Biochemical Pharmacology, Vol. 19, pp. 1251–1261. Pergamon Press. 1970. Printed in Great Britain

STUDIES ON LYSOSOMES—XIII

EFFECTS OF STILBAMIDINE AND HYDROXYSTILBAMIDINE ON *IN VITRO* AND *IN VIVO* SYSTEMS*

GERALD WEISSMANN,† PHILIP DAVIES,‡ KATHRIN KRAKAUER and ROCHELLE HIRSCHHORN§

Department of Medicine (Cell Biology and Genetics), New York University School of Medicine, New York, N.Y., U.S.A.

(*Received* 15 *May* 1969; *accepted* 22 *August* 1969)

Abstract—At concentrations between 5×10^{-5} M and 5×10^{-4} M, stilbamidine and hydroxystilbamidine retarded the release of aryl sulfatase and cathepsin from lysosome-rich fractions of rabbit liver in 0·25 M sucrose. At these concentrations, the stilbamidines had no effect upon the free activity of lysosomal hydrolases in soluble form, while at 10^{-3} M the drugs inhibited lysosomal β-glycerol phosphatase, β-glucuronidase and aryl sulfatase. Hydrolase release was inhibited by stilbamidines whether induced by simple incubation at 37° with solvents (ethanol, dimethylsulfoxide), by addition of 5×10^{-4} M retinol or 5×10^{-4} M diethylstilbestrol, or by ultraviolet irradiation. Stilbamidine also retarded release of soluble malate dehydrogenase from mitochondria present in the liver granule fraction. Stilbamidine (25 mg/kg) injected intraperitoneally antagonized the collapse of rabbit ears induced by vitamin A palmitate (500,000 i.u./kg); stilbamidine and hydroxystilbamidine inhibited phytohemagglutinin-induced transformation of human peripheral blood lymphocytes. The effects of stilbamidines in each of these systems resemble those of equivalent concentrations of cortisol and chloroquine.

THE STILBAMIDINES are members of the diamidine series of drugs used for the treatment of blastomycosis, leishmaniasis and trypanosomiasis.[1, 2] They have also been used for the palliative treatment of multiple myeloma,[3] but this application has been generally discontinued. Although interactions of diamidines with proteins, nucleic acids and lipids have been reported,[1] their exact mode of action in fungal or parasitic infections is by no means established.

By virtue of their conjugated ring systems (Fig. 1), the stilbamidines exhibit striking fluorescence, with a sharp absorption peak at 327 nmeters and an equally sharp emission peak at 394 nmeters (P. Lipsky and G. Weissmann, unpublished data). It was this property that enabled Allison[4] to describe the selective uptake and storage of stilbamidine by lysosomes, organelles which also sequester other cationic, highly fluorescent compounds such as chloroquine or acridine orange. Since the stilbamidines were readily concentrated in lysosomes, it became of interest to determine whether these agents could influence the stability of lysosomes *in vitro*. Furthermore, the effect

* Aided by grants from the National Institutes of Health (AM 08363, AM-11949) and the New York Chapter of the Arthritis Foundation.
† Career Scientist of the Health Research Council of New York (1-467).
‡ S.R.C./N.A.T.O. Fellow (1968–1970).
§ Senior Investigator of the New York Heart Association.

of these agents was studied in two systems *in vivo* in which lysosomes have been demonstrated to play an important role. Hydroxystilbamidine was studied together with stilbamidine, since the former agent is less toxic clinically;[2, 3] if their action *in vitro* were similar, then further studies *in vivo* could be conducted with hydroxystilbamidine.

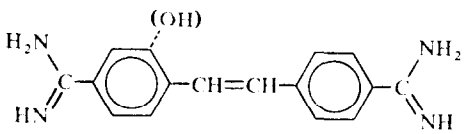

FIG. 1. (Hydroxy-)stilbamidine. The dotted line represents the position of the OH group in hydroxystilbamidine.

The studies to be reported below demonstrate that stilbamidine and hydroxystilbamidine stabilize lysosomes from rabbit liver against disruption by a variety of agents or means, at concentrations incapable of inhibiting the activity of soluble hydrolases. Moreover, they modify the collapse of rabbit ears (dissolution of cartilage matrix) mediated by excess of vitamin A palmitate, and inhibit the transformation of human peripheral blood lymphocytes induced by phytohemagglutinin.

