mor cells, it is speculated that immunization has a lethal rather than a static effect on transplanted tumors.

Discussion. McKee *et al.*(3) were unable to induce immunity by treatment with Ehrlich's ascites cells that had been killed by desiccation, freezing and thawing, mechanical grinding, and supersonic waves. The possibility exists that the physical-chemical changes of tumor antigens induced by *in vitro* x-irradiation are extensive enough to make the antigens less homologous and more antigenic for the mouse without destroying antigenic specificity. Indirect evidence for this is manifested by recent observations made in this laboratory. The agglutinins against normal Ehrlich's tumor cells are more readily produced and demonstrated in sera of animals that have been exposed to higher irradiation doses.

Summary. Pre-immunization with irradiated Ehrlich's ascites tumor cells protected mice against subsequent ip transplants of viable cells. Immunization procedures started one day after sc challenge with the tumor, significantly reduced mortality. Such post-immunization did not inhibit the rapid multiplication that follows ip transplantation of the tumor. The evidence indicated that the immunization procedures employed resulted in a lethal rather than a static effect on transplanted tumor cells.

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Passage of Bacteriophages from Mother to Foetus in the Rat.* (24885)

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During a study of tolerance to homografts produced in rats by direct injection of particulate antigens into foetuses, the question arose whether similar antigens can cross the intact placental barrier under natural conditions. Passage of antibodies and similar large molecules from mother to foetus has been shown by studies of Brambell and his associates(1). Whether larger particles can cross the placental barrier has not been answered satisfactorily. Bacteriophages offer special advantages in such a study. They are larger than protein molecules, they multiply only in specific host bacteria and their presence can easily be detected by testing for plaque forming activity, produced only by intact viable phage. Since phage is not able to penetrate and multiply in cells other than susceptible bacteria, it is free from the objection that may be raised against animal viruses, namely, that the latter may traverse the placenta by direct extension of an infectious process. The passage of coliphage and staphylococcal phage across the placental barrier was investigated by Grasset(2), who obtained no evidence for such passage in rabbits and guinea pigs; Blair and Reeves(3), using high titer phage suspensions, reported passage in the guinea pig. However, Nattan-Larrier *et al.*(4) were unable to repeat the latter results. Burckhardt(5) reported passage of coliphage from mother to foetal blood in guinea pigs. Recently Keller and Engley(6) and Keller(7) published quantitative studies on passage of *Bacillus megatherium phage* through gastrointestinal and renal barriers and through intact skin of mice.

Materials and methods. Four bacterio-

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phages of different sizes were employed. These included coliphages T3 (head diameter 47 mμ, tail length 15 mμ) and T1 (head diameter 50 mμ, tail length 150 mμ) (8); mycobacteriophages D29 (head diameter 65 mμ, tail length 125 mμ) and D32 (head diameter 80 mμ, tail length 220 mμ) (9). High titer phage suspensions, 10^8 to 10^{10} particles/ml, were prepared as described by Adams(10) for coliphage and by Sellers *et al.*(9) for mycobacteriophage. The preparations were either non-filtered lysates or lysates which had been passed through ultra-fine sintered glass filters. In addition, a few experiments were performed with phage suspensions which had been washed and purified by repeated cycles of high speed centrifugation. Long-Evans rats at different stages in second half of gestation were employed. Phage suspensions were injected by intravenous or intrauterine route, except for 2 instances of intracardiac injection. Intrauterine injections were made after opening abdomen as described by Brambell *et al.*(1). The mother was anesthetized with ether at various intervals after injection, and foetuses removed with foetal membranes intact. The membranes were washed in saline followed by 70% alcohol and dried with sterile gauze before foetal fluids were collected with sterile, finely drawn, capillary pipettes. The foetuses were then freed of all membranes and dipped in 70% alcohol or 5% Chlorox solution to free the outside of body from any contamination with phage from maternal blood. The chest of the foetus was opened aseptically and blood obtained by cardiac puncture with sterile capillary pipettes. Since the quantity of blood from each foetus was small, the majority of samples were pooled, each sample consisting of blood from 3 or 4 embryos. When foetuses were too small (10-12 days old) for bleeding, whole foetuses were ground in sterile glass homogeniser and tested for phage. In some instances, blood, peritoneal fluid, and spleen from the mother were also tested. In assaying for phage, samples (blood, foetal fluids, homogenate, etc.) were placed in flasks containing approximately 20 ml of heart infusion broth. Five ml of actively growing (log phase) culture of susceptible bacteria were then added and the flasks incubated, 4 hours for coliphage and overnight for mycobacteriophage. Presence of phage in broth culture was detected by spotting aliquots on soft agar Petri plates seeded with susceptible bacteria(10). Typical plaques indicate phage activity.

