Biochemical Pharmacology, Vol. 34, No. 17, pp. 3153–3159, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECTS OF ANTIMICROTUBULE AGENTS ON PHOSPHOLIPID METABOLISM IN RAT HEPATIC SUBCELLULAR MEMBRANES*

SALMAN AZHAR†, SHIAW-FEN HWANG and EVE REAVEN

Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305; Geriatric Research, Education and Clinical Center, Veterans Administration Medical Center, Palo Alto, CA 94304, U.S.A.

(*Received* 13 *November* 1984; *accepted* 7 *February* 1985)

Abstract—Treatment of animals with antimicrotubule drugs has been shown to cause a perplexing variety of cellular changes which, theoretically, could be the result of changes in endomembrane biosynthesis, composition or flow. In the current study we have focused on this possibility by identifying antimicrotubule drug-induced changes in the phospholipid metabolism of hepatic subcellular membranes. Young adult rats were pretreated with radiolabeled [^{32}P]orthophosphate for 12 hr, and subsequently given saline, colchicine (2.5 mg/kg body wt) or vinblastine (20 mg/kg body wt) for 4 additional hr. Afterwards, the livers were homogenized, and separate microsomal and Golgi membrane fractions were prepared and subjected to phospholipid extraction and identification using two-dimensional thin-layer chromatography. The results show that colchicine and vinblastine given *in vivo* caused specific, rapid and in some cases, dramatic changes in phospholipid turnover in different membrane fractions of rat liver. The drugs specifically increased labeling of phosphatidylinositol-4-monophosphate and phosphatidylinositol-4,5-biphosphate and decreased the radioactivity associated with phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in all fractions examined. In contrast, the antimicrotubule drugs produced a differential effect on the labeling pattern of sphingomyelin and lysophosphatidylcholine, i.e. they stimulated labeling of these phospholipids in microsomes, produced no changes in heavy Golgi fractions, and markedly increased their labeling in light Golgi fractions. These data suggest that antimicrotubule drugs restrict the incorporation of certain precursor phospholipids into forming membranes but do not affect the subsequent metabolism of these phospholipids. At the same time, the drugs appear to retard the flow of membranes from one cellular compartment to another.

Over the years, evidence has accumulated showing that antimicrotubule drugs induce a variety of changes in cells which appear, at first, to have little to do with the cytoplasmic microtubules. Many of these changes are enzymatic in nature documenting either the decrease [1–8] or the increase [9–11] in activity of seemingly unrelated enzymes. In other instances, the antimicrotubule drugs have been shown to specifically affect cholesterol and phospholipid metabolism in cells. For example, colchicine and vinblastine sulfate appear to decrease cholesterol synthesis in different cells [6, 7, 12], to decrease phosphatidylinositol turnover in lymphocytes [13], and to inhibit phosphatidylcholine synthesis in phagocytic cells [14].

It is of interest that in each of the above the described alterations have involved a change in membranes of the affected cells. This observation, plus specific instances of structural/functional changes in cell membranes caused by the antimicrotubule drugs (such as changes in capping phenomena in lymphocytes [15]), or changes in the organization and distribution of Golgi membranes in a variety of cells [16–30], has led to the idea that the antimicrotubule drugs may induce changes in the biosynthesis, composition, or flow of membranes, which may then secondarily produce the various cellular secretory changes that have been so well documented [31] in recent years. Whether microtubules themselves or some other form of microtubule protein might be involved in such a drug–membrane interaction is a separate issue to be dealt with if a cause and effect association between the drugs and membranes is firmly established.

