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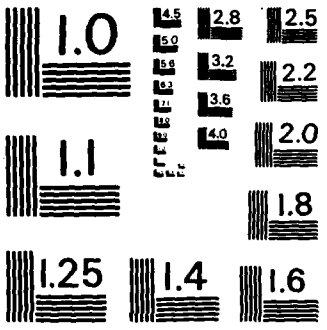

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The principal objective of the research proposed was to define the subcellular site(s) or target(s) of action of the aromatic hydrocarbon toluene. Confirmed target sites were then to be investigated in detail to elucidate possible mechanisms of toluene action in pertubating membrane structure that might be related to either an enhancement or loss in membrane function Under this problem, several test systems developed in our laboratory for toxicological evaluation of target sites of membrane active substances were employed. The basic approach was to subject each tisssue to a graded series of toluene concentrations for varying periods of time after which the material was prepared for electron microscopy under conditions develop to yield accurate and reproducible evaluations. Comparisons were to identical tissues treated in a similiar fashion in the absence of toluene. Gross and subtle morphological changes were noted indicative of an activity target using the following three test systems:

Test System 1. Primary Rat Hepatocytes in Culture,
Test System 3. Cultured BHK, KB and L Cells,
Test System 4. Outer Cap Cells of the Maize Root Tip.

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EARLY PHASE INTERACTIONS OF TOLUENE WITH MEMBRANES; A STRUCTURAL AND FUNCTIONAL EVALUATION

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Objectives

The principal objective of the research proposed was to define the subcellular site(s) or target(s) of action of the aromatic hydrocarbon toluene. Confirmed target sites were then to be investigated in detail to elucidate possible mechanisms of toluene action in pertubating membrane structure that might be related to either an enhancement or loss in membrane function.

Approach

The approach to the conduct of this investigation was organized under three problems to be distributed over approximately four years. Work was to have proceeded sequentially beginning with target identification under Problem I in the first year, proceeding to target verification under Problem II and finally to move to basic mechanistic studies under Problem III. Since this report covers only the first year or the proposed four year study, completed portions deal primarily with Problem I.

Progress under Problem I

Under this problem, several test systems developed in our laboratory for toxicological evaluation of target sites of membrane active substances were employed. The basic approach was to subject each tissue to a graded series of toluene concentrations for varying periods of time after which the material was prepared for electron microscopy under conditions developed to yield accurate and reproducible evaluations. Comparisons were to identical tissues treated in a similar fashion in the absence of toluene. Gross and subtle morphological changes were noted indicative of an activity target using the following three test systems:

Test System 1. Primary Rat Hepatocytes in Culture
Test System 3. Cultured BHK, KB and L Cells
Test System 4. Outer Cap Cells of the Maize Root Tip.

Work with Test System 2, Guinea Pig Testis Tubules, was initiated too late to be included in this report.

Considerable time was spent during the first six months of the project in exploring different methods of toluene administration in each of the different test systesm. Using radio-actively labeled toluene, we have monitored the rates at which toluene is lost from the various test systems through volatilization and into plastic ware and through normal handling and cell culture work. Normal cell culture containers and procedures are out of the question if constant conditions of toluene concentration are to be maintained. Even sealed (but not full) glass containers present problems as the toluene in the medium escapes to saturate the air above the medium. For the results obtained so far, we have compared three conditions of treatment: 1) Brief exposure to a solution containing the toluene at a known starting concentration, 2) Repeated exposures to solutions containing the toluene at a known starting concentration, and 3) Exposures in completely filled and sealed glass containers where toluene concentrations remain constant. Using methods 1 and 3, and comparing BHK and KB cells in culture, we find 100% mortality of cells between 500 and 1000 ppm toluene (Fig. 1), with 25 ppm toluene being about the lowest con-centration at which cell killing can be detected by exposure method 1. At intermediate concentrations, e.g. 100 ppm toluene, cells in

mitosis were markedly more susceptible than cells at other stages of the cell cycle (Fig. 1). This was investigated using KB cells synchronized using the mitotic shake technique. Dead cells were counted using trypan blue exclusion as the criterion for viability.

In similar experiments using L-cells, viability was maintained at 25 ppm toluene but growth was slowed. These cells were selected to begin a survey of changes in electron microscope morphology (Figs. 2 and 3). Several hundred cells have been examined as part of the subcontract with Texas A & M University. The most obvious and striking changes involve the plasma membrane at the cell surface.

Fig. 2A shows a typical untreated cell with many surface protuberances (microvilli or pseudopodia). After 15 min of exposure to 25 ppm toluene, the surface becomes smooth (Fig. 2B) with some tendency for the cells to round up and to possibly show important changes in the orientation of the Golgi apparatus by 30 min (Fig. 2C). With a single exposure, the effects are reversible and by 2 h (Fig. 3D), a nearly normal surface morphology is restored including the numerous protuberances.

