voir was allowed to return to the left ventricle by way of the lungs. The diastolic volume rapidly increased to a plateau at (6). The systolic volume decreased with one exception to (2). From (3) to (4) the output of the left ventricle was about 1000 cc. per minute. The venous return to the left ventricle was blocked at (4). At (5) the volume changes of the heart were again approximately the same as at (1). Heart rate was constant and mean blood pressure increased only about one mm. Hg. during the peak of the flow, as indicated at (4).

Records of increased output obtained by allowing the blood to return to the heart by way of the superior vena cava (before cannulating the pulmonary artery) showed marked increases in both the systolic and diastolic levels, the systolic rising well above that present when the heart was taking care of the coronary flow alone. Whether changes in pressure in the pulmonary artery or a combination of factors accounts for the results obtained requires further experimentation.

A paper presenting other experimental results and a detailed description of the apparatus is now in the process of preparation.

7471 P

Does Methylene Blue Form Methemoglobin?

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These experiments were done to help clear up the present confusion as to the action of methylene blue on hemoglobin.

Barron and Harrop[1] and Warburg, Kubowitz and Christian[2] were the first to make any quantitative studies involving the interpretation of the action of methylene blue on hemoglobin. The latter state that methemoglobin does not accumulate during methylene blue catalysis when rabbit erythrocytes are placed in a saline-PO_4-glucose solution, but that it disappears as fast as formed, is reformed, disappears again and so on in a continuous cycle. The *presence of glucose* is necessary for demonstrating this catalytic effect of methy-

[1] Barron, E. S. G., and Harrop, G. A., *J. Exp. Med.*, 1928, **48**, 207; *J. Biol. Chem.*, 1928, **79**, 65; 1929, **81**, 445; 1929, **84**, 83.

[2] Warburg, O. F., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1930, **227**, 245.

lene blue because it is the reductant by which methemoglobin is transformed back to hemoglobin. Since leuco methylene blue is autoxidizable, this completes the cycle.

It could, therefore, be predicted that since a certain amount of glucose is present in the blood stream, methemoglobin would be reduced as fast as it was formed and that no appreciable amount of it would appear until there was no longer any glucose in the blood. This was proven by injecting more than a therapeutic dose into rats in the following experiments: rats (10 in each of 3 groups) were injected intraperitoneally with (1) $NaNO_2$; (2) methylene blue; (3) saline solution or nothing. The dose of $NaNO_2$ was 1 cc. of a 2% solution; of methylene blue, 1 cc. of a 0.1% solution, both per 100 gm. body weight. Both aqueous and saline solutions were used without any difference in results. The dose of methylene blue is 10 times that used in my former experiments[3] with CN and CO. $NaNO_2$ served as samples of blood with nearly complete transformation of hemoglobin to methemoglobin. Blood was analyzed at intervals of 15 minutes, ½ hour, 2 hours and the next day. The defibrinated blood was prepared for spectrophotometric analysis by dilution to 1% in 0.4% NH_4OH aqueous solution (Ray, Blair and Thomas.[4]) This does not change the proportion of methemoglobin to oxyhemoglobin. The extinction coefficients at various wave lengths were determined spectrophotometrically, and the ratio, R, of that at 540 mμ to that at 560 mμ was compared with those plotted by Ray, Blair and Thomas for blood containing different proportions of methemoglobin and oxyhemoglobin. It is possible by this method to detect less than 2% methemoglobin in a solution of oxyhemoglobin.

Results. In the case of $NaNO_2$ even at 15 minutes, R was 1.25, indicating about 90% methemoglobin. With methylene blue, all samples showed $R = 1.65$, the same as the controls, thus indicating negligible methemoglobin formation.

To see whether dog's blood would act differently, a 23.5 kg. dog was injected *via* the femoral artery with 16 cc. of a 1% solution of methylene blue, which is equivalent to the customary clinical dose. Before injection, $R = 1.60$; 15 minutes later, 1.63; 1 hour later, 1.62, thus again indicating negligible methemoglobin formation and practically 100% oxyhemoglobin.

The same results were obtained with intravenous injections of methylene blue into rabbits. These results show that methylene

[3] Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145; 1933, **104**, 139.

