requiring, which insures germination near the surface. However, the soil-air interface is a harsh environment. As the center of radiation exchange, it undergoes the most rapid and extreme temperature changes, and with regard to the moisture factor, it is alternately bombarded by splattering raindrops and subjected to severe droughts. Therefore, any depth below the surface, however slight, will be an improvement from the standpoint of an environment for germination of seeds. If then, a seed is light-requiring, it is of some survival value for it to be red sensitive, since this property will facilitate germination at nearly the maximum depth of penetration by visible light. Of course, this will not prevent the seed from germinating on or near the surface, where the risk of early desiccation is greatest; it merely confers on the seed the capacity to germinate where this risk is diminished.

PHILIP V. WELLS*
Department of Botany, Duke University, Durham, North Carolina

References and Notes

1. E. H. Toole, S. B. Hendricks, H. A. Borthwick, V. K. Toole, *Ann. Rev. Plant Physiol.* 7, 299 (1956).
2. P. V. Wells, Ph.D. thesis, Duke University (1958).

* Present address: Department of Biological Sciences, University of California, Goleta.

16 September 1958

Critical Period for Effects of Infantile Experience on Maturation of Stress Response

Abstract. Manipulated infant rats respond to cold with depletion of adrenal ascorbic acid (AAA) significantly earlier than nonmanipulated infants. The study discussed in this report examined the critical period for infantile manipulation on the depletion of AAA. It was found that infant rats manipulated immediately following birth exhibited significant AAA depletion, whereas infants manipulated later did not exhibit depletion.

Recently it has been reported (*1*) that infant rats which had been manipulated (handled) once daily from birth responded to cold stress with a significant depletion of adrenal ascorbic acid as early as 12 days of age, whereas nonmanipulated infant rats did not show significant AAA depletion until 16 days of age. One question which arose from this study was whether the age at which the experimental treatment of manipulation was initiated is a significant factor in the accelerated maturation of the systems which result in AAA depletion with stress.

The experiment discussed in this report (*2*) was directed, therefore, toward answering the question of whether there exists a critical period in the development of the organism during which manipulation has its greatest effect on the AAA depletion response to stress. The existence of such a period seemed likely, since critical periods have been documented for many other aspects of development (*3*).

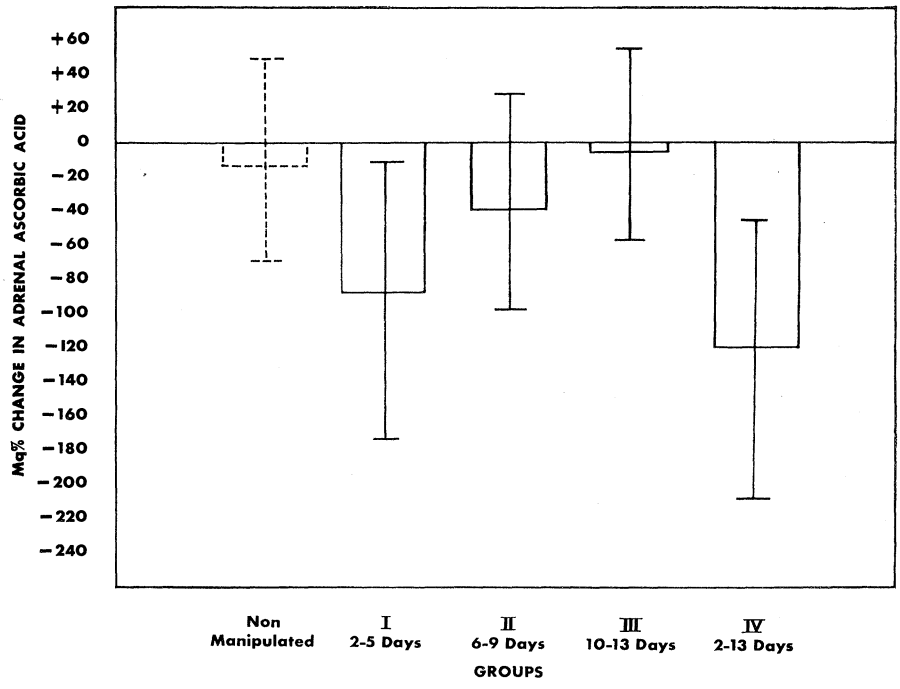

Fig. 1. Comparison of depletion in AAA in the various groups of infant albino rats of the study. The bar represents the mean depletion; the lines, the range. The dotted bar and dotted line represent untreated animals that had previously been tested.

