of different toxohormone-like fractions and for untreated mice.

Toxohormone-like fractions from mutants significantly reduced liver catalase values when injected into mice, whereas catalase values were not significantly altered in mice that were injected with toxohormone-like fractions from parent strains of the microorganisms.

From these results it can be assumed that respiration-deficient mutants of microorganisms, at least in some species, produce a toxohormone-like factor or catalase-depressing factor which has the same effect as substances produced by cancer cells.

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Species Differentiation in Fish by Electrophoretic Analysis of Skeletal Muscle Proteins

Abstract. Extracts of skeletal muscle protein from ten species of the fresh-water sunfish family were examined by the electrophoretic technique, and the resulting patterns showed characteristic specificity in each instance. Usually three major components of greater mobility were accompanied by slower moving minor components. No uniformity of mobility or of proportion of protein was evident. Thus patterns that "fingerprint" the species are evident.

The moving-boundary method of electrophoresis was adapted by Connell (*1*) to the analysis of skeletal-muscle proteins of fish. In analyses of 20 species he found a very characteristic electrophoretic pattern for each species, as well as considerable differences in component mobilities and composition. Much additional information about proteins of fish has been reviewed by Hamoir (*2*). We have extended the same method of skeletal-muscle-protein analysis to several families, genera, and species of fish native to the Great Lakes region in Michigan. In this work we have sought to ascertain whether this procedure might provide another physicochemical means of distinguishing taxonomic differences.

The fish in this study, with the exception of the white crappie (*Pomoxis annularis*), were captured during summer months from lakes and streams in the vicinity of the W. K. Kellogg Gull Lake Biological Laboratory near Battle Creek, Michigan (*3*). Shortly after capture, specimens were placed in a deep-freeze unit and stored until the analysis was to be made. To prepare the sample for electrophoresis, each specimen was thawed sufficiently to permit easy removal of the skin, and with the aid of a scalpel about 20 g of skeletal muscle was removed from a region just ventral to the dorsal fin. The muscle was thoroughly mixed in a Waring blender, at room temperature, with 20 ml of buffer composed of 0.0156*M* Na_2HPO_4 and 0.0035*M* KH_2PO_4 at *p*H 7.5 and ionic strength of 0.10. The mince was allowed to settle, and a portion of the supernatant was decanted, placed in a 15-ml centrifuge tube, and clarified for 15 minutes at 1500 rev/min in an International Clinical Model centrifuge. The sample was then dialyzed for a 12-hour period against the buffer described, and electrophoresis was carried out in the Perkin-Elmer model 38 Tiselius apparatus. All runs were carried out at ice-water temperature for 6300 seconds, under a potential gradient of 8.5 volt/cm. The protein concentration was maintained between 1 and 1.5 percent, and a 2-ml conductivity cell (constant = 0.4893) was used to measure the resistance of the dialyzed protein samples at ice-water temperature.

The electrophoretic patterns obtained from ten species representing six genera of the family Centrarchidae, the fresh-water sunfish family, are shown in Fig. 1. These patterns show a great diversity in number and location of components as well as nonenantiography between the ascending and the descending boundary curves. Electrophoretic mobilities of the discernible peaks averaged from the ascending and descending patterns clearly showed further differentiation between the species. While some minor components may be barely distinguishable, the electrophoretograms are typical for the species considered, and runs made on other individuals of the same species always give the same pattern.

There is an overall similarity in the electrophoretograms of Fig. 1 in that several major components (usually three) are present in the middle or leading range of the patterns. Each pattern is made up of a set of component mobilities different from those of any other pattern. This variation, which according to Hamoir (*2*) characterizes muscle extract from other species of fish, is very evident for the centrarchid species studied in this work. Although these protein extracts consist principally of enzymes that have the same functions in muscle glycolysis, the electrophoretic specificity of the extracts is pronounced. The patterns are quite similar to those of marine species, as given by Connell (*1*), with respect to the major components but differ in kind and proportion for the minor ones.

The minor components in the patterns of Fig. 1 seem to exist in the more slowly moving region and appear too ill-defined to warrant any special designation. Only the patterns for the

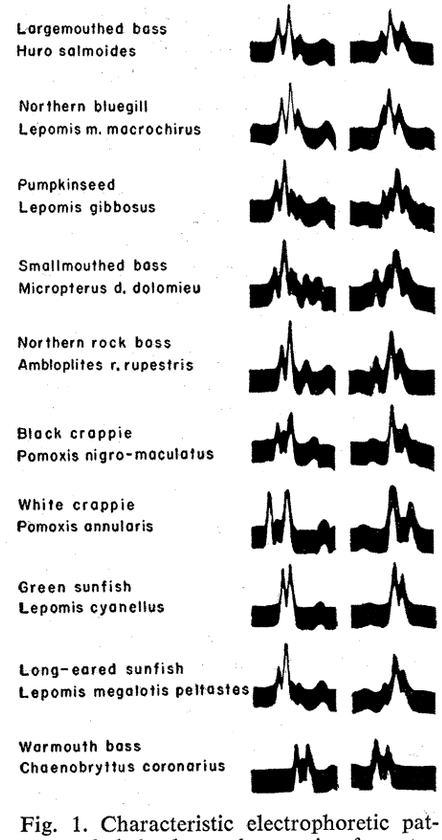

Fig. 1. Characteristic electrophoretic patterns of skeletal muscle proteins from ten species of fresh-water sunfish (family Centrarchidae). Patterns at left, ascending; at right, descending.

northern bluegill and the black crappie indicate the possible presence of a minor component of greater mobility than the major components. The extremely characteristic specificity of the electrophoretic patterns for each of the species included in this study also corroborates the observation of Connell (*1*) that the electrophoretic method of analyzing the muscle protein of fish may be a means of "fingerprinting" any species. We have noted some evidence (not included in this report) that differentiation of a taxonomic category of subspecies may be possible.

