ognition that changes in the molecular weight profile of iβ-EP occur during stimulated secretion could help to clarify conflicting findings on the molar ratio of β-EP to β-LPH (*18*); the range of reported values might reflect differences in time elapsed from the onset of stimulated secretion to collection of plasma specimens.

5) The enhancement of iβ-EP release during naloxone administration is evidence of "short-loop" feedback—previously observed only in unstressed subjects (*19*)—modulating stress-induced secretion of endogenous opioids. This finding implies that the amelioration of endotoxic shock by naloxone (*1*) occurs despite acute augmentation of already high circulating levels of β-EP and β-LPH.

6) The demonstration of late increases in CSF iβ-EP after endotoxin administration suggests that physiological stimuli, such as fever, that provoke an outpouring of iβ-EP into the periphery may occur in concert with alterations of central opioid peptide metabolism. Though not in itself evidence either for direct secretion of iβ-EP from the brain into the CSF or "leakage" of iβ-EP into the CSF through the blood-brain barrier, this last finding supports the hypothesis that certain behavioral correlates of fever may be manifestations of altered central metabolism of peptides related to iβ-EP (*20*).

DANIEL B. CARR
Endocrine Unit, Massachusetts General Hospital, Boston 02114, and *Harvard Medical School, Boston 02115*
RICHARD BERGLAND
ALLAN HAMILTON
HOWARD BLUME
Department of Neurosurgery, Beth Israel Hospital, Boston 02215, and *Harvard Medical School*
NORMAN KASTING
MICHAEL ARNOLD
JOSEPH B. MARTIN
Neurology Service, Massachusetts General Hospital, and *Harvard Medical School*
MICHAEL ROSENBLATT
Endocrine Unit, Massachusetts General Hospital, and *Harvard Medical School*

References and Notes

1. J. W. Holaday and A. I. Faden, *Nature (London)* **275**, 450 (1978); W. P. Peters, M. W. Johnson, P. A. Friedman, W. E. Mitch, *Lancet* **1981-I**, 529 (1981).
2. W. B. Cannon and J. R. Pereira, *Proc. Natl. Acad. Sci. U.S.A.* **10**, 247 (1924); E. L. Bliss, C. J. Migeon, K. Eik-Ness, A. A. Sandberg, L. T. Samuels, *Metabolism*, **3**, 493 (1954).
3. R. Guillemin *et al.*, *Science* **197**, 1367 (1977).
4. R. Bergland, H. Blume, A. Hamilton, P. Monica, R. Paterson, *ibid.* **210**, 541 (1980).
5. These samples were free of blood and were withdrawn from a cannula tip placed at level A32, 3 mm lateral to the midline, at a depth of 7 to 10 mm depending on individual sheep. Coordinates are as in P. Richards [*Atlas Stereotaxique du Cerveau du Brebis* (Institut National de la Recherche Agronomique, Paris, 1967)].
6. Preliminary studies showed this dose uniformly produced fever and hypotension lasting approximately 5 hours, but led to no fatalities even though animals received no therapy. Tolerance was avoided by not exposing animals repeatedly to endotoxin.
7. The dose of naloxone used was similar to that used in clinical settings [see (*1*)] and was unlikely to be high enough to result in blockade of nonopiate systems [see J. Sawynok, C. Pinsly, F. S. LaBella, *Life Sci.* **25**, 1621 (1979)].
8. K. Nakao, Y. Nakai, S. Oki, S. Matsubara, T. Konishi, H. Nishitani, H. Imura, *J. Clin. Endocrinol. Metab.* **50**, 230 (1980); E. Wiedemann, T. Saito, J. A. Linfoot, C. H. Li, *ibid.* **45**, 1108 (1977); D. B. Carr *et al.*, *N. Engl. J. Med.* **305**, 560 (1981).
9. S. L. Wardlaw and A. G. Frantz, *J. Clin. Endocrinol. Metab.* **48**, 176 (1979).
10. F. J. Scully, *J. Am. Med. Assoc.* **69**, 20 (1917); M. D. Altschule, A. S. Freedberg, M. J. McManus, *J. Clin. Invest.* **24**, 878 (1945).
11. J. E. Blalock and E. M. Smith, *Biochem. Biophys. Res. Commun.* **101**, 472 (1981); E. M. Smith and J. E. Blalock, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7530 (1981).
12. W. G. Clark, *Neurosci. Biobehav. Rev.* **3**, 179 (1979).
13. I. Chowers, H. T. Hammel, J. Eisenman, R. M. Abrams, S. M. McCann, *Am. J. Physiol.* **210**, 606 (1966).
14. D. S. Gann, D. G. Ward, D. E. Carlson, *Recent Prog. Horm. Res.* **34**, 357 (1978).
15. I. Lemaire, R. Tseng, S. Lemaire, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6240 (1978).
16. Y. P. Loh and B. G. Jenks, *Endocrinology* **109**, 54 (1981).
17. S. S. C. Yen and A. Lein, *Am. J. Obstet. Gynecol.* **126**, 942 (1976).
18. K. Nakao, Y. Nakai, H. Jingami, S. Oki, J. Fukata, H. Imura, *J. Clin. Endocrinol. Metab.* **49**, 838 (1979); H. Yamaguchi, A. S. Liotta, D. T. Krieger, *ibid.* **51**, 1002 (1981).
19. E. R. Levin, B. Sharp, N. V. Meyer, H. E. Carlson, *Endocrinology* **109**, 146 (1981).
20. D. Carr, *Perspect. Biol. Med.* **23**, 1 (1979).
21. We thank J. Fisher, S. Kleshinski, and J. Walsh for technical assistance, C. H. Li for ovine β-LPH, and J. T. Potts, Jr., for guidance and encouragement. Supported in part by NIH grants AM 07028-06 and AM 26252 and by a grant from the Kleberg Foundation. D.B.C. is a Daland Fellow of the American Philosophical Society.