Results. Twenty-three pregnant rats were injected with coliphage preparations and foetuses sampled to 24 hours later. Phage T3, smallest of the T phages, was employed in first experiments and results with T3 lysate are recorded in Exp. 1, Table I. Samples of mothers' blood, peritoneal fluid, and spleen contained phage. That phage remained viable in the animal (spleen) to 23 hours was interesting. Phage given directly into the uterine cavity was later found in foetal blood. Five attempts to demonstrate phage passage across the placental barrier after intravenous administration of non-filtered lysate were unsuccessful.

Non-filtered phage lysates cleared only by centrifugation were used. Since these lysates undoubtedly contained a few living bacteria containing phage which could have passed from mother to foetus in an infectious manner, experiments were performed using lysates which had been freed of all bacteria by filtration (Table I). Exp. 2 and 3 show that phage in sterile filtrates passed from mother to foetus.

Since *Escherichia coli* is a Gram-negative rod, T phage lysates contain Gram-negative endotoxins. It was possible that these toxins could affect foetal membranes so that they became more permeable to phage. Toxic effects were indeed manifest in death of embryos next to site of injection, provided assay time was delayed a few hours or more after inoculation. However, in rats injected in one uterine horn only, normal foetuses at the anterior end of uninjected horn were as likely to contain phage as foetuses in the injected horn. Dead foetuses were not used. To exclude the effects of endotoxin on passage, phage which had been washed and purified by high speed centrifugation was used (Exp. 4, Table I). Intracardiac and intrauterine injections of these preparations showed passage of phage from mother to foetus. In Exp. 5 (Table I), T1 coliphage which is larger than T3,

TABLE I. Recovery of T1 and T3 Coliphages Injected into Pregnant Rats.

Exp. No.	Inoculation	Time (hr)*	Sample tested		Phage recovery
	(T3 non-filtered lysate, titer: 10^{10})				
1	3 ml intracardiac	3½	Maternal blood	(1)†	Positive
			" peritoneal fl.	(1)	"
			Foetal blood	(1)	"
	5 ml into left uterine horn	6	Maternal blood	(1)	"
			Foetal "	(2)	"
	4 ml *Idem*	6½	" "	(4)	"
	4 ml "	23	Whole foetuses	(1)	"
			Maternal spleen	(1)	"
	(T3 filtered lysate, titer: 1.7×10^9)				
2	3 ml into left uterine horn	4	Foetal blood	(5)	3 pos, 2 neg
	3 ml *Idem*	6	*Idem*	(3)	2 " , 1 "
			Foetal fluids	(1)‡	Positive
	2 ml "	6	*Idem*	(1)	"
			Whole foetuses	(1)	"
	(T3 filtered lysate, titer: 1.7×10^9)				
3	5 ml into left uterine horn	24	Foetal blood	(4)	Negative
	2 ml into each uterine horn	24	*Idem*	(4)	3 pos, 1 neg
	(Washed T3 phage, titer: 9×10^9)				
4	1 ml intracardiac	5	Foetal blood	(3)	2 pos, 1 neg
	1.5 ml into each uterine horn	5½	*Idem*	(3)§	1 " , 2 "
	1 ml *Idem*	6	"	(6)	Positive
	2 ml into left uterine horn	7	Whole foetuses	(2)	"
	2 ml into right uterine horn	8	Foetal blood	(3)	1 pos, 2 neg
	(T1 filtered lysate, titer: 10^9)				
5	4 ml into left uterine horn	17	Foetal blood	(4)§	Positive
	4 ml *Idem*	18	Whole foetuses	(1)	"

