13. D. A. Crerar, personal communication.
14. For McDonalds Branch, $SO_4^{2-} = 1.36\ H^+ + 62.6$, $r = .66$ (significant at $P = .01$), $N = 36$; for Oyster Creek, $SO_4^{2-} = 0.9\ H^+ + 78.1$, $r = .44$ (significant at $P = .05$), $N = 26$. Concentrations are in microequivalents per liter; r is the correlation coefficient; N is the number of samples in which anion and cation equivalents and calculated and measured specific conductances agree.
15. The summer *p*H was higher than the winter *p*H, and there was no relationship between discharge and *p*H. There was no tendency for *p*H to decrease during high flow events in summer.
16. U.S. Geological Survey Water Resources Division, Trenton, N.J., compilation of *p*H values for 64 Pine Barrens streams 1951–1979.
17. B. Pyle, personal communication.

25 January 1979; revised 10 September 1979

Lens Epithelial Cell Elongation in the Absence of Microtubules: Evidence for a New Effect of Colchicine

Abstract. *Embryonic chick lens epithelial cells cultured in serum-supplemented medium elongated in the absence of microtubules after treatment with the anti-microtubule drug nocodazole. Colchicine, at concentrations lower than those that dissociate microtubules, blocks cell elongation and the associated increase in cell volume. These results indicate that an increase in cell volume, not microtubules, is responsible for lens cell elongation and suggest a previously undescribed effect of colchicine on cell volume regulation.*

Microtubules (MT's) are nearly ubiquitous cell organelles that have been associated with a wide range of cellular activities including intracellular transport (*1*), regulation of membrane function (*2*), maintenance of cell shape (*3*), ciliary and flagellar motion (*4*), chromosome movement (*5*), and active cell shape change (*6–8*). Much of the evidence for the role of MT's in these processes has come from the use of colchicine or drugs that, like colchicine, lead to the depolymerization of cytoplasmic MT's. Although it is likely that MT's play a central role in many of these activities, investigators have cautioned that in some cases the treatments used to interfere with MT structure may actually be exerting their disruptive activity through effects on other cellular structures (*8, 9*). We have found evidence which suggests that the ability of colchicine and three other anti-MT drugs to block lens epithelial cell elongation in vitro during lens fiber cell differentiation is due to their effect on cell volume regulation rather than their direct effect on cytoplasmic MT's.

Embryonic chick lens epithelial cells cultured in medium supplemented with fetal calf serum (FCS) elongate and differentiate into lens fiber cells (*8, 10*). The early phase of this elongation, in which the cells approximately double in length, can occur during nearly complete inhibition of protein synthesis (*11*). Lens epithelial cell elongation is blocked by colchicine (*11*) and the anti-MT agents vinblastine (*10*), demecolcine (Colcemid) (*12*), and podophyllotoxin (*12*). Colchicine ($2 \times 10^{-5}M$) does not, however, inhibit specialization of lens epithelial cells for δ-crystallin protein synthesis or the accumulation of δ-crystallin messenger RNA, events that are associated with lens fiber cell differentiation (*10, 13, 14*).

We have measured the volume of elongating embryonic lens epithelial cells and cells prevented from elongating with $10^{-6}M$ colchicine. Central lens epithelia from 6-day-old chick embryos (*15*) were cultured in 35-mm Falcon plastic tissue culture dishes as previously described (*10, 16*). Cell volume was determined after 0, 5, and 24 hours of culture by multiplying the mean cell length (*17*) of central epithelial cells by their mean area (*18*). Control and colchicine-treated lens epithelial cells had a similar mean cell area at 0, 5, and 24 hours of culture (*18*). Epithelial cells cultured in FCS without colchicine elongated from 10.0 ± 0.2 μm [mean ± standard error (S.E.)] at 0 hours to 19.1 ± 0.4 μm at 5 hours and 26.6 ± 0.6 μm after 24 hours. This resulted in a mean volume increase during elongation that was proportional to cell length, as shown in Fig. 1A. Colchicine ($10^{-6}M$) completely blocked the increase in cell length and the associated increase in cell volume (Fig. 1A).

