and perfusion of the brain was begun within 30 seconds. The perfusate consisted of 3 percent glutaraldehyde in 0.1*M* cacodylate buffer and 0.02 percent $CaCl_2$. After 20 minutes of perfusion, the brain, pituitary gland, eyes, and optic nerves were removed and placed in jars containing the perfusion fluid. Areas of special interest were dissected out from these tissues which were then fixed further in osmium tetroxide for 2 hours, dehydrated in graded ethanols, and embedded in Araldite after an intermediate stage in toluene. Sections 1 μm thick were cut with glass knives (0.95 cm) and stained for light microscopy (*9*). Sites of lesion formation identified by light microscopy were examined with the electron microscope in ultrathin sections prepared from the same block.

A lesion affecting the periventricular-arcuate region of the hypothalamus and essentially identical in light microscopic appearance to the form of pathology seen in mouse brain after MSG treatment (*2, 7*) was readily apparent (Fig. 1). Electron microscopic examination established that the cellular constituents primarily affected were dendrites and cell bodies of neurons. Many synaptic complexes could be found in which the postsynaptic (dendritic) component was massively dilated (Fig. 2). These processes were either empty or contained degenerating organelles and diffusely distributed particulate debris. The presynaptic component (axonal) of such complexes was usually unaffected, as were axon bundles passing through the region of injury. Many neuronal cell bodies were swollen with intracellular edema and, in some, the cytoplasmic organelles appeared to have undergone a lytic process, while nuclei showed marked alterations in chromatin pattern (Fig. 3). A mild intracellular edema of the ependyma was evident, but this was not accompanied by degenerative changes in either nuclei or intracellular organelles, and no alterations were noted in the appearance of junctional complexes between ependymal cells. No structural alterations were detected in glial or vascular components to suggest involvement of these elements in the pathological process.

The lack of symptoms in this primate infant during the time when a small percentage of its brain cells were being destroyed is evidence of a subtle process of brain damage in the developmental period, which could easily go unrecognized were it to occur in the human infant under routine circumstances. However, a high dose of MSG was used to produce brain damage in this neonatal monkey, and it was administered by the subcutaneous rather than oral route. Thus, while we have demonstrated susceptibility of a primate species to the mechanism of the glutamate effect, it remains to be seen whether this mechanism can be triggered by any set of naturally occurring circumstances. Presumably, an elevated blood concentration of glutamic acid is an important prerequisite to lesion formation.

In attempting to evaluate the risk of glutamic acid blood concentrations rising high enough to produce brain damage in the human infant, it is important to recognize that the oral dose of MSG is but one among several potential determinants of glutamic acid concentrations in the blood. Other factors, such as circadian periodicity (*10*), viral infection (*11*), immaturity of enzyme systems, rapid absorption from an empty gastrointestinal tract, and individual variations in metabolic capabilities could act in concert with a high glutamate diet to produce much higher concentrations of glutamic acid in an infant's blood than might be expected were such factors overlooked.

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Luteinizing Hormone–Releasing Activity in Hypophysial Stalk Blood and Elevation by Dopamine

Abstract. *Pituitary halves incubated in pituitary stalk plasma release more luteinizing hormone than their opposite halves incubated in plasma from peripheral blood. Glands incubated in stalk plasma from dopamine-treated rats release more luteinizing hormone than glands incubated in stalk plasma from untreated controls. Luteinizing hormone–releasing activity in stalk plasma may be due to the luteinizing hormone–releasing factor, and the secretion of luteinizing hormone–releasing factor may be controlled by a dopaminergic mechanism.*

Adrenergic and cholinergic mechanisms are thought to be involved in the regulation of gonadotropin release from the anterior pituitary (*1*). For example, monoamine oxidase (*2*) and cholinesterase (*3*) activities and the monoamine content (*4*) of the hypothalamus vary during the estrous cycle and at other times when there are changes in the production of ovarian or testicular steroids, as during pregnancy and after castration. It has been shown by means of a histochemical fluorescence technique (*5*) that monoamines are present in high concentrations in several regions of the mammalian nervous system and that adrenergic nerve terminals are especially dense in the hypothalamus near the median eminence (*6*). Recent results indicate that dopamine stimulates the release of gonadotropins from pituitaries incubated with hypothalamic tissue in vitro (*7*).

