layer chromatography of Bischel *et al.* (*13*), metachromatic spots were present at the primary solvent front only in the G_{M1}-gangliosidosis samples. Similarly, only the G_{M1}-gangliosidosis samples contained a fast-moving component that stained pink with the orcinol-sulfuric acid spray in the plain silica gel thin-layer chromatography described by Wusteman *et al.* (*14*). The purified keratan sulfate migrated slightly faster than the standard skeletal keratan sulfate in electrophoresis at *p*H 7.4. A preliminary analysis suggested that the molar ratio of sulfate to hexosamine of this compound may be significantly greater than one. Such over-sulfation may account for its faster electrophoretic mobility. My findings justify the tentative, although by no means conclusive, identification of this compound as keratan sulfate.

I conclude that (i) the degree of increase in G_{M1}-ganglioside in the viscera of patients with G_{M1}-gangliosidosis is the same as that of Tay-Sachs ganglioside (G_{M2}) in viscera from those with Tay-Sachs disease; and (ii) there are specific increases of keratan sulfate and, to a lesser degree, a sialomucopolysaccharide with a similar sugar composition in the viscera of those with G_{M1}-gangliosidosis. The viscera of patients with Tay-Sachs disease have practically no histological abnormality, whereas those from patients with G_{M1}-gangliosidosis are characteristically affected. Therefore, the histological lesions of viscera in this disorder are more likely due to the accumulation of keratan sulfate and related compounds than to accumulation of ganglioside.

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15. Dr. Bruno W. Volk, Jewish Chronic Disease Hospital, Brooklyn, N.Y., kindly provided me with all the Tay-Sachs visceral organs for this study. I thank Dr. Karl Meyer, the Belfer Graduate School of Science, Yeshiva University, New York; Dr. James H. Austin, University of Colorado Medical Center, Denver, Colo.; and Dr. David Hamerman, Albert Einstein College of Medicine, Bronx, N.Y. for their helpful advice. Dr. Meyer also kindly gave me the authentic skeletal keratan sulfate for the standard. Supported by grant R-160-67C from the United Cerebral Palsy Foundation and grant NB-03356 from the PHS.

3 January 1968

Bracken and Locust Ecdysones: Their Effects on Molting in the Desert Locust

Abstract. *Bracken contains ecdysone derivatives that are active when injected into locusts. However, when fed to the desert locust as its sole or chief diet, it does not affect molting, growth, or development. There is evidence that, in locusts, the active ecdysones are dehydroxylated to* α*-ecdysone and passed out through the gut in the feces. There is no evidence for any uptake of ecdysones from the gut.*

Substances which either are active in inducing puparium formation in Diptera (*Calliphora* and *Musca*) or have chemical structures similar to those of the insect ecdysones have been isolated from plants (*1*). Ecdysone, 20-hydroxyecdysone, and three related materials of unspecified structure are present in the pinnae of bracken [brake fern, *Pteridium aquilinum* (Linn.)]. Kaplanis and his co-workers (*2*) tentatively agreed with the supposition (*3*) that "perhaps these biologically active substances have been elaborated by the plant to interfere with the growth processes of insect predators" (by predators they meant plant-eating insects). This hypothesis is open to the simple test of feeding insects upon bracken.

The desert locust *Schistocerca gregaria* (Forskål) differs from most other Acridids in that it thrives better on a mixed diet of herbs than on grass alone. For this reason it was selected for these tests. The insects were reared individually in gauze-topped, glass jars under standard conditions (*4*). The controls were fed wheat seedlings. The experimental animals were fed air-dried fronds of autumnal bracken that were moistened immediately before being placed in the jars. At weekends, each locust fed upon bracken was given three wheat seedlings. Although the locusts were reared on these diets from 1 day after hatching until they became adult, molting was entirely normal and mortality was insignificant; less than 8 percent in any one group died.

Staal (*5*) implanted extra prothoracic gland tissue (as a source of extra molting hormone) into second-instar locusts (*Locusta migratoria*) and obtained fully formed adults after only three further molts in about 50 percent of his animals. The individuals that passed through the normal five instars produced adults with extra-long wings. In our work, all the locusts passed through the normal five instars, and the wings were shorter, not longer, in bracken-fed animals. This was related to an overall reduction in size. The difference in wing lengths was significant in the case of females: the mean for wheat-fed locusts was 4.70 $\pm$ 0.013 cm for males and 5.06 $\pm$ 0.009 cm for females; the mean for bracken-fed males was 4.42 $\pm$ 0.010 and for females 4.56 $\pm$ 0.018 cm. The weights of bracken-fed adults were significantly less than those for wheat-fed ones; for males the means were 0.832 $\pm$ 0.049 g and 1.094 $\pm$ 0.026 g, respectively; for females the means were 0.926 $\pm$ 0.018 g and 1.395 $\pm$ 0.025 g, respectively. The bracken-fed locusts

Table 1. Mean length (and standard error of the mean) of instars of locusts given different diets (based on results for at least 20 locusts per group).

