both soils, sulfate became unstable at a potential of about −150 mv; the more negative the potential below this value, the more complete was the reduction of sulfate to sulfide during the 3-week incubation. The curves relating sulfide concentration to redox potential in the two soils were almost identical. The *p*H of the two soils were about the same and changed from approximately 6.8 to 7.1 as the potential changed from +100 to −250 mv.

An interesting consequence of the reduction of sulfate to sulfide at such a low potential is that accumulation of H_2S is prevented in soils containing reducible iron, because of the precipitation of sulfide as insoluble FeS. Ferric iron is reduced to ferrous iron in the soil at a considerable higher redox potential than that at which sulfate is reduced (*5*); consequently ferrous iron is always present before sulfide is formed. Harter and McLean (*2*) recorded the production of 2000 ppm of sulfide, under waterlogged conditions, without the release of H_2S from the soil. In our study, all the sulfide was in the combined form; none was present as H_2S.

In addition to its dependence on redox potential, reduction of sulfate to sulfide in a soil or other media depends on *p*H also. Zobell (*6*) reported sulfate reduction occurring between *p*H 6.4 and *p*H 9.5 in marine bottom deposits and between *p*H 4.2 and *p*H 10.4 in poorly drained soils. Starkey and Wright (*7*) found the range of active sulfate reduction in the soil to be between *p*H 5.5 and *pH* 8.5, with the greatest accumulation occurring near *p*H 7.

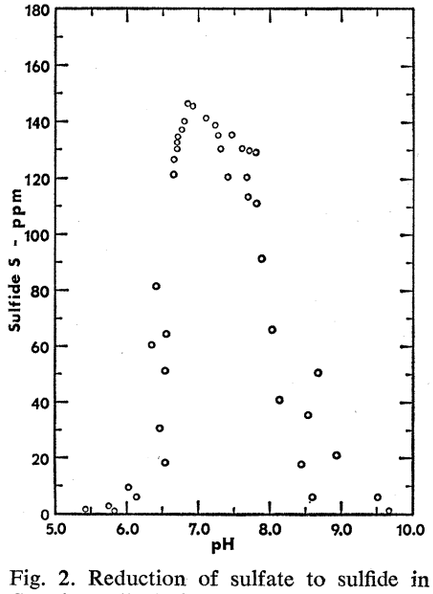

Fig. 2. Reduction of sulfate to sulfide in Crowley soil, during 28-day incubation, as affected by soil *p*H.

The effect of *p*H on reduction of sulfate to sulfide was studied with 50-g samples of a Crowley silt loam soil, initially of *p*H 5.8, which had been adjusted to a wide range of *p*H values with either HCl or NaOH. The acid-treated or alkali-treated soil, 50 ml of distilled water, 0.5 percent of finely chopped rice straw, and sulfate S at 100 ppm were placed in serum bottles and incubated for 28 days under an atmosphere of argon; the samples were then analyzed for total sulfide content. The redox potential of the highly reduced soil was approximately −250 mv. The *p*H values that we report are those measured at the end of the incubation period. The original soil contained approximately 40 ppm of sulfate sulfur.

A pronounced effect of *p*H on sulfate reduction was recorded in this experiment. The data of Fig. 2 show little reduction of sulfate to sulfide at *p*H outside the range 6.5 to 8.5. The sulfide-*p*H gradient on the acid side was especially steep, with a marked decrease in sulfide production between *p*H 6.7 and *p*H 6.2.

The restriction of sulfate reduction to the *p*H range 6.5 to 8.5 does not necessarily mean that soils with normal *p*H values, outside this range, do not support sulfate reducers. After waterlogging, the *p*H of both acid and alkaline soils tends to shift toward the neutral point as a result of chemical reactions, involving iron and manganese, that bring most waterlogged soils, regardless of original *p*H, into the *p*H range of sulfate reduction (*8*). In our study, sufficient acid or alkali was added to counteract the shift in *p*H that normally occurs.