In addition, the eletrophoretic method of analysis can be used in fish hybridization studies, in studies of phylogeny, and—to cite a very practical application—in connection with game enforcement procedures to prove illegal possession of fish fillets.

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Synergism of Malathion against Resistant Insects

Abstract. Several tris-substituted derivatives of phosphoric acid synergized the toxicity of malathion to resistant houseflies and mosquitoes. In some tests it was possible to overcome acquired resistance completely. The synergists had much less effect on the toxicity of malathion to susceptible strains of the same species.

Studies with mammals have shown that the toxicity of malathion (*1*), one of the safest of insecticides, can be increased by simultaneous administration of, or prior treatment with, a number of organic phosphates. Thus, the potent insecticide EPN (*2*) synergizes malathion against mammals (*3*), as does the noninsecticide tri-*o*-cresyl phosphate (*4*). With both compounds it is known that the effect of their synergizing action is to block the degradation of malathion through carboethoxy ester hydrolysis (*5*).

The effect of a series of phenyl phosphorus materials on the toxicity of malathion to mice has been investigated, and a correlation between the ability to synergize malathion and the ability to inhibit ali-esterase activity has been demonstrated (*6*). Recent work has shown that resistance to organophosphates in insects is associated with a decline in ali-esterase activity and a change in the nature of the ali-esterase from an enzyme (or enzymes) inhibited by organophosphates to an enzyme (or enzymes) capable of degrading them (*7*). These findings prompted us to investigate the possibility that acquired resistance to malathion in insects might be overcome through use of ali-esterase inhibitors. Experiments were conducted with several tris-substituted aromatic and aliphatic derivatives of phosphoric acid which are known to be ali-esterase inhibitors.

For these experiments 2- to 4-day-old adult female houseflies, *Musca domestica* L., of a malathion-susceptible (Orlando Regular) colony and a malathion-resistant (Grothe) colony were used. The mosquitoes used were fourth-instar larvae of a susceptible and a malathion-resistant strain of *Culex tarsalis* Coq. In the tests with flies, groups of 20 adult females were exposed to films of the insecticide with or without synergist in 1-pint glass jars. Mosquito larvae were tested by placing groups of 20 in 250 ml of water in glass jars containing the toxicants. In all tests mortality determinations were made 24 hours after initial exposure to the toxicant.

Technical-grade malathion of more than 90 percent purity was used in these studies. The synergists were samples of commercially available materials (*8*). The materials were tested on a weight-to-weight basis.

Table 1. The effect of some synergists on the toxicity of malathion to susceptible and resistant houseflies and mosquitoes.

Ratio of insecticide to synergist	Toxicity (LC_{50} in μg per jar)			
	Houseflies		Mosquitoes	
	Resistant	Susceptible	Resistant	Susceptible
	Malathion only			
	1800	17	2.4	0.025
	Malathion plus triphenyl phosphate			
1:1	80	17	0.024	.016
1:10	50	25	.025	.022
	Malathion plus tributyl phosphorotrithioate			
1:1	25	9	.030	.014
1:10	20	10	.014	.010
	Malathion plus tributyl phosphorotrithioite			
1:1	40	12	.025	.014
1:10	18	13	.015	.018

The results of experiments with several of the most effective synergists are presented in Table 1. As shown by the data, the synergists reduced the resistance to malathion of flies of the Grothe colony from about 100-fold to less than 5-fold. None of the synergists tested increased the toxicity of malathion to flies of the susceptible colony by as much as a factor of 2. In tests with mosquito larvae the same synergists completely overcame the 100-fold resistance. As with the flies, the synergists failed to produce striking increases in toxicity to larvae of the susceptible colony.

At present the effect of the synergists is not fully understood. All the synergists are known to be inhibitors of ali-esterase activity in flies and mosquitoes under both in vivo and in vitro conditions. They also synergize the toxicity of malathion to mammals (*9*).

Preliminary studies have indicated that the synergists inhibit the degradation of malathion by mosquito larvae. The most logical explanation is that the synergists inhibit the ability of the insects to degrade malathion by cleavage of the carboethoxy ester linkages. Both houseflies and mosquitoes are known to degrade malathion partially through hydrolysis of these bonds, and with *Culex tarsalis,* increased ability to degrade through carboethoxy ester hydrolysis is known to be a factor in resistance (*10*).

The results provide evidence that, at least in certain cases, acquired resistance to organophosphate insecticides can be overcome through selective inhibition of degradation mechanisms with noninsecticidal compounds.

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