

THE CATALYTIC EFFECT OF METHYLENE BLUE ON THE OXYGEN CONSUMPTION OF TUMORS AND NORMAL TISSUES

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It has previously been shown (1, 2, 3) that the addition of methylene blue, or similar reversibly oxidized and reduced dyes, increases the oxygen consumption of cell suspensions. It was also demonstrated that correlated with this increase, there is a decrease in the aerobic glycolysis* as shown by a diminished lactic acid formation (4). It was furthermore suggested (5) that the effect of the dye seemed to be proportional to the fermentative power of the cell. To test the validity of this hypothesis, use has been made of the fundamental researches of Warburg (6) on the metabolism of normal tissues and tumors. Warburg has shown there is a sharp difference between the metabolism of tumors and that of normal adult tissues. While in general normal adult tissues possess a high respiration, sufficient to check the appearance of the fermentative processes, tumors, on the other hand, have a low respiration, and consequently part of the energy necessary for the performance of cellular activities has to be provided by fermentation. The lactic acid produced in tumors may thus reach about 13 per cent of the weight of the tumor. If methylene blue and similar dyes act as catalysts for the oxidative processes of living cells only when the cells in question possess a fermentative power, these two kinds of tissues are the most appropriate material with which to test the validity of the hypothesis.

The experiments were performed on fresh rat tissues and rat tumors taken soon after death caused by a blow on the head. The tissues and tumors were cut with

* Whenever the word glycolysis is employed in this paper, it is used in the sense given by Warburg, *i.e.*, the splitting of one molecule of glucose into two molecules of lactic acid according to the equation $C_6H_{12}O_6 = 2C_3H_6O_3$.

a hand razor into thin slices, 0.4 mm. thick, to allow free diffusion of oxygen throughout the section. They were kept in a saline solution containing 0.15 gm. per cent of glucose, and a mixture of phosphate buffers, $\frac{M}{15}$ having a pH of 7.38. The oxygen consumption was determined in Warburg vessels with Barcroft manometers. The temperature of the water bath was 37.5°C.

The Effect of Methylene Blue on the Oxygen Consumption of Normal Tissues

The following normal tissues were employed: liver, kidney, brain (gray matter), spleen, and testicles. For the study of pancreas, rabbits were used. The respiration and carbohydrate metabolism of slices of these tissues have been determined *in vitro* by Warburg and his associates. Liver, pancreas and kidney possess no aerobic glycolysis. Brain and testicles are reported as having a small aerobic glycolysis. The metabolism of spleen has been determined by Murphy and Hawkins (7) and found to show an aerobic glycolysis.

In preliminary determinations, the metabolism of all these tissues was measured by the use of the well known Warburg methods, and found to be more or less like that reported by the above named investigators, except for the metabolism of rat testicles, where a QO_2 of -15.0 was found, and a QCO_2 of $+1.2$ instead of $QO_2 - 12.3$ and $QCO_2 + 7.2$ found by Warburg. In view of this discrepancy, the glucose consumption and lactic acid formation of rat testicles were determined chemically. As it can be seen from Table 1 there was no lactic acid formation, though there was destruction of glucose. The addition of methylene blue did not have any influence either on the speed of glucose consumption or in the lactic acid formation.

TABLE 1

The Effect of Methylene Blue on the Glucose Consumption and Lactic Acid Formation of Rat Testicles

Testicles continuously shaken in a saline solution containing phosphate buffer $\frac{M}{15}$ pH 7.38.

437.8 mg. testicle	Glucose in mg. per cent	Lactic acid in mg. per cent
Before incubation	114.0	66.9
2 hours after incubation control	87.0	66.4
M. B. added	87.0	66.8

