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Factors Associated with the Incidence of Infantile Diarrhea in Mice.* (20048)

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Infantile diarrhea in man and other mammals is classified as an infectious disease but searches for common, specific agents have failed. Etiology of infantile diarrhea in mice has been attributed to a Salmonella microorganism in one instance(1) and a virus in another instance(2). Both reports further indicate that a variety of factors such as temperature, diet, season, infection, and physiological condition of parents and offspring may, singly or cumulatively, be influential in determining the severity of an epidemic. Infantile diarrhea is prevalent in many colonies of experimental mice and presents a serious handicap for standardizing experimental animals.

Methods and materials. A colony of C3H mice was observed for symptoms of diarrhea in suckling animals between September 14, 1949 and January 17, 1950. More than 900 animals were born during this period of which almost 30% displayed severe diarrhea before they were 18 days old. Symptoms of diarrhea varied from a mild condition recognizable only by the finding of fecal material adhering to the under surface of the tail, to the passage of large amounts of yellowish, watery, feces. The intensity of the disease varied within litters, but the onset of symptoms usually occurred between 8 and 14 days. The mice were kept under conditions typical in our laboratory, *i.e.,* 75°F, exhaust ventilation and water and Purina Fox Checkers *ad libitum.* Clean dry pens of wood were provided on Tuesday of each week. Individual pairs of breeders were kept together at all times. Notation concerning the presence of diarrhea was made on the parent's pedigree card. The subsequent analysis of data was made on the basis of litters as units. Correlations were made (a) between litters born just before and just after clean pens were supplied (b) between litters born to females of different ages (c) between litters of different parity (d) between litters of different size and (e) between litters born in 3 successive 5-week periods. Significance of differences in these correlations was tested by the χ^2 test for homogeneity.

Results. Data were obtained from 76 pairs of C3H mice and 900 young born to them during the period of 4 months (Table I). Thirty-seven of the pairs gave birth to 62 litters, including 350 young, none of which showed appreciable symptoms of diarrhea. The remaining 39 pairs had 92 litters, which included 550 young, of which 46 litters (50%) were discarded due to severe diarrhea. The overall incidence was about 30% of the young born. Although the incidence of diarrhea remains high the colony of animals still prospers. The following tabulations and cor-

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TABLE I. Summary of Observations Made on Litters Born between 9/14 and 1/7.

	No. of pairs of parents	No. of litters observed	No. of litters affected	%
Parents which had no diarrheal litters	37	62 (350 young)	0	—
Parents which had at least 1 diarrheal litter	39	92 (550 ")	46	50

relations are based on the 92 litters born to those 39 pairs of parents which had at least one litter with diarrhea during the observation period.

The first correlation was made by grouping the 92 litters according to the part of the week in which they were born, *i.e.*, before or after the weekly supply of clean boxes was put in the colony. This was done to see if the disease might be partially attributable to birth of young animals in established but dirty pens, or in pens recently disrupted but clean. The comparison showed that 56% and 50% of young contracted diarrhea in the 2 groups respectively, therefore no obvious influence of the relative condition of the nest was demonstrated.

Correlations made between incidence of diarrhea and the age of the parents (Table II) showed an inverse relationship between the two. Animals in litters of the younger parents (60 days to 120 days) had a greater tendency to contract the disease than did litters of parents over 120 days, 58% and 40% respectively. A second correlation was made between the incidence of diarrhea and the seriation of the litter (Table III). Here also an inverse relationship existed between the frequency of the disease and the factor being correlated; the young in litters of the early parities, 1 and 2, had a greater tendency to contract the disease than did those of later parities, 3 through 6. Thus it was demonstrated that a higher incidence of diarrhea occurred in litters born to young females and in early litters. As the seriation of the litter and the age of the parents are related, more data would be necessary to separate these two factors.

Correlations made between occurrence of diarrhea and size of litter (Table IV) indicated a direct relationship between the two; the percentage of the litters affected increased directly with their size. Half of the litters of 6 or more contracted diarrhea as compared to 28% of litters of less than 6 animals. The 3 foregoing observations indicate that the physiological condition of the parents, *viz.*, age, parity, and litter size, was implicated with the appearance of the disease.

The 4-month period of observations was

TABLE II. Distribution of Diarrheal Litters in Relation to Age of Mother.

Age of parents (days)	No. of litters observed	No. of litters with diarrhea	% with diarrhea
60–90	31	18	58
90–120	19	11	
121–150	20	10	40
151–180	7	3	
181–200	3	2	
>200	12	2	

TABLE III. Distribution of Diarrheal Litters in Relation to Seriation of Litters.

Serial order of litters	No. of litters observed	No. of litters with diarrhea	% with diarrhea
1	27	15	58
2	25	16	
3	16	6	38
4	10	5	
5	9	3	
6	5	1	

TABLE IV. Distribution of Diarrheal Litters in Relation to Size of Litter.

Size of litter	No. of litters observed	No. of litters with diarrhea	%
3	8	3	28
4	7	0	
5	10	4	
6	17	7	50
7	11	5	
8	11	7	
9	5	3	
*	23	17	

* Not recorded at birth.

TABLE V. No. of Diarrheal Litters during Each of Three Periods.

5-wk periods	No. of litters observed	No. of litters with diarrhea	% with diarrhea
1 (9/4 -10/22)	23	11	48
2 (10/23-11/29)	30	12	40
3 (11/30- 1/7)	39	23	59

TABLE VI. Frequency of Diarrhea in Litters during Periods 1 and 2 and 3; Comparisons with Size and Seriation of Litters and Age of Parents.

