transformation and excretion of antipyrine as determined by plasma analysis was constant over a period of time sufficient for analysis.

Ease of analysis and lack of toxicity make antipyrine a suitable substance for use in measurement of total body water in animals.

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Infection and Immunity in Offspring of Mice Inoculated during Gestation with Murine Poliomyelitis Virus. (18051)

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A study of 100 pregnant mice, inoculated at various intervals during gestation with Col. SK murine poliomyelitis virus, has shown that there is a progressive increase in susceptibility to infection which begins with the fourth day of pregnancy and reaches a maximum during the last 4 days before parturition (1). The present paper deals with observations on the young from the inoculated mothers, the data being concerned with the infection of the fetus and the immunity of surviving offspring.

Materials and Methods. Among 100 gestating mice orally inoculated with Col.SK virus pregnancy terminated in 5 different ways (Table I). In addition to surviving young from these infected mothers, 6 litters whose mothers had been infected soon after parturition, another 6 litters produced by breeding mice with known immunity, and 4 normal litters contributed to the study of immunity in young mice.

Infection in the young. Fetal tissues, and tissues from offspring of mothers which had succumbed to infection after parturition were tested for recovery of virus by intracerebral inoculation of young mice.

Immunity of offspring. Litters were examined for their immunity at various intervals between weaning and 44 days of age by intranasal inoculation of Col.SK virus diluted 10^{-2}. Older littermates were tested by intraperitoneal inoculation of virus diluted 0.5 x 10^{-2}, a challenge which served also in investigating the development of maternal immunity. Surviving offspring of paralyzed mothers were placed with litters of other lactating mice, some of which were normal, whereas others were surviving experimental animals. When it became apparent that immunity in these fostered infants corresponded with the immunity of the fostering mother, the influence of suckling with immune mice was further investigated by means of interchanging normal baby mice and the young of immune mice during the 21-day period of nursing, and subsequently testing the fostered mice for their ability to resist infection.

In vitro test of milk for its capacity to neutralize virus. Milk collected(2) from 2 immune mothers was pooled and examined at various dilutions for its capacity to neutralize Col.SK virus. Pooled milk from 2 normal lactating mice served as control. Equal mixtures of milk and suspensions of virus were incubated at 37°C for 1 hour, placed overnight in the refrigerator, and then inoculated intraperitoneally into young mice.

Results. Outcome of pregnancy. (Table I). Eighteen viable litters, born to 100 female mice orally inoculated with Col.SK virus during gestation, were apparently normal at birth. One half of the litters were reared by their own mothers which had resisted infection. The remaining 9 litters came from mothers which became paralyzed after parturition; 4 of these litters were successfully reared by foster mothers. The rate of abortion was high among mice infected

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TABLE I.
Outcome of Pregnancy in Mice Orally Inoculated with Col. SK Virus During Gestation.

Time of inoculation of pregnant mice. Gestational days	Litters of 25 surviving mice			Litters of 75 paralyzed mice				
	Living	Devoured at term	Aborted	Living	Stillborn or died shortly	Devoured at term	*In utero* at death of mother*	Aborted
1 to 5	4	0	6	0	0	0	7	4
6 to 10	1	0	7	0	0	0	18	3
11 to 15	2	2	1	3	7	3	12	3
16 to 19	2	0	0	6	5	2	2	0
Totals	9 (36%)	2 (8%)	14 (56%)	9 (12%)	12 (16%)	5 (7%)	39 (52%)	10 (13%)

* Of the litters found *in utero*, 26 gave evidence that fetal death had preceded maternal death; 21 of these came from mice inoculated between the 1st and 10th days of gestation, inclusive.

TABLE II.
Recovery of Virus from Tissues of Young.

Time of onset of maternal symptoms	Condition of litter	Recovery of virus from tissues of fetuses or young No. of tests	
		Positive	Negative
Before parturition	Died *in utero*	13	0
At parturition	Stillborn	3	1
	Sacrificed at birth	0	1
	Died at 2 days of age*	0	1
Postpartum: 24 hrs	Sacrificed at birth	0	1
	Died at 3 days of age*	1	0
48 ''	Sacrificed at death of mother	0	2
	Died at 4 days of age*	0	1
5 days	Sacrificed at death of mother	0	1

* Fostered 2 days by normal foster mother.

during the first 10 days of gestation, varying from 72% in the case of resistant animals to 88% for those that died of the disease. With inoculation carried out after mid-term, however, abortion was comparatively rare.