In the current study, we have focused on this issue by examining the effects of two separate antimicrotubule agents on the metabolism of various phospholipids in subcellular membrane fractions of rat liver. The decision to look for changes in phospholipids was based on previous data indicating that antimicrotubule drugs have an effect on phospholipid turnover and synthesis in some cell systems [13, 14]. The decision to look for changes in specific membrane fractions (rather than in total liver homogenate) was influenced by previous studies from this laboratory [8] showing that antimicrotubule drugs have specific effects on different subcellular compartments of liver which are not seen when the whole homogenate is examined. The specific choice of studying microsomal and Golgi membrane fractions was a reflection of the fact that phospholipids are known to be synthesized in endoplasmic reticulum [32] or Golgi [32, 33] membranes and that these membranes have been shown to undergo specific

* This research was supported by the Research Service of the Veterans Administration.

† Address correspondence and reprint requests to: Salman Azhar, Ph.D., GRECC (640/182B), VA Medical Center, 3801 Miranda Ave., Palo Alto, CA 94304.

functional changes (such as loss of glycosyltransferase activity [8]) and structural changes [23, 24, 30] following *in vivo* treatment of rats with either colchicine or vinblastine. Finally, microsomal and Golgi membranes are the primary source of the limiting membranes of secretory vesicles in hepatocytes, whose specific transit to the plasma membranes is inhibited by the use of antimicrotubule drugs [30, 34, 35].

EXPERIMENTAL PROCEDURES

Methods

In vivo *labeling experiments*. Male Sprague–Dawley rats (140–160 g) were purchased from Simenson Laboratories, Gilroy, CA, fed standard laboratory chow and water *ad lib.*, and kept for at least 48 hr in a light-controlled room (12 hr cycle) prior to use. Animals were lightly anesthetized with diethyl ether, and a volume of 500 μl of carrier-free [^{32}P]orthophosphate (250 μCi) was given intravenously to each rat. Four hours later, the rats were again injected with carrier-free [^{32}P]orthophosphate (250 μCi); 12 hr after the last [^{32}P]orthophosphate injection, rats were treated with antimicrotubule drugs or injected with saline. All animals were killed 4 hr later (total time 16 hr) and the livers were removed for further processing. Livers from four to five animals were used for each experiment.

Samples from glutaraldehyde-perfusion-fixed livers were also obtained from rats treated with saline or colchicine as above and processed for electron microscopy. The hepatocyte content of visible microtubules was assessed as previously described from this laboratory [30].

Isolation of microsomal and Golgi fractions. Excised livers were placed in ice-cold 0.25 M sucrose, pH 7.0 ± 0.1, weighed and minced. The tissue was washed free of blood and then homogenized in 0.25 M sucrose (pH 7.0 ± 0.1) to give a final homogenate concentration of 20% (w/v). Although three Golgi fractions (GF_1, GF_2 and GF_3) were isolated by a modification [8] of the procedure of Ref. 36, for the current study, fractions GF_1 and GF_2 were found to show similar characteristics and for convenience these fractions were combined to give a single GF_{1+2} fraction. GF_3 was studied separately as were microsomes obtained from the load zone. All fractions were subsequently divided into "soluble (non-membrane) subfractions" and "membrane subfractions" by treatment with 0.1 M Na_2CO_3 as described by Howell and Palade [37]. The purity of the Golgi and microsome fractions was assessed by marker enzymes and electron microscopy as previously described [8].

Extraction and quantitation of ^{32}P-labeled phospholipids. All Golgi and microsomal fractions as well as the soluble and membrane subfractions were subjected to phospholipid extraction according to the combined procedures of Shukla *et al.* [38] and Billah and Lapetina [39]. Briefly, subcellular fractions (2.4 ml) were mixed with (i) 6 ml methanol-HCl (50:1); (ii) 6 ml chloroform; and (iii) 3 ml of 2 M KCl containing 5 mM EDTA and samples were vortexed intermittently for 15 min at room temperature. The suspension was briefly centrifuged to separate two phases. The upper phase was discarded and the lower phase plus interfacial material was washed successively with 0.25 ml of 1 N HCl and 3 ml of chloroform–methanol–1 N HCl (3:48:47, by vol.). The two phases were again separated, and the upper phase and interfacial material was discarded. The lower phase was washed three times with 2.5 ml of 50 mM $CaCl_2$–methanol–chloroform (47:48:3), and the chloroform phase was dried under N_2 and used for phospholipid analysis.

