

## OBSERVATIONS ON THE RELATION BETWEEN SUCKLING AND THE RATE OF EMBRYONIC DEVELOPMENT IN MICE

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### INTRODUCTION

The present paper embodies the results of two separate but simultaneously conducted experiments, both designed to elucidate further the problems presented by the lengthened gestation period in mice which are pregnant and at the same time suckling a previous litter. Daniel ('10), working with such animals, obtained results which showed an almost constant relation of one day added to the gestation period for each animal suckled; the present writer ('16), however, in a somewhat similar series of experiments, obtained results which showed no correlation between the length of the gestation period and either the number of young suckled or the number of embryos carried (cf. also table 3). This work did, however, reveal the fact that when more than two young are being suckled the implantation of embryos instead of occurring on the fifth day after fertilization usually is delayed until the fourteenth day, during which period the blastulae lie free in the lumen of the uterus. The cause of this delayed implantation was tentatively stated in that paper to be due to an inhibition of some sort exerted by the fully activated mammary glands upon the uterine mucosa. The testing out of this hypothesis was the purpose of the following experiment.

### INTERRUPTED SUCKLING AND THE RATE OF EMBRYONIC DEVELOPMENT

The program for this experiment was to take healthy female mice which had just given birth to litters, pair them for twenty-four hours with healthy males, and then remove all but one of the

suckling young at intervals varying, with different females, from one to thirteen days. One young animal was left in each case to avoid too violent a reaction upon the lactating organs, and the presence of a solitary suckling animal has been found to have no influence upon the length of the gestation period. The females were all killed on the thirteenth day after the birth of the suckling young, and any embryos then present were sectioned and their age determined by reference to a check series of known age from non-suckling females (Kirkham, '16). The results of this experiment are set forth in table 1 and should be studied in two groups. The first group, cases when the full litter was suckled one to six days, embraces a period before the next set of embryos were ready to implant, while the second group, the remainder of the table, covers an interval when the blastulae were being in-

TABLE 1

*Data of all mice used in an experiment to determine the effect on developing embryos of removing all but one of the suckling young. Unless otherwise stated, the full number of young born were suckled until removed for the purpose of the experiment. Stage of development of embryos is in terms of actual age of similar embryos from non-suckling females where thirteenth day post-partum equals twelfth day of embryonic development. All the females were killed on the thirteenth day after the birth of the suckled young*

SERIAL NUMBER	DAYS SUCKLED	NUMBER OF YOUNG	NUMBER OF EMBRYOS	STAGE EMBRYONIC DEVELOPMENT	REMARKS
				days	
J 22	1	6	6	7	1 young died
J 21	2	7	6	11	
J 26	3	6	1	12	
J 27	3	6	8	7	
J 24	4	7	3	8	
J 25	4	6	6	10	
J 18	5	10	7	11	
J 17	6	7	11	8	
J 28	6	5	5	10	1 young died
J 29	6	4	6	10	
J 16	7	8	12	6	
J 12	8	12	10	7	
J 13	9	5	6	6	1 horn uterus lost
J 10	10	7	9	6	
J 11	11	7	4+	4	
T 14	13	5	9	4	

hibited from implanting. The importance of this grouping is apparent when one considers that in the first group of cases in every instance an interval of from a few hours to several days intervened between the end of suckling by the full litter and any readiness of the embryos to implant. Embryos in the second group, on the contrary, were presumably all prepared, before the removal of the suckling young, to implant themselves, and did so as soon as the inhibition acting upon the uterine mucosa fell below a certain minimum.

Analysis of the data, with these facts in mind, reveals the interesting fact that while some sets of embryos (J 18, 21, and 26) are as fully developed as control series when no young were being simultaneously suckled, others show a lag in development of from one to four days. No such diversity of results has been found by the writer in series of embryos from non-suckling females, and furthermore, it is evidently not directly correlated with the number of young suckled (cf. J 26 and J 27), the number of embryos (cf. J 21 and J 22), or with the combination of these two numbers (cf. J 18 and J 27). There remains the explanation that the irregularity is due to an individual variation either in the strength of an inhibitory influence exerted by the mammary glands upon the uterus or in the susceptibility of the uterus to such influence. Evidently some individuals are so constituted metabolically that the inhibition is rapidly and entirely neutralized, so that if even a short interval elapses between cessation of full mammary activity and the arrival of the eggs in the uterus, implantation and further embryonic development proceed as though no young had been suckled, while in other individuals an even longer interval still leads to delay in embryonic growth. The possible existence of such an inhibitory influence of the activated mammary glands upon the uterine mucosa has previously been shown by the experiments of Adler ('12), who found that repeated injection of extracts of mammary gland into pregnant guinea-pigs and rabbits arrested the development of embryos and often produced abortion.

