human erythrocytes. Also, infectious virus was not produced when susceptible CEF cultures were treated with 50 μg/ml of 5-bromo-2-deoxyuridine 4 hours prior to inoculation with virus from tumor fluids.

The virus neutralization studies indicate that the agents isolated from all three tumors are the same virus. These data show that the serum of three tumor patients neutralized the virus taken directly from two of the three tumors, as well as the virus passaged once in CEF cultures. These studies were carried out by inoculating susceptible CEF cultures with mixtures of serum and virus and then taking the cytopathic effect as the end point of neutralization. In all neutralization studies, we maintained a constant virus concentration, and varied the serum concentrations. Serum from a fourth patient, with an identical tumor that has not yet been studied, also neutralized all three viruses. Also, rabbit and hamster antiserums prepared against EFMU-1 virus from primary tumor cultures specifically neutralized the virus derived from tumor or CEF cultures. These data suggest that the virus in the tumor and that passaged once in CEF are similar. Control studies were done with serums from normal, age-matched patients, from patients with renal adenocarcinoma of the kidney, and from animals before they were injected with the virus. Virus neutralizing antibody was not detected in any of the control serums. Also, specific antiserums against the seven known serotypes of simian foamy virus (*13*), diluted 1 : 10, did not specifically neutralize the virus.

During the past 18 months we have attempted to isolate virus directly from renal adenocarcinomas (hypernephromas), from renal tumors in cell culture, and from normal adult kidney. Thus far, we have failed to demonstrate an agent associated with these tumors by virological and morphological techniques, and we have not detected virus in normal kidney removed from patients of different ages with benign and neoplastic diseases of the urinary tract.

We believe that this is the first report of the isolation of a virus, with similar characteristics, from three malignant human tumors as well as from tumor cells in primary culture. Our initial studies suggest that this virus is not a known human RNA virus, and is therefore either a new human virus or an animal virus commonly associated with these tumors. Although we have established that EFMU has certain characteristics in common with known oncornaviruses, it is unlike known RNA tumor viruses in that it is a cytopathic agent, and it does not appear to mature by budding from the cell membrane.

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7. The abbreviations in this report are as follows: poly(rA) • $oligo(dT)_{10}$, hybrid of polyadenylate and deoxythymidylate (PL Biochemicals, Milwaukee, Wis.); poly(rC) • $oligo(dG)_{12-18}$, hybrid of polycytidylate and deoxyguanylate (Collaborative Research, Waltham, Mass.); TTP, thymidine triphosphate; dCTP, deoxycytosine triphosphate; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate (PL Biochemicals); [$^3$H]TTP (International Chemical & Nuclear Corp., Chemical & Radioisotope Division, Irvine, Calif.); [$^3$H]dGTP (Schwarz/Mann, Orangeburg, N.Y.). The assay for RNA-directed DNA polymerase was done on 20 μl of undiluted purified virus per 100 μl of a reaction mixture that contained the following final concentrations: 0.04*M* tris-HCl (*p*H 7.8); 0.06*M* KCl; 0.002*M* dithiothreitol (DTT); 0.1 percent Triton X-100; 0.001*M* manganese acetate; $1.6 \times 10^{-5}M$ [$^3$H]dTTP (specific activity, 12.5 c/mmole, 9400 count/min per picomole), and 0.02 absorbance unit (at 260 nm) of poly(rA) • $oligo(dT)_{10}$. With poly(rC) • $oligo(dG)_{12-18}$, we substituted 0.001*M* magnesium acetate and $1.6 \times 10^{-5}M$ [$^3$H]dGTP (specific activity, 12.5 c/mmole) per 100 μl of reaction mixture. To study the endogenous reaction, we used a reaction mixture that contained, per 100 μl of reaction mixture, 0.04*M* tris-HCl (*p*H 8.3); 0.06*M* KCl; 0.002*M* DTT; 0.1 percent Triton X-100; 0.001*M* magnesium acetate; $1.6 \times 10^{-5}M$ [$^3$H]dTTP (specific activity, 17.8 c/mmole; 13,400 count/min per picomole); $2 \times 10^{-4}M$ each unlabeled dGTP, dATP, and dCTP, and 20 μl of test virus. The mixtures were incubated at 37°C for 15, 30, 60, and 90 minutes. The reactions were terminated by the addition of 2 ml of cold 10 percent trichloroacetic acid (TCA) containing 0.08*M* sodium pyrophosphate. The precipitate was then collected on Millipore filters (0.45 μm), and washed with cold 5 percent TCA. The filters were dried in an oven, and the radioactivity was determined by liquid scintillation counting in 10 percent BioSolv III in Ready-Solv IV (Beckman).
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## Somatosensory Cortex: Structural Alterations following Early Injury to Sense Organs

