrotic tumors should be analyzed.

2. The veins from which blood is taken should be grown together with the tumor. The capsule rich in blood-vessels which covers the tumor and in which frequently lie veins coming from the viscera, is thrown aside.

3. The blood from tumor veins is dark. If, after the cutting out of a vein, the blood flows bright or pulses as it flows out, one of the fine arteries which often lie hardly visible beside the veins, has been cut.

4. Only those tumors are available which lie so that one can see their veins, the roots of which should be more or less visible. Searching for veins by changing the natural position of the tumor is to be avoided, because the blood-vessels are thus twisted.

5. Only a few minutes should elapse between the opening of the abdomen and the completion of blood removal. In time the tumor cools off (a small one faster than a large one), so that too little metabolism is found. The fermentation of tumor cells is almost nil at 20°.

The results of our experiments are collected in Table I (normal experiments) and Table II (tumor experiments).

The tables show that various tissues are differently supplied with glucose. Most completely supplied is the region of the jugular vein, least completely of the normal tissues the region of the portal vein. The tumor is supplied three times as poorly as the portal region. In blood flowing through a tumor the concentration of glucose falls on the average to 57 per cent of the arterial concentration. The tumor takes on the average 70 mg. of glucose from 100 cc. of blood, the

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normal tissue 2 to 16 mg. Thus the normal tissues—in resting animals—have the advantage of the tumor as far as glucose supply is concerned.

IV. Lactic Acid Formation in the Body.

The glucose which disappears as the blood passes through a tumor is partially converted to lactic acid, and partially burned to carbon

TABLE I.

	Glucose in 100 cc. of blood.		$C_0 - C$	$\frac{C_0 - C}{C_0}$
	Artery. C_0	Vein. C		
	mg.	*mg.*		*per cent*
Jugularis	99	97	2	2
Renalis	111	108	3	3
Iliaca	143	125	18	13
Portæ (fasting)	91	75	16	18

TABLE II.

	Glucose in 100 cc. of blood.		$C_0 - C$	$\frac{C_0 - C}{C_0}$
	Artery. C_0	Vein. C		
	mg.	*mg.*		*per cent*
Jensen sarcoma	92	44	48	52
" "	144	77	67	47
" "	103	31	72	70
" "	144	69	75	52
" "	120	60	60	50
" "	136	41	95	70
Average	124	54	70	57

dioxide and water. To find out how the glucose consumption is divided between respiration and fermentation, we determined the lactic acid in the tumor, and compared the increase in lactic acid with the decrease in glucose. To determine lactic acid we used a method recommended by Clausen.[5] We removed the protein after Folin-Wu,

[5] Clausen, S. W., *J. Biol. Chem.*, 1922, lii, 263.

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glucose after Van Slyke, oxidized the lactic acid to aldehyde according to the procedure of Fürth-Charnass and Embden, and titrated the aldehyde in the distillate, after Clausen, in bicarbonate solution with N/100 iodine. 1 cc. of normal blood and 0.5 cc. of tumor blood, gave with this treatment sufficiently decisive titration results. Furthermore, the lactic acid experiments corresponded in every respect with the glucose experiments. Here as well the above mentioned five precautions must be observed.

A few experiments on the lactic acid formation of normal organs may be given before the tumor experiments. Table III contains the results for five normal tissue systems of normal animals (without tumors).

TABLE III.

	Lactic acid in 100 cc. of blood.		$C - C_0$
	Artery. C_0	Vein. C	
	mg.	*mg.*	
Jugularis	16	17	+1
Renalis	28	15	−13
Iliaca	44	39	−5
Portæ	22	21	−1
Placenta (maternal placenta vein, weight of embryo, 8.7 gm.)	17	13	−4

Table III shows that in no case did we find more lactic acid in veins than in arteries. The lactic acid content of venous and arterial blood was either, within the limits of error, the same, or as in the case of kidney, was smaller than in the venous blood. Hence none of the organ systems tested formed lactic acid, nor did the placenta, which —according to an observation of Murphy and Hawkins[6]—formed lactic acid in Ringer solution containing glucose.

Thus the conception of Liebig[7] that normal tissues under normal living conditions put no lactic acid in the blood was confirmed. On the contrary, they remove it from the blood. Normal body cells form lactic acid in general only when their oxygen is cut off or their respir-

[6] Murphy, Jas. B., and Hawkins, J. A., *J. Gen. Physiol.*, 1925–26, viii, 115.
[7] Liebig, J., *Ann. chem. Pharm.*, 1847, lxii, 338.