Seventy-six infant Sprague-Dawley albino rats were used as subjects. The subjects were assigned at birth to one of four groups. For the infants in group I ($N = 20$), the treatment was initiated on the second day following birth and continued through day 5. The treatment was started on day 6 and was continued through day 9 for group II infants ($N = 20$). The treatment for the group III subjects ($N = 20$) was given from day 10 through day 13. The last group, group IV, received the treatment from day 2 through day 13. The experimental treatment was identical to that previously described (*1*) and consisted of removing the pup from the nest, placing it in a 2.5- by 3.5- by 6-in. compartment for 3 minutes, and then returning it to the nest. This procedure was followed once daily during the period assigned to the subject. At 14 days of age, approximately half the pups within each group were randomly assigned to either the stress or control condition to test for AAA depletion with stress.

The stress conditions and method of analysis for AAA are fully described in previous reports (*1*) and, therefore, will be only briefly described here. The nonstressed subjects within each group were killed by cervical spinal separation and weighed. The adrenals were removed, weighed, and assayed for AAA by the modified method of Glick *et al.* The stressed infants were subjected to a cold stress of 5°C for 90 minutes before removal of the adrenals and determination of AAA.

The results of this experiment are shown in Fig. 1 and are expressed in terms of milligrams percent change in AAA level. Change in AAA level was determined by subtracting the AAA present in the stressed animals from the mean for the nonstressed subjects.

The data clearly indicate that the age during which the infant rat is manipulated is a major variable in the effect described in this report. Only the animals in groups I and IV showed significant AAA depletion. In terms of percentage, the group I subjects showed a 25-percent depletion and the group IV subjects showed a 32-percent depletion. The depletion in AAA in the group II and group III animals (9 percent and 0 percent, respectively) did not differ significantly from that in the respective controls. Thus, in the groups (I and IV) which had been manipulated during the period directly following birth, a significant depletion in AAA is evidenced in response to cold stress at 14 days of age, whereas the groups manipulated later in infancy do not show significant AAA depletion.

Recent evidence has indicated that the early postnatal period is also critical for behavioral changes during adulthood.

Schaefer (*4*) found that handling during the first 7 days produced the greatest reduction in adult emotionality measured in terms of behavior in an open field situation. Denenberg (*5*) reports that handling during the first 10 days of life resulted in avoidance learning superior to that found when handling was initiated later. In both of these studies, the period during which the treatment was initiated includes the critical period found in the experiment discussed in this report. Whether behavioral difference can be detected in experiments with such restricted age groups as were tested in this experiment remains to be determined.

SEYMOUR LEVINE
GEORGE W. LEWIS
Columbus Psychiatric Institute and Hospital and *Ohio State University Health Center, Columbus*

References and Notes

1. S. Levine, M. Alpert, G. W. Lewis, *J. Comp. Physiol. Psychol.*, in press.
2. This investigation was supported by research grant PHS M-1630 from the National Institute of Mental Health of the National Institutes of Health, U.S. Public Health Service.
3. J. P. Scott, *Psychosom. Med.* 20, 42 (1957).
4. T. Schaefer, Ph.D. dissertation, University of Chicago (1957).
5. V. Denenberg, paper presented at the 1958 meeting of the American Psychological Association.

31 July 1958

Nonidentity of Fetuin and Protein Growth (Flattening) Factor

Abstract. Fetuin, a fetal calf serum glycoprotein, appeared to possess activity with cultured mammalian cells similar to that of a protein growth factor partially purified from adult bovine and human sera. Column chromatography, however, yielded highly purified but inactive fetuin. These results leave open questions regarding the role of this interesting and readily purified protein.

Efforts to characterize the factors present in animal sera required for the growth of mammalian cells in culture led to the partial purification of a protein from bovine and human sera (*1*). The protein, studied most extensively with a culture of human origin, Appendix A 1 (*2*), causes adherence of cells to a glass surface; only in its presence do cells assume a flattened, epithelial-like appearance; and it is required for growth. Available evidence indicates that it is a glycoprotein.

Fisher, Puck, and Sato (*3*), working independently with a different cell culture, HeLa S3, reported some of these effects with fetuin, a glycoprotein from fetal calf serum. Their interesting results not only offered a rich source of the activity [fetuin represents about 45 percent of fetal serum protein (*4*)], but also suggested a possible in vivo growth-stimulating role for a molecule with flattening factor activity. The similar activity levels of fetal calf and beef sera, however, despite the high level of fetuin in the former, raised the question whether the activity of fetuin preparations might not result from contamination with the growth factor.

