a unit. In ultraviolet absorption the exited electron may originate from either of the two sulphur atoms, and in an electrically asymmetrical disulphide there must be two separate exitation energies for electrons from the two different sulphur atoms, resulting in two UV absorption bands.

Experimental. A Beckman model DU photoelectric quartz spectrophotometer equipped with a hydrogen discharge lamp was used for the ultraviolet absorption measurements.

The synthetic methods for the acids (*cf.* p. 1710) will be published later.

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On the Effect of Ionizing Radiations on Cyanocobalamin

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The destruction of cyanocobalamin (vitamin B_{12}) by ionizing radiations has generally been estimated spectrophotometrically or microbiologically [1-4]. Smith [4] mentions in his recent article on the instability of radioactive vitamin B_{12} that absorption spectroscopy gives a moderately accurate measure of the residual vitamin B_{12}. He also states that a microbiological assay can be used as there is no evidence of a significant breakdown to other microbiologically active compounds. In our preliminary studies of the effect of γ-rays on cyanocobalamin we have been unable to correlate the changes in the absorption spectra with the microbiological activity of irradiated solutions of vitamin B_{12}. Moreover, evidence has been obtained for the formation of microbiologically active breakdown products.

Solutions of cyanocobalamin containing 5 mg per ml in a phosphate buffer of pH 5 were irradiated under aerobic conditions with γ-rays from a ^{60}Co source. The doses given were 2, 5 and 10 Mrad. The spectra of the irradiated and nonirradiated solutions of vitamin B_{12} were measured in a Beckman Model DU spectrophotometer. A tube assay with *Escherichia coli* 113—3 [5] was used for the microbiological tests. The assay media were those of Diding [6] and of Burkholder [7], to which 1.5 mg of KCN per l had been added. Growth was measured turbidimetrically after incubation of the tubes for 20 h at 37°C.

The spectra of the non-irradiated and of the irradiated solutions of cyanocobalamin are shown in Fig. 1. Concomitant with the decrease in the absorption maxima following irradiation, there was also a tendency for the peaks in the 360 and 550 mμ regions to shift towards lower wavelengths. Calculation of the degree of destruction by comparing the absorption at 361 mμ of irradiated and non-irradiated solutions indicated that 60 % of the vitamin originally present had been destroyed by a dose of 2 Mrad. According to the microbiological assay approximately 85 % was inactivated by this dose.

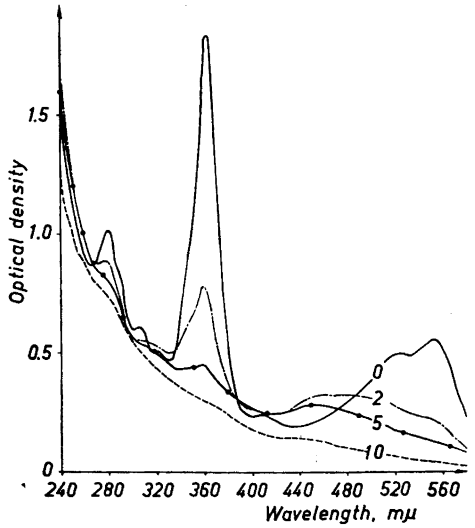

Fig. 1. Absorption spectra for solutions of non-irradiated cyanocobalamin (0), and for solutions irradiated with 2, 5 and 10 Mrad.

For the microbiological assays, series of dilutions were made of the irradiated cyanocobalamin solutions. In Fig. 2 the turbidity (optical density) of the tubes to which the solutions of irradiated vitamin B_{12} were added has been plotted against the degree of dilution. In the same figure

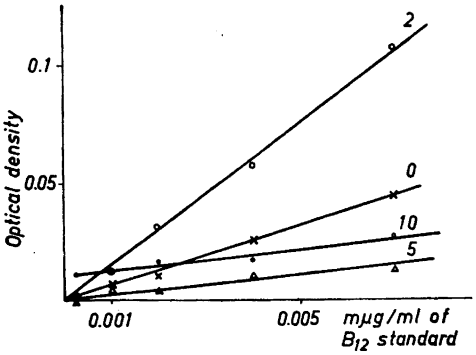

Fig. 2. The response of *E. coli* 113—3 to varying concentrations of non-irradiated cyanocobalamin (0) and to dilutions of cyanocobalamin solutions irradiated with 2, 5 and 10 Mrad.

the turbidity of the B_{12}-standards has been plotted against the concentration of the vitamin in mμg per ml. The slope of the line of the B_{12}-solution irradiated with 2 Mrad was twice as steep as that of the standard curve. The solution irradiated with 10 Mrad, however, gave a line with a slope that was less than that of the non-irradiated cyanocobalamin. As the retention of the vitamin was very low after irradiation with 10 Mrad, the latter line was obtained by adding increasing amounts of non-irradiated cyanocobalamin to the irradiated solution diluted $1:3.5 \times 10^6$. Vitamin B_{12}-solutions that had received a dose of 5 Mrad gave varying results but the dose-response line had in most cases (as in Fig. 2) a slope that was less than that for the non-irradiated vitamin. The same results were obtained irrespective of whether the simple medium of Diding or the more complex medium of Burkholder was used. If cyanocobalamin were the only microbiologically active compound remaining after irradiation one would expect the slopes of the lines to be identical.

The factor(s) responsible for the increased relative activity of the sample that had received a dose of 2 Mrad appears to be more heat labile than vitamin B_{12}.

Paper electrophoresis has revealed the presence of at least four compounds in the irradiated solutions which can support the growth of *E. coli* 113-3.

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