We then tested the ability of other MT-depolymerizing drugs to inhibit lens epithelial cell elongation, hoping to find one that would permit discrimination between the effects of these agents on cytoplasmic MT's and their possible direct effect on cell volume. One of these drugs, nocodazole, a synthetic MT-depolymerizing agent that binds to the colchicine binding site of tubulin (*19*) and is active in vivo (*20*) and in vitro (*19*), did not prevent FCS-stimulated lens cell elongation at either 1 or 10 μg/ml, although it blocked mitosis at both concentrations (*21*). Figure 1B shows the extent of lens epithelial cell elongation in the presence or absence of nocodazole at 10 μg/ml. Electron microscopic examination of lens epithelial cells cultured for 5 to 24 hours showed that, in contrast to controls (Fig. 2A), nocodazole-treated elongated cells completely lacked cytoplasmic microtubules (Fig. 2B). Nocodazole-treated cells also contained prominent bundles of 10- to 12-nm filaments similar to those described in other cell types treated with anti-MT agents (*20, 22*). Except for these differences, drug-treated cells were similar in morphology to untreated controls (*23*).

Additional evidence suggesting that colchicine does not prevent lens cells elongation by disrupting the structure or function of MT's was obtained by examining the effect on lens cells of low concentrations of colchicine. Lens epithelial cell elongation was blocked at $10^{-8}M$ colchicine and partially inhibited at concentrations as low as $10^{-10}M$. Colchicine associates with chick brain tubulin with a binding constant of 1×10^6 to 2×10^6 liter/mole (*24*). Assuming that the binding constant for chick lens tubulin is similar to that for chick brain tubulin, only a small fraction of the tubulin molecules would be bound to colchicine at drug concentrations that block lens cell elon-

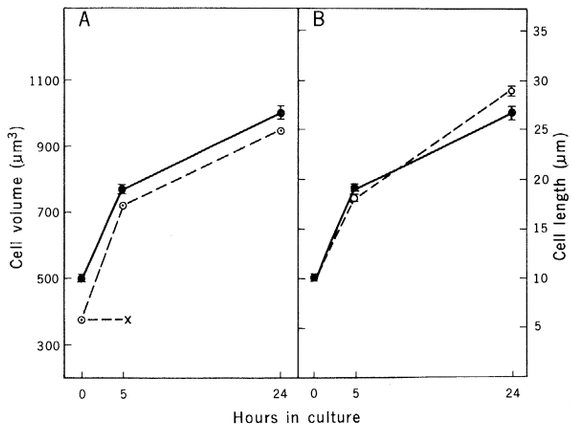

Fig. 1. (A) (●) Mean length (± S.E.) and (⊙) volume of 6-day-old embryonic chick lens epithelial cells cultured for 0, 5, or 24 hours in medium supplemented with FCS. (x) Average volume of epithelia cultured for 5 hours with FCS and $10^{-6}M$ colchicine. (B) Mean cell length of epithelia cultured as in (A) in the (○) presence or (●) absence of nocodazole (10 μg/ml).

0036-8075/79/1116-0836$00.50/0

gation. In addition, at a colchicine concentration of $10^{-8}M$, anaphase and telophase mitotic figures were seen in lens epithelial explants cultured for 5 hours in the presence of the drug. This indicates that at this colchicine concentration mitotic spindle microtubules were present and functioning. Finally, colchicine appeared to inhibit lens epithelial cell elongation in less than 1 hour after addition to the culture medium (*25*). However, the binding of colchicine to tubulin is usually a slow process, which may require 6 to 8 hours to reach equilibrium (*26*). Taken together, the low effective dose, the absence of mitotic arrest at inhibitory drug concentrations, and the rapid inhibitory action of the drug suggest that colchicine may not block lens cell elongation by binding to cytoplasmic tubulin.

Several studies have revealed the presence of microtubules in elongating cells of the lens placode (*6*), lens epithelium (*11*), and neural plate (*7, 27*), where they are most often oriented parallel to the direction of cell elongation. In all cases tested, elongation in these cells has been inhibited by colchicine and other MT-depolymerizing agents (*7, 11, 28*). These observations have led to the hypothesis that MT's may provide the motive force (*7, 11, 29*) or the cytoskeletal framework (*7*) necessary for epithelial cell elongation. This view was questioned in one case by Zwaan and Hendrix (*30*), who proposed that the elongation of the cells of the lens placode occurs by a localized crowding of cells as a result of continued cell division within an organ rudiment of restricted size. In this view, colchicine might block cell elongation in the lens placode by stopping mitosis.

