mation. The finding of ribose among these products indicates an inversion at some level in this series of reactions. The nature of the unknown Bial-reactive phosphate is of interest in this connection. Whether the triose phosphate arose from the pentose phosphate (*5*) or some other product is being investigated.

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Transplantation of Rabbit Blastocysts at Late Stage: Probability of Normal Development and Viability at Low Temperature[1]

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Following previous investigations on transplantation of rabbit ova at different stages (*1*, *2*), the following experiment was performed. Rabbit blastocysts at late stage, 6 days after mating, were recovered from superovulated does (*7*) by flushing the excised uterus with fresh rabbit serum diluted with an equal volume of 0.9% NaCl. The percentage of recovery was very high, 75%–100%, as checked by counting the number of corpora lutea. The highest number of blastocysts recovered from one doe was 62. Most of the blastocysts measured 3 mm in diam, but a few of them measured 1 mm, probably due to the large number of ova produced by superovalation. Most of them were round in shape, and the germ disk appeared in the large ones. After recovery they were kept at 30° C in a watch glass placed inside a Petri dish no more than 45 min before transplantation.

Transplantation was performed by making a 5-mm longitudinal incision on the exposed uterus and by placing the blastocysts into the uterus with a pipette 4 mm in diam. The incision was closed with catgut sutures. Fifty blastocysts were transferred to seven recipients at the sixth day of pseudopregnancy, and six of the recipients gave birth to 21 normal young genetically resembling the donors. The gestation period was 26–27 days, and the percentage of blastocysts which developed into young in the pregnant recipients was 47.7.

The blastocysts, placed in a small tube containing serum diluted with 0.9% NaCl, were stored for 1 or 2 days at 10° C (at room temperature for 30 min before storage) or at 0° C (with acclimatization at 10° C for 2 hr). They were then cultured at 38° C in a Carrel flask containing undiluted serum. The results are presented in Table 1. After storage for 1 day at 10° C about one-third of them were shrunk, their round shape lost, but practically none shrank at 0° C for 1 day. After storage for 2 days, either at 10° or at 0°, most of them were collapsed, with separation of trophoblast and albumin coat, and had sunk to the bottom of the tube. In culture, it took about 12 hr for the shrunken blastocysts, or about 24 hr for the collapsed blastocysts, to recover their round shapes. Their growth was observed by enlargement in size and appearance of the primitive streak (or neural groove in two cases) after 3 days' culture.

TABLE 1

VIABILITY OF RABBIT BLASTOCYSTS AT LOW TEMPERATURE

Storage temperature in °C	Storage time in days	No. stored	% Shrunk or collapsed	No. cultured	No. recovered	No. grown
10	1	36	36	15	11	8
	2	39	87	25	19	6
0	1	37	3	9*	5	3
	2	11	91	6	0	0

* Eight of nine shrank in culture on the first day, five of eight recovered their round shape on the second day.

It is interesting to note that no blastocysts recovered in culture after storage at 0° C for 2 days, and that some intact blastocysts after storage at 0° C for 1 day shrank in culture on the first day and recovered on the second day. Very few of them resumed their growth. A temperature of 10° C is therefore better for the storage of blastocysts at the late stage, just as in the case of ova at an early stage (*1*).

Following these observations, 18 blastocysts after storage at 0° C for 1 day (all intact) were transferred to three recipients at the fifth and sixth days of pseudopregnancy. None was diagnosed as pregnant by palpation. At laparotomy, one small swelling on the uterus (indicating maternal placental formation) of one recipient was observed. Twenty-three blastocysts after storage at 10° C for 2 days (18 shrunk, five intact ones) were transferred to three recipients. Three swellings of different sizes but without normal embryos (indicating degeneration of embryos at different stages after placental formation) were observed in two of the recipients. Twenty-eight blastocysts after storage at 10° C for 1 day were transferred to five recipients. The first, which received four blastocysts, did not become pregnant; the second, which received four intact ones, had two large swellings as well as two small swellings at laparotomy and gave birth to two young at term; the third, which received three intact and four shrunken ones, had six normal embryos, 7–9 mm, when examined 6 days later. The last two animals each gave birth to two normal young 28 days after transfer. The percentage of development of blastocysts in the pregnant does was therefore 50.

