TABLE 1

TOTAL NITROGEN, PHENYLALANINE, AND HISTIDINE IN MUCOID, HYALURONIC ACID, INSULIN, INSULIN-MUCOID, AND INSULIN-HYALURONIC ACID

Materials analyzed	Nitrogen, %	Phenyl-alanine, %	Histi-dine, %
Vitreous humor mucoid ...	12.35	3.19	2.45
Hyaluronic acid	3.73	...	...
Insulin-mucoid precipitate .	13.97	6.45	3.87
Insulin-hyaluronic acid precipitate	12.86	...	...
Insulin	14.62	7.25	4.40

ing and resuspending it in the original volume of water. Of this suspension 0.14 ml/kg was injected intradermally in seven rabbits.

The supernatant fluid in solution C, after centrifuging, was separated and called *solution D*. From it 0.14 ml/kg was injected in a control rabbit.

Blood-sugar determinations were made (by the method of Hoffmann [*2*]) before each injection and every 2 hr afterwards, and the results were plotted together with relation to time, as shown in Fig. 1. From this, it will be seen: (1) that there was no noticeable difference between the effects of solutions A and B; (2) that solution C, although slightly less effective during the first 2 hr than A or B, exerted a more prolonged effect, and for this reason the original sugar values return more slowly to their former level; (3) that solution D was still active, but in a much lower degree.

We have planned an investigation of the nature of the precipitates formed. A priori, one is tempted to identify those formed by hyaluronic acid, mucoids or mucins, and described by Meyer and Palmer, as mere artifacts. For the time being we have conducted analysis of only the total nitrogen content (by micro Kjedahl) in the two above-mentioned precipitates, and also in mucoid, hyaluronic acid, and insulin (the last precipitated from the original solution by alcohol ether). Phenylalanine and histidine in the precipitates, insulin, and mucoid were determined by Stokes *et al.* (*4*) by microbiological method. Table 1 reports the results from duplicate determinations.

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Spawning of Oysters at Low Temperatures

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The assumption by Churchill (*1*), Galtsoff (*2*), Nelson (*5*), and others that 20.0° C is the minimum temperature for spawning of the oyster (*Ostrea virginica*) in nature was first questioned when it was established that oysters in Long Island Sound were spawning at temperatures ranging from 16.4° to 18.5° C (*4*). Systematic observations conducted since then by one of us have shown that in some years not only spawning but also larval metamorphosis, or, as it is commonly called, setting, took place in Long Island Sound before the temperature reached 20° C. Reports of these observations are in the official files of the U. S. Department of the Interior, Fish and Wildlife Service. However, this evidence was not corroborated by laboratory observations.

The first direct observation on mass spawning of laboratory oysters at a comparatively low temperature was made in May 1949. These oysters were kept for a period of 90 days in trays with running sea water, the temperature of which was steadily maintained at 15° ± 1° C. Because samples of gonads taken toward the end of the 90-day period began to show that the oysters were discharging some spawn, and because examinations of the bottom deposit disclosed fertilized eggs, it was decided to add sperm and egg suspension to one tray to see if the oysters would respond to the stimulation and begin spawning. Within a minute after addition of the suspension, the first male began to spawn. He was soon followed by two other males, and after 5 min three females were also spawning. Later on more oysters of both sexes began to spawn, and finally, after about 25 min almost all 20 oysters were spawning, and continued to do so for about 2 hr.

Addition of sperm and eggs to three other trays also induced mass spawning, with oysters of both sexes discharging large quantities of eggs or sperm. Throughout spawning, the water temperature in all trays remained steadily at 15.8° C. Fertilized eggs were collected and cultured; they showed normal development.

Obviously, spawning of a certain portion of the oyster population in northern waters may begin at temperatures much lower than 20° C. It is possible that the temperatures prevailing during the period of gonad maturation may determine the temperature at which the first spawning will take place.

As Thorson (*6*) points out, many marine invertebrates may have ripe sexual products outside the breeding period, sometimes months ahead of spawning. In our waters, such is the case in *Venus mercenaria,* which possesses ripe sperm and morphologically mature ova in November, although it does not spawn until the following July or August (*3*). Therefore, a distinction should be made between the temperature high enough to permit maturation of sexual products and the temperature at which spawning is possible. Sometimes such temperatures are several degrees apart. However, as our laboratory observations indicate, by conditioning oysters for a long period at a temperature just high enough for maturation of the gonads it is possible to induce spawning without an increase in temperature, thus bringing together, or to virtually the same level, the temperatures needed for ripening of gonads and for spawning.

