counting cells on selected histological sections (*3*, *4*) is subject to error in interpretation because the result does not give information about the increase in the total number of cortical cells unless it is assumed that this increase is similar in all directions.

Clendinnen and Eayrs (*5*) have repeated some of these experiments (subcutaneous injection of pituitary growth hormone into pregnant rats) and found in the mature offspring a 20-percent increase in the cell-gray coefficient, 23-percent increase in the mean number of dendrites, and a 22-percent increase in the mean length of dendrites; these changes were statistically significant. The estimated probability of interaction between an extended axonal system and dendritic field had increased by 55 percent. These animals showed a statistically significant enhancement in the performance of cortically mediated behavior (closed-field Hebb-Williams test). Berry, Rogers, and Eayrs (*2*) subsequently suggested that the addition of the superficial laminae, and hence the degree of complexity of the cortex, may be attributable to a prolongation of the period during which mitoses may occur in the ependymal layer.

Block and Essman (*6*) used the same technique of injection into pregnant rats, but administered a smaller dose of growth hormone. The offspring at maturity showed a statistically significant improvement in extinction of a conditioned avoidance response.

Our experiments were mainly designed to correct inadequacies mentioned above (*4*). Eleven pregnant Sprague-Dawley rats were injected from the 7th till the 20th day of pregnancy with purified bovine pituitary growth hormone (*7*). The daily dose was 3 mg, which is estimated to be 9 times higher than the entire growth hormone content of a 9-mg pituitary of an adult rat (*8*). The injections were either subcutaneous (eight animals) or intravenous (through a canula permanently implanted in the jugular vein, three animals). Eight control animals received similar injections of saline. After delivery (normal and at term), 85 offspring (newborn or 20 days old) were killed; the brains were removed without cerebellum and olfactory lobes (*9*) and the wet weight of these brains was determined. Fifty-two brains were used for the determination of DNA (*10*). For histologic study the brains of rats 20 days old (four control and three experimental) were fixed in formalin, embedded in paraffin and sectioned (*11*) at 10 μ, and stained by the Nissl method with thionin at *p*H 4.6 (*12*).

Neither the subcutaneously (Table 1) nor intravenously (Table 2) injected animals showed significant increase in body weight (Tables 1 and 2). On the other hand, brain weight and, consequently, the ratios of brain weight to body weight in the experimental animals were significantly higher. Thus, the organ in which most cells (neurons) have a very short proliferation period responded to growth hormone more strongly than the rest of the body.

Cortical cell density per unit volume (by estimate from histologic preparations) at 20 days was 63 percent higher in subcutaneously injected animals than in controls; this suggests a delayed differentiation and possibly a prolonged proliferation period.

The increase in total number of brain cells in experimental animals, as compared to controls, was estimated from the increase in the total DNA per brain (*10*), on the basis of evidence that the total amount of DNA per diploid cell of a species is constant and that brain cells are essentially diploid (*10*). The increase in the total amounts of DNA per brain, and consequently, increases in total number of brain cells, followed the increases in brain weights and were statistically significant (Tables 1 and 2). The increases in DNA were lower than the estimated increases in cell density, probably because the former refer to the total number of brain cells whereas the latter refer to cortical cells only.

The neuron-glia index (ratio) (*13*) (by histologic estimate) in rats 20 days old was 71 percent higher in subcutaneously injected experimental animals than in controls. Thus, the total increase in cell number affected the cells with a short proliferation period (neurons) more than those that may continue mitoses after birth (glia).

Our data support the conclusion that the final number of cortical neurons can be significantly increased by stimulation with pituitary growth hormone administered before the neurons cease to divide.