Phospholipid analysis. Phospholipids were separated by two-dimensional thin-layer chromatography on silica gel (Redi-Coat T-2D). Plates were activated for 1 hr at 110°. Development in the first dimension was with chloroform–methanol–ammonia (65:25:4, by vol.) and in the second dimension with chloroform–methanol–acetic acid–water (30:40:10:5, by vol.) Polyphosphoinositides were separated on silica gel H thin-layer plates impregnated with potassium oxalate [40] using chloroform–acetone–methanol–acetic acid–water (40:15:13:12:8, by vol.) as solvent system. For ^{32}P-quantitation, separated phospholipids were visualized with iodine vapor prior to scraping. Identification was achieved by comparison with authentic standards Radioactivity associated with phospholipid spots was determined in 5 ml Beta Max using a Beckman LS 9000 liquid scintillation spectrometer.

Analytical methods. Protein concentrations were determined by a modified procedure of Lowry *et al.* [41] as described by Markwell *et al.* [42]. For phosphorus estimation, the silica gel from the specific lipid areas was digested with 70% perchloric acid, and the resulting inorganic phosphate was measured by the method of Bartlett [43].

Chemicals and reagents. Carrier-free [^{32}P]orthophosphate in 0.02 N HCl was obtained from Amersham. Phosphatidylinositol-4-monophosphate and phosphatidylinositol-4,5-bisphosphate were purchased from the Sigma Chemical Co. Other phospholipid standards and Redi-Coat T-2D thin-layer plates were supplied by Supulco. Precoated silica gel plates were products of E. Merck. All other reagents used were of analytical grade.

RESULTS

Distribution of phospholipid-associated ^{32}P-radioactivity in cellular membrane fractions

The total amount of ^{32}P incorporated into phospholipids was assessed by measuring chloroform extractable radioactivity in samples of whole liver homogenate. All the phospholipids appeared to be labeled to near steady-state by 10–12 hr after the first [^{32}P]orthophosphate treatment. The results in Table 1 show the relative incorporation of [^{32}P]orthophosphate into the various microsomal and Golgi fractions at these steady-state levels. When the radioactivity is expressed per gram tissue, it appears that the various microsomal fractions incorporate thirty to forty times the [^{32}P]orthophosphate of the Golgi fractions. However, when corrected for protein content, Golgi fractions exhibit higher specific activity than do the microsomal fractions. In general, GF_{1+2} fractions incorporated more radioactivity than GF_3 fractions. Also, as previously shown by Howell and

Table 1. Distribution of phospholipid-associated ^{32}P-radioactivity in hepatic microsomal and Golgi fractions*

		^{32}P-Radioactivity		
		dpm × 10^{-3}/g tissue	%	dpm × 10^{-3}/mg protein
(A)	Microsomes			
	Total fraction	2116.0		130
	Membrane subfraction	1850.0	93	157
	Soluble subfraction	167.0	7	44
	Recovery (%)	(96)		
(B)	Golgi GF_3			
	Total fraction	48.0		199
	Membrane subfraction	40.2	92	255
	Soluble subfraction	3.6	8	58
	Recovery (%)	(91)		
(C)	Golgi GF_{1+2}			
	Total fraction	60.2		274
	Membrane subfraction	49.8	89	379
	Soluble subfraction	6.4	11	79
	Recovery (%)	(93)		

* Total, membrane and soluble subfractions from microsomes and Golgi fractions were extracted and quantitated for phospholipid-associated radioactivity as described under Experimental Procedures. The results are the mean of three separate experiments.

Palade [37], the major amount of radioactivity (89–93%) was associated with the membrane portion (membrane subfraction) of each sample. Given these findings, data in subsequent tables are restricted to membrane subfractions only and are corrected for the protein content of the sample.