Infection in the young. (Table II). Fetuses recovered at autopsy from paralyzed mice were found invariably to have been infected *in utero,* whereas young mice born during the maternal incubation period of the disease as a rule escaped infection. This indicates that intrauterine infection occurs but late in the course of the maternal disease.

Immunity in the young. Resistance of young mice to intranasal infection with Col. SK virus correlated well with the development of immunity in the mother (Table III). The susceptibility of littermates examined after the age of 4 months illustrates the transient nature of the observed immunity in the young. This immunity was shown to be directly related with the immunity of the nursing mother which suckled the babies, rather than with the immunity of the natural mother (Table IV). The time required to produce immunity in baby mice through suckling with immune mothers was not determined. It was observed, however, that 5 litters which had been placed with immune mothers, not on the day of birth, but at various times between the third and eleventh days of age, were fully protected from infection. Thus, a nursing period of 10 days was sufficient to provide complete protection, and a delay of 11 days between birth and fostering with an immune mother did not preclude the development of immunity in the fostered young.

In vitro capacity of milk to neutralize virus. (Table V). Milk obtained from normal lactating mice contained no substances capable of neutralizing Col.SK virus, while

TABLE III.
Immunity of Surviving Mothers and Their Offspring.

Mothers			Offspring tested for immunity at various ages Results of inoculation with virus at:		
Time of oral infection with virus	No.	Immunity as shown by intraperitoneal test	23 to 44 days of age	66 to 68 days of age	over 119 days of age
Inoculated during pregnancy	5	Positive	0/26		4/4
	2	Negative	10/10		2/2
	1	Not tested	4/4		3/4
Inoculated soon after parturition	6	Positive	3/19*	5/7	
Inoculated during first pregnancy and rebred	3	Positive	1/12		5/6

Denominator: No. of mice inoculated; numerator: No. of mice died.
* Three mice which died comprised 1 litter.

TABLE IV.
Immunity of Young Mice Following Fostering with Normal or Immune Mothers.
(Tested between 23 and 36 days of age).

Immunity of natural mothers	Immunity of offspring. Results of intranasal inoculation with virus after suckling with:	
	Normal foster mother	Immune foster mother
Negative	7/7 (members of 3 litters)	0/16 (members of 7 litters)
Positive	5/5 (members of 2 litters)	0/6 (members of 2 litters)

Denominator: No. of mice inoculated; numerator: No. of mice died.

TABLE V.
Capacity of Milk to Neutralize Col. SK Virus *in Vitro*.

Milk		Virus dilutions	
Source	Dilution	10^{-2}	10^{-4}
Immune mice	Undiluted	S, S, S	S, S, S
	1:5	7, S, S	S, S, S
	1:10	3, 3, 4	S, S, S
	1:50	3, 3, 4	6, 8, S
	1:100	3, 4, 4	6, 7, S
Normal mice	Undiluted	2, 3, 3, 4	3, 4, 4, 5
	1:10	2, 3, 3, 3	4, 5, 5, 6

Numbers indicate incubation periods (in days) of paralyzed test mice. "S" indicates surviving test animal.

milk collected from immune mothers effectively neutralized the virus. In the absence of any other explanation, it is believed that the neutralizing principle in the milk of immune mothers was the same antibody which is usually present in the serum.

Discussion. From the data presented it would appear that murine poliomyelitis, occurring during gestation, frequently interferes with the successful outcome of pregnancy. This conclusion is based upon two observations: the low rate of productivity among susceptible animals infected during the last few days of pregnancy, and the high rate of abortion among animals inoculated before mid-term. A similarly high rate of abortion

has been observed by Byrd(3) in pregnant mice infected intracerebrally with the Lansing strain of murine poliomyelitis virus. The constant presence of virus in the fetuses of paralyzed mothers suggests the primary nature of the risk to the young *in utero* and militates against the presence of a murine placental barrier to the virus. On the other hand, the fact that living, normal litters may be born while mothers are in the incubation period of the disease indicates that embryos are well protected from infection during the initial phases of the disease process. Only one of such litters proved to be infected, and, judging from the time of onset of paralysis, probably had contracted the disease during or soon after birth rather than *in utero*.