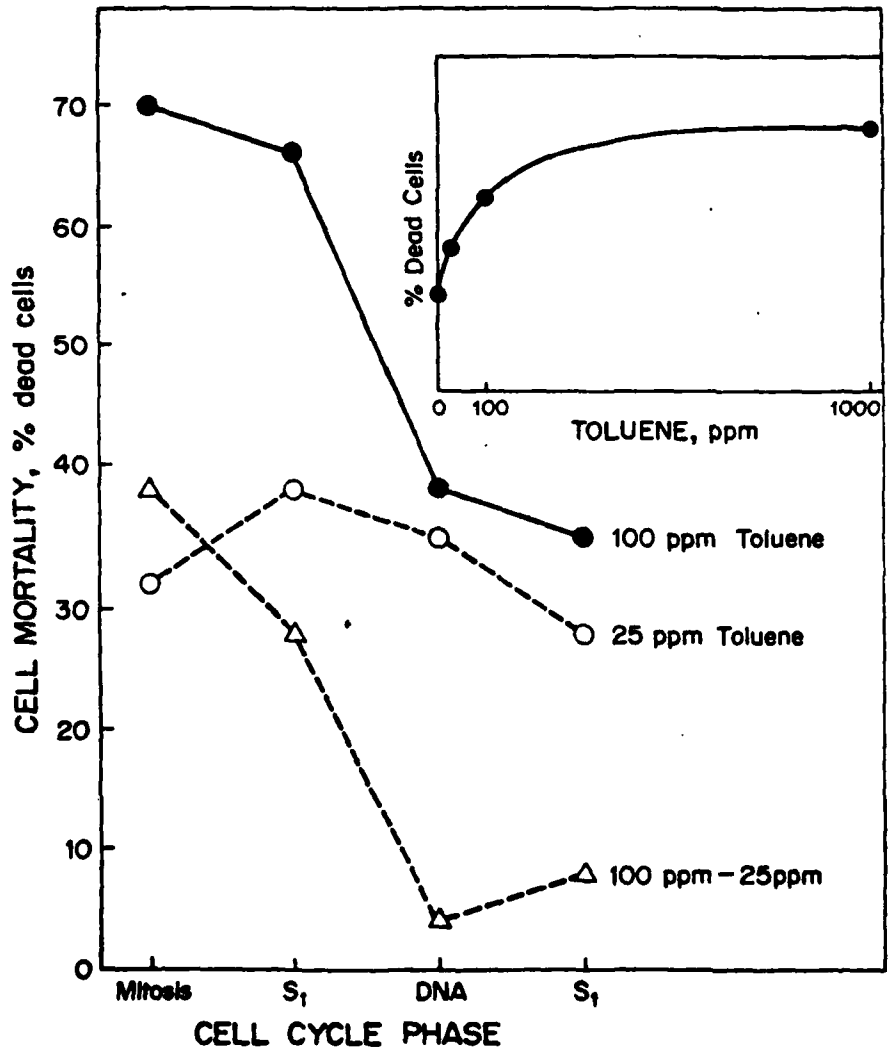

Fig. 1. Cell Mortality as a function of cell cycle phase for KB cells treated with 25 and 100 ppm toluene in the culture medium. Cells were synchronized by the miotic shake technique. Cells in mitosis appear most sensitive to the solvent for cell mortality using treatment method 1.

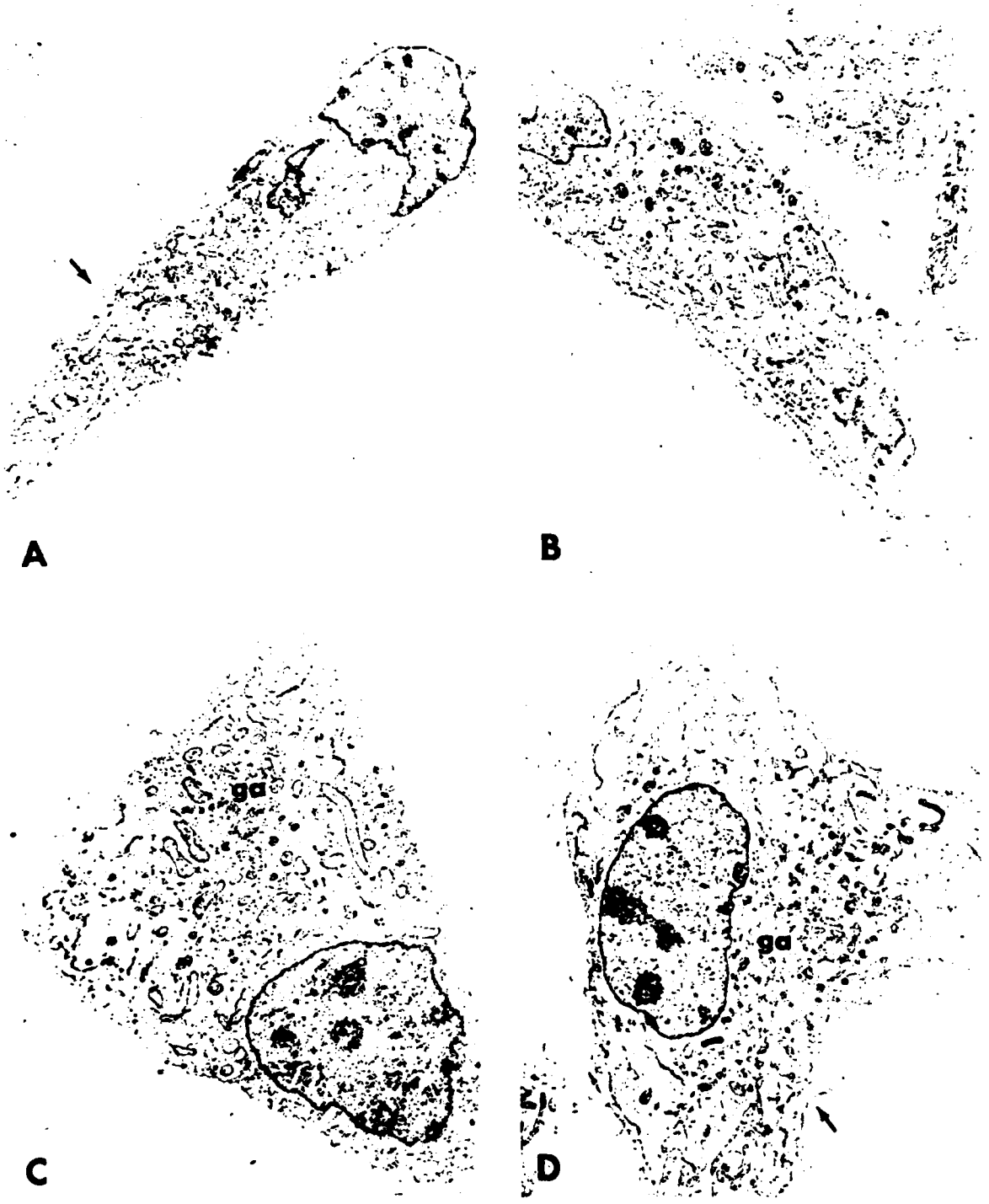

Fig. 2. Electron micrographs of L cells illustrating cell surface (plasma membrane changes observed following a single exposure to 25 ppm toluene contained in the cell culture medium. A. No toluene (control). Note numerous surface protuberances. B. 25 ppm toluene for 15 min. The cell contour is now smooth. C. 25 ppm toluene for 30 min. The cell contours remain smooth and the cells begin to round with some alterations in the form and position of the Golgi apparatus. D. 25 ppm toluene for 2 h. As the toluene is lost from the medium, the effects disappear, the normal morphology returns including the typical form and placement of the Golgi apparatus and the numerous surface protuberances. Figs. A, B and C X 6,000. Fig. D X 4,500.

