blood sugar observed in the completely sympathectomized animal was due to the direct stimulating action of ether upon the adrenals. Both adrenals were therefore removed aseptically in the completely sympathectomized animal shown in Fig. 1C. Twenty hours later, ether again was administered for one hour and no rise in blood sugar was obtained (Fig. 1D). The low fasting blood sugar in this cat made us suspect that the liver might have been deficient in glycogen, a condition which has been reported after bilateral adrenalectomy.[12] Ether was therefore administered to a completely sympathectomized cat seven hours after bilateral adrenalectomy. An increase in blood sugar (Fig. 1E) was readily demonstrable.

The mechanism of the rise of blood sugar which takes place on etherization after ruling out the sympathico-adrenal factor is unexplained.

Summary. The elevation of the blood-sugar level in cats on etherization (Fig. 1A) is considerably reduced by inactivating the adrenals and cutting the liver nerves (Fig. 1B).

The residual increase persists in the completely sympathectomized animal (Fig. 1C), and therefore does not result from the production of sympathin.

The persistent hyperglycemia does not depend upon direct stimulation of adrenal secretion by ether since it is still present when both adrenals have been removed in the completely sympathectomized cat (Fig. 1E).

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Toxic Effect of Sodium Monoiodoacetate on Trypanosomes.

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It has been demonstrated by several investigators[1-7] that mono-

[12] Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, **100**, 693.

[1] Bersin, T., *Biochem. Z.*, 1932, **248**, 3.

[2] Dickens, F., *Nature*, 1933, **131**, 130.

[3] Quastel, J. H., *Nature*, 1933, **131**, 206.

[4] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 1932, **26**, 216.

[5] Waldschmidt-Leitz, E., Wieland, L. u. Purr, A., *Z. physiol. Chem.*, 1933, **215**, 64.

[6] Rapkine, L., *Compt. Rend. Soc. Biol.*, 1933, **112**, 790.

[7] Schroeder, E. F., Woodward, G. E., and Platt, M. E., *J. Biol. Chem.*, 1933, **101**, 133.

iodoacetic acid will react with sulfhydryl groups, presumably according to the following equation—

$$RSH + CH_2ICOOH \longrightarrow R\text{-}S\text{-}CH_2COOH + HI$$

That this reaction may have important biological applications is evident from the demonstration that the poisonous effect of iodoacetic acid on the action of the enzyme glyoxalase has been shown[2] to be due to the removal of the co-ferment, reduced glutathione.[8]

It is also known that some organic arsenicals react with thiol compounds forming condensation products,[9] and according to the theory of Voegtlin arsenicals exert their toxic effect upon trypanosomes by combining with sulfhydryl groups present in and, in his opinion, essential for the oxygen consumption of these organisms. If the above considerations are correct, it would seem to follow, necessarily, that iodoacetic acid must be toxic to trypanosomes.

In vitro experiments, carried out to test the above conclusion, showed that sodium iodoacetate added to *Trypanosoma equiperdum,* suspended in the citrated blood of a rat, is in fact very toxic. A 0.001 M solution killed all the organisms as a rule in 2 or 3 minutes. This time naturally varies with the number of trypanosomes in the emulsion and with their resistance. In view of this finding it seemed desirable to test the action of iodoacetate on trypanosomes *in vivo.*

Preliminary experiments with 14 rats showed that the maximum tolerated dose of sodium iodoacetate, injected intravenously into a normal 240 gm. rat, is 0.2 cc. of a 0.25 M solution. 0.3 cc. of this solution kills about 50% of the rats of this weight. These figures are in good agreement with the findings of Hall and Field[10] on the toxicity of intraperitoneally injected iodoacetate. Subcutaneous and intramuscular injections have also been tried. The toxicity was practically independent of the mode of injection. If rats, which have been infected with *Tr. equiperdum* and which contain in their blood stream at the time of treatment one trypanosome per 10-15 red blood cells, are injected with doses of 0.2 cc. of a 0.25 M solution, their blood becomes free of trypanosomes, usually in 2-3 hours. Some of the infected rats die from this dose, as a rule, some time after the trypanosomes have disappeared from the blood stream. Thus, it seems that the infected animals are somewhat more sensitive to iodoacetate than normal rats. The blood remains free of trypanosomes for a period varying from 2 to 10 days. Trypanosomes then reap-

[8] Lohmann, K., *Biochem. Z.,* 1932, **254,** 332.