Diet	Instar length (days)			
	2	3	4	5
Wheat	5.01 $\pm$ 0.322	7.84 $\pm$ 0.341	8.08 $\pm$ 0.292	11.90 $\pm$ 0.582
Bracken	9.23 $\pm$ .610	10.90 $\pm$ .791	11.63 $\pm$ .500	18.12 $\pm$ .463
		Probability		
	$< .01$	$< .05$	$< .01$	$< .01$

also differed from the controls in that they took longer to develop (Table 1). The smaller size and longer nymphal life of the bracken-fed locusts was probably related to a dietary deficiency.

Our tests revealed no evidence that a diet of bracken interfered with the molting cycle of desert locusts. It seemed possible, however, that English bracken does not contain the ecdysones found in American samples.

We made a crude extract of bracken by extracting (three times) 300 g of dried pinnae in 6 liters of 75 percent methanol and passing the extract through a silica-gel column of activity 5. The extract was then dried and dissolved in 80 percent ethanol. Some of the extract was subjected to chromatography on activated silica gel with a mixture of chloroform and ethanol (4:1) used as solvent. Staining with vanillin and sulfuric acid revealed five stained areas, whose R_F values and colors, when compared with those given by Thompson *et al.* (*6*), who used the same system on purified ecdysones, indicated that our bracken contained α-ecdysone and some 20-hydroxyecdysone [the main constituent of β-ecdysone (*7*)].

Hoffmeister *et al.* (*8*) reported large amounts of α-ecdysone in the feces (supplied by our laboratory) of locusts (an equivalent of 200 to 750 *Calliphora* units per gram of dried feces). In contrast, we have searched for and failed to find α-ecdysone in the blood and prothoracic glands (PTG) of locusts (although at present the only methods available for detecting small quantities of naturally occurring ecdysones is by paper and thin-layer chromatographic techniques). Instead, we have found evidence of some β-ecdysone in the blood, but not in the PTG, and large amounts of a more polar ecdysone derivative in blood and PTG. This last material we have called λ-ecdysone (*9*).

The effects of injecting bracken extract into locusts were compared with those of injecting homogenates of the PTG of fifth-instar locusts dissected at a time when the gland was full of secretion. Fourth-instar locusts injected with bracken extract received various doses, but in the tests referred to here they were given 1.2 μl, which was equivalent to the extract from about 0.1 g of dried pinnae. The mean dry weight of bracken eaten by locusts of this instar reached a maximum of 0.06 g per day. We made the PTG extract by grinding up the freshly dissected and dried glands in 40 percent ethanol and filtering the

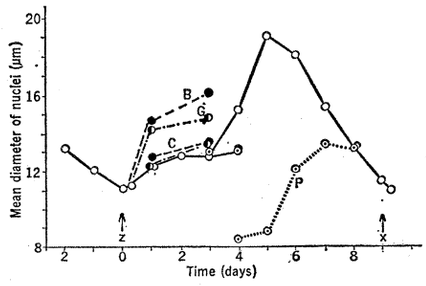

Fig. 1. Average diameter of nuclei of the prothoracic gland of nymphs of *Schistocerca gregaria* in relation to the stages of the fourth instar and molt cycle. At about day 4 in this instar, some of the peripheral cells (*P*) divide and metaphase plates can be seen. Locusts injected at the molt from the third to the fourth instar with 40 or 80 percent ethanol (*C*), prothoracic gland homogenate (*G*), or bracken extract (*B*). Each point on the graph represents the average for 60 measurements, ten from each of six locusts (standard error of means $< \pm 0.24$); *Z*, molt from third to fourth instar; *X*, molt from fourth to fifth instar.

homogenate. The locusts each received 6 μl of this extract, which contained the equivalent of about one-third of a pair of glands from fifth-instar insects.