TABLE 2
The Effect of Methylene Blue on the Oxygen Consumption of Normal Tissues

Kind of tissue	O ₂ consumption in c.mm. per hour		Per cent increase or decrease
	Before dye addition	After dye addition	
Rat kidney:			
1).....	47.6	47.3	-0
2).....	128.8	116.3	-9.7
3).....	59.0	56.2	-4.7
4).....	51.6	38.0	-26.3
5).....	63.0	46.5	-26.2
6).....	94.0	79.0	-15.9
Rat liver:			
1).....	37.2	37.5	-0
2).....	80.6	75.0	-6.9
3).....	41.7	40.3	-3.3
4).....	42.7	37.4	-12.4
5).....	54.2	52.2	-3.7
Rabbit pancreas:			
1).....	25.6	23.4	-8.6
2).....	22.0	20.6	-6.8
3).....	17.5	13.0	-25.7
4).....	23.6	19.0	-19.5
5).....	24.8	22.6	-8.9
Rat testicle:			
1).....	120.5	119.5	-0
2).....	118.9	114.9	-3.4
3).....	117.2	114.0	-2.7
4).....	140.7	140.0	-0
Rat spleen:			
1).....	31.0	33.4	+7.7
2).....	29.7	30.8	+3.7
3).....	38.3	42.1	+9.9
4).....	32.0	34.2	+6.9
5).....	34.8	37.0	+6.3
6).....	55.1	55.4	0
7).....	46.8	57.4	+22.7
Rat brain (gray matter):			
1).....	28.6	30.3	+6.0
2).....	35.3	38.6	+9.3
3).....	37.2	37.6	0
4).....	33.0	41.8	+26.6
5).....	25.8	31.9	+23.6
6).....	28.8	33.2	+15.3
7).....	29.2	31.7	+8.5

In Table 2 have been tabulated the results of the experiments on the oxygen consumption of normal tissues, and the effect of methylene blue. The values given do not refer to the weight of the tissue, but are the figures directly found. The oxygen consumption of these tissues, weighed after drying for some hours at 100°C., was found to correspond to the figures given by Warburg. In the tissues where there is no aerobic glycolysis, *i.e.*, lactic acid production, methylene blue has no catalytic effect; moreover, it exerts a definite toxic action as shown by a definite drop in the oxygen consumption. Methylene blue increases the oxygen consumption of brain tissue and spleen,

O₂

which possess an aerobic glycolysis of QCO₂ +2.5 and +2.3 respectively. Methylene blue does not increase the oxygen consumption of the tissue of rat testicles, nor does it have action on the lactic acid formation. It is interesting to note that the toxic effect of the dye observed in kidney and liver tissues is not found in testicles.

In order to provide further evidence of the action of methylene blue on the fermentation phase of cellular metabolism, the oxygen consumption of the above mentioned tissues was measured in the presence of KCN ($\frac{M}{800}$), previously neutralized to the same pH as that of the saline solution, and the effect of methylene blue determined afterwards. Cyanide is a specific respiratory poison as shown by Warburg, but it has no effect on the fermentative process which proceeds undisturbed. Incidentally, Dixon and Elliot's (8) observations on the influence of cyanides to inhibit partially the power of normal tissues to consume oxygen were confirmed.* While KCN inhibits completely the respiration of goose erythrocytes, yeast cells and *Arbacia* and starfish eggs, this inhibition is never complete in the case of normal tissues. In Table 3 are given average figures of the experiments. Liver, kidney, pancreas, and testicles, which normally did not show any increase in their oxygen consumption by the action of methylene blue, exhibit a manifest increase when the respiration is inhibited by cyanide and the glycolysis appears. In spleen, the increase of the oxygen

* After this paper had been sent to press, Howard, from Warburg's laboratory, showed that KCN inhibition of respiration is about complete when the tissues are kept in Ringer solution buffered with bicarbonate (*Biochem. Z.*, 1930, 221, 498). This recent communication does not affect the experiments related here.

consumption by the action of methylene blue is considerably enhanced by previous addition of cyanide.

Wendel (9) has reported that red blood corpuscles oxidize added sodium lactate in the presence of methylene blue. The addition of lactates to normal tissues, where the catalytic power of the dye is absent, produces neither an increase on the oxygen consumption nor a diminution of the added lactates. The determinations were made with liver, kidney and testicles, both by the manometric methods and by chemical analysis.

TABLE 3

The Effect of Methylene Blue on the Oxygen Consumption of Normal Tissues Previously Treated with KCN ($\frac{M}{800}$)

Kind of tissue	O ₂ consumption per hour in c.mm.		Per cent increase
	Before dye addition	After dye addition	
Rat kidney.....	22.9	36.3	58.5
Rat liver	13.8	16.4	18.8
Rat spleen	5.2	20.1	286.4
Rat testicle	9.2	27.6	200.0

The Effect of Methylene Blue on the Oxygen Consumption of Tumors

As a result of the observations of Warburg, repeated and confirmed by a number of investigators, it is known that tumor tissues possess a metabolism different from the metabolism of normal adult tissues. Tumors, whether taken from animals or cultivated *in vitro* show in general a low respiration compensated by an abnormally high fermentation, which is more manifest when measuring the "Pasteur Reaction" of these tissues, *i.e.*, the excess fermentation given by the following formula $= U = Q_M^{N_2} - 2(QO_2)$ where $Q_M^{N_2}$ is the anaerobic glycolysis and QO_2 the respiration. Let us add that aerobic glycolysis is not a specific feature of tumor tissues. Warburg (10) has summarized examples of normal tissues possessing aerobic glycolysis, Crabtree (11) has shown it to be a property of certain pathological overgrowths associated with intracellular viruses, and recently Neuhaus (12) has reported the presence of an appreciable aerobic glycolysis in granulation tissues.