Abstract. *In mouse somatosensory cortex there are discrete cytoarchitectonic units, called "barrels." Each barrel is related to one sensory vibrissa on the muzzle. Individual vibrissae were carefully injured at birth; 12 to 43 days later, the corresponding barrels proved to be absent. Evidently the sensory periphery has an important influence on the structure of the somatosensory cortex.*

It is of great interest to know whether long-term or permanent alterations in the somatosensory periphery may produce lasting changes in the brain. In the mouse, the vibrissal pad and its cortical representation in the somatosensory field (SI) provide a particularly good situation in which to analyze the structural dependence of the cerebral cortex on the sensory periphery (*1*).

The vibrissal pad contains about 25 large mystacial hairs each of which, along with its associated set of sensory receptors, constitutes a discrete tactile sense organ. Each vibrissa is identifiable at birth, and each is known to project to a distinct cytoarchitectonic unit in the somatosensory cortex of the opposite side. These cytoarchitectonic units, or "barrels," are confined to layer IV of the SI, a layer largely populated by small neurons. The number and arrangement of the vibrissae are constant from animal to animal, and the same is true for the barrels in the cortex. There is also a striking topological similarity in the arrangement of the vibrissae and that of cortical barrels (compare d and e in Fig. 2 with a in Figs. 1 and 2) (*2*). This topologic con-

stancy makes it possible to determine whether injury to a particular vibrissa, or to groups of vibrissae, would result in alterations in the related barrels. Whatever changes occurred could be identified by direct comparison with the neighboring normal barrels. And, where the need exists to assess the altered morphology of the entire barrel field, or to precisely evaluate changes in specific barrels, the contralateral hemisphere would provide an adequate control.

In order to determine whether early modification of the somatosensory periphery would lead to changes in the cortex, individual vibrissae were carefully injured at birth (*4*). Twelve mice were treated daily from birth to day 12 and killed on day 13. Fourteen mice were treated only at birth, and survived for periods varying from 31 to 43 days. There appeared to be no significant difference between the cortices of animals treated daily and of those treated only at birth. Each group of animals was accompanied by two littermate controls; in total, 26 white mice (Swiss, ICR) and 4 black mice (C57BL/6) were used. The animals, under sodium pentobarbital anesthesia, were perfused through the heart with 10 percent neutralized formalin dissolved in 0.9 percent NaCl. The brains were routinely embedded in celloidin and sectioned in a plane tangential to the pial surface covering the barrel field, at thicknesses varying between 30 and 100 $\mu$m. The sections were stained by a Nissl method (methylene blue chloride). To eliminate bias in interpreting the results, 22 barrel fields were recon-