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4 October 1967

Oxygen Consumption of Red and White Muscles from Tuna Fishes

Abstract. *Metabolic rates of unstimulated, minced preparations of red and white muscles from two species of Pacific tuna fishes* (Katsuwonus pelamis *and* Thunnus obesus) *were determined from respirometric measurements of oxygen consumption. Ratios of mean metabolic rates for red muscles to those of white muscles averaged 6.2 at five temperatures over the range of 5° to 35°C. Temperature coefficients* (Q_{10}'s) *for mean metabolic rates for both types of muscle were between 1.0 and 1.2 over the entire temperature range. Metabolic rates of tuna red muscles were equal to those of preparations of mixed red and white muscle from the white rat at 25° and 35°C, and were higher than the mammalian rates at lower temperatures.*

The large size of tuna fishes and their extreme sensitivity to capture and handling have made physiological studies on these fishes difficult. Only recently has adequate evidence been obtained for even such basic phenomena as the long-suspected physiological regulation of body temperature (endothermy) of several species (*1*). This paper contributes to the understanding of the metabolic basis for and the significance of this endothermy, also of the spectacularly high levels of continuous activity of these fishes. I have measured the oxygen consumption of fresh, minced, unstimulated samples of red and white muscles taken from two common species of Pacific tunas, the skipjack (*Katsuwonus pelamis*) and the bigeye tuna (*Thunnus obesus*). Similar data for mammalian (white rat, *Rattus rattus*) skeletal muscles containing both red and white fibers are included for comparison.

Adult skipjack (550 to 1200 g) and bigeye tuna (2000 to 3900 g) adapted to water at 25°C were killed by a blow on the head immediately after removal from the water. Within 2 to 3 minutes, 2- to 3-g samples of white muscle from the dorsal musculature and red muscle from the deep masses of that tissue were excised from about midway along the fishes' bodies [see both (*1*) and (*2*) for anatomical descriptions of the distributions of the muscle types]. The samples were transferred to ice-cold, covered petri dishes containing filter-paper disks moistened with Ringer solution. The tissues were

Table 1. Oxygen consumption by minced muscle preparations [cubic millimeters (STP) per gram of wet tissue per hour; mean ± standard error]. In parentheses are given the numbers of observations in each experiment; single numbers are measurements on single fish.

Temperature (°C)	Skipjack			Bigeye tuna			Rat
	White	Red	Ratio R/W	White	Red	Ratio R/W	
5	180 ± 30(5)	1100 ± 130(5)	6.2				390 ± 75(3)
15	200 ± 20(5)	1310 ± 160(5)	6.5	120,180	1000,1340		690 ± 30(4)
25	190 ± 20(5)	1500 ± 160(6)	7.8	260 ± 60(5)	1240 ± 60(5)	4.8	1240 ± 70(5)
30	260 ± 60(4)	1580 ± 180(3)	6.0	130,380	700,1520		
35	350 ± 130(5)	1540 ± 100(5)	4.4	170	600		1750 ± 70(4)

minced finely (never taking more than 1 minute) and immediately returned to the cold, covered petri dishes.

Samples (100 to 300 mg) of the minced tissue were weighed and transferred to respirometer vials containing 2 ml of Ringer solution (*3*) equilibrated at the temperature for the given experiment. Three to five samples of each muscle type were used for each fish studied. Oxygen consumption was measured with Scholander volumetric microrespirometers (*4*). Measurements were corrected to standard temperature and pressure (STP). Results reported are for the first 5 to 10 minutes after thermal equilibrium of the respirometers. Precision of replicate measurements was ± 10 percent of the mean for the group of samples. The period between excision of tissue from the fish and the beginning of measurement was almost always 20 to 30 minutes, rarely as long as 40 minutes.

Samples of thigh musculature from white rats were handled identically. These muscles, like virtually all mammalian muscles, contain both red and white fibers inextricably mixed together. White fibers generally predominate in the areas sampled.

Measurements of oxygen consumption were made over the temperature range from 5° to 35°C (Fig. 1 and Table 1). These limits were chosen because 5°C is as low a temperature as a tuna fish is ever likely to encounter in the tropical or subtropical Pacific (*5*); 35°C is no more than 2° to 3°C above the highest temperatures measured in the muscles of tunas (*1*).

Other observations made were: the freezing point depression (*6*) and chloride concentration of plasma (*7*), muscle water content (wet and dry weight), and soluble protein in muscle (*8*). Duplicate determinations were made for each fish used. Precision for all analyses was ± 5 percent of the mean for the duplicates, or better.