TABLE 4

The Effect of Methylene Blue on the Oxygen Consumption of Tumors

Kind of tumor	O ₂ consumption in c.mm. per hour		Per cent increase
	Before dye addition	After dye addition	
Human carcinoma (breast):			
1).....	17.0	21.3	25.3
2).....	24.6	33.7	37.0
3).....	26.0	31.0	19.2
Rat carcinoma (Walker 256):			
1).....	24.4	40.5	66.0
2).....	22.5	29.5	31.1
3).....	32.4	40.3	24.4
4).....	29.2	42.3	44.8
5).....	16.3	33.6	106.0
6).....	24.3	38.4	58.0
7).....	25.1	36.5	45.4
8).....	32.0	48.0	50.0
9).....	12.7	21.9	72.4
10).....	28.1	46.3	64.8
11).....	32.3	48.5	50.2
Rat adenocarcinoma No. 20:			
1).....	14.3	25.6	79.0
2).....	25.9	45.4	75.2
3).....	19.7	37.3	89.3
Rat sarcoma No. 1 (Walker):			
1).....	24.1	33.3	38.2
2).....	26.6	40.3	51.5
3).....	32.9	48.8	48.3
4).....	35.3	47.4	34.3
5).....	49.7	62.8	26.4
Rat sarcoma No. 135:			
1).....	38.7	57.0	47.3
2).....	30.5	42.5	39.3
3).....	41.3	54.5	32.0
Rat sarcoma No. 10:			
1).....	29.9	41.0	37.1
2).....	34.4	53.9	56.7
Chicken sarcoma (Rous):			
1).....	11.6	23.5	102.0
2).....	10.5	22.7	116.0
3).....	9.4	19.6	108.6
4).....	17.4	27.0	55.2
5).....	10.1	14.2	39.4

It was expected that methylene blue would exert its catalytic power in the presence of tumors, since they possess a high aerobic glycolysis. Table 4 demonstrates that such is the case. Different kinds of tumors were used in these experiments: human carcinoma, rat sarcoma, rat adenocarcinoma, and Rous chicken sarcoma with the same results, namely that there is a definite increase in the oxygen consumption of these tissues in the presence of methylene blue, an increase which is higher than the one observed in brain and spleen.

When the respiration is inhibited by cyanides, the addition of methylene blue, although increasing considerably the oxygen consumption in relation to the previous low value obtained before the

TABLE 5

The Effect of Methylene Blue on the Oxygen Consumption of Tumors, Previously Treated with KCN ($\frac{M}{300}$)

Kind of tumor	O ₂ consumption per hour in c.mm.		Per cent increase
	Before dye addition	After dye addition	
Human carcinoma	1.5	7.1	373.4
Rat adenocarcinoma (Walker No. 76).	0	15.0	∞
Rat carcinoma, mammary gland (Walker No. 256)	0.5	7.7	1440.0
Rat sarcoma (Walker No. 1)	3.8	16.5	337.0

dye is added, does not bring it back to the values observed without cyanides. There is in the action of cyanides on the oxygen consumption of tumors an interesting phenomenon which will be studied more carefully in this laboratory. KCN inhibits the oxygen consumption of tumors almost completely. In the experiments here reported the inhibition was from 100 per cent to 90 per cent in relation to the oxygen consumption of the same weight of untreated tumor. This observation is in sharp contrast to the findings of Dixon and Elliot (8), which have been confirmed in this laboratory, of the incomplete inhibition of the oxygen consumption of normal tissues by cyanides, which, in the experiments reported in Table 3, was from 70 to 85 per cent.