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ation checked (Liebig,[7] Araki,[8] Zillessen[9]). This has recently been better demonstrated by Hill, Long, and Lupton,[10] as well as Bruno Mendel.[11] Hill and his coworkers found that during forced bodily work the lactic acid of the blood increased. In this case the diffusion of oxygen into the muscle cells is not sufficient to cover the oxygen requirement of the muscle. Mendel caused want of oxygen by stoppage of the veins. He saw that after a few minutes lactic acid increased in the region of the stopped blood vessels. He pointed out that all obstruction of circulation is to be avoided in experiments on lactic acid formation.

Since the tissues tested do not, under normal conditions, set lactic acid free in the blood, but rather remove it from the blood, one must inquire from where the lactic acid in the blood of normal animals comes. Perhaps the "normal" source of lactic acid in the blood is exclusively the red blood cells, which, even when saturated with oxygen, always develop small amounts of lactic acid. In rat blood, the glycolysis of these erythrocytes would be enough to raise the lactic acid content of lactic acid-free blood to the normal in the course of 2 hours.

V. Lactic Acid Formation in the Tumor.

The results of our tumor experiments are collected in Table IV. In every case, the veins contain more lactic acid than the arteries, hence in every case lactic acid is formed as the blood passes through the tumor. The lactic acid formation was very small in Experiment 1, very large in Experiment 9. Taking the average of the other experiments, the tumor puts 46 mg. of lactic acid into 100 cc. of blood.

We have demonstrated lactic acid formation in tumors by still another arrangement. The abdomen of the tumor animal was opened, a pit made in the tumor, in which sufficient liquid for lactic acid determination collected in a few minutes. The fluid, composed of

[8] Araki, T., *Z. physiol. Chem.*, 1891, xv, 335.

[9] Zillessen, H., *Z. physiol. Chem.*, 1891, xv, 387.

[10] Hill, A. V., Long, C. N. H., and Lupton, H., *Proc. Roy. Soc. London, Series B*, 1924, xcvi, 438.

[11] Mendel, B., Werner, E., and Goldscheider, I., *Klin. Woch.*, 1925, iv, 306.

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tissue sap and blood in varying quantities, was drawn off with filter paper. In every case we found more lactic acid in the sucked up

TABLE IV.

	Lactic acid in 100 cc. of blood.		$C - C_0$
	Artery. C_0	Vein. C	
	mg.	*mg.*	
1. Jensen sarcoma	29	40	(11)
2. " "	46	80	34
3. " "	21	79	68
4. " "	27	56	29
5. " "	29	96	67
6. " "	29	82	53
7. " "	39	87	48
8. " "	28	62	34
9. " "	21	230	(209)
10. " "	44	78	34
Average			46

TABLE V.

	Lactic acid in 100 cc. of solution.		$C - C_0$
	Artery. C_0	Tumor cavity. C	
	mg.	*mg.*	
1. Jensen sarcoma	34	124	90
2. " "	20	89	69
3. " "	9	63	54
4. " "	31	106	75
5. " "	33	94	61
6. " "	26	75	49
7. " "	32	102	70
8. " "	40	118	78
9. " "	34	113	79
Average			69

fluid than in the aorta. On the average, as shown in Table V, there were 69 mg. more lactic acid per 100 cc. blood than in the aorta.

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VI. Respiration of the Tumor in the Body.

On the basis of glucose and lactic acid determination we can now estimate, if only crudely, how the glucose consumption of the tumor is divided between respiration and fermentation. The tumor takes on the average 70 mg. of glucose from 100 cc. of blood, and returns 46 mg. of lactic acid to the same amount of blood. Thus of all the glucose used $\frac{46}{70}$ = 66 per cent is used in fermentation, the remainder in respiration.

A second consequence concerns the oxygen content of the tumor veins. From 100 cc. of blood the tumor takes 70 − 46 = 24 mg. glucose for respiration. If the conditions are stationary, it must take from the same amount of blood enough oxygen to burn 24 mg. glucose; that is, 18 cc. or as much as is contained in 100 cc. of rat blood. Thus the blood on its way through the tumor gives up most of its oxygen. The providing of the tumor with glucose is bad, with oxygen still worse.

VII. Fermentation of the Tumor in the Body.

The respiration of the tumor is hardly affected by the falling off of oxygen concentration in tumor veins, because respiration is very independent of the oxygen concentration.

Fermentation, however, is easily affected by decrease in glucose concentration. According to experiments on slices of tumor, the aerobic fermentation per hour of Jensen's sarcoma is as follows:

With	0.2	per cent glucose in surrounding serum:					7	per cent of tumor weight.					
"	0.1	"	"	"	"	"	5	"	"	"	"	"	
"	0.05	"	"	"	"	"	2	"	"	"	"	"	

The fermentation varies considerably within the concentration limits 0.05 to 0.2 per cent. The maximum fermentation is at about 0.2 per cent glucose concentration.