MATERIALS AND METHODS

Preparation of large granule fraction from rabbit liver. These procedures have been described in detail before.[5] Briefly, a twice-washed, large granule fraction sedimenting between 800 *g* (10 min) and 20,000 *g* (20 min) was prepared in ice-cold 0·25 M sucrose from the livers of white, male, albino rabbits weighing no more than 1 kg. To aliquots (3 ml) of the granule fractions, agents (stilbamidines, steroids, retinol) were added in solvents (water, dimethylsulfoxide or ethanol; 0·1 ml) as indicated below. In experiments with stilbamidines (as the isethionates) and retinol, the solutions were freshly prepared each day with precautions taken to prevent direct access of light. Preliminary experiments established that stilbamidines "stabilized" best if permitted to stand at room temperature with large granule fractions for 10 min before subsequent incubation at 37° with lysosomal labilizers such as retinol or diethylstilbestrol.[6] The pH of granule fractions was 6·4 $\pm$ 0·15, and did not change upon addition of stilbamidines. After incubation, the suspensions were again centrifuged at 20,000 *g* for 20 min and enzyme activity released into the supernatants was determined. To measure the total releasable activity of acid hydrolases, other aliquots of the granule fractions were incubated with Triton X-100 (0·1 %, v/v).

Enzyme assays. β-Glucuronidase (phenolphthalein glucuronidate),[5] acid phosphatase (β-glycerol phosphate),[7] aryl sulfatase (nitrocatechol sulfate),[5] cathepsin (denatured hemoglobin, pH 3·0)[8] and malate dehydrogenase ($NADH_2$ oxidation),[5] were determined by methods previously described.[5, 7, 8]

Rabbit ear collapse. Rabbits were injected intraperitoneally (i.p.) once a day according to one of four regimens (a) 1 ml of corn oil; (b) 5×10^5 units of vitamin A

palmitate in corn oil; (c) 5×10^5 units of vitamin A palmitate + 25 mg stilbamidine; or (d) 25 mg stilbamidine in saline. Ear collapse was graded as previously described;[9] alopecia was also recorded.

Ultraviolet irradiation. This was carried out by placing large granule fractions in vessels of large surface area, fixed in a shaking water bath at 37°, 32 cm beneath a Hanovia 100 W high pressure mercury arc lamp whose rated output below 3130 Å is 250 μW per cm^2 measured at 50 cm in free air. Aliquots were removed at the indicated time, spun at 20,000 *g* for 20 min, and enzyme activities released into the supernatant were measured. Control samples were incubated in similar vessels which were covered with aluminum foil. These procedures have been previously detailed.[10]

Stimulation of human peripheral blood lymphocytes. Human peripheral blood lymphocytes were obtained as previously described.[11, 12] Lymphocytes, 15–20 $\times$ 10^6, were then preincubated overnight at 37° in 20 ml of Eagle's medium (MEMS), 20% fetal calf serum, 1 mM glutamine, 100 units penicillin and 100 μg/ml of streptomycin. Freshly prepared stilbamidine or hydroxystilbamidine in saline was added (in the dark) in 0·5-ml aliquots to final concentrations as below. Phytohemagglutinin (PHA-P, Difco), 0·2 ml, was added (zero time) either simultaneously or as indicated. Uridine-2-^{14}C (specific activity, 55·2 mc/m-mole) was added for the last 3 hr of culture. Four μc was added for cultures terminated before 4 hr and 2 μc for all longer term cultures. Cultures were terminated by washing twice with 10-ml aliquots of ice-cold saline and three times with 10-ml aliquots of 10% cold TCA. The drained precipitate was dissolved in 1·0 ml hyamine and counted in toluene PPO-POPOP in a Beckman liquid scintillation counter. Results are reported as disintegrations per minute and are the average of duplicate cultures.

Materials. Triton X-100 was obtained from Rohm & Haas, Philadelphia, Pa.; diethylstilbestrol from Steraloids, Pawling, N.Y.; retinol and vitamin A palmitate from Nutritional Biochemicals, Cleveland, Ohio. Stilbamidine was a gift of the Messrs. May and Baker, Dagenham, England, and hydroxystilbamidine was obtained from the Wm. S. Merrell Company, Cincinnati, Ohio.

RESULTS

Effect of stilbamidines on hydrolase activity. Before deciding whether a given pharmacologic agent acts to augment or inhibit the release of enzymes from lysosomes, its effect must be studied upon the activities of lysosomal hydrolases in the presence of a detergent.[10] Triton X-100 (0·1%, v/v) effectively renders the maximum activity of acid hydrolases available to substrate, and therefore the effect of stilbamidines upon the activity of four acid hydrolases (Table 1) was studied in supernatants of granule fractions exposed to detergent. At 10^{-3} M, both drugs inhibited acid phosphatase, β-glucuronidase and aryl sulfatase without affecting the activity of cathepsin. At 10^{-4} M, neither hydroxystilbamidine nor stilbamidine had significant inhibitory effect on any of the four hydrolases. In subsequent experiments, aryl sulfatase or cathepsin or both were used as markers for the release of enzymes from lysosomes.

