

ually decrease until they lose their statistical significance at 18 days of age. There remains but little doubt of the reality of these deviations, and the conclusion that a single straight line does not fit the whole period of embryonic growth seems unescapable.

It is of interest to note that a similar picture prevails in embryos incubated under the high temperature of 105° F. as revealed by similar computations involving the data of Henderson and Brody.⁷ However, here the acceleration occurs at an earlier stage, which is, perhaps, in keeping with the usual temperature effects on rates of processes. The fragmentary data of the same workers on embryos incubated at 95° F. reveal a complete distortion of the logarithmic straight line. These observations lend support to the legitimacy of disregarding the data of the earlier workers.

The significance of the finding here reported undoubtedly needs further elaboration and explanation in biochemical terms. Thus the curve of log dry weight plotted against log time, presented graphically by Glaser from Murray's⁸ data, shows a very pronounced flexure of the same type as observed here. The differentiation of energy sources during the course of embryonic growth (Needham, p. 992) may also be recalled in this connection, as may also fluctuations in glutathione concentration (Gregory, Asmundson and Goss⁹).

The purpose of this note, however, is limited to drawing attention to the fact that while individual sets of data may produce a satisfactory fit to the logarithmic straight line, small deviations in the same direction and appearing at the same time in the majority of sets of reliable data can not be disregarded. Cognizance of this situation is commended to workers in the field of embryonic growth.

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THE ROLE OF HEREDITY VERSUS ENVIRONMENT IN LIMB BUD TRANSPLANTS BETWEEN DIFFERENT BREEDS OF FOWL

USING the technique developed by Hamburger,¹ the author has transplanted 60- to 70-hour White Leghorn limb buds into the coelome of Brown Leghorn hosts of the same age. The host embryos were then allowed to develop until 15 or more days of age. Two different types of results have been obtained. In seven cases

⁷ E. W. Henderson and S. Brody, *Mo. Agr. Exp. Sta. Res. Bull.*, 99, 1927.

⁸ H. A. Murray, Jr., *Jour. Gen. Physiol.*, 9: 405, 1926.

⁹ P. W. Gregory, V. S. Asmundson and H. Goss, *Jour. Exp. Zool.*, 73: 263, 1936.

¹ Viktor Hamburger, *Jour. Exp. Zool.*, 77: 379-399, 1938.

in which the grafts became attached to the mesenteries, a normal White Leghorn leg developed upon the Brown Leghorn host. The feathers of the transplant were white, and the scales on the shank and foot were characteristically pigmented. The White Leghorn graft, therefore, differentiated according to its hereditary potentialities. The environment, in these cases, seemed to have had no influence.

These results are at variance with those of Willier and his co-workers, who have reported² that White Leghorn limb buds developed colored plumage when grafted to colored breeds. Two grafts, however, have been obtained which seem to clarify this discrepancy. In one case the graft possessed Brown Leghorn feathers and the scales of the shank were pigmented, while the foot had yellow scales typical of the White Leghorn. In this instance environmental influences have been able to suppress, almost completely, the hereditary potentialities of the graft. In another the upper part of the leg was covered with brown feathers, the lower portion with white, and the shank and foot were unpigmented. This case, therefore, was an intergrade. Both these latter grafts were exceedingly well attached to the inner body wall.

These results indicate that White Leghorn plumage develops on grafts which are attached to the mesenteries and that Brown Leghorn plumage occurs on grafts that are attached to the body wall. It is possible that the results may be explained on the basis of a diffusion gradient between host and graft. If the graft is well attached to the host and an enzyme or "color-inducing substance" reaches the transplant in sufficient amounts, the hereditary potentialities of the graft are suppressed completely and the transplant develops the plumage and pigmentation characteristic of the host. If the attachment is less secure and a smaller amount of color-inducing substance reaches the graft, an intergrade results. In cases where little or no enzyme diffuses into the graft, a typical White Leghorn leg develops on the brown host. A further analysis of this problem is in progress.

