boon and man; Corey *et al.* (*8*) showed that the bisepoxide of squalene can be enzymatically converted into the 24,25-epoxide of lanosterol; and Shishibori *et al.* (*9*) and Nelson *et al.* (*10*) demonstrated that inhibition of cyclization of squalene epoxide induced accumulation of the bisepoxide. These reports suggest that squalene bisepoxide may be a normal by-product of cholesterol biosynthesis. Lanosterol epoxide is probably converted to either 24- or 25-hydroxycholesterol.

Both squalene bisepoxide (40 mg/kg) and lanosterol-24,25-epoxide (5 mg/kg) (*16*) affected the minor branches of the pulmonary artery but not the major branches. In view of a possible delayed effect due to biogenesis, we examined the pulmonary arteries of animals killed 10 days after the third injection of either squalene bisepoxide or lanosterol epoxide. Both compounds caused a delayed response that was grossly visible at 10 days in the major branches; microscopic changes in the minor branches had progressed as shown in Table 1.

Kandutsch *et al.* (*5*) suggest that oxygenated sterols, not cholesterol, modulate the rate of biosynthesis of cholesterol. The data presented here show that oxygenated sterols can be potent angiotoxins and indicate that 25-hydroxycholesterol may occur normally in vivo (*17*).

Our data support the hypothesis that oxygenated sterols have an important role in causing injury of the arterial wall and initiating atherosclerotic lesions. They also indicate that feeding experiments with cholesterol should be evaluated with caution when the purity of the dietary cholesterol has not been controlled. Further studies are warranted to evaluate additional factors, such as high plasma lipids, on the progression of basic lesions induced by oxygenated sterols.

HIDESHIGE IMAI
Department of Pathology, Neil Hellman Medical Research Building, Albany Medical College, Albany, New York 12208
NICHOLAS T. WERTHESSEN
Biomedical Division, Brown University, Providence, Rhode Island 02912
VINAYAKAM SUBRAMANYAM
PHILIP W. LEQUESNE
ALBERT H. SOLOWAY
Department of Medicinal Chemistry, College of Pharmacy, Northeastern University, Boston, Massachusetts 20115
MASAYOSHI KANISAWA
Department of Pathology, Tokyo Metropolitan Institute of Gerontology, Tokyo 173 Japan