19 January 1982; revised 22 March 1982

Significance of Tissue *myo*-Inositol Concentrations in Metabolic Regulation in Nerve

Abstract. *Approximately 25 percent of resting energy utilization in isolated nerve endoneurium is inhibited by medium containing defatted albumin and selectively restored by arachidonic acid but is unaffected by indomethacin or nordihydroguaiaretic acid. The same component of energy utilization is inhibited by small decreases in endoneurial* myo-*inositol, which decrease incorporation of carbon-14–labeled arachidonic acid into phosphatidylinositol. The fraction of the resting oxygen uptake inhibited by ouabain is decreased 40 to 50 percent by a reduced tissue* myo-*inositol concentration or by defatted albumin. Metabolic regulation by rapid, basal phosphatidylinositol turnover is dependent on the maintenance of normal tissue* myo-*inositol concentrations.*

The significance of the high concentrations of *myo*-inositol (MI) normally maintained in most mammalian tissues (*1*) is unknown. This question has much broader import, but is central to a hypothesis that a decrease in nerve MI is a critical factor in the pathogenesis of diabetic neuropathy (*2*). *myo*-Inositol is directly incorporated into phosphatidylinositol (PI) by CDPdiacylglycerol—inositol 3-phosphatidyltransferase (PI synthetase) in the terminal step of PI synthesis (*3*). Studies with ^{32}P-labeled inorganic phosphate and labeled MI indicate that most tissues contain pools of membrane PI that undergo a rapid cycle of partial degradation and resynthesis (PI turnover) (*3*); this occurs in peripheral nerve axons (*4*). The cycle is initiated by a phospholipase C cleavage of PI and ultimately is completed by the PI synthetase reaction (*3*). The predominant species of PI in tissues is 1-stearyl-2-arachidonoyl (*3, 5*), and free arachidonic acid (AA) may also be released and reincorporated during PI turnover (*3, 6*). Rapid PI turnover in specific pools serves as a mechanism for metabolic regulation (*3, 6*).

