produce the enzyme penicillinase among strains of *S. aureus* was accomplished. Four of 19 sensitive strains were capable of accepting the penicillinase marker. The 4 acceptor strains were sensitive to lysis by phages in Group I. Four different phages were capable of participating in the transduction process.

1. Bondi, A., Dietz, O. C., PROC. SOC. EXP. BIOL. AND MED., 1944, v56, 132.
2. Rammelkamp, C. H., Maxon, T., *ibid.*, 1942, v51, 386.
3. Zinder, N. D., Lederberg, J., *J. Bact.*, 1952, v64, 679.
4. Morse, M. L., *Genetics,* 1954, v39, 894.
5. Lennox, E. S., *Virology,* 1955, v1, 190.
6. Ritz, H. L., Baldwin, J. N., *Bact. Proc.,* 1958, 40.
7. Morse, M. L., *Proc. Nat. Acad. Sci.,* 1959, v45, 722.
8. Swanstrom, M., Adams, M. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 372.
9. Szybalski, W., *Antibiot. and Chemother.,* 1953, v5, 915.
10. Fairbrother, R. W., *Lancet,* 1956, v1, 716.

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Embryonic Survival in Relation to Number and Size of Implantation Swellings in the Rabbit *Oryctolagus cuniculus.** (26727)

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The mechanisms involved in implantation of blastocysts are different from those required for embryonic or fetal survival. The attachment of blastocysts depends on 3 principal mechanisms related to myometrial muscle, adhesions and invasion; these operate in sequence but with some overlap(1). Embryonic survival depends on other factors such as genetic makeup(2). age of female output of unknown substances in the dam's blood(3) and age of gametes at fertilization (4). Hormonal requirements of implantation are also different from those of embryonic survival(5). It has been shown experimentally that deficiency in progesterone can cause embryonic death at some stages of pregnancy(6).

In polytocous mammals, embryonic mortality is usually followed by reabsorption. With a very large number of implantations complete reabsorption of the litter during pregnancy is most common among younger females(7). In the rabbit, embryonic loss is distributed in a specific pattern; 7% immediately after implantation, 66% during midpregnancy and 23% during late pregnancy (8).

The purpose of this experiment is to examine embryonic survival in relation to number of implantations and diameter of uterine swelling at implantation sites. The ova transfer technic was used to obtain rabbits with different numbers of implantations within the physiologic range of the species (normal implantation number in the rabbit 5 to 15).

Materials and methods. A total of 56 pubertal and 49 adult rabbit does of New Zealand White, California, and crossbreds were used in this experiment. Average weights were 6.3 lb for pubertal does and 7.5 lb for adults. All rabbits were brought from one local breeder who used a mild degree of inbreeding. Prior to the experiments each doe was isolated for 16 days. Twelve fertile and vasectomized bucks were also available.

Donors. The pubertal does used as donors were superovulated by gonadotrophic hormones. Pregnant mare serum was used for the priming doses and HCG was used to induce ovulation. Soon after the ovulation-inducing injection, each donor was mated to

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TABLE I. Embryonic Survival† in Relation to Number of Implantations* in Both Uterine Horns.

Items		No. of implantations*/recipient 1–5	6–10	11–16
No. of recipients		22	14	13
No. of corpora lutea on both ovaries	Range	6–14	7–16	10–15
	Mean	11.4	11.4	12.2
Total No. of implantations*		73	107	160
No. of implantations/recipients—Mean*		3.3	7.6	12.3
No. of surviving embryos†	Range	0–5	5–10	7–11
	Mean	2.0	5.6	9.2
% embryo survival	Range	0–100	25–100	47–100
	Mean‡	56	74	76

* At 8 days post coitum.
† " 15 " " .
‡ Difference between the 3 means were not statistically significant.

2 fertile bucks. Forty to 48 hours post coitum, the donors were sacrificed by bleeding and the tubes were placed in sterilized Petri dishes. The ova were recovered by flushing the tubes with a few milliliters of sterilized physiological saline and were counted under a binocular microscope (×10), mixed in a watch glass and examined carefully (×45) for their normality. Healthy 8-32-blastomere ova were selected. The procedures were carried out inside a glass cabinet in a culture room kept at 25 to 28°C.