References and Notes

1. N. Anitschkow, *Beitr. Pathol. Anat. Allg. Pathol.* **56**, 379 (1913).
2. E. Schulze and E. Winterstein, *Z. Physiol. Chem.* **43**, 316 (1904); L. F. Fieser, *J. Am. Chem. Soc.* **75**, 5421 (1953); L. L. Smith, W. S. Mathews, J. C. Price, R. C. Bachman, B. J. Reynolds, *J. Chromatogr.* **27**, 187 (1967); L. L. Engel and P. Brooks, *Steroids*, **17**, 531 (1971).
3. R. Altschul, *Selected Studies on Atherogenesis* (Thomas, Springfield, Ill., 1950), p. 135; O. J. Pollak, in *Comparative Atherosclerosis*, J. C. Roberts, Jr., and R. Strauss, Eds. (Hoeber Medical Division, New York, 1965), p. 243.
4. H. Imai, N. T. Werthessen, C. B. Taylor, K. T. Lee, *Arch. Pathol. Lab. Med.* **100**, 565 (1976).
5. A. A. Kandutsch, H. W. Chen, H. J. Heiniger, *Science* **201**, 498 (1978).
6. L. L. Smith, D. E. Ray, J. A. Moody, J. D. Wells, J. E. van Lier, *J. Neurochem.* **19**, 899 (1972); L. L. Smith and N. L. Pandya, *Atherosclerosis* **17**, 21 (1973).
7. C. J. W. Brooks, W. A. Harland, G. Steel, *Biochim. Biophys. Acta* **125**, 620 (1966).
8. E. J. Corey and S. K. Gross, *J. Am. Chem. Soc.* **89**, 4561 (1967).
9. T. Shishibori, T. Fukui, T. Suga, *Chem. Lett.*, p. 1289 (1973).
10. J. A. Nelson, M. R. Czarny, T. A. Spencer, J. S. Limanek, K. R. McCrae, T. Y. Chang, *J. Am. Chem. Soc.* **100**, 4900 (1978).
11. S. Biswas, R. P. Cook, J. D. B. MacDougal, *Br. J. Exp. Pathol.* **45**, 13 (1964); J. D. B. MacDougal, S. Biswas, R. P. Cook, *ibid.* **46**, 549 (1965).
12. Cholestan-3β,5α,6β-triol and cholestan-3β-ol-5α,6α-oxide were prepared according to L. F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.* **71**, 3938 (1949); 25-hydroxycholesterol was prepared according to T. A. Narwid, K. E. Cooney, M. R. Uskokovic, *Helv. Chim. Acta* **57**, 771 (1974); 7-ketocholesterol, L. F. Fieser, *J. Am. Chem. Soc.* **75**, 4394 (1953); Squalen-2,3,22,23-bisepoxide, synthesis patterned after E. J. Corey and S. K. Gross, *ibid.* **89**, 4561 (1967); lanosterol-24,25-epoxide, P. W. LeQuesne and R. Ramachandra, unpublished procedure.
13. A glass homogenizer (Ten Broeck) and ultrasonicator (80 kHz, 80 W) were used to obtain a uniform suspension in less than 2 minutes.
14. Y. Tajima, *Lab. Anim.* **17**, 27 (1968).
15. Pulmonary arteries develop lesions in conventional cholesterol feeding experiments [C. H. Bailey, *J. Exp. Med.* **23**, 69 (1916); M. Chuma, *Virchow's Arch.* **242**, 275 (1923); R. Schonheimer, *ibid.* **249**, 1 (1924); G. V. Child, *Arch. Pathol.* **98**, 47 (1974)]. Pulmonary arteriosclerosis can be selectively induced by repeated intravenous injections of blood clots; this method is more efficient with heterogeneous than with homologous or autologous blood [C. V. Harrison, *J. Pathol. Bacteriol.* **60**, 289 (1948); B. E. Heard, *ibid.* **64**, 13 (1952); W. A. Thomas, R. M. O'Neal, K. T. Lee, *Arch. Pathol.* **61**, 380 (1956)]. Lesions thus induced are not grossly visible and are confined to small-caliber pulmonary arteries and arterioles. Pulmonary arterioles can be extensively blocked by injecting 75-μm nonthrombotic plastic particles [W. A. Thomas, R. M. O'Neal, K. T. Lee, *ibid.* **62**, 56 (1956); R. Perez-Tamayo, H. Brandt, H. Medellin, J. Doria, *Am. Heart J.* **61**, 515 (1961)]. A single dose of such particles (200 mg/kg) was well tolerated by the rabbit. No pulmonary arterial lesions were induced despite demonstrable right ventricular hypertrophy after repeated injections. No cause was found for the sudden death after the injection of 10 mg/kg. Extensive thromboembolism with associated physiological disturbance is a possibility.
16. The squalene bisepoxide and lanosterol-24,25-epoxide were shown to be potent inhibitors of cholesterol biosynthesis by J. A. Watson (personal communication).
17. In the absence of direct data on the biosynthesis of 25-hydroxycholesterol, this pathway requires substantiation. The observed effects could be direct; that is, without involving the sterol biosynthesis system.
18. We thank A. T. James and P. Dunphy (Unilever Research, England) for assistance with the chromatography and hydroperoxide synthesis. This work was supported by NIH grants HL-14177 and HL-20993; a special grant to Visiting Researcher, Tokyo Metropolitan Institute of Gerontology; and grants N00014-76-C-0027 and N00014-77-C-0339 from the Office of Naval Research, U.S. Navy.

