Reports

Observation of Bioluminescence in the Atlantic Fish (Porichthys porosissimum)

The only North American shallow-water fishes which produce light belong to the genus *Porichthys* (family Batrachoididae). The fact that the Pacific species, *P. notatus,* will produce light is well known.

Greene (*1*) observed that *P. notatus,* when it was placed in an aquarium made alkaline with ammonia water, "exhibited a brilliant white light for about 20 minutes." Dean (*2*) stated that there were "few actual observations of living fishes." Greene and Greene (*3*) reported that *P. notatus* had a latent period of 8 to 10 seconds following stimulation and that the light lasted about 20 seconds. They also reported that adrenalin, when it was injected into the fish, activated the light-forming organs. Prosser *et al.* (*4*) stated that intermittent light of this type "is an intracellular phenomenon," but the exact mechanism is still not known. Hubbs and Schultz (*5*) gave a bibliography on this group of fishes. Harvey (*6*) states that little work additional to that of Greene and Greene has been done "chiefly due to lack of material."

There seem to be no reports of observations on light production in the Atlantic midshipman, *P. porosissimum.* Jordan and Evermann (*7*) reported that this species had been seen by fishermen "shining at night" but that they had been unable to verify the observation. The following observations were made at the Gulf Coast Research Laboratory on the night of 22 June 1957. The fish came from Mississippi Sound.

At 9 P.M., after the lights had been off for about 10 minutes, a faint glow appeared to move toward the water surface. Before the lights were turned off, one of the two midshipmen present had been cruising up and down and around the tank. There were several more short emissions of light lasting 5 to 10 seconds, but interrupted by variously longer periods of dark. One show of light was so intense that the rows of photophores on the ventral side, the row above the middle of the side, and the several rows on head and chest stood out sharply as bright lights for 15 or 20 seconds. After one or two faint emissions, nothing more could be seen even though the fish was picked up in the hand and released. The following night the aquarium was again observed for more than an hour; the fish was stimulated with the hand, but no light was seen. Except for the removal of one *Gobionellus hastatus* from the tank, conditions were essentially the same as they had been on the previous night. There was about 1 inch of white sand in the aquarium, which was 10 by 18 inches by 12 inches high. The aquarium contained two *Porichthys,* one *Hippocampus hudsonius,* and two shrimp (*Penaeus*). The salinity was 16.9 parts per thousand.

On 25 June a midshipman was placed in a liter of sea water to which about 5 ml of ammonia water had been added. The same photophores that showed brightly on the one spontaneous emission became bright to about the same intensity. The fish became very active during this time and was killed by the ammonia. After respiratory and other movements ceased, the light slowly diminished.

On 29 July tests were made to see whether other chemicals would stimulate light production. These chemicals were added slowly, a few milliliters at a time, to a gallon jar containing the fish in 1 liter of sea water which had a salinity of 26.2 parts per thousand and *p*H of 7.2.

Sodium hydroxide and ethyl alcohol were as effective as ammonia, but acetic acid failed to stimulate any visible light even though it was added until the fish had died. Light did not appear in the alcohol test until 50 ml of 95-percent alcohol had been added and the fish had lost almost all ability to move. The light continued to increase as alcohol up to 112 ml was added. In the NaOH test, maximum luminescence was reached after 73 ml of 1N solution of the alkali had been added and the *p*H had become 11.2.

Porichthys porosissimum is distributed from South Carolina to Uruguay and is found in shallow waters. It is not a particularly abundant fish, nor is it extremely rare. The only estimates of its general abundance were given by Gunter (*8*), who collected eight specimens from April to November 1941 among 78,000 fishes which were taken mostly by trawl from Texas bays. He found the species at salinities ranging from 10.3 to 35.8 parts per thousand. In Mississippi Sound the fish seems to be more abundant, and one to ten are taken in an hour's trawling. The fish is not at all delicate and exhibits the well-known toughness of the batrachoidids. It is a readily available source for workers studying the physiology of bioluminescence.

