Representative photomicrographs of three animal skeletons with somewhat analogous microstructure are shown for comparison in Fig. 1: Except for greater orientation of the pores in Fig. 1, A and B, the gross microstructural features of the three materials are similar. In human bone, the pore volume ranges from about 95 percent in regions of low calcification to as low as 10 percent in the most heavily calcified areas.

It appears possible to select from the animal kingdom a microstructure most suitable for a particular application and then to reproduce that structure in ceramic, metal alloy, or polymer materials. The disadvantages in using skeletal materials directly include the low strength and high solubility of calcite and aragonite and, in the case of the hydroxyapatite bone of humans and vertebrates, the difficulty in completely removing residual organic matter which elicits immunological reactions.

The first step in the replamineform process (meaning replicated life forms or structures) is the removal of most of the organic matter contained in the source material by immersion in a 5 percent solution of sodium hypochlorite for up to 30 hours. After the soft tissues have been oxidized, the sample is rinsed in deionized water and dried at 90°C. A stream of compressed air may be used to aid in expelling liquified organic constituents. If the specimen to be replicated is echinoderm calcite or scleractinian coral aragonite, it can be easily preformed by machining to any desired geometry—for example, cylinders, screws, nuts, bolts, and pins. For methacrylate replicas, negative copies of the structure are obtained by vacuum impregnation and subsequent polymerization of methacrylate, followed by leaching of the original calcite or aragonite with 5 to 20 percent HCl. To yield positive copies, the source material is first vacuum-impregnated with wax. The $CaCO_3$ is then removed with dilute HCl, and the wax negative is vacuum-impregnated with methacrylate. After polymerization of the methacrylate, the wax is removed by melting.

Natural microstructures can also be replicated in metal alloys such as Tichonium and Vitallium. In the case of Tichonium, the echinoderm or coral skeleton is first vacuum-impregnated with wax, and the calcite or aragonite is removed with HCl. The wax negative is then vacuum-impregnated with refractory material such as cristobalite investment (*9*), and the metal is cast by means of standard centrifugal casting techniques. The result is an exact negative reproduction of the original structure in metal. For very large specimens, vacuum casting would be desirable. Positive replicas in metal require manipulation of the intermediate copies, so that the sample invested with Kerr cristobalite is a wax positive. Kerr ivory inlay casting (regular) (*9*) was used in these preparations to assure complete removal of the wax and to preserve detail in the replications. Sintered alumina copies are prepared by vacuum impregnating the wax negative with a thixotropic slurry of 5-μm particles of α-alumina. The alumina is rendered fluid by vibratory action to facilitate the filling of the pores in the wax negative. After the wax is burned off at 400°C, the alumina is sintered at 1650°C in an air atmosphere. Replamineform copies of the structure of *Porites* in methacrylate, tin, Tichonium, and alumina (α-Al_2O_3) are shown in Fig. 2.

The implications of this process are many. The special geometric characteristics of a particular microstructure, which are difficult or impossible to create artificially, could be utilized simply by copying the substance of the source material into one having the chemical, physical, and mechanical properties necessary for a given application. New avenues might also be opened in the production of composite materials. Artificial limbs might be permanently attached by means of an implant device consisting of a central Vitallium rod (for strength) coated with porous ceramic for firm attachment of living tissue. Furthermore, source materials for replication are readily obtainable in large quantity. The genus *Porites*, for example, is one of the most successful of the reef-building corals, having a worldwide distribution in the coral reef zone. The colonies grow rapidly, with massive forms often exceeding 1 m in diameter.

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Noradrenaline Nerve Terminals in Human Cerebral Cortices: First Histochemical Evidence

Abstract. *The cerebral and cerebellar cortices of man are richly provided with varicose noradrenaline nerve terminals, which are visualized by fluorescence histochemistry of brain smears obtained by a new technique. The density of such nerves in human cortices equals that of the rat. The method permits simple and rapid analysis of noradrenergic nerves of the human cortex during routine neurosurgical operations.*

There is evidence for a cellular localization of the three monoamines—dopamine, noradrenaline (NA), and 5-hydroxytryptamine—within specific neuron systems of the brains of laboratory animals (*1*). The distribution of the bodies and terminals of monoamine nerve cells is known in detail for the rat, and the presence of these cells has been documented also in other species (*2*). The functional significance of the various monoamine neuron systems, for instance their involvement in the mechanisms of action of psychotropic drugs, is under study, yet the appearance and distribution of these neurons in the human brain has not been demonstrated. In this report we describe the

application of a recently developed smear technique (*3*) to human brain tissue. Histochemical evidence has been obtained for the presence of NA nerve terminals in the cerebral and cerebellar cortices of man.