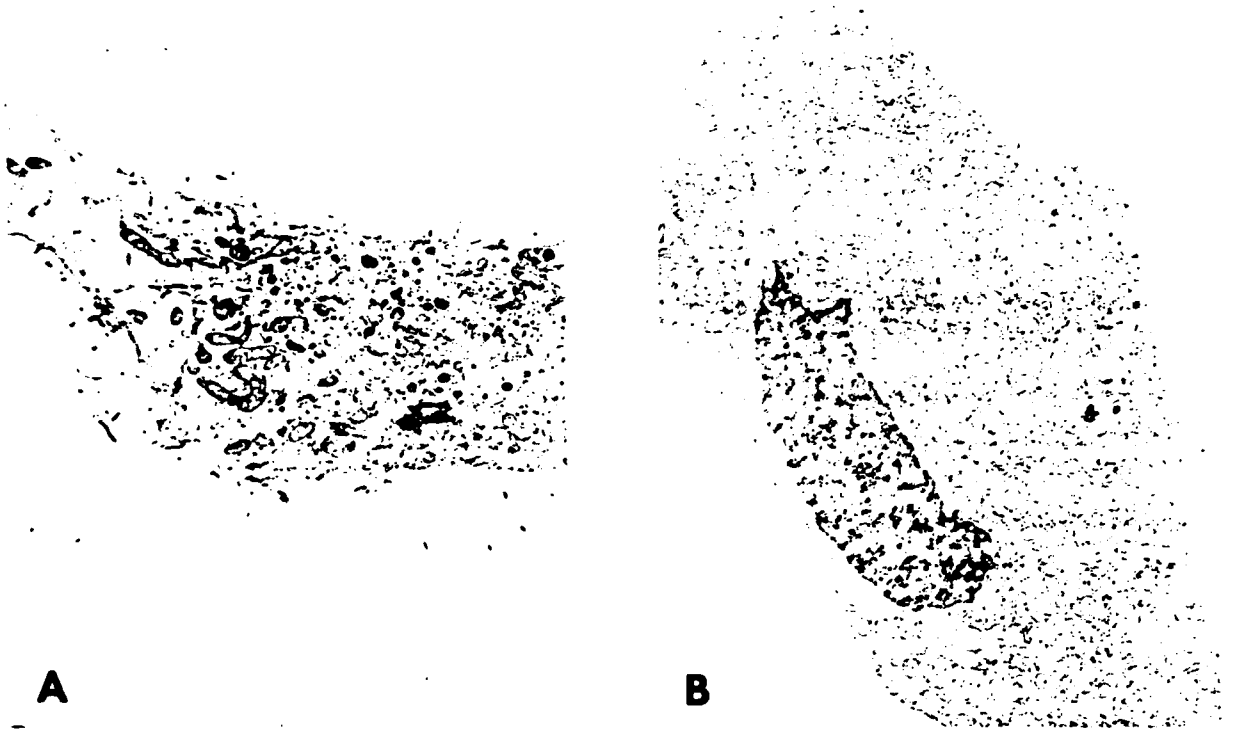

Fig. 3. Electron micrographs of L cells illustrating cell surface-plasma membrane changes observed following a single exposure to 100 ppm toluene contained in the culture medium. A. No toluene (Control). B. 100 ppm toluene for 5 min. The treated cells respond rapidly and present a surface morphology after 5 min. comparable to that observed after 30 min with 25 ppm toluene. This dose of toluene is toxic and results in many additional ultrastructural modifications involving membranes (see text). Fig. A x 4,500. Fig. B x 6,500.

With cells exposed to 100 ppm toluene, the response after 5 min was similar to that observed after 30 min with 25 ppm toluene with regard to surface morphology (Fig. 3). This concentration, which eventually is toxic, resulted in many additional ultrastructural modifications evaluated according to the criteria set forth under Problem I of this proposal. These included the characteristic distention of the nuclear envelope, dilation of the luminal space of the endoplasmic reticulum, disorganization of the Golgi apparatus and swelling of mitochondria. However, the most obvious, early, low-dose response is that described for the plasma membrane of the cell surface (Fig. 2).

A plasma membrane response has also been indicated from studies using Test System 4. In addition to subtle changes in contour, evidence for a lipid phase separation is evidenced from the formation of osmiophylic lipid globuli.

Test System 1 has proven more refractory. Cells were killed only at toluene concentrations approaching the maximum solubility in water. A similar resistance was shown by hepatocytes temperature-sensitive for growth rate and colony-forming ability but with the more rapidly growing cells showing a greater susceptibility to the solvent.

Under Problem II, work has focused primarily on the verification by biochemical analyses of some of the findings derived from Problem I. To aid in these efforts, we have developed a test system in which toluene is added to inverted jejunal segments of rat intestine to directly measure effects on plasma membrane enzymes. Segments are incubated in saline with control and toluene-treated segments in parallel. In order to maintain the toluene concentration, the toluene solution is replaced inside the gut every 10 min over a 60 min period (treatment method 2). Results using this approach show marked inhibition of the two plasma membrane enzymes thus far examined, sucrase (release of glucose) and alkaline phosphatase (release of inorganic phosphate from glycerolphosphate) by treatment with 100 ppm toluene (Fig. 4). The response is recorded within the first 5 min of toluene administration and nearly constant thereafter as long as the toluene concentration is maintained (Fig. 4). The effect is reversible for sucrase and at least partially reversible for alkaline phosphatase (Table I). If the toluene is removed, the enzymatic values return to near normal in 15 to 30 min paralleling the in vivo observation of cells with the electron microscope (Fig. 2). Electron microscope studies of the gut segments are in progress.

In other studies, we are developing procedures to use free-flow electrophoresis to decisively subfractionate primary hepatocytes and other cultured cells to facilitate enzymatic analyses of these different test systems. Cells are ruptured under hypotonic conditions and 500 psi nitrogen pressure using a Yeda press. The homogenates are then applied to VAP-IV free-flow electrophoresis unit and separated into endoplasmic reticulum-Golgi apparatus-, and plasma membrane-enriched fractions. These fractions are then placed on sucrose gradients to achieve final purification as well as provide fractions enriched in mitochondria and nuclei.