Effect of stilbamidines upon release of hydrolases by retinol. Rabbit liver granules were permitted to stand for 10 min with varying amounts of stilbamidines before exposing the suspensions to retinol (0·1 ml in ethanol), ethanol alone (0·1 ml) or Triton X-100 (0·1%, v/v). At concentrations between 5×10^{-5} M and 10^{-3} M, stilbamidine retarded cathepsin release by retinol or solvent alone, but (as above) had no effect

TABLE 1. INHIBITION OF ACID HYDROLASES OF RABBIT LIVER BY STILBAMIDINES

Enzyme	Sp. act.*	Percentage of sp. act. remaining† 10^{-4} M Stil.	10^{-4} M OH-stil.	10^{-3} M Stil.	10^{-3} M OH-stil.
Acid β-glycerol phosphatase	2·35 μm P_i	98·0	99·6	70·0	49·4
β-glucuronidase	290 μg phenolphthalein	95·8	97·8	71·8	57·3
Aryl sulfatase	149 μg nitrocatechol	103	108·0	86·0	47·5
Cathepsin	49·4 μg acid-soluble tyrosine	110·0	102·0	105·0	103·0

* Substrate released/mg protein/hr after exposure of a large granule fraction of rabbit liver to Triton X-100 (0·1%, v/v). This figure is 100%.

† After incubation of large granule fraction with Triton X-100 (0·1%, v/v) and stilbamidine (Stil.) or hydroxystilbamidine (OH-stil.) for 60 min.

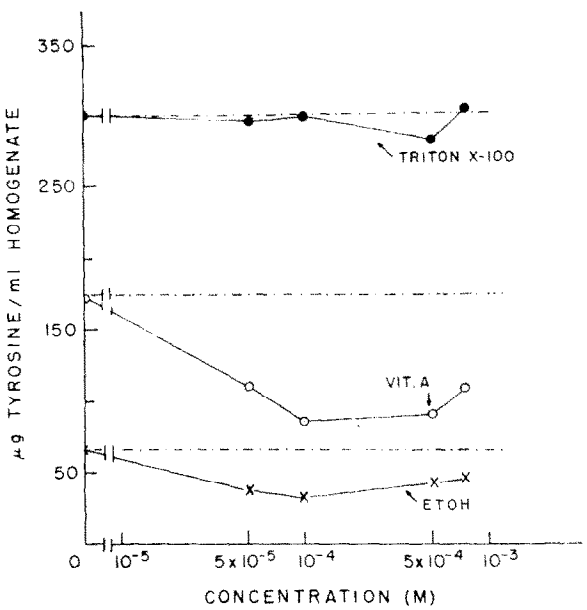

FIG. 2. Effect of stilbamidine on release of cathepsin by the detergent, Triton X-100 (0·1%, v/v), retinol (vitamin A, 5×10^{-4} M) or simple incubation with solvent (ethanol, 2%, v/v). Large granule fraction from rabbit liver in 0·25 M sucrose was incubated for 60 min with the above agents, then centrifuged for 20 min at 20,000 *g*. Cathepsin activities of supernatant were then determined with acid (pH 3·0) hemoglobin as substrate (sp. act. of Triton-treated sample; see Table 1). Dot-dashed lines refer to values obtained in the absence of drugs.

upon the activity of the enzyme when the granules had been totally disrupted by Triton X-100 (Fig. 2). Hydroxystilbamidine gave similar results, but was less effective at concentrations of 5×10^{-4} M or above (Fig. 3). These experiments indicated that 10^{-4} M concentrations of diamidines were optimum for stabilization. Therefore the effects of this concentration of the stilbamidines were repeatedly studied. Again (Tables 2 and 3), stilbamidines significantly retarded the release of cathepsin and aryl sulfatase from large granule fractions induced by solvent (ethanol) or retinol. Since

