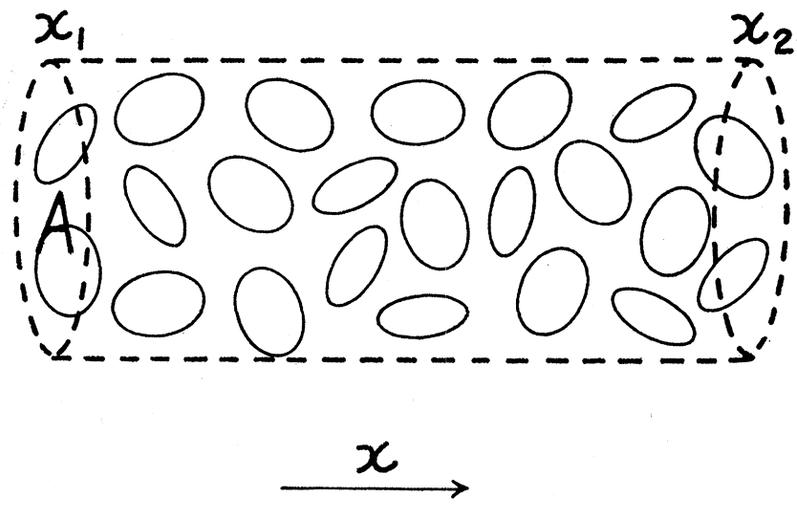

Fig. 1. Diagrammatical representation of a volume element in the diffusion layer containing oxygen-carrying particles represented by the ellipsoids.

The following three particular cases are of special interest:

1) For the diffusion of nitrogen or any other nonreacting gas, $f'(p) = 0$. Consequently, we can make the approximation that $D_o k \simeq D_i k'$ and that

$$\partial p/\partial x = (p_2 - p_1)/(x_2 - x_1) \equiv \triangle p/\triangle x = dp/dx$$

where p_1 and p_2 are the partial pressures of the diffusing gas at x_1 and x_2 respectively. Equation 5 then simplifies to the familiar form

$$q = - D_o \ (\triangle p/\triangle x)k \qquad (6)$$

2) For the diffusion of oxygen at low partial pressures, $f'(p) > k'$ or k. Hence the second integral in Eq. 5 gives rise to an increased rate of diffusion as observed by Scholander (*1*).

3) For the diffusion of oxygen under high partial pressure, $f'(p) \to 0$. Hence the diffusion rate drops back to normal, as was found experimentally.

The dependence of steady-state diffusion rate on the partial pressure of oxygen can be conveniently illustrated by considering the special case of a very concentrated layer of immobilized myoglobin or hemoglobin solution or gel or a layer of packed erythrocytes. For such a special case we have

$$v_o << v_i \qquad (7)$$

$$Dp \to 0 \qquad (8)$$

$$\partial p/\partial x = \triangle p/\triangle x = dp/dx \qquad (9)$$

Substituting Eqs. 7, 8, and 9 in Eq. 5 gives

$$q = \frac{- D_i}{x_2 - x_1}\int_{p_1}^{p_2}[f'(p) + k']dp \qquad (10)$$

For simplicity let us consider the case of myoglobin, for which $L = 1 + Kp$, $L' = K$, and $L'' = 0$; hence we have the equation

$$f(p) = cKp/(1 + Kp),$$
$$f'(p) = cK/(1 + Kp)^2 \qquad (11)$$

Substituting Eq. 11 in Eq. 10 and integrating gives

$$q = - D_i \left(\frac{\triangle p}{\triangle x}\right) \left[k' + \frac{ck}{(1 + Kp_1)\ (1 + Kp_2)}\right] \qquad (12)$$

Therefore, at constant pressure gradient, $\triangle p/\triangle x$, the diffusion rate at low partial pressures decreases as p_1 and p_2 are raised. At sufficiently high partial pressures, the second term in Eq. 12 becomes negligible; consequently, the oxygen molecules diffuse as a nonreacting gas. Similarly, the integral in Eq. 10 can be evaluated for hemoglobin, but in this case graphical and numerical methods are probably more expedient. It may also be noted that, although $f(p)$ and $f'(p)$ for hemoglobin are pH-dependent, the effect of pH on diffusion is noticeable only at low oxygen pressure when $f'(p)$ is not negligible as compared to k', which is in agreement with the observations of Hemmingsen and Scholander (*3*).

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2 December 1960

Response Latencies of Female Rats during Sexual Intercourse

Abstract. A female rat, by operating a lever, causes the presentation of a potent male rat and subsequent sexual contact. The female shows contact-response latencies that vary according to the nature of the contact. Latencies after ejaculations are longer than those after intromissions, which in turn exceed those after mounts.

