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"MSX" - THOUGHTS ON LIFE CYCLE

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Virtually all our knowledge of "MSX" to date is based on observations of "MSX" in oysters or on patterns of oyster mortality in "MSX" areas. In attempting to get at an "MSX" life cycle it seems useful to think in terms of what we know about times between exposure to "MSX" and infection, and from infection to death or recovery.

Quite a body of information has been built up over a 7-year period on times between exposure of susceptible stocks and death associated with "MSX", though we have relatively little information to break this down into exposure period before infection and incubation period from infection to death. One import in 1960 (VaJE) indicates a maximum time of 3 weeks in July between infection and death. A second 1960 group (SAC) had 5 weeks between 1st infection on 6 September and death. The shortest times to date between first exposure and death were also approximately 5 weeks in mid-summer (LC and VaJF), June 26 and 29 to August 4 and 7 respectively.

The accompanying tabulation of dates of import and first definite "MSX" kills provides a basis for estimates of effective infection periods in lower Delaware Bay.

In the summer of 1958 all tray stocks were Delaware Bay natives, brought to the Cape Shore late in June and all showed virtually simultaneous "MSX" kills in early September.

In 1959 the first definite "MSX" kills were in mid-August when all important stocks from GG (Navesink) in October 1958 to James River and Seasides brought in mid-June 1959 died simultaneously. A Connecticut stock imported on July 5 had its first "MSX" kill in mid-September. This indicates little or no important "MSX" infection between mid-October 1958 and mid-June 1959. It would appear that infection of the several stocks occurred between mid-June and 5 July and, of course, continued beyond this date to infect the Connecticut stock also.

In 1960 the infection pattern is quite different. Judging from the differences in times of 1st definite "MSX" deaths, in general, successive importations were infected in turn. An exception to this is the simultaneous kills in mid-May and early June imports, indicating that these May imports were not infected until after June first. It appears that in 1960 the "MSX" infection period extended almost continuously from mid-April at least through August. The continued build-up of infection level through the fall in the closely monitored SAC group and the subsequent history of the VaJH group also suggests continuing infections into November.

In 1961, 1962 and 1963 the time schedule was completely shifted. No oysters died with "MSX" in the same year in which imported. First "MSX" deaths occurred in the following summer and at low

levels compared with the years 1958, 1959 and 1960. Note however the mid-summer (SAM, VaJN, NF) and fall (MeB) imports. The 3 July imports showed first "MSX" kill in July, one year later and only 2 to 3 weeks ahead of spring of '64 imports! The MeB group had first "MSX" deaths in concert with most of the 1964 imports.

The 1964 imports coupled with the late 1963 imports indicate that no significant infection occurred between late September 1963 and early June 1964! Imports from 20 September 1963 to 3 June 1964 all had first "MSX" kills together after the 6th of August. The N avesink stock imported in early August had first definite "MSX" in November. One guesses that heavy infection of all stocks occurred in June and that the onset of death in the July 1963 imports was hastened by residual light infections that had occurred between mid-July and mid-September 1963.

1959
1958 and 1964 are similar in that a major infection period probably occurred in late June and not earlier in those years. All tray stocks in 1958 could also have been infected at this time, although there are no observations to limit infection to this period. The LC and VaJF imports in 1960 also indicate late June to early July infection, but in this year the infection period (s) apparently began earlier (prior to May 18).

The completely different time schedule existing in 1961, 1962, and 1963 we believe indicates a reduction in infective particle concentration in the lower Bay for those years. This bears on the question of effective size of inoculum. We have since 1958 sought an answer to the question: "Will one or a few infective "MSX" particles produce the massive general infections we find in "MSX" gapers or is a massive inoculum required?" We now believe the weight of evidence is that large doses of infective particles are required. Corollary to this would be a limited reproductive capacity of "MSX" in the oyster. Thus, even with a highly susceptible host stock, light "MSX" bombardment would require a long exposure time, inversely correlated with concentration of infective particles, to provide lethal inoculum. Depending on host-parasite interactions, "MSX" level could build up through accumulation over a period of time or a subpatent infection could persist indefinitely. Oysters under stress with low-level infection might be expected to die in shorter time if subsequently exposed to higher levels of infection.