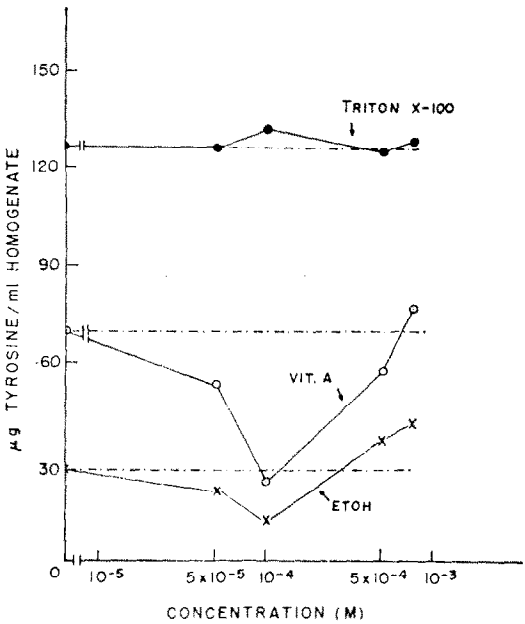

FIG. 3. Effect of hydroxystilbamidine on release of cathepsin by the detergent, Triton X-100 (0·1% v/v), retinol (vitamin A 5×10^{-4} M) or simple incubation with solvent (ethanol 2% v/v). Large granule fraction from rabbit liver in 0·25 M sucrose was incubated for 60 min with the above agents, then centrifuged for 20 min at 20,000 *g*. Cathepsin activities of supernatant were then determined with acid (pH 3·0) hemoglobin as substrate (sp. act. of Triton-treated sample; see Table 1). Dot-dashed lines refer to values obtained in the absence of drugs.

TABLE 2. EFFECT OF HYDROXYSTILBAMIDINE (10^{-4} M) ON RELEASE OF HYDROLASES FROM RABBIT LIVER GRANULES BY ETHANOL, VITAMIN A AND TRITON X-100

	Aryl sulfatase	Cathepsin
Control* (ethanol)	73·3 ± 8·1 (8)†	62·8 ± 7·1 (8)†
Vitamin A* (5×10^{-4} M)	65·6 ± 4·3 (8)†	55·0 ± 9·8 (8)†
Triton X-100* (0·1%, v/v)	98·6 ± 5·9 (12)	105·4 ± 9·2 (8)

* Incubation for 60 min; 100% = release of enzyme in absence of hydroxystilbamidine.
† $P < 0{\cdot}01$ (Wilcoxon).

TABLE 3. EFFECT OF STILBAMIDINE (10^{-4} M) ON RELEASE OF HYDROLASES FROM RABBIT LIVER GRANULES BY ETHANOL, VITAMIN A AND TRITON X-100

	Aryl sulfatase	Cathepsin
Control* (ethanol)	63·5 ± 6·3 (5)†	57·5 ± 21·2 (7)†
Vitamin A* (5×10^{-4} M)	72·9 ± 6·2 (8)†	61·3 ± 9·4 (6)†
Triton X-100* (0·1%, v/v)	97·9 ± 5·6 (8)	99·4 ± 4·3 (8)

* Incubation for 60 min; 100% = release of enzyme in absence of stilbamidine.
† $P < 0{\cdot}01$ (Wilcoxon).

incubation at 37° for 1 hr with ethanol induced release of roughly 20 per cent, while retinol (5×10^{-4} M) induced release of over 50 per cent of the total cathepsin activity of such fractions (Figs. 2 and 3), the data indicate that the diamidines antagonize enzyme release at varying levels of membrane perturbation.

To determine whether stilbamidines reacted uniquely with lysosomes, experiments were performed in which retinol-induced release of malate dehydrogenase (a soluble mitochondrial enzyme) was also determined. Retinol has been shown to act, indiscriminately as it were, on biomembranes of diverse sources, e.g. those of erythrocytes, mitochondria and lysosomes.[10, 13, 14] It is apparent (Fig. 4) that pretreatment of the

