# THE EFFECT OF METHYLENE BLUE ON THE OXYGEN CONSUMPTION AND RESPIRATORY QUOTIENT OF NORMAL AND TUMOR TISSUE[1]

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Dickens and Šimer (1930*b*), from their studies of normal and tumor metabolism, concluded that there is a definite defect in the oxidation of carbohydrate by tumor tissue, since tumors possess both high glycolysis and low oxidative carbohydrate metabolism, a combination not found in normal tissues.

Barron (1930) found that methylene blue increased the oxygen consumption of only those tissues having aerobic glycolysis, and that this catalytic effect was roughly proportional to the fermentative power of the tissue. Thus, both human and animal tumors, which constantly possess an aerobic glycolysis—usually a marked one—showed an increase in the oxygen consumption of 19.2 to 116 per cent after the addition of methylene blue, which serves, therefore, as a respiratory enzyme.

At the suggestion of Professor W. O. Fenn, *in vitro* experiments were conducted to test the possibility of supplementing the oxidative enzyme system of tumors by methylene blue so as to permit the oxidation of lactic acid and thus raise their respiratory quotient. With a normal type of metabolism the tumor cell might attain also a normal growth. The results showed, however, that the R.Q. was not increased.

## Apparatus and Method

Differential volumeters (Fig. 1) were used for the measurement of the gases (Fenn, 1927; 1928), and the procedure and technic employed were essentially those of Dickens and Šimer (1930*a*) and of Fenn (1932), with minor modifications of the apparatus and procedure.

Difficulty was experienced in using the differential volumeter of the usual design at 37.5° C. with a large capillary because at this high temperature the thin kerosene of the index drop tended to creep over the bends at the ends of the capillary into the stopcocks. Further, if the stopcocks are immersed in the water bath the grease becomes very thin and leaks more easily. To avoid these defects, the apparatus was modified so that the capillary was suspended in the water underneath the stopcocks, which remained just above the water level and in such a position that gravity tended to pull the plugs tighter into the sockets.

[1] Financial assistance by the Henry Strong Denison Foundation for this investigation is gratefully acknowledged.

The most convenient and practicable sized capillary was found to be one having a horizontal measuring portion of about 20 cm. in length, and a volume of 16 c.mm. per centimeter of length. The amount of tissue employed was increased to give a readable deflection at this low sensitivity, thus appreciably increasing the accuracy of the measurements. The experimental vessel of each volumeter was fitted with two side arms, one for acid and the other for barium hydrate. The volumes of these experimental vessels varied from 13 to 17 c.c. in different volumeters. The water bath temperature was 37.5° C. ± 0.01° C. Highly buffered phosphate-Locke's solution (Dickens and Šimer, 1930*a*) was employed with tumor tissue.

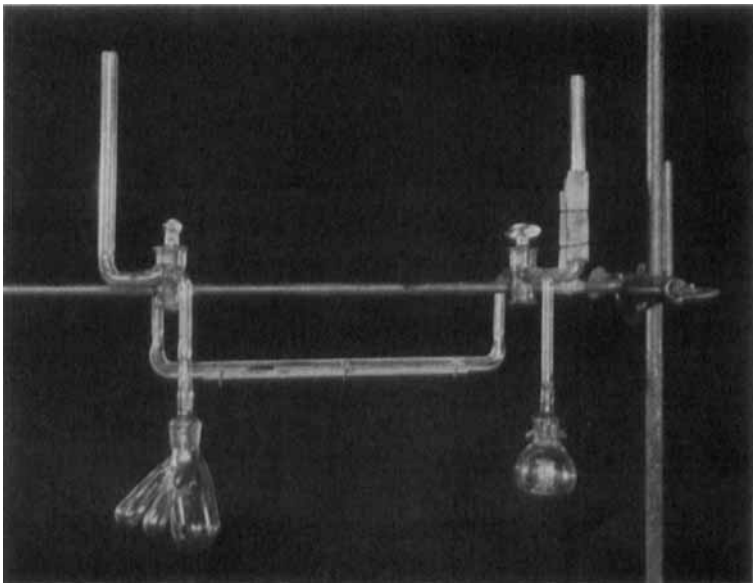

FIG. 1. DIFFERENTIAL VOLUMETER MODIFIED FOR USE AT 37.5° C.