Recipients. Three groups of recipients were available; different numbers of fertilized ova were transferred to both uterine horns of the recipients. Recipients were mated to vasectomized bucks to induce pseudopregnancy. Recipients were anaesthetized with an intravenous injection of Nembutal (Abbott). Laparotomy was performed and 3 to 20 fertilized ova were transferred into each Fallopian tube. 25,000 I.U. of penicillin were sprayed in the abdomen near the site of incision.

The abdomen was closed in one layer using chromic gut (00) (Ethicon). Recipients were laparotomized from the midline at 8 days post coitum; number and diameter of implantation sites were recorded. At 15 days p.c., recipients were sacrificed and the reproductive tract was dissected. The number of corpora lutea on both ovaries was counted and viability of implantations was determined by dissecting the fetuses and examining the heart beat. The data included 340 implantations.

Results and discussion. The number of implantations expressed as a percentage of number of ova transferred averaged 83%. Nine recipients (18% of total number recipients) had implantations in one horn only. Ten recipients (20% of total number of recipients) did not show any embryonic mortality from 8 to 15 days of pregnancy. In four recipients all implantations were degenerating at 15 days p.c.; these represented recipients with one or two implantations.

Rate of embryonic survival was based upon the number of viable embryos (at 15 days p.c.) expressed as a percentage of the number of implantations (at 8 days p.c.).

Effect of number of implantations at 8 days p.c. The recipients were grouped into 3 categories according to number of implantations in both uterine horns at 8 days p.c., namely from 1 to 5; from 6 to 10 and from 11 to 16 implantations. Average number of corpora lutea on both ovaries was similar in the 3 groups, ranging from 11.4 to 12.2. Average implantation number in the 3 groups was 3.3, 7.6 and 12.3 respectively (Table I). Average rate of embryonic survival in the 3 groups was 56, 74 and 76%. There was no significant difference between the groups as judged by analysis of variance.

However, the data of the 3 groups were then pooled and correlation and regression were calculated between number of implanta-

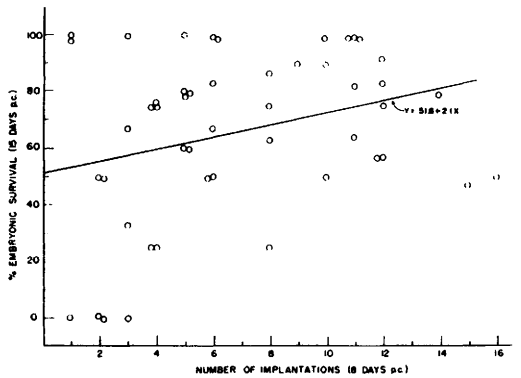

FIG. 1. Regression line between No. of implantations and embryonic survival. Each circle represents one recipient. There was a significant positive correlation.

tions and embryonic survival (Fig. 1). Rate of embryonic survival was positively correlated with number of implantations ($r = 0.290$, $n = 49$); this was significant at the 5% level. It was also noted that the location of the non-viable embryos was not related to any specific site along the uterine horn (near the tubal or cervical end) (Fig. 2).

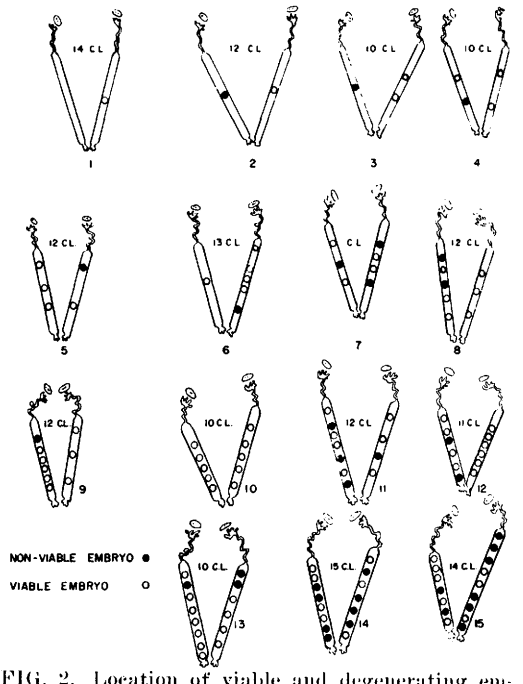

FIG. 2. Location of viable and degenerating embryos at 15 days p.c. in recipients with different numbers of implantations. Note that degeneration of embryos was not related to any specific site in uterine horn.

