The authors are greatly indebted to Dr. J. F. Tait, Worcester Foundation for Exp. Biology, for d-aldosterone-7-H^3; to Dr. R. Gaunt, Ciba Pharmaceutical Products Inc., for d-aldosterone, free alcohol; to Endocrinology Study Section, Dept. HEW, for cortisol-7-H^3 and cortisol-4-C^{14}; to Dr. V. A. Drill, G. D. Searle and Co., for spirolactone.

1. Daughaday, W. H., *Physiol. Revs.*, 1959, v39, 885.

2. Westphal, U., in *Mechanism of action of steroid hormones,* C. A. Villee and L. L. Engel (eds.), Pergamon Press, 1961, 33.

3. Firschein, H. E., DeVenuto, F., Fitch, W., Pearce, E. M., Westphal, U., *Endocrinol.*, 1957, v60, 347.

4. Ulrich, F., *Am. J. Physiol.*, 1959, v196, 752.

5. Munck, A., Scott, J. F., Engel, L. L., *Biochim. et Biophys. Acta*, 1959, v26, 397.

6. Abelson, D., Depatie, C., Craddock, V., *Arch. Biochem. Biophys.*, 1960, v91, 71.

7. Butenandt, A., Günther, H., Turba, F., *Hoppe-Seyler's Z. Physiol. Chemie,* 1960, v322, 28.

8. Pearlman, W. H., Pearlman, M. R. J., *J. Biol. Chem.*, 1961, v236, 1321.

9. Molinari, G., Lata, G. F., *Arch. Biochem. Biophys.*, 1962, v96, 486.

10. Harding, B. W., Samuels, L. T., *Endocrinology,* 1962, v70, 109.

11. DeVenuto, F., Kelleher, P. C., Westphal, U., *Biochim. Biophys. Acta,* 1962, v63, 434.

12. Davidson, E. T., DeVenuto, F., Westphal, U., *Endocrinology,* 1962, v71, 893.

13. Neville, D. M., *J. Biophys. and Biochem. Cytol.,* 1960, v8, 431.

14. Daughaday, W. H., Kozak, I., Biederman, O., *J. Clin. Invest.*, 1959, v38, 998.

15. Westphal, U., Ashley, B. D., Selden, G. L., *Arch. Biochem. and Biophys.*, 1961, v92, 441.

16. Kono, T., Colowick, S. P., *ibid.*, 1961, v93, 520.

17. Vander, A. J., Malvin, R. L., Wilde, W. S., Lapides, J., Sullivan, L. P., McMurray, V. M., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 323.

18. Sonneblick, E. H., Cannon, P. J., Laragh, J. H., *J. Clin. Invest.*, 1961, v40, 903.

19. Marinetti, G. V., Erbland, J., Stotz, E., *J. Biol. Chem.*, 1958, v233, 562.

20. Lehninger, A. L., in *Biophysical science—A Study Program,* J. L. Oncley (ed.), John Wiley & Sons, 1959, p143.

21. Willmer, E. N., *Biol. Revs.*, 1961, v36, 368.

22. Daughaday, W. H., *J. Clin. Invest.*, 1958, v37, 511.

Received March 8, 1963. P.S.E.B.M., 1963, v113.

Passage of Bacteriophage ϕX 174 Across the Placenta in Guinea Pigs.* (28374)

JONATHAN W. UHR, JOSEPH DANCIS AND MARTIN S. FINKELSTEIN
(Introduced by L. E. Holt, Jr.)
Departments of Medicine and Pediatrics, New York University School of Medicine, New York City

Previous studies(1-3) have shown that following injection of large amounts of bacteriophage into pregnant animals, trace amounts of phage could be demonstrated in foetal blood. For example, Medearis(4) injected 10^8 PFU of bacteriophage ϕX 174 into pregnant mice and recovered 0.0001% of the amount injected from the foetus; this recovered amount was not influenced by injection of pregnant animals with endotoxin or exposing them to excess CO_2. It is possible that in these experiments the very small amounts of virus found in the foetus represent contamination from maternal blood or fluids which occurred during the experimental intervention.

It was the purpose of this study to determine whether transplacental passage of bacteriophage occurs *in vivo* by excluding the possibility of foetal blood contamination by maternal fluids.

Materials and methods. Animals. Pregnant guinea pigs in each trimester of pregnancy were obtained from breeders.

Phage. The preparation and purification of ϕX has been described(5). The stock used throughout these experiments contained 6×10^{11} plaque-forming particles/ml. One

* Aided by grants from USPHS and New York City Health Research Council.

TABLE I. Appearance of Bacteriophage ϕX 174 in Foetal Blood Following Maternal Injection of Phage.

