thesis by puromycin. This suggests more strongly a direct relationship between the thyroxine effects on protein synthesis and on oxidative metabolism. Other evidence of such a relationship has been presented by Hanson, Lindsay, and Barker (*11*), who obtained an excellent correlation between the changes in oxygen consumption and amino acid incorporation into protein induced by thyroxine added to kidney slices incubated in vitro. If the action of puromycin is specifically limited to its effect on protein biosynthesis, then the results of the present studies indicate not only that the thyroxine effects on oxygen consumption and protein synthesis are directly related, but that the changes in oxidative metabolism are secondary to the effects on protein synthesis, perhaps in response to the increased energy demand.

It would be of interest to know if any other clinical symptoms, signs, or biochemical abnormalities associated with hyperthyroidism are controlled or reversed by treatment with protein synthesis blocking agents such as puromycin. If so, then the use of such drugs in doses titrated to counterbalance the peripheral action of thyroxine on protein synthesis might constitute a novel and effective means for the clinical management of Graves' disease or thyrotoxic storm.

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Terminal Oxidation in the Regulation of Heme Biosynthesis

Abstract. *Turnover of the tricarboxylic acid cycle and the rate of succinyl-coenzyme A formation may be important factors in the regulation of heme biosynthesis in liver homogenate. Acting as hydrogen acceptor, acetoacetate appears to have a unique role in influencing these metabolic processes.*

Since all known functions of heme are directly related to oxidative processes, the state of cellular oxidation might influence the metabolic regulation of porphyrin and heme biosynthesis (*1*). However, no regulatory mechanism has been described which would link directly porphyrin and heme synthesis with biological oxidation, although a number of indirect observations do suggest this association. For example, Falk *et al.* (*2*) have shown that decreasing oxygen tension, within limits, will result in increased porphyrin and heme formation by avian erythrocytes in an in vitro system. Although this system was used extensively in elucidation of the pathway of biosynthesis, the photosynthetic bacterium *Rhodopseudomonas spheroides* (*3–5*), an organism which can be induced to synthesize porphyrins at a greatly accelerated rate by growing them anaerobically in the light, has been used to study the regulation of porphyrin synthesis. Most of these studies with *R. spheroides* have been concerned with the tricarboxylic acid (TCA) cycle and with activity of 5-aminolevulinate synthetase. In an extensive investigation of factors influencing pigment formation in this organism, Cohen-Bazirre *et al.* (*6*) have concluded that the rate of porphyrin formation in the cells is governed by the state of oxidation of a carrier in the electron transport system.

Another instance where porphyrin synthesis is markedly increased above normal is in the livers of animals with experimental porphyria (*7*). This condition is induced by the administration of various chemical compounds, the most common being allylisopropylacetamide. A variety of experiments with mammals, chick embryos, tissue culture, and purified enzyme systems indicates that this type of induced porphyria reflects an inhibition in terminal electron transport (*8*). Such a conclusion suggests a possible association of porphyrin biosynthesis with biological oxidation. Results of more direct experiments linking these two processes have now been obtained.

Seconal [sodium 5-allyl-5(1-methylbutyl)barbiturate] induces experimental porphyria and is an inhibitor of reduced nicotinamide-adenine dinucleotide ($NADH_2$) oxidase (*9*), one of the electron-transport systems. The drug also has some well-defined effects on the utilization of several heme precursors by rat liver homogenate (Table 1). At a concentration of $1.8 \times 10^{-4}M$, Seconal inhibited by 45 percent the incorporation of 2,3-C^{14}-succinate into heme. In similar experiments (not shown in Table 1), 5-C^{14}-2-ketoglutarate and 2-C^{14}-glycine incorporation were inhibited by 16 and 35 percent respectively. At the same concentration, Seconal did not significantly alter the incorporation of 1-C^{14}-succinate, 4-C^{14}-5-aminolevulinate, or Fe^{59} into heme. The meaning of these different effects on the course of heme synthesis can be explained on a basis of the differing routes of incorporation of the precursors. Thus 2,3-C^{14}-succinate can incorporate C^{14} into heme either by way of the TCA cycle or by direct conversion to succinyl-coenzyme A. By contrast, 1-C^{14}-succinate can incorporate C^{14} into heme only by direct conversion of succinate to succinyl-coenzyme A, since the carboxyl groups

Table 1. Incorporation of C^{14}-precursors into heme by rat liver homogenate. Incubation mixtures contained 5.0 ml of fresh 20 percent liver homogenate in saline medium, *p*H 7.4 (*15*), 100 μmoles glycine, 1 μmole $FeSO_4$, 2 μc C^{14}-precursor (0.03 μc 4-C^{14}-5-aminolevulinate) and addenda as indicated in a final volume of 5.4 ml. Incubation was at 37°C for 30 min under air with shaking. Hemin in the incubation mixture was isolated by the method of Labbe and Nishida (*16*) with red blood cells to furnish carrier.

C^{14}-precursor	Radioactivity; heme formed in 1.0 g of liver (count/min)	
	No addition	Acetoacetate $1.85 \times 10^{-3}M$
Experiment A		
2,3-C^{14}-succinate (6.2 mc/mmole)	616	926
2,3-C^{14}-succinate + Seconal ($1.8 \times 10^{-4}M$)	339	681
Experiment B		
1-C^{14}-succinate (6.2 mc/mmole)	180	178
2,3-C^{14}-succinate (6.2 mc/mmole)	940	1790
5-C^{14}-2-ketoglutarate (3.05 mc/mmole)	162	203
4-C^{14}-5-aminolevulinate (4.0 mc/mmole)	5370	5160

Table 2. Effects of malonate on heme biosynthesis. Malonate concentration was $1.2 \times 10^{-2} M$. Other conditions were as described in Table 1.

