globulin in vivo, with subsequent cytotoxicity and tumor inhibition. It is known that guinea pig γ_2-immunoglobulin binds homologous complement and destroys target cells in vitro (*5*). Antibody plus complement also appears to be important for rejection of renal xenografts in dogs (*6*) and rejection of ascites tumor allografts in mice (*7*). Second, incubation of tumor cells in vitro with $F(ab')_2$ immunoglobulin fragments enhanced subsequent tumor growth in vivo and delayed rejection by blocking antigenic sites. It is known that noncytotoxic $F(ab')_2$ immunoglobulin fragments combine with tumor cell antigens but do not bind complement (*1*); such fragments are divalent (*8*). Combination of tumor antigenic sites with whole immunoglobulin reduces the effectiveness of the host's immune response (*9*).

Regardless of the early effect of γ_2-globulins or $F(ab')_2$ fragments in vivo, tumor size in all groups was decreasing by day 8. Both the protective action of the $F(ab')_2$ immunoglobulin fragment and the inhibitory effect of γ_2-immunoglobulin would be expected to decrease as their concentration at cell surfaces was lowered by cell division.

As shown above, the divalent $F(ab')_2$ fragment from γ_2-immunoglobulin produces immunologic enhancement, probably by blocking antigenic sites. Univalent Fab fragments from mouse γ_2-immunoglobulin may act in a similar manner (*10*). Alternatively, the $F(ab')_2$ immunoglobulin fragment could possibly enhance tumors by stimulating cell growth or increasing resistance to the host, as previously suggested for some enhancing immunogobulins (*11*); $F(ab')_2$ immunoglobulin fragments stimulate incorporation of uridine in vitro by mouse thymus cells (*12*). Immunoglobulin and its $F(ab')_2$ fragment can also induce antigenic modulation of mouse leukemia cells (*13*).

Globulins resembling antibody fragments have been isolated from human serums (*14*), small lymphocytes (*15*), and canine renal allotransplants (*16*). In guinea pigs, $F(ab')_2$ fragments depress immunoglobulin formation (*17*). Theoretically such globulins produced in vivo by synthesis or degradation and possessing the properties of immunoglobulin fragments, could alter host immune responses to pathogens, transplants, and autochthonous neoplasm.

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18. Aided by Michigan Memorial Phoenix Project 180. S.B. is a fellow of the Life Insurance Medical Research Fund.

1 August 1968; revised 4 September 1968 ■

Sterol Precursors of Cholesterol in Adult Human Brain

Abstract. *Adult human brain contains cholestanol and two series of cholesterol precursors having 30, 29, 28, and 27 carbon atoms; one has an unsaturated steroid nucleus, and the other is unsaturated in both nucleus and side chain. The ability of preparations of brain to incorporate a specific precursor into cholesterol, as well as into these sterol metabolites in vitro, indicates that cholesterol synthesis continues long after brain maturation ceases.*

It is generally assumed that in adult nervous tissue cholesterol is the only sterol present and that its metabolism is negligible after brain maturation. Although the stability of cholesterol in brain can be accounted for by its structural role, some data show that there is some turnover of cholesterol in adult brain (*1*).

Simple radioactive precursors are incorporated into brain cholesterol of mature animals in vivo and in vitro (*2*). Early workers (*3*) detected saturated sterols (cholestanol) and sterols with conjugated double bonds (*4*). Fieser and co-workers detected "Δ^7-sterols," 7-ketocholesterol, and cholestane-3β,5α, 6β-triol in human brain samples fixed in formalin (*5*).

We have identified a number of sterols related to the synthesis of cholesterol in adult rat brain (*6*). The same techniques were used to analyze the sterols in samples of adult human brain. The isolation and identification of two homologous sterol series involved in the biosynthesis of cholesterol in other tissues (*7*) is reported. The intermediary role of these sterols is supported by the incorporation of a specific precursor 2-^{14}C-mevalonate in vitro.

A portion of the anterior region of the right temporal lobe of a 44-year-old female patient undergoing surgery for an aneurism of the posterior communicating artery was obtained at the Neurosurgical Clinic, University of Milan. The time between removal and incubation of the cleaned tissue was 20 minutes, during which the tissue was maintained in cold buffer solution. A 10-g sample of minced tissue was incubated with 20 μc of mevalonic acid-2-^{14}C (*8*) in 100 ml of Krebs-Ringer phosphate buffer, *p*H 7.4, devoid of calcium ions, and in an oxygen atmosphere for 2 hours at 37°C. The procedure used for the extraction of the unsaponifiable material, acetylation of the sterol mixture, and column chromatography on silver nitrate, kieselgel G, and Celite was carried out as described (*9*). The quantities of sterols were determined by gas-liquid chromatography (GLC) and identified by GLC-mass

Table 1. Sterols identified in adult human brain.

Sterol	Amount of total sterols (%)	Amount (μg/g tissue)
C_{30} Δ^8	0.003	0.596
C_{29} Δ^8	.030	5.642
C_{28} Δ^8	.020	3.857
C_{27} Δ^8	.082	15.480
C_{30} $\Delta^{8,24}$	.004	0.708
C_{29} $\Delta^{8,24}$	.019	3.588
C_{28} $\Delta^{8,24}$	.016	3.064
C_{27} $\Delta^{8,24}$	.011	2.157
C_{27} $\Delta^{5,24}$	.020	3.838
C_{27} Δ^5	99.090	18,660.000
C_{27} Δ^{14}	0.020	3.835
C_{27} Δ^5 (7 C=O)	.009	1.626
C_{27} Δ^0	.291	54.750
Unidentified and partially identified compounds	0.384	72.380

Table 2. Incorporation of 2-^{14}C-mevalonate into sterol fractions of mature human brain. Total amount of sterols per gram of brain tissue was 19.74 mg. Total disintegrations per minute per gram of tissue was 4476, as measured at the column effluent.