Litter No.	Amt of ϕX injected into mother	Interval* (hr)	ϕX content/ 0.1 ml of mother's blood†	Avg wt of foetuses (g)	ϕX content of 0.1 ml of	
					Amniotic fluid	Foetal blood
					—	18
					1	11
1	3×10^{10}	48	3.9×10^{6}	57	156	10
					102	7
2	3×10^{10}	24	6.3×10^{6}	110	53	2
3	3×10^{10}	24	8.9×10^{6}	97	0	2
					14	7
4	3×10^{9}	18	10^{6}	77	8	2
5	3×10^{9}	18	2×10^{5}	120	—	2
					—	0
6	3×10^{9} + hyaluronidase‡	18	6×10^{5}	56	2	1
					11	0
					23	0
7	3×10^{9} + 1 μg endotoxin§	18	—	16	—	0
					—	0
					—	0
8	0	None	0	62	31	1
			0		10^{3}	0
					10^{3}	0

— Not done.
* Time between intravenous injection of phage and subsequent laparotomy.
† Sample obtained at time of Caesarean section.
‡ 225 units Wydase (Wyeth) injected intravenously with phage.
§ *S. marcescens* (Difco) injected intravenously.

ml of a 1:10 dilution of this phage preparation injected intravenously into normal guinea pigs produced no detectable pyrogenic response.

Injection of animals and Caesarean section. Pregnant animals were injected intravenously into the hind leg and 18-48 hours later were sacrificed by a blow on the head. To exclude contamination of foetal blood by amniotic fluid or maternal blood containing ϕX, a team of 3 individuals was used to perform each experiment. One individual performed the laparotomy, carefully withdrew 0.5-1 ml of clear fluid from the amniotic sac with a syringe attached to a 27 gauge needle, opened the membranes, and ligated the umbilical vessels. He then handed the foetus to a second individual who thoroughly washed each foetus and his own hands under a water tap. The washed foetus was then handed to the third individual who obtained a foetal blood sample by cardiac puncture using a syringe previously rinsed with heparin (1000 units/ml).

Assay for phage. The assay for phage was performed by the pour plate technique of Adams(6) using *E. coli C* as the indicator strain.

Results. In the first series of experiments, $3 \times 10^{9-10}$ ϕX was injected intravenously into 9 pregnant guinea pigs. The results of 7 representative injected animals and 1 control animal are shown in Table I. The highest foetal blood titers, 7-18 phage/0.1 ml, were obtained in litter No. 1 in which 3×10^{10} ϕX had been injected into the mother 48 hours before Caesarean section. Lower titers were observed in litters 2 and 3 in which 3×10^{10} phage was also injected but the interval was only 24 hours. Litters 4-6 in which 3×10^{9} phage had been injected into the mother 18 hours previously, showed from 0-2 phage particles in 0.1 ml of foetal blood. Neither hyaluronidase nor endotoxin appeared to increase the amount of ϕX that appeared in the foetal blood. Also shown is a control litter from an uninjected mother, in which phage had been injected into the amniotic fluid 5 minutes before removal and routine washing of the foetus. Two of 3 foetuses showed no detectable phage and the third animal one phage particle/0.1 ml.

These results suggested that the bacteriophage present in the foetal circulation was derived *in vivo* from the maternal circulation, but the possibility was not excluded

TABLE II. Control Experiment on the Source of Bacteriophage in Foetal Blood.

Litter No.	ϕX/.1 ml mother's blood*	Avg wt of foetuses (g)	ϕX content amniotic fluid .1 ml	Medium for washing of foetus†	ϕX content of foetal blood‡ .5 ml	.1 ml
9	1×10^6	100	—	Water	17	1
			1		117	23
			0		49	7
10	1.2×10^6	65	4	"	25	5
					44	7
11	0	45	—	ϕX	3-400	58
					20	2
					22	79
12	0	38	—	"	313	541
					500	137
13	0	28	—	"	267	337
					392	500
14§	0	60	8.8×10^4	Water	0	0
			2.3×10^5		0	0
			1.9×10^5		0	0

— Not done.

* Phage ϕX was injected intravenously. Blood sample was obtained 48 hr later immediately prior to Caesarean section.

† The foetus was either washed thoroughly with tap water or was swabbed with saline containing ϕX, 10^8/ml.

‡ Blood samples were removed separately.

§ 0.1 ml containing 3×10^7 ϕX was injected into the amniotic cavity after laparotomy and 5 min. before sampling and removal of foetus.

that contamination with maternal blood had occurred after hysterotomy. To further investigate this possibility, a second experiment was performed. Three pregnant guinea pigs were injected with ϕX intravenously prior to hysterotomy; as controls, 3 litters were delivered from non-injected animals and the foetuses were grossly contaminated by application of phage to their skin. All foetuses were bled of 0.5 and 0.1 ml whole blood using separate syringes, needles, and puncture sites. Alternately, the larger or smaller volume was withdrawn first. If the source of the bacteriophage present in foetal blood samples was, indeed, the foetal blood, then the separate withdrawal of 0.5 ml and 0.1 ml of blood should show phage counts approximately 5-fold apart. In contrast, if the phage in foetal blood resulted from contamination of the needle by phage on the foetus's skin, the results of this withdrawal procedure would not be expected to follow any particular quantitative pattern.