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5 August 1957

Tumor-Inhibiting Effects Derived from an Active Principle of Garlic (Allium sativum)

Extracts of garlic (*Allium sativum*) have been shown to contain a powerful bactericidal agent, allylthiosulfinic allyl ester (allicin) (*1, 2*). This compound is formed by the interaction of an enzyme and substrate present in garlic bulbs (*3*). The enzyme, alliinase, is liberated when the garlic bulb is crushed, and it acts on the substrate, S-allyl L-cysteine sulfoxide (alliin) as follows (*4*):

$$2\,R\text{—SO—CH}_2\text{—CH(NH}_2\text{)—COOH} \quad (\text{alliin})$$

$$\xrightarrow[\text{alliinase}]{+\,H_2O} R\text{—SO—S—}R + \quad (\text{allicin})$$

$$2\text{CH}_3\text{—CO—COOH} + 2\text{NH}_3$$

where

$$R \text{ is } \text{—CH}_2\text{—CH=CH}_2.$$

Wills (*5*) has shown that this reaction product inhibits many sulfhydryl (—SH) enzymes but that it does not affect many

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other enzymes. There is a close relationship between the structure required for bactericidal action and that required for inactivation of —SH enzymes. Thus compounds containing the —SO—S— grouping are effective in both enzyme inhibition and bactericidal activity, whereas compounds containing the —SO—, —S—S— or —S— linkages are ineffective. The —SH inactivation obtained with alkylthiosulfinic alkyl ester may be the result of a strong combination of this compound with cysteine or may be due to oxidation of —SH to —S—S— by the labile oxygen (*2, 5*).

Although most of the studies of allicin have centered upon its bactericidal action, its reactivity with —SH groups suggests that it might also have an inhibitory effect on malignant cells. An increase in —SH compounds prior to cell division has been demonstrated in a wide variety of tissues, plants, and organisms (*6*). Reduced —SH compounds stimulate cell growth and division, whereas substances which oxidize —SH to —S—S— inhibit cell division. Similar inhibition of cell division may be obtained with thiol poisons such as alkylating agents and heavy metals. Abnormalities of —SH metabolism may be implicated in malignant cell growth since a high —SH content has been demonstrated in some tumor cells (*7*). Accordingly, the effect of an alkylthiosulfinic alkyl ester on the growth of malignant tumors in animals was studied.

Since the allyl ester of allylthiosulfinic acid which is ordinarily formed in garlic extracts is unstable, the more stable diethyl analog was used in these studies. The ethylthiosulfinic ethyl ester (C_2H_5—SO—S—C_2H_5, ETHIOS) was prepared by incubating S-ethyl L-cysteine sulfoxide with alliinase. Alliinase was prepared from crushed garlic bulbs according to the modification of Wills (*5*). S-Ethyl L-cysteine sulfoxide was prepared from S-ethyl L-cysteine by oxidation with 30 percent hydrogen peroxide and crystallization from aqueous acetone. The amount of thiosulfinic ester formed was estimated by determining the amount of ammonia released from the reaction mixture after incubation with alkali in a Conway vessel.

Studies were made of the effect of these substances on the growth of sarcoma 180 ascites tumor in CFW Swiss mice (18 to 22 g). Gain in weight and time of survival were taken as an index of the number of ascites tumors formed and of the degree of malignancy produced. The mice were inoculated with a dilute suspension of tumor cells freshly drawn from donor mice. Each inoculum contained approximately 5 million tumor cells and was incubated with either normal saline or an equivalent volume of the test substance in solution for 10 to 15 minutes prior to intraperitoneal inoculation. Incubation with saline prior to inoculation was uniformly followed by successful transplantation in each of 75 control mice, as is shown by rapid weight gain and death within 16 days (Fig. 1 and Table 1).

Table 1. Effect of garlic enzyme (alliinase) and substrate (S-ethyl L-cysteine sulfoxide) on the development of sarcoma 180 ascites tumor in mice. The synthetic reaction product was ethyl thiosulfinic ethyl ester (C_2H_5—SO—S—C_2H_5; ETHIOS).

Inoculum	No. of animals	Percentage developing tumors	Survival (day)
Tumor + saline	75	100	< 16
Tumor + substrate	25	100	< 16
Tumor + enzyme	25	100	< 16
Tumor + (substrate + enzyme)	25	0	> 180
Tumor + synthetic reaction product	50	0	> 120

Similar results were obtained when the inoculum was preincubated with either the enzyme (alliinase) or the substrate (S-ethyl L-cysteine sulfoxide). However, when the tumor cells were preincubated with equivalent amounts of a solution in which the enzyme and substrate had been allowed to react, no growth of the ascites tumor was demonstrable, and there was no mortality in animals that were observed for a period of 6 months. Approximately 1.0 μmole of the enzymatically prepared ethylthiosulfinic ethyl ester was present in each inoculum. Heating the enzyme before allowing it to react with the substrate resulted in complete failure to inhibit formation of ascites tumors, the gain in weight and the mortality being identical with those obtained in the control animals.