In recent years our knowledge of the sexual behavior of the rat has increased considerably, largely because of the work of Beach and of Larsson (*1*, *2*). The great majority of the studies that these and other workers have carried out focuses primary importance on the male's behavior; the female has come in for scant attention. The experiment reported here measures the effects of single copulations on the behavior of estrus female rats, in the context of an ongoing series of copulations.

The major difficulty encountered in a detailed study of the female's behavior stems from the male's apparent dominance in the control of the intercopulatory intervals during ad libitum intercourse. In order to investigate in detail the behavioral effects of single copulations on the female, therefore, it is necessary to provide a method by which she can control the timing of the copulations. One such method is to make each copulation contingent upon some measurable *arbitrary response* by the female.

Eight albino and seven hooded female rats, ranging in age from 6 to 20 mo, were used as subjects. None of the females had been spayed; four were sexually experienced. Sexually vigorous hooded males between 3 and 6 mo old serviced the females during experimental sessions. Between sessions the animals were segregated, kept in a constant 12-hour light cycle, and fed freely.

A wooden box, 1 ft square and 15 in. high, served as the experimental space. A lever of the sort commonly used in operant conditioning studies protruded from one wall of the box. Operation of the lever activated timing and counting equipment. A buzzer on the outside of the box sounded for 1 sec after a lever-press.

The females were trained to press the lever by making presentation of the male contingent upon successively closer approximations to the desired response. The time required to train each female ranged from 15 to 90 min. At least 2 wk separated final training and the first testing session. Behavioral heat was induced by subcutaneous injections of 0.1 mg of estradiol benzoate (Progynon, Schering) 72 hr before

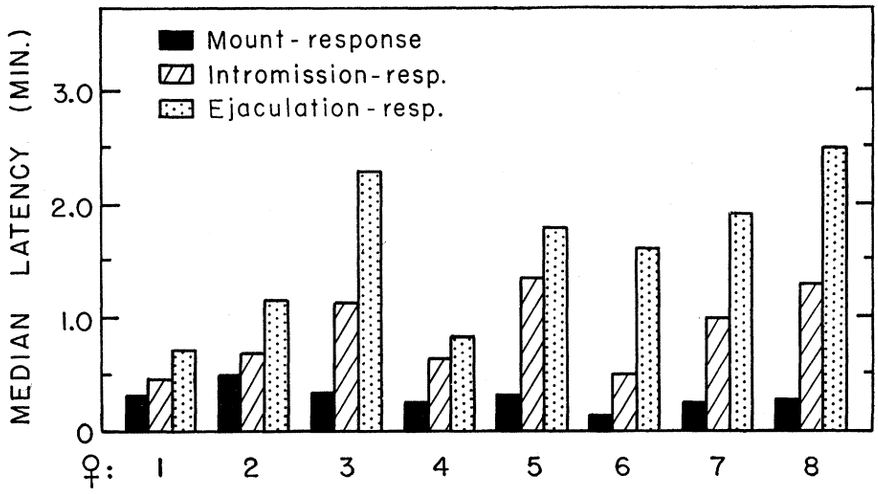

Fig. 1. Contact-response for individual animals during single sessions.

use and 1.0 mg of progesterone (Progestin, Organon, or Proluton, Schering) 6 hr before use.

During the test sessions, whenever the female pressed the lever the experimenter placed a male in the box. The male remained until copulation occurred. He was then removed and the copulation was recorded either as a mount, an intromission, or an ejaculation. The session continued for 1 hr or until the female had received five ejaculations. Tests on each female were usually spaced 21 days apart, although some sessions were run 14 days after the previous test.

Figure 1 displays the contact-response latencies of the eight females who completed at least four ejaculatory series during the experimental hour (the same eight animals are represented in Figs. 1 and 2). The data for each animal are considered individually, each bar representing the median latency after one kind of sexual event for a single session. Altogether the test was performed four times with one female, three times with another female, twice with five females, and once with four females. In each of these 21 cases the differences between medians were in the direction shown in the figure.

The bottom section of Fig. 2 displays contact-response latencies broken down with respect to performance in each of the ejaculatory series, so that each point is the average (median) for eight animals. Notice first that the latency values within a single series appear in the same order as they do when the data for each animal are considered individually: mount-response latencies are less than intromission-response latencies, which are less than ejaculation-response latencies. Now consider the time course of each of the curves. The mount-response latencies show very little change from series to series, varying but slightly around 0.3 min. The intromission-response and ejaculation-response latencies, on the other hand, show a decrease from the first to the third ejaculation, and then increase. There was only one instance of a fifth ejaculation-response latency, which is not included in the figure. Seven of the eight females represented in Fig. 2 showed minimal ejaculation-response latencies after the third or fourth ejaculation.