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On Interspecific Hybridization in *Ostrea*

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In view of numerous attempts to introduce the Japanese oyster, *Ostrea gigas,* to the Atlantic coast it is important to determine whether this species will cross with the native oyster, *Ostrea virginica,* which is found from Massachusetts to Florida.

Galtsoff and Smith (*2*) made some preliminary observations in determining whether these two species would cross. They reported that "Eggs of both species were easily fertilized by either sperm, the ensuing development resulted in apparently normal straight hinge larvae. Comparison with controls showed no increased mortality among the hybrids." Since their observations terminated at the very early straight-hinge stage, it remained undetermined whether the hybrid larvae would continue to develop normally and would finally metamorphose. The present study was undertaken to provide an answer to this question, using the methods now standard at Milford Laboratory for rearing larvae to metamorphosis.

Unfertilized eggs and active spermatozoa of *O. virginica* and *O. gigas* were obtained from mature individuals and allowed to stand suspended in aerated sea water for a short time. The egg suspensions were divided into six equal portions, after an examination had shown that they were free of casual spermatoza. To each of three portions of *O. virginica* eggs, a small volume of *O. virginica* spermatozoa was added, and the remaining three portions received the same quantity of spermatozoa from *O. gigas.* Thus, triplicate cultures of *O. virginica* ♀ × *O. virginica* ♂ and *O. virginica* ♀ × *O. gigas* ♂ were prepared. Eggs from *O. gigas* were handled in the same manner, giving triplicate cultures of *O. gigas* ♀ × *O. virginica* ♂, and *O. gigas* ♀ × *O. gigas* ♂. This assured that eggs and spermatozoa used in hybrid crosses were from the same source and therefore were equal in viability to those used in the control nonhybrid cultures. All 12 groups of fertilized eggs were then cultured under identical conditions. The experiment was repeated three times with consistent results.

In general, hybrid larvae develop quite normally to the straight-hinge veliger stage. However, contrary to the observations of Galtsoff and Smith (*2*), in most of the hybrid cultures of *O. virginica* ♀ × *O. gigas* ♂ the proportion of larvae developing to the straight-hinge stage was appreciably lower than for the corresponding control nonhybrid cultures. Furthermore, in all hybrid cultures a very high mortality became apparent about the sixth day, and almost all the larvae were dead by the tenth day. This heavy mortality in such a short period was especially striking because the hybrid larvae were entirely normal in appearance and vigorous in behavior until the onset of mortality.

The control nonhybrid cultures of *O. virginica* and *O. gigas,* on the other hand, grew quite normally, with no undue mortality, and all cultures were reared to the setting stage. The *O. virginica* cultures reached metamorphosis in 19–25 days, whereas most of the *O. gigas* cultures required 26 to 27 days to metamorphose.

As shown by these experiments, hybrid larvae died within 6–10 days after fertilization, although the nonhybrid cultures grown under the same conditions developed normally to metamorphosis. The mortality of hybrid larvae was apparently due to a lethal combination of inherited factors, that did not become active until a specific stage of development, which most larvae attained in 6–10 days, although the time may be dependent on temperature and thus may vary considerably. The possibility is not excluded, however, that in some rare cases a few of the hybrid larvae, characterized by a special genetic complex, might survive to metamorphosis or even to maturity.

Crosses of *O. virginica* with *O. lurida* were also tried, but the experiments were necessarily confined to attempts to fertilize *O. virginica* eggs with *O. lurida* spermatozoa, because many individuals of the latter species, in addition to being larviparous, are also hermaphroditic, and one cannot, therefore, be certain of exclusion of their spermatozoa. It was possible, however, to find many individuals that appeared to be true males at the time and these were stripped to obtain spermatozoa for the experiments in which active *O. lurida* spermatozoa were added to unfertilized eggs of *O. virginica.* Although the mixture of eggs and spermatozoa was held, in some cases, for as long as 8 hr, no fertilization occurred. Observations also showed that the spermatozoa of *O. lurida* did not interfere with later fertilization of these eggs by spermatozoa of their own species, even when this addition of *O. virginica* spermatozoa was as late as 8 hr after the unsuccessful attempt to fertilize the eggs with spermatozoa from *O. lurida.* Apparently the *O. lurida* sperm does not even enter the *O. virginica* egg, since it does not cause formation of a fertilization membrane or interfere in any way with the later fertilization of the egg by *O. virginica* spermatozoa. These results closely parallel those of Bouchon-Brandely (*1*), who also attempted to cross a larviparous species (*O. edulis*) with an oviparous species (*O. angulata*) and reported that there was no "evidence of successful fertilization or of development."

[1] The writer wishes to express his appreciation to Mr. C. E. Lindsay, senior biologist, Washington Department of Fisheries, for shipping us the *Ostrea gigas* and *Ostrea lurida* used in these experiments.

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