We observed that in rats the concentration of luteinizing hormone (LH) in systemic blood increases after the injection of dopamine into the third ventricle of the brain (*8*). When the anterior pituitary was perfused directly with dopamine by means of a microcannula inserted into a pituitary stalk portal vessel (*9*), so as not to involve the hypothalamus, LH release was unaffected (*8*). We now report that dopamine increases LH release by stimulating the secretion of luteinizing hormone-releasing factor (LRF).

Hypophysial stalk blood was col-

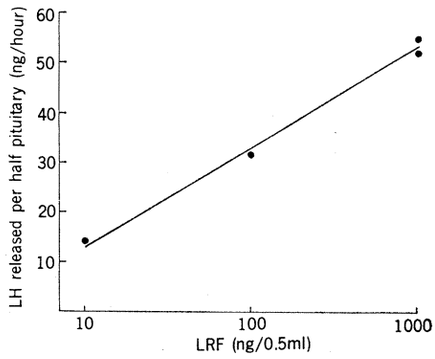

Fig. 1. Relation between purified ovine LRF and LH release by rat pituitary halves in vitro.

lected for 2 to 4 hours from male Sprague-Dawley rats weighing 350 to 450 g (*10*). Blood collected from the femoral artery of the same animals was used for control purposes. Donors of stalk blood were treated in one of two ways. At the beginning of the collection period, one group of rats was given dopamine hydrochloride (equivalent to 2.5 μg of dopamine) in 2.5 μl of isotonic saline solution by injection by means of a microcannula into the third ventricle. The cannula was inserted through the floor of the third ventricle midway between the optic chiasma and the median eminence. In the second group, the ventricular injection was omitted.

Blood plasma from 10 to 12 similarly treated rats was combined, and portions were assayed in vitro for LH-releasing activity. Anterior pituitaries from animals killed by decapitation were bisected along the midline. One half of a pituitary served as the control, and the opposite half served as the experimental tissue. All incubations were performed under an atmosphere of 95

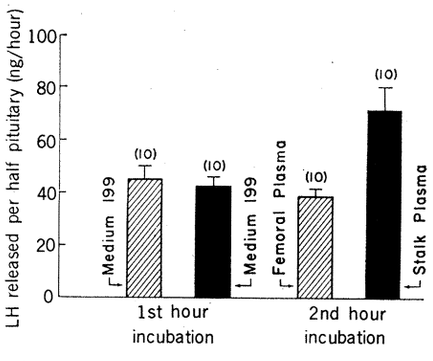

Fig. 2. Effect of hypophysial stalk plasma from untreated rats on LH release by pituitary halves in vitro. The number of halves is shown in parentheses. The vertical bar represents the standard error.

percent O_2 and 5 percent CO_2 in a metabolic shaker set for 45 rev/min and 25°C. Each pituitary half was placed in a separate flask containing 0.5 ml of a tissue culture solution (Difco medium 199, *p*H 7.2). After 1 hour, the incubation fluid was decanted; blood plasma from the femoral artery (peripheral plasma) was added to one pituitary half, and hypophysial stalk plasma was added to the opposite half of the same gland. The incubation was continued for a second hour. The quantity of LH released into the incubation medium by each pituitary half was determined by radioimmunoassay (*11*).

A satisfactory log-dose relation exists between the concentration of purified ovine LRF in the incubation fluid and the quantity of LH released (Fig. 1). Therefore, this system in vitro is adequate for the detection of LRF activity.

The concentration of LH in peripheral plasma was undetectable; however, stalk plasma from the rats treated with dopamine and from the untreated rats contained 2.1 and 3.4 ng/ml, respectively, equivalent to the standard National Institutes of Health LH-S1. These quantities were taken into account in the calculation of the final results.