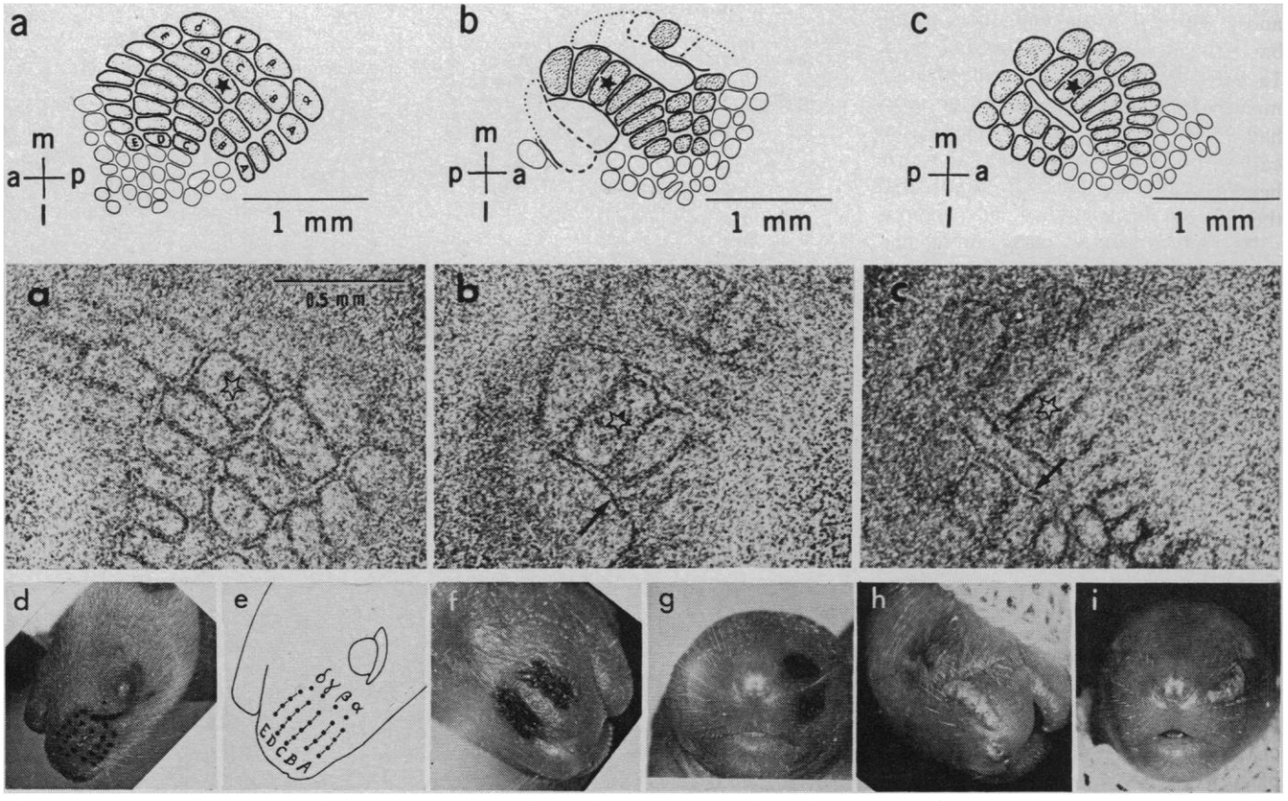


Fig. 1 (top). Three camera lucida drawings, made from three series of histologic sections of mouse somatosensory cortex (cut tangential to pial surface), depict normal and experimentally altered barrel fields. A majestic section from each series is shown in the correspondingly lettered part of Fig. 2. Shaded barrels correspond to large mystacial vibrissae. (a) The normal barrel field from a left hemisphere. A code identifying the barrels is indicated; it corresponds to our vibrissa code in Fig. 2e. (b) Field from a right hemisphere. Only barrels of row C and barrel $\beta$ remain, that is, the barrels related to the untampered vibrissal row C and to vibrissa $\beta$. In the barrelless region, there are sheetlike assemblies of cells; where striking, they are indicated by solid lines; where less clear, by dashed lines; and where barely recognizable, by dotted lines. Field shown in (a) is control for this experiment; it is taken from the left hemisphere of the same animal. (c) A right barrel field. Only barrel rows A, B, D, and E remain, that is, the barrels related to the untampered vibrissal rows A, B, D, and E. Fate of barrel $\beta$ is not clear (the experiment included injury to vibrissa $\beta$), but comparison of (c) with (a) reveals a rearrangement of barrels $\alpha$ to $\delta$. Orientations of drawings with respect to head are given by *m*, medial; *l*, lateral; *a*, anterior; *p*, posterior. Asterisks in (a), (b), and (c) identify the starred barrels in Fig. 2, a, b, and c, respectively. Fig. 2 (bottom). (a to c) Photomicrographs of barrel fields in normal and altered mouse somatosensory cortex. (d to i) Photographs, and one chart, of normal and injured muzzles; (a to c) are taken from single sections through barrel fields, cut at 40 $\mu$m (a and b) and at 100 $\mu$m (c). The sections in (a), (b), and (c) are from hemispheres contralateral to the muzzles shown in (d), (f), and (h), respectively. (a) Normal barrel field. (b and c) Experimentally altered fields. See drawings of Fig. 1 for corresponding whole barrel fields, and its legend for descriptions. In (b) and (c), arrows point to cell aggregates that occur where barrelless regions face intact barrels. The same scale is used for (a), (b), and (c). For asterisks, see legend to Fig. 1. (d) The arrangement of the large mystacial vibrissae is shown on the muzzle of a 6-day-old mouse. India ink has been applied to hair sinuses to enhance the pattern. Orientation of picture facilitates comparison with (a). Magnification approximately 1.9×. (e) Code used to identify vibrissae is shown. The five rows have been labeled A to E; the four posterior vibrissae, $\alpha$ to $\delta$; compare with barrel code in Fig. 1a. (f and h) The injuries made to vibrissal pads of newborn mice are shown in lateral view. Photographs have been oriented to facilitate comparison with (b) and (c). (g and i) Frontal views of the faces shown in (f) and (h), respectively. They allow to compare affected and control sides. The right side of the face in (g) projects to the left hemisphere shown in (a). Magnification (f to i) approximately 3.8×.

