fly, *Musca domestica,* have shown that Sevin is only about one-tenth as toxic as DDT (the LD_{50} values, for topical application, are 0.2 μg per fly for DDT and 2.3 μg per fly for Sevin). In an attempt to increase the effectiveness of Sevin, several pyrethrin synergists were investigated. Of those investigated, 2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaudecane (Sesoxane), a pyrethrin synergist discovered by Beroza (*2*) and manufactured by Shulton, Inc., was most effective. This compound has been reported as having some synergistic action for methoxychlor (*3*) and was found in our laboratories to be a synergist for DDT on German roaches. When tried with DDT on houseflies, it showed little synergism. However, Sesoxane in conjunction with Sevin gave a greatly increased toxicity for houseflies. Figure 1 shows the log dose–probit lines for DDT and Sevin, with and without the synergist, for the susceptible houseflies; dosage is expressed as the logarithm of the number of micrograms applied, and mortality is expressed in probits.

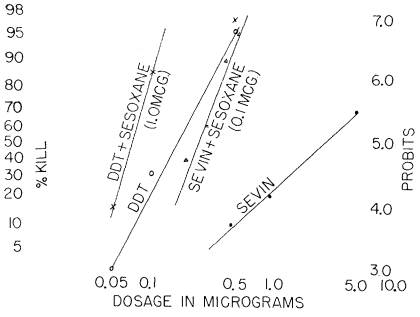

Fig. 1. Dosage mortality curves for susceptible houseflies treated with Sevin and DDT with and without Sesoxane.

Table 1. Mortality, after 24 hours, of susceptible and resistant houseflies topically treated with Sevin and Sevin-Sesoxane combinations. Twenty-five flies were treated at each point. The percentages represent the combined results of duplicate tests.

Sevin (μg/fly)	Sesoxane (μg/fly)	Kill (%)
	Susceptible	
0.5		10
1.0		20
2.0		55
5.0		67
	Parathion-resistant	
5		10
10		10
30		10
	DDT-resistant	
5		20
10		40
30		60
	Susceptible	
0.1	0.00	0
0.1	0.10	40
0.1	0.30	80
0.1	0.50	95
0.5	0.10	95
	Parathion-resistant	
1.0	0.00	0
1.0	1.00	60
1.0	5.00	100
1.0	10.00	100
	DDT-resistant	
1.0	0.00	10
1.0	0.50	100
1.0	1.00	100
1.0	5.00	100
0.1	5.00	100

Since both Sevin and Parathion are strong cholinesterase inhibitors, it may be expected that Parathion-resistant insects will also be resistant to Sevin, and this was found to be the case, as shown in Table 1. However, only a comparatively low resistance, commonly called vigor tolerance, was to be expected from DDT-resistant flies (*4*). Table 1 shows that DDT-resistant flies have about a sevenfold resistance to Sevin—that is, the LD_{50} for susceptible flies was 2.6 μg; for DDT-resistant flies, 18 μg.

Since the combination of Sevin and Sesoxane was effective on the DDT-susceptible flies, it was applied to DDT-resistant flies. This combination proved to be very effective even at small dosages. All mortalities were obtained by applying the chemicals together in acetone solutions topically to adult females. Table 1 shows the results.

The outstanding feature is the high effectiveness of the combination containing a low ratio of synergist to toxicant in contrast to the usual situation, in which large amounts of synergist are necessary.

Comparison of the rates of penetration between Sevin alone and Sevin-Sesoxane combinations shows very little difference between the two; this indicates that the effectiveness of the Sevin-Sesoxane combination is not due to increased penetration.

The effective synergistic action of Sesoxane with Sevin gives hope for control of DDT- and phosphate-resistant insects.

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Multiple Hemoglobins in Fishes

Abstract. Paper electrophoregrams of the hemoglobins from 14 species of fresh- and salt-water fish demonstrated from one to three components. The relative distribution of these fractions was determined for all species. Mobilities of the three hemoglobins of rainbow trout were calculated from moving-boundary patterns. Low anodal mobility at *p*H 8.8 was characteristic of many species examined.