Data for the bigeye tuna are less complete than those for the skipjack, since fewer fish were available. However, the basic pattern appears to be the same in the two species, with some minor quantitative differences.

At all temperatures, the activity of red muscle from tunas is much higher than that of white muscle. In the skipjack the lower ratio of activities at 35°C is probably due to sampling errors, since an analysis of variance for the two groups of white-muscle measurements at 25° and 35°C showed no statistical significance for the apparent differences between the means of these groups. The absolute values of skipjack red-muscle activity at 25° and 35°C were not significantly different statistically (by analysis of variance) from the rates for rat muscles at the same temperatures.

Metabolic rates of both types of tuna muscle are much less sensitive to temperature changes than are those of mammalian muscles. Analysis of variance shows that there were no statistically significant differences between the mean metabolic rates measured for skipjack red muscles over the entire temperature range studied. There was clearly no variation in rate over the range of 25° to 35°C [temperature coefficient (Q_{10})=1.0]. These muscles would not, however, appear to be completely insensitive to temperature. Analysis of the data at 5°, 15°, and 25°C by the method of least squares regression shows that these points can be represented by a straight line with the equation $MR = 20.6T + 992$, where MR is the metabolic rate in cubic millimeters of O_2 (STP) per gram per hour; and T is the temperature in degrees Centigrade. The slope of this line is statistically significantly different from zero ($P = .05$, determined by t-test) and is equivalent to a Q_{10} of 1.2 between 10° and 20°C. The standard error of estimate for this equation is 318.

Analysis by the method of least squares regression for the measurements at 5°, 15°, and 25°C on rat muscle results in the equation $MR = 43.8T + 113$. The slope of this line is statistically significantly larger than the slope of the line for tuna muscle ($P = .001$, determined by t-test). This slope is equivalent to a Q_{10} of 1.8 between 10° and 20°C. The standard error of estimate for this latter equation is 126.

Tuna white muscles had metabolic rates in the same range as similarly determined rates measured at 25°C for white muscles of a variety of species of nonpelagic, inshore fishes from both cool California coastal water and warm Hawaiian waters (*9*). Unlike those for muscles of inshore fishes, the metabolic rates of the tuna white muscles were completely insensitive to temperature over the entire range studied ($Q_{10} = 1.0$). Analysis of variance shows that there were no statistically significant differences between the mean metabolic rates measured for skipjack white muscles over the entire temperature range studied. Analysis of the data at 25°, 30°, and 35°C by the method of least squares regression results in an equation the slope of which is not statistically significantly different from zero.

The osmotic and chloride concentrations of the plasma of both species (Table 2) are normal for marine teleosts at ordinary temperatures (*10*). There are small, but statistically significant ($P < .001$, determined by t-test), differences between the concentrations of water in red and white muscles in both species. The red muscles in the skipjack have an average of 2.7 percent less (in the bigeye tuna, 3.2 percent less) water than the white muscles do. The concentrations of soluble protein in the two tissues differ considerably, the average ratio for red to white muscle in the skipjack being 1.8, in the bigeye tuna, 2.3. Expressed

Table 2. Blood concentration and muscle composition in tuna fishes. The mean and the standard error are given. In parentheses are the numbers of observations in each experiment.

Quantity	Skipjack	Bigeye tuna
Plasma Δ (mOsmole/liter)*	380 ± 10(5)	350 ± 20(4)
Plasma Cl^- (meq/liter)	177 ± 3(5)	189 ± 17(4)
Muscle H_2O (g/kg of wet tissue) red	757 ± 9(5)	730 ± 10(4)
Muscle H_2O (g/kg of wet tissue) white	784 ± 10(5)	762 ± 5(4)
Muscle protein (g of N per kg of wet tissue) red	14.4 ± 0.8(5)	19.8 ± 1.2(4)
Muscle protein (g of N per kg of wet tissue) white	7.8 ± 0.8(5)	8.6 ± 0.7(4)

* Δ, Freezing point depression.

on a basis of tissue dry weights these two ratios are, respectively, 1.6 and 2.0. The concentration of soluble protein in bigeye tuna red muscle is statistically significantly greater ($P = .01$ by t-test) than that of skipjack red muscle. The concentrations of protein in the white muscles of the two species are the same.