structed with the camera lucida by one investigator, who was not aware of the nature of the experiments in each case and of the number of controls involved.

Figures 1 and 2, which are derived from animals killed at 42 (a and b in Figs. 1 and 2) and 33 (c in Figs. 1 and 2) days of age, illustrate the striking nature of the results. The cases shown represent complementary experiments. In one experiment (Fig. 1b and Fig. 2, b, f, and g) all vibrissae except those in row C and the large posterior vibrissa $\beta$ were carefully injured. In the other experiment only that particular group was treated (Fig. 1c and Fig. 2, c, h, and i). By comparing the experimental barrel fields with the control field (Fig. 1a and Fig. 2a) it is obvious that the manipulation of the sensory periphery had produced a disruption of the cortical architecture in the contralateral hemisphere: there are no barrels where there were no whiskers.

The barrel field shown in Fig. 1a and Fig. 2a is normal. It appears identical to the fields of the control littermates; it is derived from the left hemisphere of the animal whose right barrel field is depicted in Fig. 1b and Fig. 2b. Most of our experimental material resulted from the pattern of injury depicted in Fig. 2, h and i; the cortical patterns were consistently altered in the manner shown in Fig. 1c and Fig. 2c. In those cases in which other combinations of vibrissae were injured, a predictable pattern of cortical changes was observed. For example, injury to vibrissal row E resulted in the disappearance of barrel row E, and so forth. In this regard, our experiments provide clear, morphological evidence for the proposed (*1*) and physiologically demonstrated (*3*) one-to-one relation between mystacial vibrissae and barrels.

Closer inspection of the barrel fields in the experimental animals revealed cell aggregates that resemble the cell-dense barrel sides. These occur where a barrelless region is apposed to intact barrels (see b and c in Fig. 2, arrows). This phenomenon made it difficult to establish whether the destruction of a single vibrissa resulted in a specific alteration in the barrel field. This difficulty was increased by the fact that the dispersion of cells in the experimentally produced, barrelless regions of layer IV is similar to that found in the central region of an intact barrel. Here and there, in barrelless patches, assemblies of cells were seen, which at times did exhibit some of the characteristics of intact barrels (see b and c in Figs. 1 and 2) (*5*). Also, where it could be determined, the overall area occupied by the barrelless zone was smaller than the region occupied by the corresponding barrels in a normal barrel field (compare c with a in Figs. 1 and 2). In some instances, remaining barrels adjacent to a barrelless region appear larger in size than their controls in the opposite hemisphere and larger than the corresponding barrels in a normal animal. It should be pointed out that the experimentally induced alterations in the barrel field can only be clearly seen in sections tangential to the cortical surface. In sections perpendicular to the pia, the overall laminar pattern of the cortex does not appear markedly altered in the barrelless regions.

