Biochemical Pharmacology, Vol. 20, pp. 2861–2870. Pergamon Press, 1971. Printed in Great Britain

DISSIMILAR EFFECTS OF ANTI-INFLAMMATORY DRUGS ON STABILITY OF LYSOSOMES FROM PERITONEAL AND CIRCULATING LEUKOCYTES AND LIVER

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(*Received* 24 *November* 1970; *accepted* 26 *March* 1971)

Abstract—Steroidal and nonsteroidal anti-inflammatory drugs were tested for their capacity to stabilize, *in vitro*, lysosomes obtained from rabbit liver and from circulating total leukocytes of rats and rabbits. Lysosome membrane stability was measured by determining the effects of drugs on the release of acid phosphatase and β-glucuronidase from lysosomes. Rabbit liver lysosomes (sedimenting in the 3500 *g* fraction) were found to be highly susceptible to membrane stabilization by chloroquine, hydrocortisone and certain nonsteroidal anti-inflammatory drugs. On the other hand, none of the drugs tested stabilized lysosomes from circulating rat or rabbit leukocytes. In fact, most of these drugs markedly labilized such lysosomes, thus resulting in enhanced release of lysosomal marker enzymes into the incubation medium. Chloroquine and glucocorticosteroids were the only anti-inflammatory drugs found to stabilize appreciably the lysosomes obtained from rabbit peritoneal polymorphonuclear leukocytes. Nonsteroidal drugs such as phenylbutazone, indomethacin, acetylsalicylic acid and flufenamic acid induced marked labilization of these lysosomes.

RECENT studies from our laboratory[1] have indicated that a crude heavy liver fraction from rat (3500 *g*) contains lysosomes which are susceptible to anti-inflammatory drug-induced membrane stabilization *in vitro*. In addition, certain experimental conditions were found to be essential in order to maintain lysosome membrane integrity *in vitro* and thereby demonstrate drug-induced membrane stabilization. Tanaka and Iizuka[2] had reported earlier that some anti-inflammatory drugs stabilize moderately lysosomes contained within a similar crude rat liver fraction.

The data presented in this report illustrate that a heavy (3500 *g*) liver fraction of the rabbit contains lysosomes that possess membrane properties similar to those of lysosomes from the heavy liver fraction of the rat in their susceptibility to drug-induced membrane stabilization. However, it appears from these preliminary studies that a heterogeneous population of lysosomes obtained from circulating leukocytes (mixture of granulocytes, lymphocytes and monocytes) of the rat and rabbit are not stabilized by anti-inflammatory drugs. Instead, the drugs markedly labilize such lysosomes.

Although a number of studies have been published dealing with lysosomes from rabbit peritoneal polymorphonuclear (PMN) leukocytes,[3–5] the capacity of anti-inflammatory drugs to induce membrane stabilization of such lysosomes has not been demonstrated. Results presented here indicate that, of all the anti-inflammatory drugs

tested, only chloroquine, hydrocortisone and paramethasone possess the capacity to stabilize rabbit peritoneal PMN leukocyte lysosomes. Most of the other drugs labilize these lysosomes.

MATERIALS AND METHODS

Drugs. Sources of drugs and preparation of drug solutions were reported previously.[1]

Preparation of rabbit liver lysosome fraction. Male albino rabbits (1 kg) were sacrificed by a blow to the head and then decapitated and exsanguinated. All subsequent procedures, including preparation of liver fractions, incubation of lysosomes and drugs, and measurement of lysosomal enzyme activities, have been described previously.[1]