[4] Ray, G. B., Blair, H. A., and Thomas, C. I., *J. Biol. Chem.*, 1932, **98**, 63.

blue in the doses given, does not cause the formation of appreciable amounts of methemoglobin in the blood of rats, rabbits or dogs *in vivo*. If methemoglobin were present after the injection of methylene blue it would have been detected by these methods.

The diminution in O_2 capacity which has been claimed to occur under these conditions proves nothing as to the presence or absence of methemoglobin, but only that under certain specified conditions certain amounts of oxygen may be liberated. Such methods cannot therefore be used in argument against the present positive proof of the absence of methemoglobin *in vivo*. Furthermore, since glucose is essential for the cyclic reduction of methemoglobin, experiments done with washed cells, or even with blood *in vitro* where glucose is absent or strictly limited in amount, are not comparable with experiments *in vivo* where methylene blue, because of a constant supply of glucose, has full scope for its catalytic action.

This error of interpretation has been made by Haggard and Greenberg,[5] Henderson,[6] and again by Richardson,[7] who measured the O_2 capacity of blood containing methylene blue and reported the formation of 8 to 12% of methemoglobin.

The concentration of methylene blue is an important factor. Warburg uses different concentrations varying as much as from 1 to 200 times the concentration of methylene blue used in my animal experiments. Different results are obtained with these different concentrations.

Williams and Challis[8] and Geiger[9] using spectrographs were not able to demonstrate the formation of methemoglobin when methylene blue was injected into humans or animals. These methods have been criticized because of their low sensitivity, but nevertheless led to correct conclusions.

Species differences in the ease of methemoglobin formation which have been reported from time to time, are quite probably due to varying concentrations or availablity of glucose in the blood stream. *In vitro* Warburg found no difference between dog, human, rabbit, guinea pig, horse, and rat bloods.

Methylene blue does not form appreciable quantities of methemoglobin in the blood stream. The explanation of the action of methylene blue in the case of CN poisoning given by Wendel,[10] Hender-

5 Haggard, H. W., and Greenberg, L. A., *J. Am. Med. Assn.*, 1933, **100**, 2001.

6 Henderson, Y., *Science*, 1933, **78**, 408.

7 Richardson, E. F., *J. Am. Med. Assn.*, in press.

8 Williams, J. R., and Challis, F. E., *J. Lab. and Clin. Med.*, 1933, **19**, 166.

9 Geiger, J. C., *J. Am. Med. Assn.*, 1933, **101**, 269.

10 Wendel, W. B., *J. Am. Med. Assn.*, 1933, **100**, 1054.

son,[6] and Haggard and Greenberg,[5] *i. e.*, that the formation of methemoglobin by methylene blue and its combination with CN removes the latter from the blood stream is untenable. Furthermore, the objection of Henderson[6] that methylene blue because of methemoglobin formation cannot be used in CO poisoning is also baseless, since the catalytic action of methylene blue as a reversible oxidation-reduction dye is responsible for recovery of the animal, and not its ability to form stoichometric equivalents of methemoglobin.

7472 P

Relative Growth in the Pacific Edible Crab, Cancer magister

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Analyses of the form in various organisms have been presented in recent years by Huxley and his students. Certain related findings incidental to a study of the life-history and growth of the Pacific Edible Crab (*Cancer magister* Dana) are here given. The data include measurements of various linear dimensions (carapace length and width, length of chela, length of the first walking leg, length and width of the sixth and seventh abdominal segments in both the male and female) of 1804 crabs. The analysis has shown the relation of various sexual differences to the onset of sexual maturity and has suggested certain correlations between body proportions and the size attained by the species.

Treated by Huxley's method of plotting the logarithm of the part on the logarithm of the whole[1] (here usually the carapace width) in most cases one or more straight lines are obtained. The slopes of these lines are the values of k, the differential growth ratio. The nature of this constant may be seen from the following formula. If the plot may be fitted by a straight line the equation of this will be

$$\log p = \log a + k \log w$$

where p represents the part considered, w the whole, here the carapace width, and a and k are constants. Transforming, this becomes

$$p = aw^k$$

The values obtained in *Cancer magister* range from 0.93 to 1.61.

[1] Huxley, Julian, Problems of relative growth, 1932.