These experiments clearly demonstrate that altering the sensory periphery may lead to profound alterations of the somatosensory cortex. The manipulations of the peripheral sense organs were intentionally carried out in very young mice, at a time that the cerebral cortex is relatively immature (*6*). It seemed likely that the effects of the experimental intervention at this early stage would be most pronounced. On the other hand, the changes in the structure of the cortex were observed when the animals were relatively mature; these changes can be regarded as permanent alterations in cortical structure. How can we explain them? Does altering the sensory periphery change the course of development that normally leads to the formation of barrels? Or does the alteration in the periphery cause changes in an already existing intact barrel field? Rice has found that barrels are not identifiable in the somatosensory cortex of the newborn mouse (*7*). This seems to favor the view that injury to the vibrissae disturbs the normal pattern of morphogenesis leading to barrel formation by (i) affecting the proliferation of cells destined for the barrels, (ii) interfering with the migration and the subsequent intracortical rearrangement of the relevant neurons, or (iii) producing a loss of neurons by abnormal cell death. However, until we have examined brains of animals with vibrissal injury at birth, which are killed at closely spaced early postnatal ages, we cannot rule out the possibility that the observed alterations in the barrel field are due to the selective degeneration of cells in barrels which initially had originated regardless of early injury at the periphery. In either case, the changes are caused by some form of anterograde transneural influence resulting from direct insult to the primary afferent neurons, or from simple loss of function, or from both. This transneuronal influence must be "transmitted" through at least two synaptic relays (in the principal nucleus of the trigeminal nerve and the arcuate division of the ventrobasal complex of the thalamus).

There is considerable evidence that transneuronal effects due to injury may occur, and that they are particularly pronounced in developing and immature nervous systems (*8*). That sensory deprivation may lead to changes in the developing cerebral cortex is also well documented, especially for the mammalian visual system. However, most of the reports on this work either have been based on physiological and behavioral observations or, from a structural point of view, have been largely concerned with subtle changes, such as partial loss of dendritic spines on cortical cells (*9*). The changes that we report here are much more outspoken, and readily identifiable even in Nissl-stained material in which only perikarya are visualized. At this point we must note that changes in the cerebral cortex of the type observed by us may not be limited to developing systems. Campbell (*10*) reported cell loss in the postcentral gyrus in an "old-standing case of amputation."

Other analytical methods should enable us to demonstrate the influence of the periphery on the circuitry of the somatosensory cortex.

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### References and Notes

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4. In most experiments, the injuries were confined to one side of the head. The vibrissae, observed with a dissection microscope, were injured in the following way. First, the individual whiskers were pulled. Then, in 11 animals (including the mouse portrayed in Fig. 2, f and g) the individual hair sinuses were cau-

terized by gently applying the fine tip of a tungsten wire loop (actually the filament of a Philips EM-200 electron microscope), minimally heated by a current from a variable, low-voltage a-c source. In 15 animals (including the mouse shown in Fig. 2, h and i) the sinuses were treated electrolytically by inserting a fine needle and passing a current from a variable d-c source (— 6½ volts); the positive lead of the source was attached to the abdomen of the animal. The short circuit adjustment of the power supply (KEPKO ABC-30-0.3M) was set at 50 ma. The two methods of injury seemed to produce identical results.

5. This observation may be explained by insufficient damage to the sense organs and the associated nerve fibers; by (partial) regeneration of nerve fibers and sense organs; or by a certain "refractoriness" of the cortex region involved to damage of the periphery under the conditions of the experiment (for example, the entire barrel field may not have the same "critical period" with regard to its vulnerability to peripheral damage). These alternatives can be experimentally tested.
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## Immunoglobulin A: Site and Sequence of Expression in Developing Chicks

Abstract. *Synthesis of immunoglobulins A, G, and M (IgA, IgG, and IgM) is prevented in chickens by embryonic treatment with heterologous antibodies to IgM when combined with bursectomy at hatching. Cells that produce IgA are seeded from the bursa of Fabricius later than cells capable of IgM and IgG synthesis; the latter do not convert to IgA synthesis outside of the bursa.*