## I. CONTROL MEASUREMENTS

The rapidity of liberation of the bound $CO_2$ and the accuracy of the measurement of the $CO_2$ with this type of volumeter were determined by control experiments using an amount of sodium carbonate calculated to give off 224.0 c.mm. of $CO_2$. Three values obtained with different volumeters were: 224.4, 224.3 and 222.5 c.mm.

Both 0.3 and 0.5 c.c. of 2.5 N HCl were employed at different times for the liberation of $CO_2$ without modifying the results obtained. Thus in one experiment the preformed $CO_2$ of slices of the same liver tissue was measured with both 0.3 and 0.5 c.c. of acid. The results showed 1.54 and 1.45 c.mm. per mg. dry weight in two trials when 0.5 c.c. was used and 1.55 and 1.77 c.mm. per mg. in two trials when 0.3 c.c. was used. Likewise in another trial the R.Q. was 0.70 with 0.5 c.c. and 0.71 with 0.3 c.c. (No. 3, Table I). The solubility of $CO_2$ in the contents of the experimental vessel after mixing the acid, $Ba(OH)_2$, and Locke's solu-

tion was 0.523 c.c. per c.c. of solution when 0.3 c.c. of acid was used and 0.498 when 0.5 c.c. was used. These figures were calculated for 37.5° C. from the data of Dickens and Šimer (1930*a*). In most experiments 0.5 c.c. of acid was employed because the $CO_2$ was liberated more rapidly than with the weaker solution, a maximal deflection being obtained in seven to fifteen minutes after tipping the acid.

TABLE I: *Control Measurements on Liver Tissue*

| Specimen No. | R.Q. | $Q_{O_2}$ | Dry Weight (mg.) | Oxygen ($mm.^3$/hr.) |
|---|---|---|---|---|
| 1 | 0.68 | 6.2 | 11.4 | 70 |
| 1 | 0.68 | 5.4 | 11.6 | 62 |
| 2 | 0.74 | 9.3 | 33.5 | 312 |
| 2 | 0.73 | 8.9 | 16.0 | 143 |
| 3 | 0.70 | 9.2 | 34.4 | 316 |
| 3 | 0.71 | 7.2 | 33.1 | 237 |

In Table I are included some preliminary duplicate determinations of the R.Q. on three different samples of liver tissues. Certain refinements were introduced in the technic later in the work, but the good agreement illustrated in these figures is typical of all the results. In Specimens 2 and 3 of Table I the amount of tissue used for the two determinations was varied in order to see whether with large amounts of tissue the diffusion rate could become a limiting factor and disturb the values obtained. This evidently was not the case.

## II. The Effect of Methylene Blue on the Oxygen Consumption

It seemed important as a preliminary step to study the effect of varying concentrations of methylene blue on the oxygen consumption of different tissues and at different times during an experiment, since the stimulating effect of the methylene blue may be only temporary.

For each sample of tissue two differential volumeters were employed, one without methylene blue for a control and the other with methylene blue in a side arm so that it could be introduced by tipping the apparatus after a short preliminary control period of about twenty minutes. Both the National Medicinal Products' (Ehrlich) and Grubler's methylene blue were employed, with no apparent difference in the results. The methylene blue solutions were freshly prepared for each experiment, by mixing the dye with the properly buffered Locke's solution.

The results are shown in Fig. 2, where the rate of oxygen consumption for different tissues [2] at various times after introducing methylene blue is plotted in per cent of the control without methylene blue. With

[2] Rat carcinosarcoma 256 was obtained from the Institute for Cancer Research, Columbia University. The Jensen rat sarcoma was obtained from the State Institute for the Study of Malignant Disease, Buffalo, New York.

all the tissues studied, methylene blue caused an initial increase in the oxygen consumption. This increase was most marked during the twenty-minute period following the addition of the methylene blue, except in the case of carcinosarcoma 256, where the increase was more prolonged and reached a maximum in the twenty to forty-minute period. The increase was also greatest with this tumor tissue, being