Small pieces of gray matter from the cerebral and cerebellar cortices and of the white matter immediately underneath were obtained during routine neurosurgical operations. In most cases, pieces of cerebral cortex were obtained during ventricular puncture before the usual coagulation of the arachnoid and the pial vessels. A corneal trepan with a diameter of 2 mm (similar to the diameter of the needle used for ventricular puncture) was gently inserted through the outer few millimeters of the brain substance. When withdrawn the trepan contained a cylinder of brain tissue several millimeters long. The cylinder was cut in pieces, and each piece used for smearing. The pial vessels were then coagulated in the usual way, and ventricular puncture was performed at this site. Taking the biopsies in this way thus caused no more harm to the brain than a usual ventricular puncture, and the time loss was minimal. In some cases tissues used for smearing were taken from resected brain material. The brain pieces were evenly smeared on an object glass with the help of a cover glass, and the smears were then allowed to dry in a desiccator overnight. All smears were analyzed for the presence of catecholamine-containing nerve fibers by the Falck-Hillarp histochemical fluorescence method (*3*).

In one case, thin slices that consisted of gray matter and a few millimeters of the underlying white matter and were obtained from a resected temporal pole were incubated in a Krebs-Ringer bicarbonate buffer with or without α-methylnoradrenaline (α-methyl-NA), 10^{-6} or $10^{-5}M$, before smearing (*4*).

Specimens of the cerebral cortex were obtained at 13 operations (Table 1). Varicosities with green fluorescence were found somewhat irregularly distributed over the whole smeared areas in most cases. The varicosities showed a varying but mostly moderate fluorescence intensity and were of varying sizes. Groups of very large and very intensively fluorescent varicosities were occasionally found. In thin smears most varicosities were separated from each other, while intact nerve fibers were found in thicker smears (Fig. 1). These fibers had a regular varicose appearance, but the size of the varicosities could vary considerably on one particular fiber. However, thin fibers with predominantly small varicosities and thicker fibers with predominantly larger varicosities could be distinguished. The fluorescent fibers were sometimes seen to branch. No catecholamine fluorescence at all could be detected in the cerebral cortex of the one deeply unconscious patient, and fluorescence was almost absent in the patient suffering from Parkinson's disease.

Table 1. Analysis by fluorescence microscopy of monoamine-containing nerves in smears from human cerebral cortices. Smears from apparently normal cerebral cortices were obtained during ventricular puncture or from resected material. Symbols are 0, no specific fluorescence; +, a few scattered fluorescent varicosities; ++, fluorescent varicosities found over the whole smeared areas; +++, a large number of fluorescent varicosities found in each field of view. The density of varicosities is similar to that of the rat cerebral cortex (Gyr. Temp. Sup., gyrus temporalis superior).

Age (year)	Diagnosis	Area	Number of smears		Nerve density
			Total	Positive	
3	Medulloblastoma	Frontal	8	8	+++
8	Neonatal asphyxia	Frontal	3	3	++
10	Tumors of thalamus and pons	Parieto-occipital	7	7	++
17	Tumor of posterior fossa	Frontal	3	3	++
37	Subarachnoid hemorrhage	Parietal	3	0	0*
37	Aneurysm	Gyr. Temp. Sup.	11	11	+++
50	Aneurysm	Frontal	12	12	++
52	Cerebral hemorrhage	Gyr. Temp. Sup.	13	13	+
55	Meningioma	Temporal	13	13	++
56	Parkinson's disease	Frontal	3	1?	+?
64	Astrocytoma	Frontal	5	3	+
65	Astrocytoma	Temporal	8	7	+
66	Meningioma	Frontal	7	7	++

* Deeply unconscious before operation.

Fig. 1. Varicose noradrenaline nerve terminals in cerebral cortex from a girl age 8. In this thick smear both intact nerve fibers and separated fluorescent varicosities are seen. Unspecific lipofuchsin-induced autofluorescence, common in older patients, is not present here. The scale line is 50 μm ($\times$ 240).

Different smears from the same cerebral sample were almost identical with regard to average density of nerve terminals and to average fluorescence intensity. In no case did smears from the same sample fall into different categories of our semiquantitative scale (Table 1).

White matter immediately below the gray matter also revealed fluorescent fibers and isolated varicosities. Longer, smoother, and fewer fibers were generally observed, with a somewhat lower fluorescence intensity as compared to the fluorescent fibers of the corresponding gray matter.

The in vitro incubation with α-methyl-NA, which is resistant to the action of monoamine oxidase, demonstrated that the catecholamine nerve fibers of the human cerebral cortex were able to actively accumulate exogenous amines in a dose-dependent way. A marked increase of the fluorescence intensity was obtained together with an increase in the number of observable fluorescent varicosities.

Two specimens from the cerebellar cortex were analyzed (normal cerebellar cortex from the tonsilla and vermis). Fluorescent varicosities were present in an amount comparable to that of the cerebral cortex. They displayed a moderate to strong fluorescence intensity. Intact varicose nerve fibers were also found and were similar to those in the rat (*3*, *5*).

