after complete formation of the protoporphyrin ring, but the enzyme system concerned is not yet known. We have investigated the role of mitochondria in this process. δ-Aminolevulinic acid was used as substrate and added to the following preparations: (i) chicken red-cell hemolysate supernatant; (ii) the same, plus rat-liver mitochondria; (iii) the whole chicken red-cell hemolysate; (iv) the whole chicken red-cell homogenate plus rat-liver mitochondria. Ferrous sulfate was added at the beginning of the incubation and also 4 hours after the beginning. It was demonstrated that addition of iron to the systems containing mitochondria brought about in each case a decrease in free protoporphyrin. It is thus very probable that mitochondria play an important role in the incorporation of iron into the porphyrin ring, but this supposition must be reexamined by means of labeled δ-aminolevulinic acid.

An attempt to localize the region of protoporphyrin formation within the living cell was made as follows: Chicken erythrocytes were incubated with glycine for 4 to 6 hours at 37°C, and the nuclear and cytoplasmic fractions were then separated by a modification of Schneider's method (*5*). It was found that protoporphyrin was present in the cytoplasm but not in the nuclei. When, however, the nuclear fraction was contaminated by mitochondria (contamination was determined by cytochrome-*c* oxidase), as is often the case when using Schneider's method, much protoporphyrin was to be found in the nuclear fraction. The livers of rabbits that had been fed Sedormid or allylisopropylacetamide were also used for this study. Much protoporphyrin was found in the mitochondria; little was found in the supernatant, microsomes, and nuclei.

These findings, together with the ones presented above, indicate that protoporphyrin can be synthesized and accumulated in the mitochondria. From the experiments described above, we concluded that mitochondria take part in three steps of porphyrin and heme biosynthesis. (i) They take part in the formation of δ-aminolevulinic acid from glycine and active succinate. Here the presence of uninjured mitochondria in the hematopoietic tissue is essential. (ii) They participate in some step or steps in the synthesis of protoporphyrin from δ-aminolevulinic acid. Here not only intact mitochondria of bone marrow, erythrocytes, liver, and mesenteric lymph nodes are active but also disrupted mitochondria or aqueous extracts of acetone powders of the mitochondria. (iii) Lastly, mitochondria appear to participate in the incorporation of iron into the porphyrin ring—that is, in heme formation.

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Vitamin B_{12} in Sewage Sludges

In view of the reports by other workers on the occurrence of an appreciable amount of vitamin B_{12} in activated sludge (*1, 2*) and on the possibility of increasing the B_{12} content of such sludge by adding small amounts of cobalt to sewage in the aeration tanks (*3*), we found it of interest to make a comparative study of the different types of sewage sludges from the point of view of vitamin B_{12} content (*4*). In this connection we examined raw sewage solids, raw sewage precipitated with chemicals such as lime and alum (0.75 g of the chemical per liter of sewage), septic sludge, and activated sludge. For all these sludges the source of the raw sewage was the same. *Epistylis* sp., a peritrichous ciliate protozoan occurring abundantly in the activated sludge tank, was also collected, repeatedly washed in clean water, and examined for its B_{12} content. For purposes of comparison, cow dung was also examined.

The sludges and other materials were treated with a few drops of 1-percent sodium cyanide and dried over a water bath. The B_{12} was extracted from the dried materials with 1-percent sodium acetate, *p*H 4.6, and the vitamin was assayed with *Lactobacillus leichmanii* ATCC 4797 as the test organism. The method employed was essentially the same as that of Skeggs *et al.* (*5*). The true vitamin B_{12} activity was determined by the alkaline destruction method. The results are given in Table 1.

Table 1. Vitamin B_{12} contents of different sewage sludges, of a sewage protozoan, and of cow dung (in micrograms of B_{12} per 100 g of the material).

Total B_{12} activity	Alkali-stable activity	True B_{12} activity (by difference)
	Raw sewage solids	
17.29	0.82	16.47
	Lime sludge	
30.48	24.72	5.76
	Alum sludge	
7.41	4.12	3.29
	Septic sludge	
32.13	22.24	9.89
	Activated sludge	
76.21	Traces	76.21
	Protozoa (Epistylis *sp.*)	
53.54	7.42	46.12
	Cow dung	
14.00	Traces	14.00

From these results it may be seen that, among the sludges, activated sludge contains the highest amount of B_{12}, although the figure given here is much lower than the figures reported for similar sludge (*1*). It is of considerable interest to note that the protozoan commonly found in activated sludge also contains an appreciable amount of B_{12}. The results also indicate the possible use of activated sludge as a supplement to the feeds of animals such as chicks and pigs.

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