Effects of antimicrotubule drugs on phospholipid-associated ^{32}P-radioactivity in membrane subfractions

In vivo treatment (4 hr) with colchicine (2.5 mg/kg body wt) and vinblastine (20 mg/kg body wt) did not affect total hepatic phospholipid labeling with [^{32}P]orthophosphate, i.e. under steady-state labeling conditions with [^{32}P]orthophosphate, phospholipids obtained from liver homogenate of control, colchicine-treated and vinblastine-treated animals showed 1.53, 1.486 and 1.61 dpm × 10^{-7} g tissue respectively. However, when phospholipids of microsomes or GF_{1+2} and GF_3 fractions were measured separately, it became clear that totally less ^{32}P-radioactivity was associated with phospholipids of the hepatic fractions from the drug-treated animals than from the control rats (Table 2). In general, the reduction in radioactivity of the fractions was small (25–35% of control values) but the reduction was consistently seen in all the membranes fractions isolated, regardless of which antimicrotubule drug was used.

Table 3 shows the effect of colchicine or vinblastine on [^{32}P]orthophosphate incorporation into individual phospholipids of the microsomal submembrane fractions. In control animals, the phospholipids most readily labeled by injecting [^{32}P] orthophosphate were phosphatidylcholine and phosphatidylethanolamine. Phosphatidylinositol-4-monophosphate, phosphatidylserine, phosphatidylinositol, lysophatidylcholine and sphingomyelins also incorporated appreciable amounts of radioactivity. Treatment of animals with colchicine or vinblastine caused a marked increase in the radioactivity of phosphatidylinositol-4-monophosphate, phosphatidylinositol-4,5-bisphosphate, sphingomyelins and lysophosphatidylcholine and a

Table 2. Distribution of phospholipid-associated ^{32}P-radioactivity in hepatic microsomal and Golgi membrane subfractions from control, colchicine- and vinblastine-treated animals*

		^{32}P-Radioactivity (dpm × 10^{-3}/mg membrane protein)		
		Control	Colchicine (2.5 mg/kg body wt)	Vinblastine (20 mg/kg body wt)
(A)	Microsomes	157	120	135
(B)	Golgi GF_3	255	195	216
(C)	Golgi GF_{1-2}	379	281	252

* Experimental details were the same as described under Table 1.

Table 3. Effects of antimicrotubule drugs on [^{32}]orthophosphate incorporation into phospholipids of hepatic microsomal fraction

Phospholipid class	^{32}P-Radioactivity (dpm × 10^{-3}/mg membrane protein)		
	Control	Colchicine†	Vinblastine†
Phosphatidylinositol-4-monophosphate	3.30 ± 0.40	9.80 ± 0.60	7.3
Phosphatidylinositol-4,5-bisphosphate	0.35 ± 0.03	0.96 ± 0.10	1.1
Phosphatidic acid	0.50 ± 0.04	0.50 ± 0.08	0.7
Phosphatidylserine	3.09 ± 0.20	2.70 ± 0.50	3.3
Phosphatidylinositol	7.60 ± 0.30	4.60 ± 0.40	6.2
Sphingomyelins	4.20 ± 0.50	8.10 ± 0.60	7.5
Lysophosphatidylcholine	3.50 ± 0.50	6.00 ± 0.50	7.0
Phosphatidylcholine	76.00 ± 3.20	46.90 ± 2.80	56.4
Phosphatidylethanolamine	24.00 ± 1.70	9.30 ± 0.70	13.6
Diphosphatidylglycerol	0.50 ± 0.06	0.40 ± 0.05	0.4

* Results from control and colchicine-treated animals represent mean (±S.E.M.) of four experiments; results from vinblastine-treated animals represent the mean of two experiments.

† Animals were treated for 4 hr with colchicine (2.5 mg/kg body wt) or vinblastine sulfate (20 mg/kg body wt).

decrease in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. Antimicrotubule agents produced no significant changes in phosphatidic acid, phosphatidylserine or diphosphatidylglycerol.