Recently, interest in PI turnover has centered on its role in regulating free AA levels and prostanoid metabolism (*6*) and its relationship to the regulation of cytoplasmic free Ca^{2+} (*3, 7*); a role in regulating Na^+,K^+-adenosinetriphosphatase (ATPase) in some tissues is also postulated (*3*). The regulatory functions ascribed to rapid PI turnover in specific pools requires the largely unnoted assumption that the resynthesis of PI in these pools is normally independent of the MI concentration, although most PI synthetases have a relatively high Michaelis constant (K_m) for MI—for instance, 1.5 m*M* for guinea pig brain PI synthetase (*8*). We found evidence that in an endoneurium preparation approximately 25 percent of resting energy utilization, including a component of Na^+,K^+-ATPase activity, is regulated through PI turnover by mechanisms that do not appear to involve prostanoid metabolism. The activity of this fraction of energy utilization is acutely and reversibly inhibited by depletion of free AA or by small decreases in tissue MI, which decrease [1-^{14}C]AA incorporation into PI approximately 40 percent. Regulation of this fraction ap-

0036-8075/82/0827-0848$01.00/0

pears to be mediated by rapid PI turnover in small, discrete PI pools and to be dependent on the maintenance of normal MI concentrations at these sites.

Composite axonal and Schwann cell metabolism was studied in endoneurium derived from a preparation of the major fascicle of rabbit tibial nerve by removing its perineurial membrane (*9*). [The perineurial membrane surrounding each nerve fascicle bounds an endoneurial compartment; it is a diffusion barrier and a component of the blood-nerve barrier which regulates the composition of the endoneurial fluid surrounding the axons and Schwann cells (*10*).] Endoneurium preparations maintain a nearly steady state of energy metabolism (stable O_2 uptake, lactate production, and ATP and phosphocreatine) for at least 2 hours when incubated in a standard medium (*9*). The standard medium consists of Krebs-bicarbonate buffer, *p*H 7.4 at 37°C, continuously gassed with 5 percent CO_2 in air, and containing 5.0 m*M* glucose, 0.50 m*M* MI, and 4.5 percent dialyzed, defatted bovine serum albumin (BSA). In endoneurium glucose is the major physiological fuel and respiration provides 99 percent of the energy requirements (*9*); thus the rate of energy utilization can be assessed by O_2 uptake during incubations with glucose under near-steady-state conditions. In endoneurium from normal rabbits, the initial MI concentration, 5.88 ± 0.47 mmole/kg, is 100 times that in plasma and remains stable during incubation in the standard medium, which contains the MI concentration present in rabbit cerebrospinal fluid (*9*). The medium contains 4.5 percent defatted BSA to prevent any change in water content during incubation (*9*); this should deplete endogenous free fatty acids (*11*) but does not affect the availability of substrate for energy provision (*9*). However, use of BSA with a low free fatty acid content in standard medium results in an approximately 20 percent higher rate of endoneurial energy utilization, which more closely approximates the rate in the fascicle preparation from which the endoneurium is derived (*9*). The perineurial membrane is a diffusion barrier to albumin in life (*10*) and protects the endoneurium in the fascicle preparation from exposure to albumin during incubation. This suggested that there is a component of endoneurial energy utilization that is inhibited by depletion of endogenous free fatty acids and restored by BSA with a low free fatty acid content. This was shown to be related to the depletion and replenishment of free AA.

