since this is a stable isotope. Hemin crystals were first obtained from the whole blood. By means of a micro-Kjeldahl process the nitrogen was extracted from the hemin in the form of an ammonium salt which was then treated in an evacuated system with hypobromide so as to release the nitrogen in gaseous form. The gas was compressed into sample bottles suitable for introduction into a mass spectrometer by means of which the ratio of $N^{14}N^{15}$ to $N^{14}N^{14}$ was determined. The probability of forming a $N^{15}N^{14}$ molecule is proportional to $2n_{14}n_{15}$ where n_{15} and n_{14} are the numbers of heavy and light nitrogen atoms in the sample. Likewise, the probability of forming a $N^{14}N^{14}$ molecule is proportional to $n_{14}n_{14}$. The ratio of $N^{14}N^{15}/N^{14}N^{14}$ will be therefore $2n_{15}/n_{14}$. It follows that the percentage of N^{15} in the sample is

$$\% N^{15} = \frac{100n_{15}}{n_{14}+n_{15}} = \frac{100}{2/r+1}$$

where r is the ratio measured on the mass spectrometer. The percentage of N^{15} excess is obtained by subtracting 0.368 (the percentage of N^{15} occurring naturally in matter) from this number. These procedures are essentially those described by Rittenberg *et al.* (*5*).

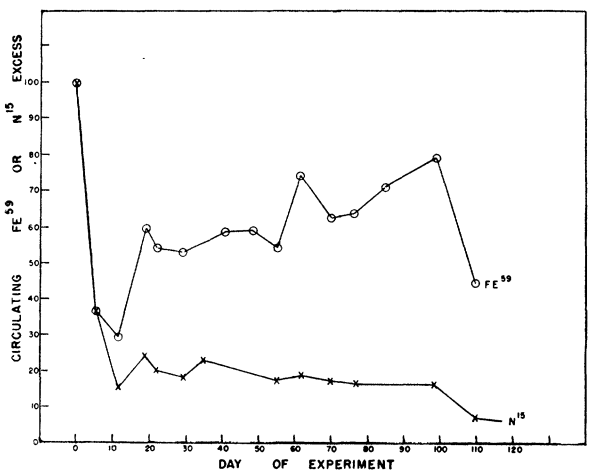

FIG. 2.

The results of the experiments are shown in Fig. 2. The circulating radioactivity as deduced from red cell mass determinations and radioactivity measurements was calculated at intervals of several days and is shown by the upper curve in the figure. The amount of activity just before the injection of the acetylphenylhydrazine was arbitrarily chosen as 100%. As usual, allowance has been made for sampling loss and the natural decay of the iron. The amount of circulating iron decreases sharply with the destruction of the dog's erythrocytes, but again there is remarkable reutilization as the dog regenerates its red cell mass.

In the case of the nitrogen it is the product of the percentage excess of N^{15} and the red cell mass which is of interest. This we refer to as the circulating N^{15} excess in Fig. 2 where it is plotted as a percentage of the initial value. Evidently the destruction of the red cells results in a permanent loss of N^{15} since there is no measurable reutilization.

It is clear that by using an isotope of nitrogen rather than of iron to follow the fate of transfused red cells, the problems resulting from reutilization can be avoided. Moreover, since it is a stable element it is not subject to the restrictions of a long half-life radioactive tracer such as C^{14}—nor is it hampered by the uncertainties of the agglutination technique sometimes used to follow red cells which is a statistical method and depends upon the strength of the serums used. It must be noted, however, that extension of this technique to tracing of transfused cells in human beings, although feasible, will be difficult because of the high excess of N^{15} required in the donor. Because of the inherent difficulties and uncertainties of other methods of following transfused red cells and in view of the results of this experiment, it is believed that N^{15} can become an important tool in the nation's vital research program for improved blood preservation.

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The Effect of Streptomycin on Oxygen Uptake and Viability of Resting Suspensions of *Escherichia coli*

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The inhibitory effect of streptomycin on the respiration of "resting" bacterial suspensions has been noted by several observers (*1–4*). Work in this laboratory has indicated that there is a discrepancy between the killing effect of streptomycin on bacterial cells and respiratory inhibition by this drug. The experiments reported in this preliminary note may be of interest from the standpoint of studying the effects of metabolic inhibitors on resting cell suspensions.

Simultaneous experiments, using the same suspension of *E. coli* (841),[1] were set up to compare the effects of streptomycin on oxygen uptake and viability. The results of such an experiment are presented in Fig. 1.

[1] The organisms were grown for 24 hr with aeration at 37° C in a synthetic medium prepared as follows: $(NH_4)_2SO_4$ 2.5 g, KH_2PO_4 2.0 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, glucose 10.0 g (autoclaved separately), tap water 200 ml, distilled water to 1000 ml, pH adjusted so that after autoclaving pH was approximately 7. After harvesting, the cells were washed twice with sterile distilled water, suspended in sterile distilled water, and refrigerated at 4° C for 24 hr before use.