We have found that, unlike the neural plate (*7, 31*) and lens placode cells (*30, 32*), lens epithelial cells stimulated to elongate in vitro increase in volume during elongation. We have further shown that this elongation may occur in the absence of cytoplasmic MT's and continued cell division. We therefore propose that at least one mechanism of cell elongation in the cultured lens epithelium is crowding of adjacent cells due to an increase in cell volume. Colchicine and the other anti-MT agents that prevent elongation might do so by disrupting the mechanism responsible for this volume increase. Preliminary estimates of lens epithelial and fiber cell volumes (*33*) suggest that lens fiber cells have volumes at least eight times greater than those of the epithelial cells from which they develop, suggesting that increase in cell volume may be an important element in lens fiber formation in the intact lens.

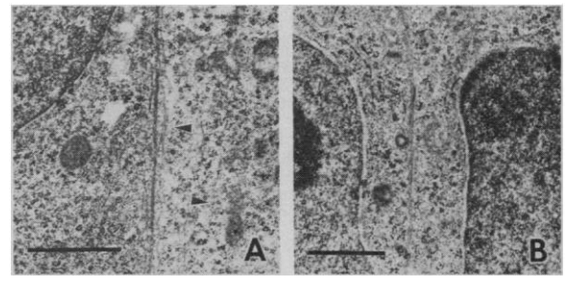

Fig. 2. Embryonic chick lens epithelia were cultured for 5 hours in medium supplemented with FCS in the (A) absence or (B) presence of nocodazole (10 μg/ml). (A) In untreated elongating lens epithelial cells, long MT's (arrowheads) are aligned parallel with the lateral plasma membranes of adjacent cells as well as throughout the cytoplasm. (B) In drug-treated cells, MT's are not seen. Epithelia were fixed for 1 hour at room temperature in 0.05*M* Pipes buffer (Sigma Chemical Company), *p*H 6.35, 2.5 percent glutaraldehyde, 2 m*M* EGTA, 1 m*M* guanosine 5'-triphosphate, 1 m*M* $MgSO_4$ (388 mOsm) in order to increase visualization of microtubules (*47*). They were postfixed in 1 percent OsO_4 in 0.15*M* sodium cacodylate, *p*H 7.4, for 1 hour at 4°C, washed, dehydrated in ethanol, embedded in Epon, and sectioned for electron microscopy. Sections were stained with uranyl acetate and lead citrate. Scale bar, 1 μm.

The plasma membrane is a likely site for the inhibitory action of colchicine on increases in cell volume. Colchicine has been shown to bind to membrane components (*34*), at least one of which is likely to be membrane tubulin (*35*), and to inhibit nucleoside transport through the plasma membrane (*36*). Lumicolchicine, a photoderivative of colchicine, inhibits nucleoside transport (*36*) but does not lead to MT depolymerization (*37*) or bind appreciably to tubulin (*37*). We therefore cultured lens epithelial cells in medium containing FCS and lumicolchicine (*38*) to determine if colchicine-sensitive volume regulation occurs by a mechanism similar to the one involved in inhibition of nucleoside transport. Under these conditions lumicolchicine did not inhibit embryonic lens epithelial cell elongation (*39*).

Microtubules have been implicated in biological processes if those processes are inhibited by colchicine and other anti-MT agents but not by lumicolchicine (*9*, pp. 169, 300, 321; *40*). In the experiments described above these criteria are fulfilled, yet our experiments have shown that MT's are not essential for and probably not involved in lens cell elongation. These results show that anti-MT drugs may act by mechanisms other than those already identified, and this possibility should be considered when interpreting data on their site of action.

We have presented evidence for an effect of colchicine that appears to be distinct from its ability to depolymerize cytoplasmic MT's and to inhibit nucleoside transport. This effect seems to be the inhibition of an activity, presumably associated with the plasma membrane, that is responsible for fluid transport (*41*) into the lens epithelial cells during cell differentiation in vitro. It has been shown that colchicine and vinblastine inhibit vasopressin-induced water transport in the toad urinary bladder (*42, 43*) and water transport in the rat intestine (*44*) and that colchicine blocks estrogen-stimulated fluid uptake in the rat uterus (*45*). Lumicolchicine does not block hormone-stimulated fluid uptake in either toad bladder (*42, 43*) or rat uterus (*45*). Although these effects have been tentatively associated with the depolymerization of MT's, they may be more closely related to the action of colchicine we have described above (*46*).