Paired samples of endoneurium were incubated for 30 minutes in standard medium and then for 10 minutes in similar medium or medium containing albumin-bound AA in molar AA/albumin ratios of 0.022 to 1.11. This range was selected since it should result in extremely small increments in tissue-associated free AA as the molar ratio is increased, particularly relative to that resulting from exposure to free AA in medium lacking albumin (*11*). Albumin-bound AA had no effect on O_2 uptake at a molar ratio of 0.022, but over the range 0.11 to 1.11 it caused an acute increase in O_2 uptake, which was relatively constant in magnitude, 22 to 31 percent (Table 1). This effect requires live tissue and glucose as substrate. The increased O_2 uptake remained stable for at least 90 minutes and was not accompanied by changes in ATP or phosphocreatine (data not shown). The effect of albumin-bound AA was not reproduced by albumin-bound γ-linolenic acid (Table 1) and, as expected from previous studies (*9*), albumin-bound palmitate acutely depressed O_2 uptake even at a low molar ratio (Table 1). The effect of albumin-bound AA was not inhibited by indomethacin (10^{-4} *M*) or by nordihydroguaiaretic acid (10^{-4} *M*) (Table 1). Replenishing endoneurial free AA over a restricted range results in an increased near steady state of energy utilization that does not appear to require AA metabolism by cyclooxygenase or lipoxygenase pathways. The magnitude of the increase in energy utilization is not directly related to the molar ratio of AA to albumin, or presumably to the level of tissue-associated free AA, once a threshold is exceeded.

Table 1. Effects of albumin-bound arachidonic, γ-linolenic, and palmitic acids on O_2 uptake in endoneurium that had been incubated for 30 minutes in standard medium. Uptake was determined after a 10-minute incubation and compared with that of paired controls incubated in standard medium. Values are means or mean changes ± standard errors for paired experiments. Probabilities were determined by Student's *t*-test for paired comparisons; N.S., not significant.

Oxygen uptake (mmole/kg per hour)			*N*	*P*	Molar ratio of free fatty acid to albumin
Control sample	Test sample	Difference (mean ± S.E.M.)			
		Albumin-bound AA			
18.1	19.6	+1.5 ± 1.9	6	N.S.	0.022
17.8	21.8	+4.0 ± 1.3	7	<.05	0.11
17.7	23.0	+5.3 ± 1.3	7	<.01	0.22
17.7	23.2	+5.5 ± 1.7	10	<.01	1.11
		Albumin-bound γ-linolenic acid			
18.0	19.4	+1.4 ± 1.4	6	N.S.	0.22
		Albumin-bound palmitic acid			
18.6	16.5	−2.1 ± 0.5	6	<.01	0.07
		*Albumin-bound AA plus indomethacin**			
18.8	22.4	+3.6 ± 0.7	7	<.01	0.22
		Albumin-bound AA plus NGDA†			
18.7	22.7	+4.0 ± 1.1	8	<.01	0.22

*Indomethacin (10^{-4}*M*) present during preincubation and incubation and present in control. †NGDA (nordihydroguaiaretic acid) (10^{-4}*M*) present during preincubation and incubation and present in control.

The effect of albumin-bound AA (Table 1) was demonstrated under conditions in which the initial tissue MI concentration was kept stable by the 0.50 m*M* MI present in standard medium (*9*). Endoneurium can be partly depleted of its initial MI content by incubation in medium lacking MI. In samples prepared and equilibrated (for 10 minutes) in standard medium, approximately 6 percent of the tissue MI is present in the extracellular (inulin) space. When samples were equilibrated in standard medium lacking MI and their MI was compared with that in paired samples after a subsequent 2-hour incubation in medium lacking MI, there was a 16 percent decrease; the mean change in eight paired experiments was −0.75 ± 0.22 mmole/kg ($P < .02$). In standard medium, which rapidly depletes endogenous free AA, the initially decreased rate of O_2 uptake is unaffected by a subsequent decrease in tissue MI of roughly 22 percent over 2 hours. Oxygen uptake was compared in paired samples after equilibration in standard medium and after a 2-hour incubation in standard medium lacking MI; the mean change in seven paired experiments was −0.9 ± 1.5 mmole of O_2 per kilogram per hour, and ATP and phosphocreatine were also unaltered. However, the effect of albumin-bound AA on O_2 uptake cannot be demonstrated in endoneurium previously incubated for 30 minutes in standard medium lacking MI to reduce the initial MI content approximately 10 percent. When paired samples were incubated in this way and albumin-bound AA was added to one sample during a subsequent 10-minute incubation, there was no effect on O_2 uptake

