action in the media with the reversing agent could not have occurred. Growth was not restored by placing the seeds in water following the 48-hour treatment with the various mixtures tested.

On the basis of the results obtained, it appears that CDAA inhibits certain sulfhydryl-containing enzymes that are involved in respiration. It further appears that it affects a mechanism even more intimately connected with growth, possibly oxidative phosphorylation.

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28 October 1955

Evidence for a Negative-Feedback Mechanism in the Biosynthesis of Isoleucine

Recent developments in automation have led to the use in industry of machines capable of performing operations that have been compared with certain types of human activity. In the internally regulated machine, as in the living organism, processes are controlled by one or more feedback loops that prevent any one phase of the process from being carried to a catastrophic extreme. The consequence of such feedback control can be observed at all levels of organization in a living animal—for example, proliferation of cells to form a definite structure, the maintenance of muscle tone, and such homeostatic mechanisms as temperature regulation and the maintenance of a relatively constant blood sugar level. Because of the complexity of so many biological systems, it is often difficult to postulate a mechanism on the molecular level that would serve in a regulatory function.

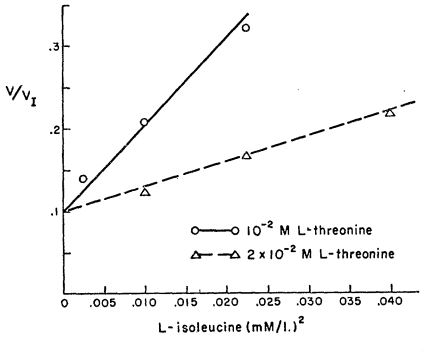

Fig. 1. Competitive inhibition of L-threonine deamination of L-isoleucine. Each point is calculated from an average of duplicate test systems in which keto acid formation from L-threonine was determined. The conditions were the same as those in Table 1 except the substrate and inhibitor concentration.

Less complex systems for study of internal regulation can be found in the orderly synthesis of protoplasmic components during the growth of bacteria. A simple, though typical, example is the effect of L-isoleucine on the L-threonine requirement of threonineless mutants of *Escherichia coli*. It has been established that a portion of the *L*-threonine that is supplied in the medium is converted to L-isoleucine (*1*). In the presence of the latter, this conversion does not occur, and L-isoleucine itself is preferentially utilized (*1*) with an accompanying sparing effect on L-threonine (*2*). Exogenous L-isoleucine might effect its own utilization by (i) reversing the equilibrium along the biosynthetic chain or (ii) by specifically inhibiting any of the several enzymatic steps along that chain. However, because of the irreversibility of L-threonine deamination, which is probably the first step in the conversion of L-threonine to L-isoleucine (*3*), only an inhibition of this step could account for the sparing effect.

Examination of crude extracts of *E. coli* strain K-12 has revealed that L-isoleucine is indeed a strong inhibitor of this reaction (*4*). The data in Table 1 show the degree of specificity of this reaction. It can be seen that L-isoleucine was about 100 times as inhibitory as the structurally similar amino acid, L-leucine. It has been observed that, of the amino acids tested, only L-isoleucine has a sparing effect on the L-threonine requirement of strain 12B14, a threonineless mutant of *E. coli*.

Preliminary kinetic studies with L-threonine dehydrase activity in crude extracts indicate that the inhibition caused by L-isoleucine is competitive. However, in order for the data to fall in straight lines when they are plotted according to the method of Ebersole *et al.* (*5*), it is necessary to square the inhibitor concentration (Fig. 1). When the double reciprocal plot of Lineweaver and Burk (*6*) is employed, it is necessary to square the substrate concentration. This property of the data would be expected if the enzyme combined with 2 molecules of substrate or inhibitor (case II of Lineweaver and Burk). Further experiments are in progress in an effort to decide whether this peculiar kinetic behavior is apparent or real.