Both Wigglesworth (*10*) and Wells (*11*) have described cyclical changes in the size of the nuclei of the PTG of bedbugs (*Cimex*) and of cotton stainers (*Dysdercus*). Using freshly dissected glands of the desert locust, we have studied the corresponding cycle in this species (Fig. 1). Injection of PTG homogenate immediately after the molt results in a significant increase ($P<.01$) in the size of the nuclei of the PTG of the recipient, which was generally measured 24 hours later (Fig. 1). Both Williams (*12*) and Karlson (*13*) have suggested that PTG hormone is capable of stimulating the gland, but the mechanism may be more complicated than this and may involve positive feedback [Etkins (*14*)] in which ecdysone stimulates the release of brain hormone. The only other tissue homogenate that we have found to alter the size of the nuclei of the PTG is one containing activation hormone of the brain. We have injected fourth-instar locusts with the bracken extract and obtained changes in the size of PTG nuclei that are comparable with those resulting from injection of fresh extracts of the PTG (Fig. 1).

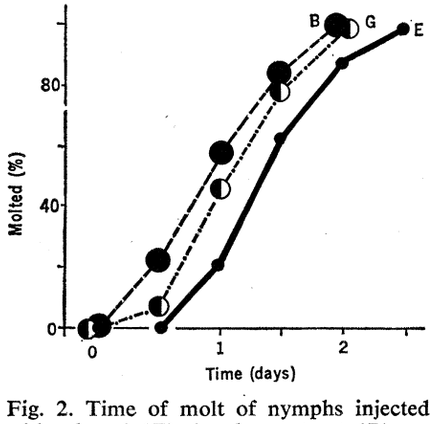

Fig. 2. Time of molt of nymphs injected with ethanol (*E*), bracken extract (*B*), or prothoracic gland homogenate (*G*). (Results based on observations for at least 20 locusts per group.)

Both the homogenate of PTG and the bracken extract, when injected immediately after the third molt, advanced the fourth molt by about 12 hours (Fig. 2). We conclude that the bracken contains materials that have the properties of ecdysones, yet when ingested they have no effect on molting. Whatever function the ecdysones have in bracken and other plants, it is unlikely, in our view, that it is a form of defense against insects. The presence of large amounts of α-ecdysone in the locust feces suggests that the gut will not take up ecdysones into the blood. Indeed this must also be true for predacious insects that prey on larval insects (for example, larval coccinellids feeding on nymphal aphids).

α-Ecdysone appears to have little effect on physiological processes in locusts, and we believe it to be an excretory metabolite of the hormone (*8*). Locusts would therefore excrete ecdysones by dehydroxylation. This is in conflict with the view of Thompson *et al.* (*6*) that excretion in the tobacco horn worm (*Manduca sexta*) is by hydroxylation.

Since the desert locust is a tropical insect of the deserts and semideserts, neither bracken (which is confined to temperate lands) nor any related fern is present in its normal habitat. However, other Acridids abound in places where bracken grows. It is unlikely that different Acridids have radically different ecdysones; results similar to ours might therefore be expected with any grasshopper with sufficiently catholic tastes to accept bracken as food. Even the desert locust would not eat bracken when wheat was present, so it seems likely that it contains feeding deterrents. Indeed, it is avoided by cattle, sheep, and horses, in which it appears to cause poisoning if ingested.

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25 January 1968

DDT Reduces Photosynthesis by Marine Phytoplankton

Abstract. *Concentrations of DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] as low as a few parts per billion in water reduced photosynthesis in laboratory cultures of four species of coastal and oceanic phytoplankton representing four major classes of algae, and in a natural phytoplankton community from Woods Hole, Massachusetts. Toxicity to diatoms increased as cell concentration decreased. This inhibition may be of ecological importance.*

Recently it has become apparent that DDT (*1*) and its derivatives are among the most widely distributed synthetic chemicals on Earth. They are found not only in soils (*2*), runoff water (*3*), air, and rainwater (*4*), but also in most animals analyzed from widely diverse parts of the world, including Antarctica (*5*, *6*). Residues of DDT were recently reported in marine organisms of both the Atlantic and Pacific oceans, including pelagic birds at the top of wholly oceanic food chains (*7*); this and other evidence suggest widespread contamination of marine plankton by these chemicals. While components of the zooplankton have been shown to be highly susceptible to DDT (*8*, *9*), very little is known of its effects on phytoplankton. Since a substantial part of the world's photosynthesis is performed by phytoplankton (*10*), interference with this process could be important to the biosphere.