To test this possibility, highly purified fetuin was prepared. The protein was first precipitated from fetal calf serum with ammonium sulfate according to the initial step of the procedure of Fisher et al. (*3*). Electrophoretic analysis of this preparation showed that about 75 percent of the protein resided in a single peak whose mobility was the same as that of fetuin. After dialysis for 24 hours against sodium phosphate buffer (0.01*M*, *p*H 7.1), the ammonium sulfate fraction was applied to a column of DEAE-cellulose (type 20, height 10 cm, diameter 1.8 cm) according to the procedure of Sober *et al.* (*5*). Elution was carried out with sodium phosphate buffer solutions containing increasing concentrations of NaCl. Protein was estimated by the method of Lowry *et al.* (*6*); flattening factor activity was estimated by determination of the lowest concentration of each fraction which caused Appendix A 1 cells to adhere to a glass surface and induced the attached cells to assume an epithelioid shape (*1*).

The results of the fractionation procedure are illustrated in Table 1. As can be seen from Table 1, eluates 1 and 2 contained 74 percent of the recovered protein but only 6 percent of the recovered activity. On the other hand, most of the recovered activity appeared in eluate 4, which represented less than 12 percent of the protein.

To show that the inactive, peak-protein fractions contained fetuin, one of them (eluate 2) was examined ultracentrifugally and electrophoretically. As is shown in Fig. 1, ultracentrifugation yielded a single, symmetrical peak and the sedimentation constant ($Sw^{20} = 2.73$) was in good agreement with that of fetuin [$Sw^{20} = 2.60$ (*7*)]. In confirmation, electrophoretic analysis revealed a peak whose area was greater than 90 percent of the total area and which showed a mobility of -5.1×10^{-5} cm^2 v^{-1} sec^{-1} [under the same conditions (*8*), the mobility of fetuin in fetal serum was found to be -4.9×10^{-5}].

Ultracentrifugal examination of the active fraction, eluate 4, was complicated by its low protein concentration. However, two well-defined peaks were revealed. The major peak, representing about 80 percent of the total protein, was asymmetric and sedimented at a rate ($Sw^{20} \approx 3$) similar to that of fetuin, while the minor component sedimented

Table 1. Chromatographic separation of fetuin and flattening factor. The eluents contained sodium phosphate buffer (0.025*M*, *p*H 7.1) except for eluate 4, which had instead 0.05*M* KH_2PO_4. In addition, the eluents contained 0.05, 0.075, 0.10, or 0.5*M* NaCl for eluates 1 to 4, respectively.

Fraction	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg of protein)
Fetal serum	600	29,620	49.4
Ammonium sulfate	175	10,580	60.5
Pass through	1.0	0	0
Eluate 1	41.8	0	0
Eluate 2	85.0	250	2.9
Eluate 3	25.2	525	20.8
Eluate 4	19.8	3,390	171

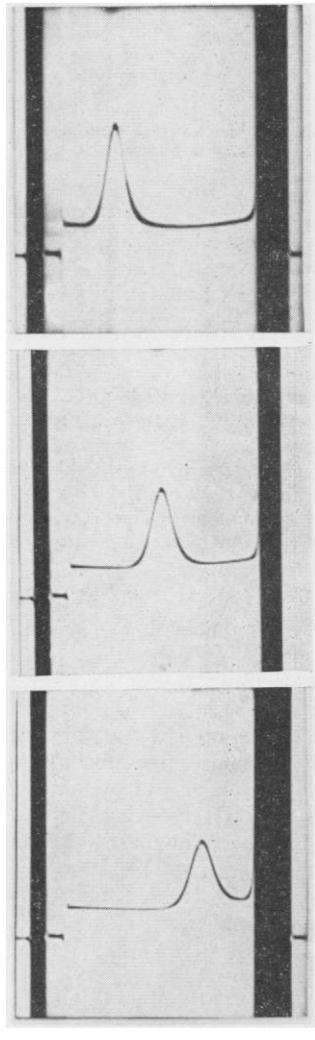

Fig. 1. Ultracentrifugal analysis of chromatographically purified fetuin. The protein, 10 mg/ml, was dissolved in sodium phosphate buffer (pH 7.1, $\Gamma/2 = 0.21$) and centrifuged at 59,780 rev/min. The photographs, from top to bottom, were taken 66, 126, and 186 minutes after maximum speed had been reached. The bar angle was 70 deg.