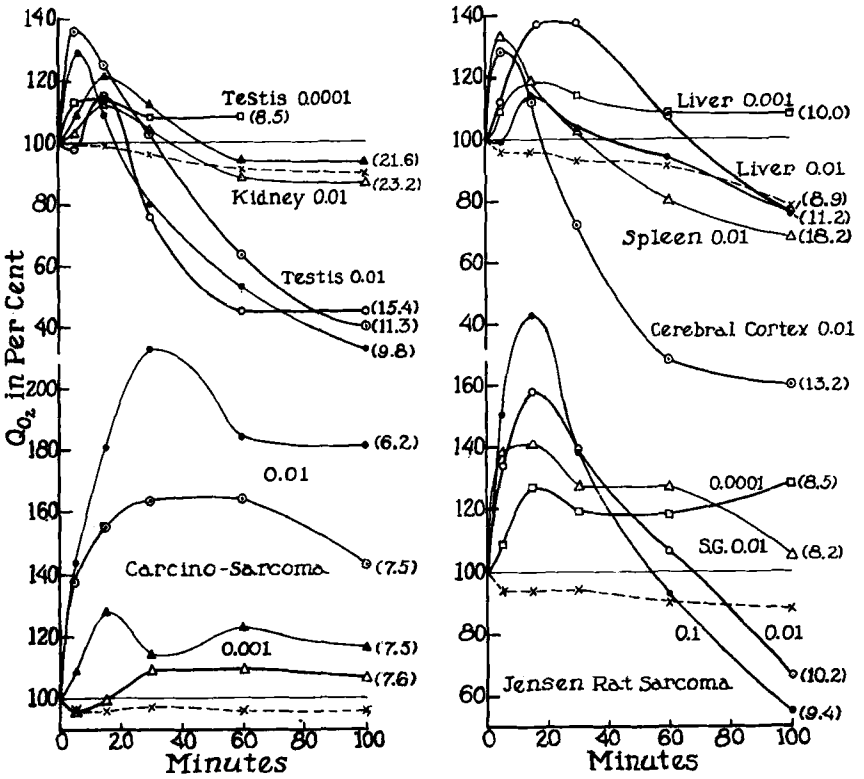


FIG. 2. OXYGEN CONSUMPTION AT VARIOUS TIMES AFTER THE ADDITION OF METHYLENE BLUE IN PERCENTAGE OF THE CONTROL VALUE WITHOUT METHYLENE BLUE

The dotted line shows the decrease in oxygen consumption of tissues without methylene blue in per cent of the initial rate. Rates for both the methylene blue and the control tissues were corrected to a common initial rate of oxygen consumption. Concentrations of methylene blue are shown on the graphs together with the initial rates of oxygen consumption (in parentheses) expressed in c. mm. $O_2$ per mg. dry weight per hour. Abscissae represent minutes after dumping methylene blue. Each point represents the middle of a period, the last two being 40 minutes each. One of the Jensen rat sarcoma graphs represents an experiment on a slowly growing tumor and is marked S.G.

85.1 per cent, whereas the greatest increase of the normal tissues was shown by spleen, 26.1 per cent. It is noteworthy that with 0.01 per cent methylene blue the increase was initially greater than with weaker solutions but did not last so long and usually gave way to a decrease at the end of the experiment. Thus in testis a solution of 0.01 per cent methylene blue caused a 16.5 per cent increase, on the average, in the zero to twenty-minute period but a 61.6 per cent decrease in oxygen consumption in the eighty to one hundred and twenty-minute period. In

