mouse. Certain strains are highly lethal to the mouse and autopsy reveals the presence of viable organisms in such tissues as lung and liver (Mergenhagen, unpublished observations). The mechanism behind this specific induced state of resistance and some of the discrepancies of these results with findings of others require further investigation.

Summary. Vaccination with viable or heat-killed oral anaerobic gram negative cocci (genus *Veillonella*) increased the resistance of a strain of Swiss-Webster mice to the lethal effects of veillonella endotoxin. Protection was best conferred with serologically specific endotoxin prepared from the strain of veillonella used for vaccination. Utilizing weight loss to sublethal dosages as a measure of biological effects of endotoxin, the duration and extent of weight loss in vaccinated mice is also considerably reduced as compared to nonvaccinated animals. Vaccination with heat-killed veillonellae significantly protected *B. pertussis*-sensitized mice against veillonella endotoxin, but not against lethal doses of *E. coli* endotoxin, histamine, or serotonin. Vaccination of mice with another oral gram negative bacterium (*Fusobacterium*) did not protect pertussis-sensitized mice against the lethal effect of veillonella endotoxin.

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1. Schaedler, R. W., Dubos, R. J., *J. Exp. Med.*, 1961, v113, 559.

2. Mergenhagen, S. E., *J. Dent. Res.*, 1962, in press.

3. Mergenhagen, S. E., Zipkin, I., Varah, E., *J. Immunol.*, 1962, v88, 482.

4. Mergenhagen, S. E., Hampp, E. G., Scherp, H. W., *J. Infect. Dis.*, 1961, v108, 304.

5. Kind, L. S., *Bact. Rev.*, 1958, v22, 173.

6. Beeson, P. B., *J. Exp. Med.*, 1947, v86, 39.

7. Perreault, M., Schweinburg, F. B., Levenson, S., Israel, J., Fine, J., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 470.

8. Abernathy, R. S., Bradley, G. M., Spink, W. W., *J. Immunol.*, 1958, v81, 271.

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"Differential Cleavage Rate" in 2-day Litter Mate Rabbit Embryos.* (27448)

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In multicellular organisms the sizes of cells, other than ova, vary only between certain relatively narrow limits; body size is primarily a reflection of cell number rather than cell size. In most mammalian species, the greatest but not the most rapid growth occurs after the end of fetal period proper, during the later phases of the whole developmental cycle when there is comparatively little change of form (1).

One of the most significant leads in analysis of growth and size in rabbits was reported in connection with breed differences in size between Flemish-giant and small Polish(2). The differences were traced back to early embryonic stages, even though there are no differences in size of ova at the time of ovulation. Similarly, Venge(3) has noted differences in cleavage rate of fertilized ova between large and small sized breeds. At comparable stages, the average number of blastomeres and the blastocyst diameters is greater in large sized breeds than in small breeds(4). At 6½ days of pregnancy, rabbit blastocysts, within the same breed, weigh from 15 to 33 mg(5). Various physio-genetical mechanisms account for size differences in different species. In the rat, the time interval between cleavages from the second to the sixth day of

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gestation is not constant, and in fact varies over a wide range even among embryos from the same litter. Byerly *et al.*(6), studying hybrids in birds, found egg size to be more correlated to body size, than genetic constitution.

The term "differential cleavage rate" used here designates the presence of fertilized ova at different stages of cleavage simultaneously *within the same animal* (Fig. 1). This phenomenon, reported in the rabbit(7), rat(8) and other laboratory mammals, should not be confused with the occurrence of ova at different stages of development in the same animal as in superovulated cows. The latter case is a reflection of a long interval (3 to 6 days) between the rupture of the first and last ovulation(9) and consequently the ova are shed and fertilized at different times. In naturally ovulated and superovulated rabbits, ovulation of follicles occurs within a few hours.

Materials and methods. Donor (pubertal) and recipient (adult) does of the New Zealand large breeds were used. The donors were superovulated by injections of P.M.S. and H.C.G. as described by Hafez(10). They were then bred to fertile bucks and autopsied 2 days *post coitum* (*p.c.*) and the embryos flushed from the Fallopian tube. The embryos obtained from each donor were divided, on the basis of number of blastomeres, into slow- and fast-cleavage groups. The embryos were placed in 2-ml test tubes in a 1:1 serum:saline mixture and stored at 10°C, after slow cooling, (1°C/min) for 48 hr. The recipients were mated to vasectomized bucks and at the time of transfer of embryos, the recipients were at a stage of pseudopregnancy comparable with the stored embryos.