Additionally, we have developed a method using Percoll gradients, to prepare highly purified fractions of lyso-

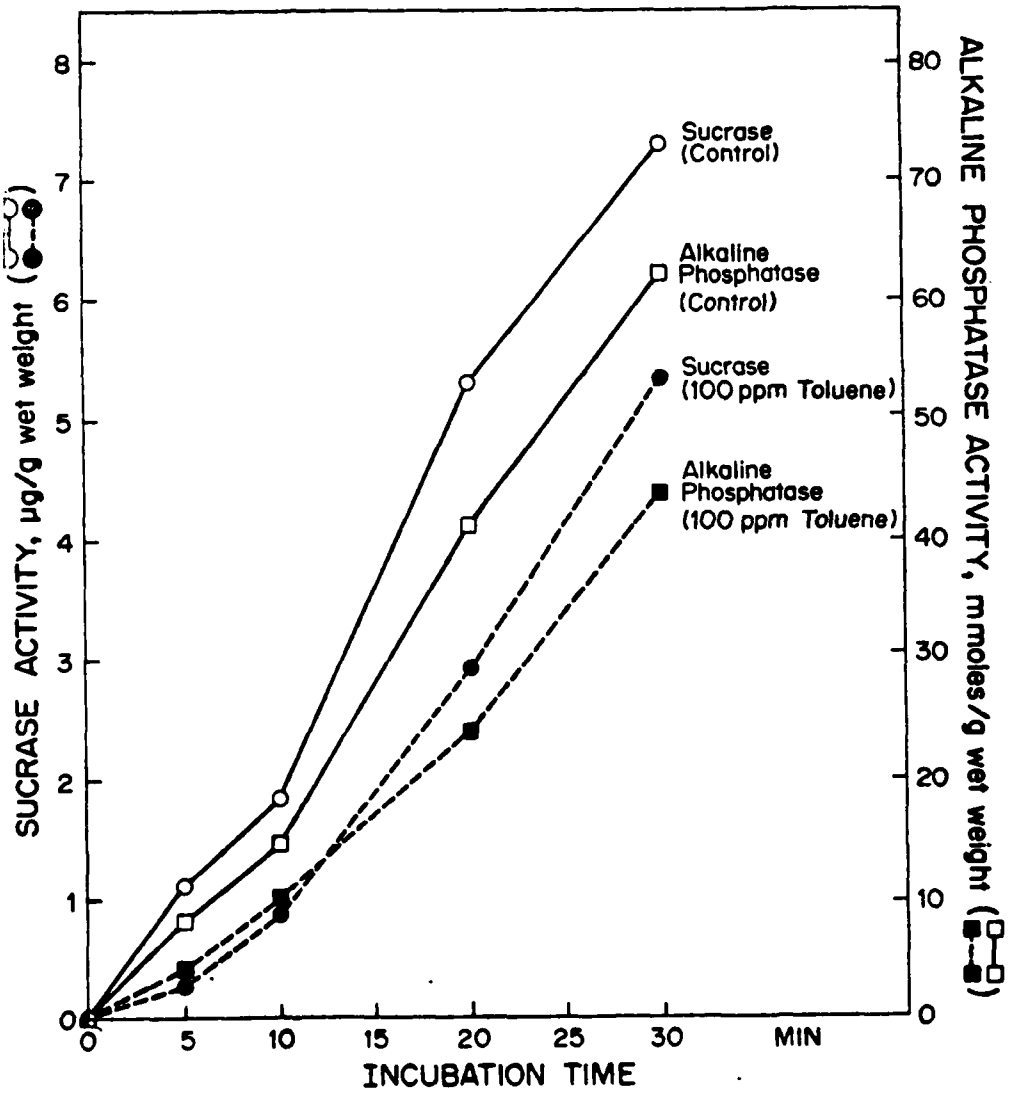

Fig. 4. Response of two plasma membrane enzymes, sucrase and alkaline phosphatase, of inverted jejunal segments of rat intestine to 100 ppm toluene. Inhibition is rapid and sustained as long as the toluene concentration is maintained to confirm a rapid, early and at least partially reversible (Table [illegible]).

somes from the same starting homogenates of liver as now provide Golgi apparatus, endoplasmic reticulum, plasma membrane, mitochondria, nuclei and peroxisome fractions. These fractions have been used in studies to monitor toluene effects of the structure and function of liver lysosomes.

Table I

Reversibility of toluene (100 ppm) inhibition of sucrase and alkaline phosphatase in inverted jejunal segments of rat intestine

Plasma membrane enzyme	% of control	
	15 min toluene	15 min recovery
Sucrase	78	108
Alkaline phosphatase	45	60

Under Problem II, biochemical analyses of isolated cell fractions prepared from rat liver are also well under way. A recently described one homogenate fractionation procedure was used (Croze and Morré, J. Cell. Physiol. 119, 46-57, 1984) which allows for the routine preparation of all of the major cell components from rat liver in useful yield and fraction purity. At 500 ppm, toluene has shown no effect on any of the enzymes of Golgi apparatus, lysosomes or mitochondria thus far tested (Table II). There was, however, an activation of the glucose-6-

Table II

Effect of a 15-30 min incubation with 500 ppm toluene on marker enzyme activities of each of the major cell components isolated from rat liver homogenates.

Marker Activity	Toluene	Homogenate Spec. Act., µmoles/h mg protein ± std. deviation	Major Cell Component	Spec. Act. of Major Cell Component, µmoles/h/mg pro ± std. dev.
5'-Nucleotidase	None	4.4 ± 0.6	Plasma Membrane	40.7 ± 0.6
	500 ppm	4.3 ± 0.3		40.7 ± 2.6
Galactosyltransferase				
Endogenous	None	0.01 ± 0.01	Golgi apparatus	1.8 ± 0.0
acceptor	500 ppm	0.007 ± 0.007		2.0 ± 0.2
Exogenous	None	7.0 ± 5.0		86 ± 4
acceptor	500 ppm	6.0 ± 2.1		76 ± 3
Nucleoside phosphate diphospatase	None	1.2	Golgi apparatus	3.8
	500 ppm	1.2		3.5
Latent acid phosphatase	None	0.5 ± 0.04	Lysosomes	17.5 ± 0.7
	500 ppm	0.5 ± 0.04		17.5 ± 0.6
Succinate-INT dehydrogenase	None	3.1 ± 0.1	Mitochondria	11.5 ± 0.7
	500 ppm	2.9 ± 0.6		11.0 ± 2.3
Glucose-6-phosphatase	None	2.0 ± 0.1	Endoplasmic Reticulum/Nuclear Envelope	4.2 ± 0.2
	500 ppm	2.5 ± 0.3		6.4 ± 0.4

Results are averages of duplicate determinations from a minimum of 3 different membrane preparations except for galactosyltransferase which was from one preparation and nucleoside phosphate diphosphatase which was from two preparations. Incubations were for 15 min with 5'-nucleotidase, nucleoside phosphate diphosphatase and succinate-INT-dehydrogenase, 20 min for latent acid phosphatase, and 30 min for glucose-6-phosphatase and galactosyltransferase.

phosphatase with 500 ppm toluene was noted as a clear exception (Table III). This membrane bound enzyme is notoriously sensitive to denaturation and a stabilizing effect of toluene could be of some practical interest to biochemists working with the enzyme. Additional studies are planned to characterize this interesting response and to determine its molecular basis.