TABLE II: *Effect of Methylene Blue on R.Q. of Normal and Tumor Tissues*

| Tissues | Durations of Experiment (minutes) | | Control R.Q. | Methylene Blue R.Q. | | Difference (per cent) |
|---|---|---|---|---|---|---|
| | Control | Methylene Blue | | 0.0001% | 0.01% | |
| Testis | 101 | 107 | 0.94 | 1.00 | | + 6.4 |
| | 103 | 95 | 0.97 | 1.01 | | + 4.1 |
| | 134 | 125 | 0.97 | 0.97 | | 0.0 |
| | 121 | 126 | 0.90 | 0.90* | | 0.0* |
| | 206 | 211 | 0.98 | | 0.86 | −12.3 |
| | 150 | 161 | 0.93 | | 0.87 | − 6.1 |
| | 180 | 230 | 0.93 | | 0.90 | − 3.2 |
| | 131 | 136 | 0.95 | | 0.89 | − 6.3 |
| | 161 | 252 | 0.96 | | 0.89 | − 7.3 |
| Average | | | 0.95 | 0.99 | 0.88 | |
| Spleen | 105 | 125 | 0.86 | 0.84 | | − 2.3 |
| | 127 | 142 | 0.87 | 0.88 | | + 1.2 |
| | 105 | 107 | 0.85 | | 0.93 | + 9.4 |
| | 88 | 75 | 0.86 | | 0.89 | + 3.5 |
| | 234 | 240 | 0.90 | | 0.87 | − 3.3 |
| Average | | | 0.87 | 0.86 | 0.90 | |
| Cerebral cortex | 52 | 72 | 0.93 | 0.97 | | + 4.3 |
| | 102 | 99 | 0.98 | 0.98 | | 0.0 |
| | 106 | 114 | 0.90 | | 1.14 | +26.7 |
| | 79 | 91 | 0.96 | | 0.96 | 0.0 |
| | 60 | 48 | 0.99 | | 0.91 | − 8.1 |
| Average | | | 0.95 | 0.98 | 1.00 | |
| Kidney | 96 | 80 | 0.83 | 0.82 | | − 1.2 |
| | 77 | 75 | 0.84 | 0.90 | | + 7.1 |
| | 99 | 85 | 0.82 | | 0.90 | + 9.8 |
| | 84 | 63 | 0.83 | | 0.91 | + 9.6 |
| | 88 | 80 | 0.85 | | 0.89 | + 4.7 |
| | 127 | 98 | 0.85 | | 0.90 | + 5.9 |
| Average | | | 0.84 | 0.86 | 0.90 | |
| Liver | 110 | 125 | 0.55 | 0.57 | | + 3.6 |
| | 74 | 80 | 0.63 | 0.62 | | − 1.6 |
| | 131 | 127 | 0.81 | 0.85* | | + 4.9* |
| | 85 | 62 | 0.67 | | 0.77 | +14.9 |
| | 87 | 60 | 0.67 | | 0.84 | +25.4 |
| | 144 | 139 | 0.75 | | 0.85 | +13.3 |
| | 145 | 105 | 0.82 | | 1.09 | +33.0 |
| | 112 | 88 | 0.85 | | 0.94 | +10.6 |
| Average | | | 0.72 | | 0.90 | |

other cases there is no decrease observed at the end of the experiment, but merely a smaller increase. This is particularly the case with carcinosarcoma 256 and in general with solutions of methylene blue of 0.001 or 0.0001 per cent.

TABLE II—*Continued*

| Tissues | Durations of Experiment (minutes) | | Control R.Q. | Methylene Blue R.Q. | | Difference (per cent) |
|---|---|---|---|---|---|---|
| | Control | Methylene Blue | | 0.0001% | 0.01% | |
| Rat carcinosarcoma 256 | 122 | 152 | 0.85 | 0.87 | | + 2.4 |
| | 121 | 138 | 0.89 | 0.85 | | − 4.5 |
| | 114 | 124 | 0.90 | 0.96 | | + 6.7 |
| | 134 | 99 | 0.87 | | 0.85 | − 2.3 |
| | 224 | 188 | 0.89 | | 0.81 | − 9.0 |
| | 319 | 329 | 0.90 | | 0.81 | −10.0 |
| | 94 | 98 | 0.90 | | 0.79 | −12.2 |
| | 107 | 95 | 0.95 | | 0.94 | − 1.1 |
| Average | | | 0.89 | 0.89 | 0.84 | |
| Jensen rat sarcoma, rapidly growing | 193 | 190 | 0.83 | 0.83 | | 0.0 |
| | 177 | 174 | 0.82 | 0.78* | | − 4.9* |
| | 100 | 67 | 0.88 | | 0.86 | − 2.3 |
| | 99 | 112 | 0.79 | | 0.74** | − 6.3** |
| Average | | | 0.83 | | | |
| Jensen rat sarcoma, slowly growing | 139 | 102 | 0.90 | | 0.87 | − 3.3 |
| | 143 | 122 | 0.99 | | 0.87 | −12.1 |
| Average | | | 0.95 | | 0.87 | |
| Mouse carcinoma, Buffalo No. 3 | 109 | 93 | 0.90 | 0.88* | | − 2.2* |
| | 279 | 274 | 0.75 | | 0.75 | 0.0 |
| Average | | | 0.83 | | | |
| Mouse sarcoma 180 | 72 | 86 | 0.92 | 0.93 | | + 1.1 |
| | 71 | 76 | 0.97 | | 0.92 | − 5.2 |
| Average | | | 0.95 | | | |

Mouse carcinoma Buffalo No. 3 obtained from the State Institute for the Study of Malignant Disease, Buffalo, N. Y.