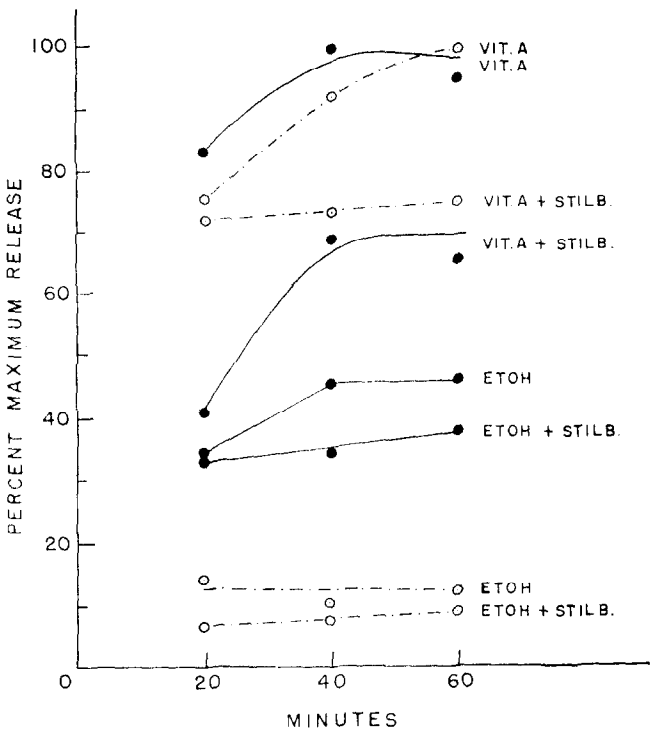

FIG. 4. Effect of stilbamidine (10^{-4} M) on release of cathepsin (●—●) and malate dehydrogenase (⊙—·—⊙) from large granule fractions of rabbit liver by retinol (vitamin A, 5×10^{-4} M) or incubation with solvent (ethanol, 2·0%, v/v). Results are expressed as percentage of maximum activity released by vitamin A (see Fig. 2 dot-dashed line).

liver granules by stilbamidine retarded vitamin A-induced release of malate dehydrogenase. At 10^{-4} M (Fig. 4), stilbamidine retarded release of cathepsin from large granule fractions of rabbit liver at 20, 40 and 60 min. Indeed, as in the case of lysosomal hydrolases, stilbamidine-treated granules released less malate dehydrogenase into the supernatant after simple incubation with solvent (lowest line, Fig. 4).

Effect of stilbamidines upon release of acid hydrolases by diethylstilbestrol and ultraviolet irradiation. The above experiments indicated that the diamidines protected lysosomes and mitochondria against the effects of incubation with retinol or ethanol.

To determine whether the spectrum of protection extended to other agents capable of disrupting lysosomes, granule fractions were exposed to diethylstilbestrol. This steroid is one of several which disrupts biomembranes, including those of lysosomes.[15] Hydroxystilbamidine, at 10^{-4} M, effectively retarded release of aryl sulfatase both by diethylstilbestrol (Fig. 5) and its solvent (dimethylsulfoxide). Furthermore, the effect of stilbamidine upon the ultraviolet-mediated release of aryl sulfatase was tested.

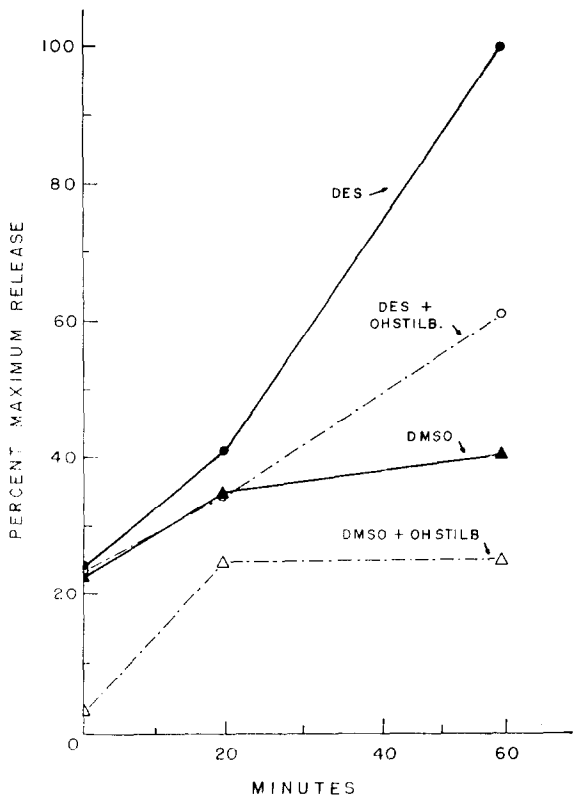

FIG. 5. Effect of hydroxystilbamidine (10^{-4} M) upon release of aryl sulfatase from large granule fractions of rabbit liver induced by diethylstilbestrol (DES, 5×10^{-4} M) or incubation with solvent (DMSO, 2·0%, v/v). Results are expressed as percentage of maximum activity released by steroid.

Ultraviolet irradiation induces lipid peroxide formation in such suspensions, with subsequent release of hydrolases from injured, membrane-bound granules.[10] As with other procedures, enzyme release provoked by ultraviolet irradiation (Fig. 6) was diminished if the granule fractions had been pretreated with stilbamidine.

Effect of stilbamidine on rabbit ear cartilage collapse and hair loss. Previous studies have documented that the depletion of cartilage matrix induced by excess of vitamin A is mediated by the release of lysosomal cathepsins from chondrocytes.[16, 17] Although the exact mechanism of hydrolase release remains unclear, both cartilage matrix depletion in intact animals (manifest by the gross collapse of rabbit ears) and similar lesions in embryonic bone rudiments *in vitro* proceed via a lysosomal mechanism.