14 May 1979; revised 27 August 1979

γ-Glutamyl Transpeptidase in Isolated Brain Endothelial Cells: Induction by Glial Cells in vitro

Abstract. *The endothelia of microvessels isolated from mouse brain by mechanical means are rich in γ-glutamyl transpeptidase; however, the enzyme often disappers when the cells migrate or proliferate from the microvessel isolates. In an endothelial cell line derived from similar isolates and negative for γ-glutamyl transpeptidase, the enzyme could be induced in the endothelial cells when they were cocultured with glial cells. Thus there may be a requirement for continuous induction of γ-glutamyl transpeptidase in brain microvessels by adjacent glial cells.*

γ-Glutamyl transpeptidase (γ-GTP) (E.C. 2.3.2.2) has been demonstrated histochemically in brain capillary endothelium, choroid plexus epithelium, and in several extracranial locations, such as the epithelial brush border of the proximal convoluted tubules of the kidney and portions of intestinal epithelium (*1–4*). We have found a similar distribution of γ-GTP-rich areas in the mouse; however, cerebral vascular endothelium is the only endothelium in this animal that contains histochemically detectable concentrations of γ-GTP.

When mouse brain capillaries are isolated by a mechanical dispersion and filtration technique (*5, 6*), they retain high concentrations of γ-GTP in the endothelium (Fig. 1a), yet the enzyme is often lost from the endothelial cells as they migrate or proliferate from the microvessel isolates. An endothelial cell line ME-2 (Fig. 1b), derived from similar mouse isolates (*6*), is also negative for γ-GTP (Fig. 1c); however, the enzyme can be induced in these endothelial cells by coculturing them with C_6 rat glioma cells (Fig. 1, d to f). The enzyme induction depends on the density of C_6 cells. Similar γ-GTP induction cannot be accomplished with cell-free conditioned medium derived from C_6 cells.

The possible role of γ-GTP in the differentiated function of blood-brain barrier endothelium is of interest because the enzyme is implicated in amino acid transport: high concentrations of γ-GTP are associated with areas where high

0036-8075/80/0208-0653$00.50/0

Table 1. Summary of culture conditions used in γ-GTP induction experiments. Monolayers of ME-2 endothelial cells on glass cover slips were cultured for various periods of time (results for 24 and 96 hours are given here) and under a variety of conditions. The ME-2 monolayers were then subjected to enzyme histochemistry (see Fig. 1) and the reaction produced scored as (0) not visible, (1) light, (2) moderate, and (3) heavy.

ME-2 cell culture condition	24 hours	96 hours
Cocultured upside down on x-irradiated C_6 cells of high density*	0, 1	3†
Cocultured upside down on x-irradiated C_6 cells of low density‡	0	0, 1
Cocultured upside down on x-irradiated C_6 cells but separated from them by ≥ 0.7 mm	0	0
Cocultured upside up on x-irradiated C_6 cells	0	0
Cocultured upside down on x-irradiated ME-2 cells	0	0
Cultured upside down in an empty dish	0	0
Cultured upside up (negative controls)	0	0
Cultured in 72-hour conditioned medium free of C_6 cells	0	0

*That is, multilayered in plateau phase at the time of x-irradiation. †Exposure to cycloheximide reduces 3 to 1. ‡That is, in low, log phase, approaching a monolayer at the time of x-irradiation.

rates of amino acid transport occur. Several lines of evidence (*1, 2, 4*), including the demonstration that γ-GTP can function as an integral part of the γ-glutamyl cycle (*7*) and that the γ-glutamyl cycle participates in amino acid transport, support this concept (*8*). Orlowski *et al.* (*2*) have suggested that γ-GTP may play a role in the transfer of at least some amino acids across the blood-brain barrier. However, it should be pointed out that amino acid transport is only one of several possible functions of γ-GTP and glutathione.

In a true capillary in the central nervous system, the endothelium and surrounding glial cells are separated only by a basement membrane. This unique anatomical relationship is coincident with the localization of the enzyme and the selective amino acid barrier or transport function. We hypothesize that it is the glial cell that provides the inductive force for endothelial γ-GTP. We reason that the cerebral endothelial γ-GTP requires an inductive force that is absent in ME-2 monolayer cultures since (i) when migrating and proliferating endothelial cells from cerebral capillary isolates as well as the endothelial cell line ME-2 are isolated or removed from other cell types, including glial cells, they are negative for γ-GTP, and (ii) it is known that this enzyme is inducible in other organs, such as the liver. In addition, Savage *et al.* (*9*) demonstrated that glial cells can exert a tropic influence on human umbilical cord endothelial cells by altering their cytomorphology when in glial-endothelial cocultures.

