mal saline since exhaustive washing with such a solution greatly reduced the amount of radioactivity in the poisoned samples. Saline washing also decreased the radioactivity in the control samples. Repeated washing with acetone did not appreciably decrease the radioactivity. When sections of aorta were held at 60°C for 30 minutes prior to incubation, their incorporation was slightly less than the control samples.

Since the radioactive cholesterol in the incubation medium was in suspension, it was decided to examine the response of the tissue to cholesterol in solution. An ether solution of 24 mg of H^3-cholesterol was added with vigorous stirring to 80 ml of Hanks' balanced salt solution containing 0.1 ml of Sterox SE (Monsanto Chemical Co., Boston, Mass.). After 4 hours of stirring and aeration, a clear ether-free solution was obtained to which was added 20 ml of pooled serum. Incorporation of radioactivity into aorta fragments from this medium was somewhat lower than when the suspension of cholesterol was used, but the same relative increase in the presence of the poison mixture was observed.

When sections of rabbit inferior vena cava were examined in the same system, no significant difference was observed between the control and poisoned samples in the amount of radioactivity taken up from the medium.

A study was performed to determine the effect of different types of serum on the incorporation. Both atherosclerotic human serum and rabbit plasma gave the same amount of incorporation as normal human serum in the absence of the poisons. The increase in incorporation due to KF or NaCN was much more pronounced in the presence of rabbit plasma than when human sera were used.

The data would indicate that prevention of the deposition of cholesterol in rabbit aorta is due to some system poisoned by cyanide and fluoride.

Summary. The *in vitro* incorporation of H^3-cholesterol into surviving rabbit aorta is increased by cyanide and fluoride. This effect is not shown by vena cava tissue. Incubation in rabbit plasma results in a greater stimulation than when either normal or atherosclerotic human serum was used. The data supports the hypothesis that an energy-requiring system functions to prevent deposition of cholesterol from the serum into the rabbit aorta.

1. Duff, G. L., MacMillian, G. C., Richie, A. C., *Am. J. Path.*, 1957, v33, 845.
2. Siperstein, M. D., Chaikoff, I. L., Chernick, S. S., *Science*, 1951, v113, 747.
3. Bailey, J. M., Gey, G. O., Gey, M. K., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 686.
4. Lazzarini, A. A., *Circulation*, 1960, v22, 656.
5. Chobanian, A. V., Hollander, W., *ibid.*, 1960, v22, 676.
6. Wilzbach, K. E., *J. Am. Chem. Soc.*, 1957, v79, 1013.
7. Doherty, J. E., Perkins, W. H., Shapira, J., *J. Lab. Clin. Med.*, 1962, in press.

Received December 22, 1961. P.S.E.B.M., 1962, v109.

Transfer and Decline of Maternal Infectious Canine Hepatitis Antibody in Puppies.* (27305)

L. E. CARMICHAEL, D. S. ROBSON AND F. D. BARNES
(Introduced by James A. Baker)
Veterinary Virus Research Institute and Biometrics Unit, Department of Plant Breeding, Cornell University, Ithaca, N. Y.

Specific immunity to infectious disease in the newborn is largely a consequence of the transfer of antibodies from the immune dam to her offspring(1,2,3). In the dog, an immune bitch bestows this immunity upon her puppies by transferring antibodies *in utero* and through colostrum(4). Passive immunity so acquired is temporary, for its dura-

* Supported by a research grant from Nat. Inst. Health, U.S.P.H.S.

tion depends upon the actual amount of antibody transferred and upon the subsequent rate of decline. Loss of antibody by metabolic decay and, to some extent, dilution due to growth of the puppy, gradually eliminates this protection supplied by the mother.

Quantitative studies of factors influencing the immune response to canine distemper have shown that puppies derive their supply of maternal antibody primarily through colostrum and that, within the first day, nursing puppies attain almost the same level of circulating antibody as the mother. The decline begins immediately and proceeds at a virtually constant rate of 50% every 8.4 days. Until a critically low antibody level is reached, however, puppies enjoying this antibody protection cannot be infected or actively immunized with live distemper virus vaccines. Passive protection against infection now is recognized as a major factor interfering with distemper vaccination in puppies(5).

Interference with vaccination against infectious canine hepatitis (ICH) was also inferred when a laboratory tested live ICH tissue-cultured vaccine failed to produce the expected immunization rate in puppies(6). Analysis of the antibody status of these puppies at time of vaccination revealed that the nonrespondents showed higher antibody levels at time of vaccination than puppies that responded. Age did not appear to influence the response rate, because successful immunization of puppies without maternal protection had been accomplished as early as 20 hours after birth. These field observations prompted further studies on factors influencing the immune response to ICH virus, and the present study was designed to characterize quantitatively the phenomenon of maternally transferred ICH antibody.