Catecholamine-containing nerve cell bodies and varicose nerve fibers have been found previously in human fetal brain (*6*). The results reported here demonstrate the presence of catecholamine-containing nerve terminals in the cerebral and cerebellar cortices of children and adults. We found no principal qualitative differences in appearance between the catecholamine nerve fibers in rat and those of man with the smear technique; this suggests that the knowledge about the fluorescence histochemistry of catecholamine neuron systems in laboratory animals might apply to the cerebral cortices of man.

The specificity and high sensitivity of the Falck-Hillarp fluorescence method have been discussed (*7*), and a change in fluorescence intensity represents a change in the NA content of adrenergic nerves (*8*). With the smear technique, optimal conditions for detection of fluorescent nerves in sparsely innervated brain areas are obtained. The smear technique has been used to quantitate the effect of minor tranquilizers on NA turnover in the rat cerebral and cerebellar cortices (*9*), and there seems to be a linear relation between the endogenous amount of NA in the rat cerebral cortex and the fluorescence intensity estimations obtained with the smear technique on coded slides (*10*). Thus, this technique should also permit quantification of nerve endings and fluorescence intensities in various clinical conditions in man.

No major regional differences in the distribution of NA nerve terminals in the rat cerebral cortex have been reported (*11*). In our study of the human brain, fluorescent terminals were found in the cortices of the frontal, temporal, and parietal lobes as well as in the parieto-occipital area (Table 1). Therefore, the lack of fluorescent terminals in one case might reflect the serious condition of this patient.

In the rat, the catecholamine nerve terminals in the cerebral and cerebellar cortices are of the NA type (*12*), and they have a common origin in the NA nerve cell bodies of the locus ceruleus (*13*). There is thus reason to believe that the nerves with green fluorescence in the human cortices are of the NA type.

The results of the initial experiments suggest that fluorescence histochemistry of brain smears may offer new possibilities to the study of central monoamine neurons in man. Apart from obtaining the necessary background of anatomy, we found with this technique that disturbances in nerve terminal frequency as well as in monoamine content may be directly related to various clinical conditions.

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Triacylglycerols Characteristic of Porpoise Acoustic Tissues: Molecular Structures of Diisovaleroylglycerides

Abstract. *More than two-thirds of the triacylglycerols from the acoustic tissues of the porpoise* (Tursiops gilli) *consist of 2 moles of isovaleric acid for every 1 mole of long-chain acids. Cranial blubber, which has no distinct acoustic function, does not contain these unusual glycerides. The presence of large amounts of diisovaleroylisopentadecanoylglycerol suggests that this structure may be particularly important in sound transmission through lipid-protein matrices.*

Porpoise acoustic tissues, such as those from the melon and mandibular canal, are composed of unusual wax esters and triacylglycerols rich in isovaleric acid and long-chain iso acids (*1*, *2*). However, blubber taken from an area of the head in close proximity to the melon (cranial region) but having no distinct acoustic function contains primarily straight-chain unsaturated acids (*3*) characteristic of normal mammalian adipose tissue (*4*).

The unusual composition of the acids in acoustic tissues has evoked an interest in the molecular structure of the triacylglycerols. At present, data on cetacean acoustic tissues are limited to analyses of the carbon numbers of hydrogenated triacylglycerols from the beluga whale (*Delphinapterus leucas*) (*5*). For current interdisciplinary studies on bioacoustics (*6*), a better understanding of the structural and biochemical role of lipid in the acoustic tissues is of paramount importance. We report here the analyses of individual triacyl-

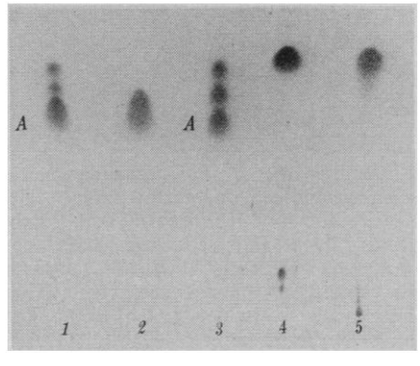

Fig. 1. Thin-layer chromatography of triacylglycerols on silica gel. Solvent: a mixture of hexane and diethyl ether (90 : 10, by volume); indicators: 0.5 percent solution of iodine in chloroform and starch solution. Fractions *A* do not stain as well as the more unsaturated upper fractions. Thus, visual estimation of relative amounts of the zones is misleading. *1*, Melon triacylglycerols from *Tursiops gilli*; *2*, diisovaleroylmyristoylglycerol; *3*, mandibular canal triacylglycerols from *Tursiops gilli*; *4*, trioleoylglycerol; and *5*, blubber triacylglycerols from *Tursiops gilli*.