Mouse sarcoma 180 obtained from the Rockefeller Institute for Medical Research.

* 0.001% methylene blue, not included in average R.Q.

** 0.10% methylene blue.

## III. EFFECT OF METHYLENE BLUE ON THE R.Q.

In these experiments, the methylene blue was placed in contact with the tissue just before the experimental vessel was flushed out with oxygen, immediately after which the differential volumeter was placed in the water bath.

The results of R.Q. measurements on different tissues with and without the addition of methylene blue are collected in Table II. Percentage change in R.Q. as well as the duration of the experiment are also included. In general, 0.01 per cent methylene blue raised the R.Q. of all tissues studied with the exception of testis and tumor, where

the R.Q. was decreased. Liver presented the greatest average percentage increase in R.Q. of any tissue studied, 20.2. In most cases the more dilute methylene blue solutions of 0.001 and 0.0001 per cent caused a slight increase in R.Q. even in testis and tumor.

Variations in the duration of the experiments in the same tissue group produced no significant effect upon either the control or the methylene blue R.Q. measurements (Table II). Thus, even a threefold difference in the time of contact of the methylene blue and the tissue caused no appreciable variation in the R.Q.

TABLE III: *Comparison between the Aerobic Glycolysis and the Changes in Oxygen Consumption and Respiratory Quotient Caused by 0.01 Per Cent Methylene Blue*

| Tissue | Average Change in $Q_{O_2}$ | | Aerobic Glycolysis $Q^{O_2}_{CO_2}$ | Average Change in R.Q. |
|---|---|---|---|---|
| | 0–20 min. | 80–120 min. | | |
| | per cent | per cent | | per cent |
| Kidney | +14.3 | − 9.4 | 0 | + 7.5 |
| Liver | +17.0 | −23.8 | 0 | +20.2 |
| Testis | +16.5 | −61.6 | + 2.0 | − 7.0 |
| Spleen | +26.1 | −34.3 | + 2.3 | + 3.2 |
| Cerebral cortex | +20.6 | −79.5 | + 2.5 | + 6 2 |
| Carcinosarcoma 256 | +85.1* | +42.2** | | − 6.9 |
| Jensen rat sarcoma | +46.1 | −33.7 | +20 | − 2.3 |
| Jensen rat sarcoma, slo wly growing | +39.8 | + 5.3 | | − 7.7 |
| Mouse sarcoma 180 | | | +16 | − 5.2 |

$$Q^{O_2}_{CO_2} = \frac{\text{mm.}^3 \text{ extra carbonic acid, formed in oxygen}}{\text{mg. tissue} \times \text{hours}}$$

* $Q_{O_2}$ for 20 to 40 minute period.
** $Q_{O_2}$ for 120 to 160 minute period.

Himwich, Fazikas and Hurlburt (1933) have published a few measurements showing a decrease in R.Q. by 0.005 per cent methylene blue, especially in cerebral cortex and testis.

## IV. Effect of Methylene Blue and Sodium Bromacetate on Jensen Rat Sarcoma in Vivo

It appeared to be of some importance to test the effect of methylene blue on the tumor already established in the host. Brooks (1933) reported that the injection of methylene blue directly into tumors of rats and mice appeared to favor regression of the tumors. Since Lundsgaard (1930) observed that either bromacetic or iodoacetic acid blocked the fermentative process without affecting the respiratory rate, it appeared worth while to try this chemical also on the growing tumor.

Toxic doses were reduced until it was found that a young adult rat could just tolerate a daily subcutaneous or intraperitoneal injection of 2.0 c.c. of a 0.10 per cent solution of methylene blue for a period of at least two or three weeks, and a daily dose of 1.0 c.c. of 0.67 per cent

neutralized sodium bromacetate for an approximately equal period. More than 1.0 c.c. per day of the latter chemical produced marked diarrhea.

Rats bearing Jensen rat sarcoma were injected intraperitoneally with the above doses at a distance from the tumor, beginning on the seventh day after tumor implantation, when a tumor was just palpable, and continuing for fifteen days. Controls received 2.0 c.c. of a 0.85 per cent sodium chloride solution.

