brown adipose tissue removed from the interscapular brown fat pad of 21 rabbits there was an average increase of 2.099 (SE 0.45) μeq/g hour beyond that of nonstimulated controls.

These observations indicate that both white and brown adipose tissue respond to nerve stimulation. The response of white fat is dependent on nerves of sympathetic origin, since it is decreased by the presence of dibenamine and completely prevented by sympathectomy, timed to allow nerve degeneration.

The isolated nerve-fat preparation has been used to explore some additional factors which effect this neuroeffector system (*10*), and it should facilitate further investigation of this mechanism (*11*).

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Tryptophan- and Indole-Excreting Bacterial Mutants

Abstract. *Prototrophic mutants of* Escherichia coli *capable of producing indole and tryptophan were obtained by selecting for resistance to antimetabolites. An anthranilic acid auxotroph grown under conditions which would derepress the tryptophan synthetase system would also accumulate substantial quantities of* L*-tryptophan.*

The usual method of obtaining microbial cultures which synthesize substantial amounts of amino acids is by screening, through trial and error, isolates from soil samples or random mutants from a known culture. Adelberg (*1*) has suggested a more rational method which is based upon the use of substances antagonistic to the desired metabolite.

An extension of such an approach is being applied in our laboratory to obtain bacterial mutants which can produce substantial quantities of extracellular L-tryptophan. *Escherichia coli* K-12 was subjected to ultraviolet (UV) radiation and then was plated on modified M-63 (*2*) agar containing 10 mg of 5-methyltryptophan and 2.0 g of glucose (instead of glycerol) per liter. Of the 5-methyltryptophan-resistant mutants which developed, several were constitutively derepressed with respect to the tryptophan synthetase system (*3*). The mutant with the greatest activity of tryptophan synthetase was treated again with ultraviolet radiation and plated on modified M-63 agar containing 300 mg of anthranilic acid per liter; this substance has been reported to inhibit the conversion of anthranilic ribonucleotide to indole-3-glycerol phosphate (*4*). Such treatment might select mutants with a synthesizing enzyme for indole-3-glycerol phosphate that has altered sensitivity to anthranilate or mutants more completely derepressed than the parent in some or all of the enzymes that produce tryptophan.

The resulting doubly resistant mutant cross-fed with an auxotroph of *E. coli* that responded to either indole or trytophan; it did not cross-feed with an auxotroph which responded only to tryptophan (*5*). Treatment of the doubly resistant mutant with ethylmethanesulfonate (EMS) (0.03 ml/2 ml of culture) produced mutants which would cross-feed with the tryptophan-responding auxotroph. When one of these EMS-produced mutants, chosen because of its large zone of cross-feeding, was grown while being shaken at 37°C on M-63 broth containing glycerol, an indole concentration of 300 mg/liter resulted after 20 hours of incubation. The final turbidity of the culture was 350 Klett units with a 640-mμ filter. Approximately 50 mg of tryptophan per liter were also present at this time (*6*). It is probable that the indole is derived from tryptophan by the action of the enzyme tryptophanase because the addition of serine had no effect on the accumulation of tryptophan. Although a similar procedure was applied to *Aerobacter aerogenes* in order to obtain derepressed mutants which lacked tryptophanase, and which would therefore accumulate only tryptophan, such efforts have not yet been successful.

When an auxotrophic mutant of *E. coli* unable to synthesize anthranilic acid (*E. coli* T3; *7*) was grown with growth-limiting amounts of anthranilic acid (3 mg/liter), thus causing derepression of the tryptophan synthetase system, and then was harvested by centrifugation and suspended (turbidity of 300 Klett units) in double-strength M-63 broth plus 1 g of anthranilic acid per liter, L-tryptophan accumulated; a peak concentration of 100 mg/liter was reached at 4 hours. Under these conditions, tryptophanase was induced and the tryptophan was degraded to indole, which accumulated to 300 mg/liter in 10 hours. If chloramphenicol (5 mg/liter) was added simultaneously with the excess anthranilic acid, synthesis of the induced enzyme tryptophanase was prevented, and tryptophan accumulation continued to a peak of 250 mg per liter in 24 hours. At this time less than 1 mg of indole per liter was present.

Previous workers have failed to detect such substantial tryptophan or indole excretion, even with constitutively derepressed or feedback-insensitive mutants. *Escherichia coli* 5 MTR-6 (*8*), a 5-methyltryptophan-resistant mutant insensitive to feedback inhibition, which excreted approximately 1 mg of indole per liter, was repressible, while Yanofsky (*9*) found that several strains of *E. coli* and hybrids of *Shigella* and *E. coli* which produced high levels of tryptophan synthetase excreted no tryptophan because of a partial block at some early step in the synthesis of tryptophan. Thus, it appears that in the case of prototrophs a single mutation either to constitutive deprepression or to insensitivity to feedback inhibition may not be sufficient to cause significant accumulation of the end product of the pathway. On the other hand, single-mutation auxotrophs blocked after the branch point of the pathway (*E. coli* T3) may readily accumulate the end product if supplied with limiting amounts of the precursor, thereby causing derepression; then a surplus of precursor is added under nongrowing conditions (*10*).

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10. *Escherichia coli* mutant T3 was furnished by Dr. C. Yanofsky, and mutant 5 MTR-6 by Dr. H. S. Moyed. We appreciate stimulating talks with Dr. B. Magasanik. Work supported in part by the Nutrition Foundation.