To test this hypothesis in vitro, a coculture scheme was used that focused on two cell types, the ME-2 endothelial cell derived from mouse brain microvessel isolates (*6*) and the C_6 glioma derived from a rat glial tumor (*10*). In this theme, C_6 glioma cells were seeded in petri dishes and were arrested by 6000 R of x-irradiation after they had reached the desired degree of confluency. These feeder layers of C_6 cells were used either in coculture or to condition media. ME-2 cells were cocultured with C_6 cells, ME-2 cells, or in empty dishes as controls, and were oriented upside up or upside down for close cell-to-cell interaction. All frozen sections, microvessel isolates, and cell monolayers that were to be tested histochemically for γ-GTP were placed on glass cover slips unless otherwise mentioned. Histochemical determinations for γ-GTP were made on acetone-fixed frozen sections, capillary isolates, and cell monolayers by using γ-

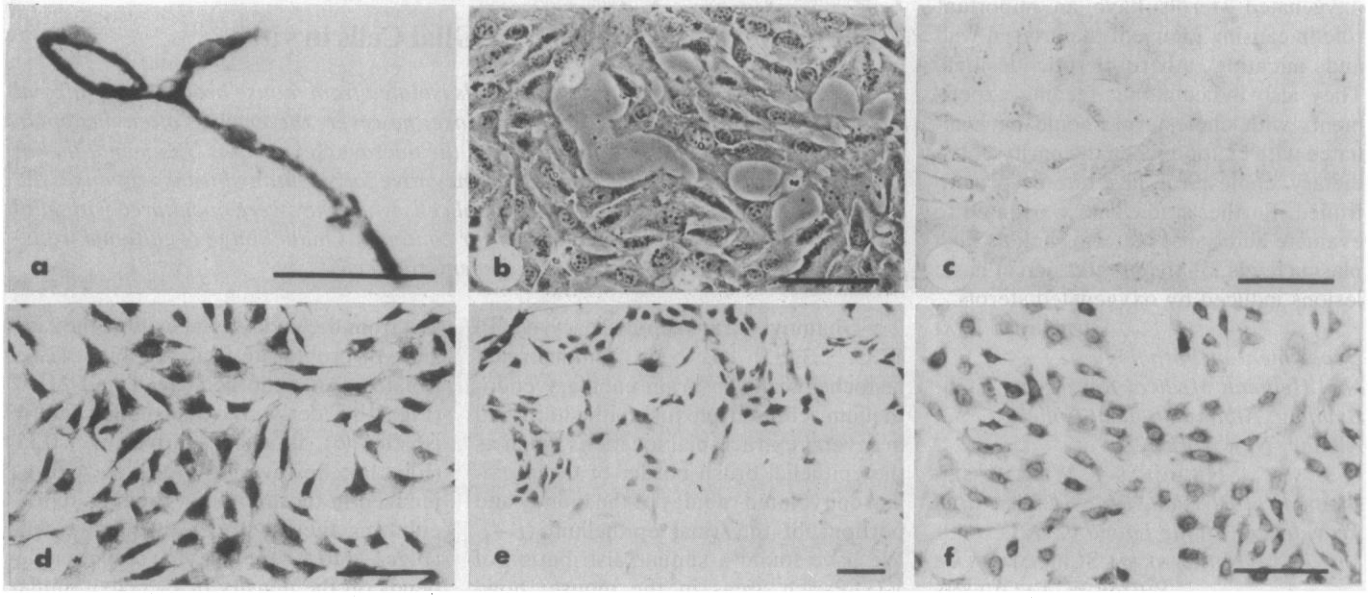

Fig. 1. Enzyme histochemistry for γ-GTP in isolated mouse brain capillaries (a) and in monolayers of mouse endothelial cells (ME-2) derived from similar isolated brain capillaries (c to e); (b) unstained phase-contrast control. Acetone-fixed preparations were rinsed twice in distilled water and incubated for 1.5 hours at 37°C in a reaction mixture of 0.5 m*M* γ-glutamyl-4-methoxy-2-naphthylamide (substrate), 20 m*M* glycylglycine (acceptor), and 0.05 percent Fast Blue BB (indicator) in 25 m*M* PO_4 buffer saline (*p*H 7.4) containing 0.25 percent dimethyl sulfoxide. After the indicator was chelated with 0.1*M* $CuSO_4$, the preparations were mounted in Aqua-mount (Lerner). (a) Isolated mouse brain capillary positive for γ-GTP; (b) phase-contrast micrograph of a typical monolayer of ME-2 cells in late log phase; (c) a similar ME-2 monolayer negative for γ-GTP; (d) ME-2 monolayer, cocultured upside down on x-irradiated C_6 monolayers for 24 hours, showing a positive reaction for γ-GTP; (e) ME-2/C_6 coculture similar to (d), except parts of the C_6 monolayer were removed prior to coculture. The ME-2 cells in the upper part of this frame (which overlay C_6 cells) were positive for γ-GTP, whereas ME-2 cells in the lower part of the frame (which overlay a C_6 cell-free area) were negative for the enzyme; (f) after ME-2 monolayers were cocultured with C_6 cells for 24 hours, the ME-2 cells were separated from the C_6 cells and maintained for an additional 72 hours as a single cell type. The enzyme is still present (scale bars, 50 μm).

glutamyl-4-methoxy-2-naphthylamide, glycylglycine (free base), and Fast Blue BB as artificial substrate, peptide acceptor, and indicator, respectively (*11*).