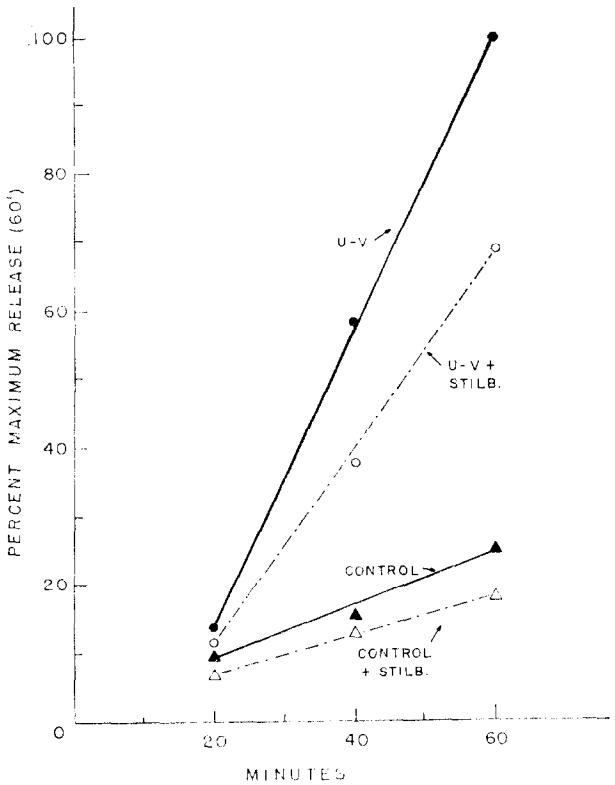

FIG. 6. Effect of stilbamidine (10^{-4} M) upon release of aryl sulfatase from large granule fractions of rabbit liver by mercury arc irradiation (u.v.). Results are expressed as percentage of maximum activity released by irradiation; controls are non-irradiated samples.

TABLE 4. EFFECT OF VARIOUS TREATMENTS ON COLLAPSE OF RABBIT EAR CARTILAGE AND HAIR LOSS

		Day					
		1		2		3	
Daily treatment	No. of rabbits	Ear droop*	Hair loss*	Ear droop	Hair loss	Ear droop	Hair loss
Corn oil (control) (1 cc)	4	0	0	0	0	0	0
Vitamin A in oil (5×10^5 i.u.)	8	0	0	6	5	6	8
Vitamin A + stilbamidine (5×10^5 i.u.) (25 mg/kg)	8	0	0	0	2	2	4
Stilbamidine	4	0	0	0	0	0	0

* Judged as in reference 9.

Therefore ear cartilage collapse was induced in rabbits by means of i.p. vitamin A. As with dosage regimens involving retinoic acid (i.p. or orally administered)[9] or vitamin A palmitate (orally),[10] gross collapse of rabbit ears was induced in most animals (Table 4). This was accompanied by severe loss of hair, a finding which is one of the earliest visible results of the induction of hypervitaminosis A.[9, 10] In the

majority of animals treated with stilbamidine concurrently with vitamin A, these effects were delayed, and only two showed any evidence of ear collapse. Neither the animals injected with vehicle alone nor those injected with stilbamidine showed any gross lesions in cartilage or skin.

Effects of stilbamidines upon lymphocyte transformation. Since earlier studies had suggested that redistribution of lysosomal hydrolases (but not of mitochondrial dehydrogenases) is associated with the early stages of lymphocyte transformation, we examined the effect of stilbamidine and hydroxystilbamidine in this system.[11, 12] To measure the stimulation of lymphocytes by phytohemagglutinin (PHA), incorporation of uridine into RNA was determined. At concentrations of 5×10^{-4} M and 10^{-4} M, stilbamidine and hydroxystilbamidine inhibited uridine incorporation into acid-precipitable material by approximately 20 per cent during the first 3 hr after PHA stimulation (Table 5). However, hydroxystilbamidine (5×10^{-4} M) inhibited RNA

TABLE 5. EFFECT OF STILBAMIDINE AND HYDROXYSTILBAMIDINE UPON URIDINE-2-^{14}C INCORPORATION INTO ACID-PRECIPITABLE MATERIAL BY CULTURED HUMAN PERIPHERAL BLOOD LYMPHOCYTES*

Drug	Drug present (hr)	Control	Control + drug (5×10^{-4} M)	PHA	PHA + drug concn (M) 5×10^{-4}	1×10^{-4}	5×10^{-5}
Hydroxy-stilbamidine	0–3	3864	4213	8494	6813	7151	8998
	24–27	4056	3087	40,224	32,266	35,093	38,091
	24–27	5607	4647	80,627	64,543	76,793	78,904
	0–27	2256	1870	58,808	23,258	40,674	48,956
	0·5–4	4843	3535	7351	5571		
	(−)0·5–3	4767	2729	8201	6729		
Stilbamidine	0–3	4715		9343	7544	8264	8834

