Reports

Marine Fungus Infecting Eggs and Embryos of Urosalpinx cinerea

This paper reports a fungus infestation of ova within the egg cases of the marine gastropod *Urosalpinx cinerea* (Say), the common oyster drill (*1*). This observation is important since it offers a possible biological control measure for this snail, which is one of the most destructive predators of young oysters. *Urosalpinx cinerea* is currently being studied by the U.S. Fish and Wildlife Service and other groups in an effort to develop methods for controlling its predation. This fungus is one of the few natural controlling factors known for this gastropod.

Fungus infestation in drill egg cases was first observed in cases taken from our outdoor tidal tanks, in which oyster drills are kept for laboratory study. Figure 1 shows an egg case, with its outer membrane removed, containing four dense masses which were once healthy ova and now are infected with fungus growth.

This fungus was isolated from the infested cases and was cultured by H. S. Vishniac (*2*), who thinks that it is a new form resembling *Sirolpidium zoophthorum*. She believes that this fungus belongs to the order Lagenidaiales and resembles the Sirolpidiaceae but lacks the septa which characterize this family, as defined by Sparrow (*3*). Because the fungus also resembles *Plectospira dubia* (Atkins), a marine fungus capable of infecting crustacean eggs, a pure culture of our fungus was sent to D. Atkins (*4*). She was successful in infecting the eggs of the oyster crab, *Pinnotheres*, with our isolate but is of the opinion that it is not the same as *P. dubia*.

Preliminary experiments conducted with a culture isolate demonstrated that this fungus will infect ova within the egg cases of *U. cinerea* in sterilized sea water, at 20°C, with a salinity of 21 parts per thousand. Moreover, ova do not have to be moribund for the infestation to develop. In one experiment, a flask containing 200 ml of sterile sea water and 20 washed egg cases containing ova and early gastrulae was inoculated with 3 ml of the cultured isolate. One hundred-percent infection occurred within 24 days, although the controls had developed from ova to protoconchs with no infestation.

In a similar experiment, 12 egg cases, of which four contained ova, four, veliger larvae, and four, protoconchs, were used. At the end of 28 days the four egg cases which initially contained protoconchs had released them; the veliger larvae developed to protoconchs; but the egg cases that contained ova were infected and did not develop. Normal development, with no infections, occurred in the control.

In early experiments, in which infected egg cases were used as an inoculum, only egg cases that contained ova through veliger stages were infected, not the older egg cases that contained protoconchs. Hence, infection appears to be confined to the ova and early developing stages and, in this respect, resembles the infecting behavior of *P. dubia* on crustacean eggs, inasmuch as *P. dubia* is likewise less pathogenic for the prezoeal and zoeal stages than it is in the early stages of the egg development (*5*).

Biotic control of the drill by using a trematode parasite which causes castration in snails has been suggested by Cole (*6*). The only other known biotic factor which might be considered as a means of control is a tube-dwelling amphipod, which was found to form mud tubes in drill egg cases and presumably destroys the ova of the drills on the West Coast (*6*). Hence, the fungus infestation here described presents another biotic factor which may be considered for control of *U. cinerea*. However, the practicability of control by the use of a fungus is doubtful, since any control measures in the field would demand the creation of environmental conditions conducive to infestation and dissemination. On the other hand, if this fungus can be carried and spread by the drills themselves, it may offer a specific, natural infecting agent capable of reducing efficiently the population density of the oyster drill.

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Fig. 1. An egg case of *Urosalpinx cinerea* with its outer membrane removed to show four dense masses of fungus-infested ova (× 10).

References and Notes

1. I wish to thank V. L. Loosanoff, for his assistance in pursuing this problem, and C. A. Nomejko, for preparing the photomicrograph. I also wish to thank H. S. Vishniac, for preparing the fungus isolate, and D. Atkins, for allowing me to refer to her correspondence with Vishniac.
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3. F. K. Sparrow (Univ. of Michigan Press, Ann Arbor, 1943), p. 785.
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Regulation of Liver Cholesterol Synthesis by Lymph Cholesterol

Very little is known about the homeostatic mechanism(s) regulating the blood and tissue cholesterol levels in the animal body. It has been reported that the endogenous synthesis of cholesterol is, in part, regulated by the intake of dietary sterol. In the dog and rat who have previously been fed cholesterol in their diet, there is a depression of liver cholesterol synthesis, both *in vivo* and *in vitro* (*1*). In addition, fasting has been shown to reduce markedly the rate of cholesterol synthesis in the rat (*2*).

Of particular interest to the atherosclerosis problem is the endogenous synthesis of cholesterol and its reabsorption. It has been suggested, on the basis of recent findings (*3*), that reduction of endogenous synthesis and also of reabsorption of endogenous cholesterol may lower the cholesterol level in the blood and tissues. In connection with studies on tissue cholesterol and cholesterol ester synthesis being carried out in our laboratories, an interesting observation was made on the effect of removal of

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endogenous cholesterol on cholesterol synthesis. This report describes these findings (*4*).

Rats with a thoracic lymph fistula were prepared and given saline to drink. Twenty-four hours after the operation, the lymph-fistula animals and normal fasted rats were given intraperitoneally a tracer dose of 30 μc of 1-C^{14}-acetate. The animals were sacrificed at 1 hour, and the liver and blood were removed. The total cholesterol was isolated by extraction, hydrolysis, and digitonin precipitation (*5*). The digitonides were plated and C^{14} activity was determined in a windowless gas flow counter and corrected for self-absorption.