In order to determine the effects of DDT on photosynthesis by marine phytoplankton, species important as food organisms, representing four classes of phytoplankton, were chosen from laboratory cultures maintained at Woods Hole Oceanographic Institution (WHOI). These included the diatom *Skeletonema costatum*, isolated from Long Island Sound (WHOI clone "Skel"); the coccolithophore *Coccolithus huxleyi* from the Sargasso Sea (32°10′N,64°30′W; "BT-6"); the green alga *Pyramimonas* sp. from the Sargasso Sea (33°11′N,65°15′W; "13-10 Pyr"); and the neritic dinoflagellate *Peridinium trochoideum* ("Peri"). In addition, water from Vineyard Sound (Woods Hole) was tested, containing a typical neritic phytoplankton community dominated by various diatoms.

Fluorescent lights (5000 lux) on a cycle of 14 hours light and 10 hours dark were used at 17°C. The four laboratory cultures were grown axenically in half-strength medium "f" (*11*), an enriched sea water. In 125-ml erlenmeyer flasks, 50-ml portions of medium were inoculated to yield the appropriate cell concentrations, and these were cultured for 24 hours. This initial culture period was omitted with *Pyramimonas* and the Vineyard Sound water; the latter was filtered through 50-μ mesh to remove zooplankton and was enriched to "f/100." To each flask 1 or 2 μl of pure *p,p′*-DDT (*1*) in an ethanolic solution was then added to yield the desired concentrations of DDT. Control flasks received equal amounts of pure ethanol, with no detectable effect on results. After culturing for 20 to 24 more hours, ^{14}C-bicarbonate (*12*) was added, and the algae were illuminated for 4 to 5 more hours. A few controls were run in darkness. Cultures were then collected on 0.8-μ Millipore filters, by use of slightly reduced pressure, and washed three times with filtered sea water; the filtered cultures were dried and radiation was counted. The radioactivity retained by the filtered cells is related to the amount of carbon fixed by photosynthesis (*13*).

Cell concentrations were chosen to approximate densities found in nature. To facilitate qualitative comparisons between the four phytoplankton species, cell concentrations were calculated to yield approximately equal total cell areas at the end of the experiment. Cell area roughly correlates with metabolic activity in phytoplankton (*14*).

The data from these experiments are shown in Fig. 1; dark uptake of ^{14}C

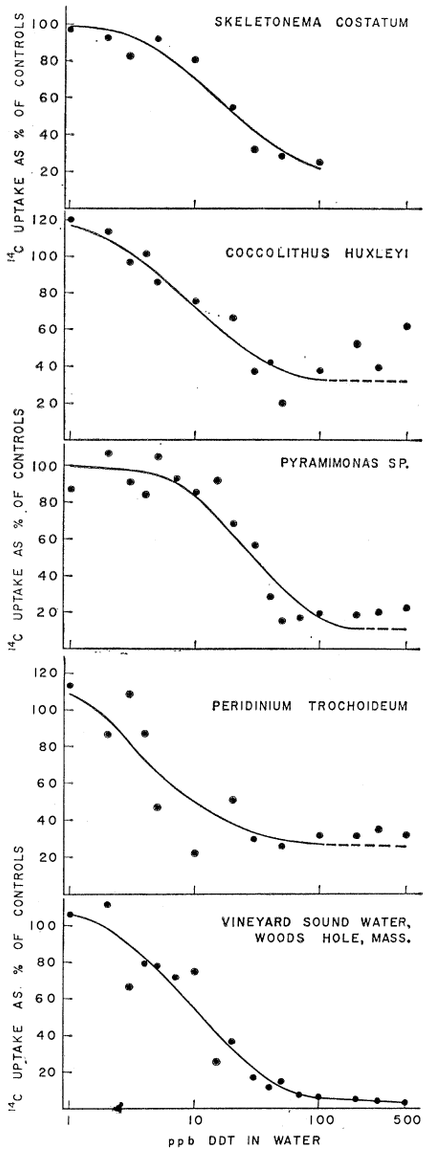

Fig. 1. Photosynthesis by phytoplankton at various concentrations of DDT, measured by uptake of ^{14}C (percentages) relative to uptake by controls. The following are, respectively, calculated cell concentrations (per milliliter) at the end of each experiment, mean counts per minute (cpm) for controls, uptake in the dark (percentages) relative to uptake by controls, and probabilities that a negative linear regression is random: *Skeletonema*, 1600, 1390 cpm, 1.1 percent, $P = .052$; *Coccolithus*, 4920, 1435 cpm, 39.7 percent, $P = .00074$; *Pyramimonas*, 2100, 564 cpm, 22.8 percent, $P = .032$; *Peridinium*, 240, 533 cpm, 53.4 percent, $P = .059$; Vineyard Sound water (as obtained), 6200 cpm, 0.6 percent, $P = .0011$.