both leaf and stem rust by the studies of pustule development (Table 1).

Thus CO_2 concentration of the atmosphere significantly affected stomatal penetration by *P. graminis* and neither stomatal opening nor direct stimulation of the fungus by light were major limiting factors. In contrast, neither removal of atmospheric CO_2 nor increase to a 5 percent concentration materially influenced penetration by *P. recondita*. Therefore it is possible that the major influence of light on penetration of *P. graminis* is exerted through its effect on the atmospheric CO_2 concentration within the leaf, which increases through respiration in darkness and is reduced by photosynthesis in the light, and that *P. recondita* penetrates independently of light, because of its insensitivity to the effects of CO_2 within the limits of concentration in the wheat leaf. Whether the effects of CO_2 on *P. graminis* are exerted directly on the rust fungus or indirectly through the host is not yet known (*6*).

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22 April 1963

Suppression of the Development of Female Mating Behavior by Estrogen Administered in Infancy

Abstract. *The administration of estradiol benzoate subcutaneously to 4-day-old female rats resulted in reduced mating in response to estrogen and progesterone in adulthood.*

It is reasonably clear that sexual differentiation in mammals is influenced by fetal sex hormones. Male hormones, in particular, seem to be critical for the appropriate development of the Wolffian duct system, and, ultimately, of male external genitals. Only recently has the influence, on adult sexual behavior, of hormones secreted or administered during the embryonic, fetal, and neonatal periods begun to be studied.

Phoenix *et al.* (*1*) found that the administration of testosterone to pregnant guinea pigs resulted in female offspring which, in adulthood, exhibited reduced female sexual behavior and enhanced male sexual behavior. Using the rat, Barraclough and Gorski, and Harris and Levine (*2*) have shown that the application of testosterone to the 5-day-old female leads to the failure of spontaneous mating activity at maturity. Further, treated females fail to mate if castrated and treated with exogenous estrogen and progesterone. In addition, Harris and Levine have found that comparable effects prevail if the male rat is treated with estrogen during infancy. At maturity the male fails to copulate even if administered normally adequate doses of testosterone propionate.

These studies suggest that the neural structures which determine sexual behavior may be deleteriously affected by heterotypical hormones acting during a critical stage of their development. To date, the effects of homotypical hormones administered during infancy in a single injection have not been studied. The present investigation was designed to help fill this gap.

Nineteen female rats from a locally maintained, randomly bred, pigmented strain were treated on the fourth day after birth. Fourteen of the females were injected subcutaneously with 200 μg of estradiol benzoate in 0.2 ml of mineral oil. The five control females were given 0.2 ml of mineral oil (*3*). Subjects were reared in groups until 95 days of age, when they were ovariectomized and caged in pairs. Mating tests began at approximately 130 days of age.

Subjects were primed with estradiol benzoate, 50 μg at 72 hours and 25 μg at 48 hours prior to each set of mating tests. Five hours before testing they were given 1.0 mg of progesterone. This procedure has been found to reliably induce receptivity in spayed females. At the appropriate time after the final priming injection a female was placed with a male in a cylindrical glass observation cage for a 15-minute period. Each individual was tested twice, with an inter-test interval of 14 days. During each test the observer recorded, for the male, the frequency of mounts without intromissions, intromissions, and ejaculations, and for the female, the frequency of assuming the mating posture (lordosis) and the number of times the female kicked her hind legs at the male. The kicking response is an index of sexual refractoriness.

Table 1. Mating responses of female rats treated with estrogen in infancy. Abbreviations: χ^2, chi-square analysis of percentage data; F, analysis of variance of frequency data; *p*, probability; n.s., not significant.

Sexual responses	Estrogen treated ($N = 14$)		Oil treated ($N = 5$)		χ^2	F
	% of females	Mean frequency	% of females	Mean frequency		
By male:						
Mount	100	13.0	100	9.1	*	1.4 (n.s.)
Intromission	36	0.68	100	4.6	6.1 ($p < .025$)	35.6 ($p < .001$)
Ejaculation	21	0.11	80	0.80	5.4 ($p < .05$)	*
By female:						
Lordosis	29	0.46	100	12.8	7.5 ($p < .01$)	*
Kicking	100	5.2	40	1.0	10.0 ($p < .01$)	7.2 ($p < .025$)

* Not analyzed because of nonnormality of distributions.

Table 1 shows the percentage of each group which exhibited the various sexual responses *at least once* during the two tests, and the frequency of the responses averaged over both tests. Males mounted all females in both groups, and with similar frequencies, but were unsuccessful in achieving intromission with the females treated with estrogen in infancy. When mounted by males, the estrogen-treated females did not show lordosis; instead, on 40 percent of the mounts the female responded by kicking the male. The reduced lordosis frequency accounts for the low intromission and ejaculation frequencies among treated females. Control females, given only oil in infancy, assumed the lordosis posture when mounted by the male and permitted intromission and ejaculation. Except for the frequency with which the females were mounted by males, the two groups of females differed significantly in their sexual behavior according to analysis of variance and chi-square tests. The indiscriminate mounting by the males probably reflects only the extreme vigor of these animals. The absence of intromission does not reflect estrogen-induced changes in genital morphology as the vaginae of these animals appear normal.

Thus, estrogen treatment at the appropriate dose level during the neonatal period acts, as does testosterone, to reduce the sexual responsiveness in females which is normally induced by estrogen and progesterone. Although it seems likely that estrogen treatment acts to alter the responsiveness to hormones of those neural cells which determine sexual behavior, it must be admitted that the actual mode of action of the estrogen administered in infancy is obscure.

