

orrhage exclude operative technique as a contributing cause in the results described.

To summarize briefly: (1) Removal of both ovaries on the first day of menstruation had no apparent effect on that period. (2) Double ovariectomy or injury to large follicles dated toward the end of, or immediately after, the follicular phase of the cycle was followed by apparently typical menstrual bleeding. These experimental menses appeared from 5 to 13 days before the expectation as calculated by the length of previously observed cycles and by Corner's mode<sup>2</sup> for cycle length of 27 days. (3) External coloring and swelling disappeared after double ovariectomy.

From these data it is concluded that menstruation is probably due to an absence of follicular hormonal stimulus after it has been acting for a certain period of time. In this connection it should be noted that the presence of the follicular hormone has been demonstrated in human *corpora lutea*.<sup>3</sup> It is quite possible that the corpus of the monkey may also continue to secrete the follicular hormone, thus postponing the onset of menstruation.

### 3003

#### Extirpation experiments upon the embryonic forelimb of the rat.

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The technique of intrauterine surgery by which experimental methods may be applied to mammalian embryos has recently been described.<sup>1</sup> The results of a preliminary investigation show that rat embryos, operated within the last eight days of development, generally survive the operation, remain viable and are born with the rest of the litter. It is possible to rear such animals although it is difficult to do so.

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<sup>2</sup> Corner, George W., *Carnegie Inst. Cont. to Emb.*, 1923, xv, 73.

<sup>3</sup> Allen, Edgar, *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 303.

<sup>1</sup> Bors, Ernst, *Roux's Arch. f. Entw.*, 1925, iii.

<sup>2</sup> Gerard, R. W., *Proc. Am. Soc. Physiol.*, 1926.

<sup>3</sup> Nicholas, J. S., *Anat. Record*, 1925, xxxi, 4.

The first point of attack to which this method is being applied is the study of the factors influencing limb development. The first phase of this problem, the capacity for regeneration, forms the basis of this communication.

The anesthetized pregnant animal is placed upon the operating table with the dorsum toward the operator. An incision is made extending from the costal line to the anterior margin of the hind leg, through the dorsal skin and musculature of the body wall. This admits the operator to a field in which the embryos are clearly visible through the uterine wall. This method of approach obviates excessive handling of the viscera and consequent operative shock.

The incision through the uterine wall is made just large enough to permit manipulation of the limb and its extrusion through the opening. The limb is then excised at the determined level by means of fine iridectomy scissors, ligature or cautery.

*Series I—Complete amputation of the limb at the scapulo-humeral junction.* (See Table I.) The limb subsequent to the twelfth day of development shows all essential details. The manus is well formed, the digits even in the youngest stages are clearly outlined at the time of operation.

This series comprising 104 operated animals (which in every case were permitted to complete the gestation period subsequent to the operation) gave absolutely negative results in regard to the power of regeneration possessed by the embryo of this form during the final third of the gestation period. There is superficial healing and the wound is closed within a relatively short time after the operation. The processes involved in healing are purely superficial in nature in all cases examined, being limited to the layers of the body covering. The deeper tissues belonging to the limb and to its girdle show no excessive proliferation.

*Series II—Amputation of the limb at the junction of the arm and forearm.* Similar results were obtained to those in the preceding series. Healing is more complete. A pad of connective tissue is formed over the stump and a callus is formed at the extremity of the bone wounds. No other indications of regenerative capacity are found. These results are based upon the gross study of 32 cases preserved at birth and two cases which have undergone further development, one to 90 days of age. In this single individual there is nothing to add to the conditions

found in those taken at birth except that a bursa has developed in the connective tissue forming the pad of the stump.

*Series III—Amputation of the manus at the radio-ulner articulation.* A series of 18 cases, see table, shows the same healing power but the complete absence of regeneration of lost parts.

*Series IV—Amputation of digits.* The tissues removed at operation must be regarded as having the greatest prospective potency, from the standpoint of tissue age, of any of the series. The digits are among the last elements of the appendage to undergo complete development and should therefore retain their

Series I—Total amputation of arm.

Age of embryo in days.	Days before birth.	Number of cases.
14	7	14
15	6	16
16	5	7
17	4	22
18	3	16
19	2	11
20	1	18

Series II—Amputation of the limb at the junction of the arm and forearm.

Age of embryo in days.	Days before birth.	Number of cases.
15	6	6
16	5	3
18	3	9
19	2	10
20	1	6

Series III—Amputation of the manus at the radio-ulner junction.

Age of embryo in days.	Days before birth.	Number of cases.
14	7	8
16	5	4
18	3	3
19	1	3

Series IV—Amputation of digits.

Age of embryo in days.	Days before birth.	Number of cases.
14	7	4
15	6	2
17	4	1
20	1	1

embryonic potency longer than those tissues entering into arm or forearm development at any given stage.

In so far as the gross study of the animals of this series is concerned, they show no more than do the preceding series concerning the potentiality of regeneration. Tissue proliferation and the steps in the healing of wounds in the embryo are now being studied histologically. These studies will form a separate communication.

The present results show that in rat embryos, operated subsequent to the fourteenth day of gestation, there is not regeneration sufficient to restore an amputated limb. The embryonic age at the time of operation represents two-thirds of the gestation period in this form. If, therefore, there exists a period in the rat embryo during which it is capable of reforming or replacing an amputated limb this potency must be restricted to a stage earlier than those here recorded.

### 3004

#### Fixed base in gastric juice.

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The authors in a study of the effects of pyloric obstruction in rabbits<sup>1</sup> noted a large loss of fixed base as well as of chloride ion into the stomach. Gamble and Ross<sup>2</sup> found sodium in vomited stomach secretions from a dog, following experimental obstruction of the pylorus, to the extent of about one-half the equivalence of the chloride ion loss. The experiments to be here reported were undertaken for the purpose of determining whether or not fixed base is contained to an appreciable extent in gastric juice produced under conditions permitting normal gastric function. So far as we are aware the only published measurements of

<sup>1</sup> Gamble, J. L., and McIver, M. A., *J. Clin. Invest.*, 1925, i, 531.

<sup>2</sup> Gamble, J. L., and Ross, S. G., *J. Clin. Invest.*, 1925, i, 403.