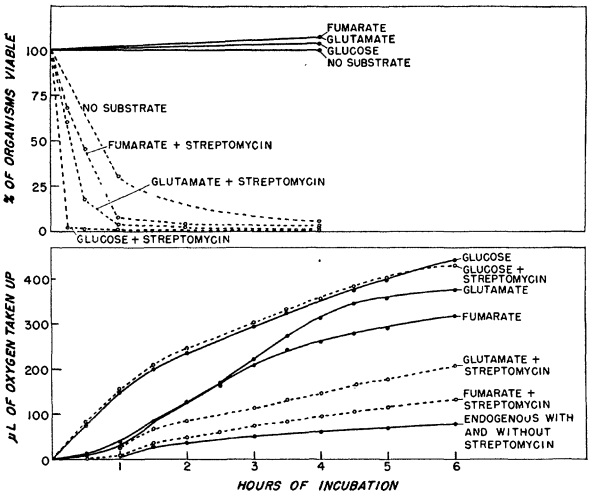

FIG. 1. Effect of streptomycin on respiration and viability of *E. coli*. The oxygen uptake studies are shown in the lower portion of the figure; conventional Warburg techniques were used. Fifty-milliliter Erlenmeyer flasks were used for the viability tests, shown in the upper part of the figure. The same suspension of cells was used in both instances and the ratios of cells to substrate and to streptomycin were kept the same, as follows: cells, 0.5 mg/ml (dry weight) containing approximately 2×10^9 viable cells; substrates, 5 μmoles; streptomycin, (dihydrosulfate) 100 μg/ml; phosphate buffer pH 7, 0.0036 *M*. The experiments were conducted at 37° C, with agitation to insure adequate oxygenation. Aliquots were removed at intervals for pour plate counting in heart infusion agar (Difco) with 1% glucose.

Streptomycin is noted to have no effect on endogenous respiration or on respiration in the presence of glucose, whereas there is a marked inhibition of oxygen uptake with fumarate and glutamate as substrates. Noticeable inhibitory effects become apparent after 1 to 2 hr of incubation. In the absence of streptomycin there is a lag period with glutamate and fumarate before the maximum rate of oxygen uptake occurs, whereas there is no lag period in respiration with glucose as substrate.

Killing of the cells by streptomycin is most rapid in the presence of glucose as substrate, with over 99% of the cells being killed in the first 15 min. Despite this fact, however, oxygen uptake with glucose as substrate continues in an unaffected fashion. The rates of killing are less rapid with glutamate and fumarate as substrates and least rapid in the absence of added substrate. The killed cells could not be revived by repeated washing in an attempt to remove the streptomycin. Similar experiments using streptomycin-resistant and streptomycin-dependent variants of this strain of *E. coli* showed no killing or respiratory inhibition by streptomycin.

These results indicate that streptomycin can kill susceptible coliform cells in the resting state in the absence as well as in the presence of inhibitory effects on respiration. The inhibition of oxygen uptake by streptomycin seems to bear no particular relation to the killing effect of this drug but rather to the substrate being metabolized. In view of the lag phase demonstrated in Fig. 1 in the oxygen uptake curves with fumarate and glutamate as substrates, respiratory inhibition with these two substances by streptomycin may simply represent an interference with adaptive enzyme formation, as earlier suggested by other workers (*5*).

It is not possible to say categorically that the cells have already been killed before plating because viability can be determined with certainty only by subculture. The streptomycin may merely be "fixed" in the bacterial cells during the period of exposure and "death" may occur following the initiation of metabolic activities concerned with growth when the cells are transferred to a suitable culture medium. Perhaps streptomycin renders the cells "sterile," that is, they are not dead but cannot reproduce themselves.

In any case it is fair to say that the fixing of streptomycin to the susceptible site in the cell, with subsequent death of the cell, occurs most rapidly in the presence of metabolizable substrates and least rapidly with endogenous metabolism. The rate of fixing of the drug to the cells appears to be directly related to the rate or degree of metabolism of the cells.

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The Effects of Wind-Drift of Weed-Killer on Some Puerto Rican Trees

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For several years one of the junior writers has been noticing the toxic effects of 2-4-D and other weed-killers on susceptible species of cultivated plants such as Sea Island cotton, *Gossypium barbadense*, and papaya, *Carica papaya*. The damage to these plants is particularly noticeable when they grow near fields of sugarcane in Puerto Rico, where weed-killers are used to control weeds.

Last year at the Isabela Substation an entire papaya grove of about 600 plants was seriously affected by 2-4-D sprayed 2000 yd or more to the east. The fine spray of the weed-killer carried by the trade winds from the northeast was powerful enough to affect all the papaya plants.

Since January 1952, the writers have been making field observations and taking numerous photographs of the effects of weed-killers on our flora. Detailed observations will be published elsewhere in the near future. However, it is of interest to note that two common species of trees in Puerto Rico serve as an