During the first hour, when medium 199 was the incubation fluid, the spontaneous release of LH was 45 ± 5.1 (mean and S.E.; $N = 10$) and 43 ± 3.9 ng/hour by one set of hemipituitaries (Fig. 2) and 46 ± 6.0 and 50 ± 4.5 ng/hour by another set (Fig. 3), which shows close agreement among the groups when the conditions were identical. When plasma was substituted for medium 199, the rates of LH release by pituitary halves in peripheral plasma were 39 ± 3.4 and 41 ± 5.6 ng/hour, respectively (Figs. 2 and 3), which was essentially the same as that released in the first hour when medium 199 was the incubation fluid. The pituitary halves incubated in stalk plasma from the untreated animals released 72 ± 8.4 ng/hour or 1.8 times as much LH as those incubated in peripheral plasma (Fig. 2). The differences between the responses of the paired halves were statistically significant ($P < .01$). The halves incubated in stalk plasma from the dopamine-treated rats released 178 ± 36.6 ng/hour or 4.3 times as much LH as the halves incubated in peripheral plasma (Fig. 3). Again, the differences between the responses of the paired samples was significant (P

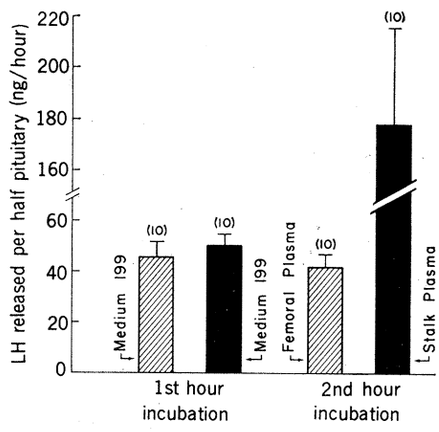

Fig. 3. Effect of hypophysial stalk plasma from dopamine-treated rats on LH release by rat pituitary halves in vitro. The number of halves is shown in parentheses. The vertical bar represents the standard error.

$< .01$). Also, the quantity of LH released by the glands incubated in stalk plasma from the dopamine-treated rats was significantly greater than that released by pituitary halves incubated in stalk plasma from untreated animals ($P < .01$).

These observations show that in the rat pituitary stalk plasma contains LH-releasing activity and the activity in stalk plasma is increased when dopamine is injected intraventricularly. The LH-releasing activity is attributed to LRF, the release of which may be regulated by a dopaminergic mechanism.

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Human Diploid Cell Transformation by DNA Extracted from the Tumor Virus SV40

Abstract. *The DNA isolated from simian virus 40 (SV40) can transform human fibroblast cells in tissue culture. Clonal lines of DNA-transformed human cells have been obtained that have properties characteristic of cells transformed by whole virus. They all contain SV40 T-antigen, and infectious virus can be recovered by cocultivation. This is the first demonstration of a permanent genetic alteration produced in human cells by purified DNA.*

Simian virus 40 (SV40), a small, DNA-containing tumor virus, has been shown to induce a morphological transformation of various cell types, including human cells, in tissue culture (*1*). The virus-transformed cells are characterized by their epithelioid morphology and lack of sensitivity to contact inhibition of cell division (*2*). They contain SV40-specific messenger RNA (*3*), and infectious virus can generally be recovered (*4*). The in vitro transformed cells are tumorigenic in the appropriate animal host (*1*). SV40-infected cells produce a new antigen, the T-antigen, that can be detected in the cell nucleus (*5*). The production of this antigen persists in transformed cells and in tumor cells.

The nucleic acid extracted from SV40 was shown by Gerber to be infectious for green monkey kidney cells (*6*). The observation by McCutchan and Pagano (*7*) that the infectivity of SV40 DNA could be markedly enhanced with diethylaminoethyl-dextran led us to study the effect of SV40 DNA on human cells. The studies described below demonstrate that infectious nucleic acid can enter and permanently alter the genetic makeup of a normal human cell.