It would seem that the interaction between L-isoleucine and L-threonine dehydrase constitutes a negative-feedback loop that could permit the biosynthesis of isoleucine to proceed only when the level of L-isoleucine in the medium or in the metabolic pool has been reduced to a very low level. The biological consequences of this interaction are being studied further in order to decide whether or not the inhibition of L-threonine deamination by L-isoleucine is in fact an important controlling mechanism in biosynthesis.

Table 1. Specificity of inhibition of threonine deamination. In addition to these amino acids at the indicated concentrations, the assay system contained 40 μmoles PO_4 buffer at *p*H 8.0, 10 μg crystalline pyridoxal phosphate, 10 μmoles L-threonine and *E. coli* extract with 2 to 3 mg of bacterial protein in a total volume of 1 ml. The reaction mixture was incubated for 20 minutes at 37°C. The extent of deamination was followed by measuring keto acid production by the method of Friedemann and Haugen (*7*).

Amino acid and concn.	Inhibition (%)
L-Aspartic, $10^{-2}M$	30
L-Alanine, $10^{-2}M$	0
L-Valine, $10^{-2}M$	0
L-Leucine, $10^{-2}M$	55
DL-Homoserine, $10^{-2}M$	0
L-Methionine, $10^{-2}M$	0
L-Isoleucine, $10^{-2}M$	100
L-Isoleucine, $10^{-4}M$	52

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21 October 1955

Studies on 4APP: Antineoplastic Action in vitro

In the course of screening experiments for antineoplastic compounds, an isomer of adenine, 4-aminopyrazolo(3,4-d)pyrimidine (4APP) has shown differential cellular damage to several malignant tissues in culture. Antineoplastic activity of this compound has recently been found by Skipper *et al.* (*1*) on adenocarcinoma 755 in mice. The present report (*2*) pre-

sents preliminary data from *in vitro* studies.

The test material included the strain HeLa, a human cervical carcinoma; primary cultures of the B-3 adenocarcinoma of the mouse that had been carried by repeated transplantation in the strain C57 B1/10J; skin and heart tissues of mouse embryos; renal papillae of adult mice; and preputial skin from a human infant.

All cultures were set up in roller tubes. The composition of nutrient media and the procedure of setting up cultures were essentially the same as described by Hsu (*3*) except that a single cover slip was used for each tube. After the tissues had been established in culture for at least 4 days, the fluid phase was replaced with fresh nutrient that contained various concentrations of 4-aminopyrazolo(3,4-d)pyrimidine. The chemical was incorporated in the medium by simple suspension that was freshly prepared every time. Addition of the agent does not change the *p*H of the nutrient. The controls received fresh nutrient only. Duplicate or triplicate tubes were used with each concentration for each series. The cultures were then returned to the incubator. After 24 hours of treatment, they were fixed with pure methanol and stained with May-Grunwald-Giemsa. In two experiments, the agent was added to the initial fluid to test possible inhibition of growth.

The stained slides were examined by inspecting more than 1000 nuclei from each type of culture at each concentration. This was done in the same way described by Biesele (*4*) except that, in the HeLa cultures, the reading was made in the middle area of the outgrowth alone. The proportions of mitotic cells and pyknotic nuclei were selected as criteria for the estimation of cellular damage.

The experimental results are summarized in Table 1 (percentage of mitotic figures) and Table 2 (percentage of pyknotic nuclei). It can be readily seen that this compound produced appreciable damage to the cells of the HeLa strain. Mitotic figures dropped drastically, and practically all the mitotic figures encountered at concentrations of 0.1 mmole or higher were abortive metaphases. On the other hand, pyknotic nuclei increased considerably in the treated cultures, and among the nonpyknotic nuclei many showed other signs of degeneration. The B-3 adenocarcinoma seemed to be more resistant than the HeLa strain, but the increase of pyknotic nuclei after treatment with high concentrations was evident.

