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Toxic Action of Hydrogen Peroxide on Trypanosomes and a Note on Chemotherapeutic Mechanism.

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We previously described[1] the toxic action of auto-oxidized aminophenol and of quinone upon trypanosomes and ascribed the action of aminophenols to the formation of quinoid compounds. However, another possible mechanism seemed worthy of consideration. During oxidation processes by molecular oxygen (auto-oxidation) intermediary peroxides are formed which readily hydrolyze in the presence of water forming H_2O_2.* Both the organic peroxides and the hydrogen peroxide may have a toxic action on trypanosomes. The nature of the supposed organic peroxide concerned in aminophenol oxidation is unknown, hence this could not be studied, but it was relatively easy to study the toxic action of H_2O_2 on trypanosomes.

We found that trypanosomes, *Tr. equiperdum,* suspended in Locke solution containing 0.5% glucose were immobilized almost immediately when the added H_2O_2 reached the concentration of 1/30,000. A trypanosome emulsion obtained in the manner outlined in the previous paper was prepared. A solution of hydrogen peroxide was made by diluting 0.1 cc. of Merck's 30% H_2O_2 to 100 cc. with 0.85% NaCl solution. A mixture was made containing 0.6 cc. of the Locke solution, 0.1 cc. of the H_2O_2 solution and 0.1 cc. of 0.85% NaCl solution with 0.2 cc. of the trypanosome emulsion. After 5 minutes no motility was observed. After 10 minutes 0.2 cc. of the fresh rabbit plasma was added and 5 minutes after this slight motility was seen. The addition of catalase or of fresh serum inhibits this toxicity to a certain extent. It was also found that trypanosomes obviously damaged by H_2O_2 will recover after the addition of fresh serum, the catalase content of which decomposes the H_2O_2. Since trypanosomes themselves contain catalase, the action of hydrogen peroxide naturally depends also on the

[1] Reiner, L., and Leonard, C. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 946.

* H_2O_2 formation has been demonstrated recently during an auto-oxidation which was catalysed by methylene blue. (Wendel[2].)

[2] Wendel, W. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 624.

density of trypanosomes present in the emulsion. In this connection it is interesting to note that there exists an optimum density of the emulsion for keeping trypanosomes alive *in vitro* a comparatively long time. The trypanosomes die off quicker in emulsions containing more or less than this optimum number. It is possible that the lower limit of this optimum range is conditioned by the fact that, in the course of the metabolism of the trypanosomes, peroxides are produced to a relatively greater extent if the oxygen content of the milieu is high, *i. e.*, the rate of oxygen consumption is low, the quantity of trypanosomes being relatively small.† Yorke, Murgatroyd and Hawking[3] have pointed out recently that the toxic action of arsenicals is greater in emulsions containing only a few trypanosomes than in dense emulsions. As we have found that only a small percentage of the available arsenical is bound to the trypanosomes, the exhaustion of the arsenical solution by adsorption cannot be the cause of this phenomenon of lessened toxicity when the number of trypanosomes per cc. is great. It seems probable that the strong reducing action of the dense emulsions is concerned in some way with this phenomenon. For example, this might act to free more sulfhydryl groups of the proteins both of the parasites and of their medium. It has been shown that the presence of such groups in the medium acts to shield the parasites by preventing the adsorption of arsenicals.[4, 5]

The formation of H_2O_2 during the auto-oxidation may be considered an accessory mechanism of toxicity of aminophenol solutions and of solutions of arsphenamine derivatives which contain these groups. But one would expect the catalase content of the blood normally to destroy H_2O_2 as quickly as it formed, hence this factor would seem of secondary importance only. It is questionable whether the same can be said of organic peroxides, if these are formed in the course of oxidation of the agent. Heavy metal compounds often poison enzyme action and one might expect arsenicals to exert an inhibitory influence on catalase and hence permit of

† Attempts to demonstrate peroxide formation in trypanosome emulsions have failed so far. This may be because the benzidine test used is not sufficiently sensitive to demonstrate the small amounts which may be present, although they still may damage trypanosomes. 1/30,000 H_2O_2 solutions give a faint benzidine test, yet kill trypanosomes in a few minutes.

[3] Yorke, W., Murgatroyd, F., and Hawking, F., *Ann. Trop. Med. and Parasitol.*, 1931, **25**, 351.

[4] Voegtlin, C., Dyer, H., and Leonard, C. S., *Public Health Rep.*, 1923, **38**, 1882.

[5] Reiner, L., Leonard, C. S., and Chao, S. S., *Arch. Internat. Pharmacodynamie*, in press.

greater H_2O_2 toxicity. Santesson[6] observed that at a concentration of 0.15%, neoarsphenamine faintly speeded catalase action, and below this concentration the arsenical exerted practically no effect. As the concentrations found in the blood stream after injection of chemotherapeutic doses of neoarsphenamine are of the order 0.001-0.01%, the catalase of the host and parasite should function normally to protect the parasites from the H_2O_2 formed in their metabolism or during the auto-oxidation of the arsenical. Santesson[7] proposed that tissue H_2O_2 assists in converting neoarsphenamine into "Arsenoxide".

Conclusion. Hydrogen peroxide is toxic to trypanosomes. The formation of traces of H_2O_2 under conditions where the catalase content of the system is low cannot be excluded. Such concentrations, although not detectable, might be sufficient to exert some damaging action on trypanosomes. While this factor is probably negligible *in vivo,* it still might be involved with the rate of the —AsO formation and thus concerned indirectly with the chemotherapeutic activity of arsenicals (Santesson).

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Effect of Adenine and Caffeine Injections on Behavior of Rats in a Circular Maze.

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Albino rats were trained in a large circular maze until they were able to run from the periphery to the center in the shortest time possible and without making an error. Such trained animals were then given different doses of caffeine and adenine separately and also in combination; the running time, the number of errors, and the general behavior of the rats were noted at various intervals thereafter. Thirty young adult rats, weighing from 100 to 200 gm., were used in these experiments. The dosages varied from 1 to 5 mg. per hundred gm. weight of the animals; the drugs were administered by intraperitoneal injection. In other experiments, the different drugs were given to large rats through a stomach tube; and in

[6] Santesson, S. K., *Arch. f. Physiol.,* 1915, **32**, 405.

[7] Santesson, S. K., *Arch. f. Physiol.,* 1923, **43**, 55.