reported a red to purple material as a component of the root of the cotton plant; the color here described is apparently related to this material. Drueding also reported a yellow material obtained from the bark of the root. Although none of the workers established the identity of the red material, Wayne called it *Gossypic* acid due to its acidic nature. The red-purple pigment has been obtained in this laboratory by precipitation from the alcoholic extract of mature cotton plants with an excess of 10 per cent. sodium hydroxide, washing with slightly alkaline 80 per cent. alcohol, dissolving in water, and precipitating with hydrochloric acid; this gelatinous material was then washed with water. It dries to an amorphous powder of a red-purple color. When dissolved in 80 per cent. alcohol, it responds to the qualitative tests of the anthocyanins as given by Onslow.[5]

Although the yellow to orange material of the alcoholic extracts of the woody tissue of the root is less susceptible to isolation, qualitative tests applied to these extracts indicate the presence of a pigment which displays characteristics of the flavone and flavonol pigments. Early in the season the high-nitrogen fertilizers produced a higher concentration of the yellow than the high-phosphate, but as the season advanced the highest concentrations of the yellow of the woody tissue and the red of the bark were both produced by the same fertilizer treatment.

There has been observed a general correlation of the pigmentation of the cotton root with the physiological age of the plant, the reaction of the soil, the effect of fertilizers and the incidence of cotton root rot as observed in the field.

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THE DORSOVENTRAL AXIS OF THE FORELIMB BUD IN AMBLYSTOMA MICROSTOMUM

THE following experiments were performed to determine the stage of development at which the dorsoventral axis of the forelimb bud of *Amblystoma microstomum* is established. It has been known since Harrison's studies[1] that the dorsoventral axis of a limb may not be determined until much later than the anteroposterior axis. Swett[2] showed that in *A. punctatum* the former axis in the forelimb bud is not irreversibly established until stage 35 (Harrison's stages), whereas in *A. tigrinum* Hollinshead,[3] using grafts only two somites in diameter, found that it is not until stage 38 that all grafts retain their prospective asymmetry.

The forelimb bud of *A. microstomum* is first visible externally as a rounded elevation beneath somites 3, 4 and 5 at stages 35 to 36. Round disks of tissue 3 to 3½ somites in diameter were removed from this region at stages varying from 27 to 34. In order to reverse only one axis, left limb buds were transplanted to the right flank in a location midway between the fore and hind limbs. This orientation resulted in an inversion of the dorsoventral axis, while retaining for the anteroposterior axis a normal relationship.

In all, 104 operations were performed, but in 30 cases the host died. In those surviving, 9 grafts were resorbed and 25 produced limbs which were too imperfect to be interpreted. The remaining 40 cases formed supernumerary limbs: 17 with the asymmetry reversed and 23 were inverted left limbs with asymmetry not reversed.

Of the eleven positive cases operated on at stages 27 to 31, ten had reorganized the dorsoventral axis and formed right limbs (Table 1). The eleventh case, performed at stage 29, formed a double limb, the primary member of which was an inverted left limb. It is believed that either this case was an error or that the transplant was larger than 3½ somites. No inverted limbs were obtained in the seven positive cases transplanted at stages 30 and 31.

TABLE 1
SHOWING THE DETERMINATION OF THE DORSOVENTRAL AXIS IN AMBLYSTOMA MICROSTOMUM

Stage	Total	Harmonic right	Inverted left
27–29	4	3	1
30	2	2	0
31	5	5	0
32	12	7	5
33	9	0	9
34	7	0	7
Totals	**39**	**17**	**22**

Stage 32 proved to be the transitional stage. In about half of these cases there developed right limbs and in the other half, inverted left limbs. This variation may be explained on the basis of slight differences in the size of the graft or in the age of the donor.

At stage 33 there were nine grafts which developed limbs and all nine had retained their prospective asymmetry. The dorsoventral axis had been established at the time of the operation and had continued its devel-

[1] R. G. Harrison, *Jour. Exp. Zool.*, 32: 1–136, 1921.

[2] F. H. Swett, *Jour. Exp. Zool.*, 47: 385–439, 1927.

[3] W. H. Hollinshead, *Jour. Exp. Zool.*, 73: 183–194, 1936.

[3] C. C. Drueding, *ibid.*, 8: 245–246, 1877.

[4] F. B. Power and H. Browning, *The Pharmaceutical Journal and Pharmacist*, 93: 420–423, 1914.

[5] "The Anthocyanin Pigments of Plants," 2nd edition, 1925, Chapter 4, by M. W. Onslow.

opment according to its original orientation. Digits 3 and 4 were formed on the ventral border, indicating that this was the ulnar side, and as development progressed the palm of the graft turned dorsally.

