may have a similar explanation, while the increase observed in distemper may be due to the liberation of this enzyme from disintegrating leucocytes, since Roche[4] has shown these cells to contain this enzyme.

7206 C

Methemoglobin and Methylene Blue as Cyanide Antagonists.

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The action of methylene blue in restoring the respiration of cyanide inhibited tissues (Gerard[1]) has led to the clinical and apparently successful use of the dye as an antidote for cyanide poisoning (Brooks[2]). It has been urged that methylene blue acts in the intact organism not directly by substituting in the cells for the inactivated respiratory enzymes, but indirectly by removing the cyanide and releasing the enzyme. Cyanide would thus be removed by the formation of the stable cyanmethemoglobin, from it and methemoglobin; formed in turn from the blood pigment by methylene blue (Wendel[3]). An antagonism of methemoglobin and cyanide has been demonstrated on rat tissues (Rosenthal and Voegtlin[4]).

It seemed of interest, in this connection, to determine the action of methemoglobin and methylene blue, with cyanide, on isolated invertebrate tissues. Ciliated gill tissue of the quahog *(Venus mercenaria)* was isolated in sea water and its oxygen consumption followed at 22°C. in Warburg manometers. Alkali in the inset, by absorbing CO_2 and HCN, led to a rise in pH, but always less than 0.5.

The normal Qo_2 (cmm. O_2 per hour per gm. fresh weight) averaged 375 (26 experiments) during the first hour in sea water, about 10% less for the second. Cyanide led to typical inhibition, though rather high concentration was needed. As per cent of the normal, average (4 series of experiments) values in cyanide were: M/11,000,

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[1] Gerard, R. W., *Physiol. Rev.*, 1932, **12**, 504.

[2] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

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[4] Rosenthal, S. M., and Voegtlin, C., *Public Health Reports*, 1931, **46**, 521.

66; M/5500, 58; M/1100, 56; M/550, 55; M/110, 16. Methylene blue in 0.09% (M/350) concentration, increased the respiration in sea water by one-third. (One-tenth this concentration was without effect, even after cyanide.) Respiration inhibited by M/110 cyanide to 16% normal was more than doubled (to 38%) by the dye, though still depressed. This dye concentration did, however, accurately restore to normal the respiration inhibited (to 57%) by M/1100 cyanide (4 experiments). It is to be noted that considerable restoration occurred even when the added dye was of lower molarity than the cyanide, indicating its separate action as a substitute catalyst in the cell.

Methemoglobin, on the contrary, only affected cyanide inhibition when an excess of the pigment was present. Ox erythrocytes in Ringer were treated with amyl nitrite, washed repeatedly and suspended in Ringer or sea water at approximately blood volume. Probably most of the hemoglobin had been oxidized, so that the suspension, containing about 15% methemoglobin was roughly M/500. It was diluted 11 times as used. Such a suspension had no influence on the respiration of gill tissue. (A similar suspension of hemoglobin cells increased respiration some 25%. This could not have been due in large degree to better oxygenation of the solution in the presence of the carrier. Cyanide inhibition was not affected). With sufficient methemoglobin, the cyanide inhibition was completely reversed or, if the pigment was added with the cyanide, prevented. As estimated above, one mol. of the pigment neutralizes 2 to 4 of cyanide. (Some cyanide distills into the alkali cup in time, so that after an hour or two the smaller methemoglobin concentrations are effective.) Results are given in Table I.

TABLE I.

Concentration		O_2 consumption in % of control		Final color
Cyanide M/	Methemoglobin M/	Before adding Methb.	After adding Methb.	
—	5500	100	95*	brown
5500	5500	62	100	reddish-brown
1100	5500	57	71	red-brown
550	11000	55	62	bright red
”	5500	”	60	” ”
”	2200	”	67	red, brown tinge
”	1100	”	100	reddish-brown

* Each value is the average of 4 experiments.

That a simple chemical reaction of methemoglobin with cyanide is involved is further suggested by the facts that, (1) the methemoglobin remains in the erythrocytes so that it could not readily act

upon the gill cells, and (2) only when the brownish color of the methemoglobin remains after mixing with cyanide is the inhibition removed; when an excess of the cyanide converts all the pigment to the bright red cyanmethemoglobin, inhibition remains.

Under the conditions of these experiments, therefore, it appears that methylene blue antagonizes cyanide inhibition by substituting for the cyanide poisoned respiratory enzyme; methemoglobin by chemically removing the active cyanide and freeing the poisoned enzyme. In the intact vertebrate, methylene blue may well act indirectly via the formation of methemoglobin.

7207 C

Electrical Stimulation of the Hypothalamus.

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Evidence has been presented that stimulation of the hypothalamus causes pupillary dilatation,[1, 2] increase in blood pressure,[3, 4] cardiac arrhythemia and ventricular extrasystoles,[4] and contraction of the bladder.[5] The suggestion has been made that the parasympathetic and sympathetic divisions of the autonomic system are separately represented in the hypothalamus.[6, 7] We have shown that the mechanism yielding pupillary dilatation is not sharply localized at one point but is widespread throughout the hypothalamus and that the most marked reactions are obtained from the lateral part in the region occupied by the medial forebrain bundle.[2]

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