Sterols	Sterols in tissue (μg/g)	Radioactivity (%)
Δ^5 (cholesterol)	18,600	46.87
$\Delta^{8,24}$ ($C_{30,29,28,27}$)	95	12.00
Δ^8 ($C_{30,29,28,27}$)	255	5.10
$\Delta^{5,24}$ (desmosterol)	4	2.11
Δ^0 (cholestanol)	55	1.80
Other unsaponifiable components		32.12

spectrometry, by comparison with authentic standards (*10*). The column separation was performed in two steps, one column being used for separating the major portion of cholesterol and the remaining sterols being applied to a second column for further fractionation. All the sterols of the Δ^8 and $\Delta^{8,24}$ series with 30, 29, 28, and 27 carbon atoms were identified (Table 1). However, we cannot exclude the possibility that minute amounts of Δ^7-sterols were present and could not be detected under our experimental conditions. The C_{30} sterols, lanosterol ($\Delta^{8,24}$) and dihydrolanosterol (Δ^8), had not been previously detected in either adult rat brain or developing chick brain (*6*), and it is believed that improvement of the technique due to separation on two columns permitted the identification of these additional sterols in adult human brain. Another sterol not previously identified in nervous tissue was C_{27} (Δ^{14}) which may be suspected to take part in the pathway of cholesterol synthesis. The origin of 7-ketocholesterol, also detected by Fieser *et al.* (*5*), is not known.

Cholesterol accounts for about 99 percent of the total sterols in the analyzed brain; the second major sterol is cholestanol, C_{27} (Δ^0). Desmosterol, C_{27} ($\Delta^{5,24}$), which is quantitatively very important in developing human (*11*) and animal (*12*, *13*) brains, and represents 0.8 percent of the sterols in mature rat brain (*6*), is present in a very low concentration in adult human brain. The sterols of the Δ^8 series occurred in larger percentages than those of the $\Delta^{8,24}$ series.

No more than one-half of the radioactivity eluted from the column is attributable to cholesterol, and the specific activity is very low because the cholesterol pool is large (Table 2). The sterols of the $\Delta^{8,24}$ series, in spite of their lower concentration, show higher incorporation of mevalonate when compared with the Δ^8 series.

Although a precise calculation of specific activities is not always possible because separation of some of the homologous sterols is incomplete, it is clear that the specific activity of the $\Delta^{8,24}$ series is the highest. These data confirm our hypothesis that sterols with a double bond in the lateral chain are intermediates of cholesterol synthesis in the brain. The labeling of desmosterol is low, in contrast with the data obtained from developing brain (*6*, *12*) and brain tumors (*14*), further suggesting a special function for this sterol in immature and undifferentiated nervous tissue.

The identification of labeled intermediates of cholesterol biosynthesis together with the labeled cholesterol indicates that human brain retains the ability to synthesize cholesterol throughout life.

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15. Supported in part by the Association for the Aid of Crippled Children, and by PHS (grant 5 RO 1 NB-04202-06).
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26 August 1968; revised 29 October 1968

Biologic Precipitation of Fluorite

Abstract. *X-ray diffraction patterns show that the statoliths of marine mysid crustaceans are composed of fluorite, and that this mineral is also a principal phase of the gizzard plates of some tectibranch gastropods. A phosphatic phase is also indicated by chemical analyses in the gizzard plates, but its crystallochemical characterization has not been feasible by x-ray diffraction. The occurrence of fluorite in mysid statoliths confirms the earlier interpretations based on insufficient documentation. Fixation of fluorine in hard tissues of marine invertebrates is extensive in the shelf seawaters and minor in the bathyal zone of the oceans.*

Information on the kinds of crystalline compounds precipitated by marine organisms is still very limited. This is borne out by discoveries of the minerals magnetite, goethite, lepidocrocite, and francolite in hard tissues of marine invertebrates, all previously thought to be solely of inorganic origin (*1*). Questions necessarily arise concerning the proper identification of some biologically precipitated minerals reported. One of these, fluorite (CaF_2), is stated to occur in the statoliths of marine mysid crustaceans (*2*, *3*) and in the spicules of a nudibranch (Mollusca) (*4*). The presence of fluorite was deduced initially from elemental determinations indicating that the mysid statoliths and nudibranch spicules were rich in calcium and fluorine (*2*, *4*). Microchemical tests subsequently performed on statoliths of another mysid species failed to detect fluorine, and the mineral matter was stated to consist of calcium carbonate (*5*). Fluorite was again reported from the statolith of still another mysid species (*3*). Identification of fluorite was obtained with the aid of x-ray diffraction, but no data on the *d*-spacings and line intensities of the diffraction were published to support the mineral determination. X-ray diffraction patterns obtained in a later study from spicules of the same nudibranch species failed to show the presence of a crystalline substance, thus excluding the possibility that it is fluorite (*6*).

We identified the mineral fluorite as one of the mineral constituents in the gizzard plates of some tectibranch gastropods. To determine whether this constitutes the first unquestionable occurrence of biologically precipitated