certain environmental conditions and nutritional balance this injury was caused primarily by urea itself.

More recently a new lot of urea caused injury more severe than that observed with another lot of urea, in spite of the fact the plants were under identical environmental conditions and had the same nutritional status. This new lot of urea, along with urea from other sources and chemically pure urea, was analyzed to ascertain whether there were any differences in the amounts of contaminants present. The only major variation in chemical composition was in the biuret content. Biuret ($NH_2CONHCONH_2 \cdot 4/5H_2O$) (*1*) is chemically related to urea (H_2NCONH_2) and is formed on heating urea. Biuret was determined by the method of Robinson and Hogden (*2*) modified only in the manner of estimating the color intensity of the copper-biuret complex. The absorption spectra of the copper complex of biuret synthesized according to Haworth and Mann (*3*) and of biuret in different ureas were identical, maximum absorption being at 560 mμ determined in a Beckman model DU spectrophotometer, in agreement with that previously obtained (*2*).

In order to compare the effect of urea fertilizers from various sources with the effect of synthetic biuret on pineapple plants, a preliminary test (two replicates per treatment) was made. Table 1 presents the treatments, the amounts of biuret found in urea from various sources, and the relative toxicity of these products. Toxicity is measured in terms of amount of leaf-tip dieback and chlorosis. Application of the urea in this test was made on 14 July 1953 to 40 7-mo-old pineapple plants per treatment. Toxicity measurements were made 2 mo later.

The results from this test indicated that there was a positive correlation between the toxicity symptoms and the amount of biuret, whether added to the urea or present as a contaminant. Urea containing 3 percent or more of biuret produced dieback and yellowing. A spray containing biuret alone also produced dieback and leaf yellowing.

Table 1. Effect of biuret and of urea from different sources on chlorosis and leaf-tip dieback of pineapple plants.

Urea		Biuret ($NH_2CONHCONH_2 \cdot 4/5H_2O$)					
		Contaminant		Added	Total		
Source	Rate* (g/40 plants)	Content (%)	Rate (g/40 plants)	Rate (g/40 plants)	Rate (g/40 plants)	Average chlorosis index†	Average leaf-tip dieback (cm)
C.P.	270	Trace	Trace	0	Trace	55	0.0
A	270	0.4	1.1	0	1.1	50	.0
B	270	3.0	8.1	0	8.1	63	2.5
C.P.	270	Trace	Trace	16.3	16.3	83	3.5
C	270	6.0	16.3	0	16.3	95	4.0
No urea		0	0	32.4	32.4	100	4.3

* Applied as a 4.2-percent aqueous solution.
† Chlorosis index: the higher the number, the yellower the plant.

Biuret injury from urea foliar sprays has not been reported in the literature so far as is known to us. Experiments conducted in the USDA by Armiger, Breen, and Starostka (*4*) indicated that soil applications of 30 lb of biuret nitrogen (73.6 lb of biuret) per acre decreased yield of perennial ryegrass in the first clipping but not in the second clipping.

The results of this preliminary test suggest that leaf-tip dieback and chlorosis of pineapple plants sprayed with urea are caused primarily by the biuret found in urea and not by urea itself.

References and Notes

* Published with the approval of the director of the Pineapple Research Institute of Hawaii as technical paper No. 222.
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Crayfish-Reddening Effect of Vertebrate Adrenal Cortical Extract

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Adrenal cortical extract (ACE) has been observed to have a pronounced reddening effect upon crayfish pigmentation. A total of 361 locally collected crayfish [*Orconectes virilis* (Hagen)] ranging in length from 2.4 to 5.7 cm were kept in individual finger bowls, each containing 250 ml of tap water (*1*). The water was changed daily. Approximately half of these animals received daily dosages of 0.05 ml adrenal cortical extract (Upjohn, 50 dog units/ml) in the water, administered just after changing the water. The untreated animals remained as controls. After about 3 wk of ACE treatment, all the experimental animals appeared quite red or orange, whereas all untreated controls retained normal brown coloration.

Subgroups of experimental and control crayfish were kept at room temperature or at 4.5°C, were starved or fed bits of beef, had their eyestalks removed or remained unoperated as controls. Variations in these factors had little influence on the crayfish-reddening effect of ACE and, therefore, are not discussed in detail here. In these experiments, ACE did not significantly prolong postoperative life or alter the acceleration of molting brought about by removal of the eyestalks. Destalked crayfish treated with ACE strikingly exhibited the reddish coloration.

