EPIDEMIC DIARRHEAL DISEASE OF SUCKLING MICE

III. The Effect of Strain, Litter, and Season upon the Incidence of the Disease*

By F. SARGENT CHEEVER, M.D., and J. HOWARD MUELLER, Ph.D.

(From the Department of Bacteriology and Immunology, Harvard Medical School, Boston)

(Received for publication, May 29, 1948)

An epidemic diarrheal disease of 2 to 3 weeks old suckling mice in the breeding colony of this laboratory has been described in previous papers (1, 2). No recognized bacterial pathogen was isolated from these animals and inflammatory changes in the large and small bowel were minimal or absent. The finding of intranuclear inclusion bodies in the epithelial cells of the villi of the small intestine (2) strengthened the supposition that the disease might be fundamentally one of viral origin. Before this theory could be subjected to adequate experimental trial an unfortunate accident in the animal house wiped out practically the entire colony.

During the course of our work it was brought to our attention that diarrheal disease of suckling mice was endemic in the colonies of several other breeders. It was determined to introduce one or more of these strains of mice into our laboratory in order to study the occurrence of the diarrheal syndrome in these animals with an eye to determining whether the etiology of the disease was single or multiple. Furthermore it was of primary importance to determine the relative resistance or susceptibility of the various strains; for if a highly susceptible strain were discovered it would greatly facilitate the carrying out and evaluation of transmission experiments. Conversely the finding of a relatively resistant strain would permit us to supply the number of mice required by the other activities of the laboratory with a minimum of loss.

EXPERIMENTAL

The only survivors of our original (Harvard) stock that remained after the accident in the Animal House were nine females and one male which had been transferred prior to the event to an isolated room in the Medical School. Here they had been kept in close contact with a littler of strain C white mice obtained from the National Institute of Health, Bethesda, Maryland, through the kindness of Dr. H. B. Andervont. Diarrheal disease had been endemic among the suckling mice of this colony for some time. When it was realized that further work under conditions identical with those that had prevailed in 1944–45 was impossible because the few survivors of the original stock had already been exposed to the possibility of superinfection from an extraneous source, it was decided to introduce additional strains of mice without further delay in order to broaden the scope of the experiment. The four strains finally selected were:

* Aided by a grant from The National Foundation for Infantile Paralysis.

1. The so called Harvard strain of Swiss white mice bred in our own laboratory.

2. The C strain of white mice raised at the National Cancer Institute, National Institute of Health, Bethesda, Maryland.[1] Epidemic diarrheal disease of suckling mice was prevalent in this laboratory, but this strain had been found to be relatively resistant to it.

3. The CFW strain of Swiss white mice raised by Carworth Farms. Diarrheal disease was reported to be present among the suckling mice of this colony.

4. The Schwentker strain of Swiss white mice raised by Mr. Victor Schwentker of Tumblebrook Farms, Brant Lake, New York. So far as was known this colony was free of diarrheal disease.

Attempts to establish a fifth strain, the C3H strain of brown cancer mice from the National Institute of Health,[1] failed because of the extreme susceptibility of the young mice to fatal diarrheal disease, and the tendency of the adults to develop spontaneous tumors.

These mice were quartered in a common room, cared for by the same attendant, and fed the same diet. No precautions were taken to prevent the spread of infection from one strain to another, although each continued to be bred as a pure line strain.

By the study of the colony over the course of a year, it was hoped to investigate the following problems:

1. The relative susceptibility of the four strains to the disease or group of related diseases.

2. The general validity of our observation in one strain (Harvard) that first litters were significantly more susceptible than later ones.

3. The seasonal incidence of the disease or diseases in the five strains. Experience in at least one other breeding colony had suggested that the incidence of the obvious signs of disease was significantly lower during the summer months.

25 to 28 females of each strain were selected and bred. Following the weaning of the first litters the mothers were bred again. This process was repeated until each female had produced five litters.[2] Each breeder was properly identified and a careful record was kept of the history of the successive litters.

Diarrheal disease appeared promptly among the suckling mice of all five strains and continued with varying degrees of severity throughout the course of the year. The disease in all strains appeared indistinguishable from that seen in the original stock. The same sharply limited age incidence was noted. Once more no recognized bacterial pathogens were isolated and again inflammatory lesions of the large and small bowel were minimal or absent. As has, however, been previously reported (2), no intranuclear inclusion bodies were noted, but in contrast to our original observations many of the mice showed *cytoplasmic* inclusion bodies in the epithelial cells of the small intestine. These pathological findings are reported in detail in an associated communication (3).