In reciprocal transplants, Brown Leghorn limb buds transplanted to White Leghorn hosts, three different types of results were obtained. In several cases typical Brown Leghorn feathers developed upon the transplanted limb, and the shank and foot possessed the typical pigmentation of the Brown Leghorn. These results confirm the findings of Willier, who obtained similar results with skin grafts. In three cases the environment has suppressed, or at least retarded, the development of the brown feathers since 15-day-old transplants possessed all white feathers or else only a few feathers were pigmented.

² B. H. Willier, Mary E. Rawles and E. Hadorn, *Proc. Nat. Acad. Sci.*, 23: 542-546, 1937.

In one fifteen-and-one-half-day transplant which was attached to the mesenteries of the host, the outer portion of the graft was covered with brown feathers, the inner portion possessed white plumage, and the characteristic brown pigment was lacking in the lower leg and foot. Furthermore, typical Brown Leghorn feathers covered the greater portion of the right wing of the host. Although this latter condition may have been due to a somatic variation, Dr. Harry L. Kempster, professor of poultry husbandry at the University of Missouri, joins me in the view that the pigmentation of the wing feathers has been induced by the graft. This case seems to indicate a mutual interaction between the host and transplant or between the hereditary constitution and the environment.

A diffusion gradient apparently does not explain these latter cases, since some well-attached grafts developed colored plumage and other less well-attached transplants possessed white feathers. Experiments are in progress which may shed additional light on the problem.

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NEW OBSERVATIONS ON THE EFFECTS OF CALCIUM DEPRIVATION¹

THE authors have observed that a profound neurological disturbance develops in growing rats maintained on diets very low in calcium (0.01 to 0.02 per cent.) which has heretofore not been associated with a deficiency of calcium. The neurological picture, which is quite complex, is suggestive of a diffuse lesion involving the cortex, basal ganglia, spinal cord and peripheral nerves.

The nervous condition is best demonstrated by subjecting the experimental animals to short and mild galvanic shocks from an induction coil after they have been on the low calcium ration for six weeks or more. This stimulus causes the rats to collapse. The rats remain conscious, but they respond poorly and sluggishly to all stimuli. They show little ability to right themselves or to grasp objects with their paws. The fore limbs remain relatively normal, but there is always a paralytic foot drop of one or both hind limbs. Immediately after the onset of the collapse, the tail becomes anesthetic, while the head and trunk appear to be hyperesthetic. After a lapse of about 24 hours, the head and trunk also become anesthetic. The normal propulsive locomotion of these animals is greatly altered, and they generally show a retropulsive response which is not observed in the normal rat.

The effects of the disturbance appear to be rever-

sible. The degree of prostration increases the longer the animals are kept on the calcium deficient diet. Severely depleted animals remain in a state of prostration for long periods of time and, as a result, usually die of inanition. Less severely deficient animals recover from the prostration after a varying period of time, even if they are still kept on the low calcium ration. Recovery, however, is hastened by feeding the control diet to the injured animals.

A condition similar to that which is induced by the galvanic stimulus may develop spontaneously in animals that have been maintained on the experimental diet for a period of from 9 to 14 weeks. The effect of the spontaneous collapse is very severe and the animals usually die within a few days.

Visible hemorrhage occurs frequently. In the central nervous system it has been observed in the cerebrum, spinal cord and the circle of Willis. This finding suggests that the neurological disturbance may well be a secondary effect of the vascular pathology. Hemorrhagic areas also have been noted in the lungs, gastrointestinal tract, bladder, bone and in the muscles of the gluteal region.

Chemically, the calcium deficient animals exhibit a low blood calcium, which was found to vary from 4.4 to 6.6 mg per 100 ml of serum.

Tetany does not occur in animals when merely reared on the low calcium rations. Tetany can be induced in thyro-parathyroidectomized rats by placing them on the low calcium diet. It takes 4 to 6 weeks to develop on this regimen. The tetanic spasms can be induced with a galvanic shock or, even better, with the hissing sound from an air jet.

Another point of considerable interest is that, although the bony skeleton is almost completely decalcified, the teeth appear to be relatively well calcified.

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