Table III

Glucose-6-phosphatase activity of rough endoplasmic reticulum stimulated by 500 ppm toluene with both freshly prepared membranes and with membranes stored for 24 h at ice bath temperature.

Cell Fraction	Toluene	Specific activity, µmoles/h/mg protein Fresh	After 24 h	Ratio, toluene/control Fresh	After 24 h
Total homogenate	None	2.0 ± 0.1	0.3 ± 0.1		
	500 ppm	2.5 ± 0.3	0.45 ± 0.05	1.25	1.5
Endoplasmic reticulum	None	4.2 ± 0.2	1.3 ± 0.2		
	500 ppm	6.4 ± 0.4	3.5 ± 0.8	1.5	2.7

In metabolic labeling experiments, we have utilized slices from livers of 200 g male Wistar rats as an additional test system to begin to monitor toluene effects on biochemical parameters of internal membranes. A dosing schedule was developed using a sealed chamber. At the end of the toluene treatment (15, 30 or 60 min), radioactive precursors (e.g. ^{3}H-leucine) are administered for an additional 20 min to measure metabolic activity. Then each of the various membrane fractions are isolated and analyzed for incorporation of radioactivity (nuclei, Golgi apparatus, endoplasmic reticulum, lysosomes, mitochondria, and plasma membranes) (Fig. 5 and Table IV).

As illustrated in Fig. 5, incorporation of radioactivity from ^{3}H-leucine into the various membrane fractions is reduced considerably in Golgi apparatus, endoplasmic reticulum and plasma membrane by pretreatment with 500 ppm toluene A maximum effect on Golgi apparatus and plasma membrane was obtained with a pretreatment time of 30 min whereas with the endoplasmic reticulum, inhibition was recorded just with toluene preduring the 20 min time of isotope incorporation (Fig. 5).

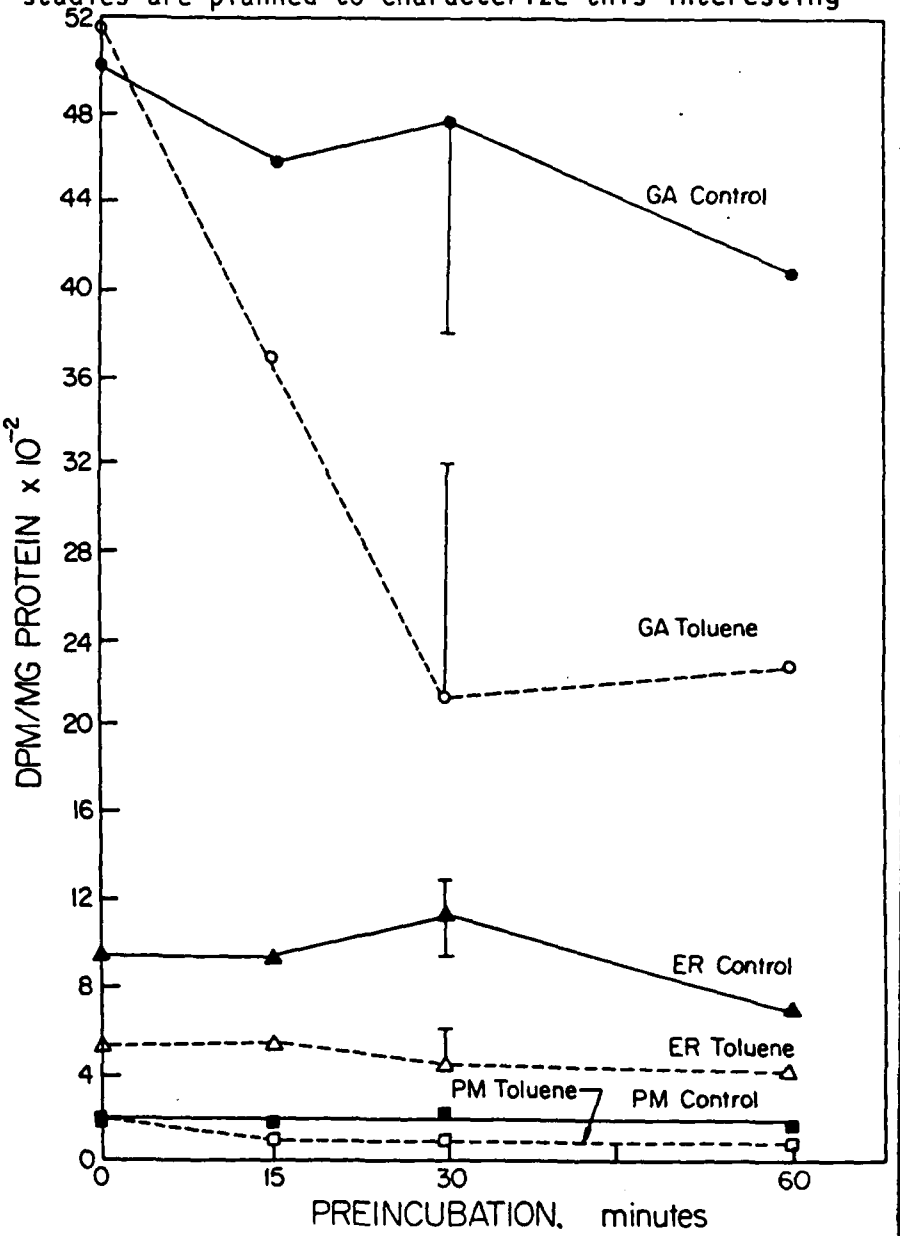

Fig. 5. Time of preincubation with 500 ppm toluene o a subsequent 20 min incubation with ^{3}H-leucine of ra liver slices in vitro at 37°. A maximum effect with Golgi apparatus (GA) and plasma membrane (PM) was ob- tained with a 30 min preincubation whereas with endoplasmic reticulum (ER), an effect was observed

Table IV

Effect of 30 min toluene preincubation (500 ppm) on subsequent incorporation during 20 min of ^{3}H-leucine into rat liver slices *in vitro* at 37°. Results are the average of duplicate or triplicate determinations of 3 different membrane preparations from 3 different animals ± standard deviation (S.D.) membrane preparations.