Since the great majority of deaths that occurred among the suckling mice

[1] The C strain and the C3H strain of mice now being bred in our laboratory each originated from a single litter of mice sent us by Dr. H. B. Andervont of the National Institute of Health. Our experience with them therefore may not be entirely representative of the parent strain.

[2] During the course of the year an occasional female was eliminated because of unrelated disease, tumor, or comparative sterility.

during the 2nd and 3rd weeks of life were obviously due to the effects of the diarrheal disease, the percentage of mice of any given litter that were weaned formed a fair index of the severity of the process. Mice dying during the 1st

TABLE I

Per Cent of Mice Weaned, by Strain

Strain	Born	Weaned	Per cent
Schwentker	924	546	59.1 ± 1.62
Harvard	821	589	71.7 ± 1.57
C	845	527	62.4 ± 1.67
CFW	987	501	50.8 ± 1.59
Total	3,577	2,163	60.5 ± 0.82

TABLE II

Per Cent of Mice Weaned, by Litter

Litters	Born	Weaned	Per cent
1st	603	221	36.7 ± 1.97
2nd and 3rd	1,599	956	59.8 ± 1.22
4th and 5th	1,375	986	71.7 ± 1.41
Total	3,577	2,163	60.5 ± 0.82

TABLE III

Per Cent of Mice Weaned, by Month

Period	Born	Weaned	Per cent
Aug.–Sept., 1946	144	125	87.7 ± 2.74
Oct.–Nov., 1946	654	240	37.1 ± 1.89
Dec.–Jan., 1946–47	997	606	60.8 ± 1.55
Feb.–Mar., 1947	868	469	54.0 ± 1.69
Apr.–May, 1947	604	454	75.1 ± 1.76
June–July, 1947	252	222	88.1 ± 2.04
Aug.–Sept., 1947	58	47	81.0 ± 5.15
Total	3,577	2,163	60.5 ± 0.82

week of life were excluded from the calculations. To the number of mice dying between the 7th day and the 22nd day of life (the time at which the young mice were weaned) were added those relatively few mice that had to be sacrificed at the time of weaning because of the presence of severe obstipation, the commonest complication of the disease. This figure represented the total number

TABLE IV

Per Cent of Mice Weaned, by Strain, Litter, and Date of Birth

	1946 Aug.-Sept.			1946 Oct.-Nov.			1946–47 Dec.-Jan.			1947 Feb.-Mar.			1947 Apr.-May.			1947 June-July			1947 Aug.-Sept.		
	B.	W.	Per cent	B.	W.	Per cent	B.	W.	Per cent	B.	W.	Per cent	B.	W.	Per cent	B.	W.	Per cent	B.	W.	Per cent
Schwentker																					
1st				113	10	8.8															
2nd and 3rd				136	77	56.5	292	239	81.8												
4th and 5th							20	18	90.0	257	123	47.8	106	79	74.5						
Harvard																					
1st	86	76	88.3	83	38	45.7															
2nd and 3rd				94	40	42.5	184	126	68.5	86	52	60.4									
4th and 5th							38	37	97.4	97	81	83.5	147	133	90.5	6	6	100.0			
C																					
1st	24	23	95.8	49	19	38.9	70	29	41.4												
2nd and 3rd	5	1	20.0	77	44	57.1	144	76	52.8	93	73	78.5	67	31	46.2						
4th and 5th							52	33	63.5	78	39	50.0	104	85	81.7	82	74	90.2			
CFW																					
1st	29	25	86.3	45	0	0.0	32	1	3.1	72	0	0.0									
2nd and 3rd				57	12	21.0	128	30	23.5	98	52	53.1	89	58	65.1	49	45	92.0			
4th and 5th							37	17	45.9	87	49	56.3	91	68	74.7	115	97	84.4	58	47	81.0

TABLE V

Per Cent of Mice Weaned, by Strain

(Period and litter constant)

