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29 August 1963

## Pea Aphid: Rearing on a Chemically Defined Diet

Abstract. *Pea aphids,* Acyrthosiphon pisum *(Harr.), reared for two successive generations on a diet made of 23 amino acids and amides, 11 water-soluble vitamins, 35 percent sucrose, salts, and cholesterol, remained apterous. They grew and developed during the first generation almost as well as those grown on pea plants, with very little mortality.*

Information on the rearing of aphids on synthetic diets is very limited. Various attempts to feed these insects on gels or liquids, sometimes under pressure, and with the use of various types of membranes, usually ended in failure, the insects surviving for about 1 week (*1*). Recently, a first limited but significant success was reported (*2*) on the artificial feeding and rearing of an aphid (*Myzus persicae*) on a chemically defined diet. Although high mortality occurred, a few nymphs transformed into diminutive adults after 16 to 17 days of rearing. This diet, when fed to the pea aphid, *Acyrthosiphon pisum* (Harr.), supported little growth and resulted in the death of all nymphs within 1 week. During the past year, we have developed a chemical diet which gives pea aphid growth rates and development almost equivalent to those obtained on pea plants, with almost no mortality during the first generation.

The type of cage used is similar to one already described (*3*), except that it is larger and better ventilated; the plaster of Paris in the bottom is replaced by a wet cotton wad, and the aphids are further confined within an inverted cone of nylon. The aphids have access to the liquid diet by way of a natural rubber (*3*) or a Parafilm (*2, 4*) membrane which they "puncture" to feed. One of the diets that has so far given satisfactory results is as follows.

L-Amino acids and amides (mg): alanine, 100; arginine, 400; asparagine, 300; aspartic acid, 100; cysteine, 50; cystine, 5; γ-amino butyric acid, 20; glutamic acid, 200; glutamine, 600; glycine, 20; histidine, 200; isoleucine, 200; leucine, 200; lysine mono hydrochloride, 200; methionine, 100; phenylalanine, 100; proline, 100; serine, 100; threonine, 200; tryptophan, 100; tyrosine, 20; valine, 200; and DL homoserine, 800 mg to make a total of 4315 mg of amino acids and derivatives. The diet also contained the following vitamins (mg): ascorbic acid, 10.0; biotin, 0.1; calcium pantothenate, 5.0; choline chloride, 50.0; folic acid, 1.0; i-inositol, 50.0; nicotinic acid, 10.0; p-aminobenzoic acid, 10.0; pyridoxine hydrochloride, 2.5; riboflavin, 5.0; and thiamine hydrochloride, 2.5, to make a total of 146.1 mg. In addition the following were added, cholesterol benzoate, 2.5 mg; $K_3PO_4$, 500.0 mg; $MgCl_2 \cdot 6H_2O$, 200.0 mg; salt mixture No. 2 USP, 5.0 mg; sucrose, 35.0 g; water (to make) 100.0 ml; at *p*H, 7.3 to 7.4.

The choice and quantity of each amino acid and amide in the diet was based on the average concentration of these compounds in pea-aphid blood and honeydew (*5*), but the diet contained twice the concentrations measured. Arginine, cysteine, histidine, lysine, and tryptophan, not reported in pea-aphid blood and honeydew, were added to the diet. The vitamins and salt mixtures described (diet I) were, with slight modifications and additions, similar to those used for *Myzus persicae* (*2*). From this diet I, a diet II was prepared by reducing the total concentration of amino compounds and vitamins from 4.46 to 2.12 percent. In addition, a diet IV was prepared identical to that used for *Myzus persicae* (*2*) containing 18 percent sucrose and 2.25 percent amino compounds and vitamins; finally, diet III was prepared identical to diet IV, but its sucrose content was raised to 35 percent. The addition of the salts resulted in slight turbidity, especially in diet I, and was probably due to the formation of small amounts of poorly soluble magnesium phosphate. Contamination by microorganisms was minimized by pasteurizing diets and renewing them in the feeding cages every 4 to 5 days. Membranes were dipped for a few minutes into 70 percent ethanol, dried, and used immediately thereafter.

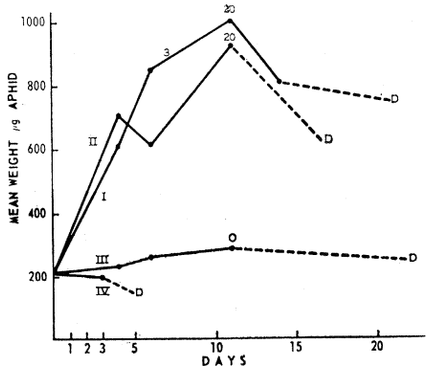


Fig. 1. Growth curves of nymphs of pea aphids reared from first-instar on four chemical diets (I–IV). The arabic numbers above each curve indicate the number of adults produced out of 20 nymphs. *D* = death of the last aphid.

The four diets were fed to groups of 40 first-instar nymphs of pea aphids averaging 215 µg per nymph; nymphs were maintained at 20°C, high humidity, and under a 16-hour photoperiod. On the 7th day of the test, live aphids on diets I, II, and III numbered respectively 36, 35, and 28; the numbers were further reduced to 20 aphids per diet. The results on growth and development (Fig. 1) show that all nymphs on diets I and II reached the adult stage by the 11th day of feeding, increasing their weight by 333 to 450 percent. None of the nymphs on diets III and IV reached the adult stage, and the maximum average weight gain on diet III was only 36 percent. Adults reared from first-instar nymphs on diets I and II gave birth to numerous nymphs and then died after 17 to 21 days of feeding. On other similar diets, some aphids survived for as long as 30 days; on pea plants, they usually died 30 to 40 days after birth. In another test, the first 30 nymphs born from aphids reared on diet I from first instar to the adult stage were also kept on the same diet. At birth, these second-generation nymphs weighed 60 to 70 µg each, and many of them transformed after 17 days into small second-generation adults weighing 450 to 500 µg each, thus representing an average of 630 percent weight increase. It is also significant that the test nymphs, obtained from a pea-aphid culture and capable of producing winged forms under certain conditions, remained apterous during two generations on the artificial diet. Furthermore, aphids feeding on diet I almost always remained immobile and apparently well "satisfied," even though no substances were

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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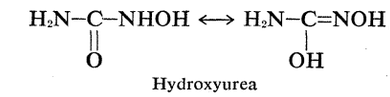
23 September 1963

## Hydroxyurea: Mechanism of Action

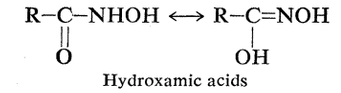
Abstract. *Acetohydroxamic acid has been identified, by paper chromatography, in the blood of three patients with chronic myelogenous leukemia on hydroxyurea therapy. This suggests that the drug is hydrolyzed yielding hyroxylamine, which then cleaves thioesters, in particular acetyl-coenzyme A.*

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*).

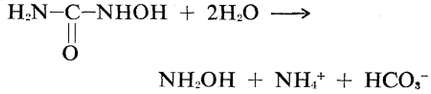


Hydroxyurea



Hydroxamic acids

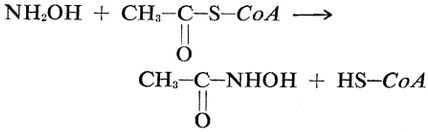
The substitution of the amino group for the alkyl residue of other hydroxamic acids suggested that this compound might be particularly labile and might undergo spontaneous or enzymatic hydrolysis in vivo to generate free hydroxylamine:



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 × $10^{-5}M$,