Preparation of lysosomes from rat and rabbit circulating leukocytes. Rats (300 g, male Sprague–Dawley, Charles River) were anesthetized lightly with ether and 10 ml blood was withdrawn by direct cardiac puncture using a heparinized syringe. A similar volume of blood was obtained from unanesthetized rabbits (male albino, 2–3 kg) via an ear vein. Whole blood was first allowed to stand at 25° for 30 min and then centrifuged at low speed (250 *g*) for 4 min at 25° in a Clinical International table-model centrifuge. Plasma, the buffy white layers of leukocytes, and platelets were carefully removed and centrifuged at 1000 *g* (for 5 min at 4°) in a Sorvall RC 2-B centrifuge. Supernatants were discarded and the pellets resuspended each in 10 ml of cold 0·45 M sucrose–0·04% glycogen–0·02 M tris acetate, pH 7·4, contained in 15-ml Ten Broeck tissue grinders. Standard loose-fitting glass pestles were employed manually to disrupt the intact cells by executing ten complete strokes of the pestle. Homogenates were then centrifuged at 1000 *g* (for 10 min at 4°) to sediment broken and intact cells. Pellets were discarded and the supernatants centrifuged at 15,000 *g* (for 20 min at 4°). The resulting supernatants were discarded and the pellets, containing lysosomes, were resuspended each in 4 ml of cold 0·45 M sucrose–glycogen buffer according to the following procedure. First, a siliconized glass rod was employed to disperse the pellet in the suspending medium. Then the 4 ml of heterogeneous suspension was sucked up into a 5 ml siliconized pipette and blown out gently a total of three times to make a fine homogeneous suspension.

The granule pellets prepared by the procedures described constitute a heterogeneous population of organelles or particles including lysosomes which were obtained from a mixture of circulating leukocytes and platelets. No attempts were made to separate and isolate individual white cell types. A leukocyte differential count, employing Tetrachrome stain (MacNeal) or Giemsa stain (Paragon), was performed on the resuspension (0·45 M sucrose–glycogen buffer) of the buffy white layers containing leukocytes, platelets and some erythrocytes. Suspensions of rat blood cells contained $1{\cdot}2 \times 10^4$ total leukocytes per mm^3 of final suspension (10 ml). Expressed as per cent of total leukocytes, the differential white cell count revealed 26 per cent neutrophils, 70 per cent lymphocytes, 2 per cent monocytes and 2 per cent eosinophils. Only a trace of basophils (less than 0·5 per cent) was found. Suspensions of rabbit blood cells contained $8{\cdot}8 \times 10^3$ total leukocytes per mm^3 of final suspension (10 ml). Expressed as per cent of total leukocytes, the differential count revealed 49 per cent neutrophils, 38 per cent lymphocytes, 7 per cent monocytes, 4 per cent basophils and 2 per cent eosinophils. The blood platelets were also present in both the rat and rabbit leukocyte suspensions. The mean per cent values indicated above did not vary appreciably from animal to animal.

The procedures employed in the preparation of the final suspensions containing leukocyte-derived lysosomes were monitored microscopically beginning with the white cell differential. Homogenization of cell suspensions resulted in the disruption of about 86 per cent (82–90 per cent) and 78 per cent (73–83 per cent) of total leukocytes obtained from rat and rabbit respectively. Supernatants obtained after centrifugation (1000 *g*) contained no appreciable amounts of intact or broken cells, nuclei or cellular membrane debris. The final suspensions of intracellular granules were homogeneous (no agglutinated masses of granules) and free of cellular debris. No ultrastructural studies on these suspensions were performed.