The functional differences between the red and the white muscles of fishes have been studied and speculated upon many times by many authors. Bone (*11*) has carefully reviewed the literature and concludes, as Arloing and Lavocat first did in 1875 (cited by Bone), that the red muscles provide the sole motive power for swimming at slow and cruising speeds by all fishes that have been studied. The white muscles provide a power reserve used only for rapid movements. Bone's own electrophysiological and pharmacological studies of sharks fully support this position, as do electrophysiological studies on Hawaiian skipjack tunas (*12*). A variety of structural and metabolic differences are correlated with this functional difference (reviewed in *11*). The most important metabolic difference is that red muscle depends primarily on aerobic oxidative processes for energy, while muscle depends primarily on anaerobic processes.

The highest and best regulated intramuscular temperatures in bigeye and yellowfin (*Thunnus albacares*) tunas occur in and are centered around the deep masses of red muscles about midway along the bodies of these fishes (*1*). Carey and Teal (*1*) measured temperatures as high as 32°C in red muscle of bigeye tuna taken from 20°C water. Steep, declining, radial temperature gradients existed in white muscle and the more superficial red muscles surrounding the deeper masses of red muscle.

From my data and these other observations a reasonably coherent picture of the locomotory metabolic activities of tunas (at least for skipjack) can be drawn. The very high rate of oxidative metabolism in the red muscles provides the energy required for both the high cruising speeds of these fishes (*13*) and the maintenance of high temperatures in the muscles. The thermoregulatory abilities of the fishes assure that muscle temperatures remain high so that the energy supply for swimming will be maximum. The temperature independence of red muscle metabolism between 25° and 35°C assures that even rather large variations in absolute levels of thermoregulation will not seriously affect this maximum energy supply. This low Q_{10} for red-muscle metabolism between 5° and 25°C assures that fairly high rates of energy supply for normal swimming will continue even if the fishes encounter sufficiently low water temperatures (either at great depths or near the northern and southern limits of their ranges) that they are no longer able to maintain high temperatures in their deep red muscles.

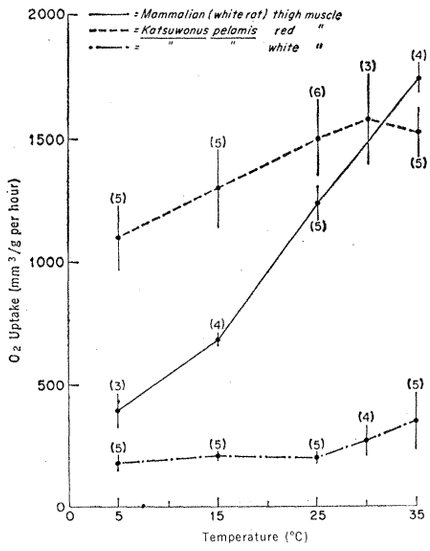

Fig. 1. Oxygen consumption (corrected to STP) of minced skeletal muscle samples from skipjack (*Katsuwonus pelamis*) and white rat (*Rattus rattus*) at various temperatures. Points indicate means for groups of measurements involving numbers of animals indicated. Vertical lines ± 1 S.E.

The complete temperature independence of the (largely nonthermoregulated) white muscles ensures constant availability of extra energy for sudden efforts (escape from predators, chasing prey) irrespective of ambient temperature. The primary dependence of the white muscles on anaerobic metabolism may, however, complicate this situation. I have no data on the temperature dependence of anaerobic metabolism in these muscles. The fact that yellowfin tuna are capable of bursts of rapid swimming at speeds up to at least 75 km/hr (*14*) demonstrates that substantial extra energy is available from the white muscles.

I know of only one other published set of observations of rates of oxygen consumption of red and white muscles from fishes. Wittenberger and Diaciuc (*15*) studied freshwater carp (*Cyprinus carpio*) muscles at 21°C. Their experimental procedures were somewhat different from mine, so their absolute values may not be exactly comparable. However, as would be expected for a relatively sluggish species, rates of oxygen consumption were low [red muscle: 280 mm³/g of tissue (wet weight) per hour; white muscle: 150 mm³/g of tissue (wet weight) per hour; both figures are means for all their observations on "normal" fish]. Rates for red muscle, under all circumstances studied, were very nearly twice those for white muscle. A particularly important observation was that rates measured on both types of muscles taken from fishes electrically stimulated to strong movements for 10 to 20 minutes averaged 40 to 50 percent above normal values. On this basis my data on unstimulated tuna muscles are probably minimum estimates only.

