pression of cardiac contractility. It appears plausible also to conclude that the stores of norepinephrine or epinephrine in the myocardium are important in maintaining normal contractility. It is suggested that the myocardial catecholamines may be released in small quantities under normal conditions to affect the rate of the pacemaker and contractility and serve as humoral agents for the regulation of normal cardiac function.

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Interference with Feedback Control: a Mechanism of Antimetabolite Action

Abstract. The action of an enzyme essential for tryptophan biosynthesis is inhibited by tryptophan and also by an analog of tryptophan. Similarly, histidine and one of its analogs inhibit the action of an enzyme essential for histidine biosynthesis. A mutant resistant to the histidine analog produces an apparently altered enzyme which is insensitive to both the analog and histidine.

Structural analogs of amino acids, purines, and pyrimidines have generally been considered to inhibit growth by interfering competitively with the *incorporation* of the corresponding normal metabolites into essential components of the cell. Evidence has now been obtained that analog action, in certain instances, may be explained by an alternative mechanism in which the analog inhibits the *biosynthesis* of the normal metabolite. Such a mechanism of analog action was suggested by recent observations concerning the control of biosynthetic reactions. Several metabolites, including valine (*1*), isoleucine (*1*), proline (*2*), and cytidine 5′-phosphate (*3*), have each been found to inhibit an enzymatic reaction necessary for its own biosynthesis. This type of inhibition constitutes a negative feedback system which permits the metabolite to regulate its biosynthesis (*1*). It was considered possible that an analog might act by mimicking the specific inhibitory effect of the corresponding metabolite.

This possibility has been confirmed by the finding that DL-6-fluorotryptophan (6-FT) (*4*), as well as tryptophan itself, is a potent inhibitor of the condensation of anthranilic acid with phosphoribosylpyrophosphate, a reaction essential (*5*) for the biosynthesis of tryptophan (Table 1). Similarly, histidine and its analog DL-2-thiazole alanine (2-TA) (*4*) are inhibitors of the synthesis of "compound III," an essential intermediate (*6*) in the biosynthesis of histidine (Table 2). DL-6-Fluorotryptophan is as effective as tryptophan for the inhibition of the condensation of anthranilic acid with phosphoribosylpyrophosphate. On the other hand, 20 times as much 2-TA as histidine is necessary for the inhibition of "compound III" synthesis. The difference in the effectiveness of the two analogs as enzyme inhibitors may be partially reflected in the difference in their bacteriostatic effects on *Escherichia coli* W. Colony diameter is reduced by 50 percent in the presence of $2 \times 10^{-7} M$ 6-FT, whereas 3500 times that amount of 2-TA is required for a similar reduction.

If such an inhibitory effect of an analog on the action of an enzyme necessary for the biosynthesis of the corresponding normal metabolite is indeed responsible for bacteriostasis, then the development of resistance might be accompanied by a decreased sensitivity of the affected enzyme to inhibition by the analog. An alteration of this type has been found in a mutant selected for resistance to $2 \times 10^{-2} M$ 2-TA. The enzymatic synthesis of "compound III" by extracts of this organism is completely insensitive to 2-TA (Table 2) (*7*).

In addition to this change, the enzyme in the mutant has also lost sensitivity to histidine (Table 2), and the organism excretes a compound provisionally identified as histidine. The parent strain, on the other hand, produces precisely enough histidine to meet its needs and does not excrete histidine. These observations provide evidence that the inhibition by histidine of the enzymatic synthesis of "compound III" is responsible for the precision of the feedback control of histidine biosynthesis in the parent strain. Cohen and Adelberg (*8, 9*) have reported excretion of other amino acids by mutants resistant to a variety of analogs, and it seems possible that the loss of feedback control which they postulate involves a mechanism similar to that described here.

The observations that both 6-FT and 2-TA mimic the specific inhibitory effects of their corresponding normal metabolites support a mechanism of analog action involving interference with the control of biosynthetic reactions. If the bacteriostatic effect of 2-TA on the parent strain is the result of such interference, as represented by the inhibition of the enzyme synthesizing the histidine precursor, "compound III," then in the mutant the insensitivity of this enzyme to inhibition by 2-TA accounts for the observed resistance. However, it is also possible that the bacteriostatic action of 2-TA is due to competition with histidine for incorporation into macromolecules. In this case the insensitivity of the enzyme to histidine, leading to the overproduction of this competitive metabolite, could account for the resistance of the mutant to 2-TA. It is hoped that

Table 1. Inhibition of the condensation of anthranilic acid (AA) with phosphoribosylpyrophosphate (PRPP) by tryptophan and by its analog, 6-FT. Phosphoribosylpyrophosphate was generated *in situ* from adenosine triphosphate and ribose-5-phosphate (PRPP kinase is present in excess in these extracts). The rate of condensation was determined according to the method of Yanofsky (*5*); 0.01 ml of an extract of *E. coli* W (12 mg of protein per milliliter) was used.

Inhibitor and concn. (M)	Inhibition of AA, PRPP condensation (%)
None	0*
L-tryptophan (5×10^{-5})	45
L-tryptophan (5×10^{-4})	62
6-FT (5×10^{-5})	40
6-FT (5×10^{-4})	80

* In the absence of an inhibitor 10.0 mμmole of anthranilic acid was metabolized in 10 minutes.

Table 2. Inhibition of "compound III" synthesis. The rate of synthesis was determined by a previously described method (*6*); 0.2 ml of extract (12 mg of protein per milliliter) was used. Extracts were prepared by sonic oscillation from a mutant resistant to DL-2-thiazole alanine (2-TA) and from the wild type, *E. coli* W.