In GF_3 fractions (Table 4), the most radioactive phospholipid was again phosphatidylcholine followed by phosphatidylethanolamine. However, in this fraction, sphingomyelins were six times more heavily labeled than it was in the microsomal fraction (see Table 3). Considerable radioactivity was also observed in other phospholipids, though phosphatidylinositol-4,5-bisphosphate was less labeled than in the microsomal fraction. Treatment of animals with colchicine or vinblastine caused an increase in labeling of phosphatidylinositol-4-monophosphate, phosphatidylinositol-4,5-bisphosphate and a decrease in the labeling of phosphatidylcholine and phosphatidylethanolamine. No changes occurred in the radioactivity of any other phospholipids including lysophatidylcholine or sphingomyelins.

Results presented in Table 5 show the labeling pattern of phospholipids in GF_{1+2} fractions from control, colchicine- and vinblastine-treated animals. Again, the phospholipid most readily labeled by *in vivo* [^{32}P]orthophosphate administration was phosphatidylcholine. Phosphatidylethanolamine and sphingomyelins also exhibited considerable radioactivity. The labeling pattern of phosphatidylserine, phosphatidylinositol, lysophosphatidycholine and phosphatidylinositol-4-monophosphate was similar to that of the GF_3 fraction. As before, administration of antimicrotubule agents resulted in a significant increase in labeling of phosphatidylinositol-4-monophosphate and phosphatidylinositol-4,5-bisphosphate, and a decrease in radioactivity associated with phosphatidylcholine and phosphatidylethanolamine. In contrast to the results in the other fractions, both colchicine and vinblastine caused an

Table 4. Effects of antimicrotubule drugs on [^{32}P]orthophosphate incorporation into phospholipids of hepatic Golgi GF_3 fraction

Phospholipid class	^{32}P-Radioactivity* (dpm × 10^{-3}/mg membrane protein)		
	Control	Colchicine	Vinblastine
Phosphatidylinositol-4-monophosphate	3.80 ± 0.60	9.3 ± 1.30	6.00
Phosphatidylinositol-4,5-bisphosphate	0.15 ± 0.04	0.5 ± 0.06	0.50
Phosphatidic acid	0.40 ± 0.04	0.5 ± 0.06	0.60
Phosphatidylserine	7.40 ± 0.40	6.9 ± 0.60	6.00
Phosphatidylinositol	8.40 ± 0.30	6.9 ± 0.10	6.10
Sphingomyelins	25.80 ± 3.10	25.1 ± 2.40	25.40
Lysophosphatidylcholine	7.80 ± 1.40	7.3 ± 0.60	7.90
Phosphatidylcholine	95.00 ± 5.20	66.2 ± 6.30	72.50
Phosphatidylethanolamine	30.00 ± 1.70	21.7 ± 2.30	22.50
Diphosphatidylglycerol	0.50 ± 0.03	0.3 ± 0.03	0.34

* Experimental details are as in Table 3.

Table 5. Effects of antimicrotubule agents on [^{32}P]orthophosphate incorporation into phospholipids of hepatic Golgi (GF_{1+2}) fraction

Phospholipid class	^{32}P-Radioactivity* (dpm × 10^{-3}/mg membrane protein)		
	Control	Colchicine	Vinblastine
Phosphatidylinositol-4-monophosphate	9.90 ± 0.70	19.50 ± 2.30	12.20
Phosphatidylinositol-4,5-bisphosphate	0.21 ± 0.04	0.48 ± 0.05	0.35
Phosphatidic acid	0.27 ± 0.03	0.28 ± 0.04	0.34
Phosphatidylserine	7.90 ± 0.30	5.90 ± 0.90	5.30
Phosphatidylinositol	11.10 ± 0.80	8.90 ± 0.80	8.30
Sphingomyelins	37.70 ± 0.70	22.80 ± 1.50	22.60
Lysophosphatidylcholine	15.90 ± 1.30	11.30 ± 1.10	11.70
Phosphatidylcholine	163.00 ± 6.40	121.00 ± 2.40	103.00
Phosphatidylethanolamine	43.00 ± 1.60	28.50 ± 8.10	25.30
Diphosphatidylglycerol	0.50 ± 0.06	0.50 ± 0.04	0.60

* Experimental details are as in Table 3.

actual decrease in two additional phospholipids (sphingomyelins and lysophosphatidylcholine) in the GF_{1+2} fractions.