Radioactivity; in heme formed in 1.0 g of liver (count/min)			
No addition	Malonate	Aceto-acetate	Malonate + aceto-acetate
	2,3-C^{14}-succinate		
384	211	443	408
	1-C^{14}-succinate		
79	82	81	69

are lost during metabolism through the cycle (*10*). Together with these observations, it should be noted that 2,3-C^{14}-succinate is incorporated into heme by passing through two NAD-dependent enzymatic reactions (malate dehydrogenase and 2-ketoglutarate oxidase). 2-Ketoglutarate is incorporated into heme by conversion to succinyl-coenzyme A through the action of the latter enzyme system. In the incorporation of radioactivity into heme by 1-C^{14}-succinate, 4-C^{14}-5-aminolevulinate, and Fe^{59}, no known NAD-dependent reactions occur. Thus, it would appear that these effects of Seconal can be explained as an inhibition of $NADH_2$ oxidation.

A consistent change observed in the livers of porphyric animals has been an increase in the acetoacetate concentration (*8*). This important metabolite is an excellent oxidant for $NADH_2$ as catalyzed by β-hydroxybutyrate dehydrogenase. Therefore, if Seconal inhibits heme synthesis indirectly in liver homogenates by blocking $NADH_2$ oxidation, then the inhibition should be reversed by acetoacetate; and this is the effect observed (Table 1). In fact, even in the absence of Seconal, acetoacetate at a concentration of $1.85 \times 10^{-3} M$ stimulated the synthesis of heme from 2,3-C^{14}-succinate by as much as 100 percent. The stimulation from 2-ketoglutarate and glycine to heme was somewhat less; and there was no effect of acetoacetate on 1-C^{14}-succinate, 4-C^{14}-5-aminolevulinate, or Fe^{59} incorporation into heme.

Acetoacetate may further influence the utilization of succinate by another mechanism. Succinic dehydrogenase is competitively inhibited by malonate, resulting in a block of the TCA cycle and inhibition of the incorporation of 2,3-C^{14}-succinate into heme. This malonate inhibition appeared to be reversed completely by acetoacetate (Table 2). The utilization of 1-C^{14}-succinate was unaffected by malonate, implying that there may be a NAD-dependent, malonate-insensitive pathway for the oxidation of succinate, a pathway that would be stimulated by acetoacetate. This, of course, is speculative since the reduction of NAD by succinate is not yet fully understood. Chance and Hollunger (*11*) have suggested several possible mechanisms by which this process might occur. Acetoacetate may be quite specific in affecting heme synthesis since no other hydrogen acceptor has yet been found which behaves similarly.

Accordingly, Seconal probably acts by inhibiting the TCA cycle, an effect that would be expected from impairment of $NADH_2$ oxidation. Acetoacetate, on the other hand, appears to behave in the opposite manner by increasing $NADH_2$ oxidation and hence perpetuation of the TCA cycle, making tenable the hypothesis that porphyrin and heme synthesis is regulated, partially at least, by the rate of turnover of the TCA cycle. A sustained high level of succinyl-coenzyme A may participate in the induction of 5-aminolevulinate synthetase (*3, 12*) in much the same way that 5-aminolevulinate induced 5-aminolevulinate dehydratase (*4, 13*). Undoubtedly the activities of these two enzymes play important roles in regulating porphyrin biosynthesis.

An apparent paradox in the effects of Seconal should be noted. In liver homogenates the drug inhibits porphyrin and heme synthesis; but after injection into an animal, it induces porphyria and thereby increases porphyrin and heme synthesis. These differing responses to Seconal can be reasonably explained by assuming that the drug in both instances still inhibits $NADH_2$ oxidation. However, the intact cell, with its compartmentalization, might behave quite differently from an homogenate in response to this inhibition (*14*).

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References and Notes

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Radium-226 in Human Diet and Bone

Abstract. *The mean radium-226 content of samples of vertebrae from New York City and San Francisco is 0.032 and 0.026 picocuries of radium-226 per gram of calcium, respectively. The ratio of these two mean values is associated with a similar ratio of radium-226 to calcium found in the diet at the two locations. A plot of the radium-226 content of bone as a function of age indicates that there is no marked difference in levels of bone radium with age.*

In the past few years, perhaps spurred by studies of Sr^{90} from weapons fallout, there has been renewed interest in the Ra^{226} content of man in relation to his diet. A knowledge of the Ra^{226} content of the biosphere can lead to an understanding of a significant part of our environmental radioactivity. In addition, it is of interest in that Ra^{226} in man is probably close to being in equilibrium with the environment, as other investigators indicate and this study further corroborates. This relationship is not true of Sr^{90}.

In 1962, this laboratory determined the dietary Ra^{226} levels for three cities in the United States: New York, Chicago, and San Francisco (*1*). The average daily intake, as determined from some 180 samples of all foodstuffs including water, was found to be 2.3, 2.1, and 1.7 pc of Ra^{226} per day or 2.2, 2.0, and 1.6 pc/g of Ca, respectively.

In this current work, samples of