In cocultures in which ME-2 cells were incubated upside down on C_6 cells, γ-GTP could be demonstrated in ME-2 cells within 24 hours (Fig. 1d). When the length of coculture incubation or the number of C_6 cells in the feeder layer or both were varied, the appearance and intensity of γ-GTP reaction were dependent on time and C_6 cell density. The enzyme concentration, as indicated by intensity of staining, increased with increasing incubation time and, for a standard induction period (24 or 96 hours), the enzyme concentration increased with increasing C_6 cell density. If cycloheximide (100 μg/ml) was present during coculture conditions that would normally give a high concentration of γ-GTP in ME-2 cells, the enzyme concentration could be reduced (Table 1); however, the enzyme was never completely abolished. In similar ME-2/C_6 cocultures in which parts of the C_6 monolayer were removed prior to coculture, the enzyme was detectable only in ME-2 cells that lay directly over C_6 cells; ME-2 cells that lay over empty areas of the dish were negative for γ-GTP (Fig. 1e). This result indicates that an intimate relationship is needed between the two cell types in order to induce the enzyme in the ME-2 cells.

In other experiments, the ME-2/C_6 cocultures were separated after 24 hours and the ME-2 cells maintained as a single cell type for various periods of time after being cocultured; the enzyme was shown to persist for at least 72 hours after coculture (Fig. 1f). However, in ME-2 monolayers 72 hours after coculture, there began a disappearance of γ-GTP on a per cell basis at a time coincident with renewed proliferation in the ME-2 cells. We interpret this to indicate that γ-GTP needs constant induction. All other coculture conditions used did not induce γ-GTP (Table 1).

We propose that the brain capillary endothelial cells require an inductive force to maintain high levels of γ-GTP activity. In coculture the glial cell can provide such an inductive force to the ME-2 cells, provided the two cell types are in close contact, a relationship that mimics the microanatomy in vivo. To our knowledge, this is the first demonstration of the glial cell inducing an enzyme in another cell type. This type of cellular interaction may be crucial to the normal development of the blood-brain barrier. It should be noted that the induction process is not species-specific, since the C_6 glial cells are of rat origin whereas the ME-2 vascular endothelial cells are isolated from the mouse. These findings pose many interesting questions related to mechanisms of interaction between a variety of cell types.

LAWRENCE E. DEBAULT
PASQUALE A. CANCILLA
Department of Pathology and
Arteriosclerosis Specialized Center of Research, College of Medicine, University of Iowa, Iowa City 52242