Fraction	Toluene	Dpm/mg protein ± S.D.	Ratio: Toluene/Control ± S.D.
Total Homog.	None	13,928 ± 590	1.01 ± 0.07
	500 ppm	14,114 ± 1,419	
Golgi Pellet	None	1,477 ± 272	0.40 ± 0.22
	500 ppm	597 ± 202	
Golgi Apparatus	None	9,604 ± 2,462	0.45 ± 0.15
	500 ppm	4,359 ± 2,588	
Supernatant	None	44,256 ± 7,961	0.95 ± 0.10
	500 ppm	42,195 ± 7,282	
Plasma Membrane	None	261 ± 76	0.56 ± 0.23
	500 ppm	146 ± 42	
Mitochondria	None	471 ± 145	0.44 ± 0.20
	500 ppm	208 ± 80	
Nuclei	None	673 ± 195	1.18 ± 0.76
	500 ppm	792 ± 509	
Nuclei-II	None	466 ± 266	0.74 ± 0.12
	500 ppm	343 ± 170	
ER_0*	None	1,201 ± 1,076	1.13 ± 0.78
	500 ppm	1,355 ± 1,293	
ER_1**	None	1,483 ± 1,076	0.26 ± 0.18
	500 ppm	380 ± 137	
ER_2#	None	1,810 ± 1,672	0.47 ± 0.29
	500 ppm	885 ± 375	
ER_{Ave}##	None	1,590 ± 190	0.38 ± 0.11
	500 ppm	610 ± 237	

* Material collecting at the homogenate/1.3 M sucrose interface of the ER gradients. Composed of light membranes: Golgi apparatus elements (fragments), sinusoidal plasma membrane, mitochondrial outer membranes

** Membranes from the 1.3/1.5 M sucrose interface of the ER gradients. So-called "less-rough" endoplasmic reticulum. Drug-induced smooth endoplasmic reticulum membranes would enter this fraction.

Material collecting at the 1.5/2.0 M sucrose interface of the ER gradient. Composed of rough ER.

Average of Golgi pellet, ER_1 and ER_2.

The dose dependency shown in Fig. 6 and Table V indicated a near maximum response at 500 ppm for a 30-min pre-incubation. Values for 0 ppm (Control) are the same as for Table IV.

As seen throughout these data (Tables IV-VI), at 500 ppm toluene, endoplasmic reticulum exhibited a marked and rapid reduction in the amount of ^{3}H-leucine incorporated. Subsequently, reductions were seen as well in Golgi apparatus and plasma membrane. Incorporation into nuclei was much less affected while incorporation into mitochondria was affected similarly to that of endoplasmic reticulum. Labeling of lysosomes was investigated in a separate series of experiments and was found not to be affected by toluene.

In subsequent studies, an incubation time of 30 min (Fig. 5; Table IV and Table IV) and a toluene concentration of 500 ppm (Fig. 6; Table V) were judged to provide an optimum response in the test system using rat liver slices.

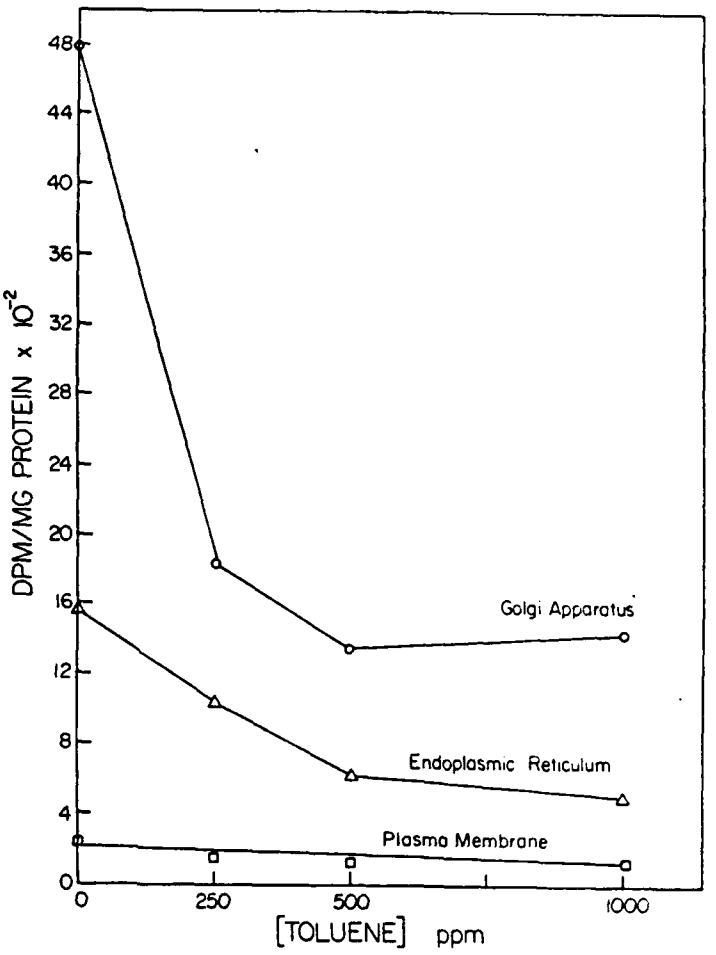

Fig. 6. Effect of toluene concentration during a 30 min preincubation on a subsequent incubation with ^{3}H-leucine of rat liver slices in vitro at 37°. A maximum effect was obtained at 500 ppm with a 30 min incubation.

Table V

Effect of toluene concentration during a 30 min preincubation on subsequent incorporation during 20 min of ^{3}H-leucine into rat liver slices in vitro at 37°. Results are single determinations except for 0 ppm (= control) which are the average of 3 different preparations ± standard deviations.