* Purified lymphocytes were preincubated in complete media overnight (see text). PHA-P was added at 0 time, the drug added for the time intervals indicated, and uridine-2-^{14}C added for the last 3 hr of culture (see text for details). Results are given as dpm's and represent the mean of duplicate cultures.

synthesis by 20 per cent whether present for 3 hr at the onset of stimulation or for 3 hr at 24–27 hr after stimulation, a time at which these cells are entering the DNA synthetic phase. No difference could be observed between results obtained by addition of hydroxystilbamidine for 3·5 hr beginning 30 min before PHA or those obtained by addition of hydroxystilbamidine for 3·5 hr beginning 30 min after PHA. Hydroxystilbamidine (5×10^{-4} M) apparently inhibited RNA synthesis in unstimulated control cells to the same extent as in stimulated cells.

DISCUSSION

The studies detailed above indicate that stilbamidines inhibit release of acid hydrolases from lysosome-rich fractions of rabbit liver. Although this effect was studied chiefly after granule fractions were exposed to retinol *in vitro,* it could also be demonstrated when hydrolases were released by other agents or procedures. These included simple incubation at 37° in the presence of trace amounts of solvent (ethanol, dimethylsulfoxide), addition of diethylstilbestrol, or ultraviolet irradiation. At the

concentration at which optimum "stabilization" was noted (10^{-4} M), neither stilbamidine nor hydroxystilbamidine inhibited the activity of aryl sulfatase or cathepsin, once granules had been entirely disrupted by means of the detergent, Triton X-100. These observations suggest that diamidines acted by inhibiting the release of enzymes from membrane-bound particles rather than by directly interacting with hydrolases. Stilbamidines prevented release of hydrolases both by relatively mild disruptive procedures (incubation with solvents) and by more drastic means (ultraviolet irradiation, retinol). It would therefore appear unlikely that nonspecific or salt-like precipitates formed by the diamidines with newly solubilized enzymes accounted for failure of hydrolases to appear in the 20,000 *g* supernatants. Were this the case, treatments which rendered relatively little enzyme activity soluble (ethanol, dimethylsulfoxide) should have been antagonized more effectively than such harsher treatments, as with retinol. Indeed, the observation that concentrations of approximately 10^{-4} M were optimum for "stabilization" against several agents indicates that the stilbamidines exert a single kind of effect upon granules. However, these studies also suggest that stilbamidines act upon several sorts of biomembranes, since they also serve to retard release of dehydrogenases from mitochondria.

The means whereby stilbamidines serve to protect lysosomes against disruption *in vitro* are as yet unknown. Allison[4] has reported that stilbamidine, like many drugs, dyes and carcinogens, is selectively taken up and concentrated within lysosomes. Indeed, several other drugs useful in trypanosomiasis share this property with stilbamidine; these include the anionic agent, suramin.[4] Neither Dr. Pierre Jacques (personal communication) nor our laboratory has been able to demonstrate that suramin stabilizes the membranes of lysosomes, although this anionic trypanocide acts to inhibit several acid hydrolases *in vivo* and *in vitro*. It is therefore possible that the cationic groupings of stilbamidines are crucial for interaction with lysosomal membranes. Indeed, Barrett and Dingle[18] have isolated an anionic component from isolated lysosomes which can bind other cationic, fluorescent compounds such as acridine orange. Further experiments will have to determine whether this anionic structural component of lysosomes is responsible for stilbamidine–lysosome interactions or whether (like cortisol or chloroquine) the stilbamidines interact with discrete lipids which bound the organelles.[19] Elson,[20] for example, found that phospholipids antagonized the bacteriostatic actions of diamidines, and suggested that the drugs might have membrane activity. Experiments in which the stilbamidines antagonized two biological events, mediated at least in part by lysosomes, are capable of several interpretations other than that the diamidines acted directly on lysosomal membranes. Indeed, retinol-induced cartilage degradation and PHA-induced lymphocyte transformation may not involve simply the direct discharge of lysosomal contents into cytoplasm or surrounding tissues.[21] Lysosomes function as part of a vacuolar system (see review in reference 22) in which the shuttle and flow of the various subgroups of lysosomes (primary lysosomes, heterophagic vacuoles, autophagic vacuoles) may regulate the local access of packets of hydrolases to portions of the cell interior or tissue fluids. Recent studies with two well-studied lysosomal "stabilizers" *in vitro* have shown that cortisol may indeed modify merger of lysosomes with heterophagic vacuoles,[23] and that chloroquine induces the formation of autophagic vacuoles *in vivo*.[24] Thus the capacity of a pharmacologic agent to stabilize lysosomes *in vitro* may not directly reflect its only site of action in the vacuolar system. Indeed, although effects similar to

