

strated by a coupled oxidation of alcohol in the presence of catalase⁵. As an artificial ascorbic oxidase it had a Q_{O_2} (μ l. oxygen per mgm. dry weight per hr.) at pH 7.2 and 39° C. of about 10,000. Hydrogen cyanide inhibited this catalysed oxidation of ascorbic acid by combining with modified ferri-cytochrome *c*, thus preventing its reduction, while carbon monoxide, by combining with modified ferrocyclochrome *c*, prevented its oxidation. The carbon monoxide inhibition was somewhat light-sensitive. This reaction was also inhibited by methyl isocyanide and nitrosobenzene. Modified cytochrome *c* also catalysed the decomposition of hydrogen peroxide, being itself destroyed in this reaction. Cyanide inhibited this catalysed decomposition of hydrogen peroxide.

The result of this experiment agrees with an observation of Keilin and Hartree⁶ that when cytochrome *c* is made autoxidizable it loses its catalytical activity in biological systems.

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Estimation of 'Hetrazan' in Body Fluids

THE estimation of 'Hetrazan' (1-diethylcarbonyl-4-methyl piperazine hydrochloride), which has been recently introduced for the treatment of human filariasis due to *Wuchereria bancrofti*¹, may be carried out by a 'dye-laking' method, similar in principle to the methods developed for organic bases by Brodie and Udenfriend².

Blood serum or plasma, or other body fluid, is made strongly alkaline with one-fifth of its volume of 10 N sodium hydroxide and extracted with two to three times its volume of ethylene dichloride. The ethylene dichloride layer is separated by centrifugation and clarified by passage through a small Whatman No. 41 filter paper. It is then shaken for a few minutes with one-fifth of its volume (more may be required for urine) of 0.05 per cent Bromthymol blue solution. This solution is made in phosphate buffer at pH 7.0. Part of the Bromthymol blue combines with the 'Hetrazan' base and dissolves in the ethylene dichloride, giving a yellow colour. The intensity of this colour is then compared with that of a standard solution of 'Hetrazan' carried through the same procedure.

Where the concentration of 'Hetrazan' is low (less than 1 μ gm./ml.) the colour is too pale for accurate colorimetry. If a micro-colorimeter is available, a measured volume of the ethylene dichloride is extracted into 2 ml. of N sodium hydroxide and the resulting blue colour compared with a standard solution. Alternatively, a blue colour may be developed in the ethylene dichloride by addition of 0.1 N alcoholic caustic potash and sufficient ethanol to prevent turbidity.

If whole blood is extracted, or the proteins removed by trichloroacetic acid, normal bloods give a

blank value of about 1 μ gm./ml. Further, trichloroacetic acid precipitation removes about 20 per cent of added 'Hetrazan'. Therefore, although it may be more tedious, it is preferable to extract serum or plasma.

Urines show a blank value of about 5 μ gm./ml., but occasionally, in concentrated urines, the blank may reach 20 μ gm./ml.

The method is sufficiently sensitive to measure a blood concentration of 'Hetrazan' of 1 μ gm./ml. Like most of these methods, it lacks specificity, but has been found useful in following the concentration in blood and urine after oral administration of the drug.

A full account of the results of these experiments will be published later. Typically, ingestion of 10 mgm./kgm. body-weight of the hydrochloride results in a maximum plasma concentration of about 5-7 μ gm./ml., reached in three hours. The level falls slowly to zero in 24 hours, during which period about 20 per cent of the drug is excreted in the urine. A further small amount is excreted during the next 24 hours, after which no more 'Hetrazan' can be detected.

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Mass Therapy with 'Hetrazan' as a Control Measure for Bancroftian Filariasis on St. Croix, American Virgin Islands

IN the spring of 1948 a mass treatment programme with 'Hetrazan' was conducted on the island of St. Croix, with the object of reducing microfilaræmia in persons infected with *Wuchereria bancrofti* to sub-infective levels for mosquitoes. A survey of 977 people of all age groups revealed an infection-rate of 15.9 per cent, using 60 c.mm. blood samples. After preliminary observations were made on a group of known positives treated with varying oral doses of 'Hetrazan', the dosage selected for island-wide therapy was 100 mgm. tablets for adults and 53 mgm. tablets for children more than five years of age, administered three times daily for seven days. Before distributing tablets, the programme was explained to civic leaders, school teachers, and in general to all inhabitants of the island, through press notices, handbills and individual and collective talks with the people. Systematic distribution of the 'Hetrazan' was then carried out on an island-wide basis, first within the schools, and then in the towns, villages and rural communities. During a three-week period, 7,781 individual treatment units were distributed, and before leaving St. Croix 2,520 units were left at the two hospitals. The population of St. Croix is between 12,000 and 14,000. Supervised treatment, under the control of teachers, was carried out in all schools; children under school-age were not supplied with tablets. With the exception of one group of adults in a home for the aged, all adults were trusted to take their tablets as directed without supervision.

Upon returning to St. Croix approximately one year later (June 1949) as many of the control group as could be found were examined for microfilaræ, and re-surveys were made in six selected communities. Of 65 control patients examined, 82 per cent were negative for microfilaræ, and a reduction of 98 per