Since the demonstration of abnormal hemoglobin in man by Pauling *et al.* (*1*), electrophoretic studies have been carried out on many other species. The occurrence of multiple hemoglobins has been reported in other mammals (*2*), in birds (*3*), and in amphibians and reptiles (*4*). Manwell (*5*) has recently reported a difference in alkaline denaturation between adult and postlarval hemoglobins from a teleost fish (*Scorpaenichthys marmoratus*). Examination of fish hemoglobins should therefore reveal some differences which might be species characteristic and perhaps even race dependent.

Samples of fish blood were collected in heparinized tubes, either by cardiac puncture of the larger fish or by amputating the peduncle of smaller fish and collecting the droplets of blood from the severed caudal vein and artery. The red cells were separated from the plasma by centrifugation and washed three times with 0.9-percent sodium chloride. The hemoglobin solutions used for analysis (*6*) were prepared by adding 2 volumes of distilled water to 1 volume of packed red cells. After standing overnight in the cold, the hemolyzates were centrifuged at 25,000*g* for 1 hour. The supernatant

Table 1. Relative distribution of fish hemoglobin components. Values are averages from at least two fish of each species.

Electrophoregram	Percentage of total hemoglobin (%)		
	Highest mobility	Medium mobility	Lowest mobility
	Steelhead trout		
E	54–56	22–23	22–23
	Rainbow trout		
F	54–58	21–23	21–23
	Blueback salmon		
G	51–53		47–49
	Shad		
H	66–70		30–34
	Largemouthed bass		
I	60–64		36–40
	Brook trout		
J	56–64	18–22	18–22
	Silver salmon		
K	50–60		40–50
	Chinook salmon		
L	50–60		40–50

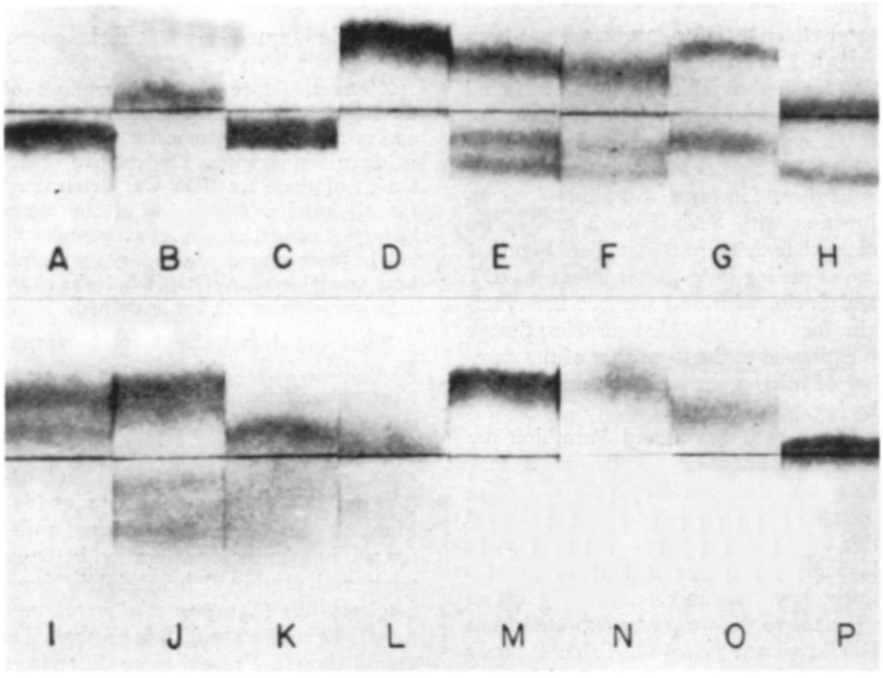

Fig. 1. Separation of fish hemoglobins in Veronal buffer (*p*H 8.8; ionic strength, 0.05): (A) *Entosphenus tridentatus* (Pacific lamprey); (B) *Lepomis macrochirus* (bluegill); (C) *Cyprinus carpio* (carp); (D) human hemoglobin A; (E) *Salmo gairdneri* (steelhead trout); (F) *S. gairdneri* (rainbow trout); (G) *Oncorhynchus nerka* (blueback salmon); (H) *Alosa sapidissma* (shad); (I) *Micropterus salmoides* (largemouthed bass); (J) *Salvelinus fontinalis* (brook trout); (K) *Oncorhynchus kisutch* (silver salmon; (L) *O. tshawytscha* (chinook salmon); (M) human hemoglobin A; (N) *Squalus acanthias* (dogfish shark); (O) *Ophiodon elongatus* (ling cod); and (P) *Ptychocheilus oregonensis* (Columbia squawfish).