* Time between inoculation and sampling.
† Numbers in parentheses indicate No. of separate samples tested.
‡ Refers to a mixture of amniotic and exocoelomic fluids.
§ Samples were tested after foetuses had been delivered by the mother.

was inoculated into pregnant rats. This phage also was recovered from foetuses. T phages crossed the placental barrier in 74% of all samples tested after intrauterine injection of the mother.

In Exp. 4 one animal delivered her young 5½ hours after receiving purified T3 phage. The blood of some of these newborn animals contained phage. Also, blood from foetuses born 17 hours after the mother had been injected with T1 filtered lysate, contained phage (Exp. 5, Table I).

In further studies, 6 pregnant rats were inoculated with D29 mycobacteriophage and 5 animals with D32 mycobacteriophage (Table II). After intravenous injection these phages were not found across the placental barrier. Following uterine injection, phage activity was found in 6 out of 14 samples in the case of D29 and in 3 out of 9 samples in the case of D32. No deleterious effects on foetuses were observed when mycobacteriophages were injected into the uterine horn.

Discussion. Apparently, high concentrations of phage must reach the uterine cavity before passage into the foetus becomes evident. Therefore, the probability of similar large particles reaching the foetus under natural conditions is remote; nevertheless, it is of considerable interest to find that it is possible for them to do so. Because quantitative assays were not performed, the actual numbers of phage particles which passed the placental barrier are not known.

The possibility of contamination of foetal samples with phage from maternal circulation is an important source of error in such studies. Even when phage was injected into the uterine lumen it could later be recovered from samples of maternal blood. Therefore, ex-

TABLE II. Recovery of D29 and D32 Mycobacteriophages Injected into Pregnant Rats.

Exp. No.	Inoculation	Time (hr)*	Sample tested		Phage recovery
		(D29 non-filtered lysate, titer: 5×10^9)			
1	3 ml intravenous	4½	Maternal blood	(1)†	Negative
			" peritoneal fl.	(1)	"
			Foetal blood	(3)	"
	4 ml "	7½	Maternal blood	(1)	Positive
			" spleen	(1)	"
			" peritoneal fl.	(1)	Negative
			Foetal blood	(1)	"
	Idem	24	Maternal spleen	(1)	"
			" peritoneal fl.	(1)	"
			Foetal blood	(5)	"
		(D29 filtered lysate, titer: 8×10^8)			
2	3 ml into right uterine horn	5	Amniotic fluid	(1)	Negative
			Foetal blood	(5)	1 pos, 4 neg
	2 ml into each uterine horn	6	*Idem*	(4)	3 " , 1 "
	2 ml into left uterine horn	6½	Amniotic fluid	(2)	1 " , 1 "
			Foetal blood	(1)	Negative
			Whole foetuses	(1)	"
		(D32 filtered lysate, titer: 3×10^8)			
3	2 ml into left uterine horn	4	Whole foetuses	(2)	1 pos, 1 neg
	Idem	4½	Foetal blood	(2)	1 " , 1 "
	"	5	*Idem*	(2)	Negative
	"	5½	Amniotic fluid	(1)	"
			Foetal blood	(2)	1 pos, 1 neg
	"	6	*Idem*	(1)	Negative

* Time between inoculation and sampling.
† Numbers in parentheses indicate No. of separate samples tested.

treme care had to be exercised during sampling to avoid spurious results. The possibility of contamination and use of inoculation routes other than uterine may explain some of the conflicting results of earlier workers.

