introduced specifically to stimulate feeding activity. In preliminary tests, the green-apple aphid, *Aphis pomi*, and another species, *Macrosiphum rudbeckiae*, survived on diet I for as long as 15 days, with maximum weight gains of 160 percent.

Similar results were obtained with either stretched natural rubber, or stretched or unstretched Parafilm as feeding membrane. Parafilm membrane can however be manipulated more easily. Numerous salivary sheaths were formed on the inside of the membranes and copious quantities of honeydew were excreted by the feeding aphids. The different results from the four diets (Fig. 1) suggest that the success obtained with diets I and II was due mainly to a better distribution in the quality and quantity of each amino compound, which approximated that found in pea-aphid blood and honeydew (*6*).

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6. We thank St. Georges Morin for technical assistance.

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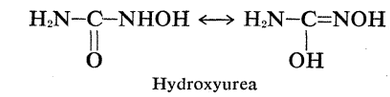
Hydroxyurea: Mechanism of Action

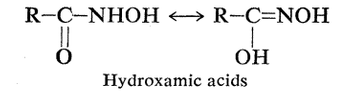
Abstract. *Acetohydroxamic acid has been identified, by paper chromatography, in the blood of three patients with chronic myelogenous leukemia on hydroxyurea therapy. This suggests that the drug is hydrolyzed yielding hyroxylamine, which then cleaves thioesters, in particular acetyl-coenzyme A.*

Hydroxyurea is a century-old compound (*1*) which has recently been introduced into cancer chemotherapy because of its high degree of activity against mouse leukemia L1210 (*2*). Clinical experience suggests that the major therapeutic benefit of this drug is in the treatment of chronic myelogenous leukemia, where it regularly reduces the white count and spleen size upon either oral or intravenous administration with little gastrointestinal or other toxicity (*3*).

The compound is one of the hydroxamic acids (carbaminohydroxamic acid), which are generally stable in neutral solution but are known to be hydrolyzed by mineral acids to yield a carboxylic acid and hydroxylamine (*4*).

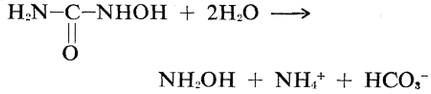


Hydroxyurea



Hydroxamic acids

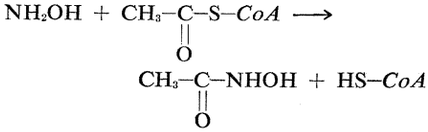
The substitution of the amino group for the alkyl residue of other hydroxamic acids suggested that this compound might be particularly labile and might undergo spontaneous or enzymatic hydrolysis in vivo to generate free hydroxylamine:



Thus, at *p*H 1 the color reaction of a 1m*M* concentration of acetohydroxamic acid in 2 percent $FeCl_3$ was completely stable for at least 3 hours, whereas the color reaction of 20-fold this concentration of hydroxyurea faded very rapidly and was completely gone within 20 minutes. Hydroxyurea is known to undergo gradual decomposition on standing in dry form and in acid solution (*5*), and has been found to be metabolized by urease, although the products of the reaction have not been identified (*6*).

At physiologic *p*H, the hydroxylamine formed would be a rather specific agent for the cleavage of thioesters (and acyl phosphates) and has been widely used in biochemical studies for this purpose (*7*).

As a test of this hypothesis, a search was made for acetohydroxamic acid in the blood of leukemic patients receiving the drug, since acetyl-coenzyme A is the prime thioester in mammalian tissues:



Blood specimens were obtained from a patient in the blastic stage of chronic myelogenous leukemia receiving 100 mg of hydroxyurea per kilogram of body weight per day intravenously, and from two ambulatory patients with the disease receiving 25 to 30 mg per kilogram of body weight per day orally.

Table 1. Approximate R_F values of hydroxamic acids.

Aceto-hydroxamic	Plasma extract	Hydroxyurea
Butanol, water saturated		
0.52	0.52	0.19
Phenol, water saturated		
0.75	0.75	0.50
Isobutyric acid, water saturated		
0.55	0.55	0.52
Butanol, isopentanol, formic acid, water (2:2:1:3)		
0.40	0.40	0.21
Butanol, formic acid, water (3:1:3)		
0.50	0.50	0.32

These were separated into red cell, white cell, and plasma components by the method of Fallon *et al.* (*8*). The aqueous solutions of the plasma fractions, after extraction with ether, were made up to 75 per cent ethanol and centrifuged to remove precipitated protein. The aqueous ethanol was evaporated to dryness at 50°C under reduced pressure (15 mm-Hg), the residue was extracted with warm ether, and the ether solution was filtered, concentrated, and used for descending paper chromatography.

In each case a major chromatographic spot was obtained which stained reddish-purple with $FeCl_3$ and showed no difference in mobility when chromatographed against or in combination with pure acetohydroxamic acid, prepared by a modification of the method of Jeanrenaud (*9*), in five solvent systems. In contrast, the compound was easily distinguished from hydroxyurea by R_F values as shown in Table 1, and also by the greenish color the hydroxyurea develops when sprayed with $FeCl_3$. It has also been satisfactorily separated from its chromatographically closest congener, γ-hydroxybutyrohydroxamic acid (*10*). In the specimen of the patient in blastic crisis, an additional spot with the mobility of hydroxyurea was also present. The spots were absent from control plasmas, obtained from untreated patients with chronic myelogenous leukemia and taken through the same procedure.