Inhibitor and concn. (M)	Inhibition of "compound III" synthesis (%)	
	Wild type extract	Resistant mutant extract
None	0*	0*
L-Histidine (1×10^{-4})	59	< 1
L-Histidine (2×10^{-4})	73	< 1
L-Histidine (3×10^{-2})		< 1
2-TA (8×10^{-4})	28	< 1
2-TA (2×10^{-3})	57	< 1

* In the absence of an inhibitor 74 and 39 mμmole of "compound III" were synthesized in 15 minutes by extracts of the sensitive and resistant strains respectively.

studies of the kinetics of histidine biosynthesis by the mutant and by the parent strain will permit us to determine which of these mechanisms is responsible for the bacteriostatic effect of 2-TA (*10*).

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Isolation of Gelatin from Ancient Bones

Abstract. The isolation and characterization of gelatin from 12,000-year-old deer antlers is described. Use of gelatin from ancient bones for carbon-14 dating may improve the accuracy of the dating procedure because gelatin is not likely to be contaminated by extraneous carbon.

Estimates of the age of buried objects based on their carbon-14 content has been of great value in anthropology. We believe that gelatin from ancient bones might be used for such dating with advantage. Charcoal has been the material most extensively used by anthropologists for dating. It is assumed that carbon produced by pyrolysis of ancient materials could not subsequently be contaminated with contemporary carbon. Charcoal, however, is not always pure carbon (*1*) but is, rather, a complex organic material. It presents a large surface area and may absorb other organic substances.

It is necessary to separate the organic and inorganic carbon fractions of bone before the count is made, because calcium carbonate in the mineral matrix may exchange with contemporary carbon dioxide of the air or ground water (*2*). Furthermore, ancient bones are porous and are capable of absorbing organic material from the soil.

Approximately 80 percent of the carbon of bone is collagen carbon, constituting 8.7 percent of air-dried bone. Collagen is soluble in hot water, in which it dissociates into a soluble protein gelatin. With time, the amount of this collagen decreases. However, appreciable amounts of collagen may be found in bone up to 100,000 years old (*3*). Gelatin is 18 percent nitrogen. The alpha-amino nitrogen of gelatin is 14 percent. In humic acids the alpha-amino nitrogen is between 0.2 and 1.5 percent (*4, 5*). The hydroxyproline content of gelatin is 13.5 percent; of humic acids, less than 0.05 percent.

We have attempted to prepare gelatin from approximately 10 g of deer antler (*6*). The deer antler was the remainder of radiocarbon sample Y-158, approximately 12,000 years old (*5*). Ten grams of pulverized antler were extracted with three 50-ml portions of 10 percent Versene, *p*H 7.05, in a boiling water bath for 1-hour periods with occasional shaking. The pooled extractions were dialyzed on a rocking dialyzer against distilled water at 2°C for 72 hours with eight water changes. After the dialysate had been concentrated in a vacuum to 50 ml, the solution was made 5 percent in trichloroacetic acid and allowed to stand overnight at 2°C. The precipitate was removed by centrifugation, and the supernatant was dialyzed as before. The gelatinous precipitate which formed was filtered and dried. It weighed 249 mg.

The material collected was dissolved in H_2O at room temperature with the addition of a small amount of NaOH which brought the *p*H of the solution to between 4.0 and 4.5. A 1-percent solution of this material gelled at 2°C.

The molecular weight of this gelatin was estimated by the viscosity procedure of Pouradier and Venet (*7*). A molecular weight of 41,000 was obtained. Such a molecular weight is not significantly different from that of commercial gelatins isolated from fresh bone.

Hydroxyproline determinations (*8*) on material dried in a vacuum oven at 100°C for 24 hours indicated that this material contained 12.9 percent hydroxyproline (the usual literature value is 13.5 percent) and 17.4 percent nitrogen (the usual literature value is 13 percent). The carbon content was 96.0 percent of theoretical. The material did not contain detectable amounts of tyrosine (*9*) or uronic acid (*10*).

This material was apparently gelatin of a purity of at least 96 percent. We feel that carbon-14 dating of purified gelatin would be as reliable as charcoal dating, or possibly more so, since analytical evidence may be obtained that the organic material is what it appears to be, namely bone protein (*11*).

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Proposed System of Terminology for Preparations of Adrenocorticotropic Hormone

Abstract. During the past few years there has been a considerable amount of confusion with respect to the terminology used for adrenocorticotropins (ACTH) isolated from the pituitary glands of various species. In this paper (*1*) a unified system of nomenclature for these hormones is proposed. It is hoped that this system will furnish readily and at a glance, from the terminology itself, pertinent information about the source of the particular preparation as well as the chemical formula of any active degraded product derived from the natural hormone.

Some confusion among investigators is usually unavoidable with respect to the term or name used to designate a biologically active substance derived from natural products before it is isolated in pure form and before its structure is known. The confusion is compounded when preparations of the same substance isolated from tissues of different species differ chemically but have similar biological effects. The terminological problem becomes even more complicated when the pure substances can be modified chemically without loss of biological activity. The present state of the terminology used to designate the adrenal-stimulating substance from the adenohypophysis is a case in point.

Since the discovery of the existence of adrenal-stimulating activity in pituitary glands by Smith in 1927 (*2*), many names have been proposed for the hormone: adrenotropic hormone, adrenocorticotropic hormone, corticotropic hormone, adrenotropin, corticotropin, adrenocorticotropin, and ACTH. In the past few years, adrenal-stimulating pep-