Preparation of lysosomes from rabbit peritoneal PMN leukocytes. Lysosomes from rabbit peritoneal PMN leukocytes were prepared essentially according to the method reported by Cohn and Hirsch.[4] Briefly, leukocytes were harvested from peritoneal exudates obtained from male albino rabbits (3 kg) 4 hr after 100 ml of 0·1% (w/v) glycogen in sterile saline had been injected. Heparinized saline (100 ml) was injected intraperitoneally through a 13 gauge hypodermic needle and the syringe disconnected from the needle. By careful application of pressure to the abdomen, PMN leukocytes were obtained in turbid suspension (75–100 ml) with a minimum of erythrocyte contamination. Over 95 per cent of the leukocytes obtained in this manner were polymorphonuclear. Monocytes were usually absent and when present constituted less than 2 per cent of the total leukocyte population. The suspension (containing a total of about 1×10^8 cells) was centrifuged at 250 *g* (for 5 min at 4°). The cell pellet was resuspended in cold 0·35 M sucrose–0·02 M tris acetate, pH 7·4 (containing 25 U.S.P. units of heparin/ml), and the suspension centrifuged again as above. The resulting pellet was resuspended in 15 ml of 0·35 M sucrose buffer (heparinized) and a 25-ml narrow mouth pipette was employed (by vigorously sucking in and blowing out the suspension a total of three times) to liberate intact granules from the leukocytes. This procedure resulted consistently in the disruption of about 70 per cent (65–75 per cent) of the intact PMN leukocytes. The suspension was centrifuged at 600 *g* (for 10 min at 4°) to sediment intact cells, nuclei and other cellular debris and the resulting supernatant was centrifuged at 7500 *g* (for 15 min at 4°). The granule pellet, containing lysosomes, was resuspended in 6 ml of 0·35 M sucrose–0·02 M tris acetate, pH 7·4, containing 25 U.S.P. units of heparin per ml. A 5 ml pipette was employed (by gently sucking in and blowing out the heterogeneous suspension a total of three times) to obtain a fine homogeneous suspension. This final suspension containing lysosomes was examined microscopically and found to be virtually free of contaminating cells and cellular debris. No ultrastructural examinations of the granule suspensions were made.

Assays of lysosome membrane stability. Labilization or stabilization of lysosome membranes was ascertained by determining the release of lysosomal marker enzymes. Aliquots (0·2 ml) of each of the various lysosome suspensions were added to glass tubes containing 2 ml of the indicated sucrose–tris acetate buffer, pH 7·4, at 25°, with or without added drug and incubated at 37° for 15 min in a metabolic shaker set at 75 agitation cycles per min. Incubation was terminated by high speed centrifugation (27,000 *g* for 15 min at 4°) after transfer of the contents from glass tubes to 15-ml polyethylene tubes. One-ml aliquots of resulting supernatants were employed for the measurement of lysosomal enzyme activity.

Lysosomal enzyme assays. Details of the methods employed to measure acid phosphatase (EC 3.1.3.2, orthophosphoric monoester phosphohydrolase) and β-

TABLE 1. EFFECT OF DRUGS ON STABILITY OF RABBIT LIVER LYSOSOMES *IN VITRO*

Drug	Marker enzyme	Per cent inhibition of release of enzyme*			
		10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
Phenylbutazone	βG†	81‡	31	15	0
	AP†	80	35	10	0
Oxyphenbutazone	βG	42	15	0	0
	AP	40	15	0	0
Indomethacin	βG	23	42	15	0
	AP	20	40	10	0
Acetylsalicylic acid	βG	36	21	3	0
	AP	40	20	0	0
Flufenamic acid	βG	26§	22	33	11
	AP	30§	30	35	10
Mefenamic acid	βG	0	0	0	0
	AP	0	0	0	0
Meclofenamic acid	βG	26	7	0	0
	AP	25	3	0	0
Niflumic acid	βG	78	48	19	0
	AP	80	50	20	0
Chloroquine	βG	100	82	33	20
	AP	100	90	40	25
Hydroxychloroquine	βG	82	45	18	0
	AP	80	45	16	0
Hydrocortisone	βG	47	28	14	0
	AP	50	30	16	0
Paramethasone	βG	55	31	11	0
	AP	58	32	12	0
Ibufenac	βG	0	0	0	0
	AP	0	0	0	0
Gold sodium thiomalate	βG	0	0	0	0
Imuran	βG	0	0	0	0
	AP	0	0	0	0
Chlorpromazine	βG	24§	0	0	0
	AP	25§	0	0	0
Tripellenamine	βG	0	0	0	0
	AP	0	0	0	0

* Aliquots (0·2 ml) of 3500 *g* liver fraction and drugs were incubated in 2 ml of 0·18 M sucrose–0·04 M tris acetate, pH 7·4, for 15 min as described previously. Data represent the means of two separate experiments. Values varied by less than 10 per cent of the corresponding mean. Values of 20 per cent or greater were significant ($P < 0·05$).