Table 6 summarizes the various effects of the antimicrotubule drugs on the labeling pattern of phospholipids in the various fractions studied; for simplicity, only the effects of colchicine are illustrated. Colchicine treatment specifically increased labeling of phosphatidylinositol-4-monophosphate and phosphatidylinositol-4,5-bisphosphate and decreased the radioactivity associated with phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol in all fractions examined. In contrast, colchicine produced a differential effect on the labeling pattern of sphingomyelins and lysophosphatidylcholine in Golgi (GF_{1+2}), Golgi GF_3 and microsomal membrane subfractions, i.e. it stimulated ^{32}P-labeling in microsomes, produced no changes in GF_3, and markedly decreased radioactivity in GF_{1+2} fractions. The turnover of other phospholipids was not affected significantly by colchicine.

As a corollary to these observations, it should be noted that essentially no microtubules were visible in hepatocytes of the colchicine-treated animals although the microtubule content of hepatocytes from control animals was similar to that found previously (0.03% of cytoplasm) in our laboratory [30].

DISCUSSION

The present study indicates that treatment with colchicine and vinblastine sulfate *in vivo* caused specific, rapid and, in some cases, dramatic changes in phospholipid turnover in different fractions of rat liver. Several points are of interest. First, the phospholipid changes observed in the microsomal and/or Golgi fractions (of antimicrotubule drug-treated rats) were not apparent when total liver homogenate of the same animals was examined. This suggests that the drug-induced phospholipid changes were present in only certain membranes (possibly those that represented only a small percentage of total hepatic membranes) and that the effects were diluted beyond the point of recognition when the phospholipids of whole cells were measured.

Second, the results show that, within any given cell fraction, it was primarily the membranes themselves, and not the soluble content of the membrane vesicles, which showed the drug-induced changes. This is recognized when one realizes that the soluble

Table 6. Summary of colchicine effect on [^{32}P]orthophosphate labeling of phospholipids in hepatic membranes*

Phospholipid class	% of Control		
	Microsomes	Golgi GF_3	Golgi GF_{1+2}
Phosphatidylinositol-4-monophosphate	274	333	229
Phosphatidylinositol-4,5-bisphosphate	297	245	197
Lysophosphatidylcholine	171	92	71
Sphingomyelins	193	100	60
Phosphatidylinositol	61	82	80
Phosphatidylcholine	62	69	74
Phosphatidylethanolamine	39	73	67

* Data were computed from Tables 3 to 5.

portions of the fractions represented 7–11% of the total fraction phospholipids (Table 1) [37], while in some instances (i.e. in phosphoinositide metabolism) the observed changes in specific phospholipids represented two to three times control levels (Table 6).

The third point of interest is that antimicrotubule drugs produced a general decline in membrane-associated phospholipid radioactivity in all the fractions measured, whereas individual phospholipids within that group showed strikingly different patterns. For example, the polyphosphoinositides were equally increased in all three subcellular fractions, while phosphatidylinositol itself was decreased in the same three fractions; this occurred while the accumulation of still another phospholipid, lysophosphatidylcholine, increased in one fraction (microsomes) and decreased in another (Golgi GF_{1+2}).