Table 2. Effects of albumin-bound AA and of MI on O_2 uptake in endoneurium samples that were preincubated in medium lacking MI and containing defatted albumin to reduce their initial MI content and deplete endogenous FFA. In all instances the medium, Krebs-bicarbonate buffer, *p*H 7.4, contained 5.0 m*M* glucose. Additions to the medium were AA −, 4.5 percent defatted albumin; AA +, albumin-bound AA with a molar ratio of 0.22; MI −, no MI; and MI +, 0.50 m*M* MI. Values for O_2 uptake are means or mean differences ± S.E.M. for a group of paired experiments.

Group	Addition to the medium								Oxygen uptake (mmole/kg per hour)			*N*	*P*
	Preincubation (30 minutes)				Incubation (10 minutes)								
	Control		Test		Control		Test		Control	Test	Difference (mean ± S.E.M.)		
	AA	MI	AA	MI	AA	MI	AA	MI					
A	−	−	−	−	−	−	+	−	17.1	18.1	+1.0 ± 0.8	6	N.S.
B	−	−	−	+	+	−	+	+	20.1	27.5	+7.4 ± 0.8	6	<.001
C	−	−	−	−	+	−	+	+*	19.7	22.6	+2.9 ± 0.4	5	<.01
D	−	−	−	−	−	−	−	+*	19.1	18.5	−0.6 ± 1.0	7	N.S.

*MI absent from medium during 10-minute incubation, but 1.0 m*M* MI added to medium immediately before recording O_2 uptake in an O_2 monitor.

(Table 2, group A). When paired samples were incubated for 30 minutes in standard medium with or without 0.50 m*M* MI and then incubated with albumin-bound AA, the sample in which the initial tissue MI was maintained had a higher rate of O_2 uptake (Table 2, group B). When paired samples were incubated in standard medium lacking MI for 30 minutes, then incubated in medium lacking MI but containing albumin-bound AA to replenish a free AA pool, and transferred into similar medium in O_2 monitor chambers, the addition of 1.0 m*M* MI to one sample caused a significant increase in its O_2 uptake, which was apparent within 1 to 2 minutes (Table 2, group C). In contrast, addition of 1.0 m*M* MI had no effect on O_2 uptake in tissue with a decreased MI content in medium containing defatted albumin (Table 2, group D). Thus, the same component of resting energy utilization appears to be inhibited by depletion of free AA or by a decrease in endoneurial MI.

