Its metabolic function remains obscure, although it is suspected to have a lipotropic effect independent of that of choline (*4*).

Inconsistent results have been obtained in attempts to produce symptoms of inositol deficiency in animals. In mice, alopecia was produced in approximately half the animals (*5*); and in rats also inositol caused a positive growth response and prevented and cured a generalized alopecia (*6*). More recently, however, inositol was not found to have a demonstrable effect in the growth of rats on an amino acid-sucrose-corn oil-salt-vitamin diet (*7*). Similarly conflicting results have been reported with respect to its possible relationship to the "spectacle eye" syndrome in rats (*8*). A growth-promoting effect has been noted in cotton rats (*9*); and the omission of both inositol and PABA from the diet of golden hamsters led to the death of some of the animals (*10*). In man there has been no evidence of an inositol requirement.

The demonstration of its vitamin function in animals or man is complicated by its possible production by the intestinal flora (*11*) and by its partial biosynthesis (*12*) and is further complicated by the reported dependence of its effects on the presence or absence of other B vitamins (*13*).

Under these circumstances, the present demonstration that inositol is an essential growth factor for a wide variety of human cell lines in tissue culture is of particular significance. The effective concentrations, higher than those of most of the other essential vitamins, are of the same order of magnitude as the choline requirement of the mouse fibroblast. Whether inositol is used as a metabolite —for instance, for incorporation into phospholipides—or whether it functions as a vitamin remains to be determined, as does the degree to which it can be replaced by its isomers or by related compounds. To date, no compound has been found capable of substituting for myo-inositol.

Contrary to some of the results obtained in animals, the presence of pantothenic acid in the medium did not obviate the need for inositol, and the addition of PABA similarly had no qualitative or quantitative effect. It is of interest also that, although inositol has been reported to have a carcinolytic action in experimental animals (*14*), in the present experiments it was essential for the growth of five of the seven tumor lines tested.

When inositol was added to the 26 essential factors previously identified, every cell line here described could be grown for long periods in a medium supplemented only with serum protein. It thus becomes possible to determine the specific amino acid and vitamin requirements of all these human cell lines, deriving from a variety of normal and malignant tissues, with a view to ascertaining possible quantitative or qualitative differences.

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Biochemical Action of CDAA, a New Herbicide

One of the more active herbicides that has been discovered since the introduction of 2,4-dichlorophenoxyacetic acid is α-chloro-*N,N*-diallylacetamide (CDAA) (*1*). This herbicide, which has only recently been announced, is grass-specific and preemergent. It is also extremely selective, affecting only certain members of the monocotyledons and dicotyledons. For example, when it is applied at the appropriate dosage, it will destroy giant foxtail and pigweed in either corn or soybean fields without injuring the crop.

The mode of action of such a selective herbicide is of sufficient interest to warrant studies of the respiratory processes in moderately susceptible and susceptible plants (wheat and ryegrass, respectively). Germinating seeds of wheat and ryegrass were treated with CDAA at 10 ppm in the absence and presence of sulfhydryl-containing compounds. The data in Table 1 on oxygen uptake, changes in respiratory quotients, and growth show that ryegrass seeds are more susceptible to CDAA than wheat seeds. This finding is in agreement with results obtained from greenhouse studies. The possibility exists that the site and/or mode of action of CDAA in wheat is different than it is in ryegrass since the respiratory quotient of wheat was increased in the presence of this herbicide, whereas the respiratory quotient of ryegrass was markedly decreased.

The reversal studies suggest that sensitive sulfhydryl enzymes in both wheat and ryegrass are inhibited by CDAA, and this reaction could be related to the classical alkylation of sulfhydryl groups by iodoacetate and iodoacetamide (*2–3*). The reversal produced by calcium pantothenate was attributed to its conversion to coenzyme A. Reversal studies with coenzyme A per se were not significant; however, this may have been caused by an inability of the nucleotide to penetrate the seed at the proper site.

Since the growth of wheat and ryegrass seedlings was almost completely inhibited despite the observed effects on the respiratory quotients and respiration rates, a simple detoxification of CDAA by inter-

Table 1. Influence of α-chloro-*N,N*-diallylacetamide (CDAA) on the respiration, respiratory quotients, and growth of germinating wheat and ryegrass seeds. The respiratory quotients were determined according to the "direct method" of Warburg (*3*).

Compound* and concn. (ppm)	O_2 uptake [μlit/hr 100 mg of tissue (wet wt.)]	Respiratory quotient	Cotyledonary growth after 120 hr (mm)
Wheat			
Control	49.0	0.96	16.9
CDAA (10)	38.0	1.12	0.8
GSH (15)	42.0		15.5
CDAA (10) + GSH (15)	45.0		1.0
Ryegrass			
Control	16.0	1.00	21.3
CDAA (10)	3.4	0.62	0
GSH (15)	17.4	0.96	20.6
CDAA (10) + GSH (15)	18.8	0.94	0
Ca-pan (15)	16.8	1.02	19.3
CDAA (10) + Ca-pan (15)	11.0	1.05	0
α-lipoic acid (4)	16.0	1.02	21.5
CDAA (10) + α-lipoic acid (4)	11.5	1.07	0

* The abbreviations represent the following: GSH, reduced glutathione; Ca-pan, calcium pantothenate.

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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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.

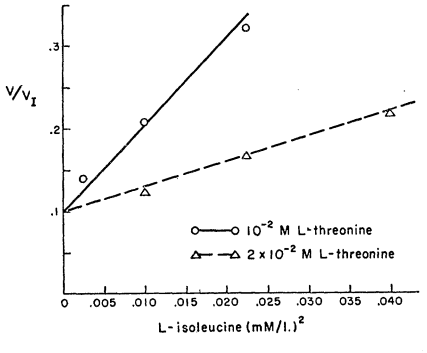

Fig. 1. Competitive inhibition of L-threonine deamination of L-isoleucine. Each point is calculated from an average of duplicate test systems in which keto acid formation from L-threonine was determined. The conditions were the same as those in Table 1 except the substrate and inhibitor concentration.

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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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-