This hypothesis of a "limited infection" would provide a reasonable basis for failures to date to infect oysters experimentally with tissues of infected oysters. Experimental inoculation with "initial gill lesions" might succeed where all other attempts have failed.

Certain of our tray studies in which susceptible stocks have been mixed with "MSX" infected oysters, indicate pretty conclusively that proximity to infected oysters does not increase intensity or the time schedule of infection. Our observations on initial lesions in gill, palp and suprabranchial chambers indicate the the infective

particles are not carried directly from infected to uninfected oysters.

Assuming that the oyster provides for a necessary part of the "MSX" life cycle, demonstrable periods of loss of "MSX" from oysters might provide valuable clues on life cycle stages. The consistently lowered"MSX" incidences in early spring without corresponding mortalities is suggestive; but the frequent return of infections to the same level within one or two months also indicates the probability that "MSX" was present throughout and was not recognized. On the other hand, the oyster may be a "dead end" for "MSX".

Observations on Blood of "MSX" Infected Oysters

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During the period June-December 1964, a new diagnostic technique for "MSX" based on preparation and study of oyster blood has been developed. Compared with histological section the blood technique has proved more sensitive in determining "MSX" infections, except in cases of localized lesions, as in the initial invasion of gill epithelium.

The blood studies show clearly that most of the "MSX" plasmodia are not in leucocytes. Since earlier tissue studies have shown that "MSX" is not generally in tissue cells it is suggested that "MSX" is not primarily an intracellular parasite but moves freely within the blood spaces.

Comparing blood cell types from oyster to oyster, "MSX" infection is associated with drastic shifts in numbers and ratios of various morphological types.

Histochemical Observations on "MSX"

John L. Myhre

A consistent pattern of Feulgen positive structures is found in "MSX." Development of the plasmodia is associated with a regular series of changes in shape and intensity of the Feulgen-positive structures. As shown by a Giemsa stain, the Feulgen-positive structures also correlate with a very basic substance which may be a protamine.

Recent Incidence of "MSX" in Delaware Bay

Eric Rifkin

Long-term studies of "MSX" incidence on Delaware Bay oyster grounds dating back to 1958 have been continued during 1964. A comparison of "MSX" incidence and non-drill mortality for three plantings in a high-salinity area is presented: Mortalities for a 1961 planting of Delaware Bay seed remained low though "MSX" incidence was as high as 75% in late summer, and, after an early fall low, rose sharly in late fall to the 50% level. By August, infection levels in a 1964 planting of James River stock (Horsehead Bar planted June 1) had reached 30% and in spite of cumulative mortalities approximating 35% by December, "MSX" incidence rose to 75%. "MSX" incidence data on the third planting, a 1964 planting of Delaware Bay seed, although not as complete, show comparable incidence values with the Virginia stock but the mortalities to date are about one-third as great.

Six other grounds sampled in late September showed "MSX" incidence levels of 0 to 20%. The sharp increases in "MSX" incidence on the three grounds discussed here followed this early fall low.

Mortalities and their Causes in Experimental Oyster Populations on the Cape Shore of Delaware Bay for 1964

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Monitoring of oyster populations on the Cape Shore continued through 1964 for the seventh consecutive year. Beginning in mid-summer there was a significant change in the mortality pattern as compared with similar periods from 1961-1963.

Spring imports of James River and Navesink River susceptibles had cumulative mortalities of 67% and 82% respectively from August to November with monthly rates between 20-40%. This compares closely with levels attained in similar stocks in 1959 and 1960. Initial mortalities occurred first in the 1963 James River imports late in July and this group reached a 77% cumulative level. Old tray stocks (imports prior to 1961) maintained relatively low levels throughout the period, their maxima being below 5% per month.

A large portion of this mortality in new stocks appears to be associated with "MSX." Newly imported susceptible stocks had gaper incidences between 80 and 100% for the July-November period. Old tray stocks never exceeded a 50% "MSX" level in gapers and much of their mortality can be attributed to Dermocystidium. This pattern is essentially the same in old tray stocks for similar periods from 1961-63. The "MSX" levels in gapers of newly-introduced susceptibles were considerably higher than those for similar stocks from 1961-63. Dermocystidium remained at a low level in these new imports and probably was a minor cause of mortality.