The viability of blastocysts at different stages *in vitro* may not be the same. Most of the blastocysts which

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were recovered from the uteri 4 days after mating shrank in serum within 15 min at 30° C, but none of the large 6-day-old blastocysts shrank under this condition. It seems that 4-day-old blastocysts are more delicate than 6-day-old ones.

TABLE 2

DEVELOPMENT OF TRANSFERRED RABBIT OVA OR BLASTOCYSTS AT DIFFERENT AGES AND AFTER STORAGE AT 10° C FOR 1 DAY*

Age of ova in days	Ova transferred	Recipients used	Recipients pregnant	Young obtained	% Development of all ova transferred	Site of recovery and transfer
1	239	24	21	130	54	From tubes
Stored	94	7	4	35	37	to tubes
2	76	7	5	17	22	From tubes to uteri
3	132	11	5	41	31	From tubes
Stored	103	7	6	38	37	or uteri to uteri
4	167	17	13	71	43	From uteri
Stored	138	8	7	26	19	to uteri
6	50	7	6	21	42	From uteri
Stored	28	5	4	12	43	to uteri

* Transplantation at the corresponding stage of ova and corpora lutea.

In order to show the probability of normal development of transferred ova or blastocysts at different stages, and after low temperature storage, the data published previously (*2*) and the data accumulated in a recent study on the development and fate of transferred ova in relation to the ovulation time of recipients (*3*) were pooled and are presented in Table 2. After direct transfer to the portion of the tract normal for a given stage, the percentage of transferred ova developing into young varies from 31% (3-day ova) to 54% (1-day ova), indicating no very great variation in transplantability from stage to stage. Although storage at low temperature may reduce the viability of transferred ova (e.g., 4-day blastocysts), this is not invariable, so that 24-hr storage under the conditions we have employed appears to have no markedly deleterious effect. The low percentage of development in the case of 2-day-old ova is probably owing to the fact that ova were recovered from the tubes but transferred to the uteri.

The development of transferred ova in rabbits (*5, 8, 9*), in rats (*6*), and in mice (*4*) has been reported. So far as the writer is aware, this is the first report on the successful transplantation of blastocysts at a late stage. Technically, the recovery of tubal ova and transplantation of ova to the tubes of recipients require delicate surgery, whereas the recovery of ova or blastocysts from the uterus and transfer to the uterus of a recipient can be performed without surgery in large farm animals. The present study demonstrates this possibility. These findings have an application in the experimental study of early development in mammals.

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Brucella Agglutinin-blocking Phenomenon in Bovine Sera[1]

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Recently Griffitts (*1*) demonstrated the existence of an agglutinin-blocking property, sometimes called "incomplete antibody," in sera from known cases of human brucellosis. His work was prompted by reports of Wiener (*5*), Race (*4*), Levine (*3*), and others on the agglutinin-blocking phenomenon in sera of individuals sensitive to the Rh factor, and by the absence of agglutinins in significant concentration in a number of individuals known to have brucellosis.

The agglutination test is probably the most extensively used diagnostic procedure in both human and bovine brucellosis. Its diagnostic usefulness in bovine brucellosis may be even greater than in human brucellosis, since certain critical titers have been established designating an individual animal as a reactor, suspect, or nonreactor. Therefore, the existence of agglutinin-blocking substances in bovine sera could be of considerable diagnostic—and economic—importance. Although this blocking phenomenon has been encountered in human brucellosis sera (*1*), reports of its occurrence in bovine sera have not appeared in the literature. The following experiments demonstrate the presence, in significant concentration, of such agglutinin-blocking substances in bovine sera.

A study of the agglutinating and agglutinin-blocking properties was made on the sera of animals of five bovine herds. Three separate tests were carried out on each serum at repeated intervals: First, a double-dilution tube-agglutination test with dilutions in 0.9% saline beginning with a dilution of 1: 2. The last tube contained no serum and served as a control. The total volume of serum dilution-antigen mixture per tube was 1.0 ml. Tubes were incubated at 37° C for 2 hr and read after remaining in the refrigerator overnight. Second, an agglutinin-blocking test, consisting of the addition to each tube in the tube-agglutination test of 0.1 ml of known complete *Brucella abortus* rabbit antibody in a dilution such that complete agglutination occurred in the saline controls after further incubation at 37° C for 2 hr and refrigeration overnight. Third, a rapid macroscopic agglutination test, with the

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