contains some RNA (pyronin and Azure B stainable; ribonuclease and trichloroacetic acid extractable) as well. The small amounts of cytoplasmic RNA are localized close to the nucleus. In corresponding deuterated cells, the nucleus is larger and appears to contain greater amounts of DNA; more striking than this, however, is the occurrence of far larger amounts of cytoplasmic RNA which is much more widely distributed throughout deuterated cells (Fig. 1, *A* and *B*).

The widespread dissemination of nucleic acids throughout the deuterated cells is also observed in *Chlorella vulgaris*. The chloroplasts of *C. vulgaris*, unlike those of *S. obliquus*, contain (according to the previously mentioned criteria) both DNA and RNA. The presence of larger amounts of cytoplasmic nucleic acids in deuterated *C. vulgaris* as compared to the nondeuterated organisms is clearly visible in Fig. 1 (*C* and *D*).

The basophilia shown in these photomicrographs is caused by both DNA and RNA. In *C. vulgaris* DNA fills the nucleus and occurs at the periphery of the chloroplast. Ribonucleic acid occurs in the same regions as DNA, but is also found more centrally in the chloroplasts and in nonchloroplast cytoplasm. This is true of both deuterated and normal cells. However, the nucleic acid-containing structures of deuterated algae are larger and stain more intensely than do their hydrogen counterparts.

Deuterated algae generally show increased heterogeneity of chloroplast shape. The typical single chloroplast of the nondeuterated cells was, in the deuterated cells of both *S. obliquus* and *C. vulgaris*, replaced by a great many varieties of chloroplast shape and number.

Some individuals in both deuterated and nondeuterated *C. vulgaris* appear to be multinuclear. Occasionally a nuclear configuration which resembles a telophase was observed in *C. vulgaris*. This configuration was observed with so much greater frequency in deuterated cells than in nondeuterated cells that it seems as though nuclear division had been delayed by deuteration. Since in these cells telophase is the easiest stage of mitosis to recognize, it cannot be said at this time that other stages of mitosis are not also affected by deuteration. That the nucleic acid–containing structures have grossly distorted shapes in deuterated algae may be only a reflection of the larger amounts of nucleic acid present, but it is probable that other factors are involved.

Although mitosis appears to be delayed in the algae, it is clearly not prevented. The situation here is clearly different from the arrest of mitosis of *Arbacia* eggs caused by high concentrations of D_2O as described by Gross and Spindel (*5*). But even in the case of the green algae, the effects of isotopic substitution are already evident on the cytochemistry of the organisms (*6*).

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Staining of Skin with Dihydroxyacetone

Abstract. The reaction of skin with dihydroxyacetone to produce a brown "artificial tan" appears to proceed through combination with free amino groups in skin proteins, and particularly by combination of dihydroxyacetone with the free guanido group in arginine.

Much interest centers around the mechanism of staining of skin by solutions of dihydroxyacetone, a three-carbon keto sugar. Our interest in this sugar arose from observations on excretion of it following ingestion of the substance in a diagnostic test for glycogen-storage disease (*1*). Urine specimens obtained from children after dihydroxyacetone-tolerance tests (*2*) gave several conspicuous spots on chromatograms resolved in isopropanol–ammonia–water solvent treated with diazotized sulfanilic-acid sodium-carbonate reagent (Pauly reagent). No reaction was observed when the same reagent was applied to duplicate chromatograms resolved in butanol–acetic acid–water solvent. This reagent ordinarily reacts with substances which contain an active hydrogen, an aromatic phenol or amine, or substances with imidazole nucleus. An area at R_F 75 (in isopropanol–ammonia–water) turned brown on standing a short time in air, more rapidly on heating. It seemed likely that a reaction occurred between dihydroxyacetone and ammonium hydroxide to form new compounds.

Analyses, by the micro-Kjeldahl method, of areas of the chromatogram which gave positive reactions with Pauly reagent and blank areas indicated that the substance at R_F 75 contained nitrogen. The stability of the reaction products was confirmed when the substances were eluted from chromatograms run in isopropanol–ammonia–water and rechromatographed in butanol–acetic acid–water solvent with no apparent change. Reactions of two principal substances derived from interaction of dihydroxyacetone and ammonium hydroxide are given in Table 1.