A human fibroblast strain (F.R.), obtained from a skin biopsy specimen of a clinically normal adult female, was tested. It had previously been shown to have high susceptibility to transformation by SV40 (*8*). Other cells studied included green monkey kidney cells, the mouse cell line Balb/3T3 (*9*), and a line of rat kangaroo fibroblasts. Cultures were maintained in 50-mm plastic petri dishes in Dulbecco's modification of Eagle's medium supplemented with 10 percent calf serum. For experiments with whole virus, a pool of plaque-purified SV40 small-plaque virus (SV-S) (*10*) was used.

Small-plaque SV40 DNA (*11*) was extracted with a mixture of chloroform and isoamyl alcohol in the presence of sodium dodecyl sulfate and sodium perchlorate (*12*), and the double-stranded, twisted circular DNA (DNA I) was isolated by equilibrium density centrifugation in cesium chloride containing ethidium bromide (*13*). One μg of DNA I was found to correspond to 3.0×10^4 plaque-forming units.

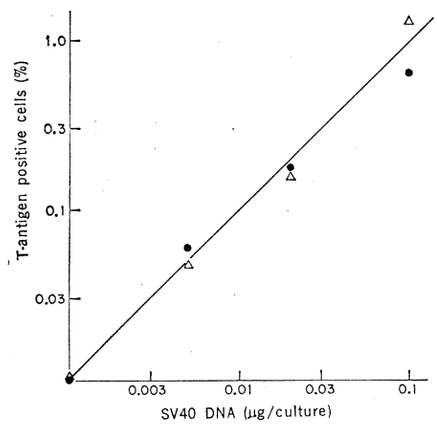

Fig. 1. Induction of SV40 T-antigen in monkey and human cells by SV40 DNA; relationship between micrograms of SV40 DNA I per infection and the fraction of T-antigen positive cells. Each point represents the data from two separate experiments; at least 20,000 cells were scored for each point. Triangles, green monkey kidney cells; dots, human diploid fibroblasts.

Cells in the logarithmic phase of growth were washed twice with serum-free medium and incubated with 0.2 ml of fluid containing SV40 DNA and 300 μg/ml of diethylaminoethyl-dextran in Earle's balanced salt solution without sodium bicarbonate. After 30 minutes at room temperature with frequent gentle agitation, the cultures were washed twice and fresh medium was added. Within 24 hours the cells were transferred both to dishes containing cover slips for SV40 T-antigen studies and to dishes at appropriate dilutions for the transformation assay (*8*). Following exposure to SV40 DNA the cells were maintained in medium supplemented with 0.5 percent rabbit antiserum to SV40.

Cells containing SV40 T-antigen were stained by the indirect fluorescent antibody method (*5*). The fraction of T-antigen positive cells was scored at 72 hours, a time at which, under our conditions, the maximum number of positive cells was seen either with intact virus or with DNA. In a typical test 20,000 to 40,000 cells were counted to determine the fraction of cells that were T-antigen positive. Infectious virus was recovered from DNA-transformed clones by cocultivation with green monkey kidney cells in the presence of ultraviolet-inactivated Sendai virus (*4*).

Human cells and green monkey kidney cells were infected with serial dilutions of DNA I. Figure 1 shows that the percentage of SV40 T-antigen positive cells rose with the concentration of DNA for both monkey and human cells. The efficiency of infection was comparable in spite of the fact that whole virus is over 100 times more efficient at inducing T-antigen in monkey cells than in human cells. The same efficiency of T-antigen induction by DNA was also found with Balb/3T3 cells, and with rat kangaroo cells. In the latter case the cells were fully resistant to T-antigen induction by whole virus.

Transformed colonies were first observed in DNA-infected human cultures 2 to 3 weeks after infection. The transformation frequency was assayed at 3 to 4 weeks. In their tissue culture properties the transformed colonies that developed were typical of SV40-transformed cells. The cells were epithelioid,