Tuna red muscles are exceptions to the general rule that tissues of the generally "cold blooded" (ectothermous) lower vertebrates possess much lower metabolic rates than do tissues of the generally "warm blooded" (endothermous) higher vertebrates. Indeed, skipjack red muscles are much higher in activity than the thigh muscles of rats adapted to room temperatures near 20°C, when the muscles of both are studied at relatively low temperatures. The red muscles of a variety of other, nonscombrid, pelagic species of fishes appear to be similar (*16*).

Mammalian muscles containing only red fibers may also be like the tuna red muscles. However, I know of no such mammalian muscles.

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3. The Ringer solution used contained: (i) 70 parts of solution I containing in final concentration 110 m*M* NaCl, 2 m*M* KCl, 1 m*M* $CaCl_2$, 2.5 m*M* $NaHCO_3$, and 0.1 m*M* Na_2HPO_4, (ii) 10 parts of buffer solution II containing 13 parts of 21 m*M* Na_2HPO_4 and one part of 160 m*M* KH_2PO_4; (iii) four solutions of organic substrates dissolved in solution I: five parts of glucose, 300 m*M*; four parts of sodium pyruvate, 160 m*M*; seven parts of sodium succinate, 100 m*M*; four parts of sodium-L-glutamate, 160 m*M*, all at a *p*H of the final solution of 7.0 to 7.2. This Ringer solution is a slight modification of that used with goldfish (*Carassius auratus*) tissues by D. R. Ekberg [*Biol. Bull.* **114**, 308 (1958)]. Extensive experiments with minced muscle preparations from several species of fishes, both freshwater and marine, have shown that no statistically significant differences in rates of oxygen consumption result, under our conditions, when the osmotic concentration, ratio of monovalent cations to divalent cations, absolute concentration of divalent cations, ratio of buffer salts, or concentrations of organic substrates are changed. Stability of the preparation and its survival is enhanced by increased concentrations of organic substrate and increased volumes of Ringer in the respirometer chambers.

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17. I thank J. C. Marr, J. J. Magnuson, M. D. Rayner, and the staff for cooperation and provision of facilities and tuna fishes at the Kewalo Basin Laboratory, Bureau of Commercial Fisheries, U.S. Fish and Wildlife Service, Honolulu, Hawaii; T. A. Pryor, J. E. Randall, and J. Ellis for laboratory facilities at the Oceanic Institute, Makapuu Point, Oahu; K. S. Norris for important logistic support; and N. Packard, B. Bartholomew, R. Sahara, and K. Yuen for technical assistance. Supported by the Zoology Fisheries program at the University of California, Los Angeles, and NSF grants GB-3584 and GB-5661.

3 November 1967

Venom of the Scorpion Vejovis spinigerus

Abstract. *The chemical composition of lyophilized venom from* Vejovis spinigerus *is reported. At least 13 distinct bands were obtained on disc electrophoresis; on Sephadex G-50, four major peaks were found. The lethal activity was associated with the second peak. The intravenous lethal dose, median (lethal for 50 percent of inoculated group), of the crude venom was 4.87 milligrams per kilogram of body weight. Neither the crude venom nor its fractions had a deleterious effect on neuromuscular transmission, reflex discharge, or antidromic inhibition. Crude venom evoked simultaneous changes in systemic arterial, venous, and cisternal pressures.*

The scorpion, *Vejovis spinigerus* (Wood), 1863, is found throughout Arizona, in southwestern New Mexico, in parts of Southern California, and in northern Mexico including Baja California (*1*). It is responsible for numerous stingings to humans, although such injuries usually produce little more than transient pain at the site of the sting, and on occasions some localized swelling, paresthesia, and tenderness. On rare occasions a few vesicles may develop around the wound. Systemic manifestations are very rare.

We now report on certain chemical and physiopharmacological properties of the venom of this species.

Approximately 450 *Vejovis spinigerus* were milked (*2*) over a 24-month period. The scorpions were collected from Portal, Tucson, and Wickenburg, Arizona, and from Imperial and Riverside counties in Southern California. An additional lot was supplied by H. L. Stahnke of Tempe, Arizona. All samples were lyophilized immediately after collection and were stored in the dark at 5°C until used. The individual lots were assayed separately and in combinations.