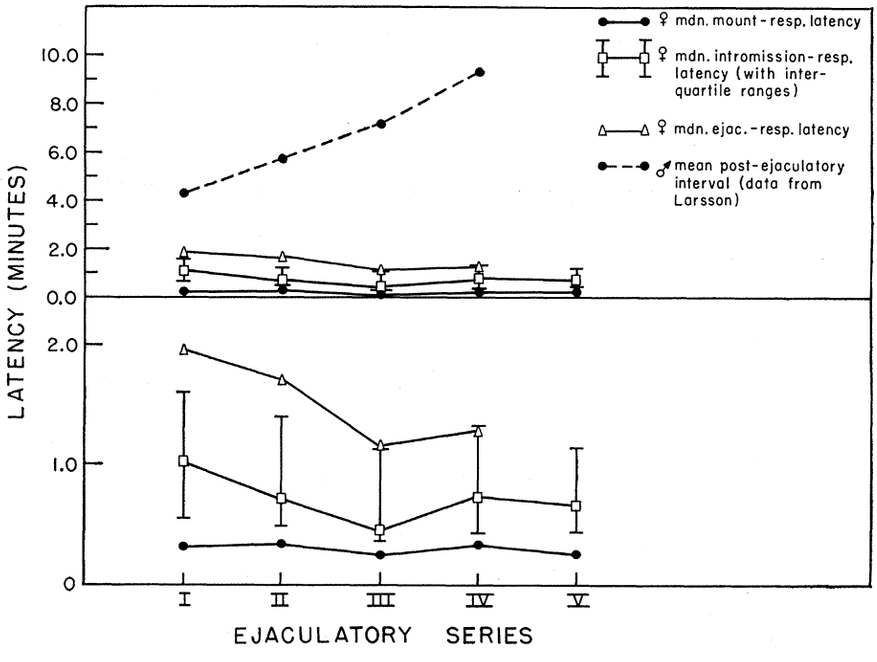

Fig. 2. Contact-response latencies as a function of ejaculatory series. The upper section of the figure repeats the lower section on a different ordinate scale and includes Larsson's data.

Figures 1 and 2 reveal the order in which female rats return to copulation-producing behavior after different sexual events. Male rats, copulating ad libitum, return to copulation in the same order. Secondly, there is some indication that female intromission-response latencies decrease in the middle of an experimental session. Males show decreased intercopulatory intervals after the first ejaculation. It is clear that the females' increased activity is not simply a reflection of a change in the male's condition, because fresh males were used for each ejaculatory series.

The broken line in the top part of Fig. 2 represents Larsson's data on the postejaculatory intervals for males who achieved four ejaculations during an hour test (*2*). For comparison, the female data are repeated in this part of the figure. Successive ejaculations cause progressively greater postejaculatory intervals in the male, while the female shows a decrease in ejaculation-response latencies toward the middle of the session. There is also a considerable difference in the absolute magnitudes of the two measures. Possible strain differences preclude a detailed comparison of this aspect of these data to those of Larsson. In observations of his own colony, however, Larsson has noted that the female's postejaculatory refractory period is "almost negligible" when compared to that of the male (*2*, p. 36). This observation is in agreement with the comparison shown in the figure.

In summary, it has been found that there are well-defined similarities and differences between the behavior of the male rat as observed during ad libitum intercourse and the behavior of the female as measured by the arbitrary

response technique. These relations exist within a single ejaculatory series and also in the dynamics of response associated with the progression from ejaculation to ejaculation (*3*).

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1 December 1960

Sparing of Folinic Acid by Thymidine

Abstract. Thymidine, which is inactive when added alone to *Pediococcus cerevisiae* (*Leuconostoc citrovorum*) 8081, was found to increase its growth in the presence of folinic acid. The addition of thymidine increased both the sensitivity of the folinic acid assay and the total growth yield. The mechanism of the synergistic effect of thymidine is briefly discussed.

Folinic acid (leucovorin) is known to meet the requirement of *Pediococcus cerevisiae* (*Leuconostoc citrovorum*) 8081 for the citrovorum factor, whereas the related pteroylglutamic acid is very inefficient (*1*). Although thymidine has been shown to supply the citrovorum factor for this organism, much larger quantities (10,000 times more than leucovorin) were required (*2*).

Wood and Hitchings (*3*) have shown recently that cell-free extracts of *P. cerevisiae* catalyze the transformation of pteroylglutamic acid into the citrovorum factor. They explained that the inefficiency of pteroylglutamic acid as a growth factor, in contrast to folinic acid, is due to its inability to permeate the cell membrane of *P. cerevisiae*.