There has apparently been a return to the epizootic conditions observed from 1958-60 though the cause for this shift remains obscure.

THE MSX EPIZOOTIC ON MARUMSCO BAR, POCOMOKE SOUND, MD.

C. AUSTIN FARLEY

SUMMARY: Comparison of MSX epizootics within a marginal to submarginal salinity range has been provided by analysis of data collected during four years of sampling of the same natural population of oysters. MSX incidence increased abruptly in September of 1963 and has continued at a high level up to the present time. This increase coincides with a period of dry weather in the last two years which caused higher summer and fall salinities. Random samples of 50 oysters have been collected since September of 1963 on a biweekly basis. Studies of these samples indicate at least five types of infection as given in the following proposed classification:

A. Initial Infection --infections only in gill or palp tissues.

B. Intermediate Infection --infections in gills, gonad tubules, gut wall and surrounding tissue, and diverticula.

C. Advanced Infection --all tissues infected, heavy host response present, gametes destroyed, recessive growth evident, no food organisms in gut.

D. Terminal Infection --very heavy infections, host response pienotic or absent.

E. Recovery --plasmodia concentrated near or in mantle epithelium or in fibrocytic pustules. Shell pustules sometimes evident. Return of gonadal development and feeding response.

MSX-implicated mortalities appear to occur in June, September-October, and January through April. New infections were found most commonly in June and August-September but appeared to occur whenever oysters were filtering. Advanced infections were most common from September through December. Recovery was most noticeable from January through April and July through October.

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Agglutination of Erythrocytes by Oyster Blood

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Normal cell-free oyster blood agglutinates erythrocytes of several vertebrate species. The degree of reactivity of the agglutinating substance varies with the type of erythrocyte tested and the previous history of the donor oyster. The active material is heat sensitive, non-dialyzable and is adsorbed by erythrocytes. Chemical tests indicate that this substance is protein.

Published reports of hemagglutinating substances of molluscan origin indicate that they are not gamma globulins and, hence, are not classical antibodies. Whether or not this humoral factor is important in resistance to infectious agents is not known, but it can be used as an experimental tool to determine the ability of oysters to respond to antigenic stimuli.

Studies on Oyster Serum

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Delaware Bay oysters were bled either from the heart or the muscle sinus. The blood was pooled, freed of leucocytes, and analysed for protein and amino acids.

Protein levels were determined by Kjeldahl analysis. Oyster serum protein (OSP) was precipitated with 10% trichloroacetic acid at 40°C. Filtered TCA supernatant and diluted whole serum were analysed for nitrogen; the difference between these values represented the protein-N content of the serum pool. A "Spinco" amino acid analyser indicated the nitrogen content of OSP to be 16.862%. Thus, the protein levels of the serum pools were calculated by applying the factor 5.93 (reciprocal of 16.862%) to the protein-N values. The mean serum protein concentration for 158 oysters was 17.67 mg/ml.

Two uncommon amino compounds were found free in oyster serum in the highest quantities: taurine (0.226 uM/ml) and beta-alanine (0.120 uM/ml). Reports in the literature suggest that the role of free taurine in marine invertebrates may be osmoregulatory (Simpson, et. al., Biol. Bull. 117, 1959) or as a phosphagen in the form taurocyamine (Chem. Abst. 58, 1963, 772a). Its role in the oyster has not been investigated in this laboratory. Most unexpected was the appearance of taurine at a level of 0.025 mM/g on the chromatogram of an acid hydrolysate of OSP. To our knowledge, there is but one published account of taurine as a protein substituent (Chem. Abst. 51, 1957, 8876a), where it is reported in the tuberculin protein of some mycobacteria. OSP is rich in histidine (0.510 mM/g), aspartic acid (0.320 mM/g), and glycine (0.220 mM/g); also present are the aromatic amino acids, phenylalanine and tyrosine, responsible for the marked UV absorption of OSP noticed in this laboratory. Although tryptophan is destroyed on acid hydrolysis, its presence was suggested by humin in the hydrolysates.