Samples from a pooled lot of 150 mg of venom were assayed for total protein (*3*) [additional determinations were made on several smaller lots (*4*)]; nonprotein nitrogen (NPN) and total nitrogen (*5*); monosaccharides and disaccharides (*6*); sodium (*7*, p. 1140), potassium (*7*, p. 1140), and calcium (*7*, p. 1133). The proteolytic (*8*, p. 167), cholinesterase (*8*, p. 73), amylase (*8*, p. 118), phosphodiesterase (*9*), and L-amino acid oxidase (*10*) activities were also determined.

A sample of venom and serotonin were chromatographed simultaneously at 20°C on Whatman No. 31 paper in a system composed of *n*-butanol, glacial acetic acid, and water (12 : 3 : 5 by volume). The venom (10 mg/ml) was dissolved in deionized water and applied to the paper in spots, the amount of venom increasing by 20-μg increments from 20 to 120 μg. The serotonin was applied in 3*N* acetic acid in spots of 1, 2, 4, and 8 μg. After chromatography, the paper was dried in air, examined under ultraviolet light, dipped in Ehrlich reagent [10 percent *p*-dimethylaminobenzaldehyde in a mixture of 12*N* HCl and acetone (1 : 4 by volume)], and redried; and the developed spots were noted.

The lyophilized venoms of scorpions from Portal (two samples, 27.5 mg and 59.7 mg), Tucson (one sample, 42.6 mg), and southeastern California (one sample, 36.8 mg) were chromatographed separately on Sephadex G-50. The gel was prepared in a 0.1 percent NaCl solution, washed repeatedly, and then added as a slurry to a glass column (2.54 by 50 cm) to a height of 25.5 cm, and equilibrated at 5°C; the venom was then added in 3 ml of solvent. Chromatography was carried out at 5°C, with 0.1 percent NaCl as the eluent; 3-ml samples were collected on an automatic fraction collector.

All fractions were analyzed for absorbance at 260 and 280 mμ in a Beckman DB spectrophotometer. Portions were taken for protein determination (*3*), with bovine serum albumin (Armour) as a standard protein. Both pooled and individual samples of the

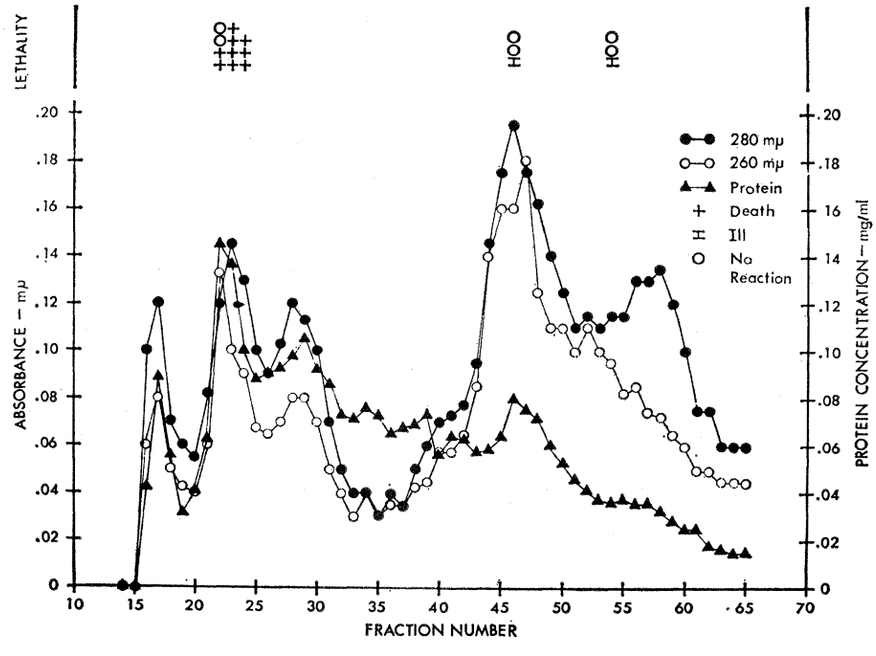

Fig. 1. Elution pattern of *Vejovis spinigerus* venom by gel filtration on Sephadex G-50.