These observations led to the conjecture that the staining of skin by dihydroxyacetone might occur through combination with basic groups of skin proteins, or with free amino acids on the surface of the skin. Aqueous solutions of the sugar with ammonium hydroxide alone produced a brown color. The reactions with amino acids and related compounds are shown in Table 2. Arginine was the most reactive, with the appearance of a dark brown color within 30 minutes. Mixtures of dihydroxyacetone with glycine, lysine, and histidine also gave brown to yellow colors.

Chromatography of the mixtures of amino acids with dihydroxyacetone showed that while a positive test for arginine was obtained with ninhydrin, there was no reaction with aniline-phthalate, a sensitive reagent for sugars, at the position usually occupied by dihydroxyacetone. However, in the mixture of arginine and dihydroxyacetone another compound was formed which was positive both with ninhydrin and aniline-phthalate. In the case of glycine at least two additional ninhydrin-positive, aniline-phthalate-positive substances were formed. These reaction products, unlike those obtained from dihydroxyacetone and ammonium hydroxide, did not give a strong positive test with Pauly reagent. Similar observations were made of reaction mixtures of lysine, alanine, proline, and hydroxyproline, even though in

Table 1. Reactions of substances obtained from combination of dihydroxyacetone (DHA) and ammonium hydroxide.

	Substance		DHA
	A	B	
R_F INH*	0.75	0.88	0.70
R_F BuAc†	.48	.70	.60
Pauly reagent	Red-orange	Red	–
Ninhydrin	–	–	–
Aniline-phthalate	–	–	+
Phosphomolybdate	–	–	+
Arsenomolybdate	–	–	+
Brown color on standing in air	+	–	+
N (micro-Kjeldahl)	+	–	–

* Solvent: isopropanol-ammonia-water.
† Solvent: butanol-acetic acid-water.

Table 2. Reactions of aqueous solutions of amino acids in dihydroxyacetone.

Arginine	Immediate yellow; dark brown in 30 min
Glycine	Yellow after 60 min; dark brown in 6 hr
Histidine	Yellow after 60 min; brown in 12 hr
Lysine	Yellow after 60 min
Tryptophane	Yellow after 12 hr
Threonine	Yellow after 12 hr
Alanine	Faint yellow after 12 hr
Valine	No color change
Leucine	No color change
Phenylalanine	No color change

some cases no color change was evident. Additional ninhydrin-positive aniline-phthalate–positive substances were formed in small amounts.

Epidermal proteins have a very high content of basic amino acids, arginine, lysine, and histidine (*3*). Amino acids in sweat (determined by microbiological assay), listed in order of decreasing concentration, are arginine, histidine, threonine, valine, leucine, isoleucine, lysine, phenylalanine, and tryptophane (*4*). Dreizen and associates reported melanoidin formation between degradation products of glucose (dihydroxyacetone and glyceraldehyde) and the organic fraction of tooth structure (*5*). Interaction between amino acids and sugars has been shown to be the cause of browning that occurs in storage of dried foods (*6*). Richards was able to isolate the enolic form of N-(carboxymethyl)-amino-1-deoxyfructose as an intermediate in the browning reaction between glycine and *d*-glucose (*7*). Our data suggest that reactions similar in nature occur between dihydroxyacetone and amino acids. The reaction proceeds more rapidly with the highly reactive dihydroxyacetone.

The presence of arginine and other basic amino acids in skin proteins in relatively high concentration has led to the interesting application of this browning reaction between dihydroxyacetone and skin proteins in cosmetic lotions (*8*).

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Chromatographic Comparison of Scorpion Venoms

Abstract. The venom of seven species of scorpions was subjected to two-dimensional chromatographic analysis. Six major components were defined and tentatively correlated with the physiological activity of the venoms.

The venom of *Centruroides sculpturatus* Ewing and *Centruroides gertschi* Stahnke has the distinction of exhibiting a severe neurotoxic effect, while that of other scorpions of the Southwest produces little or no systemic effects. These two species also differ in this respect from other species of United States centruroidian scorpions, even though some of the other species morphologically resemble them closely (*1*). *Centruroides sculpturatus* and *C. gertschi* venom also has the distinction of being lethal to man in quantities injected during the process of a single sting.