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Cysteine Stimulation of Glucose Oxidation in Thyroid Tissue

Abstract. *Oxidation of* D-*glucose-C*^{14} *by bovine thyroid slices was stimulated by cysteine, 2-aminoethanethiol, and cystamine. Amino acids tested, other than cysteine, were not stimulatory, except tyrosine and arginine to a slight degree. The pattern of stimulation resembled that brought about by thyroid-stimulating hormone. Stimulation of labeled* CO_2 *production was greater when glucose-1-C*^{14} *rather than glucose-6-C*^{14} *was the substrate.*

Thyroid-stimulating hormone (TSH) can be inactivated by oxidation. It can then be partly reactivated by exposure to reducing substances (*1*). Some of the reducing substances used in these reactivation experiments produce goiters, but others do not, and cysteine has been reported to belong to the second category (*2*). Since such reducing substances which do not produce goiters have never been shown to exert a direct action on the thyroid in the absence of TSH, it is of interest that cysteine and several related substances exert an action resembling that of TSH on thyroid slices in vitro.

Fresh beef thyroids obtained from local slaughterhouses were brought immediately to the laboratory in ice-chilled beakers. They were stripped of adventitial tissue and sliced on a Stadie-Riggs hand slicer into pieces about 0.5- to 1.0-mm thick weighing approximately 75 mg. These slices were then placed in 25-ml erlenmeyer flasks fitted with removable glass center wells and containing 2 ml of Krebs-bicarbonate medium, 2.0 mg of D-glucose, and 0.50 μc of D-glucose-1- or -6-C^{14} (*3*). The flasks were flushed for 20 seconds with a mixture of 95 percent O_2 and 5 percent CO_2, and incubated for 45 minutes at 37°C while being shaken. At the end of the incubation, 1 ml of "Hyamine" base was added to the removable center well and 0.2 ml of 10*N* H_2SO_4 to the medium in each flask. The flasks were then shaken for another hour at room temperature. The "Hyamine" was transferred to crystallite vials and counted in a Tri-Carb liquid-scintillation counter. The results are expressed as counts per minute per gram of wet weight of thyroid tissue. The TSH used in these experiments ("Thytropar," Armour) had a specific activity of approximately 1 U.S.P. unit per mg.

Table 1. Radioactivity recovered as $C^{14}O_2$ from D-glucose-1-C^{14}. Results are given in 10^2 counts per minute per gram of tissue, wet weight. Each set of figures represents the mean ± the standard deviation for three to five flasks.

Control	TSH*	L-Cysteine, $10^{-2}M$	Cystamine-2HCl, $10^{-2}M$	2-Amino-ethanethiol-HCl, $10^{-2}M$
		Experiment 1		
350 ± 55	673 ± 225‡	621 ± 138§	803 ± 85‡	1,185 ± 189‡
		Experiment 2		
965 ± 84	1,161 ± 135†	1,248 ± 68†	1,400 ± 79‡	2,285 ± 67§
		Experiment 3		
317 ± 15	497 ± 76†	404 ± 89†	573 ± 99‡	1,012 ± 313‡

* TSH concentration: 0.25 U.S.P. units per vessel. † $p < 0.05$. ‡ $p < 0.01$. § $p < 0.001$.

The results in Table 1 indicate that $10^{-2}M$ cysteine stimulates glucose oxidation (25 experiments) in thyroid tissue as does $10^{-2}M$ 2-aminoethanethiol (7 experiments) and $10^{-2}M$ cystamine (5 experiments). Other amino acids tested as controls were arginine, tyrosine, aspartic acid, proline, methionine, lysine, leucine, tryptophane, serine, and alanine. Tyrosine and arginine sometimes gave slight but statistically significant stimulation of glucose oxidation; the others appeared to be inert or inhibitory. All amino acids were tested at a concentration of $10^{-2}M$. It has been reported that tyrosine was inert in such a system (*4*), but it was tested then only at a concentration of $10^{-4}M$. Although occasionally the combined stimulation in the presence of both TSH and cysteine was greater than that from either alone, we were unable to demonstrate an additive or synergistic effect of the two substances. The pattern of stimulation from cysteine resembled that resulting from TSH, since oxidation of glucose-1-C^{14} to $C^{14}O_2$ was accelerated more than oxidation of glucose-6-C^{14} (four experiments). When the incubation time was shorter, stimulation by cysteine was evident within 5 minutes, another resemblance to TSH (*5*).

The action of TSH on thyroid slices has been considered fairly specific, since other known hormones of adenohypophysis do not show corresponding activity (*5*). Similar stimulation is seen with epinephrine, norepinephrine, serotonin, and acetylcholine (*6*); however, a number of other biologically active substances (for example, glucagon, insulin, methimazole, triiodothyronine, histamine, thiolhistidine, and others) have been tested and do not stimulate glucose oxidation under these conditions (*7*). It has been postulated that all of the stimulatory substances act by increasing the availability of oxidized triphosphopyridine nucleotide though perhaps by several different mechanisms (*8*). While this may be the case with the thiols mentioned in this paper, the exact mechanism by which such stimulation would take place is not immediately apparent. A purely preservative action of these thiols cannot be ruled out by our data, but the several similarities to TSH stimulation suggest that the mechanisms may be alike in the two cases.

These results emphasize the importance of proper controls when reducing agents are used as stabilizing agents during the preparation or storage of TSH (*9*).

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