Effect of diameter of implantation sites at 8 days p.c. The diameter of implantation sites is indicated here as the "index of uterine swellings" (I.U.S.). This index, calculated for each litter, represented the average diameter of implantation sites (uterine swellings) which survived from 8 to 15 days p.c. The "index of uterine swellings" for the 3 groups of recipients was 8.8, 9.4 and 9.4 mm respectively (Table II and Fig. 3).

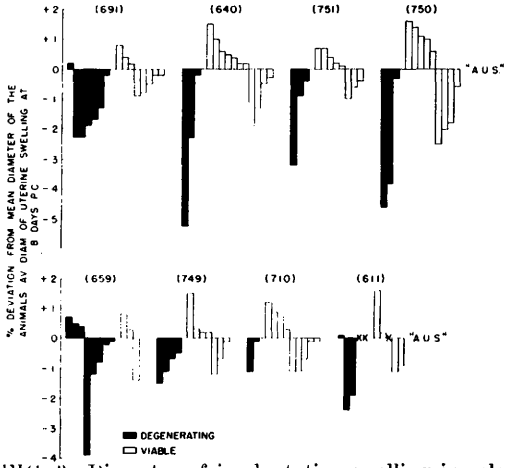

FIG. 3. Diameter of implantation swelling in relation to embryonic survival at 15 days post coitum. The 2 horizontal lines "A.U.S." represent mean diameters of uterine swellings (at 8 days p.c.) which eventually were detected as viable embryos at 15 days p.c.; the lines represent avg for each litter. Each embryo is represented by one column. The columns represent % deviation of swelling diameter (8 days p.c.) from the mean of its litter. Eight litters are represented; each litter included both degenerating and viable embryos. For instance, No. 691 had 1 degenerating embryo with a swelling diameter larger than the mean; and 6 swellings less than the mean; 3 viable embryos with swelling diameter larger than the mean and 5 swellings less than the mean. Most of the degenerating embryos had a smaller diameter of uterine swellings at implantation. × indicates swelling of a diameter to the average.

It appears that, within litters of average size, embryonic survival is not primarily related to number of implantations. There is a "ceiling value" for the number of implantation sites which can be successfully maintained. Beyond a certain level, the mortality tends to fall on the "litter" as a unit rather than on individual embryos, which by competition *inter se* prejudice each other's survival(8). This phenomenon seems more pronounced in excessively large litters in

TABLE II. Embryonic Survival in Relation to Diameter of Swellings at Implantation Site.

Items		No. of implantations/recipient 1–5	6–10	11–16
Total No. of implantations*		75	228	138
Index of implantation swelling (I.U.S.)†	Range	5.5–13.0	6.6–12.8	7.1–12.7
	Mean	8.8	9.4	9.4
Uterine swellings which did not survive until 15 days p.c.				
% over mean of I.U.S.		17	23	12
% below " " "		80	75	83
% equal to mean		3	2	5

* At 8 days post coitum.
† Diameter of swellings of implantations at 8 days p.c. which survived until 15 days p.c.

which the placental development may be adversely affected or the normal vascular supply is inadequate. It is possible that the "ceiling value" for embryonic survival varies with breed, strain and individual as well as it varies with species. Among women, it is well known that some individuals tend to abort repeatedly(9).

In 75 to 83% of the cases, the embryos degenerating at 15 days p.c. had at 8 days p.c., implantation diameters less than the average diameter of uterine swellings at 8 days p.c. This may indicate that most, but not all, the small implantations at 8 days p.c. are susceptible to embryonic death. However, the results do not show whether embryonic death at this stage is a result of retardation in placental development or that degeneration occurs very close to implantation with subsequent retardation of placental development.

Unilaterally ovariectomized females gestate the normal number of live embryos per pregnancy(10): since in such pregnancies, one uterine horn is empty, the average number of embryos gestated in the other horn is doubled. In the mouse, when the number of implantations in one horn is increased artificially above the normal level by ova transfer, embryonic survival is reduced in the other horn so that on an average the total number in the 2 horns combined does not exceed average litter size of the breed(7,11).

Attempts to increase litter size of a small-sized breed of rabbits by increasing the number of ova transferred were unsuccessful and, in fact, produced opposite effects(12). However, in superovulated sheep, with the increasing number of implants, number of surviving embryos reached a ceiling at a level of about 4 per pregnancy(13). This is nearly three times the normal litter size of sheep. The effects of endogenous and exogenous levels of steroid hormones on embryonic survival is still not fully understood.

