cept for their remarkably low potassium content, these basalts are very similar to typical tholeiites from Hawaii and from many other areas (*9, 10*).

The basalt PV 17 is texturally and compositionally similar to the basalt cored from the experimental Mohole (Tables 1 and 2). The experimental Mohole basalt (sample EM) contains 0.16 percent K_2O, and PV 17, 0.26 percent K_2O. There are no obvious signs of leaching or extensive alteration of these basalts that might induce a secondary loss of potassium. It is probable that the low potassium content of these basalts is a primary feature. Basalts from both of these sites tend to be uniformly crystalline, medium-grained, and have a diabasic texture.

Other compositional features of interest in the basalts analyzed to date are the wide range and occasional high values of Ti, P, H_2O, and the ratio of Fe_2O_3 to FeO. Numerous workers have attempted to relate the degree of hydration and oxidation of basalts to subaerial or subaqueous extrusion and to textural features (*11*). These relations are bound to be complicated by nonequilibrium processes and products. Hence, generalizations are dangerous. Extreme hydration and oxidation of fine-grained and glassy submarine basalts from the dredge hauls are common and widespread, presumably both as a primary feature and as a function of aging and weathering. There is no obvious, simple correlation between texture and degree of oxidation and hydration in the least weathered basalts as is frequently suggested (*11*). It seems probable, however, that most fine-grained and glassy submarine basalts become increasingly hydrated and oxidized within a few thousand or tens of thousands of years after their extrusion. We are separating plagioclases and pyroxenes from these basalts for age dating in an effort to clarify some of these problems.

The glassy to distinctly porphyritic nature of many samples permits the complete separation of phenocrysts and groundmass. Analyses of the separates permits studies of the partitioning of elements between the coexisting phases, the course of crystallization, magmatic differentiation, as well as age studies. In many of the basalts, olivine, plagioclase, pyroxene, and iron oxides appear to precipitate within a relatively short temperature interval (*11*). This is indicated by the fact that all four phases appear in basalts of appropriate composition that contain more than 85 percent glass and crystallites. Other basalts deviate from this pattern. The most striking example is the aluminous basalt PV 148. Therein, the plagioclase phenocrysts form up to 45 percent of the rock and the remainder consists of microcrystalline groundmass and glass. Very possibly sample PV 148 is an example of a crystal accumulate in which plagioclase has been locally concentrated.

The optical properties, brownish-pink color, and chemical composition of pyroxene in the alkali basalts indicate that subcalcic augite and titanoaugites predominate. The augite in sample PV 77 contains 2.5 percent TiO_2, 7 percent Al_2O_3, and 22 percent CaO. The olivine is 65 percent forsterite. Compared to the total rock, the groundmass of sample PV 77 is enriched in alkalis, especially Na, as well as Fe and has a higher ratio of Fe_2O_3 to FeO. In contrast, it is slightly impoverished in silica. Hence, the trend in the composition of the liquid with increasing crystallization was toward an even more alkalic basalt (*12*).

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Reversal of Thyroxine-Induced Hypermetabolism by Puromycin

Abstract. *Previous studies have demonstrated that in addition to its effects on metabolic rate, thyroxine stimulates protein biosynthesis. The administration of puromycin, a drug which blocks protein synthesis and, therefore, the thyroxine effect on protein synthesis, acutely reverses the hypermetabolism induced in rats by prior administration of thyroxine and restores the oxygen consumption of the thyrotoxic rats to the euthyroid level. The results suggest that a larger fraction of the total body basal oxygen consumption in hyperthyroidism is related to the process of protein synthesis than in the euthyroid state and that the calorigenic effect of thyroxine is secondary to its effect on protein synthesis.*

Previous studies in this laboratory have demonstrated that L-thyroxine administered either in vivo or in vitro stimulates the rate of amino acid incorporation into microsomal protein in cell-free rat liver homogenates (*1, 2*). More recently it has been found that hyperthyroidism induced by L-thyroxine administration results in increased amino acid incorporation into protein in the liver, kidney, and heart in the intact animal (*3*), but has no effect on incorporation in the brain, testis, and spleen, all organs in which oxygen consumption is also unaffected in hyperthyroidism (*4*).

In their initial report of the thyroxine effect on amino acid incorporation into protein, Sokoloff and Kaufman (*1*) suggested that the "acceleration of metabolic rate characteristic of thyroxine action may be secondary to the stimulation of energy-requiring reactions such as protein synthesis." The results of the present studies offer support for this possibility. Administration

Table 1. Effects of puromycin on metabolic rates of normal and thyrotoxic rats. Δ, difference in milliliters of oxygen per 100 grams body weight per hour.