those of stilbamidines upon PHA-induced transformation and retinol-induced cartilage matrix depletion can be achieved by cortisol or chloroquine,[7,16] this correspondence could well be fortuitous. Since diamidines react well with polynucleotides,[1] their effects on lymphocyte transformation would be equally well explained by virtue of their interference with transcription, translation, or both.

Whatever the mechanism whereby stilbamidines exert their effects in such intact systems, these studies serve to indicate that these agents share with cortisone or chloroquine the capacity to stabilize lysosomal membranes *in vitro*. Moreover, lysosomes appear to constitute the chief sites within the cytoplasm in which both stilbamidine and chloroquine are concentrated. It would be of great interest were the stilbamidines to share other features with cortisol or chloroquine, especially their relative therapeutic efficacy in inflammatory diseases.

Acknowledgements—The experiences of Dr. I. Snapper with stilbamidines in multiple myeloma first attracted our attention to these agents, while the experiments of A. C. Allison on the localization of stilbamidine in lysosomes by fluorescence optics suggested a study of their effects on the membranes of lysosomes. We are grateful for the assistance of Dr. Mark Hoekenga of the Wm. S. Merrell Co., Cincinnati, Ohio, in supplying us with hydroxystilbamidine.

REFERENCES

1. E. B. Schoenbach and E. M. Greenspan, *Medicine, Baltimore* **27**, 327 (1948).
2. I. Snapper, L. McVay and B. Schneid, *Am. J. Med* **15**, 603 (1953).
3. I. Snapper, *J. Mt Sinai Hosp.* **15**, 156 (1948).
4. A. C. Allison, in *The Interaction of Drugs with Subcellular Components of Animal Cells* (Ed. P. N. Campbell), p. 218. Churchill, London (1968).
5. G. Weissmann, W. Troll, B. L. Van Duuren and G. Sessa, *Biochem. Pharmac.* **17**, 2421 (1968).
6. G. Weissmann, in *The Interaction of Drugs with Subcellular Components of Animal Cells* (Ed. P. N. Campbell), p. 203. Churchill, London (1968).
7. R. Hirschhorn, K. Hirschhorn and G. Weissmann, *Blood* **30**, 84 (1967).
8. G. Weissmann, I. Spilberg and K. Krakauer, *Arthritis Rheum.* **12**, 103 (1969).
9. L. Thomas, R. T. McCluskey, J. Li and G. Weissmann, *Am. J. Path.* **42**, 271 (1963).
10. G. Weissmann and L. Thomas, *Recent Prog. Horm. Res.* **20**, 215 (1964).
11. G. Brittinger, R. Hirschhorn, S. D. Douglas and G. Weissmann, *J. Cell Biol.* **37**, 394 (1968).
12. R. Hirschhorn, G. Brittinger, G. Weissmann and K. Hirschhorn, *J. Cell Biol.* **37**, 412 (1968).
13. J. T. Dingle and J. A. Lucy, *Biochem. J.* **84**, 611 (1962).
14. J. A. Lucy, M. Luscombe and J. T. Dingle, *Biochem. J.* **89**, 419 (1963).
15. G Weissmann, *Biochem. Pharmac.* **14**, 537 (1965).
16. G. Weissmann and L. Thomas, *Clin. Invest.* **42**, 661 (1963).
17. J. A. Lucy, J T. Dingle and H. B. Fell, *Biochem. J.* **79**, 500 (1961).
18. A. J. Barrett and J. T. Dingle, *Biochem. J.* **105**, 20P (1967).
19. A. D. Bangham, M. M. Standish and G. Weissmann, *J. molec. Biol.* **13**, 253 (1963).
20. W. O. Elson, *J. biol. Chem.* **154**, 717 (1944).
21. G. Weissmann, in *Lysosomes* Vol. I (Eds. H. B. Fell and J. T. Dingle), p. 276, North Holland, Amsterdam and London (1969).
22. C. deDuve and R. Wattiaux, *Am. Rev. Physiol.* **28**, 435 (1966).
23. L. Merkow, M. Pardo, S. M. Epstein, E. Verney and H. Sidransky, *Science, N.Y.* **160**, 79 (1968).
24. M. E. Fedorko, *Lab. Invest.* **18**, 27 (1968).