Materials and methods. Animals. Dogs used in this study came from 2 sources: (1) a purebred beagle colony maintained by the Cornell Institute, and (2) a cooperating commercial kennel whose vaccination program has been administered by this laboratory for the past 4 years. All dogs were beagles, and a record was kept of each animal's identity, age, sex and immunization history.

Collection of specimens. From 48 to 72 hours after whelping, a blood sample was collected from the jugular vein of each bitch and her puppies, because tests of 8- to 12-hour puppy sera showed that the full complement of antibody had not yet been acquired. Additional sera were collected at weekly or bi-weekly intervals for periods up to 14 weeks. Also, colostrum was collected within 24 hours after whelping and, from certain bitches, daily for the first week and at weekly intervals thereafter for one month. All samples were frozen and stored at −20°C until tested. In all instances samples from each litter group were tested on the same day.

Serological tests. For neutralization tests, 5-fold dilutions of serum or colostrum were prepared in 0.15 M phosphate-buffered saline, pH 7.4, and heated for 15 minutes at 60°C, although heat-labile inhibitors for ICH virus have not been found in dog sera. An equal volume of ICH virus diluted in medium 199 (Difco) to contain 100 to 300 $TCID_{50}$ per 0.1 ml then was added to each serum dilution. Serum-virus mixtures were incubated at 36°C for 45 to 60 minutes and then 0.2 ml portions of each dilution mixture were inoculated into 4 tubes of dog kidney cell cultures. Tests finally were read when virus control tubes containing 100 to 300 $TCID_{50}$ showed specific viral degeneration, usually after 5 or 6 days. Endpoints were estimated by the Reed-Muench method. As an additional control, a standard reference serum was included in each test series. The standard error of neutralization titers of 11 tests of the control serum was $\log_{10} \pm 0.2$ with a range of 0.5.

Results. The relationship between the antibody titers of 13 bitches and their progeny, as measured by the neutralization test, is shown in Table I. Puppy antibody titers did not vary markedly within litter groups, with the notable exception of 2 puppies (litters 3 and 8) which failed to nurse during the first day of life.

It is seen that puppy antibody titers are linearly related to the antibody titer of the dam (Fig. 1). The linear regression of $\log_{10}$ puppy titer on $\log_{10}$ mother's titer can be expressed as: $\log_{10}$ puppy titer $= -.16 + .92$ $\log_{10}$ mother's titer. Colostral antibody titers generally appear to be higher than dam serum

TABLE I. Relationship between Maternal ICH Antibody Titer and Puppy Antibody Titers 48 to 72 Hours after Birth.

Litter	Titer		
	Dam Serum*	Colostral	Puppy
1	2.9	4.4	3.0
			3.3
			3.2
			2.5
2	3.3	4.5	4.0
			3.2
3	2.5	3.9	2.5
			< .7
			3.0
			2.9
			2.8
4	2.5	2.5	1.8
			1.7
			1.3
			1.7
			1.3
			1.7
			1.8
			1.3
5	< .7	< .7	< .7†
6	4.4	3.6	4.2†
7	1.9	2.6	2.1†
8	2.3	3.2	1.9
			2.1
			1.8
			< .7
9	< .7	< .7	< .7
			< .7
			< .7
10	3.9	NT	3.5
			3.0
			3.0
			3.2
			3.2
11	2.3	2.3	1.8
			2.1
			2.1
			2.3
			1.8
12	4.5	3.9	4.4
			4.4
			4.2
			4.5
			4.5
			3.8
13	3.9	NT	3.2
			3.2
			3.2
			2.8
			2.5
			2.8

* Titer expressed as $-\log_{10}$ serum dilution that neutralized 100-300 $TCID_{50}$ of virus.

† Serum pool titer from 9 dogs (litter 5), 8 dogs (litter 6), 5 dogs (litter 7). These data not used in computing regression equation (Fig. 1).

NT means no titer.

titers; however, correlation is poor. The heterogenous and viscous nature of colostrum naturally results in considerable sampling error. Nevertheless, a general relationship could be established between the antibody levels of bitch's sera, colostrum, and puppy's sera. Fig. 2 shows a typical series of observations on a bitch and her progeny. Here it can be seen that the colostral antibody titer decreases rapidly during the first week, then reaches a level that remains constant for an indefinite period of at least 30 days. Puppy titers, on the other hand, decline at a uniform rate. Fig. 3 shows the regression of mean titers of 3 litters of puppies. One litter (litter II) shows a significantly lower mean antibody level at 12 hours after birth than 2 weeks of age, indicating that the full complement of passive antibody had not yet been acquired. By 72 hours maximal titers seem to have been obtained.

