

only a trace of a diffuse and generalized staining after prolonged incubation. Control experiments, confirming the specificity of the cytochemical method used herein for LDH have been described (6).

With phenazine methosulfate, and the application of the "incubation mixture film" method, LDH activity is demonstrable in dystrophic muscle fibers. In the absence of the methosulfate, dystrophic fibers remain unstained or stain only faintly. This may explain the failure of Fennel and West (2), who did not use this agent, to observe marked differences in the staining of dystrophic and normal muscles.

Phenazine methosulfate transfers the electrons from NADH to nitro blue tetrazolium and thus can substitute for the low content of endogenous NADH-diaphorase in "white" muscle fibers (3, 6). This essential role of the methosulfate in revealing cytochemically the LDH activity in normal "white" muscle fibers has been demonstrated previously (3) and also here in Figs. 1 and 2. It may therefore, be assumed that in the dystrophic muscles (Figs. 3 and 4) the majority of dystrophic fibers, which stain only in the presence of phenazine methosulfate, are of the "white" type. "White" fibers depend primarily on glycolysis for their energy metabolism (7), and if they were the fibers primarily involved in muscular dystrophy, the glycolytic activity would be expected to be affected in the dystrophic muscles. McCaman (1) has presented biochemical evidence of such an effect on glycolysis in dystrophic muscles.

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Antarctic Asteroid *Odontaster validus*: Constancy of Reproductive Periodicities

Abstract. *The fact that samples of Odontaster validus from the Balleny Islands (67°S) and Robertson Bay (71°S) closely resembled reproductively samples taken from McMurdo Sound (77°S) indicates reproductive synchrony in this species over much or all of their circumcontinental antarctic distribution. This synchrony suggests that the reproductive periodicities of O. validus are both adapted to and synchronized by the summer period of phytoproduction and that neither light nor temperature changes have any direct synchronizing role.*

The reproductive periodicities of the omnivorous antarctic asteroid *Odontaster validus* Koehler in two populations at McMurdo Sound (77°S, 166°E) have been worked out in detail (1). The populations were in reproductive synchrony, the beginning of gametogenesis (differentiation of spermatocytes or oocytes from gonial cells) occurring between May and March, with a peak of activity between August and January. Oocyte growth to full growth required between 18 and 24 months, while the differentiation of spermatozoa from spermatocytes required about 9 months. Spawning occurred during midwinter and late winter (June to mid-September). These periodicities probably result from adaptations to ensure a summer food supply for the slowly developing demersal bipinnaria larvae.

The main difference between the two populations was related to difference in amounts of available summer plant food. Different ice conditions resulted in much more phytoproduction at one locality, Cape Evans, than at the other, McMurdo Station, and the animals at Cape Evans produced about twice as many gametes as did those at McMurdo Station. Although food in quantitative terms did not effect the timing of reproduction, it was still considered likely that the marked seasonality of phytoproduction could synchronize the reproductive periodicities.

A sample taken in October from 12 m at Hallett Station (72°S, 170°E), 560 km north of McMurdo Sound, was in reproductive synchrony with the McMurdo Sound samples. Difference in light between Hallett Station and McMurdo Sound, as well as similar difference between Cape Evans and McMurdo Station, made photoperiodic control of the reproduction of *O. validus* very unlikely, but more observations were needed, especially from more northerly locations.

Slight but definite seasonal changes in sea temperatures were noted in McMurdo Sound. Because of the im-

portance of changes in sea temperature to reproductive periodicities of north-temperate species, and because of the apparent unimportance of photoperiods and quantitative food differences, it was suggested that the slight seasonal changes in sea temperature might be important in the synchronization of reproduction in *O. validus*.

On 12 February 1965, eight specimens of *O. validus* were dredged from 274 m off Young Island, Balleny Islands (66°37'S, 162°38'E), nearly 1200 km north of McMurdo Sound. Three specimens were also dredged on 27 January 1965 from 274 m in Robertson Bay (71°24'S, 170°10'E), near Hallett Station. The gonads of these specimens were fixed for histologic preparation, as the McMurdo Sound samples had been treated. The reproductive conditions of all gonads were very similar to those collected from less than 40 m in McMurdo Sound during late January and February 1961. Three of the specimens from the Balleny Islands and two of those from Robertson Bay were males; their testes were filled with growing spermatogenic papillae containing mostly spermatids and spermatocytes, like comparable 1961 samples from McMurdo Sound. The females in both samples had two size groups of oocytes: one below 75 μ in diameter, with a mean diameter of about 30 μ ; the other ranging between about 80 and 130 μ in diameter. These oocyte size frequencies were almost identical with those in comparable 1961 samples from McMurdo Sound (Fig. 1).