We began to study the relationship of thymidine to folinic acid to elucidate the role of folic acid derivatives in the metabolism of *P. cerevisiae*. In the initial stage of this work we found that thymidine increased growth in the presence of folinic acid, but had no growth-promoting activity in its absence. The present report is confined to the elucidation of this interesting finding.

Figure 1 shows the effect of the addition of thymidine to graded amounts of folinic acid (*4*). At low concentrations of folinic acid (50 $\mu\mu$g/ml), the addition of thymidine caused a fourfold increase in growth, while at higher concentrations (200 $\mu\mu$g/ml) the increase was twofold; even at the highest concentration used (600 $\mu\mu$g/ml) the addition of thymidine resulted in a heavier growth than when folinic acid alone was added. The effective concentrations of thymidine were between 0.1 to 5 μg/ml. However, in the absence of folinic acid no visible growth was obtained even with concentrations of thymidine as high as 50 μg/ml.

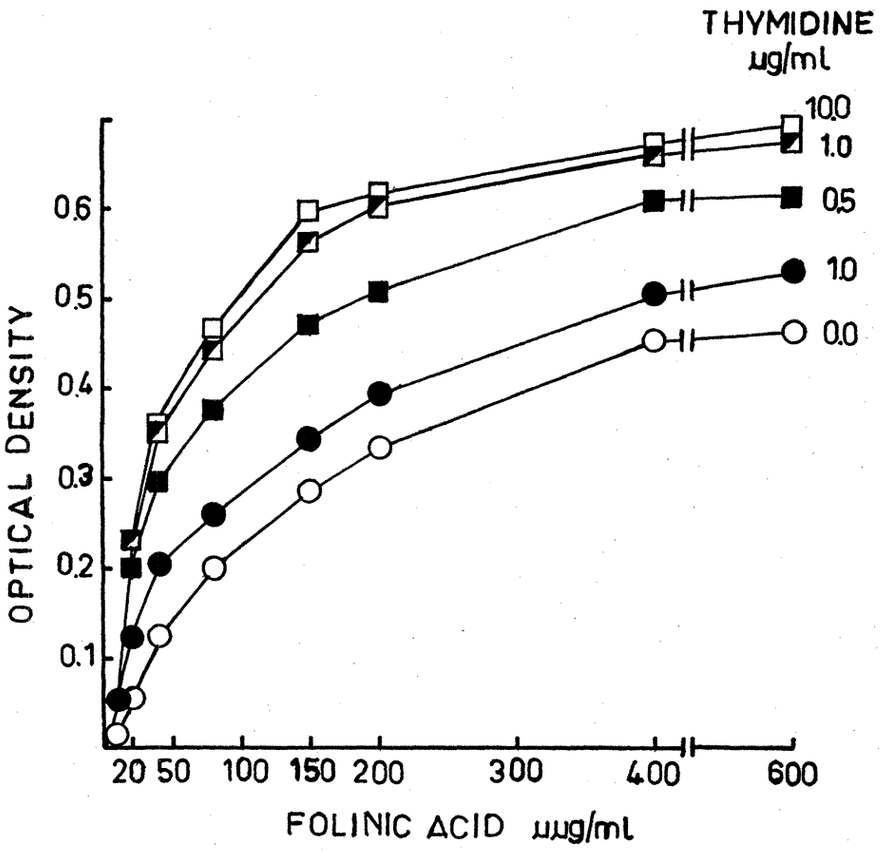

Fig. 1. Effect of thymidine on the growth response of *P. cerevisiae* (*L. citrovorum*) 8081 to graded amounts of folinic acid. Growth, expressed as optical density, was measured in the Coleman Junior spectrophotometer at 650 mμ after incubation for 48 hr at 37°C.

Thymidine was also found to increase the growth of *P. cerevisiae* in the presence of high concentrations of pteroylglutamic acid. In the presence of thymidine (1 μg/ml), visible growth occurred at an acid concentration of 0.2 μg/ml, whereas 10 times as much (2 μg/ml) was required to obtain similar growth in the absence of thymidine (*5*).

The medium was that of Toennies *et al.* (*6*), and the strain was obtained from the American Type Culture Collection.

The strain that shows the sparing effect of folinic acid by thymidine seems to be different from the original *P. cerevisiae* 8081 (*2*). Apparently, mutants of this organism are obtained rather easily. Nichol (*7*) selected strains that responded to lower concentrations of pteroylglutamic acid and which were found to display an increased sensitivity towards aminopterin.

The mechanism of the thymidine effect is not clearly understood at present. It would appear, however, that in this strain of *P. cerevisiae* folinic acid not only acts as a catalyst for the synthesis of thymidine but also for some other essential metabolite. Thus, the addition of thymidine spares folinic acid for its other functions.

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24 March 1961