DAVID C. BEEBE
DOUGLAS E. FEAGANS
E. JOAN BLANCHETTE-MACKIE
MARTIN E. NAU
Department of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014

References and Notes

1. L. I. Rebhun, *Int. Rev. Cytol.* **32**, 93 (1972); P. Weiss and A. C. Taylor, *Proc. Soc. Exp. Biol. Med.* **55**, 77 (1944); D. L. Price and J. W. Griffin, in *Amyotropic Lateral Sclerosis*, J. M. Andrews, R. T. Johnson, M. A. B. Brazier, Eds. (Academic Press, New York, 1976), pp. 1-32; M. Schliwa, in *Microtubules and Microtubule Inhibitors*, M. Borgers and M. DeBrabander, Eds. (North-Holland, Amsterdam, 1975), pp. 215-218.
2. G. M. Edelman, J. L. Wang, I. Yahara, in *Cell Motility*, R. Goldman, T. Pollard, J. Rosenbaum, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1976); I. Yahara and G. M. Edelman, *Ann. N.Y. Acad. Sci.* **253**, 455 (1975); R. D. Berlin, *ibid.*, p. 445; J. M. Oliver, *J. Reticuloendothel. Soc.* **19**, 389 (1976).
3. D. W. Fawcett and F. Witebsky, *Z. Zellforsch. Mikrosk. Anat.* **62**, 785 (1964); O. Behnke, *Int. Rev. Exp. Pathol.* **9**, 1 (1970); D. W. Fawcett, W. A. Anderson, D. M. Phillips, *Dev. Biol.* **26**, 220 (1971); J. R. McIntosh and K. R. Porter, *J. Cell Biol.* **35**, 153 (1967).
4. W. S. Sale and P. Satir, *J. Cell Biol.* **71**, 589 (1976); P. Satir, in *Cilia and Flagella*, M. A. Sleigh, Ed. (Academic Press, London, 1974), pp. 131-142.
5. S. Inoue, in *Cell Motility*, R. Goldman, T. Pollard, J. Rosenbaum, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1976), pp. 1317-1328; J. R. McIntosh, Z. Cande, J. Snyder, K. Vanderslice, *Ann. N.Y. Acad. Sci.* **253**, 407 (1975).
6. B. Byers and K. R. Porter, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 1091 (1964).
7. B. Burnside, *Dev. Biol.* **26**, 416 (1971); *Am. Zool.* **13**, 989 (1973).