		Periods 1 and 2	Period 3
Size of litter	1–5 young	5/23†	2/7
	6–9 "	10/26	12/18
	*	8/9	9/14
Serial order of litters	1–2	15/31	16/21
	3–6	8/22	7/18
Age of parents (days)	60–120	13/30	10/23
	>120	16/20	7/19

* Not recorded at birth.
† Numerator = litters with diarrhea; denominator = litters observed.

divided into 3 portions of approximately 5 weeks each and the number of litters with and without diarrhea tabulated for each period. The results of the grouping are shown in Table V. There was an increase from 48 and 40% of the litters affected in each of the 2 early periods to 59% in the last period, *i.e.*, during December and the first week of January.

Discussion. The data collected for a 4-month period from a colony of C3H mice in which diarrhea was endemic indicated that the physiological condition of the parents, *viz.*, age of parents, seriation of litters, and size of litters, had influence upon the occurrence of diarrhea in their offspring. Increased incidence of diarrhea in December may have been due partially to birth of a larger proportion of a) early parities b) large litters and/or c) litters born to young females. Even when litters in early parities and from young parents were compared, the incidence of diarrhea was higher in December, period 3. Table VI shows that during this period there was also a higher incidence of the disease in litters born to older females and in litters of small size (3 young or less). A temporal factor evidently was present therefore, during the period observed, but this was superimposed upon other contributing factors which have been classified under physiological condition of parents. The higher incidence of the disease in December suggests that season might influence its expression. This may lead to reduction or elimination of the disease by more rigid control of the seasonal factors.

The condition of the pen in relation to cleanliness, as indicated by the part of the week during which the young were born, was found not to be associated with a difference in the incidence of diarrhea. The data show that neither the soiled condition of the pen nor the neglect of newborn at or near time of birth differentially affected the incidence of diarrhea.

Some factors possibly related to the disease which were not examined include change of food formula with new shipments, variations in the supply of bedding and introduction of infectious agents.

Summary. 1. Observations on 900 C3H mice born to 76 parents showed that 30% of the young contracted diarrhea severe enough to make it necessary that they be discarded. The 46 litters so affected comprised 50% of the offspring from 39 pairs of mice. 2. Observations on the 39 pairs of parents which had one or more litters afflicted with diarrhea showed that the litters born before and after clean boxes were supplied were not differentially susceptible to diarrhea. 3. Correlations between the incidence of diarrhea and age of parents, and seriation of litters and size of litters, showed that each of these had some influence on the numbers of litters showing symptoms of diarrhea. Litters from young parents having early parities and large litters showed highest incidences of the disease. The physiological condition of the parents common to these three factors seems to be an important consideration. 4. A higher incidence of the disease was observed in December than in either October or November. Part of the increased incidence in December was due to a greater number of large litters, early parities and younger parents. Superimposed upon physiological condition of the parents were other factors which were tentatively called

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Effect of Butazolidin[R]* upon the Fate of I^{131} in the Rat. (20049)

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During the course of the evaluation of other drugs upon the thyroidal accumulation of iodine in patients, it was observed that a patient being given butazolidin[R] had a very low I^{131} thyroid uptake. In this study rats were used in order to measure more completely the effect of this drug upon iodine metabolism.

Methods. Young albino rats were given butazolidine[R] by subcutaneous injection at dosage levels ranging from 8 to 200 mg/kg body weight. In some groups of animals this was followed immediately by a tracer dose of 5 μc of I^{131} intraperitoneally. No additional iodide was added in these studies; thus the administration of the I^{131} served only to label the iodide pool of the body. The total amount of iodide present in 5 μc of I^{131} is calculated to be less than 1×10^{-3} μg. As will be noted in Table I, the uptake of I^{131} by control animals varied as much as 100%. Although the experimental and control animals for each group are of the same lot and origin, both Sprague Dawley and Slonaker strains of rats were employed in the several experiments combined in Table I. In our hands, Sprague Dawley rats recently received from Wisconsin have much higher thyroid uptake of I^{131} than that observed in rats bred locally. We believe that this merely represents a smaller iodide pool in rats imported from the Midwest since this higher uptake is reduced to that seen in local rats after a period of several weeks in their new environment. These animals were sacrificed 20 hours following I^{131} administration. Other groups of rats were given the drug daily for periods of 10 to 16 days in which the last injection of the drug was followed by 5 μc of I^{131}. After sacrifice selected organs of all of the rats were measured for I^{131} with a scintillation counter in which the error in measurement was less than $\pm 4\%$. The geometry of the scintillation counter was such that 8% of all of the γ disintegrations occurring in the sample was observed as counts. The beta particles associated with I^{131} decay were completely absorbed with a 200 mg cm^{-2} aluminum filter and were not seen by the counter. Because of this, the wet tissues were counted directly for I^{131} by placing them in metal dishes 5 cm from the crystal of the counter. Corrections for mass absorption or small differences in geometry were not necessary when the samples were counted in this manner. The tissues of animals which were given butazolidine[R] for longer periods of time were also examined for pathological changes and compared to the controls.

Results. The data as summarized in Table I show that the administration of butazolidine[R] once to rats will reduce the amount of a test dose of I^{131} accumulated by the thyroid. The degree of reduction is directly related to the quantity of the drug given. More of the I^{131} given was excreted in the urine of the animals receiving the drug than by the controls. This amounted to 167, 171, 191, 400% of normal for the 1, 4, 10 and 20 mg dose levels respectively. Relatively less of the I^{131} tag was observed in plasma, kidney, GI tract plus contents, liver and skin in the group receiving 20 mg of the drug and it was observed to be about 50% of normal as shown in Table II.

* Butazolidin[R] is 3,5-dioxo-1,2-diphenyl-4-n butyl-pyrazolidin. Butazolidin[R] was synthesized and supplied by Geigy Co., New York.