fluid, after centrifugation, was stored at −10°C. Paper electrophoresis was performed in a Spinco model R apparatus at 6 ma for 6 hours; S and S 2043A paper with sodium barbital buffer (*p*H 8.8; ionic strength, 0.05) was used. Preliminary experiments had established that maximum separation occurred under these conditions. The electrophoregrams were scanned photometrically by means of a Photovolt densitometer, model 525, equipped with either a 420 or a 540 mμ filter. Moving-boundary electrophoresis of dialyzed hemoglobins was carried out at 10 ma in sodium barbital buffer (*p*H 8.6; ionic strength, 0.1) in a Perkin-Elmer model 38 apparatus operating at approximately 180 v.

In Fig. 1 the mobilities at *p*H 8.8 of hemoglobins (7) from 14 species of fish (8) are compared with that of human hemoglobin A, which served as a reference standard. Although many samples have been examined in this manner, no gross differences in mobilities have been detected in a given species with regard to sex, age, or race.

Percentage distribution of the hemoglobin, as determined by planimetry of the densitometer recordings, is given in Table 1 for those species possessing more than one hemoglobin component. In general, the largest component appears to have the highest mobility.

It is interesting to note that many fish hemoglobins are characterized by a low anodal mobility at *p*H 8.8 and are consequently shifted to the cathode side of the origin by the electroosmotic buffer flow. Mobility measurements made from moving-boundary electrophoresis patterns showed, for example, that at *p*H 8.6 the mobilities of the three hemoglobin components of rainbow trout were -2.39×10^{-5}, -1.24×10^{-5}, and -0.70×10^{-5} cm²/v sec. The low mobility at this *p*H and apparent high isoelectric point associated with many fish hemoglobin fractions may prove to be a characteristic unique among vertebrates.

Although the physiological significance of the occurrence of multiple hemoglobins in fishes is at present not known, subsequent investigation may establish a correlation of this heterogeneity with phylogeny.

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6. No major protein contaminant was found either in boundary patterns or in dyed paper strips.
7. Preliminary studies showed no difference in mobilities of fish carbonmonoxyhemoglobin and oxyhemoglobin; consequently, all of the work described in this report was carried out with oxyhemoglobin.
8. We are indebted, for specimens, to C. H. Elling and A. Henslip, Bonneville Experimental Laboratory, North Bonneville, Wash.

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Analysis of Gas in Biological Fluids by Gas Chromatography

Abstract. Combining a vacuum extraction method with gas chromatography makes possible accurate, reproducible determination of small quantities of permanent gases contained in biological fluids. One useful application is demonstrated by determining the oxygen tension in 1 ml of human plasma.

Chromatography as a technique for separation of closely related substances has been known for many years. However, the more recent use by James and Martin (*1*) of gas-liquid partition chromatography has resulted in a renewed interest in the technique and the development of modern instrumentation which makes possible rapid separation and accurate quantitative analysis of most of the biologically important permanent gases when they are in the gas phase. However, it has not previously been possible to analyze the permanent gases in terms of their concentration in biological solutions, for two reasons: (i) the columns used for separation of permanent gases are adsorption columns and lose their useful characteristics when they are wet, and (ii) attempts to rapidly volatilize gases in biological solutions have been unsuccessful because of the coagulation of protein and because of the oxidation and the resultant change in gas concentrations which occur with heat. These problems have been solved by combining a vacuum extraction method with the technique of gas chromatography.

The instrumentation and apparatus consist of a gas chromatograph (*2*), a 1-mv, 1-sec full-scale strip-chart recorder (*3*), and a Van Slyke volumetric extraction chamber (*4*), the waste arm of which is connected to the carrier-gas stream of the gas chromatograph proximal to the chromatograph column. Helium is used as the carrier gas.

An aliquot of the fluid is admitted to the Van Slyke reaction chamber via a between-line Van Slyke pipette and washed in with mercury. The upper three-way stopcock is closed, the lower