Although these measurements tell us that these antimicrotubule drugs induced a variety of changes in membrane phospholipids, they do not explain the mechanism by which these changes occur. We do not yet know whether the drugs directly affected the membranes of a given region of the cells (altering biosynthesis or degradation of membrane phospholipids) or whether the drugs altered the movement of membranes from one region of the cell to another. Indeed, in the current experiments, there are suggestions that both events occurred. For example, Table 6 indicates that lysophosphatidylcholine showed increased labeling in microsomes, identical labeling in Golgi GF_3 fractions, but diminished labeling in GF_{1+2} fractions, respectively, as compared to similar fractions from control animals. Insofar as biosynthesis of membranes containing lysophosphatidylcholine is thought to occur in the endoplasmic reticulum [32], increased or decreased biosynthesis (associated with normal membrane movement) probably would have resulted in similar levels of increased or decreased labeling in all the examined fractions. The fact that the increased labeling occurred only in the first compartment (representing the endoplasmic reticulum) and declined stepwise in fractions representing cell compartments into which the endoplasmic reticulum membranes normally flow [44] suggests that the membranes of the drug-treated rats did not move normally into the secondary compartments. The fact that labeling of phosphatidylcholine (the parent compound of lysophatidylcholine) was itself decreased in the microsomal fraction suggests that phosphatidylcholine continued to be converted to the lysophosphatidylcholine form (the movement of which was inhibited) but that the synthesis of phosphatidylcholine itself was probably diminished (or its degradation enhanced) by the use of antimicrotubule drugs. In general, the data with all the various phospholipids measured in this report are consistent with the view that antimicrotubule agents restrict the incorporation of crucial precursor phospholipids into forming membranes, but that once the phospholipids are formed their conversion to other metabolic forms is normal. At the same time, the drugs appear to retard the flow of certain membrane-associated phospholipids from one cellular compartment to another.

Finally, there is the issue of whether the antimicrotubule drugs produce these multiple changes in membrane phospholipids through direct action on the membranes or through some secondary action of microtubule protein. Although we cannot answer this question, we are mindful of the fact that in this study two structurally different antimicrotubule drugs were used (which have in common only their abilities to bind to microtubule protein [45]) and, in every respect, both drugs produced precisely the same membrane phospholipid changes. Whether these results have anything to do with the fact that microtubule proteins, under *in vitro* conditions, bind avidly to certain phospholipids in hepatic microsomal and Golgi membranes [46] remains to be seen.

