Table 1. Production of hexosamine by cultures of rabbit subcutaneous tissue.

Tissue	Medium	Hexosamine contents (mg)									
		3 days					4 days				
		Initial	Final			Change (%)	Initial	Final			Change (%)
		(tissue)	Tissue	Medium	Total		(tissue)	Tissue	Medium	Total	
Living	Hanks' solution with 25 percent ox serum ultrafiltrate	0.374	0.215	0.440	0.655	+ 75	0.263	0.166	0.450	0.616	+134
		.492	.362	.393	.755	+ 54	.254	.253	.465	.718	+182
		.366	.189	.171	.360	− 2	.215	.284	.312	.596	+177
		.286	.344	.445	.789	+175	.234	.250	.380	.630	+169
		.251	.415	.497	.912	+263	.418	.217	.459	.676	+ 62
Heat inactivated	Hanks' solution with 25 percent ox serum ultrafiltrate	.450	.348	.084	.432	− 4	.400	.218	.119	.337	− 16
		.398	.230	.087	.317	− 20					
Living	Hanks' solution (with glucose)	.545	.565	.431	.996	+ 83	.193	.199	.370	.569	+191
		.300	.232	.259	.491	+ 64					
Living	Hanks' solution without glucose	.355	.237	.175	.412	+ 16	.452	.239	.087	.326	− 28
		.391	.273	.151	.424	+ 9					

hundred milligram tissue samples were divided equally into three flasks and were maintained without significant proliferation. All flasks contained 50 units of penicillin and 50 μg of streptomycin (*7a*) per milliliter of medium. At the end of the period of incubation, the sterility of each culture was established by subcultures on blood agar and Sabouraud's mediums. The tissue fragments were separated from the medium by filtration. The tissue residue and an aliquot of the medium were hydrolyzed in 2*N* HCl for 15 hr, and the total amounts of hexosamine were determined following their isolation on Dowex-50 (*7*).

Whole serum or embryo extracts could not be used as nutrients, since they contain relatively large amounts of hexosamine. Hanks' balanced salt solution (*8*), with or without ox serum ultrafiltrate, was used as the suspending medium, since both were found to be free of hexosamine; hence, the only hexosamine in the culture was the amount initially present in the tissue and that synthesized during incubation. The combined hexosamine content of tissue and medium was significantly increased after 48 hr or more of incubation in either medium (Fig. 1).

When the glucose was omitted from Hanks' solution, no increase in total hexosamine occurred (Table 1). When the tissue was inactivated by heating at 100°C for about 15 min prior to incubation, no increase in the total hexosamine occurred. In control cultures in which no synthesis occurred, hexosamine diffused out of the tissue into the medium, as was evidenced by the fact that the total hexosamine content of tissue and medium corresponded with that estimated to be present in the original tissue sample.

Histological studies made on tissues cultivated under the specified experimental conditions but not used for hexosamine determinations revealed that some necrosis occurred in the tissue fragments early and increased progressively during incubation. The progressive tissue damage may explain why most of the hexosamine production occurred in the first 48 hr.

Synthesis of hexosamine by rabbit synovial tissue was also demonstrated by incubating infrapatellar fat pads that are lined by synovial cells. No increase in hexosamine was found when muscle tissue was used.

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Toxicity to Pineapple Plants of Biuret Found in Urea Fertilizers from Different Sources*

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During investigations involving the spraying of urea on pineapple plants, it has been observed that in some cases injury to the plants occurred. This injury consists of leaf-tip dieback, which is separated from the normal tissue by a zone of yellow tissue. Dieback varying from 1 to 12 cm has been observed. In extreme cases there has been yellowing of the edges of the lower leaves. In the past, the assumption has been made that under

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.

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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-