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References and Notes

1. J. B. Angevine and R. L. Sidman, *Nature* **192**, 766 (1961); short-axoned neurons (microneurons) may be an exception in that they may still proliferate after birth [J. Altman and G. D. Das, *Nature* **207**, 953 (1965)].
2. M. Berry, A. W. Rogers, J. T. Eayrs, *ibid.* **203**, 591 (1964).
3. S. Zamenhof, *Growth* **5**, 123 (1941).
4. ———, *Physiol. Zool.* **15**, 281 (1942).
5. B. G. Clendinnen and J. T. Eayrs, *J. Endocrinol.* **22**, 183 (1961).
6. J. B. Block and W. B. Essman, *Nature* **205**, 1136 (1965).
7. Supplied by the Endocrinology Study Section, NIH, as prepared in the laboratory of A. E. Wilhelmi, Emory University.
8. Y. Ishida, A. Kuroshima, C. Y. Bowers, A. V. Schally, *Endocrinology* **77**, 759 (1965).
9. The term "brain" used hereafter refers to such a brain without cerebellum and without olfactory lobes.
10. S. Zamenhof, H. Bursztyn, K. Rich, P. J. Zamenhof, *J. Neurochem.* **11**, 505 (1964).
11. The sections used were A24 to A27, as suggested by W. Zeman and J. R. M. Innes, *Craigie's Neuroanatomy of the Rat* (Academic Press, New York, 1963), p. 183.
12. W. F. Windle, R. Rhines, J. Rankin, *Stain Technol.* **18**, 77 (1943).
13. K. R. Brizee, J. Vogt, X. Kharetchko, *Progr. Brain Res.* **4**, 136 (1963).
14. Supported by NIH grant HD-01331.

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Transmigration of Lymph Nodes by Tumor Cells

Abstract. *When either V2, Brown-Pearce, or Walker tumor cells were perfused at low pressure into the afferent lymphatic of popliteal lymph nodes or were injected into the foot pads of rabbits, they rapidly appeared in lymph draining from the node. This finding indicates that lymph nodes are not the effective barrier to dissemination of tumor cells they had previously been assumed to be.*

Virchow in 1860 (*1*) first proposed the theory that lymph nodes act as a barrier to particulate matter in lymph. The effectiveness of nodes as traps for inert particles (India ink, gum acacia-graphite, and other materials) (*2*), bacteria (*3*), viruses (*4*), and red blood cells (*5*) has been repeatedly evaluated under a variety of experimental conditions. As a result of opinion and inference from such investigations, there exists, with few exceptions (*6*), the generally held belief that lymph nodes provide an effective barrier to the passage of tumor cells. Neither Yoffey and Courtice (*7*), Ruszнyák, Földi, and Szabó (*8*) nor Willis (*9*) in their extensive reviews allude to a single experiment in which tumor cells were employed to test this function of the lymph node. Aside from the report of Zeidman and Buss (*10*), the literature is to our knowledge completely devoid of evidence obtained by such in-

vestigation to substantiate this concept. Zeidman and Buss noted in 1954 that when V2 or Brown-Pearce carcinoma cells were injected into the afferent lymphatic of the rabbit popliteal lymph node and the nodes were removed 1 to 42 days later, only 2 of 30 animals demonstrated growth of tumor in pelvic nodes. This suggested to them that the popliteals as well as other lymph nodes were an effective, albeit temporary barrier to the further spread of cancer. The failure to observe tumor growth at sites other than in the injected node is, in our opinion, not conclusive evidence that transnodal passage of tumor cells failed to occur. Consequently, we have reinvestigated this aspect of tumor spread.

The afferent and efferent lymphatics of the popliteal lymph nodes of anesthetized rabbits were identified with or without the use of Evans Blue dye previously injected into the foot pad. With minimal trauma and no manipulation of the node, these vessels were cannulated with polyethylene tubing (PE-50). The cannula in the afferent lymphatic was connected by a Y connection to a 1-ml syringe which, by means of constant perfusion with a Harvard pump, delivered a suspension of either V2, or Brown-Pearce, or Walker tumor cells. The last tumor grows only in rats. The other limb of the Y tube was connected by polyethylene tubing to a pressure transducer, strain gauge, and recorder. In 20 such preparations 0.5 to 1.0 ml of a tumor cell suspension containing from 0.5 to 20 $\times$ 10^6 cells were delivered during a 30- to 60-minute period. Pressures were constantly monitored and were maintained below 50 mm-Hg—for the most part less than 30 mm—during infusion. In some animals the efferent lymphatic was cannulated prior to tumor cell delivery and in others the efferent catheter was inserted 2 hours after injection. Proof that afferent lymph vessels did not circumvent test nodes was obtained by dye studies with injected dyes and anatomical dissections. Lymph was obtained after tumor cell injection with and without saline infusion into the afferent vessel. It was collected in tubes containing citrate and formalin and permitted to fix for 24 hours. Samples were passed through 5-μ Millipore filters which were subsequently stained with hematoxylin and eosin, mounted on glass slides, and examined for cells. Tumor cells morphologically identical with those of the parent tumors were observed in one or more of the lymph samples collected from 15 such preparations. They were present in the efferent lymph following perfusion of as few as 500,000 cells and at pressures as low as 10 mm-Hg. All three types of tumor cells were found in efferent lymph.