REFERENCES

1. J. Myren, G. C. Luketic, R. Ceballos, G. Sachs and B. I. Hirschowitz, *Am. J. dig. Dis.* **11**, 394 (1966).
2. M. I. Cohen and H. McNamara, *Am. J. dig. Dis.* **15**, 247 (1970).
3. J. J. Herbst, R. Hurwitz, P. Sunshine and N. J. Kretchmer, *J. clin. Invest.* **49**, 530 (1970).
4. T. F. Race, I. C. Paes and W. W. Faloon, *Am. J. med. Sci.* **259**, 32 (1970).
5. T. Chajek, O. Stein and Y. Stein, *Biochim. biophys. Acta* **380**, 127 (1975).
6. J. M. Ottery, *Fedn Eur. Biochim. Soc. Lett.* **64**, 346 (1976).
7. J. J. Volpe, *J. biol. Chem.* **254**, 2568 (1979).
8. S. Azhar, S. F. Hwang and E. P. Reaven, *Biochem. J.* **212**, 721 (1983).
9. O. R. Affonso, E. Mitidieri and G. G. Villela, *Nature, Lond.* **193**, 64 (1962).
10. S. Mookerjea, J. W. Marshall, J. M. Collins and S. Ratnam, *Biochem. biophys. Res. Commun.* **78**, 309 (1977).
11. K. Oda and Y. Ikehara, *Biochim. biophys. Acta* **640**, 398 (1981).
12. S. Chen and A. Y. Kou, *Biochem. biophys. Res. Commun.* **97**, 1140 (1980).
13. R. R. Schellenberg and E. Gillespie *Biochim. biophys. Acta* **619**, 522 (1980).
14. M. C. Pike, N. M. Kredich and R. Synderman, *Cell* **20**, 373 (1980).
15. J. M. Oliver and R. B. Zurier, *J. clin. Invest.* **57**, 1239 (1976).
16. P. Neve, C. Williams and J. E. Dumont, *Expl Cell Res.* **63**, 457 (1970).
17. G. Rossman, P. Dukor and R. B. Zurier, *Nature New Biol.* **231**, 131 (1971).
18. S. Moskalewski, H. Thyberg, S. Lohmander and U. Friberg, *Cell Res.* **95**, 440 (1975).
19. J. Seybold, W. Bieger and H. G. Kern, *Virchows Arch path. Anat. Histol.* **368**, 309 (1975).
20. C. Hindelang-Gertner, M. Stoeckel, A. Porte and F. Stutinsky, *Cell Tissue Res.* **170**, 11 (1976).
21. S. Moskalewski, S. J. Thyberg and J. Friberg, *J. Ultrastruct. Res.* **54**, 304 (1976).
22. S. Hoffstein, I. M. Goldstein and G. Weissman, *J. Cell Biol.* **73**, 242 (1977).
23. C. Patzelt, D. Brown and B. Jeanrenaud, *J. Cell Biol.* **73**, 578 (1977).
24. E. P. Reaven and G. M. Reaven, *J. Cell Biol.* **75**, 559 (1977).
25. M. DeBrabander, J. Wander, T. Mosselmans, G. Geuens and P. Drohmans, *Biol. Cell.* **31**, 127 (1978).
26. C. M. Knudson, B. H. Stemberger and S. Patton, *Cell Tissue Res.* **195**, 169 (1978).

27. R. B. Kelly, C. Oliver and H. R. Huad, *Cell Tissue Res.* **195**, 227 (1978).
28. C. Stock, J. F. Launay, J. F. Grenier and J. Bauduin, *Lab. Invest.* **38**, 157 (1978).
29. J. Thyberg, S. Moskalewski and U. Friberg, *Cell Tissue Res.* **193**, 247 (1978).
30. E. Reaven and G. Reaven, *J. Cell Biol.* **84**, 28 (1980).
31. P. Dustin, *Microtubules*, p. 284. Springer, Berlin (1978).
32. R. M. Bell and R. A. Coleman, *A. Rev. Biochem.* **49**, 459 (1980).
33. B. Jergil and R. Sundler, *J. biol. Chem.* **258**, 7968 (1983).
34. C. M. Redman, D. Banerjee, K. Howell and G. E. Palade, *J. Cell Biol.* **66**, 42 (1975).
35. S. Busson-Mabillot, A-M. Chambaut-Guerin, L. Ovtracht, P. Muller and B. Rossignol, *J. Cell Biol.* **95**, 105 (1982).
36. J. H. Ehrenreich, J. J. M. Bergeron, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **59**, 45 (1973).
37. K. E. Howell and G. E. Palade, *J. Cell. Biol.* **92**, 822 (1982).
38. S. D. Shukla, R. Coleman, J. B. Finean and R. H. Michell, *Biochem. J.* **179**, 441 (1979).
39. M. M. Billah and E. G. Lapetina, *J. biol. Chem.* **257**, 12705 (1982).
40. J. Jolles, H. Zwiers, A. Dekker, K. W. A. Wirtz and W. H. Gispen, *Biochem. J.* **194**, 283 (1981).
41. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
42. M. A. K. Markwell, S. M. Hass, L. L. Bieber and N. E. Tolbert, *Analyt. Biochem.* **87**, 206 (1978).
43. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
44. M. G. Farquhar and G. E. Palade, *J. Cell Biol.* **91**, 77s (1981).
45. L. Wilson, *Ann. N.Y. Acad. Sci.* **253**, 213 (1975).
46. E. Reaven and S. Azhar, *J. Cell Biol.* **39**, 300 (1981).