Information of the type illustrated in Fig. 3 was obtained from 8 litters, all of which exhibited the same constant rate of antibody decline. The difference between titers at any fixed age can be expressed as a proportional relationship between puppy titer and dam titer and, on the log titer scale, this functional relationship at age T weeks became (Fig. 4):

$$\text{puppy titer} = \text{dam's titer} - 0.25\ T_{(\text{age in weeks})}$$

Using this formula, the half-life of maternally transferred antibody is approximately 9 days. The correlation between the observed mean titers and those predicted by this equation is 0.984.

Discussion. Maternal ICH virus neutralizing antibody has been shown to be quantitatively transferred from immune mothers to their puppies provided that the puppies ingest colostrum during the first day or 2 of life. No virus neutralizing antibodies were detected in the colostrum of non-immune mothers or in neonatal puppy sera from non-immune mothers. Colostrum from immune mothers, however, showed antibody levels that were high initially and which tended to decline rapidly after the second day following whelping. Although correlation between maternal serum and colostral antibody was poor,

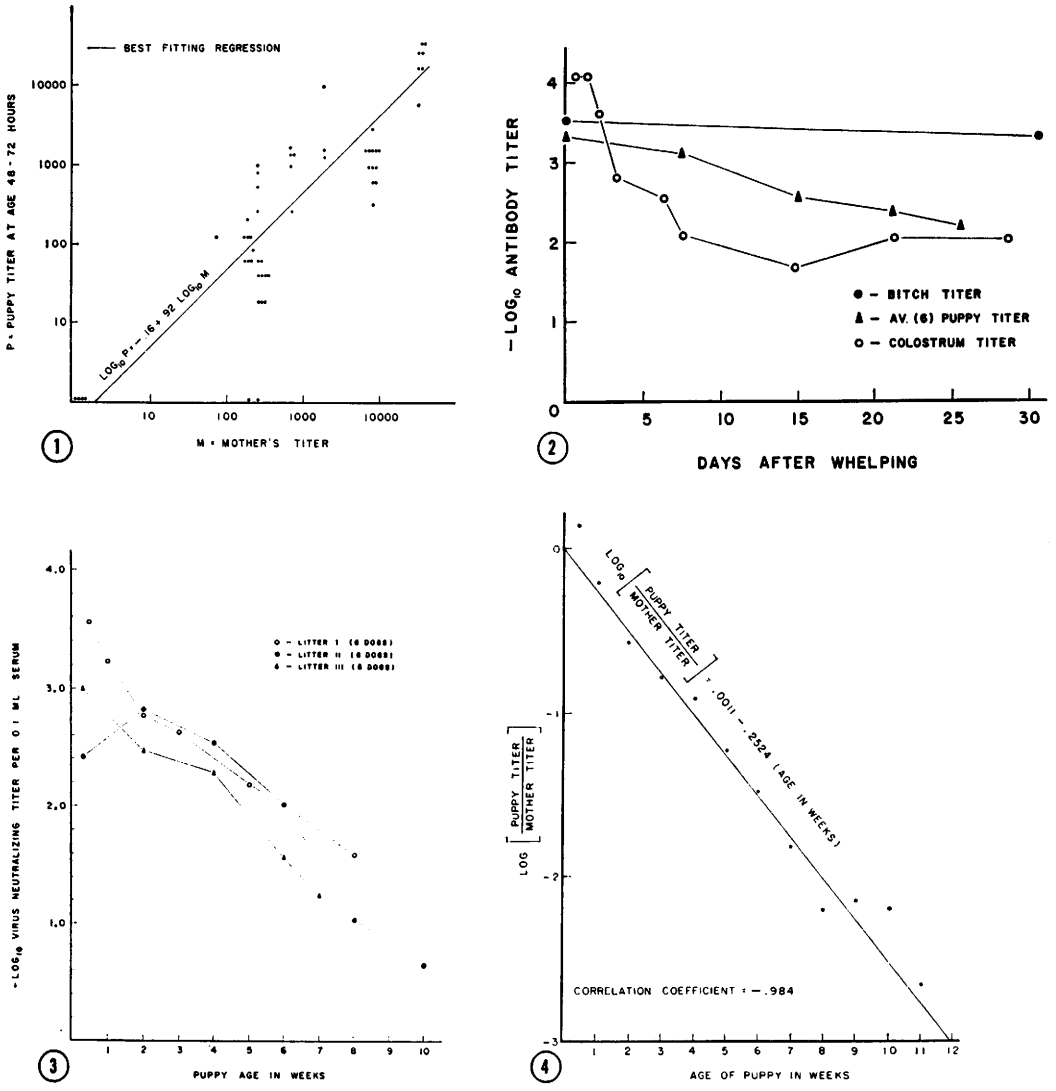

FIG. 1. Relationship between mother ICH antibody titers and newborn puppy titers.

