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Inhibition by Glucose of Methemoglobin Formation.

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The purpose of these experiments is to show that injection of glucose prevents the formation of methemoglobin in animals, or if methemoglobin is already present, it can by the same means be reduced to hemoglobin which is then readily oxygenated to oxyhemoglobin.

Methemoglobin was produced experimentally in rabbits by injections of 0.15 gm. of $NaNO_2$ for every kg. of body weight. This dose converted 15% of the blood-hemoglobin into methemoglobin in a few minutes. This is less than the theoretical effect predicted by Wendel[1] for this dose, owing no doubt to the constant presence of glucose normally in the blood stream. Glucose was used in amounts of 1 cc. to 2 cc. of a 1% solution for every kg. body weight. All solutions were made up fresh with 0.9% NaCl and injected intravenously into one ear of the rabbit; blood samples were taken from the other ear at intervals thereafter.

The spectrophotometric method was used for determining the proportions of methemoglobin in blood. This method is sensitive to less than 2%. The ratio, R, of the extinction coefficient at $\lambda = 540$ mμ to that at $\lambda = 560$ mμ, as found by Ray, Blair and Thomas[2] indicates the per cent of methemoglobin present. All blood samples were diluted to 1% with 0.4% NH_4OH. No difference in readings was noted when water was used as a diluent. The thickness of the layer measured was 1 cm. Five animals were used in each group. The probable error of the readings was a fraction of 1%. The method is also valuable because determinations can be made so quickly that chemical changes following collection are minimized.

Results *in vivo*. Methemoglobin was produced in the rabbit as indicated above, the R value found being 1.56 which indicates 15% conversion to methemoglobin. Glucose was then injected. Five minutes later the R value was 1.66 which indicates complete reconversion of methemoglobin to oxyhemoglobin. The controls which had not received glucose still showed 15% methemoglobin present. If glucose is injected before the nitrite, no methemoglobin can be demonstrated even after several hours. Injections of saline in the

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

[2] Wendel, W. B., *J. Am. Med. Assn.*, 1933, **100**, 1054.

place of glucose have no effect on methemoglobin formation or its change to oxyhemoglobin.

Results *in vitro* with sheep blood also showed that methemoglobin formation is delayed if glucose is present, or if already formed, the addition of glucose will reconvert a part of it to oxyhemoglobin. The following experiment will illustrate this: to 4 cc. of washed r.b.c. suspended in 0.9% saline solution, there was added .05 cc. of M/20 phenylhydroxylamine, freshly prepared. R was then found to be 1.42, indicating 48% methemoglobin. This solution was divided into 2 parts: glucose was added to one part, and to the other part only saline. The glucose-containing blood now had only 18% methemoglobin present ($R = 1.53$) while that with a saline alone remained unchanged at 48% ($R = 1.42$). It has not been found possible to reduce *in vitro* all the methemoglobin to oxyhemoglobin. This was also found by Warburg, Kubowitz and Christian,[3] who state that unknown reactions take place.

Similar effects can be demonstrated using $NaNO_2$ instead of phenylhydroxylamine. If glucose be added before the methemoglobin-producing substances, the formation of methemoglobin is delayed and the sample is still reddish when the control is brown.

These results show that glucose is effective in preventing methemoglobin formation, or after formation, in reducing it to hemoglobin which can then form oxyhemoglobin. It is suggested that the presence of glucose in the blood stream is responsible for the often observed failure of various agents to produce the expected proportion of methemoglobin. It is also suggested that injections of glucose be used clinically in cases where methemoglobin is present either as a result of pathological conditions or as a result of poisoning by such methemoglobin-producing substances as aniline dyes, nitrites, etc.

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Action of Respiratory Catalysts and Inhibitors on Oxygen Consumption by Nitella.

EDWARD ROSS. (Introduced by S. C. Brooks.)

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The material used consisted of young, actively growing coenocytic cells of *Nitella clavata* collected from a large outdoor pool. The

[3] Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1931, **242**, 170.