Litters	Schwentker	Harvard	C	CFW
	per cent	*per cent*	*per cent*	*per cent*
1st litter				
Aug.–Sept., 1946		88.3	95.8	86.3
Oct.–Nov., 1946	8.8	45.7	38.9	0.0
Dec., 1946–Jan., 1947			41.4	3.1
2nd and 3rd litters				
Oct.–Nov., 1946	56.5	42.5	57.1	21.0
Dec., 1946–Jan., 1947	81.8	68.5	52.8	23.5
Feb.–Mar., 1947		60.4	78.5	53.1
Apr.–May, 1947			46.2	65.1
4th and 5th litters				
Dec., 1946–Jan., 1947	90.0	97.4	63.5	45.9
Feb.–Mar., 1947	47.8	83.5	50.0	56.3
Apr.–May, 1947	74.5	90.5	81.7	74.7
June–July, 1947			90.2	84.4

of deaths attributed to the disease. To it was added the number of mice successfully weaned from each litter in order to obtain the total number of mice exposed to the risk of death due to the disease.

Since the purpose of the experiment was to determine the effect of strain of mice, of litter (first as opposed to later ones), and of season upon the mortality of the weanling mice, the data were first analyzed from these points of view.

TABLE VI

Per Cent of Mice Weaned, by Litter

(Strain and date constant)

Month	1st Litter mice weaned	2nd and 3rd litter mice weaned	4th and 5th litter mice weaned
	per cent	*per cent*	*per cent*
Schwentker			
Oct.–Nov., 1946	8.8	56.5	
Dec., 1946–Jan., 1947		81.8	90.0
Harvard			
Oct.–Nov., 1946	45.7	42.5	
Dec., 1946–Jan., 1947		68.5	97.4
Feb.–Mar., 1947		60.4	83.5
C			
Oct.–Nov., 1946	38.9	57.1	
Dec., 1946–Jan., 1947	41.4	52.8	63.5
Feb.–Mar., 1947		78.5	50.0
Apr.–May, 1947		46.2	81.7
CFW			
Oct.–Nov., 1946	0.0	21.0	
Dec., 1946–Jan., 1947	3.1	23.5	45.9
Feb.–Mar., 1947	0.0	53.1	56.3
Apr.–May, 1947		65.1	74.7
June–July, 1947		92.0	84.4

In Table I, the percentages of mice weaned are given by strain. The Harvard strain appears to be the most resistant and the CFW mice the least so; the individual differences noted between these four groups are statistically significant except in the case of the Schwentker and C strains.

When grouped according to litter as in Table II, significant differences appear between first litters as opposed to second and third, and to fourth and fifth litters. Approximately 1 of 3 suckling first litter mice survived to be weaned, slightly better than 1 of 2 second and third litter mice, and nearly 3 of 4 fourth and fifth litter mice. These differences are in agreement with our previous experience.

The results according to season of year are given in Table III, in which the figures are grouped in bimonthly periods according to date of birth. A high percentage of mice were successfully weaned during the late summer months of 1946. This was followed by a sharp rise in the mortality rate during the autumn months. A relatively gradual and somewhat irregular recovery occurred during the winter and spring months and the percentage of mice weaned during the summer of 1947 was high. The differences noted are for the

TABLE VII

Per Cent of Mice Weaned, by Date of Birth

(Litter and strain constant)

Litters	1946 Aug.-Sept.	1946 Oct.-Nov.	1946-47 Dec.-Jan.	1947 Feb.-Mar.	1947 Apr.-May	1947 June-July	1947 Aug.-Sept.
Schwentker							
1st							
2nd and 3rd		56.5	81.8				
4th and 5th			90.0	47.8	74.5		
Harvard							
1st	88.3	45.7					
2nd and 3rd		42.5	68.5	60.4			
4th and 5th			97.4	83.5	90.5		
C							
1st	95.8	38.9	41.4				
2nd and 3rd		57.1	52.8	78.5	46.2		
4th and 5th			63.5	50.0	81.7	90.2	
CFW							
1st	86.3	0.0	3.1	0.0			
2nd and 3rd		21.0	23.5	53.1	65.1	92.0	
4th and 5th			45.9	56.3	74.7	84.4	81.0

most part statistically significant although less striking and consistent than those presented in Tables I and II.

As was anticipated all three factors appeared to influence the results. Each one was analyzed separately in an effort to determine its relative importance. The over-all results are tabulated in Table IV, and the individual analyses in Tables V, VI, and VII.

When litter and date of birth are comparable (Table V) the only significant strain difference that appears with any degree of consistency is the relatively low percentage of CFW mice weaned. This is particularly striking in the instance when first litters of the four strains are compared, and hardly less so when second and third litters are studied. In the case of fourth and fifth

litters the differences between strains are less consistent and frequently are not statistically significant.