† βG, beta-glucuronidase; AP, acid phosphatase.

‡ Actual extinction values for controls (incubations of aliquots of liver suspension without drugs) were 0·34–0·39 (β-glucuronidase) and 1·18–1·28 (acid phosphatase).

§ These values signify per cent increase in release of marker enzymes.

glucuronidase (EC 3.2.1.31, β-glucuronide glucuronohydrolase) activities were described previously.[1]

RESULTS

Effect of drugs on stability of rabbit liver lysosomes. The data in Table 1 indicate that certain steroidal and nonsteroidal anti-inflammatory drugs prevent the release of acid phosphatase and β-glucuronidase from granules (lysosomes) present in the 3500 *g* crude rabbit liver fraction. Chloroquine, flufenamic acid and niflumic acid were among the more potent agents found, while mefenamic acid, ibufenac, gold and Imuran were inactive. Chlorpromazine and tripellenamine, drugs which are not antirheumatic agents, did not stabilize lysosomes.

TABLE 2. EFFECT OF INCUBATION TIME, SUCROSE CONCENTRATION AND pH ON STABILITY OF RAT BLOOD LEUKOCYTE LYSOSOMES *IN VITRO*

Incubation time (min)	Release of acid phosphatase*		
	0·025 M sucrose†	0·050 M sucrose†	0·100 M sucrose†
0	0·15 ± 0·01‡ (17)§	0·11 ± 0·01 (13)	0·11 ± 0·01 (13)
15	0·37 ± 0·04 (42)	0·24 ± 0·02 (27)	0·14 ± 0·01 (16)
30	0·46 ± 0·04 (52)	0·30 ± 0·03 (34)	0·17 ± 0·02 (19)

* Expressed on the basis of total unsedimentable enzyme activity, which was determined by incubation of appropriate aliquots of lysosome suspension in 2 ml of 0·2% Triton X-100–0·05 M tris acetate, pH 7·4, for 30 min. Extinction values (405 mμ) for total enzyme activity were 0·88 ± 0·07 (mean ± S.E.) for three experiments.

† Incubation media contain sucrose in 0·05 M tris acetate, pH 7·4.

‡ Values are extinctions (405 mμ) expressed as the mean ± S.E. from three separate experiments.

§ Numbers in parentheses signify per cent of total unsedimentable enzyme activity and were calculated directly from the extinction values.

Stability of rat blood leukocyte lysosomes in vitro. The data in Table 2 indicate that lysosomes from a mixture of total leukocytes of rat blood are unstable and undergo gradual labilization at 37° in sucrose buffer over an incubation period of 30 min. These granules possess minimum stability in 0·025 M sucrose buffer at pH 7·4, about 50 per cent of total unsedimentable acid phosphatase activity having been released after 30 min of incubation at 37°.

In the experiments involving leukocyte lysosomes, determinations of total unsedimentable enzyme activities were conducted by incubation of appropriate aliquots of lysosome suspension in 0·2% (v/v) Triton X-100 in 0·05 M tris acetate, pH 7·4, at 37° for 30 min. Under these conditions, practically complete solubilization of enzymes measured was obtained at the end of a 15–30 min incubation period. Further solubilization of enzymes could not be achieved when incubations were conducted at 4° for up to 24 hr.