In phage-injected animals, all of 5 foetuses showed larger amounts of ϕX in the 0.5 ml compared to the 0.1 ml sample (Table II). Moreover, the expected quantitative relationship was demonstrated in 4 of 5 animals, keeping in consideration the experimental error of the assay on the basis of a single plate and an extremely low phage count. In contrast, in the *in vitro* phage-contaminated, unwashed foetuses, there was no consistent quantitative relationship in amount of phage between the 2 samples. As before, the routine wash of foetuses in which the amniotic fluid had been heavily contaminated prevented phage contamination of foetal blood. These findings led to the conclusion that there had been transplacental passage of bacteriophage into the foetal circulation.

Discussion and summary. These studies indicate that a virus particle, bacteriophage ϕX 174, incapable of infecting mammalian cells, can cross the placenta and gain access to the foetal circulation. The possibility of *in vitro* contamination of foetal blood samples during cardiac puncture of the foetus can be excluded since: a) the washing procedure of the foetus was shown to be effective after deliberate contamination of the foetus's skin; b) after injection of phage into pregnant guinea pigs, separate bleedings of 0.5 ml and 0.1 ml from the foetal heart usually showed a 5-fold difference in amount of ϕX in the 2 samples; this difference was not observed in deliberately contaminated

foetuses from pregnant guinea pigs *not* injected with ϕX. It is unlikely that endotoxin contamination of phage preparations was responsible for the transplacental passage of virus because of virtual absence of biologically detectable endotoxin in the preparations of phage employed and the failure of an injection of endotoxin with phage to increase the amount of phage found in the foetal circulation. There was no apparent difference in phage transfer between foetuses of markedly different weights, 56-120 g.

The route of transplacental passage of phage is as yet uncertain. The failure to find a gradient in concentration from amniotic fluid to foetal blood (the latter sometimes had a higher phage concentration) suggests that phage is not transferred by this route. Previous studies of placental transfer of large proteins indicate that the splanchnopleure is the principal transfer organ in guinea pigs(7).

The question arises as to whether the placenta is an effective barrier in preventing intrauterine viral infections. The following findings are relevant to this question: a) in the guinea pig, the difference between maternal and foetal blood virus concentrations was approximately 10^5-10^6 and transfer usually was not demonstrated when maternal ϕX concentration was below 10^7/ml; b) the ϕX particle is an unusually small virus (approximately 250 angstroms in diameter). It is possible that its size or other particular properties(8) facilitates its passage into the foetal circulation.

These findings suggest that the placental barrier is usually an effective one in the presence of viremia of moderate intensity, provided, of course, that the placenta is free of infection and pathology. However, if there is a sufficiently high concentration of virus in the maternal circulation, passage of virus into the foetus may occur. Whether or not infection results will depend on a variety of factors, including the capacity of the placenta (9) and the foetus(10) to develop an immune response against the virus.

1. Blair, J. E., Reeves, D. L., *J. Inf. Dis.*, 1928, v42, 440.

2. Burckhardt, K., *Arch. Ges Virusforsch.*, 1940, v1, 140.

3. Kulangara, A. C., Sellers, M. I., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 207.

4. Medearis, D. N., Jr., *Am. J. Dis. Child.*, 1962, v104, 573.

5. Uhr, J. W., Finkelstein, M. S., Baumann, J. B., *J. Exp. Med.*, 1962, v115, 655.

6. Adams, M. H., in *Methods in Medical Research,* Year Book Publishers, Chicago, 1950, 2.

7. Brambell, F. W. R., Hemmings, W. A., Henderson, M., in *Antibodies and Embryos,* London, Althone Press, 1951, 5.

8. Brambell, F. W. R., Hemmings, W. A., Oakley, C. L., Porter, R. R., *Proc. Roy. Soc. Lond.* (sec. B) 1960, v151, 478.

9. Dancis, J., Samuels, B., Douglas, G., *Science,* 1962, v136, 382.

10. Silverstein, A. M., Uhr, J. W., Kraner, K. L., Lukes, R. J., *J. Exp. Med.*, 1963, v117, 799.

Received March 11, 1963. P.S.E.B.M., 1963, v113.

The Fluorescent Antibody Technique Applied to Titration and Identification of Antigens in Solutions or Antisera.* (28375)

FIORENZO PARONETTO (Introduced by Hans Popper)
Department of Pathology, The Mount Sinai Hospital, New York City

The fluorescent antibody technique has been used principally for localization of antigen or antibody in tissues or for identification of micro-organisms. The high sensitivity of the technique is caused by the fluorescent label and probably also by the ability to detect or use non-precipitating as well as precipitating antibody(1); the sensitivity is

* Supported by grants from Nat. Inst. of Arthritis and Metab. Dis., USPHS and by U. S. Army Medical Research and Development Command contract.