	Dpm/mg protein			
Fraction	0 ppm	250 ppm	500 ppm	1000 ppm
Total Homogenate	13,926 ± 590	15,550	12,632	14,162
Golgi Pellet	1,477 ± 272	385	375	646
Golgi Apparatus	9,604 ± 2,462	3,671	2,714	3,020
Supernatant	44,256 ± 7,961	47,240	45,500	33,846
Plasma Membrane	261	182	146	146
Mitochondria	471 ± 145	198	133	292
Nuclei	673 ± 195	352	1,350	675
Nuclei-II	386 ± 205	444	147	750
ER_0	1,201 ± 198	1,074	2,667	1,100
ER_1	1,483 ± 1,076	1,222	483	224
ER_2	1,810 ± 1,672	(612)	1,287	666

Table VI

Effect of time of preincubation with 500 ppm toluene on a subsequent 20 min incubation with ^{3}H-leucine of rat liver slices *in vitro* at 37°. Values are single determinaitons except for 30 min which are the average of 3 different preparations ± standard deviations.

Fraction	Toluene	0 min	Ratio	15 min	Ratio	30 min	Ratio	60 min	Ratio
Total homog.	None	15,939	1.01	14,160	1.13	13,928 ± 590	1.01	10,735	1.04
	500 ppm	16,129		16,053		14,114 ± 1,459		11,127	
Golgi pellet 851 0.68	None	1,493	0.57	1,182	0.72	1,477 ± 272	0.40		
	500 ppm	841		846		597 ± 202		575	
Golgi appar.	None	3,450	1.11	9,207	0.80	9,604 ± 2,462	0.45	16,550	0.56
	500 ppm	3,816		7,407		4,359 ± 2,588		9,205	
Supernatant	None	45,429	1.05	46,680	0.99	44,256 ± 7,901	0.95	34,038	1.14
	500 ppm	47,736		46,365		42,195 ± 7,282		38,893	
Plasma memb.	None	236	1.04	216	0.81	261	0.56	179 358	0.73
	500 ppm	246		176		146		262 131	
Mitochondria	None	360	0.74	432	0.44	472 ± 145	0.44	341	0.79
	500 ppm	268		246		208 ± 80		268	
Nuclei	None	755	0.4	589	0.86	673 ± 195	1.18	186	1.05
	500 ppm	321		378		792 ± 509		194	
Nuclei-II	None	660	0.4			466 ± 266	0.74	276	1.23
	500 ppm	433				343 ± 190		223	
ER_0	None	1,123	1.0	1,193	1.67	1,201 ± 198	1.13	658	1.12
	500 ppm	1,097		1,994		1,355 ± 1,293		734	
ER_1	None	756	0.57	1,075	0.26	1,483 ± 1,076	0.48	783	0.53
	500 ppm	394		615		380 ± 137		374	
ER_2	None	600	0.55	485	0.55	1,810 ± 1,672	0.47	502	0.59
	500 ppm	369		275		855 ± 375		300	

To determine if the toluene response observed above was translated into an effect on the flow dynamics of the intracellular membranes, i.e., transfer of membrane from endoplasmic reticulum to plasma membrane via the Golgi apparatus, classical flow kinetics were determined also using this test system. Results depicted in Figures 7 and 8 show that the kinetic parameters of transfer from endoplasmic reticulum to Golgi apparatus, passage through the Golgi apparatus, and transfer from the Golgi apparatus to the plasma membrane are little affected by toluene if at all.

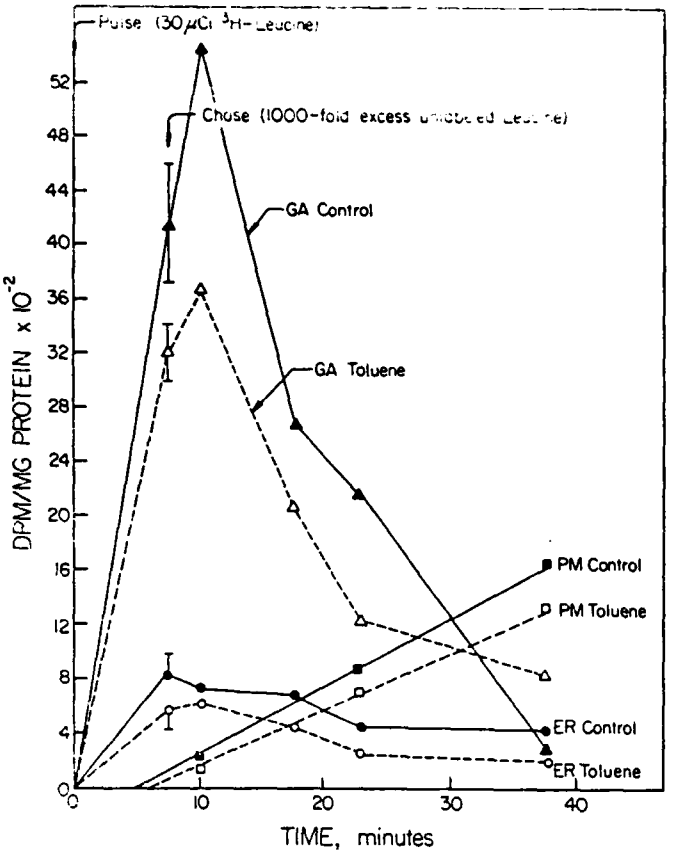

Fig. 7. Flow kinetics of membrane and secretory proteins labeled with a 7.5 min pulse of ^{3}H-leucine and a subsequent chase with 1000-fold excess non-radioactive leucine following a 30 min preincubation with 500 ppm toluene. GA -Golgi apparatus. ER - endoplasmic reticulum. PM - plasma membrane. Solid curves are control values. Dotted curves are for liver slices preincubated for 30 min with 500 ppm toluene.