Effect of drugs on stability of rat and rabbit blood leukocyte lysosomes. Anti-inflammatory and other drugs were tested and found to decrease the stability of rat blood leukocyte lysosomes *in vitro* (Table 3). Indomethacin and chlorpromazine were the most potent labilizing agents found. Most of the other drugs tested were also found to labilize these lysosomes. Hydrocortisone and paramethasone did not labilize and, in fact, displayed a slight tendency to stabilize these granules. Similar results were obtained for the study employing lysosomes from rabbit blood leukocytes (Table 4).

TABLE 3. EFFECT OF DRUGS ON STABILITY OF RAT BLOOD LEUKOCYTE LYSOSOMES *IN VITRO*

Drug	Per cent increase in release of acid phosphatase*			
	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
Phenylbutazone	133†	60	0	0
Oxyphenbutazone	95	39	0	0
Indomethacin	180	77	25	0
Acetylsalicylic acid	111	10	0	0
Flufenamic acid	125	60	15	0
Mefenamic acid	10	0	0	0
Chloroquine	84	70	26	0
Hydrocortisone	5	5	(5)‡	0
Paramethasone	(8)	(6)	5	0
Ibufenac	16	0	0	0
Chlorpromazine	152	121	37	0
Promazine	117	67	17	0
Chlorpheniramine	60	5	0	0

* Incubation medium: 0·05 M sucrose–0·05 M tris acetate, pH 7·4. Incubations were conducted at 37° for 15 min as described previously. Each value represents the mean of three separate experiments. Values varied by no more than 10 per cent of the corresponding mean. Values of 15 per cent or greater were significant ($P < 0·05$).

† Actual extinction values (405 mμ) for controls (incubations of aliquots of lysosome suspension without drugs) were 0·26 ± 0·02 (mean ± S.E.).

‡ Values in parentheses signify per cent inhibition of release of enzyme.

Stability of rabbit peritoneal PMN leukocyte lysosomes in vitro. The data presented in Table 5 reveal that a time-dependent release of lysosomal marker enzymes from rabbit PMN leukocyte lysosomes at 37° can be obtained using sucrose–tris acetate, pH 7·4. Acid phosphatase and β-glucuronidase are released from lysosomes at similar rates when incubated in 0·12 or 0·18 M sucrose buffer for periods up to 60 min. Since more consistent results were obtained, 0·18 M sucrose buffer was employed for all experiments involving the use of drugs.

Effect of drugs on stability of rabbit peritoneal PMN leukocyte lysosomes. Chloroquine, hydrocortisone and paramethasone were the only anti-inflammatory drugs found to stabilize PMN leukocyte lysosomes *in vitro* (Table 6). Most of the other drugs were found to labilize these lysosomes. Chlorpromazine, in particular, displayed potent lytic properties *in vitro*. Mefenamic acid and ibufenac exerted no appreciable effect on lysosome stability in this system.

TABLE 4. EFFECT OF DRUGS ON STABILITY OF RABBIT BLOOD LEUKOCYTE LYSOSOMES *IN VITRO*

Drug	Per cent increase in release of acid phosphatase*			
	10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
Phenylbutazone	104†	50	5	0
Oxyphenbutazone	58	22	0	0
Indomethacin	191	132	23	5
Acetylsalicylic acid	65	24	4	0
Flufenamic acid	130	59	24	0
Mefenamic acid	22	0	0	0
Chloroquine	83	58	24	0
Hydrocortisone	(23)‡	(9)	0	0
Paramethasone	(22)	(5)	0	0
Ibufenac	4	6	0	0
Chlorpromazine	192	150	84	8
Promazine	139	56	0	0
Chlorpheniramine	104	91	9	0

* Incubation medium: 0·05 M sucrose–0·05 M tris acetate, pH 7·4. Incubations were conducted at 37° for 15 min as described previously. Each value represents the mean of two separate experiments. Values of 20 per cent (increase or inhibition) or greater were significant ($P < 0·05$).

† Actual extinction values (405 mμ) for controls (incubations of aliquots of lysosome suspension without drugs) were 0·35–0·39.