Preincubation of tumor cells with the ethylthiosulfinic ethyl ester synthetically prepared by oxidation of diethyl disulfide with perbenzoic acid (*8*) was also effective in preventing tumor growth. Successful inhibition was obtained with 0.1 μmole of the synthetically prepared ester per inoculum. Iodoacetate, which also inhibits some thiols, did not prevent formation of ascites tumors or death when preincubation was carried out with 1 μmole per inoculum.

Intravenous injections of the ethylthiosulfinic ethyl ester into mice previously inoculated with sarcoma 180 ascites tumor cells delayed the onset of malignant ascites tumors and in some instances completely prevented their formation and the death of the mice. Five micromoles of the ester were injected intravenously into mice daily for 7 days, the first injection being given 24 hours after intraperitoneal inoculation with sarcoma 180 ascites tumor cells. When the inoculum was 5 million ascites cells per animal, no ascites tumors developed during this period of time, but malignant ascites tumors developed rapidly after intravenous administration of the ester was discontinued. When the tumor inoculum was decreased to 1 million cells per animal, 40 percent of the animals failed to develop ascites tumors even when intravenous injection of the ester was discontinued.

The effect of the ethylthiosulfinic ethyl ester on incorporation of a radioactive sulphur (S^{35}) amino acid by leukemic leukocytes *in vitro* was also studied. Leukemic leukocytes were incubated with the thiosulfinic ester for 30 minutes; the cells were then centrifuged and washed, and S^{35} L-cysteine was added. Following incubation, the uptake of S^{35} was determined by methods previously described (*9*). Addition of increasing amounts of either the enzymatically formed or synthetic thiosulfinic ester resulted in a progressive decrease in the uptake of S^{35}. With 2.5 μmole of the ester per milliliter of whole blood, the uptake of S^{35} was reduced to 10 percent of the control values.

It is evident that ethylthiosulfinic ethyl ester may have tumor-inhibiting effects when malignant cells are placed directly in contact with this compound prior to inoculation. The inhibitory effect of the thiosulfinic ester on tumor growth when not placed directly in contact with this compound is suggestive but not conclu-

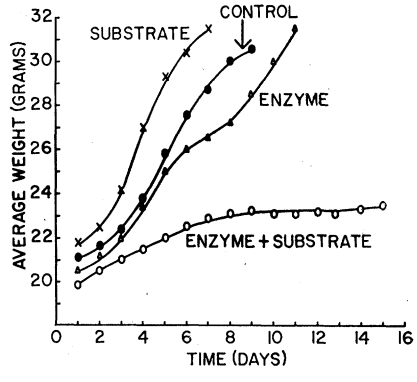

Fig. 1. Effect of garlic enzyme and substrate on growth of sarcoma 180 ascites tumor in mice. Preincubation of the inoculum with saline (control) results in rapid growth and death of all animals within 16 days. Preincubation with the enzyme (alliinase) or substrate (S-ethyl L-cysteine sulfoxide) results in a similar rapid growth of the tumor and death of all the animals within 16 days. When the enzyme was allowed to react with the substrate, and the inoculum was preincubated with the reaction mixture, no tumor growth occurred, and the animals remained alive during a 6-month observation period.

sive. Both the bactericidal and tumor inhibitory effects appear to be related to the presence of the —SO—S— linkage and may be the result of —SH inactivation by direct combination or by oxidation of —SH to —S—S—. The decreased uptake of S^{35} L-cysteine by leukemic leukocytes may also be related to —SH inactivation. These effects on malignant cells by an agent which inactivates —SH groups are further suggestive of the importance of —SH metabolism in neoplasia (*10*).

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3 September 1957

Biquartimin Criterion for Rotation to Oblique Simple Structure in Factor Analysis

The last 5 years have seen much effort on the part of workers in the field of factor analysis to develop a completely analytical method for rotating axes to what Thurstone (*1*) called "simple structure." Such a method would supplant the largely subjective, graphical methods which have been in wide use for at least 20 years. In 1953 I published (*2*) a method for the general case of oblique axes, but the results were not satisfactorily close to those achieved by the best graphical methods. At about the same time Saunders (*3*), Neuhaus and Wrigley (*4*), and Ferguson (*5*) independently proposed what Neuhaus and Wrigley called the "quartimax" method, which yielded an approximation to simple structure under the restriction of orthogonality. This method is mathematically equivalent to my method, under the stated restriction. Kaiser (*6*) showed that part of my solution can be achieved by the use of a characteristic equation; he also presented (*7*) a further criterion for the orthogonal case, called the "varimax" criterion since it depends on maximizing the variance of squared factor loadings. Pinska and Saunders (*8*) suggested a variant of their criterion for the oblique case, and Kaiser (*9*) generalized his varimax method for the oblique case.