In cases of moderate to severe venenation, *C. sculpturatus* and *C. gertschi* venom will cause severe drooling, so that the patient loses large quantities of fluids, and characteristic convulsions.

Normally the venom of *Vejovis spinigerus* (Wood) and *Hadrurus arizonensis* (Ewing) apparently produces only a local reaction in the form of a swelling and sometimes ecchymosis at the site of the sting. In severe cases of venenation by these species, reactions of a systemic effect were experienced. This led Palmer (*2*) to investigate the effects of these venoms in larger doses than are injected during the natural stinging process. Among other things, he found that *Vejovis spinigerus* venom in a lethal dose would produce only slight salivation but severe convulsions, while *Hadrurus arizonensis* venom produced severe drooling without convulsions.

The present work is an attempt to characterize chromatographically the proteinaceous components of the venom of seven of these species. The venom from each was collected, lyophilized, weighed, and reconstituted with sterile water to give the concentrations, in milligrams per milliliter, shown in Table 1.

Two - dimensional chromatograms were run on Whatman No. 1 paper squares at 6°C (43°F) with two parts of *N*-propyl alcohol to one part of 1-percent ammonium hydroxide as the first solvent and one part of 1-percent phenol to one part of 1-percent ammonium hydroxide as the second solvent. Two drops from a tuberculin syringe were used as inoculum for all runs.

The ninhydrin-positive pattern was revealed by spraying the strips with 0.2-percent ninhydrin solution in 95-percent ethanol to which 5-percent 2,4,6-collidine was added just before use (*3*).

Table 1. R_F values for seven species of scorpions.

Concn. (mg/ml)	R_F*	R_F†	Component group
	Hadrurus hirsutus (Wood)		
11.5	0.27	0.94	*D*
	.42	.96	*C*
	.65	.90	*B*
	.88	.85	*A*
	Hadrurus arizonensis (Ewing)		
9.9	.46	.96	*C*
	.70	.84	*B*
	.86	.96	*A*
	Centruroides sculpturatus Ewing		
4.63	.12	.97	*E*
	.16	.58	*F*
	.85	.91	*A*
	Centruroides gertschi Stahnke		
0.72	.12	.91	*E*
	.85	.83	*A*
	Centruroides pantheriensis Stahnke		
6.0	.84	.79	*A*
	Centruroides vittatus (Say)		
5.8	.82	.71	*A*
	Vejovis spinigerus (Wood)		
5.6	.90	.71	*A*

* In *N*-propyl alcohol and NH_4OH.
† In phenol and NH_4OH.

The R_F values obtained for the seven species are given in Table 1. Figure 1 shows the distribution pattern of the venom for these seven species. It is apparent that the venom components fall into six areas as designated in Fig. 1.

While some uniformity of venom components is to be expected, the variation in the effect of the sting of differ-

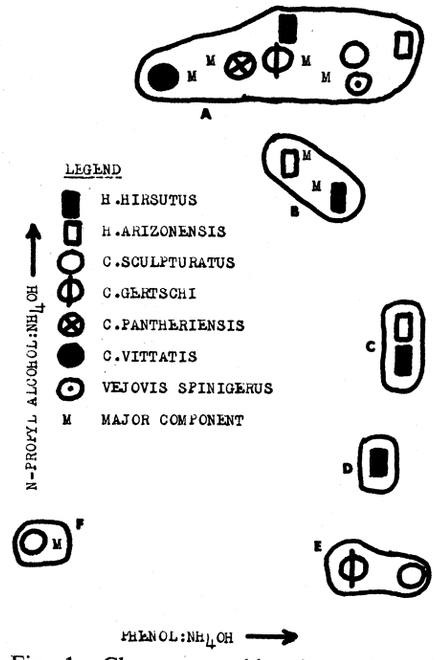

Fig. 1. Chromatographic distribution of scorpion venom into groups. See Table 1 for R_F values.