When strain of mice and date of birth are comparable the influence of litter upon percentage of mice weaned (Table VI) is less marked but still reasonably consistent, and many of the individual differences are statistically significant. For all strains at nearly all times of the year the mortality in first litter mice was higher than in second and third litter ones, which in turn presented a higher mortality than did fourth and fifth litter mice.

When the mice are grouped according to date of birth with strain and litter comparable (Table VII) there is in the case of the CFW strain a fairly consistent pattern which bears a close resemblance to that of Table III. A high percentage of the mice born in the summer of 1946 survived to be weaned. In the autumn of 1946 the percentage dropped precipitously. The periods of Feb. to Mar., 1947, and Apr. to May, 1947, showed a gradual rise in the percentage of mice weaned. Again a low mortality was observed during the summer months of 1947. Although but few of the individual differences are statistically significant the general trend is quite consistent. In the case of the other strains the evidence is less satisfactory. The strain C mice present a picture somewhat similar to that shown by the CFW strain but the trend is less consistent and the individual differences smaller. The data furnished by the Harvard and Schwentker strains are too fragmentary to permit the drawing of conclusions.

DISCUSSION

Our investigations have clearly confirmed the report that suckling mice belonging to the colony of the C3H strain were highly susceptible to diarrheal disease. The stock was freed of detectable infection by the delivery of the young by Caesarian section at term, and the subsequent nursing of these animals by presumably disease-free females belonging to another strain under conditions of rigid isolation. This colony is increasing slowly in size and has remained free of disease for nearly a year.

Among the four strains of mice that were observed during the course of a year significant differences were limited to the CFW strain. A lower percentage of these mice were weaned fairly consistently in comparison to the others. The three more resistant strains failed to show consistent or significant differences in the percentage of mice weaned.

The effect of litter was definite and consistent. For all four strains followed throughout the course of a year first litters fared worse than second and third ones while fourth and fifth litters showed the highest percentage of mice weaned. A possible explanation for this finding is that the breeding females may develop some degree of active immunity against the disease as a result of sustained contact with it, and that this immunity may be passed on in some degree to the young either *in utero* across the placental barrier or by the milk or colostrum.

This passively acquired immunity may persist long enough to give some protection to the weanling mice during the first 3 weeks of life. Further evidence suggesting that this does occur has been reported (1). If this hypothesis is correct artificially induced active immunization of females prior to pregnancy may be of assistance in controlling the disease in the mouse colony.

The effect of season seemed clear cut only in the case of one strain (CFW); the highest percentage of mice weaned were observed during the summer months, and the lowest during the autumn and early winter. No consistent pattern was noted among the other strains. Further work along these lines is necessary before the relative importance of season as it affects the incidence of the disease can be properly assessed.

The question of the relationship of the present disease to that seen in our original stock during the period 1944–46 remains unsettled. Although the obvious manifestations were similar in both outbreaks the finding of intranuclear inclusion bodies in one as opposed to cytoplasmic ones in the other suggests that the diarrheal syndrome may be caused by more than one viral agent. This point is taken up in greater detail in a following paper (3) which deals with the occurrence of the cytoplasmic inclusion bodies.

SUMMARY

Four strains of mice—Harvard, Schwentker, CFW (Carworth Farms), and C (National Cancer Institute)—have been kept and bred under identical conditions over the course of a year in order to determine the effect of strain, of litter, and of season upon the incidence of diarrheal disease among the suckling mice.

1. Significant strain differences were limited to CFW mice. A consistently lower percentage of these mice were weaned in comparison to the others.

2. The effect of litter was definite and consistent: In all four strains first litters fared worse than second and third ones, while fourth and fifth litters showed the highest percentage of mice weaned.

3. The effect of season seemed clear cut only in the case of one strain CFW; the highest percentage of mice weaned were observed during the summer months and the lowest during the autumn and early winter.

BIBLIOGRAPHY

1. Cheever, F. S., and Mueller, J. H., *J. Exp. Med.*, 1947, **85**, 405.
2. Pappenheimer, A. M., and Enders, J. F., *J. Exp. Med.*, 1947, **85**, 417.
3. Pappenheimer, A. M., and Cheever, F. S., *J. Exp. Med.*, 1948, **88**, 317.