The Relation between the Catalytic Power of Methylene Blue and the Aerobic Glycolysis of Cells and Tissues

In an attempt to correlate, in a quantitative way, the relation between the catalytic power of methylene blue, and the fermentative power of the tumors studied, the aerobic glycolysis was determined, employing the methods of Warburg. The data have been tabulated in Table 6. The figures given for the aerobic glycolysis of normal tissues have been taken from Warburg's data (13), excepting that the aerobic glycolysis of spleen has been taken from the data of Murphy and

TABLE 6

Relation between the Aerobic Glycolysis, and the Catalytic Power of Methylene Blue on the O₂ Consumption of Tumors and Normal Tissues

Kind of tumor or tissue	Per cent increase or decrease due to M. B. (O ₂ consumption)	Aerobic glycolysis
Kidney.....	-13.8	0
Liver.....	-5.2	0
Pancreas.....	-13.9	0
Spleen.....	+8.2	+2.3
Brain.....	+12.7	+2.5
Human carcinoma.....	+27.2	+9.2
Rat carcinoma (Walker).....	+55.7	+17.7
Rat adenocarcinoma (Walker).....	+81.7	+22.5
Rat sarcoma (Walker No. 1).....	+39.7	+11.3
Rat sarcoma (Walker No. 135).....	+39.5	+12.1
Chicken sarcoma (Rous).....	+84.2	+21.4

Hawkins. The data on the aerobic glycolysis of Rous chicken sarcoma have also been taken from the last mentioned authors. It can be seen in this table that methylene blue exerts its catalytic power only on cells or tissues possessing aerobic glycolysis. There is evidently a rough proportionality between the catalytic power of the dye and the fermentative power of the cell or tissue.

DISCUSSION

It has been established (5) that the following factors influence the catalytic power of reversibly oxidized and reduced dyes on the cellular oxidative processes: the permeability of the cell membrane to the

dye; and the reduction potential of the dye, since the speed of this activation is a function of the speed at which the dye is reduced by the cell, and the speed at which the leucodye is oxidized by the atmospheric oxygen. From the experiments reported in this paper it can be said that the fundamental factor is the fermentative power of the cell or tissue. Thus normal adult tissues have, in so far as the microscopic sections can reveal, the same permeability for methylene blue as tumors; they possess the same reducing power as shown by Voegtlin, Johnson and Dyer (14). Nevertheless, while the dye has no action or even exerts a toxic effect on the respiration of normal adult tissues when deprived of aerobic glycolysis, the dyes show their catalytic power on cells or tissues possessing a fermentative power as expressed by their aerobic glycolysis. Methylene blue has no effect on the oxygen consumption of most normal adult tissues because they do not produce lactic acid under aerobic conditions. The high respiration of the cell checks the fermentative power. When the respiration of these tissues is inhibited, by the addition of a specific respiratory poison, namely KCN, and the fermentation appears, methylene blue exhibits its catalytic power, increasing the oxygen consumption of these tissues, normally inactive to the action of the dye.

The effect of methylene blue on the oxygen consumption of tumors is a further confirmation of this point of view. It is known that the respiratory power of the tumors is limited; as a consequence, and, according to the brilliant conceptions of Pasteur (15), because respiration is performed at the expense of fermentation, the fermentative process in aerobic conditions appears to provide the energy necessary for the maintenance of the cellular metabolism. Neoplasms therefore provide the necessary conditions for the action of methylene blue. An extremely interesting question is raised here, a problem which will be investigated shortly. If it is possible to change the metabolism of tumor tissues by the addition of methylene blue in such a way that the tissue shows an increased respiration, as well as a decreased aerobic glycolysis with a consequent shift of the Pasteur Reaction towards the negative side, it means, that, by the action of the dye, the metabolism of these tumors is shifted towards the direction of the metabolism of normal adult tissues. By using a small concentration of methylene blue on cultures of tumors, and growing them for several generations in a medium containing the dye, possibly the characteristic

tumor metabolism, with its low respiration and high fermentation, might be changed permanently into one showing the characteristics of normal tissues.

The constant relation between the fermentative power of cells and tissues, and the increase of their oxygen consumption after the addition of methylene blue is so general and has been tested with such a variety of cells and tissues, that it seems justifiable to suggest the use of methylene blue as a test for the fermentative power of cells and tissues.

CONCLUSIONS

1. Methylene blue has no catalytic effect on the oxygen consumption of those normal adult tissues which do not possess aerobic glycolysis. The dye increases the oxygen consumption of these tissues when their respiration has been inhibited by the addition of KCN and their fermentative power thus brought into action.

2. Methylene blue increases the oxygen consumption of normal tissues having aerobic glycolysis, and of tumors.

3. The effect of methylene blue is roughly proportional to the fermentative power of tissues.

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