Slow-cleaving and fast-cleaving embryos obtained from a given donor were transferred into 2 Fallopian tubes of one recipient; the right and left tubes being used alternatively in the series for the slow-cleaving embryos. Four to 6 embryos were transferred in each Fallopian tube. The recipients were laparotomized at 8 days *p.c.* and the number and diameter of implantations were recorded. The recipients were then autopsied 29 days *p.c.* and number, weight and sex of viable fetuses were recorded. The results were evaluated statistically by the analysis of variance.

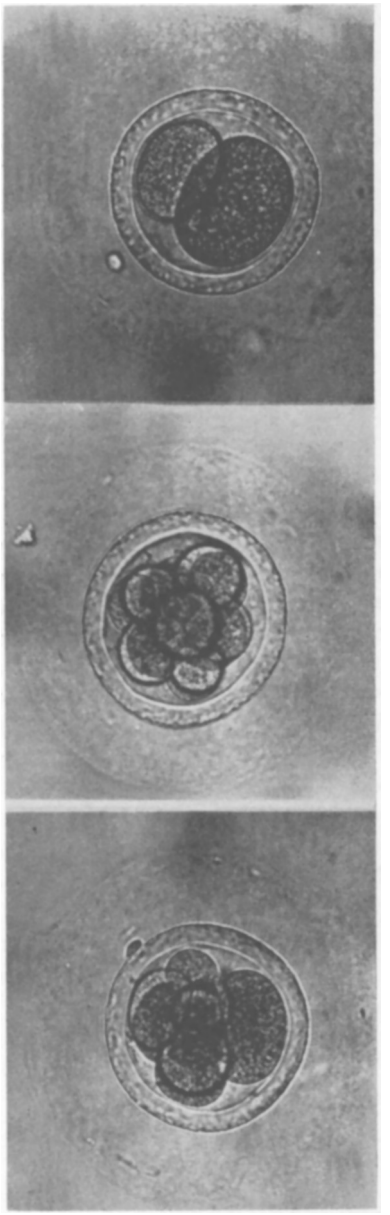

FIG. 1. Embryos recovered from doe No. 21, sacrificed 49 hr *post coitum* showing wide variations in rate of cleavage. Variation in cleavage rate of ova within the same doe takes place in does after natural mating or superovulation.

Results and discussion. The average number of blastomeres per embryo was 7 for the slow-cleaving group and 15 for the fast-cleaving group. The percentage of embryos which implanted was significantly higher in the fast-

TABLE I. Implantation and Fetal Development as Related to Pre-implantation Cleavage Rate (Donors and recipients were at 2 days *p.c.* at time of embryo transfer).

Items	Cleavage rate of transferred embryos		Statistical significance
	Slow	Fast	
Total No. of embryos transferred	60	62	—
Av No. of blastomers/embryo	7	15	—
Embryos implanted† (%)	38	77	*
Avg diameter of implants‡ (mm)	8.7	10.0	NSD
Fetal survival§ (%)	52	75	NSD
Avg fetal wt at 29 days *p.c.* (g)	41.3	44.7	NSD
Male:female ratio of fetuses	1:1.0	1:1.08	—

*Significant at 5% level.
NSD—no significant difference.
† Total number of embryos implanted at 8 days *p.c.* expressed as a percentage of total embryos transferred.
‡ Average length and width of uterine swellings at 8 days *p.c.*
§ Total No. of viable fetuses at 29 days *p.c.* expressed as a percentage of implantations at 8 days *p.c.*

cleaving than in the slow-cleaving group (Table I). It is possible that certain proportion of the so-called slow-cleaving embryos were either non-viable or in early stages of degeneration. It may be concluded that the viability of fast-cleaving embryos, as judged by survival after storage at 10°C for 48 hr, is higher than that of slow-cleaving embryos. There was no indication that fast-cleaving embryos yielded more males than slow-cleaving embryos.

There were no significant statistical differences between the two groups with respect to average diameter of uterine swellings at 8 days *p.c.*, percentage of fetal survival or fetal weight at 29 days *p.c.* The data on the diameter of implantations and fetal weight was expressed according to the number of implants per horn, for both the slow- and fast-cleaving groups (Table II). Space-wise, the slow-cleaving embryos had an advantage since there were less implantations than for the fast-cleaving embryos; this advantage was not demonstrated in fetal development. In most cases, the diameter of implants at 8 days *p.c.* and the fetal weight at 29 days *p.c.* was higher for the fast- than for the slow-cleaving embryos. The possible effect of overcrowding should be excluded from the present experiment since the number of implants per horn never exceeded six.

It is emphasized here that the classification

TABLE II. Diameter of Implantation Swellings and Fetal Weight of Slow- and Fast-Cleaved Embryos in Relation to Number of Implantations in one Uterine Horn.