Ultracentrifugation (Spinco Model-E, An-D rotor) of serum dialysed against phosphate buffer (pH 6.6, 0.02M) revealed one rapidly sedimenting component present in high concentration, another slower component of considerably lower concentration, and a possible third component of intermediate velocity. The sedimentation constant (s) at 20 °C and 44,770 rpm of the major component was 31.40 S (Svedbergs); for the minor component, 19.55 S. From these high s values, it is apparent that OSP consists of some very large molecules indeed, although exact molecular weights cannot be calculated without knowing diffusion coefficients. An attempt to ascertain the diffusion coefs. of OSP by the serological method of Allison and Humphrey (Immunology, 3, 1960) is underway in this laboratory.

Once oyster serum has been better characterized, it is hoped that some of its properties will provide a diagnostic index to pathological changes in the animal.

A Report on Studies of MSX

by
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Preparations have been in process for the development of a fluorescent anti-serum, in ponies, specific for MSX. When viewed under the ultra-violet microscope, a positive reaction causes the MSX to fluoresce against a dark background of oyster tissue. It would be possible to use this technique as a quick diagnosis for the presence of MSX and to observe stages in the life cycle of MSX previously unrecognized as being MSX.

The first of our procedures was to develop a pony anti-oyster serum that was later to serve to inhibit antibody production to oyster tissue in a second pony used to produce an anti MSX serum.

Dehydrated, powdered oyster tissue, from New England, a region of the U.S. thought to be free of MSX served as antigenic material.

Photographic evidence of the specificity of this immuno-fluorescent technique is presented in a reaction between fluorescein isothiocyanate conjugated anti-serum and oyster sections. A blocking of this reaction is accomplished by pre-treatment of the oyster sections with non-labeled anti-serum. Plates I and II

Oysters known to contain large amounts of MSX were homogenized, filtered through several layers of cheesecloth, mixed with ten times their volume of pony, anti-oyster serum and reacted at 37°C for 3 hours. This material was centrifuged and observed to stratify, giving a light brown layer overlying a cream colored layer that was shown to be MSX. This layer was mixed with more anti-oyster serum, centrifuged and was observed to still contain a rather pure suspension of MSX. When this layer was washed in 0.85% saline, the MSX were completely lysed. A comparative treatment of the MSX suspension with 3% saline demonstrated that the parasites were abnormally shrunken, crenated, and eventually disrupted.

Chart I shows an experiment which, through the help of Dr. Rosenfield and his staff at Oxford, demonstrated the effects of three different salinities on survival of the parasites and oysters. It is indicated under the experimental conditions that the parasitized oysters succumbed more readily to the high and low salinities than did healthy oysters. Secondly, an intermediate salinity isotonic to the area from where the oysters were taken appeared to support the parasitized oysters. This is an interesting case where a parasite living in a host that is essentially isotonic internally to its environment is limited in distribution by its direct response to environmental factors.

This observation is given in its preliminary form since it may be highly significant in view of our interest concerning trophic transmission in the absense of any recognizable spore or cyst state and may account for negative transmission in the spore or cyst state.

Salinity tolerance would be a decisive factor in its distribution and may well indicate the potential of the parasite as a hazard in other oyster beds.

At the termination of the above salinity experiments, the remains of five heavily infected oysters from the 2% salinity group that were not used for histological studies were placed in approximately 150 cc

of pony, anti-oyster serum of a high titer. These were frozen, returned to Hiram, homogenized, and allowed to react at 37°C for three hours. This homogenate was used as an antigen in a second pony in which an antibody was produced against the MSX present without a response being stimulated to the oyster tissue. The serum was exposed for 1 hr. at 37°C to powdered oyster tissue to further remove any non-specific reaction against the oyster tissue and the ubiquitous bacteria therein. Photographs III and IIIa, and IV and IVa demonstrate fluorescent parasites that were later stained with toludine blue-O, to demonstrate the presence of a specific MSX reaction.

Chart I Showing the Effects of Salinity on MSX and Host Reaction.

Salinity	N oysters introduced	N oysters dead prior to term. of exp.	% inf. of dead	Survivors N	Survivors N inf.	Survivors % inf.
1%	20	7	71%	13	3	23%
2%	20	3	66%	17	8	47%
3%	20	5	100%	15	4	27%