Influence of Pregnancy in Mice on the Course of Infection with Murine Poliomyelitis Virus. (17730)

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It is generally believed that a majority of human beings exposed to the virus of poliomyelitis contracts the disease in sub-clinical form, only a small minority developing the characteristic paralytic type of infection. Some workers have suggested that those who escape paralysis may be protected by an "autarceologic" type of host resistance, the exact nature of which has not yet been established. Physiological fluctuations in the individual's resistance to the disease are illustrated in the possible enhanced susceptibility of women during pregnancy which has been noted by many clinical observers. A number of authors have recently attempted to evaluate by empirical or statistical methods the increased risk of infection with poliomyelitis during pregnancy(1-7). Although records of more than 380 cases may be found in the medical literature, information of epidemiological value is frequently lacking and, in many instances, inconsistent. The geographical distribution of these cases includes 3 continents, North America, Europe and Australia, the great majority originating in the United States and in the Scandinavian countries. In time, they cover a period of observation extending over 48 years. The small number included in any single outbreak has been insufficient to form a basis for adequate statistical analysis. In all events, the paucity of the data, the incomplete recording of cases and the purely empirical approach have only added to the uncertainty. Nevertheless, a general opinion has been formed that pregnancy predisposes to paralytic poliomyelitis(1,2,6-10) and, more particularly, that the pregnant woman is prone to develop the disease in its more severe manifestations, especially during the final trimester of gestation(3,4,5,11,12). On the other hand, occasional observers maintain that pregnancy alters neither the susceptibility to the disease(13) nor its course(2,14-16). A belief that women during the first trimester of pregnancy may actually be somewhat more resistant to infection was based upon disproportionately small numbers of cases which had been reported for the earlier part of pregnancy(10).

Experimental exploration of this problem was undertaken by Weaver and Steiner(17) who infected groups of pregnant and non-pregnant cotton rats with the Armstrong-Lansing C-R strain of poliomyelitis virus. Since resistant animals were found only in the group

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which had been infected during the first trimester of gestation, it was concluded that early pregnancy represents a period of increased resistance. The experiment was so designed that only an increase of resistance could be demonstrated, precluding the detection of any possible enhancement of susceptibility during the later stages. The present work was initiated in order to study experimentally the influence of pregnancy, throughout its entire course, upon the susceptibility of the white mouse to infection with murine poliomyelitis virus.

Experimental. The Virus. The infectious agent used throughout was the Col. SK strain of murine poliomyelitis virus. Now approaching the 400th serial passage in white mice, the strain has maintained its high infectivity for rodents and cynomolgus monkeys when injected by neural or extraneural routes.

The mice. The experimental animal was the Swiss white mouse, Rockland strain. Female mice weighing from 24 to 26 g were examined daily to establish the oestrous cycle(18). Upon the appearance of pro-oestrus each mouse was placed overnight with a normal male mouse. When one half of the mice had been successfully mated, the other half was set aside, unmated, to serve as control animals. Thus, the control group consisted of non-pregnant female mice of comparable age, which would also have been of comparable weight had it not been for the changes associated with pregnancy. A smaller number of mice was permitted to come to term; these were infected during the first 5 days after parturition. The experiment was planned so as to study the susceptibility of the mouse for each day of pregnancy throughout the entire gestation period. Since it was impossible to obtain, at one time, 100 pregnant mice suitably distributed in respect to their periods of gestation, the pregnant mice were infected, along with their non-pregnant controls, in small groups at various stages of pregnancy.

Infection. The inoculum was prepared from freshly harvested brains of young mice sacrificed at the height of paralysis. The brains were triturated and suspended in physiological saline at a dilution of 10^{-1}. After light centrifugation the supernatant was employed for further dilution. Orally infected mice received by gavage 0.1 ml of virus suspension at a concentration of 10^{-2}, an infection which, it was estimated, would approximate 50% lethality in the control group of mice. Apart from a total of 211 mice which were infected by feeding the virus, 2 smaller groups, comprising 19 pregnant and 19 non-pregnant animals, were infected by the intravenous route, each mouse receiving via the tail vein 0.1 ml of virus diluted 10^{-5}.

The mice were examined daily over a period of 3 weeks for the appearance of typical symptoms of paralysis or encephalitis. When mice were found dead and symptoms had not been observed, the viral cause of death was confirmed by subpassage of the brains in at least 3 young mice by the intraperitoneal route, such brains having previously been shown to be bacteriologically sterile when cultured upon ordinary laboratory media. In all, 40 mice (27% of those which died) failed to exhibit typical symptoms.

Results. Infection by the Oral Route. Of a total of 100 orally infected pregnant mice, 75 died of the infection, while in a group of 100 non-pregnant mice, 45 succumbed to the disease. When the gestation period is divided into approximate quarters of 5 days each, the following mortality rates are observed (Table I): first quarter, 52%; second quarter, 72%; third quarter, 85%; fourth quarter, 88%. Eleven mice inoculated during the first 5 days post partum had a mortality rate of 46%. The incubation period of the disease is defined as the interval between the day of inoculation and the day upon which unequivocal symptoms first appeared, or, in the absence of symptoms, the day of death. The average incubation period in the 75 pregnant mice was 5.4 days, whereas in the 45 control animals it was 6.1 days. For the 4 quarters of pregnancy the respective figures were 6.7, 4.9, 5.3 and 5.5 days. The five mice of the post partum group had a mean incubation period of 6.7 days.

Infection by the Intravenous Route. Of

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TABLE I.
Mortality Among Pregnant and Non-pregnant Mice Inoculated with Col. SK Virus.