Similar results were obtained at stage 34. In all positive cases the formation of the hand indicated that the asymmetry had not been reversed.

Several limbs transplanted at stages 33 and 34 have been sectioned. The asymmetry of the pectoral girdle was reversed; a harmonic right girdle had developed from a left limb rudiment. The supernumerary appendage was an inverted left limb with a normally oriented right girdle.

We conclude that the dorsoventral axis of the forelimb bud of *A. microstomum* is determined in a manner similar to that of other species of the genus which have been studied. The dorsoventral axis for 3 somite grafts is partially determined at stage 32 and becomes firmly established at stage 33. These experiments are being continued.

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THE RETICULO-ENDOTHELIAL SYSTEM AND THE CONCEPT OF THE "ANTI-HORMONE"[1]

NUMEROUS investigations have shown that, following prolonged administration of certain endocrine preparations, loss of sensitivity to the injected extract will eventually result. Collip and his associates believe that normally hormonal effects are kept in balance by the presence of specific anti-hormones and that these appear in excess when animals are chronically treated with various endocrine extracts. Another group claims that this response is of immunity reaction nature and believes that the inhibitory substances are antibodies. One way of determining which of the two interpretations is correct would be to administer such hormone preparations to animals from which a large portion of the reticulo-endothelial system, intimately concerned with antibody formation, has been removed. Since the spleen is the most concentrated source of reticulo-endothelial tissue in the body and since the ratio of its weight to body weight is relatively great in the rat, we have compared, in our first studies, the response of young immature splenectomized and normal hooded litter mate female rats to daily injections of 10 R.U. pregnancy urine extract (Follutein or Antuitrin S).

In the controls, the ovary size reaches a maximum after approximately 10 to 15 days of treatment and then regresses, becoming normal in size within two to three months, despite continued injection. The ovaries of the splenectomized rats, however, continue to grow, attaining weights approximately 2 to 3½ times that of the injected controls, 20 to 30 days after splenectomy. This increase in weight is due almost entirely to an increase in the size and, in many cases, the numbers of corpora lutea. With continued treatment the ovaries of the splenectomized animals begin to regress rather rapidly after about 30 days, due, we believe, to the establishment of a compensatory mechanism, in the form of a hyperplasia and increased activity of the remainder of the reticulo-endothelial tissue.

The vaginal smears of the control injected animals show a condition of almost continual estrus for as long as one month after beginning injections. The smears of the splenectomized rats, although indicative of estrus for a few days after the opening of the vaginal orifice, soon become almost of the complete diestrus type and remain that way for at least a month following onset of treatment. We interpret these results as follows. The injected normal immature rat, because its reticulo-endothelial system is intact, soon produces the inhibitory principle for the luteinizing factor believed to be present in the pregnancy urine extracts. The animal's own pituitary, however, is continually producing follicular stimulating substance for which no anti-substance is formed and so the animal remains in constant estrus. This finds corroboration in sections of ovaries of all our treated controls where large follicles are always present for at least 1½ months following beginning of treatment. The splenectomized immature rat, being deprived of a large amount of reticulo-endothelial tissue, produces for a time a smaller quantity of the inhibitory substance for the luteinizing principle. More and larger corpora result, and even though mature follicles are still present, the animal lapses into diestrus because of the greater preponderance of progesterone over estrin in the circulating blood.

It is of extreme importance that the rats employed be completely free of all traces of *Bartonella muris*, a latent infection quite prevalent in rats,[2] since this necessarily affects reticulo-endothelial activity. The following experiments indicate the importance of taking this factor into account.

(1) Unoperated immature Bartonella carrier rats in response to daily injections of 10 R.U. pregnancy urine extract for 20 days develop luteinized ovaries as large as those which are present in 20-day treated Bartonella free splenectomized animals. That this response is due most likely to reticulo-endothelial block-

[1] For chief references on the subject, refer to the papers by: J. B. Collip, *Jour. Mt. Sinai Hosp.*, 1: 28, 1934; G. H. Twombly, *Endocrinol.*, 20: 311, 1936; P. A. Katzman, N. J. Wade and E. A. Doisy, *Endocrinol.*, 21: 1, 1937; K. W. Thompson and H. Cushing, *Proc. Roy. Soc.*, B, 121: 501, 1937; I. W. Rowlands, *Proc. Roy. Soc.*, B, 121: 517, 1937.

[2] D. Perla and J. Marmorston, "The Spleen and Resistance." Williams and Wilkins Co. 1935.