was essentially *nil.* In 10 animals treated with the fresh crystoid and 14 with the amorphous ficin, trophozoites of *E. histolytica* in the wall of the large bowel quite uniformly became dwarfed and inactive, and at times had died *in situ.*

Summary. The unpreserved, refrigerated crude latex was consistently the most efficient parasiticide tested, the preserved latex somewhat less effective. A 10% solution of the freshly opened crystoid compared favorably with the refrigerated latex but exposure to air rapidly diminished its potency. The amorphous ficin, while relatively stable, proved to be only about half as efficient as the fresh crystoid. The most specific action of these products occurred on whipworms, provided the large bowel was previously free of feces. The effect on hookworms was almost uniformly less satisfactory. Considerable amebostatic and, at times, amebicidal action was demonstrated.

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Determination of Ascorbic Acid in Whole Blood.*

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It has been found that ascorbic acid is oxidized by the oxygen set free from oxyhemoglobin when whole blood is deproteinized with HPO_3, and that this oxidation can be prevented by displacing the oxygen of oxyhemoglobin with CO, N_2, and H_2.[1-3] Butler and Cushman[4] have reported a method for the determination of ascorbic acid in whole blood involving saturation of the blood with CO and precipitation of the proteins under an atmosphere of CO.

We have found that a very convenient method to prevent the oxidative effect of oxyhemoglobin is alternate removal of oxygen by evacuation and treatment with CO_2 under pressure. A combination of these two steps gives an efficient and practical way of reducing oxyhemoglobin which is adaptable to clinical laboratory use.

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1 Lemberg, R., and Legge, J. W., *J. Proc. Roy. Soc. N. S. Wales,* 1938, **72**, 62.

2 Lemberg, R., Cortis-Jones, B., and Norrie, M., *Biochem. J.,* 1938, **32**, 149.

3 Huzita, A., Ebihara, T., and Numata, I., *Biochem. Z.,* 1939, **301**, 245.

4 Butler, A. M., and Cushman, M., *J. Clin. Invest.,* 1940, **19**, 459.

Five cc of blood are pipetted into a cylindrical separatory funnel with a mark at 20 cc, containing 2 drops of octyl alcohol as an antifoam. The glass stopper of the funnel has a removable clamp by means of which it may be held in position. The funnel is connected by means of pressure tubing to the single outlet on one side of a 3-way stopcock. One of the other outlets is connected to a vacuum line through a manometer, and the third outlet is connected to a tank of CO_2 with a manometer and a gas reservoir in the line to give it a larger volume and thus minimize the pressure changes. We have used a volleyball for the reservoir and found it very satisfactory. The funnel is then evacuated to a pressure of about 5 mm Hg until the evolution of gas stops. We have found that 2 minutes is usually sufficient time to do this. After filling the funnel with CO_2, both the stopper and stopcock are opened and CO_2 run through to wash out any residual air remaining in the funnel. The clamp is then placed on the stopper and CO_2 run in at a pressure of about 76 cm Hg above atmospheric pressure for 5 minutes. During evacuation and CO_2-pressure treatment the funnel is rotated gently and continuously. *It is very important to spread the blood in a thin layer over the inner surface of the funnel during the evacuation and saturation.* The evacuation and saturation are repeated twice. After the third saturation, the funnel is removed from the CO_2 pressure line. Five cc of distilled water, from which *all O_2 has been removed* with a stream of CO_2, is then introduced into the funnel to lake the blood. The stopper is immediately replaced and the contents of the funnel mixed. Ten cc of 10% HPO_3, from which the O_2 has been removed with CO_2, is then introduced, the volume is made up to the 20 cc mark, and the contents of the funnel are thoroughly mixed by shaking and filtered through a dry filter paper.

The filtrate obtained in this manner is analyzed for reduced ascorbic acid by a modification of the method of Mindlin and Butler.[5] The galvanometer of the photoelectric colorimeter (Evelyn) is set to read 100 with a tube of blank dye solution which has been completely decolorized with ascorbic acid. A blank is run by measuring 4 cc of 3% HPO_3 into a colorimeter tube, adding 4 cc of the indophenol-acetate solution and reading in the colorimeter. The indophenol-acetate solution contains 17 g of $NaC_2H_3O_2 \cdot 3H_2O$ and approximately 15 mg of indophenol per liter.

Four cc of the filtrate are measured into a colorimeter tube, the tube is inserted into the colorimeter and 4 cc of indophenol-acetate

[5] Mindlin, R. L., and Butler, A. M., *J. Biol. Chem.*, 1937-38, **122**, 673.

solution are added. The galvanometer is read 15 and 30 seconds after addition of the dye. A few crystals of ascorbic acid are added and the reading of the decolorized solution is taken after 5 minutes.

The final pH of a mixture of equal volumes of the dye solution and filtrate as described above is about 3. At a pH appreciably lower than this, as shown by Bessey,[6] there is decolorization of the dye by hydrogen ion, and at higher pH values the decolorization of the dye by interfering substances (principally sulfhydryl) is appreciable. Bessey[6] carries out this determination at pH 3.5-3.6. Mindlin and Butler[5] use pH 4.1. We have found pH 3 to be optimum for this reaction.

The amount of ascorbic acid in the sample is given by the following equation:

$$\text{Mg Ascorbic Acid} = K[\log (Gs_{15} - (Gs_{30} - Gs_{15})) + (2 - \log Gd) - \log Gb]$$

where K is the constant (0.086, Mindlin and Butler[5]) determined by analyzing standard solutions of ascorbic acid in 3% HPO_3, Gs_{15} and Gs_{30} are the 15 and 30 second readings, respectively, Gd is the decolorized reading of the sample and Gb is the reading of the blank solution. The first term of the equation includes a correction for the drift in the galvanometer reading produced by the non-ascorbic acid reducing substances released when the erythrocytes are hemolyzed. The second term is a correction for any turbidity which the solution may have. We have found that filtrates which to the eye appear to be perfectly clear will not give a reading of 100 when decolorized, and therefore this correction is necessary

In 9 analyses of normal human blood, and normal and scorbutic guinea pig blood to which pure ascorbic acid was added, the following recoveries of the added ascorbic acid were obtained: 90, 92, 98, 99, 102, 102, 103, 108 and 110%.

Summary. A method for the determination of ascorbic acid in whole blood is proposed. The oxidation of ascorbic acid by oxyhemoglobin is prevented by reduction of the oxyhemoglobin by alternate evacuation and treatment with CO_2 under pressure before deproteinization with HPO_3.

[6] Bessey, O. A., *ibid.*, 1938, **126**, 771.