Incorporation of [1-^{14}C]AA into PI was compared in paired samples that were incubated for 30 minutes in standard medium with and without 0.50 m*M* MI and then for 90 minutes in similar medium containing albumin-bound [1-^{14}C]AA (molar ratio, 0.11; specific activity, 2 μCi/μmole). [The tissue was rinsed in medium containing defatted BSA and rapidly blotted, frozen, and powdered in liquid N_2. The powder was extracted twice with 50 volumes of chloroform and methanol (2:1) with 2.5*M* KCl to maintain a 5 percent aqueous component. Unlabeled PI was added as carrier. The extract was partitioned with 0.20 volume of 0.25*M* KCl; the lower phase was washed five times with upper-phase solvents containing 0.25*M* KCl (*12*) and dried under N_2. PI was isolated by two-dimensional thin-layer chromatography on silica gel G (*13*) and scraped into a liquid scintillation vial; 15 ml of Aquasol-2 was added and the samples were counted with an external standard.] Incorporation of [1-^{14}C]AA into PI in the samples that maintained their initial MI concentration averaged 4.78 ± 0.46 μmole/kg. This was 60 percent higher than in the samples partially depleted of MI (3.00 ± 0.37 μmole/kg); the mean change in seven paired experiments was +1.78 ± 0.32 μmole/kg per 90 minutes ($P < .005$). The increased O_2 uptake induced by albumin-bound AA in tissue depleted of endogenous free fatty acids appears to require incorporation of AA into PI at sites of PI formation in which MI becomes limiting when the initial MI concentration is decreased. An increase in PI formation of roughly 1.78 μmole/kg per 90 minutes is required to restore this component of energy utilization, but the energy required for an increase in PI formation of this magnitude is at least 1000 times too small to account for the changes in O_2 uptake induced by albumin-bound AA (Table 1) or by a decrease in tissue MI (Table 2, group B). These changes in O_2 uptake probably result from changes in the rates of energy-requiring processes that are regulated through PI turnover in pools in which it is rapidly restricted by depleting free AA or by small decreases in total tissue MI. This suggests that small, discrete, rapidly turning over pools are involved, and that these are at sites in which MI is critically reduced by incubation in medium lacking MI. Axons are thought to contain much lower MI concentrations than Schwann cells (*14*); whether incubation in medium lacking MI preferentially depletes a small axonal MI pool remains to be clarified.

The fraction of resting energy utilization that is related to Na^+,K^+-ATPase activity was estimated from the percent decrease in resting O_2 uptake caused by $10^{-4}M$ ouabain within 30 minutes. [In rabbit vagus nerve $10^{-4}M$ ouabain causes a 31 percent fall in resting O_2 uptake in 30 minutes and a 40 percent fall in 1 hour (*15*).] In paired endoneurium samples incubated in standard medium containing albumin-bound AA (molar ratio, 0.22), ouabain caused a 30.3 ± 2.3 percent decrease in O_2 uptake ($N = 6$). In samples incubated in standard medium the lower resting O_2 uptake was decreased only 14.8 ± 3.4 percent by ouabain ($N = 7$). In samples incubated (for 30 minutes) in standard medium lacking MI and then incubated with albumin-bound AA without MI, ouabain caused a 16.7 ± 3.6 percent fall in O_2 uptake ($N = 13$). A component of Na^+,K^+-ATPase activity is included in the energy-requiring processes that are regulated through rapid PI turnover in endoneurium.

The normal MI concentration in nerve endoneurium appears to be required to prevent a restriction on PI synthetase activity at sites of rapid PI turnover involved in metabolic regulation. This may be one function of the high MI concentrations normally present in most tissues, and could explain the provision of mechanisms to maintain these concentrations when the diet lacks MI (*1*). Our data also suggest the existence of a mechanism which could contribute to the pathogenesis of diabetic complications in nerve and other tissues in which the maintenance of normal MI concentrations is impaired (*2*).

DAVID A. SIMMONS
ALBERT I. WINEGRAD
DONALD B. MARTIN
George S. Cox Medical Research Institute, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia 19104

References and Notes

1. M. A. Stewart, M. M. Kurien, W. R. Sherman, E. V. Cotlier, *J. Neurochem.* **15**, 941 (1968).
2. D. A. Greene, P. V. DeJesus, Jr., A. I. Winegrad, *J. Clin. Invest.* **55**, 1326 (1975).
3. J. N. Hawthorne and M. R. Pickard, *J. Neurochem.* **32**, 5 (1979); B. W. Agranoff and J. E. Bleasdale, in *Cyclitols and Phosphoinositides*, W. W. Wells and F. Eisenberg, Jr., Eds. (Academic Press, New York, 1978), p. 105.