According to Table II the average arterial glucose concentration of tumor animals amounts to 0.12 per cent, the average of tumor veins 0.054 per cent. It follows from this that the fermentation of tumor cells varies in different parts of the tumor. Tumor cells at

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the point of entrance of a capillary ferment about 5 per cent of their weight in glucose per hour. Tumor cells near the venous end of a capillary ferment in the same time only 2 per cent of their weight. Fermentation decreases in the direction of capillary blood-flow, and amounts to about 3.5 per cent of the tumor weight per hour.

Since this is half of the maximum possible fermentation of tumor cells, it must be possible to double the fermentation by raising the arterial glucose concentration. But merely raising the *arterial* glucose concentration to 0.2 per cent is not enough to call forth the maximum tumor fermentation. It is necessary to raise the glucose content of tumor *veins* to 0.2 per cent. We achieved this by injecting 2 cc. of a 25 per cent glucose solution in the caudal vein of a tumor rat. After 20 minutes we found:

Glucose content of aorta blood 0.342 per cent.
" " " tumor vein blood 0.207 per cent.

Thus the glucose consumption in 100 cc. of blood was 135 mg. This corresponds well with our prediction. It is almost twice as much as the tumor consumed with normal blood-sugar concentration.

VIII. The Effect of Want of Glucose.

Silberstein,[12] von Witzleben,[13] and Rondoni[14] have published experiments which make it seem likely that relationships exist between fermentation and tumor-growth. Insulin checks the growth, glucose accelerates it. We can understand the effects if we consider that slight changes in the blood-sugar concentration affect fermentation considerably, because the tumor is badly supplied with glucose. Experiments with slices of tumor *in vitro* give in this respect a false picture of the sensitivity of fermentation in the body. If for example we reduce the glucose content of serum surrounding a slice of tumor from 0.1 per cent to 0.05 per cent, the aerobic fermentation of the tumor is halved. But if we vary the arterial glucose content within the same limits, fermentation is not halved, but, as can be easily shown,

[12] Silberstein, F., *Wien. klin. Woch.*, 1925, xxxviii, 356.
[13] von Witzleben, H. D., *Klin. Woch.*, 1925, iv, 2115.
[14] Rondoni, P., *Klin. Woch.*, 1926, v, 465.

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it is reduced to one-quarter, on account of the bad provision for the tumor.

Different from the question of checking tumor growth is the problem of killing off tumor cells in living animals. Tumor cells obtain the oxygen necessary for respiration from the blood circulation. Consequently they can exist without glucose. Even if it were possible to remove the blood-sugar entirely in living animals, the life of the tumor would not be threatened.

To support this statement, we kept tumor animals at very low blood-sugar content in insulin convulsions for hours, and then measured the metabolism of pieces of tumor so treated. We found respiration and fermentation nearly normal, a proof that the main part of the tumor cells was intact.

IX. The Effect of Want of Oxygen.

In order to kill tumor cells in living animals through want of energy it is necessary, as in experiments *in vitro*, to stop respiration as well as fermentation. Nature itself cares for the latter. If we consider a tumor which is traversed by series of parallel capillaries, cut through the middle perpendicular to the line of the capillaries, we obtained two tumor halves, of which we will call one the "arterial," the other the "venous" half. On account of the poor provision of glucose, fermentation in the venous half is not sufficient to maintain, in the absence of oxygen, the life of the cells. If it is possible to check respiration in the venous half, then that half must die out.

We have carried out experiments in this direction. Tumor rats were kept in an atmosphere of 5 volume-per-cent oxygen with small pressure of ammonia to prevent acidosis.

After 40 hours treatment the tumor animals were killed, the tumors removed, and their metabolism measured *in vitro*. The results showed that most of the tumor cells were killed off. Respiration and fermentation of most of the tumors were almost nil, only a thin outer shell showing normal metabolism.[15]

Thus the anticipated effect was achieved, but it was far greater than expected. Clearly the suffocation of the venous half of the tumor resulted in the killing off of most of the arterial half.

[15] Recent experiments show that these results can be obtained after much less than 40 hours treatment (after several hours).

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We explain this by the assumption that want of oxygen in the venous part of the tumor kills not only the tumor cells but also the cells of the capillary. The result must be that the tumor capillaries become non-conducting, so that the arterial tumor half is injured.

X.

At first it seems paradoxical that cells which can live by fermentation can be killed off by want of oxygen. But there is really no contradiction here. Yeast cells, as well as tumor cells, can be killed through want of oxygen; in both cases only when the sugar required for fermentation is lacking.