## CONSTANT NUMBER SUCKLING AND THE DEVELOPMENT OF EMBRYOS

The second method of attacking the problem of prolonged gestation in suckling female mice was to determine whether or not, if the number of young suckled was constant, the stage of embryonic development could at any given time after fertilization be theoretically determined. Four was chosen as the constant number, since it was neither the smallest size of litter known to prolong gestation nor, on the other hand, was it such a large number as to prevent the use of nearly all of the available material.

Females who had just given birth to litters of four or more young had the male already present removed, together with any excess number of young. This was done the morning following the birth of the litter, and at varying intervals thereafter the females were killed, the eggs or embryos obtained from these females were sectioned, and their stage of development determined, the assembled data being shown in table 2.

The first notable feature in table 2 is the delay in implantation, no implanted embryos being found before the twelfth day after ovulation, a confirmation of work published by the writer in a previous paper ('16). After the twelfth day of gestation there appears the same lack of correlation, as noted above in connection with table 1, between their stage of development and the time of ovulation, and since if degeneration is going to occur it always takes place at the stage of four or five days' development, this possible factor can be ruled out. In other words, we have here proof of the fact that the irregularities in the rate of development of embryos in females which are simultaneously pregnant and suckling is due, only in small measure, if at all, to the number of young suckled, for if the number suckled and the rate of embryonic development were correlated the data in table 2 should indicate a regular correlation between stage of development and age of embryos, and such is not the case.

The explanation of this failure to correlate the ratio of embryonic development in suckling females with either the size of the

TABLE 2

*Data of all mice used in an experiment to determine the effect on developing embryos of the suckling of a fixed number of young. In this experiment all litters within twelve hours of their birth were reduced to four young. Stage of embryonic development in terms of actual age of similar embryos from non-suckling females*

SERIAL NUMBER	SIZE OF LITTER	AGE OF SUCKLED YOUNG	STAGE EMBRYONIC DEVELOPMENT	DELAY
		<i>days</i>	<i>days</i>	<i>days</i>
T 75	4	3	2	0
T 11	4	4	3	0
T 70	4	5	4	0
T 24	4	6	4	1
T 63	8	9	4	4
T 67	8	10	4	5
T 43	4	11	4	6
T 42	4	12	4	7
T 66	5	13	4	8
T 62	8	14	4	9
T 15	4	15	10	4
T 54	5	16	7	8
T 65	6	17	9	7
T 60	5	18	5	12
T 56	4	19	6	12
T 68	8	20	14	5
T 73	4	21	18	2
T 61	5	22	9	12
T 57	6	23	13	9
T 59	8	24	11	12
T 71	7	26	17	8
T 72	9	26	19	6

litter or with any other factor is to be sought in the first part of this paper, where the irregular influence of the termination of suckling by a full litter is conclusively shown. Add to this the fact that under normal circumstances the suckling young begin to eat solid food at about the time implantation occurs in females which are simultaneously pregnant, and the evidence here presented all indicates that after full suckling ceases, whether by removal of the litter or weaning matters not, the inhibition to implantation is withdrawn or overcome at a widely different rate in different females. Therefore, even when the number suckled is the same, the sets of embryos show no constant rate of develop-

TABLE 3

*Data regarding the length of the gestation period in mice simultaneously pregnant and suckling young. The gestation period in non-suckling females averages twenty days*

NUMBER SUCKLED	GESTATION PERIOD OF EMBRYOS	DELAY	REMARKS
	<i>days</i>	<i>days</i>	
1	20	0	A male was present with each female when the young were born, and in each case the male was removed 24 hours later
1	20	0	
2	19	0	
2	20	0	
3	20	0	
3	29	9	
3	30	10	
4	31	11	
4	30	10	

ment, nor, as a consequence, is the period of gestation a matter that can be figured out in advance, except with broad limitations (table 3).

#### SUMMARY

1. In mice simultaneously suckling and pregnant the removal of all but one of the suckling young at any time during the first six days after the birth of the suckling litter leads in some instances to implantation of the embryos as soon as they reach the uterus; in other instances the implantation is more or less delayed. These varying results can be correlated neither with the time of removal nor with the number of young taken away.

2. In mice simultaneously suckling and pregnant the removal of all but one of the suckling young at any time from seven to fourteen days after the birth of the suckling litter regularly results in implantation being delayed, but the exact extent of this delay can be correlated neither with the exact time of removal nor with the number of young removed, although removal during the earlier part of the period in question does hasten implantation as compared with the time required if the young had continued to suckle.

3. The facts in the above paragraphs justify the statement that full activity of the mammary glands is the chief cause of

delayed implantation in the case of mice which are suckling young, and also that this influence of the mammary glands is subject to marked individual variation.

4. The suckling by pregnant female mice of the same number (four in this experiment) of young will not necessarily lead to either synchronous development of embryos or the same length of gestation periods. The only explanation that can be offered at the present time for this lack of uniformity is the individual variation, noted in the preceding paragraph, of the inhibition from the mammary glands or in the strength of the counteracting forces, probably due to metabolic idiosyncrasies.

#### LITERATURE CITED

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