4. R. M. Gould, R. J. Lasek, P. S. Spencer, in *Cyclitols and Phosphoinositides*, W. W. Wells and F. Eisenberg, Jr., Eds. (Academic Press, New York, 1978), p. 535.
5. R. M. Bell and R. A. Coleman, *Annu. Rev. Biochem.* **49**, 459 (1980).
6. R. L. Bell, D. A. Kennerly, N. Stanford, P. W. Majerus, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3238 (1979); P. J. Marshall, J. F. Dixon, L. E. Hokin, *ibid.* **77**, 3292 (1980).
7. R. H. Michell, *Biochim. Biophys. Acta* **415**, 81 (1975); M. J. Berridge and J. N. Fain, *Biochem. J.* **178**, 59 (1979).
8. J. A. Benjamins and B. W. Agranoff, *J. Neurochem.* **16**, 513 (1969).
9. D. A. Greene, A. I. Winegrad, J.-L. Carpentier, M. J. Brown, M. Fukuma, L. Orci, *ibid.* **33**, 1007 (1979); D. A. Greene and A. I. Winegrad, *Diabetes* **28**, 878 (1979); *ibid.* **30**, 967 (1981).
10. P. K. Thomas and Y. Olsson, in *Peripheral Neuropathy*, P. J. Dyck *et al.*, Eds. (Saunders, Philadelphia, 1975), vol. 1, p. 168.
11. A. A. Spector and J. E. Fletcher, in *Disturbances in Lipid and Lipoprotein Metabolism*, J. M. Dietschy, A. M. Gotto, Jr., A. A. Ontko, Eds. (American Physiological Society, Bethesda, Md., 1978), p. 229.
12. J. Folch, M. Lees, G. H. Sloane Stanley, *J. Biol. Chem.* **226**, 497 (1957).
13. E. Yavin and A. Zutra, *Anal. Biochem.* **80**, 430 (1977).
14. H. Kusuma and M. A. Stewart, *J. Neurochem.* **17**, 317 (1970).
15. J. M. Ritchie and R. W. Straub, *J. Physiol. (London)* **304**, 109 (1980).
16. Supported in part by U.S. Public Health Service grant T32 AMO7314 and gifts from the Ware Foundation and R. J. Reynolds Industries.

14 December 1981; revised 8 March 1982

Anomalous Patterns in Cultured Cell Monolayers

Abstract. *Gridlike patterns of differing cell density were observed in evenly seeded cell monolayers. Such patterns were obtained in five of six cell lines tested, suggesting widespread occurrence. The mechanism appears to involve small, transient temperature changes related to incubator tray structure. The very short time course of appearance of the patterns implicates attachment rather than growth as the critically affected factor. Impaired adhesion or directed sedimentation resulting from thermally induced microcurrents in the medium are the two most likely mechanisms.*

Achieving a uniform distribution of cells in a culture vessel is of primary importance in cell culture. Such basic considerations as plating efficiency and contact inhibition of cell growth are directly related to the density with which suspended cells settle out and attach to the culture dish. In addition, in many cell lines function at high cell densities differs from function at low densities. Mullin *et al.* (*1*) found that confluent but not subconfluent cultures of LLC-PK_1 cells actively accumulated α-methyl-D-glucoside and that the development of morphological polarity in this line (appearance of apical microvilli) was also correlated with culture density. Cereijido *et al.* (*2*) found that monolayers of MDCK cells seeded at superconfluent densities developed transepithelial resistance more rapidly when the cells were derived from confluent cultures than when they were derived from subconfluent cultures. The investigators suggested that information for synthesizing tight junctions was not present in the subconfluent cultures. Similarly, cilia development in rat liver cells (*3*) and the appearance of a 60,000-dalton cell surface protein in endothelial cell cultures (*4*) have been correlated with high cell density. In many nonepithelial cell lines, attainment of cell confluence and density-dependent growth inhibition is associated with decreased transport activity. Decreased transport of K^+, phosphate, nucleosides, and sugars have all been observed as cultures become confluent (*5*). The existence of cell populations of nonuniform density could thus introduce a significant error into investigations of a number of parameters.