ACE-reddened animals were examined more closely with the aid of a binocular microscope at 40 × magnification. Bright red chromatophores appeared to domi-

nate the field of observation. There remained scattered irregular dark patches, but many areas were completely devoid of any dark pigment. The effect was more pronounced on smaller animals but was noticeable on all crayfish that received ACE. Control animals exhibited normal chromatophore distribution, black chromatophores being prominent as well as those of reddish hue.

In order that the crayfish-reddening effect of ACE might be described more precisely, uropods were removed from normal crayfish and placed in water in spotplate depressions. The uropods from each animal were separated, one exopodite and one endopodite serving as the experimental material and their opposite members as the control. Each of the former received 0.05 ml of ACE in the water, while the controls received additional water or van Harreveld's balanced-ion solution (*2*). Both groups were examined at intervals with the aid of a compound microscope at 100× magnification. The entire experiment was repeated more than a dozen times. During a 12-hr observation period, no changes were observed in the colored chromatophores on ACE-treated uropods. The black chromatophores, however, became generally gray in appearance, and irregular patches of black granules appeared. The dark chromatophores appeared to have been bleached or denuded of pigment, without noticeable change in degree of pigment dispersion. No observable changes occurred in the chromatophores of control uropods. The crayfish-reddening effect apparently results from destruction of dark pigment, leaving unaltered red chromatophores to create the dominant visual impression.

Although the effect of ACE on arthropod chromatophores has not been reported previously, there is considerable published evidence that adrenal cortical substances may influence pigmentation in vertebrates. Desoxycorticosterone suppresses the formation of melanin, inhibits melanophore differentiation, and causes melanophores to degenerate in explants of chick-embryo skin (*3*). Black or brown rats turned gray as a result of dietary vitamin-B deficiency and resumed deposition of melanin upon adrenalectomy, but the effect is suppressed by exogenous ACE or desoxycorticosterone (*4, 5*). It has been observed for many years that human patients with Addison's disease show varying degrees of darkening or pigmentation, presumably as a result of adrenal cortical insufficiency (*6*). It seems reasonable to hypothesize that the adrenal cortical mechanisms that suppress or destroy pigment in vertebrates and in the crayfish are basically similar.

References and Notes

1. Tap water at the university is not chlorinated and is not toxic to aquatic organisms.
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Absorption of l-Glutamic Acid

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The possibility that the salts of l-glutamic acid have a different physiological effect than the acid has been raised by Waelsch (*1*). Pond and Pond (*2*) reported that a given dose of the acid raised the plasma level of glutamic acid less markedly but over a longer period of time than did an equivalent dose of either the sodium or potassium salt. Mayer-Gross and Walker (*3*) found that the oral administration of as much as 100 g of l-glutamic acid hydrochloride was not efficacious in arousing patients from insulin hypoglycemia, whereas the administration intravenously of 20 g of glutamic acid as sodium glutamate was.

A survey of the literature on l-glutamic acid administered either to animals or to man shows that three forms of the compound have been used: the salt of sodium or potassium, the unneutralized acid, and the hydrochloride. These three compounds differ markedly in solubility (*4*) and, thus, might be expected to be absorbed at different rates. In order to test this hypothesis, equivalent amounts of the three compounds were given to human subjects in a fasting condition. Blood samples were drawn routinely before the ingestion of the test substance and at 30, 60, 90, 120, and 180 min afterward; in four cases samples were also taken at approximately 4 and 5 hr. Plasma glutamic acid levels were determined by the method of Prescott and Waelsch (*5*). Since 16 such tolerance tests had been completed previously with 15 g of the monosodium salt of glutamic acid in tomato juice and water (*6*), the same dosage level and mode of administration were used for the other compounds.

The unneutralized glutamic acid was given twice to each of four subjects who had previously shown typical responses to the administration of sodium glutamate. In the first test of the unneutralized acid, in each of the four subjects, it was given as a sludge with tomato juice followed by water *ad libitum*. It was felt, however, that the ingestion of the acid with or immediately after food would more nearly approach the condition existing in the treatment of mentally defective children and that the presence of food might aid in the solution and absorption of the acid. Therefore, in the second test the acid was given with a liberal breakfast of hot cereal, eggs, milk, tomato juice, and toast, with water being allowed *ad libitum*. In three cases, the administration of food with the acid raised the absorption slightly but not at all to a level comparable to that of the salt. In the fourth individual no rise in blood level occurred after ingestion of the acid with or without food. In only one case was there any indication of a delayed rise, even though in two cases blood was drawn as late as 5 hr after the ingestion of the acid. The rises in these cases with unneutralized acid are similar to those reported by Bessman *et al.* (*7*) with a dose of 1 g/10 kg of body weight. One subject who