Time of inoculation	Oral inoculation of 0.1 cc virus diluted 10^{-2}			Intravenous inoculation of 0.1 cc virus diluted 10^{-5}		
	Mortality	%	Avg incubation time, days	Mortality	%	Avg incubation time, days
During gestation:						
Days 1 to 5	11/21*§	(52)	6.7	†		
" 6 to 10	21/29	(72)	4.9	5/ 8*	(63)	7.4
" 11 " 15	28/33	(85)	5.3	‡		
" 16 " 19	15/17	(88)	5.5	10/11	(91)	4.8
Total:						
All pregnant mice	75/100	(75)	5.4	15/19	(79)	5.7
During first 5 days post partum	5/11	(46)	6.7			
Normal non-pregnant female mice	45/100	(45)	6.1	10/19	(53)	6.1

* Denominator: No. of mice inoculated; numerator: No. of mice died.
† Animals from first 10 days of pregnancy pooled.
‡ Animals from last 9 days of pregnancy pooled.
§ During first 3 days of pregnancy, 11 mice inoculated, of which 4 died (36%).

19 pregnant mice inoculated by the intravenous route, 15 (79%) died with an average incubation period of 5.7 days; 10 (53%) of 19 non-pregnant mice died with an incubation period of 6.1 days. Because of the small number of animals used in this part of the experiment, composite values for only the 2 halves of the gestation period are computed. Thus, 5 (63%) of the 8 mice infected on or before the 10th day died, whereas 10 (91%) of the 11 mice inoculated after the 10th day succumbed. The mean incubation periods for the 2 groups were 7.4 and 4.8 days respectively.

Discussion. Judging from the observed mortality rates and average incubation periods, white mice develop a gradual increase in susceptibility to oral infection with Col. SK virus beginning with the 4th day of gestation. Maximal susceptibility is reached toward the end of pregnancy, since the mortality rate at that time was very nearly twice that observed for the group of non-pregnant mice. The low mortality rate (36%) for mice inoculated during the first 3 days of pregnancy may be taken to indicate that such mice are at least as resistant as the controls, and possibly more so. Therefore the results agree well with the earlier observations of Weaver and Steiner(17) on infection with the Lansing strain in pregnant cotton rats, *i.e.* that early gestation may represent a period of increased resistance.

Since young mice, similarly infected, almost invariably die of the disease, the increased susceptibility of the adult mouse during gestation suggests a return to the maximal susceptibility characteristic of youth, and a temporary loss of the non-specific type of resistance associated with age. The transient nature of the phenomenon is illustrated by the fact that mice which are infected soon after parturition have a mortality rate which approximates closely that of non-pregnant adult females. That susceptibility to poliomyelitis may, in some way, depend upon disturbances in the endocrine balance is not a new thought. Aycock(19), for instance studied the possibility that changes in estrogen levels may be responsible for the frequent occurrence of the disease among pregnant women. When castrated immature female monkeys were treated with estrogenic substance they were found to be more resistant than untreated castrates. In these experiments the animals had been infected by the intranasal route, and it was held that changes in the nasal mucosa, resulting from elevated estrogen levels in the treated monkeys, might have inhibited passage of the virus. On the

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other hand, the average urinary estrogen levels of chronic poliomyelitis patients were found to be higher than those observed in normal individuals. The conflicting and confusing results prompted Aycock to conclude that "endocrinopathy sought as a basis of autarceologic susceptibility does not lie in a simple deficiency in elaboration of estrogenic substance, but rather in some discrepancy in its economy." A similar conflict has been noted in the case of the pregnant woman(3) and it has been suggested that fluctuations in resistance during gestation may depend upon variations in relative dominance of the manifold endocrine constituents rather than upon quantitative changes of any individual hormone in the system(20). However, the argument in favor of a protective function of chorionic gonadotropin in early pregnancy would appear to stand upon less conflicting evidence contributed by other investigators(17,21).

More recently, the influence of various endocrine substances was studied anew by Anderson and Bolin(22) in mice orally infected with the MM strain of murine poliomyelitis virus. While stilbestrol or progesterone treatment afforded a high order of protection, mice treated with t-propionate (presumably testosterone-propionate) were only partially protected, and those receiving desoxycorticosterone-acetate were as susceptible as the untreated animals. Again, it was suggested that the protective effects might have been due to alterations in the permeability of mucous surfaces preventing entry of the virus. In our work, however, there was no significant difference in the results which could be ascribed to the route of inoculation, whether oral or intravenous. Thus, our own observations serve to emphasize once more the possibility that the state of pregnancy may be associated with a systemic increase in susceptibility to poliomyelitis, irrespective of any changes that may exist at the portal of entry.

Conclusions. Pregnant mice inoculated with the Col. SK strain of murine poliomyelitis virus by the oral route showed a definite increase in susceptibility to infection as compared with non-pregnant female control animals. Beginning with the 4th day of pregnancy, mortality increased progressively and reached, with the last 4 days of gestation, a rate almost twice that of the control group. Immediately after parturition there was a rapid return of resistance to the non-pregnant level. The mechanism responsible for the greater susceptibility of the pregnant mouse does not seem to depend wholly upon alterations in the permeability of the intestinal mucosa during gestation, since a smaller group of mice infected by the intravenous route exhibited a similar increase in susceptibility.

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Further Observations on the "Animal Protein Factor." (17731)

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A previous publication(1) from this laboratory has shown that an "animal protein factor" supplement (Fraction 1) made from cultures of *Streptomyces aureofaciens* produced a growth response in chicks which was greater than the maximum growth response obtainable with vitamin B_{12}. Distillers' solubles, fish meal, fish solubles and dried brewers' yeast did not produce additional growth

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