Immunoglobulins of the IgM class are synthesized first by developing lymphoid cells of the plasma cell line (B cells) in chickens, guinea pigs, and man (*1*). The following observations suggest that, in chickens, virgin B cells committed to the synthesis of IgG arise within the bursa of Fabricius from cells that formerly made IgM: (i) Removal of the bursa sufficiently early in embryonic life permanently prevents development of all immunoglobulin-producing cells (*2*); (ii) IgG-containing cells are found first in the midst of IgM-containing cells within the bursa and, later, individual cells containing both of these classes can be identified frequently in the bursa, but rarely in the spleen (*1*); (iii) embryonic treatment with heterologous antibodies to IgM ($\mu$ chain–specific) suppresses synthesis of IgG as well as of IgM; (iv) IgM-producing cells appear unable to convert to IgG synthesis outside of the bursa (*3*, *4*). At the time of these studies IgM and IgG were the only major classes demonstrated in birds. On the basis of (i) information on the phylogeny and ontogeny of immunoglobulins, (ii) combinations of immunoglobulins seen in cultured human cells, and (iii) patterns of immunoglobulin deficiencies observed in humans, we predicted that cells committed to IgA synthesis also arise from cells that formerly made IgM by a genetic switchover mechanism having the following sequence, IgM→IgG→IgA (*5*). In support of this hypothesis, treatment of mice with antibody to IgM from birth to maturity suppressed synthesis of all immunoglobulin classes (*6*), but the conversion sequence for IgA and IgG heavy chain genes has not been analyzed experimentally. A third immunoglobulin class that is similar to mammalian IgA has been found in chickens (*7*). We now describe studies on the origin of cells committed to synthesis of this class of immunoglobulin and their developmental relationship to B cells committed to IgM or IgG synthesis.

An antiserum specific for chicken IgA was prepared by a modification of the procedure of Lebacq-Verheyden *et al.* (*7*). Pooled bile was dialyzed to remove the salts, filtered over a G-25 Sephadex column, and concentrated. An immune precipitate was then prepared by mixing at equivalence bile and goat antibody to chicken light chains. The washed precipitate, which contained substantial amounts of IgA and only trace amounts of other immunoglobulins was used to immunize goats. The resulting antiserum was rendered specific by repeated passage over immunoadsorbent columns bearing agammaglobulinemic serum, purified IgM, or purified IgG (*1*). When the antibody to IgA was tested by immunoelectrophoresis, a single line was obtained with normal chicken serum and no reaction was detectable with agammaglobulinemic serum, purified IgM, or IgG as antigens. Our antiserum to bile IgA formed a precipitin line with an additional element in bile as described (*7*). Purified antibodies to IgM or IgG, used in our earlier studies (*1*), did not recognize either serum or bile IgA.

The concentration of IgA in the serum of developing chicks was measured by single radial immunodiffusion (*8*). Comparisons were made to pooled serum from normal adult chickens. Minimum amounts of detectable IgA were comparable to a 10 percent dilution of this standard. While lack of a purified serum IgA standard prevented precise quantitation of IgA in serum, dilution studies indicate that the concentration of circulating IgA is far less than the concentrations of IgG and IgM in serum. IgA was not detected

Table 1. Effects of treatment of embryos with heterologous antibodies to IgM or bursectomy at hatching (or both) on serum IgM, IgG, and IgA in chicks at 4 months of age.

| Test group | No. | Serum immunoglobulin* | | |
|---|---|---|---|---|
| | | IgM* (mg/100 ml) | IgG* (mg/100 ml) | IgA† (%) |
| Normal chickens | 6 | 124 ± 57 | 307 ± 108 | 212 ± 353 |
| Bursectomy at hatching | 9 | 136 ± 66 | 492 ± 283 | 36 ± 39‡ |
| Antibody plus bursectomy | 17 | 0 | 0 | 0 |

* Means ± S.D. are given in milligrams per 100 ml and minimum detectable levels were 2 and 5 mg per 100 ml, respectively. † Means ± S.D. are given as percent of a normal adult serum pool, and the detection threshold was 10 percent. ‡ Serum IgA was undetectable in four of nine birds in this group; IgG deficiency (6 mg per 100 ml) occurred in one of nine birds.