Conversely, all four sets of nonmalignant tissues showed relatively indifferent response to the treatment at all concentrations tested. There was neither a significant drop of mitotic indices nor a great increase of pyknosis. Indeed, the mitotic figures represented all phases and showed no abnormalities.

Table 1. Percentage of mitosis following treatment with suspension of 4-aminopyrazolo(3,4-d)pyrimidine. Each sample is based on observation of at least 1000 cells. All concentrations are in millimoles.

Tissue	Culture period (day)	Time of exposure before fixation (day)	Concentration									Control
			4	2	1	0.5	0.1	0.05	0.01	0.005	0.001	
Neoplastic												
HeLa	5	1	0.4	0.8	0.5	0.3						4.0
HeLa	5	1	0.4	0.6	0.1	0.1	0.6					4.0
HeLa	5	1			0.4	0.6	1.3	3.1				2.8
HeLa	5	5	0.0	0.0	0 0	0.0	0.0	0.0	0.6	1.0	2.6	3.3
B-3	31	1	0.0	0.0	0.0	0.0	0.0					0.1
B-3	5	1	0.2	0.0	0.1	0.1	0.0					0.4
B-3	5	5	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.4	0.6
Normal												
Mouse embryo skin	5	1	0.6	1.4	1.0	2.3	1.7		1.8			1.3
Mouse embryo heart	5	1	1.2	0.7	1.3	1.1	0.8					1.3
Mouse renal papilla	7	1	0.0	0.0	0.0	0.0	0.1					0.0
Human preputial skin	9	1	1.2		0.9	1.2	1.1		1.5			1.0

Table 2. Percentage of pyknotic nuclei following treatment with suspension of 4-aminopyrazolo(3,4-d)pyrimidine. Each sample is based upon observation of at least 1000 cells. All concentrations are in millimoles.

Tissue	Culture period (day)	Time of exposure before fixation (day)	Concentration									Control
			4	2	1	0.5	0.1	0.05	0.01	0.005	0.001	
Neoplastic												
HeLa	5	1	35.2	19.4	13.8	5.6						1.6
HeLa	5	1	33.7	12.2	7.1	7.4	2.6					0.2
HeLa	5	1				7.9	5.6	4.2	3.6			1.1
HeLa	5	5	100.0	100.0	100.0	87.2	72.9	65.2	34.4	32.1	4.3	1.0
B-3	31	1	11.3	6.5	2.3	3.4	3.3					1.4
B-3	5	1	9.3	3.9	2.4	2.0	1.5					1.1
B-3	5	5	100.0	100.0	100.0	100.0	100.0	91.2	4.4	2.5	0.5	0.7
Normal												
Mouse embryo skin	5	1	1.3	1.2	1.9	0.6	0.4		1.0			0.3
Mouse embryo heart	5	1	0.5	0.3	0.3	0.1	0.1					0.4
Mouse renal papilla	7	1	2.7	1.9	1.6	1.3	2.0					2.4
Human preputial skin	9	1	1.5		1.0	1.5	0.9		0.4			1.5

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26 October 1955

Crystal Structure of Turnip Yellows Virus Protein

An important problem in the study of purified proteins deals with the role of the salt in the solution from which most crystallize. This can be approached in a new way by the methods we now have (*1*) for observing with the electron microscope the molecular distribution in some of these crystals. The protein (*2*) of the turnip yellows virus is a particularly suitable material because it crystallizes readily both from ammonium sulfate and from salt-free alcoholic solutions.

In the present experiments, shadowed evaporated-carbon replicas were made from these two types of crystal. Those from salt appear under the optical microscope as moderately well-developed isotropic octahedra. At electron microscopic magnifications, their surfaces are very uneven and are often covered with extraneous material, but it is not hard to see and measure the molecular arrangements on the octahedral (Fig. 1) and the occasional cubic faces that they display. The structure is evidently a cubic close-packing of spheres with a particle-to-particle distance of about 360 A and a