Quantitative estimation of acetohydroxamic acid in the plasma by comparison of color intensity with that of known amounts of the pure substance with the Spinco Analytrol desitometer indicates levels of 2 to 5 × $10^{-5}M$,

which would result from the decomposition of 1 to 10 percent of the hydroxyurea administered, and the formation of a corresponding amount of hydroxylamine. This is well below the mouse LD_{50} for hydroxylamine (*11*), which has also been shown to have antitumor activity (*12*). In patients receiving hydroxyurea intravenously and orally, recovery of the hydroxyurea in the urine averaged about 50 percent and in no case exceeded 80 percent of the administered dose (*6*).

In one case the red-cell fraction was treated by the procedure used for plasma after the cells were lysed by freezing and thawing, and this fraction also contained acetohydroxamic acid. Assuming that the red cell contains 64 percent water (*13*), the concentration in relation to red-cell water was the same as that in the serum, suggesting that the metabolite is freely diffusible through cell membranes.

From a biochemical viewpoint, the major metabolic effect of this type of drug interference would be diminution in oxidative phosphorylation, with consequent effects due to a diminished total concentration of adenosine triphosphate (ATP), or perhaps specific lowering of mitochondrial ATP (*14*). Although it seems surprising that interruption at such a central point of cell catabolism should be peculiarly effective in chronic myelogenous leukemia, the same situation obtains in the unique value of arsenite in the therapy of this disease. This agent, which is now known to block the formation of acetyl-coenzyme A by preventing the participation of α-lipoic acid in the pyruvic oxidase reaction (*15*), was once widely used and highly recommended in the treatment of this blood dyscrasia (*16*) and remains valuable experimentally (*17*).

The acetyl-coenzyme A cleavage formulation does not exclude the additional attack by the hydroxylamine generated upon other areas of cell function. It has been reported that hydroxyurea simulates the action of hydroxylamine in fragmenting mammalian and bacteriophage DNA (*18*); this type of action may be particularly pertinent in explaining the occasional case of megaloblastosis that develops during treatment with large doses of hydroxyurea (*19*). Hydroxylamine is also well known as a very potent inhibitor of catalase (*11*).

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Inhibition of Adhesiveness and Aggregation of Dissociated Cells by Inhibitors of Protein and RNA Synthesis

Abstract. *Mutual adhesiveness and aggregation of dissociated embryonic retina cells in suspension cultures are suppressed rapidly and reversibly by puromycin, an inhibitor of RNA-dependent protein synthesis; and at a slower rate, irreversibly, by actinomycin D, an inhibitor of RNA formation. The results imply that biosynthetic processes are involved in the mechanism of reattachment of these cells.*

Clarification of mechanisms controlling mutual adhesion and aggregation of embryonic cells is an essential step towards detailed analysis of cell interactions, differentiation, and histogenesis. Critical studies on various aspects of cell contact have become possible through procedures for reaggregation of single cells, in suspensions prepared by tryptic dissociation of embryonic tissues (*1*). Evidence from such studies pointed to a correlation between adhesiveness of the dissociated cells and metabolic activity (*2*) and suggested that their histological reattachment and aggregation might require resynthesis of cell-binding constituents removed from the cell surface or from intercellular spaces in the course of dissociation (*3*). The indications that adhesive properties of cells are influenced by changes in the metabolic states raised the question whether suppression of biosynthetic processes in dissociated embryonic cells might not affect their adhesiveness. The data summarized here provide evidence that the capacity of dissociated embryonic cells to readhere and to aggregate is indeed inhibited by suppressing protein synthesis at the RNA level with puromycin (*4*) and with actinomycin at the DNA-RNA level (*5*).

Cell suspensions were obtained from the neural retinas of 10-day chick embryos by our method of tryptic dissociation (*1*). The dispersed cells were rinsed in a calcium- and magnesium-free salt solution with deoxyribonuclease (30 μg/ml) added to minimize incidental clotting (*3, 6*). The cells were aggregated in flasks by the rotation procedure (*1*) whereby the cells are rapidly swept into contact independently of individual mobilities or proliferation; mutually adhesive cells aggregate, others remain single or form small, loose clusters. Aggregability in terms of number, size, and rate of formation of aggregates provides a measure of cell adhesiveness with reference to the particular conditions (*1, 3*). Samples (3 ml) of cell suspension (2×10^6 cells/ml) in culture medium (Eagle's basal medium with 1 percent glutamine, 10 percent horse serum, and penicillin-streptomycin) with and without the inhibitors were distributed into 25-ml erlenmeyer flasks, gassed with a mixture of 5 percent CO_2 and air, and rotated on a gyratory shaker at 70 rev/min. All cultures were incubated at 37°C and scored after 1, 2, 4, 6, 12, and 24 hours of rotation. The results are based on more than 200 cultures.

Figure 1 shows the increase in size of aggregates in control cultures (*a*), and the suppression of cell aggregation by puromycin (*b*) and actinomycin D (*c*). In puromycin the block to aggregation occurred within the first