FIG. 2. Relationship between serum ICH antibody titer of mother and her colostrum and titer of her puppies during nursing.

FIG. 3. Decline of maternally transferred antibody in puppies. Each point represents mean titer of n observations.

FIG. 4. Relationship between age of puppy and puppy's titer expressed as percentage of mother's titer.

principally because of the nature of colostrum, bitches with high serum antibody titers also possessed high colostral antibody titers. It is not known how long antibody persists in bitchs' milk, but at least 1% of the initial amount was still present 30 days after whelping. It appears, then, that a mutually complementary mechanism operates to afford puppies specific immune protection, for the antibody in the bitchs' milk begins to decline precipitously approximately the same time that the nursing puppy has received its full portion of maternal antibody. The time at which a puppy no longer is able to assimilate colostral antibody has not been determined precisely, but it appears that the maximal amount has not been acquired by 12 hours, while the full complement seems to have been received by 72 hours after birth.

Quantitative studies on the relationship

between ICH virus neutralizing antibody levels and immunity (or susceptibility) will be presented elsewhere. However it has been previously shown that puppies with ICH antibody titers less then 0.7 (1:5) regardless of age can be immunized successfully with adequate live virus vaccines(6). Also it has been shown that puppies with antibody levels above a critical range are refractory to immunization. The inference can be made, therefore, that puppies which are susceptible (or immune) to vaccine strains of ICH virus also would be susceptible (or immune) to virulent virus. Our ultimate objective is to predict with reasonable confidence, before it is born, the age at which a puppy would be susceptible to infection (immunization) because vaccination is not effective until a puppy has lost essentially all of its maternal antibody. This idealized goal for controlling disease already has been realized with canine distemper by the use of a nomograph which predicts the age at which to vaccinate a puppy on the basis of a maternal blood sample taken at the time of whelping(7). This procedure has proven over 95% successful in field tests conducted over the past 3 years. For such a device, maternal and puppy antibody levels must be correlated, and rate of passive antibody decay must be known. Here it has been shown that maternal and puppy ICH antibody levels are correlated ($r = 0.82$), and that the regression of puppy titer on time is regular. The relationship between the ICH antibody titer of an immune bitch and her puppies is in remarkable agreement with that previously determined for distemper, for the regression of log puppy titer/log mother titer on time (weeks) is essentially the same in both instances(5).

Using the *in ovo* neutralization test for distemper antibody, Gillespie reported that the half-life of distemper maternal antibody is 8.4 days. In this study, the antibody half-life, as measured by the ICH virus neutralization test, is 8.6 days, which seems in good agreement with measurements that employed I^{131}-labeled canine gamma globulin(8). In the latter study the half-life was ascertained to be 8.1 ± 1.2 days.

Summary. Maternal serum antibody titers have been shown to be correlated with puppy serum antibody titers when the full complement of maternal antibody, principally in the form of colostrum, has been acquired. Maximal titers were estimated to occur at or before 72 hours, but not 8 to 12 hours after birth. The relationship between puppy antibody titers and the maternal antibody titer could be expressed as: $\log_{10}$ puppy titer $= -.16 + .92 \log_{10}$ mother titer. The rate of maternal antibody decline in puppies was regular, and the half-life of ICH virus neutralizing antibody was calculated to be 8.6 days. A relationship also was established between the puppy titer as a proportion of the $\log_{10}$ maternal titer, and rate of antibody decay. This could be expressed as: Puppy titer = Mother titer $-.25$ T (age in weeks).

1. Brambell, F. W. R., *Vet. Record,* 1958, v70, 1060.

2. Hemmings, W. A., Brambell, F. W. R., *Brit. Med. Bull.,* 1961, v17, 96.

3. Galfand, H. M., Fox, J. P., DeBlanc, D. R., Elveback, L., *J. Immunol.,* 1960, v85, 185.

4. Gillespie, J. H., Baker, J. A., Burgher, J., Gilman, G., *Cornell Vet.,* 1958, v49, 158.

5. Baker, J. A., Robson, D. S., Carmichael, L. E., Gillespie, J. H., Hildreth, B., *Proc. Animal Care Panel,* 1961, v11, 234.

6. Carmichael, L. E., Doctoral Thesis, Cornell, 1959.

7. Baker, J. A., Robson, D. S., Gillespie, J. H., Burgher, J., Doughty, M. F., *Cornell Vet.,* 1959, v49, 158.

8. Dixon, F. J., Talmadge, D. W., Maurer, H., Deichmiller, M. P., *J. Exp. Med.,* 1957, v96, 313.

Received December 27, 1961. P.S.E.B.M., 1962, v109.