8. J. Piatigorsky, *Ann. N.Y. Acad. Sci.* **253**, 333 (1975).
9. P. Dustin, *Microtubules* (Springer-Verlag, New York, 1978), p. 168.
10. J. Piatigorsky, D. C. Beebe, P. Zelenka, L. M. Milstone, T. Shinohara, *Colloq. Inst. Natl. Sante Rech. Med.* **60**, 85 (1976).
11. J. Piatigorsky, H. de F. Webster, M. Wollberg, *J. Cell Biol.* **55**, 82 (1972).
12. Unpublished results from our laboratory.
13. D. C. Beebe and J. Piatigorsky, *Dev. Biol.* **59**, 174 (1977).
14. L. M. Milstone and J. Piatigorsky, *ibid.* **43**, 91 (1975); L. M. Milstone, P. Zelenka, J. Piatigorsky, *ibid.* **48**, 197 (1976).
15. Obtained from Truslow Farms, Chestertown, Md.
16. Lens epithelia were cultured as described in (*10*) except that the explants were oriented with the lens capsule upward and the epithelial cell monolayer positioned between the capsule and the surface of the culture dish.
17. Cell length was determined on living epithelia by focusing on the apical and basal cell surface and measuring the distance between these focal planes. Measurements were made with a micrometer attached to the stage of a Zeiss inverted microscope equipped with Nomarski interference contrast optics (Baltimore Instrument Co.). Measurements were made at the center and at four equally spaced points 125 μm from the center of each epithelial explant. Cell lengths were confirmed by direct measurement with an ocular micrometer after the explants were fixed, embedded in glycol methacrylate (JB-4 embedding kit, Polysciences) and sectioned at 2 μm with a Dupont Sorvall JB-4 microtome.
18. Mean cell area was determined on fixed epithelia after staining with Gill's hematoxylin 2 (Polysciences). By use of an ocular reticule, a 2768-μm^2 area was superimposed on the cells at the center of the epithelia and the number of nuclei lying within this area was counted. Mean cell area was taken as 2768 μm^2 divided by the number of nuclei. Average cell areas for control epithelia were: zero time, 37.2 $\pm$ 0.7 μm^2; 5 hours, 37.6 $\pm$ 0.9 μm^2; and 24 hours, 35.7 $\pm$ 0.9 μm^2 (mean $\pm$ S.E.).
19. J. Hoebeke, G. Van Nijen, M. DeBrabander, *Biochem. Biophys. Res. Commun.* **69**, 319 (1976).
20. M. DeBrabander, R. M. I. Van de Veire, P. Aerts, M. Borgers, P. Janssen, *Cancer Res.* **36**, 905 (1976); M. DeBrabander and J. C. Wanson, *J. Cell Biol.* **70**, 87a (1976); M. DeBrabander and M. Borgers, *J. Cell Sci.* **19**, 231 (1975).
21. Unpublished observations from our laboratory.
22. H. Holtzer, S. Fellini, N. Rubenstein, J. Chi, K. Strahs, in *Cell Motility*, R. Goldman, T. Pollard, J. Rosenbaum, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1976), pp. 823–840.
23. Details of the ultrastructure of nocodazole-treated and control lens epithelia will be presented elsewhere.
24. L. Wilson and J. Bryan, in *Advances in Cell and Molecular Biology*, E. J. DuPraw, Ed. (Academic Press, New York, 1974), pp. 21–72.
25. See (*8, 11*) for data suggesting that chick lens epithelia elongating in FCS-supplemented medium stop elongating soon after treatment with colchicine and do not shorten after continued culture in the drug. In our experiments, the average cell length of lens epithelia cultured in $10^{-6}M$ colchicine for 5 hours was 10.4 μm. Since epithelial cells that were 10.0 μm long at the time of explantation averaged 13.7 μm after culture for 1 hour in FCS-supplemented medium without added colchicine, it is evident that the drug acts within the first hour after explantation.
26. L. Wilson, J. R. Bamburg, S. B. Mizel, L. M. Grisham, K. M. Creswell, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 158 (1974).
27. P. Karfunkel, *Dev. Biol.* **25**, 30 (1971); *J. Exp. Zool.* **181**, 289 (1972).
28. R. Pearce and J. Zwaan, *J. Embryol. Exp. Morphol.* **23**, 491 (1970); J. Zwaan, personal communication.
29. R. H. Warren and B. Burnside, *J. Cell Biol.* **78**, 247 (1978).
30. J. Zwaan and R. W. Hendrix, *Am. Zool.* **13**, 1039 (1973).
31. M. B. Burnside and A. G. Jacobson, *Dev. Biol.* **18**, 537 (1968).
32. M. S. McKeehan, *J. Exp. Zool.* **117**, 31 (1951).
33. Unpublished observations from our laboratory.
34. L. Wilson, *Biochemistry* **9**, 499 (1970); B. Bhattacharyya and J. Wolff, *J. Biol. Chem.* **250**, 7639 (1975).
35. B. Bhattacharyya and J. Wolff, *Nature (London)* **264**, 576 (1976).
36. S. B. Mizel and L. Wilson, *Biochemistry* **11**, 2473 (1972).
37. L. Wilson and M. Friedkin, *ibid.* **6**, 3126 (1967).
38. Lumicolchicine was prepared by ultraviolet irradiation of solutions of colchicine ($4 \times 10^{-5}M$) in ethanol. Irradiation continued until the absorbance of colchicine at 350 nm decreased to 20 half-values of the original absorbance.
39. Epithelia were 19.9 $\pm$ 0.9 μm long after 5 hours in 15 percent FCS and 20.6 $\pm$ 0.7 μm long in the presence of $10^{-5}M$ lumicolchicine. Epithelia cultured in 15 percent FCS with $10^{-5}M$ colchicine were 10.9 $\pm$ 0.2 μm long after 5 hours.
40. G. M. Edelman, I. Yahara, J. L. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 442 (1976); R. D. Berlin, in (*2*).
41. Volume expansion during lens cell elongation is assumed to be due to net fluid transport, since elongation can occur in the absence of protein synthesis. However we do not wish to imply that a direct increase in water permeability is necessarily the cause of this volume increase.
42. A. Taylor, R. Maffly, L. Wilson, E. Reaven, *Ann. N.Y. Acad. Sci.* **253**, 723 (1975).
43. A. Taylor, M. Mamelak, H. Golbetz, R. Maffley, *J. Membr. Biol.* **40**, 213 (1978); W. A. Katchadorian, *Kidney Int.* **10**, 587 (1976).
44. D. Rachmilewitz, R. Fogel, F. Karmeli, *Gastroenterology* **74**, 1981 (1978); E. Beubler, F. Lembeck, M. Sweditsch, *Naunyn Schmiedebergs Arch. Pharmacol.* **301**, 195 (1978).
45. G. I. Fujiimoto and G. A. Morrill, *Biochim. Biophys. Acta* **538**, 226 (1978).
46. Cytochalasin B, a drug thought to inhibit the function of actin-containing microfilaments, blocks vasopressin-stimulated water transport in the toad bladder (*42*) and also inhibits serum-stimulated embryonic lens epithelial cell elongation in vitro (J. Piatigorsky, personal communication). This is another instance of parallel drug effects in these two tissues and may indicate a similar mechanism of volume regulation.
47. R. B. Luftig, P. N. McMillan, J. A. Weatherbee, R. R. Weihing, *J. Histochem. Cytochem.* **25**, 175 (1977).
48. We thank J. Piatigorsky for helpful comments, L. Wilson for advice in preparing lumicolchicine, and B. Lachey for assistance in preparing the manuscript.