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

[10] Hall, V. E., and Field, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29,** 360.

pear. Another dose of iodoacetate may be given and it again clears the blood stream for about the same length of time. This treatment has to date been repeated 7 times and the animals are apparently in good health with the exception of some inflammation and soreness at the place of injection (the tail). These results show that iodoacetate is also very toxic to trypanosomes *in vivo*. Furthermore, they indicate that compounds like iodoacetic acid offer some possibility of development to practical chemotherapeutic agents.

In regard to the mechanism of iodoacetate action, the results just quoted would appear to justify the prediction reached in the introduction. There are, however, differences in the action of iodoacetate and of arsenicals. Thus, the toxic action on trypanosomes *in vitro* could not be prevented or overcome by the addition of sulfhydryl in the form of cystein or thioglycollate solutions. Concentrations up to 0.02 M (a 20 fold excess) were used. Arsenicals are detoxified in analogous experiments. Similarly, the toxic effect on rats or on trypanosome infections in rats could not be prevented or overcome by the intravenous injections of these thiol compounds. This is again in contrast to the results with arsenic.[9, 11] If the conditions are comparable, our results are also somewhat in contrast to the results of Quastel and Wheatley,[4] who found that the inhibiting action of iodoacetate on glucose oxidation by brain tissue could be much decreased by the addition of cystein, glutathione or sodium thiosulphate.

Another point of difference between arsenicals and iodoacetate is the behavior of an arsenic fast strain of trypanosomes toward the 2 compounds. The concentration of neoarsphenamine which kills the resistant strain used in a certain time is about 16 times that of the concentration which kills the normal strain in the same time. Iodoacetate is, however, just as toxic to a resistant as to a normal strain. If both reagents exert their toxic effects on rat and on protozoon solely by combining with thiol groups, the results indicate that the reactions are different in some respects.

Schroeder, Woodward and Platt[7] have stated that the action of iodoacetate on yeast is not due solely to combination with thiol groups. There is also some evidence that this is true for muscle. Thus, Lohmann[12] has shown that its efficiency in inhibiting lactic acid production from glycogen is far greater than its efficiency in inhibiting glyoxalase action. He has also shown that the former

[11] Reiner, L., and Leonard, C. S., *Arch. Int. Pharmacodynamie et Therap.*, 1932, **43**, 49.

[12] Lohmann, K., *Biochem. Z.*, 1933, **262**, 152.

effect remains after a muscle extract has been dialyzed free of glutathione and cannot be removed by the re-addition of glutathione. Maschmann and Helmert[13] have found that the enzymes, kathepsin and papain, are both inhibited by iodoacetate and cannot be reactivated by thiol compounds. Bersin and Logemann[14] have obtained the same results with papain. Meyerhof and Kiessling[15] have presented evidence that one effect of iodoacetate on muscle is to prevent the reaction of pyruvic acid and glycerine phosphate. They also add further evidence to the finding that the effect of sodium fluoride on muscle is to prevent the formation of pyruvic acid from glyceric acid monophosphoric ester. It seemed of interest, therefore, to test the toxicity of sodium fluoride to trypanosomes *in vitro*. It was found to be more than 100 times less toxic than iodoacetate.

As a general conclusion from these experiments, it seems that if the iodoacetate reacts with thiol groups in trypanosomes either the result of this is different from the supposed action of arsenic with these groups, or the iodoacetate, in addition to its reaction with thiol groups, acts by another mechanism.

Summary. 1. Sodium monoiodoacetate is highly toxic to *Trypanosoma equiperdum.* 2. The blood stream of rats infected with trypanosomes can be freed from these organisms by the intravenous injection of tolerated doses of sodium iodoacetate. The blood remains free for a period varying from 2 to 10 days after which trypanosomes reappear. The treatment may be repeated. 3. The toxic action of iodoacetate upon either rats or trypanosomes could not be counteracted by a 20-fold excess of thiol compounds. 4. Arsenic resistant strains of trypanosomes are not resistant to iodoacetate. 5. The mechanism of the action of iodoacetate is discussed.

[13] Maschmann, E., u. Helmert, E., *Z. physiol. Chem.*, 1933, **220**, 199.

[14] Bersin, T., u. Logemann, W., *Z. physiol. Chem.*, 1933, **220**, 209.

[15] Meyerhof, O., u. Kiessling, W., *Biochem. Z.*, 1933, **264**, 40.