To eliminate the possibility that results were an artifact of the perfusion, Brown-Pearce and V2 rabbit tumor cell suspensions (1 to 5 $\times 10^6$ cells in 2 ml of saline) were injected into the foot pad after the efferent lymphatic of the popliteal node had been cannulated. Lymph was collected and treated in a manner similar to that described above. Tumor cells were evident in all of ten such preparations within 60 minutes after inoculation; not infrequently tumor cells were observed in lymph collected 10 minutes after inoculation. In many, cells appeared not only singly, but in clumps.

Extensive investigation is under way to quantitate the transnodal passage of such cells, to evaluate the effect of nodal alteration, and to equate cell viability following its egress from the node. The data obtained from these experiments prompts the conclusion that while tumor cells *may* be sequestrated in the lymph node, they also traverse that structure.

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References and Notes

1. R. Virchow, *Cellular Pathology*, F. Chance, trans. (Lippincott, Philadelphia, 1863).
2. R. K. Gilchrist and V. C. David, *Ann. Surg.* **108**, 621 (1938).
3. C. E. Drinker, M. E. Field, H. K. Ward, *J. Exp. Med.* **62**, 849 (1934).
4. J. M. Yoffey and E. R. Sullivan, *ibid.* **69**, 133 (1939).
5. C. A. Buckman and W. M. Stahl, Jr., *Surg. Forum* **14**, 116 (1963); A. Engeset, *Lancet* **1962-I**, 324 (1962); C. F. Silvester, *Amer. J. Anat.* **12**, 447 (1912).
6. E. Goldman, *Proc. Roy. Soc. Med. Surg. Sec.* No. 1, 1, 1 (1907); J. M. Yoffey, *Brit. Med. J.* **2**, 1052 (1932).
7. J. M. Yoffey and F. C. Courtice, *Lymphatics, Lymph and Lymphoid Tissue* (Harvard Univ. Press, Cambridge, Mass., 1956), pp. 94–110.
8. S. Rusznyák, M. Földi, G. Szabó, *Lymphatics and Lymph Circulation* (Permagon New York, 1960), p. 35.
9. R. A. Willis, *Pathology of Tumours* (Butterworth, London, ed. 3, 1960), pp. 167–172; *The Spread of Tumours in the Human Body* (Butterworth, London, 1960).
10. I. Zeidman and J. M. Buss, *Cancer Res.* **14**, 403, (1954).
11. Supported by PHS grants CA-05716, CA-06670, HE-07822, and American Cancer Society grant P-142. The technical assistance of Joyce Mitchell, E. Rodis, and R. Pellegrini is acknowledged.

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Maternal Effect in Dental Traits of the House Mouse

Abstract. *The size of the second and third mandibular molar teeth of inbred mice is altered if the mice are fostered on a different strain. The dental change is opposite in direction to that of body weight. The prenatal and postnatal components of variance are correlated with the developmental histories of the two tooth types.*

The maternal effect in mammals is generally regarded as the nongenetic influence of a dam on her progeny. It includes a prenatal element associated with the egg cytoplasm or the uterine environment or both; it may also include a postnatal element associated primarily with the lactational performance of the dam. Its presence has been demonstrated in several mammalian species, most particularly with respect to measures and correlates of overall body size (*1*). Maternal effects acting on a specific anatomical system independently of overall body growth or on a specific morphogenetic event have only rarely been demonstrated in mammals (*2*).

With respect to quantitative traits in an isogenic strain, a maternal effect will result in a correlation of sibs and, perhaps, in a regression of offspring on dam. The differences between sibships in an isogenic strain in a controlled macro-environment may be considered as due principally to differences in the micro-environment or "noise" acting via the maternal physiology. In genetically variable populations, the maternal effect may include, in addition to the noise component, the effect of differences in maternal physiology consequent to the variation in the maternal genotypes. This effect is, of course, nongenetic with respect to the traits of the offspring.

Only recently has the relative importance of the maternal effect on dental dimensions of a genetically variable mammalian population been determined. In a randomly bred strain of house mice approximately 16, 17, and