During a routine check of cell growth on collagen-coated Nuclepore filters in plastic petri dishes, we observed a gridlike growth pattern. Areas of heavy cell growth surrounded evenly spaced patches of low cell density. The growth pattern appeared to correspond to the structure of the incubator tray on which the petri dishes had been placed during incubation, with areas of low cell density corresponding to holes in the tray and areas of higher density corresponding to the metal framework (Fig. 1, A and B). This correspondence between cell growth pattern and incubator tray design was previously observed with HeLa cells by Puck (*6*). The phenomenon was attributed to an extreme sensitivity of cell growth to small transient temperature differences presumed to be created between holes in the tray and the framework when the incubator door was opened.

To examine the mechanism of the observed patterning of cell growth in more detail we suspended trypsinized LLC-PK_1 cells in Eagle's minimum essential medium (Flow) containing 10 percent fetal bovine serum (Flow) and seeded them at different densities into 60-mm-diameter Falcon petri dishes (*7*). The cultures were incubated at 37°C in a humidified 5 percent CO_2 atmosphere in a water-jacketed incubator (capacity, 0.15 m^3) with perforated aluminum shelves. At the end of each experiment the cells were fixed with absolute methanol for 30 minutes and stained with Giemsa. For experiments in which cell density was assayed by DNA analysis, cells were grown on collagen-coated Nuclepore filters. Halved filters were

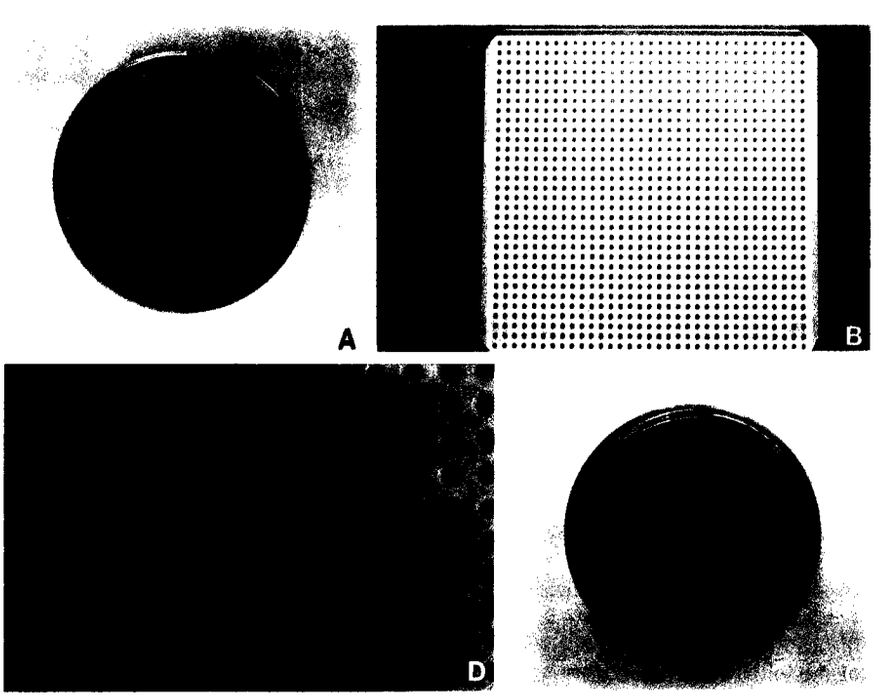

Fig. 1. (A) Patterned cell growth in a 60-mm-diameter petri dish. (B) Tray on which the dish was placed during incubation. (C) Petri dish not exhibiting patterning. This is typical of dishes resting on glass plates during incubation. (D) Sheet of liquid crystals (sensitivity, 35° to 40°C) on an incubator tray that has just been removed from an incubator at 39°C (color spectrum 35° to 40°C: black, brown, red, yellow, green, blue, and black).

0036-8075/82/0827-0851$01.00/0