As far as our data go, there is a close correspondence between resistance of the young and immunity of the mouse which suckles them. Similar results were obtained by Orskov and Andersen(4) who found that litters were passively protected against oral infection with Theiler-FA virus or with Col. SK virus when mothers had previously been vaccinated with the corresponding virus. No cross immunity was observed in these tests. In work with MM virus, Curley and Gordon (5) and Anderson and Bolin(6) have demonstrated the passive nature of immunity in the young and its brief duration, and have shown that such resistance is associated with the immunity of the mouse which suckles the young(7). Transfer of antibody by way of ingested milk was postulated as the mechanism responsible for the passive protection of young mice, although the transplacental route was also considered. The latter mechanism, however, granted a protection of much shorter duration(7). While, in the mouse, passive transfer of immune bodies of various types undoubtedly does occur *in utero*, the most important route in this species is thought to be by way of the milk(8,9). In view of the constant agreement obtained by *in vivo* experiments, the *in vitro* demonstration of virus neutralizing substances in the milk of immune mice is not surprising. Furthermore, the fact that milk obtained from normal mice exhibited no neutralizing capacity establishes the specific nature of the phenomenon.

Summary. 1. From 100 mice, orally infected with Col.SK virus at various times during the period of gestation, only 18 viable litters were obtained. These litters were equally divided among 25 surviving animals and 75 animals which succumbed to the disease.

2. The rate of abortion for mice inoculated during the first half of pregnancy, regardless of their capacity to resist infection, was high, being 72% for surviving animals and 88% for those which died, since two-thirds of the litters found *in utero* at the time of maternal death gave evidence of having predeceased the mother.

3. Fetuses obtained at autopsy consistently contained demonstrable amounts of virus.

4. Living young, when delivered at the time of onset of maternal symptoms, had apparently escaped infection, while a majority of stillborn young yielded virus.

5. Surviving offspring of susceptible mothers, born during the maternal incubation period, were apparently normal and usually escaped infection.

6. Litters of immune mothers, with one exception, were fully protected against intranasal infection between weaning and the age of 44 days. When tested between 66 and 68 days of age only 2 of 7 young were resistant, while after 119 days there was no evidence of immunity.

7. By means of experiments in which the offspring of immune and normal mice were interchanged during the period of suckling, immunity in the young was shown to be re-

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lated to the observed immunity in the nursing mother.

8. Milk collected from normal mice contained no antiviral substances while milk from immune mice was capable of neutralizing the virus *in vitro*.

9. The data indicate that the protection of young mice is passive in nature and probably brought about by the ingestion of antiviral substances (antibodies?) in the milk of immune mothers. There is no evidence in this work to suggest that the offspring of infected mothers may acquire a state of active immunity through inapparent infection.

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Effect of Purified Hyaluronidase on Growth of Sarcoma 37 in the Mouse. (18052)

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Several studies of the effect of hyaluronidase on the growth and invasiveness of experimental tumors have been reported with contradictory results ranging from enhancement (1,2) to inhibition(3,4) and no effect(5,6,7). In all these studies crude preparations and undetermined doses of enzyme were administered. It seemed desirable, therefore, to carry out further studies with massive doses of a highly purified hyaluronidase of the quality used as a therapeutic and diagnostic agent.*

Materials and methods. One hundred and sixty-three male Swiss mice weighing between 17 and 20 g were implanted subcutaneously near the axilla with fragments of sarcoma 37 measuring approximately 2 sq mm.† The animals were injected daily either intravenously or subcutaneously with physiological salt solution (PSS), hyaluronidase dissolved in PSS, or heat inactivated hyaluronidase in PSS, as outlined in Table I. In Group VII the tumor implant consisted of a standardized macerated suspension of a 7 days old tumor mixed with 20 mg hyaluronidase. The subcutaneous injections were administered apart from the immediate site of tumor implantation. The hyaluronidase* was prepared from bull testes and assayed 6300 turbidity reducing units per mg of nitrogen. Inactivated hyaluronidase was prepared by heating the enzyme at 60°C for 2 to 6 hours. All injections were begun either on the day of implantation or on the seventh day following implantation. Thirteen to 16 days after implantation the mice were killed and the tumors dissected out and wet weighed. Sections of the following organs and tissues were examined for metastases by Dr. W. E. Ehrich of the Philadelphia General Hospital: liver, lungs, thymus gland, kidneys, salivary and mesenteric lymph nodes, adrenal glands, thyroid gland, salivary glands, pituitary gland, and bone marrow.

Results. Intravenous administration. In Group I in the control series of mice, which received PSS only, the tumors grew to a size which was significantly larger than those in mice which had received 10 mg hyaluronidase daily from the day of implantation. In Group

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* Wydase, Wyeth.

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