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15 May 1963

Respiration of Heart Muscle as Affected by Oxygen Tension

Abstract. *Isolated cat papillary muscles were exposed to gas mixtures containing carbon dioxide and oxygen. In muscles exposed during the second of three periods to 25 percent oxygen, the volume of oxygen consumed* (Q_{O_2}) *was depressed by 50 percent, but returned to the control level during a third period at 98 percent oxygen. The work capacity was not significantly altered if the partial pressure of oxygen was low.*

The close correlation usually found between blood flow and oxygen consumption in various organs and tissues of the body has usually been interpreted to mean that blood flow is adjusted to the demand for oxygen. However, as discussed previously (*1*), evidence has been accumulating that the reverse may also be true, that is, the blood flow may normally impose a limitation on oxygen uptake (*2*). This evidence suggests that some oxidative metabolic reactions may "run-free," simply generating heat, if blood flow exceeds a certain minimum level.

We have attempted to test the hypothesis in vitro by making the assumption that it is the partial pressure of oxygen (pO_2) rather than some other constituent of the blood which is the limiting factor. If the Q_{O_2} (mm^2 O_2 STP/mg [dry wt.] per hour) of the isolated papillary muscle from the cat heart varied directly with the pO_2 in the medium without affecting the capacity to perform mechanical work, the hypothesis would be tenable, although far from proven.

The techniques used have been described previously in detail (*1*, *3*). Briefly, each of two muscles was mounted in a respirometer which allowed the simultaneous measurement of oxygen consumption and mechanical work. A third respirometer served as a control. The respirometers contained bicarbonate-buffered medium held at a constant temperature of 31.4 ± 0.01°C, and at a *p*H of 7.3 ± 0.1. Pardee's solution (*4*) was used in the "absorbed" cups to maintain the pCO_2 at about 2 percent. Since the gas phase consisted of 4 to 5 cm^3, little change in pO_2 with time might be expected. Even so, for technical reasons, the pO_2 in the chambers may have been slightly lower than in the aerating gas.

A day's run, which lasted about 7 hours, consisted of three periods of 2 hours each. Each period was preceded by 15 minutes of gassing with 2 percent CO_2 and either 98 or 25 percent O_2. After the gas was turned off, an hour was allowed for stabilization, after which O_2 readings were taken for three or more 20-minute intervals. The constancy of the 20-minute readings suggested that a steady state had been attained. At the end of the first gassing with 98 percent O_2 and hourly thereafter, the muscles were stimulated to contract 10 to 15 times (stimulation frequency of 30 per minute) while lifting a near maximal load, and the work for each contraction computed. The work capacities reported below are, in order, those measured after the first gassing with 98 percent O_2, and at the end of the second and third periods. At the end of the experiment, the muscles were measured, dried overnight at 100° C, and weighed.

In a series of 13 control muscles (Fig. 1, light stippling) exposed to 98 percent O_2 in the three periods, the Q_{O_2} and work capacity changed little with time.

In the experimental series of 18 muscles (Fig. 1, dark stippling) the Q_{O_2} fell significantly when the muscles were exposed in the second period to 25 per cent O_2. Upon regassing with 98 percent O_2 in the third period the Q_{O_2} returned to the control level, within the limits of error, suggesting that no damage to respiratory enzymes had occurred in 25 percent O_2 (see *5*). On the other hand, the work capacity of the muscles in 25 percent O_2 did not fall significantly, indicating that the contractile mechanism was not impaired.

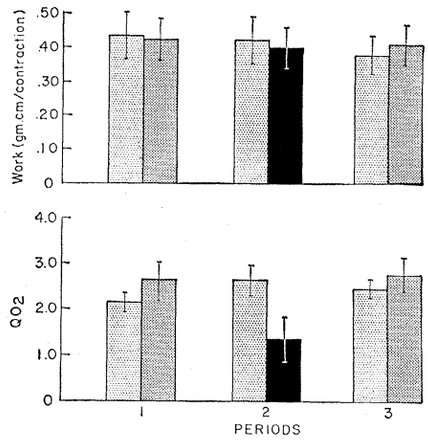

Fig. 1. Mean work capacity (by brief test) and Q_{O_2} (average of three or more 20-minute intervals succeeding stabilization) from 13 muscles (light stippling) exposed to 98 percent O_2 throughout; and 18 muscles (dark stippling) exposed in the middle period to 25 percent O_2 (solid block). Bars indicate the standard error ± 1.

In view of the hypothesis that the pO_2 may limit the Q_{O_2} it might be expected that muscle size would be a critical factor. Indeed, very thick muscles did have a lower Q_{O_2} (Fig. 2, top). On

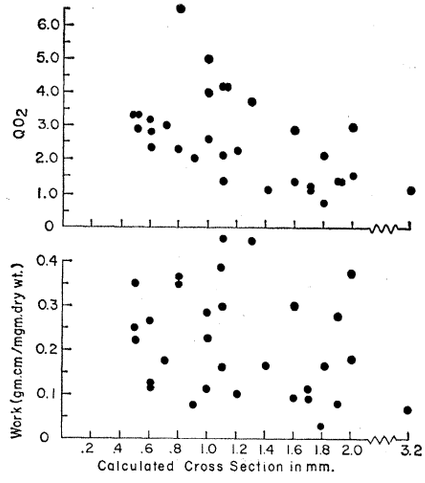

Fig. 2. (Top) Q_{O_2}, average of periods 1 and 3 at 98 percent O_2) plotted against the approximate cross-sectional area, determined from the ratio of the calculated wet weight to the length, where the wet weight to dry weight ratio was assumed to be 4.1 (*14*). (Bottom) Mechanical work and approximate cross-sectional area.