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7. *Thyonella gemmata* hemoglobin was crystallized by the following method, which has also been used successfully on the sea cucumbers *Thyone briareus, Cucumaria elongata, C. miniata*, and *Molpadia intermedia*. Most of the hemoglobin is precipitated between 35 and 45 percent saturation with ammonium sulfate at 0°C, at *p*H 7.0 to 7.5. The precipitate is dissolved in approximately 10 volumes of cold potassium phosphate buffer (ionic strength, 0.6; *p*H 7.5), and the hemoglobin solution is dialyzed against distilled water at 0°C. Within from 12 hours to, at the most, 3 days good-sized crystals are obtained. Oxyhemoglobin, CO-hemoglobin and methemoglobin can be crystallized. In my studies the hemoglobins were kept under CO except where destined for oxygen equilibrium measurements. The hemoglobin heterogeneity is not altered by crystallization.
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9. Molecular-weight data might also be explained by the interconvertibility of hemoglobin components I_a, I_b, I_c and I_d in terms of successive dissociation of a tetramer, although the data are still equivocal. An experiment on a preparation of hemoglobin from the "thin" sibling species was performed by Dr. C. D. Trader, Dept. of Chemistry, Florida State University. Only a single sedimenting component appears in "thin" hemoglobin examined in the analytical ultracentrifuge; the molecular weight estimated from the corrected sedimentation constant is 41,000. This is too high for the expected molecular weight of the hemoglobin dimer (32,000 to 34,000) and too low for that of the tetramer (66,000 to 68,000). The unusual molecular weight may result from either (i) a polypeptide chain size different from that of the 16,000 to 17,000 unit typical of all vertebrate and some invertebrate hemoglobins, or (ii) a sufficiently rapid dissociation and association of subunits so that a single boundary is observed in sedimentation equilibrium. Whatever the explanation for the anomalous molecular weight, it does not detract from the significance of the studies on polypeptide-chain type, although it means that at present it is not possible to tell if the multiple hemoglobins are dimers (A_2, AB, AC, etc.) or tetramers (A_4, A_2B_2, A_2C_2, etc.), or some mixture.
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Stimulation of the Proliferation of Cortical Neurons by Prenatal Treatment with Growth Hormone

Abstract. *Subcutaneous or intravenous injections daily of purified bovine pituitary growth hormone into pregnant rats from the 7th till the 20th day of pregnancy (total dose 36 mg) resulted in offspring with unchanged body weight but with significant increases in brain weight, brain DNA content, cortical cell density, and ratio of neurons to glia.*

This report deals with the problem of whether the final number of cortical neurons can be experimentally increased for the eventual purpose of studying a possible correlation between such an increase and behavior.

Conceivably, such an increase in number could be achieved by stimulating mitoses during the period in which neurons are still capable of dividing (proliferation period). In mice and rats this period ceases around birth (*1*, *2*). Conceivably such stimulation will be more effective for cells with a short proliferation period (neurons) than for cells for which this period extends longer throughout life. Pituitary growth hormone was chosen as the proper stimulant since this hormone is known to stimulate protein synthesis and to improve nitrogen retention. Nitrogen retention may also result in stimulation of synthesis of other essential nitrogenous constituents of the cell, such as nucleic acids.

These concepts were supported by experimental evidence. Previous studies (*3*, *4*) have demonstrated that subcutaneous injections of bovine pituitary growth hormone into tadpoles (*3*) or pregnant rats (*4*) resulted in a statistically significant increase in brain weight and in number of brain cells (of tadpoles or of rat offspring, respectively). These experiments had certain inadequacies which, at that time, could not be rectified. One was the possible side effects of the impurities in the growth hormone preparations that were then available; another was that the finding of an increase in total number of cells, which also included glia, did not prove that the number of neurons themselves has increased. Still another was that

Table 1. Offspring of rats injected subcutaneously with bovine pituitary growth hormone from the 7th till the 20th day of pregnancy.

Newborn				20-day-old	
Weight (g)		Brain wt/ body wt	Total DNA per brain (μg)	Cortical cell density (10^6 cells)	Neuron-glia index†
Brain	Body				
Control rats					
0.156 ± .006*	6.25 ± .47	0.0247 ± .0019	438 ± 37	1.9 ± .05	1.87 ± .25
Experimental rats					
0.182 ± .024	6.4 ± .71	0.0285 ± .0017	495 ± 32	3.1 ± .15	3.2 ± .32
Increase (% of control)					
17	2.4	15.4	13	63	71
Probability					
.004	.60	< .001	< .001	< .001	< .001

* Standard deviation. † Estimates on 35 (5625 μ^2) grids per slide (2nd and 3rd layer), 21 slides (*11*, *12*).

Table 2. Offspring (newborn) of rats injected intravenously with bovine pituitary growth hormone from the 7th till the 20th day of pregnancy.

Weight		Brain wt/body wt	Total DNA per brain (μg)
Brain (g)	Body (g)		
Control			
0.152 ± .008*	5.98 ± .44	0.0248 ± .0017	426 ± 37
Experimental			
0.197 ± .018	6.06 ± .62	0.0329 ± .0011	530 ± 19
Increase (% of control)			
30	1	33	24
Probability			
< .001	.66	< .001	< .001

* Standard deviation.

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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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-