‡ Values in parentheses signify per cent inhibition of release of enzyme.

DISCUSSION

Recent studies have suggested that anti-inflammatory drugs stabilize lysosomes from crude heavy (3500 *g*) rat liver fractions *in vitro*.[1,2] The membrane-stabilizing effects *in vitro* of glucocorticosteroids and chloroquine on lysosomes obtained from light mitochondrial rabbit liver fractions have been reported previously.[6–8] The present study demonstrates that steroidal and nonsteroidal anti-inflammatory drugs stabilize lysosomes from a heavy fraction of rabbit liver. For example, using the 3500 *g* rabbit liver fraction, glucocorticosteroids, chloroquine, phenylbutazone, flufenamic acid, niflumic acid and indomethacin were found to stabilize lysosomes *in vitro*. Mefenamic acid, meclofenamic acid, ibufenac, gold and Imuran were inactive. The latter drugs also exerted no appreciable effect on rat liver lysosomes.[1] It was pointed out in past studies[1,2] that the 3500 *g* fraction of rat liver contained latent hydrolases (lysosomes) that were contaminated with mitochondria and possibly other organelles. The 3500 *g* fraction from rabbit liver is very similar in this regard.

In contrast to the membrane-stabilizing action of anti-inflammatory drugs on liver lysosomes, no such effect was observed on lysosomes from rat or rabbit blood leukocytes. However, it is important to note that in the case of rat and rabbit blood leukocytes, the data reflect the actions of drugs on lysosomes from the total leukocyte population. The lysosome granule preparations represent a complex heterogeneous mixture of organelles derived primarily from neutrophils and lymphocytes (very few monocytes were present). No attempts were made to separate individual cell types. Most of the nonsteroidal anti-inflammatory drugs displayed a marked capacity to labilize lysosomes from the total leukocyte population of rat and rabbit blood. The glucocorticosteroids, hydrocortisone and paramethasone, did not labilize these lysosomes and, in fact, demonstrated significant membrane-stabilizing effects at higher

TABLE 5. STABILITY OF LYSOSOMES FROM RABBIT PERITONEAL POLYMORPHONUCLEAR LEUKOCYTES *IN VITRO*

Incubation time (min)	Marker enzyme	Release of enzyme* 0·12 M sucrose†	Release of enzyme* 0·18 M sucrose†
0	βG‡	0·08 ± 0·005§ (22)‖	0·06 ± 0·004 (17)
	AP‡	0·16 ± 0·02 (13)	0·13 ± 0·01 (11)
15	βG	0·19 ± 0·02 (57)	0·16 ± 0·02 (46)
	AP	0·47 ± 0·04 (39)	0·36 ± 0·04 (30)
30	βG	0·23 ± 0·03 (68)	0·18 ± 0·02 (52)
	AP	0·77 ± 0·06 (64)	0·61 ± 0·04 (51)
60	βG	0·29 ± 0·04 (84)	0·23 ± 0·02 (68)
	AP	0·92 ± 0·12 (77)	0·78 ± 0·09 (65)

* Expressed on the basis of total unsedimentable enzyme, which was determined by incubation of appropriate aliquots of lysosome suspension in 2 ml of 0·2% Triton X-100–0·05 M tris acetate, pH 7·4, for 30 min. Extinction values (mean ± S.E.) for total enzyme activity were 0·34 ± 0·02 (β-glucuronidase) and 1·22 ± 0·11 (acid phosphatase).

† Incubation media contain sucrose in 0·05 M tris acetate, pH 7·4.

‡ βG, beta-glucuronidase; AP, acid phosphatase.

§ Values are extinctions expressed as the mean ± S.E. from three separate experiments.