	No. of implants/horn		Slow embryos — No. of implants/horn				
			0	1	2	3	4
Avg diameter of implants (mm)	Fast embryos	1				10.8 (8.2)	
		2	8.0 (L) 10.6 (L)	8.5 (6.5)			
		3					10.9 (7.9)
		4					14.2 (10.9)
		5		8.4 (7.4)	10.4 (8.3)		9.0 (9.2)
		6	10.0 (L)	11.2 (10.3)	8.1 (8.1)		
Avg fetal wt at 29 days *p.c.* (g)	Fast embryos	1		50.6 (42.8)			
		2	46.5 (L) 61.2 (L)		44.8 (40.8)		
		3					40.6 (37.8)
		4		34.4 (34.6)			
		5		49.2 (44.8)	40.6 (45.6)		
		6	42.1 (L)	48.8 (49.2)			

Figures in parentheses represent "slow" embryos. The other figures represent "fast" embryos. "L" indicates complete loss of embryos.

of embryo into slow- and fast-cleaving is based only on morphological criterion, *i.e.*, microscopical examination of litter mate embryos before transfer. (In the rabbit, the interval from one cleavage to the other varies from 6 to 16 hr.) In some cases the difference between the slow- and fast-cleaving embryos was one cleavage rate; in such cases the difference in number of blastomeres at 2 days *p.c.* may be due to some embryos being ready to cleave while others were completely cleaved. In other cases, however, the difference between slow- and fast-embryos was 3 or 4 cleavages.

In the rat, differences were found between embryos in rate of cleavage and there was a tendency for development of retarded embryos to be accelerated with time so that, by 120 hr *p.c.*, most of the viable ova had reached the blastula stage(8). Fetal size in the rabbit has been correlated with the concentration of glutathione in the fetus at birth, greater concentration being associated with higher growth rate(11,12,13). Further comparative studies are needed to evaluate the relationship between the number of blastomeres, at a given stage, in relation to subsequent rate of fetal development.

Summary. Two-day litter mate embryos were obtained from superovulated rabbits, divided into slow- and fast-cleaving according to the number of blastomeres per embryo, and stored in 1:1 serum:saline at 10°C for 48 hr. Slow- and fast-cleaving embryos obtained from one donor were transferred into the 2 Fallopian tubes of one recipient. The recipients were laparotomized at 8 days *p.c.* and autopsied at 29 days *p.c.* The viability of fast-cleaving embryos, as judged by per cent implants after storage, was higher than in slow-cleaving embryos. In most cases, 2-day embryos with more blastomeres had larger implants and heavier fetuses than embryos with less blastomeres; these differences were not statistically significant.

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1. Berrill, N. J., in *Analysis of Development*, 1955, Willier, B. H., Weiss, P. A., Hamburger, V. (eds.), Saunders, Philadelphia, p620.

2. Painter, T., *J. Exp. Zool.*, 1928, v50, 441.

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

4. Castle, W. E., Gregory, P. W., *J. Morphol.*, 1929, v48, 81.

5. Lutwak-Mann, C., *Proc. 3rd Internat. Congr. Anim. Reprod.* 1956 (Cambridge) Sec. I, 50.

6. Byerly, T. C., *J. Morphol.*, 1930, v50, 341.

7. Hafez, E. S. E., *Intern. J. Fertil.*, 1961, v6, 393.

8. Ishida, K., *Tohokyo J. Agric. Res.*, 1954, v5, 1.

9. Hafez, E. S. E., Sugie, T., *Proc. 4th Internat. Congr. Anim. Reprod.* 1961 (The Hague) Physiol. Sec., 26.

10. Hafez, E. S. E., *J. Reprod. Fertil.*, 1961, v2, 163.

11. Gregory, P. W., Goss, H., *J. Exp. Zool.*, 1933, v66, 335.

12. Goss, H., Gregory, P. W., *ibid.*, 1935, v71, 311.

13. Lerner, I. M., Gregory, P. W., Goss, H., PROC. SOC. EXP. BIOL. AND MED., 1936, v35, 283.

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Misleading Reductions of Serum Sodium and Chloride Associated with Hyperproteinemia in Patients with Multiple Myeloma. (27449)

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Conventional units which depict concentrations of electrolytes, clinically, in blood plasma or serum are milliequivalents per liter (meq/l), millimoles per liter (mM/l), milliosmoles per liter (mosm/l) and mg per 100 ml. Albrink *et al.*(1) have shown that under circumstances of extreme hyperlipemia (marked lactescence) spurious

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