Table 1

	No. of rats	Age (days)	Body weight (g)
With hypothalamic lesions	42	37·2 ± 5·98	75 ± 21·1
Sham operated	12	41·0 ± 2·38	100 ± 5·8
Normal controls	83	44·0 ± 4·75	108 ± 14·1

tions, but the precocity of the appearance of the phenomenon in the operated rats compared with normal controls is demonstrated both by the number of individuals (in six rats the vagina opened before they were 30 days old: in one on day 26, in one on day 27, and in four on day 29) and by the mean value for all rats. In normal rats we never observed this phenomenon earlier than day 32, regardless of season. Oddly enough, the opening of the vagina in sham operated rats was also significantly advanced (Table 1). A lag in the rate of gain of body weight regularly follows the injury to the hypothalamus, but this does not seem to affect seriously the precocity of the opening of the vagina. At death or after repeated laparotomies, that is, any time between 3 and 12 months after birth, the ovaries of all the rats in which the vagina opened precociously contained numerous corpora lutea, but this holds for an even larger number of rats in which the vagina opened within the interval characteristic for normal controls.

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[1] Donovan, B. T., and van der Werff ten Bosch, J. J., *Nature*, **178**, 745 (1956)
[2] Bogdanove, E. M., and Schoen, H. C., *Proc. Soc. Exp. Biol. and Med.*, **100**, 664 (1959).
[3] Elwers, M., and Critchlow, V., *Amer. J. Physiol.*, **198**, 381 (1960).
[4] Gellert, R. J., and Ganong, W. F., *Acta Endocrinol.*, **33**, 569 (1960).
[5] Horowitz, S., and van der Werff ten Bosch, J. J., *Acta Endocrinol.*, **41**, 301 (1962).
[6] Halász, B., and Gorski, R. A., *Endocrinology*, **80**, 608 (1967).

Continued Cleavage of Fertilized Bovine Ova in the Rabbit

FERTILIZED sheep ova have been shown to continue cleavage to the early blastocyst stage after transfer to the reproductive tract of the female rabbit[1]. This *in vivo* culture technique has facilitated the long distance transport of sheep ova[2]. This report suggests that a similar technique may be applied to cattle ova.

During 1967, cattle ova were recovered from the fallopian tubes of one hundred and forty cattle after superovulation treatment with placental gonadotrophins. Culture studies were made on five hundred of these ova. *In vitro* culture of ova in media based on cattle blood serum has not been successful. This agrees with previous reports[3]. Continued but limited cleavage to the 24-blastomere stage has been achieved in bovine follicular fluid. This supports the findings of other work[4].

In transferring cattle ova to the pseudo-pregnant rabbit, we have had limited success after using cattle serum as the recovery and transfer medium. We transferred one hundred and five fertilized ova using serum; fifty-one of these developed further. The maximum cleavage stage reached was nineteen blastomeres. We had better results after using bovine follicular fluid for recovery and transfer.

Seven sexually mature cattle were treated with 3,000 IU pregnant male serum gonadotrophin (PMSG) to induce superovulation on the sixteenth day of the oestrous cycle. The cattle were slaughtered 3 days after breeding and injection of 2,000 IU human chorionic gonadotrophin (HCG). Fifty-six of the fifty-nine ova recovered from these cattle were fertilized; thirty-two of these were used in transfers to seven pseudo-pregnant rabbits. Within 20 min of the slaughter of the cattle we began to recover the ova from their fallopian tubes using bovine follicular fluid. We then studied the ova within a temperature controlled cabinet (26·7° C).

Table 1. DEVELOPMENT OF CATTLE OVA IN THE RABBIT

Heifer identn.	Rabbit identn.	Fertilized ova Transferred	Fertilized ova Recovered	Initial cell stages	Final cell stages	Culture period (h)
98	*B*43	6	5	× 5 − × 10	4 × 80 + 1 × ruptured ovum	98
104	*B*7	6	1	× 4 − × 10	1 × 60 +	95·5
112	*B*9	4	2	× 6 − × 10	1 × 100 + 1 × 19	98
114	*B*5	4	3	× 8 − × 16	2 × 100 + 1 × degenerate ovum	94
124	*B*42	3	3	× 7 − × 8	3 × 90 +	94
129	*B*6	1	1	× 8	1 × 90 +	96
139	*B*28	8	4	× 5 − × 10	1 × 90 + 3 × 100 +	116·25

The fertilized ova were transferred in bovine follicular fluid to the ligated fallopian tubes of the rabbits, which had been injected 20–47 h previously with 30 IU of HCG. Four days later, the rabbit oviducts were removed and nineteen ova were recovered. Results of the transfers given are in Table 1. Seventeen ova had developed to the early blastocyst stage; in one instance the zona pellucida had ruptured and one ovum was degenerate. After recovery from the rabbits, the ova were fixed in acetic acid alcohol and stained with lacmoid solution. The high power of the microscope was used to estimate the number of nuclei in each blastocyst. On the basis of evidence for the early stages of the bovine ovum[5], the stages of cleavage reached by the cultured ova seem compatible with normal development.

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[1] Averill, R. L. W., Adams, C. E., and Rowson, L. E. A., *Nature*, **176**, 167 (1955).
[2] Adams, C. E., Rowson, L. E. A., Hunter, G. L., and Bishop, G. P., *Proc. Fourth Int. Congr. Anim. Reprod. (Hague) Physiol. Sect.*, 381 (1961).
[3] Brock, H., and Rowson, L. E., *J. Agr. Sci.*, **42**, 479 (1952). Dowling, D. F., *J. Agr. Sci.*, **39**, 374 (1949). Hafez, E. S. E., Sugie, T., and Gordon, I., *J. Reprod. Fert.*, **5**, 359 (1963). Pincus, G., *Proc. First Nat. Egg-Transfer Breed. Conf., Texas*, 18 (1951).
[4] Thibault, C., *Ann. Biol. Anim. Bioch. Biophys.*, **6** (2), 159 (1966).
[5] Hamilton, W. J., and Laing, J. E., *J. Anat.*, **80**, 194 (1946).

Effect on the Guinea-pig Taenia Coli of the Substitution of Strontium or Barium Ions for Calcium Ions

THE spontaneous discharge of the taenia coli of the guinea-pig is not inhibited by even a high concentration of tetrodotoxin (5×10^{-6} g/ml.), but it is blocked by a low concentration of ionic manganese (0·5 mM)[1], which is known to be a specific inhibitor of the calcium spikes in crustacean muscle[2] and also in barnacle muscle fibres[3]. These facts suggest that in the smooth muscle of taenia the membrane current during the action potential is carried principally by calcium ions. This would explain earlier findings that spike generation continues in solutions which are deficient in or free from sodium[4–6] and that