Expt. No.	Oxygen consumption* (ml O_2/100 g body weight per hour)				L-Thyroxine effect				Puromycin effect			
	Before puromycin		After puromycin		Before puromycin		After puromycin		Normal		Thyrotoxic	
	Normal	Thyrotoxic	Normal	Thyrotoxic	Δ	%	Δ	%	Δ	%	Δ	%
1	184	260	150	204	+ 76	+ 41	+ 54	+ 36	− 34	− 18	− 56	− 22
2	202	276	186	184	+ 74	+ 37	− 2	− 1	− 16	− 8	− 92	− 33
3	220	394	220	220	+ 174	+ 79	0	0	0	0	− 174	− 44
4	199	332	170	199	+ 133	+ 67	+ 29	+ 17	− 29	− 15	− 133	− 40
5	181	287	159	229	+ 106	+ 59	+ 70	+ 44	− 22	− 12	− 58	− 20
6	195	278	170	157	+ 83	+ 43	− 13	− 8	− 25	− 13	− 121	− 44
Mean	197	304	176	199	+ 107	+ 54	+ 23	+ 15	− 21	− 11	− 106	− 34
SE	± 6	± 20	± 10	± 11	± 16	± 7	± 14	± 9	± 5	± 3	± 19	± 4
p†					< .01		> .1		< .01		< .01	

* Corrected to standard temperature and pressure. † Determined by method of paired comparison.

of the inhibitor of protein synthesis, puromycin, acutely lowers the metabolic rate in thyrotoxicosis to the normal level and eliminates the difference in total body oxygen consumption observed in normal and thyrotoxic rats immediately prior to the puromycin treatment.

Recent studies on the mechanism of the thyroxine stimulation of protein synthesis have localized the effect to the step involving the transfer of soluble-RNA-bound amino acid to microsomal protein (*5*). Puromycin inhibits protein synthesis by inhibiting this step (*6*). It is believed to compete with aminoacyl-sRNA and interrupts the normal sequence of aminoacyl-sRNA incorporation into the template leading to the formation of smaller peptide chains (*7*). Inhibition of protein synthesis by puromycin would of necessity then block the thyroxine stimulation of protein synthesis. If the increased metabolic rate in thyrotoxicosis were secondary to the thyroxine effect on protein synthesis, then blockade of protein synthesis could reasonably be expected to remove the increased oxygen consumption in hyperthyroidism; on the other hand, if the calorigenic effect of thyroxine were primary to or independent of its action on protein synthesis, then the increased metabolic rate in hyperthyroidism would then be maintained, at least in part, for some period of time after the block of protein synthesis. These considerations formed the basis of the experimental design of these studies.

Normal male Osborne-Mendel rats weighing between 75 and 130 g were paired for age and weight. One member of each pair received almost daily intraperitoneal injections of 200 μg of Na L-thyroxine dissolved in 1 ml of 0.01*N* NaOH for 6 to 13 days; the other received equivalent volumes of the NaOH solution alone. Paired animals were treated identically through all subsequent procedures in accordance with the paired experimental design. The doses of thyroxine employed were more than adequate to produce substantial increases in amino acid incorporation into protein (*1*, *2*). Such large doses were used to maximize the possibility of finding residual differences in oxygen consumption between the normal and thyrotoxic animals after puromycin treatment. The animals were fasted 16 to 24 hours prior to the initial measurement of total body oxygen consumption. Oxygen consumption was measured over a 30-minute period by means of a small animal spirometer (model 160, Custom Engineering and Development Co., St. Louis) and corrected to values at standard temperature and pressure. Immediately after the initial measurement of metabolic rate, 20 mg of puromycin was administered intraperitoneally in two equally divided doses 45 minutes apart, and measurement of oxygen consumption was then repeated 1 hour after the initial dose. The timing and doses of puromycin were selected to achieve at least 90-percent inhibition of protein synthesis (*8*).

The results are presented in Table 1. Before puromycin treatment the thyrotoxic rats exhibited the expected increase in metabolic rate. Puromycin treatment produced a small but significant fall of about 11 percent in the metabolic rate of the euthyroid rats, but in the thyrotoxic rats puromycin decreased the metabolic rate by about 34 percent to a level not significantly different from that of the euthyroid rats either before or after puromycin treatment. In effect, the puromycin completely reversed the thyroxine effect on oxygen consumption and eliminated the difference in metabolic rates of normal and thyrotoxic animals. These results demonstrate that the interruption of protein synthesis blocks the action of thyroxine on metabolic rate and suggest that the calorigenic effect of thyroxine is secondary to its effect on protein synthesis. Furthermore, they suggest that a larger fraction of the total body basal energy utilization in hyperthyroidism is related to the process of protein synthesis than in the euthyroid state.

It was recently reported that inhibition of protein synthesis by puromycin or actinomycin D in hypothyroid rats prevents the reversal of the hypometabolic state by simultaneously administered triiodothyronine (*9*). Such a study, however, fails to provide sufficient evidence to imply a direct relationship between the thyroxine effects on oxygen consumption and protein synthesis. In hypothyroidism there are undoubtedly alterations in the levels of enzymes involved in oxidative metabolism (*10*), and interruption of protein synthesis during the latent period of action of triiodothyronine may prevent a reorganization of enzyme levels necessary to permit the expression of triiodothyronine action on oxygen consumption. Indeed, it is not unlikely that in many deficiency states inhibition of protein synthesis prevents recovery during specific replacement therapy, even if the deficient agent has no function directly related to protein synthesis. In the present studies, however, the thyroxine effect on oxidative metabolism was being fully expressed up to the time it was acutely interrupted by the acute inhibition of protein syn-

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