No significant difference in the tumor growth rates, percentage of

TABLE IV: *Effect of Methylene Blue and Bromacetate on Growing Jensen Rat Sarcoma*

| Controls | | Methylene Blue | | Bromacetate | |
|---|---|---|---|---|---|
| Grew | Regressed | Grew | Regressed | Grew | Regressed |
| 6 | 10 | 5 | 8 | 6 | 6 |
| 37% | 63% | 38% | 62% | 50% | 50% |

tumor regressions, or survival period of the hosts was measurable between the different groups (Table IV). More animals would be necessary to obtain more exact statistical evidence, but the above results did not seem promising enough to warrant them.

## DISCUSSION

As already mentioned, Barron (1930) has attempted to show that methylene blue stimulates the respiration only in those tissues which show aerobic glycolysis. In Table III, therefore, a comparison has been made between the percentage stimulation of the oxygen consumption during the initial period, as found in Fig. 2, and the aerobic glycolysis as given by Barron (1930) and Fujita (1928, for testis). In general it is true that the tumor tissues which show the greatest degree of aerobic glycolysis show also the greatest increase in the oxygen consumption. Kidney and liver, however, have no aerobic glycolysis and are nevertheless stimulated by methylene blue. The stimulation of liver by methylene blue is as great as that of testis, which has an aerobic glycolysis of + 2.0.

Table III also summarizes for the same tissues the effect of 0.01 per cent methylene blue on the R.Q. as taken from Table II. In this respect also, there is some rough correlation, since kidney and liver, which have no aerobic glycolysis, show the greatest increase in R.Q., while tumor tissues with the greatest aerobic glycolysis show the greatest decrease in R.Q. Spleen and cerebral cortex with a small amount of aerobic glycolysis show an intermediate effect on the R.Q. Testis differed from all other normal tissues and resembled the tumor tissues in having a definitely decreased R.Q.

The primary object of these experiments was to find some substance which would make the metabolism of tumor tissue more normal by

enabling it to burn lactic acid and thus raising its R.Q. It appears from the results that methylene blue is not such a substance. If it decreases aerobic glycolysis of tumor tissue as it does that of mammalian and avian erythrocytes (Barron and Harrop, 1928), then this effect is not attributable to a better burning of lactic acid but rather to more abundant oxidative energy due to an increase of the type of metabolism which is already going on, *i.e.*, an increased combustion of substances other than lactic acid.

## Summary

1. The differential volumeter, as modified by Fenn, was adapted for the *in vitro* measurement of oxygen consumption and respiratory quotient of various mammalian tissues, both normal and tumor.

2. The addition of relatively strong concentrations (final concentrations of 0.01 per cent and higher) of methylene blue to normal and transplantable tumor tissues produced an early increase in the oxygen consumption in every case, and a later decrease in all five of the normal tissues studied, and in all but two of the tumor tissues, the latter two, rat carcinosarcoma 256 and a slowly growing Jensen rat sarcoma, showing a stimulated rate throughout the experiment, which usually lasted about two or three hours.

3. Methylene blue concentrations of 0.001 and 0.0001 per cent (final concentrations) stimulated the oxygen consumption of all tissues tested to a less extent than did the higher concentrations, but the increase over the control lasted throughout the experiment, and the stimulation became even more pronounced toward the end of the experiment with the tumor tissues.

4. Methylene blue in final concentration of 0.01 per cent raised the respiratory quotient of spleen, cerebral cortex, kidney, and liver to an average percentage difference of 3.2 to 20.2 in ascending order, and lowered the respiratory quotient of testis and all the tumor types studied.

5. The 0.0001 and 0.001 per cent concentrations of methylene blue usually raised the respiratory quotient of most of the tissues slightly, even with testis and tumor.

6. Methylene blue and bromacetic acid, injected into rats bearing Jensen rat sarcoma, at a distance from the tumor, produced no significant effect on the growth rate of the tumors.

## Conclusions

1. There is a rough, direct proportionality between the rate of aerobic glycolysis and the initial stimulative power of methylene blue on the oxygen consumption of various normal and tumor tissues.

2. Methylene blue (0.01 per cent) produced a poisoning effect on the oxygen consumption after the early stimulation in all tissues except in two tumor types which showed a stimulative effect for relatively long periods of time.

3. There is a rough inverse relationship between the degree of aerobic glycolysis and the effect of methylene blue on the respiratory quotient.

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