Summary. Variable numbers of fertilized healthy ova (3 to 20) were transferred into 49 recipient rabbits of a synchronous stage of pseudopregnancy. The recipients were laparotomized at 8 days *post coitum* (p.c.) to record number and diameter of implantation sites. At 15 days p.c., the recipients were killed and number of viable embryos recorded. In 3 groups of recipients the number of implantations at 8 days p.c. was 3.3, 7.6 and 12.3 and rate of embryonic survival was 56%, 74% and 76% respectively with no significant difference between the groups. Embryonic survival tended to be positively related to number of implantations. Nonviable implantations were not located at specific sites along the uterine horn. Diameter of implantation swellings was not related to number of implantations per recipient. In 75 to 83% of the cases, the degenerating embryos (15 days p.c.) had implantation diameters (8 days p.c.) less than the average of viable implantation swellings.

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1. Boving, B. G., *Ann. N. Y. Acad. Sci.*, 1959, v75, 700.
2. Perry, J. S., *J. Reprod. Fertil.*, 1960, v1, 71.
3. Hammond, J., *Fertility*, Chap. 21, *Mashall's*

Physiology of Reproduction. Edited by A. S. Parkes, 1952, vII, p648.

4. Blandau, R. J., *Ann. N. Y. Acad. Sci.*, 1954, v57, 526.

5. Hafez, E. S. E., Pincus, G., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 531.

6. Smithberg, M., Runner, M. N., *J. Exp. Zool.*, 1956, v133, 441.

7. McLaren, A., Michie, D., *J. Exp. Biol.*, 1959, v36, 381.

8. Adams, C. E., *J. Reprod. Fertil.*, 1960, v1, 36.

9. Vignes, H., *Physiologie Obstetricale*, Paris, 1923, p184.

10. Hollander, W. F., Strong, L. C., *J. Exp. Zool.*, 1950, v115, 131.

11. McLaren, A., Michie, D., *J. Exp. Biol.* 1956, v33, 394.

12. Venge, O., *Acta Zool.*, 1950, v31, 1.

13. Robinson, T. J., *J. Agric. Sci.*, 1951, v41, 6.

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Propagation of Junin Virus, the Etiological Agent of Argentinian Hemorrhagic Fever, in HeLa Cell Cultures. (26728)

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During the late fall and early winter of 1958, an epidemic outbreak of a hemorrhagic, febrile illness occurred in the province of Buenos Aires, Argentine Republic. From the blood of several patients, strains of presumably one virus were isolated by Parodi *et al.*(1) and Pirosky *et al.*(2). Studies by these workers showed that the virus, which has been named Junin, was pathogenic for guinea pigs, newborn mice, and chick embryos. Their reported observations, however, as well as work in these laboratories, indicate that serial propagation of Junin virus in these species is not without difficulties. The incubation period in 1-day-old mice is from 10 to 15 or more days; furthermore, numerous animals survive inoculation at various serial dilutions of virus, with resulting irregular titrations.

Other workers(3) have also reported failure of Junin virus multiplication in monkey kidney tissue cultures and KB and HeLa cells. Nevertheless, it was decided to attempt propagation of the virus in HeLa cells according to methods that have been successfully applied in these laboratories to the study of arthropod-borne viruses(4,5).

Materials and methods. Virus: The present studies were done with strain *XJ* of Junin virus, supplied by Dr. Parodi. Similar investigations with strains *R5* and *F*, supplied by Dr. Pirosky, have yielded preliminary results closely resembling those obtained with strain *XJ*.

Immune sera. Immune sera against Junin virus were produced in mice and rabbits. Mice were given 3 injections of a 10% suspension of infected newborn mouse brain, the first 2 injections intracerebrally at a 3-week interval and the 3rd intraperitoneally; mice were bled 8-15 days after the last injection. Rabbits were inoculated intravenously with 0.5 ml of a 10% suspension of infected mouse brain in physiological saline once a week for 3 consecutive weeks, and exsanguinated 1 month after the last inoculation. Immune sera against other viruses were prepared in the same manner to serve as controls.

Tissue cultures. The stable cell line, strain HeLa (Gey), used throughout was obtained from The Tuskegee Institute in 1954 and has been transferred regularly, first at Division of Laboratories and Research, New York State Dept. of Health, Albany, and since 1957 in these laboratories.

The cells were grown in 160-ml French square bottles in a growth-promoting medium consisting of 45% Hanks' balanced salt solu-

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