The similarity among the samples from the Balleny Islands, Robertson Bay, and McMurdo Sound in late January and February, as well as among the earlier October samples from Hallett Station and McMurdo Sound, indicates that reproduction in individuals of *O. validus* is in synchrony throughout much or all of their circumcontinental antarctic distribution. Such synchrony is not surprising because the winter breeding sea-

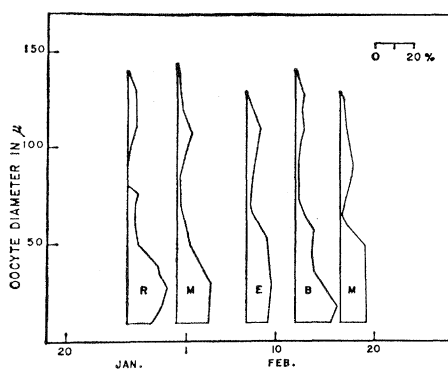


Fig. 1. Averaged oocyte size frequencies in the ovaries of *O. validus* collected in 1961 from McMurdo Sound [McMurdo Station (M) and Cape Evans (E)] and in 1965 from Robertson Bay (R) and the Balleny Islands (B).

son of *O. validus*, as found at McMurdo Sound, is most probably an adaption synchronizing the presence of the slowly developing demersal bipinnaria larvae with phytoproduction, which occurs almost exclusively during the summer months throughout antarctic waters.

The difference in light conditions between the Balleny Islands and McMurdo Sound is even more striking than between Hallett Station and McMurdo Sound; the sun never sets for more than 24 hours at the islands, while it is below the horizon for over 3 months at McMurdo Sound. Moreover, at 274 m it seems unlikely that perceptible light reached the animals on the islands' site, except perhaps in midsummer. Thus it seems certain that the reproductive periodicities of *O. validus* are not under direct photoperiodic control.

Reserpine: Inhibition of Olfactory Blockage of Pregnancy in Mice

Abstract. Failure of pregnancy in newly mated female mice exposed to fresh urine from alien males is prevented by administration of reserpine, at 6.25 micrograms per day per female, on days 1 to 5 post coitum—that is, throughout the period of exposure to male urine and for 2 more days. Since reserpine is known to suppress the inhibitory center in the hypothalamus controlling the release of prolactin, inhibition by reserpine of the blockage of pregnancy provides a strong direct indication of hypothalamic mediation in the male-induced failure of pregnancy in mice.

Recent reports (1) indicate that male urine is the immediate source of the pheromones that cause failure of ovulation in newly mated female mice closely exposed to alien males (2, 3). Unlike that of normal females,

The water temperature at 247 m near the Balleny Islands site was $+0.8^{\circ}\text{C}$, while it was 1.4°C at Robertson Bay; the former temperature was higher than any recorded at the McMurdo Sound collecting sites, which ranged between -2.2° and -1.4°C . Such temperature differences make it also unlikely that changing sea temperatures play any role in synchronizing reproduction of *O. validus*.

Onset of the summer phytoproduction period, however, appears to be very well defined throughout antarctic waters. In McMurdo Sound the main bloom of summer phytoplankton begins quite suddenly in December at McMurdo Station (2) and Cape Evans (3). At Mawson also ($67^{\circ}36'S$, $62^{\circ}53'E$), at nearly the same latitude as the Balleny Islands, the summer phytoplankton bloom begins in December (4). Since individual *O. validus* are in reproductive synchrony throughout their distribution, as indicated by the Balleny Islands sample, it seems most likely that phytoproduction itself somehow acts directly to synchronize reproduction.

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Table 1. Inhibition in newly mated Parkes-strain females, by injection of reserpine, of male-induced blockage of pregnancy. One form of treatment was exposure (Exp) of the females to fresh urine of CBA males. Some controls were injected with normal saline. Numbers of subjects appear in parentheses.

Treatment	Females (No.)	
	Returned to estrus	Remained pregnant or pseudo-pregnant
Exp + reserpine (54)	8	46
Exp + saline (54)	44	10
None (21)	3	18

drogen-dependent gland (4). Blockage of pregnancy can be prevented in females closely exposed to males or male urine by the administration of exogenous prolactin (5, 6) or progesterone (6), or by the presence of a functioning ectopic pituitary graft (6). Hence the immediate endocrine cause of the blockage of pregnancy appears to be the failure of secretion of prolactin by the anterior pituitary, with consequent failure of corpus luteum development and with return of the female to estrus as if no mating has occurred.

This report deals with the inhibitory effect of reserpine, injected in the females, on the male-induced blockage of pregnancy in newly mated mice. All breeding mice were outbred albinos of the Parkes strain. Females were separated from males when vaginal plugs were found (day 0) and were housed singly; 24 hours later (day 1 of pregnancy) they were: (i) exposed to fresh urine from 12 CBA males (7) on days 1 to 3 and injected with reserpine (Serpasil, Ciba; $6.25 \mu\text{g/day}$ each) on days 1 to 5—that is, throughout the period of exposure to male urine and for two more days; (ii) exposed to similar urine on days 1 to 3 and injected with normal saline, 0.5 ml/day each, on days 1 to 5; or (iii) left undisturbed. Vaginal smears from all females were examined daily, and a return of vaginal cornification within day 7 post coitum was taken to indicate failure of pregnancy (2, 6).

The results (Table 1) clearly demonstrate that reserpine can suppress the male-induced failure of pregnancy. Reserpine is known to suppress the inhibitory center in the hypothalamus that controls the release of prolactin (8). Moreover, administration of reserpine inhibits the gonadotrophic cells and stimulates the luteotrophic cells of the anterior pituitary (9). Thus it is