These experiments do not provide information on the mechanism of phage passage. However, it appears likely that simple diffusion across membranes may be ruled out since electron microscopic examination of the yolk-sac membrane and placenta(11) has not shown pores which would allow passage of such large particles. The electron micrographs have shown abundance of microvilli at the free border of epithelial cells of yolk-sac splanchnopleure. It is probable that passage of phage particles is accomplished by some active method of transport, possibly by pinocytosis of membrane cells.

Since bacteriophage is infectious only when intact, it can be concluded that these particles reached the foetal circulation in an undegraded state. This means that the placental barrier in the rat does not prevent passage of particles as large as D32 mycobacteriophage.

Summary. T1 and T3 coliphages and D29 and D32 mycobacteriophages were injected into pregnant rats. Examination of foetuses and foetal fluids showed presence of phage, which demonstrates that intact particles of relatively large size can cross the placental barrier in the rat. Phage injected into maternal blood rarely reached the embryo. However, when phage was inoculated into the uterine lumen, passage to the foetus was demonstrated in three-fourths of test samples using coliphage, and in one-third of samples using mycobacteriophage. It appears that large concentrations of phage in the vicinity of yolk-sac splanchnopleure are required before passage becomes apparent; this suggests that passage of similar particles from mother to foetus does not frequently occur in nature.

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Metabolic Studies of Vitamin B_{12} (Depinar) (24886)

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When a large dose of Vit. B_{12} is given parenterally, a major portion is excreted in urine and therefore is not available for metabolic functions. Hence, it is desirable to explore any preparation containing cyanocobalamin which reduces such rapid excretion. This report presents evidence that both in experimental animal and man, Vit. B_{12} administered by either intraperitoneal or intramuscular route as its tannate derivative (Depinar) is excreted much more slowly in urine and maintained at significantly higher levels in blood than when B_{12} is given in aqueous form. Data are presented to demonstrate that Vit. B_{12} Co^{60}-tannate administered to the rat results in increased organ content of the radioactive component and presumably due to Vit. B_{12} molecule itself(1).

Methods. Animal experiments. Ten male adult rats of McCollum strain were divided into 2 groups of approximately equal average weight. One group was administered 0.84 μg of Co^{60} B_{12}-tannate (Co^{60} "Depinar"). The second received 0.84 μg of Co^{60} B_{12} (both of 180 μc/mg specific activity) intraperitoneally. Animals were housed in individual metabolism cages. Urine was collected daily under toluene. Urine samples and washings were evaporated to 50 ml on steam bath in graduated 100 ml brown bottle. After 20 days the 10 rats were sacrificed. Liver, kidney and intestines were preserved in frozen state and subsequently were individually homogenized in stainless steel cup on Waring Blender. Radioactivity of urine samples and of total organ homogenates was measured by well scintillation counter. *Human Vit. B_{12} serum levels.* Male, healthy adults were administered either 500 μg of crystalline Vit. B_{12} or 500 μg of Vit. B_{12} as its tannate derivative (Depinar). Blood was obtained by venipuncture prior to administration as well as 8, 24, 72, 144, 216, 504 and 672 hours (4 weeks) after administration of Vit. B_{12} or "Depinar." Serum obtained by centrifugation was stored in frozen state until assayed for Vit. B_{12} by microbiologic assay with *Lactobacillus leichmannii* ATCC 4797 according to method of Skeggs and co-workers(2). All urine was collected for duration of study and aliquots were analyzed at 6, 24, 72, 96, 124, 144 and 168 hours by above technic. *Materials.* "Depinar" is a Vit. B_{12} tannic acid derivative prepared and kindly supplied by Thompson & Hecht of Armour & Co. Radioactive Vit. B_{12}, Co^{60} Vit. B_{12} (Co^{60} B_{12}) preparations employed for these tests were kindly supplied by Dr. Chas. Rosenblum of Merck & Co. Specific activity of 180 μc/mg. "Resting" cells of *Lactobacillus leichmannii* ATCC 4797 was described previously(3).

Results. In Fig. 1, urinary excretion of