This report (*10*) presents the "biquartimin" criterion for simple structure in the oblique case. When applied to several "school problems" such as Thurstone's box problem (*1*, p. 229), it yields results which appear to be closer to graphical solutions than those yielded by other analytical approaches. The complete evaluation of this and other methods awaits the development of parallel high-speed computational systems and their application to a wide variety of data.

To introduce the biquartimin criterion, it may help to refer to my original method (*2*) as the quartimin, since it depended upon the minimization of the sum of the cross-products of squared factor loadings and thus involved terms of the fourth degree. Let the $n \times m$ matrix of the initial factor loadings be denoted F, where n is the number of variables and m is the number of factors. Then, the quartimin method finds a transformation matrix Λ such that it will be true of the elements v_{jp} of the resulting matrix $V = F\Lambda$ that

$$\sum_{p<q}^{m} \sum_{j=1}^{n} v^2_{jp} v^2_{jq} = \text{a minimum},$$

where $j = 1,2, \ldots n$, and $p, q = A,B, \ldots m$. The rationale offered for the quartimin criterion depended on the fact that simple structure requires a maximum number of zero or near-zero entries in V.

Kaiser's (*9*) generalization of his varimax criterion to the oblique case might be called the *covarimin* criterion, since it requires that the sum of the covariances of squared factor loadings be a minimum; that is, that

$$\frac{1}{n} \sum_{p<q}^{m} \sum_{j=1}^{n} v_{jp} v_{jq} = \text{a minimum},$$

where

$$v_{jp} = (v^2_{jp} - \overline{v^2_{jp}})$$

The covarimin criterion is closely related to the quartimin criterion but corrects for the mean value of the squared factor loadings. Thus, the latter utilizes the deviations of squared factor loadings from zero while the former utilizes deviations from their mean value.

Experimentation with the quartimin and covarimin criteria as applied to several sets of real or hypothetical data revealed that the presence of factorially complex variables created almost precisely opposite disturbances, the quartimin axes being too highly correlated and the covarimin axes being too much separated. The centroids of corresponding quartimin and covarimin transformation vectors proved to be very close approximations to the results of graphical solutions, but this type of solution (although otherwise acceptable) was rejected because it would entail more than twice the normal amount of computation and the possibility of difficulty in identifying corresponding vectors.

Table 1. Data for Thurstone's "box problem" (*1*, p. 229): transformation matrix (Λ) obtained by the analytical biquartimin method as compared with that obtained by Thurstone by graphical methods.

	X	Y	Z
	Biquartimin criterion		
I	.450	.434	.473
II	−.862	.237	.597
III	.234	−.869	.648
	Thurstone's solution		
I	.483	.466	.479
II	−.834	.254	.560
III	.267	−.847	.675

The biquartimin criterion combines the advantages of the quartimin and covarimin methods by requiring that the expression

$$\sum_{p<q}^{m} \left[\sum_{j=1}^{n} v^2_{jp} v^2_{jq} + \sum_{j=1}^{n} v_{jp} v_{jq} \right]$$

be a minimum. It doubly satisfies the requirement of parsimony (*5*) in that the sum of cross-products of squared factor loadings must be minimized along with the sum of cross-products of deviations of squared factor loadings from their mean values.

In one of several possible computational schemes, the biquartimin criterion can be expressed as the sum of the off-diagonal elements of a symmetric matrix composed of quadratic forms—that is,

$$\sum_{p<q}^{m} \lambda_p H_q \lambda'_p = \text{a minimum},$$

where λ_p is a transformation vector of Λ and

$$H_q = 2n \sum_{j=1}^{n} (\lambda_q F'_j F_j \lambda'_q) F'_j F_j - (\lambda_q F' F \lambda'_q) F' F,$$

where F_j is the jth row of F. The solution for the minimum value must be made iteratively. For any one stage of the iterations, designate the vector to be solved for as x and any one of the remaining vectors as r, then determine

$$\lambda_x (\Sigma H_r) \lambda'_x = \text{a minimum}$$

by determining λ_x as the latent vector corresponding to the smallest latent root of the symmetric matrix (ΣH_r). (In