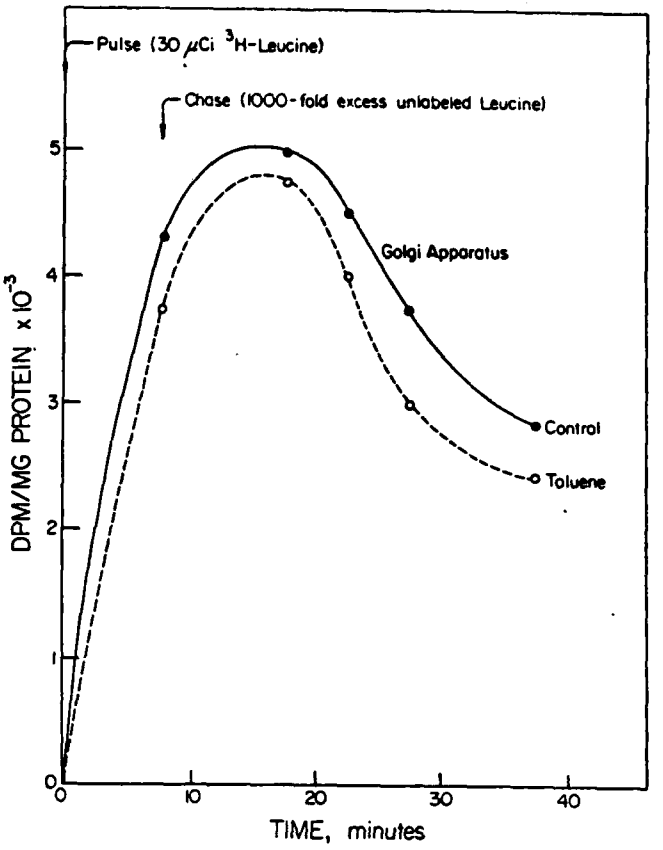

Fig. 8. As in Fig. 7 but only flow kinetics through the Golgi apparatus were determined. Slices prepared from livers of mature, male rats were preincubated 30 min with and without 500 ppm toluene. After the preincubation, 30 μCi ^{3}H-leucine was added ± 500 ppm toluene for 7.5 min (the pulse). After 7.5 min, the slices were removed from the radioactivity, washed and placed in 1000-fold excess leucine (± 500 ppm toluene), the chase, for the times indicated.

Whereas, overall incorporation during the 7.5 min pulse is less, there is no difference in the rate of passage through the Golgi apparatus (Figs. 7 and 8) or in the time or rate of appearance in the plasma membrane (Fig. 7).

The basis for the inhibition of incorporation is presently under investigation. The inhibition is rapid and occurs, apparently, at the level of the rough endoplasmic reticulum. Experiments are planned to attempt to distinguish between inhibition of incorporation of protein synthesis on cytoplasmic vs. membrane bound polyribosomes and to more precisely determine the level of translational control involved. An alternative interpretation based on complete or nearly complete inhibition of protein synthesis in outer cell layers by toluene with much less of an effect on inner cell layers is also being investigated.

Table VII

Effect of treatment of liver slices for 30 min with 500 ppm toluene on lysosome integrity as determined by measurement of latent acid phosphatase. Latent acid phosphatase was defined as the specific activity (μmoles/h/mg protein) of the hydrolysis of sodium p-nitro-phenylphosphate in preparations where membrane were lysed with the detergent Triton X-100 minus the specific activity of the same preparations in isotonic sucrose prior to lysis.

Fraction	No. of determinations	Toluene	Latent acid phosphatase, μmoles/h/mg pro ± std. dev.
Total homogenate	7	None	0.54 ± 0.2
		500 ppm	0.54 ± 0.3
10,000 g pellet (crude lysosomes)	4	None	2.2 ± 1.2
		500 ppm	1.39 ± 0.9
Purified lysosmes (Percoll gradients)	8	None	14.29 ± 4.4
		500 ppm	12.65 ± 2.1

To study the effects of toluene on liver lysosomes, a procedure to purify lysosomes by centrifugation in Percoll gradients was adapted for use in this study. Lysosomes isolated from liver slices preincubated for 30 min with 500 ppm toluene showed some tendency toward decreased latency, i.e. increased lysosomal lability (Table VII). In contrast, 500 ppm toluene added directly to preparations of purified lysosomes or to crude preparations containing lysosomes had little or no effect (Table VIII). The effect on lysosome lability of Table VII is small, not statistically significant. It may be the result of toluene toxicity to some of the cells of the outer layers of the liver slices and not a general response of liver lysosomes to toluene treatment.

Table VIII

Effect of toluene (500 ppm) added directly to preparations of total homogenate, crude lysosomes (10,000 g pellet) or purified lysosomes (Percoll gradients). Assays were as for Table VII except that the toluene was present during the 20 min incubation with the enzyme and the tissue was not pretreated with toluene prior to the preparation of the isolated fractions.

Fraction	Toluene	Latent p-nitrophenyl phosphatase, μmoles/h/mg protein ± std.dev.
Total homog.	None	0.5 ± 0.04
	500 ppm	0.5 ± 0.04
10,000g	None	0.88 ± 0.07
	500 ppm	0.87 ± 0.07
Lysosomes (Percoll grad.)	None	17.5 ± 0.8
	500 ppm	17.5 ± 0.6

To determine the effect of toluene on biogenesis and turnover of lysosomes, pulse-chase experiments were conducted similar to those described above for endoplasmic reticulum and Golgi apparatus. While the findings are not as extensive as for the other membrane fractions, it would appear that toluene has no strong effect on this parameter of lysosome function either (Table IX).

Table IX

Results of pulse-chase experiments to determine the effect of toluene on lysosome biosynthesis and turnover in liver slices preincubated for 30 min with or without 500 ppm toluene and subsequently incubated with ^{3}H-leucine (total of 30 µCi) for 7.5 min as a pulse followed by transfer to 1000-fold excess non-radioactive leucine (chase) ± toluene for the times indicated.

Pulse time	Chase time	Toluene	Lysosomes, dpm/min/mg protein
7.5 min	0 min	None	7,404
		500 ppm	8,048
10.0 min	0 min	None	10,625
		500 ppm	5,200
7.5 min	10 min	None	1,224
		500 ppm	2,000
7.5 min	15 min	None	1,428
		500 ppm	1,481
7.5 min	30 min	None	305
		500 ppm	353

Summary

The cell nucleus is little affected by toluene either ultrastructurally, by leucine incorporation or from incorporation of radioactive thymidine (data not shown). Also little affected by toluene are Golgi apparatus (analysis of marker enzymes and flow kinetics of membranes and secretory proteins) and lysosomes (analysis of marker enzymes, latency, biosynthesis and turnover). Endoplasmic reticulum (ribosomes?) show a high dose response (500 ppm/30 min) both in terms of ability to incorporate radioactive leucine and in the activity of one marker enzyme in liver. A similar response is shown by mitochondria although a more detailed enzymatic analysis and functional analysis must be carried out. The most sensitive cell component is the plasma membrane showing a morpohological response at 25 ppm and a response in terms of enzymatic activity at 100 ppm and treatment times of between 5 and 30 min. Thus the plasma membrane is indicated as the most likely single primary target for toluene intoxication.

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