References and Notes

1. Z. Albert, M. Orlowski, Z. Rzucidlo, J. Orlowska, *Acta Histochem.* **25**, 312 (1966).
2. M. Orlowski, G. Sessa, J. P. Green, *Science* **184**, 66 (1974).
3. K. Renkawek, M. R. Murray, M. Spatz, I. Klatzo, *Exp. Neurol.* **50**, 194 (1976).
4. For reviews, see M. Orlowski [*Arch. Immunol. Ther. Exp.* **11**, 1 (1963)] and S. B. Rosalki [*Adv. Clin. Chem.* **17**, 53 (1975)].
5. K. Brendel, E. Meezan, E. C. Carlson, *Science* **185**, 953 (1974).
6. L. E. DeBault, L. E. Kahn, S. P. Frommes, P. A. Cancilla, *In Vitro* **15**, 473 (1979).
7. M. Orlowski and A. Meister, *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1248 (1970); S. Prusiner, C. W. Doak, G. Kirk, *J. Cell. Physiol.* **89**, 853 (1976).
8. For a review, see A. Meister, *Science* **180**, 33 (1973).
9. K. E. Savage, R. L. Suddith, P. S. Baur, H. T. Hutchison, *J. Cell Biol.* **70**, 91 (Abstr.) (1976).
10. P. Benda, J. Lightbody, G. Sato, L. Levine, W. Sweet, *Science* **161**, 370 (1968).
11. G. G. Glenner, J. E. Folk, P. J. McMillian, *J. Histochem. Cytochem.* **10**, 481 (1962); S. Samuels, *J. Theor. Biol.* **64**, 729 (1977).
12. We thank L. Yoder, Y. Wu, S. Frommes, and G. Glass for their assistance. Part of this material was presented at a satellite meeting to the Second World Congress for Microcirculation, Galveston, 27–31 July 1979. Supported by Heart and Lung Institute grants 14230-07 and 14230-08.

16 July 1979; revised 10 September 1979

Electrophysiological Signs of Split-Second Decision-Making

Abstract. *When young adults detected auditory stimuli at split-second intervals, different components of the event-related brain potentials showed markedly different speeds of recovery. The P_3 component (latency 300 to 350 milliseconds) was fully recovered at intervals of less than 1.0 second, while the N_1–P_2 components (latencies 100 to 180 milliseconds) were markedly attenuated with stimulus repetition even at longer interstimulus intervals. Thus, the N_1–P_2 recovers much more slowly than a subject's ability to evaluate signals, whereas the P_3 appears to be generated at the same high rates as the decision processes with which it is associated.*

Stimuli in all modalities elicit a succession of time-locked electrical waves from the human brain that can be recorded at the scalp as the evoked potential or event-related potential (ERP). The earlier waves in the ERP are generally regarded as exogenous or stimulus-bound, because their properties are determined primarily by the physical attributes of the stimulus. A number of components with longer latencies (including the P_3 wave at 300 to 500 msec) are highly sensitive to the processing demands of the task; these are considered to be endogenous in that they reflect cognitive operations triggered by relevant or significant stimuli (*1*).

Attempts to correlate human ERP components with specific psychological processes have met with varying success. In the case of exogenous ERP's, many of the psychophysiological relationships that have been reported are severely disrupted when the rate of stimulus delivery is changed (*2*). For example, the amplitudes of the long-latency components of the auditory evoked potential (N_1 and P_2 occurring at 100 and 180 msec, respectively) covary with perceived loudness when the interstimulus interval (ISI) is constant (*3*); however, shortening the ISI below about 10 seconds progressively reduces N_1 and P_2 amplitudes without a corresponding change in loudness (*4*) or reaction time (*5*).

The temporal recovery properties of endogenous ERP components have not yet been systematically investigated. Insofar as they index human cognitive processes, however, they should exhibit short refractory periods or recovery cycles like those of the cognitive operations with which they are associated.

We have examined the recovery properties of the P_3 wave, a long-latency (300- to 500-msec) endogenous potential associated with cognitive operations like decision-making, stimulus evaluation, and sensory detection (*1*, *2*). We now report that the P_3 wave exhibits a recovery cycle that parallels the speed with which accurate decisions can be made in a signal-detection paradigm, a recovery cycle much shorter than those reported for other long-latency ERP components.

We elicited sequences of P_3's by requiring subjects to tally near-threshold or suprathreshold (70-dB sound level) tones presented at short ISI's. As many as three such tones (all 1.0 kHz and 50 msec long) could be presented on a single trial during a 1200-msec interval (*6*).

Each trial began with a faint warning flash, which was followed by three positions where tones might or might not be presented, according to a random sched-

0036-8075/80/0208-0655$00.50/0