The results, expressed as specific activity of the isolated cholesterol, are shown in Table 1. There was an eightfold increase in the specific activity of the liver cholesterol of the lymph-fistula rat over that of the normal rat. In the plasma, the specific activity of the cholesterol of the lymph-fistula rat was approximately 13 times that of the normal rat. In addition, there was no significant change in the total cholesterol content of the liver and plasma in the lymph-fistula animal.

The amount of cholesterol present in a 24-hour sample of lymph from a fasting rat is 8 to 10 mg. This represents sterol derived from the hepatic lymph and from reabsorption of cholesterol secreted in the bile and from the intestinal mucosa. It appears that the removal of this amount of endogenous cholesterol (8 to 10 mg) during a 24-hour fast leads to a pronounced increase in the rate of cholesterol synthesis in the liver. Moreover, the ratio of the specific activity of the plasma cholesterol to that of the liver cholesterol in the two types of animals suggests that there is a more rapid release to the plasma of newly synthesized cholesterol in the lymph-fistula animals.

These findings provide further evidence that the homeostatic control of cholesterol synthesis is sensitive to fluctuations in the cholesterol supply. Exogenous cholesterol depresses synthesis; in the present experiments, the "bleeding out" of 8 to 10 mg of endogenous cholesterol by way of the lymph fistula produced a marked increase in synthesis. Since exogenous cholesterol and a part of the endogenous supply of cholesterol enter the plasma by way of the thoracic duct, a major factor controlling the rate of cholesterol synthesis in the liver may be the amount of cholesterol entering the plasma by this lymph route. These experiments also pose a question whether the removal of endogenous cholesterol would be effective in lowering the levels of blood and tissue cholesterol.

Table 1. Liver cholesterol synthesis in 1 hour from 1-C^{14}-acetate in normal and lymph-fistula rats. There were four animals in each group.

Group	Specific activity (count/min mg of cholesterol)		Total concn. of cholesterol	
	Liver	Plasma	Liver (%)	Plasma (mg %)
Normal	359 ± 142	128*	0.219	58
Lymph fistula	2935 ± 293	1627 ± 173	0.212	54

* Plasma was pooled.

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References and Notes

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4. This work was supported in part by research grants from the American Heart Association and the National Institutes of Health (H-1897 and H-2746). A paper describing our more extensive findings is in preparation.
5. A procedure for a refined gravimetric sterol method was kindly furnished by W. M. Sperry.

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Histochemical Demonstration of Reactivation of Acetylcholinesterase in vivo

Pyridine-2-aldoxime methiodide (2-PAM) and certain other oxime and hydroxamic acid derivatives can reactivate acetylcholinesterase *in vitro* following its inactivation by diisopropylfluorophosphate (DFP) and other irreversible alkylphosphate anticholinesterase agents (*1, 2*). The oximes can also protect animals against the lethal effects of the alkylphosphate anticholinesterases and reverse their neuromuscular blocking action (*3–6*). Although reported findings suggest that the mechanism of the protective action *in vivo* is dependent at least in part on reactivation of acetylcholinesterase, evidence for this has been found only with diaphragmatic muscle (*7*). An alternative explanation is that the protective effect of 2-PAM and similar agents may be due largely to direct combination between the oxime and the anticholinesterase agent in the body before the latter has reacted with the enzyme (*2, 8*), since in most studies the oxime has been injected intraperitoneally prior to or shortly after subcutaneous injection of the anticholinesterase agent (*3, 5*).

Recent work has indicated that the total acetylcholinesterase of neurons may consist of "functional" acetylcholinesterase, external to a lipoid-like membrane, and internal or "reserve" acetylcholinesterase; the latter may represent more recently synthesized enzyme which is stored within the endoplasmic reticulum (*9*). The pharmacological actions of anticholinesterase agents are probably due to inactivation of the former (*10*). If this represents only a small portion of the total acetylcholinesterase, its inactivation and subsequent reactivation might not be detectable by usual homogenate techniques. Furthermore, only limited quantities of quaternary agents, such as 2-PAM, might penetrate the blood-brain barrier and have access to the central nervous system.

In order to determine whether 2-PAM can reactivate acetylcholinesterase of nervous tissue and skeletal muscle *in vivo,* the following study was undertaken (*11*). Cats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally) and given atropine sulfate (3 mg/kg, intraperitoneally) and 20 μmole (3.68 mg) of diisopropylfluorophosphate per kilogram, (intravenously) (freshly diluted from a 0.1*M* stock solution in anhydrous propylene glycol). Four cats served as diisopropylfluorophosphate controls. Six received intravenous injections of 2-PAM at the doses and time intervals indicated in Table 1. After the cats had been sacrificed by intravenous administration of air, the stellate and ciliary ganglia and a portion of intercostal muscle were removed and sectioned immediately at 10 μ (ganglia) or 20 μ (muscle) on the freezing microtome. Sections were placed on slides and incubated in acetylthiocholine medium for 5, 10, 20, 40, and 80 minutes, after which they were developed with $(NH_4)_2S$, gold-toned, dehydrated, and mounted, (*12*). Staining was compared

Table 1. Schedule of intravenous administration of 2-PAM and sacrifice of anesthetized cats following intravenous administration of diisopropylfluorophosphate (DFP) (20 μmole/kg).

Cat No.	2-PAM (μmole/kg)	Time of administration after DFP (min)	Time of sacrifice after DFP (min)
1			35
2			35
3			78
4			79
5	20	5	35
6	40	5	35
7	20	20	35
8	40	20	35
9	40	63	78
10	40	64	79