8 March 1979; revised 13 April 1979

Triglyceride Concentrations: The Disaccharide Effect

Abstract. *The mean 24-hour or integrated concentration of triglyceride is significantly higher when dietary sucrose is provided rather than an equivalent amount of its component monosaccharides, glucose and fructose. In contrast, the plasma triglyceride concentration after a 12-hour fast is not significantly different.*

Plasma concentrations of triglyceride are influenced by a variety of factors, including the amount and composition of dietary carbohydrate. Sucrose has repeatedly been reported to result in elevated concentrations of triglyceride in humans and animals (*1*), an effect usually ascribed to the fructose component of sucrose. Michaelis and co-workers (*2*) found that lipogenic enzymes in rat liver were induced to a greater extent when the animals were fed sucrose than when they were fed equivalent amounts of glucose and fructose, the monosaccharide components of the disaccharide sucrose. This phenomenon has been termed the disaccharide effect. The metabolic scope of the disaccharide effect has been expanded to include differences in serum triglyceride and free fatty-acid concentrations after fasting (*3*); differences in serum insulin concentrations, food efficiency, and relative fat pad size (*4*); and differences in hepatic microsomal enzyme activities (*5*). Documentation of the disaccharide effect has heretofore been limited to studies with various strains of rats (*2–5*). This report indicates that when normal human subjects consume diets containing sucrose, the mean 24-hour (or integrated) triglyceride concentrations are significantly higher than when the subjects are fed diets that are identical except that fructose and glucose are provided as monosaccharides.

The design, sample collection, assay methods, and statistical evaluation used in this study are analogous to those described in detail earlier (*6*). Significant features include the use of a diet sequence that results in all subjects ingesting both test diets in a balanced crossover design, and continuous collection of blood over an entire 24-hour period as a series of 48 half-hour integrated collections. The withdrawal system allowed normal activity and consumption of the test diets while the blood samples were collected. Eight normal males aged 24 to 27 were studied on the tenth day of ingestion of the test diets. The study protocol was approved by the University Committee on Research Involving Human Beings. Informed consent was ob-

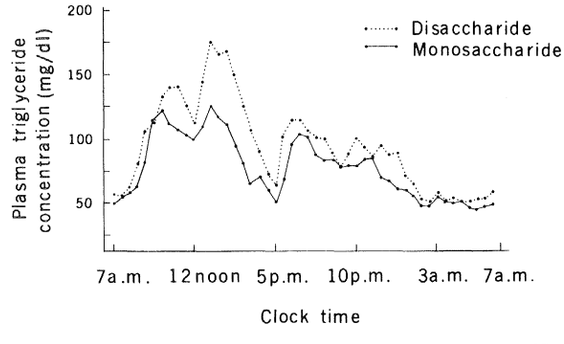

Fig. 1. Mean diurnal triglyceride patterns for two diets that varied in the form of fructose. The disaccharide diet (sucrose) and the monosaccharide diet (glucose and fructose) each provided 11.3 percent of total calories as fructose.

0036-8075/79/1116-0838$00.50/0