‖ Numbers in parentheses signify per cent of total unsedimentable enzyme activity and were calculated directly from the extinction values.

concentrations in the case of rabbit blood leukocyte lysosomes. Differences between rat and rabbit blood with regard to the actions of these steroids on leukocyte lysosomes may be a reflection of differences in white cell type between the two species. For example, the neutrophil–lymphocyte ratio for rat blood is about 0·37, while it is 1·3 for rabbit blood. Lysosomes obtained from rabbit peritoneal PMN leukocytes (almost entirely neutrophils) were stabilized markedly by hydrocortisone and paramethasone. These data suggest that the smaller membrane-stabilizing effects observed with rabbit blood and possibly rat blood were due primarily to actions on lysosomes from neutrophils.

Although chloroquine stabilized lysosomes from rabbit peritoneal PMN leukocytes, only a labilizing effect was observed on lysosomes from a mixture of leukocytes from rabbit or rat blood. The heterogeneity of cell types in these blood leukocyte preparations makes interpretation of the data difficult, if not impossible. Chloroquine may have a preferential membrane-stabilizing action on lysosomes from a specific white cell type (i.e. neutrophils), and such an action may be masked by membrane-labilizing effects on lysosomes from other cells. However, no attempts were made to examine

TABLE 6. EFFECT OF DRUGS ON STABILITY OF LYSOSOMES FROM RABBIT PERITONEAL POLYMORPHONUCLEAR LEUKOCYTES *IN VITRO*

Drug	Marker enzyme	Per cent inhibition (stimulation) of release of lysosomal enzyme*			
		10^{-3} M	10^{-4} M	10^{-5} M	10^{-6} M
Phenylbutazone	βG†	(55)‡	(20)	0	0
	AP†	(47)	(16)	0	0
Oxyphenbutazone	βG	(40)	(14)	0	0
	AP	(36)	(12)	0	0
Indomethacin	βG	(80)	(30)	(10)	0
	AP	(60)	(17)	0	0
Acetylsalicylic acid	βG	(30)	(12)	0	0
	AP	(27)	(11)	0	0
Flufenamic acid	βG	(52)	(26)	(8)	0
	AP	(46)	(17)	(10)	0
Mefenamic acid	βG	0	0	0	0
	AP	0	0	0	0
Chloroquine	βG	48	33	20	5
	AP	42	31	18	0
Hydrocortisone	βG	70	45	15	0
	AP	68	50	25	0
Paramethasone	βG	63	50	12	0
	AP	61	41	16	0
Ibufenac	βG	(8)	(0)	(0)	(0)
	AP	(13)	(0)	(0)	(0)
Chlorpromazine	βG	(120)	(75)	(30)	(10)
	AP	(106)	(60)	(23)	(0)
Promazine	βG	(70)	(25)	0	0
	AP	(67)	(18)	0	0
Chlorpheniramine	βG	(26)	0	0	0
	AP	(31)	(5)	0	0

* Incubation medium: 0·18 M sucrose–0·05 M tris acetate, pH 7·4. Incubations were conducted at 37° for 15 min as described previously. Each value represents the mean of three separate experiments. Values varied by no more than 10 per cent of the corresponding mean. Values of 15 per cent (inhibition or stimulation) or greater were significant ($P < 0·05$).

† βG, beta-glucuronidase; AP, acid phosphatase.

‡ Actual extinction values (mean ± S.E.) for controls (incubations of aliquots of lysosome suspension without drugs) were 0·19 ± 0·02 (β-glucuronidase) and 0·39 ± 0·04 (acid phosphatase).

each individual cell type from blood because of technical difficulties encountered in their isolation and separation.

The lysosome membrane-labilizing actions of nonsteroidal anti-inflammatory drugs (except chloroquine) were consistently observed with the various leukocyte preparations employed. Phenylbutazone, indomethacin, acetylsalicylic acid and flufenamic acid labilized lysosomes obtained from either a mixture of leukocytes of rat and rabbit blood or from PMN leukocytes of rabbit peritoneal exudates. The pharmacologic actions of these drugs on leukocyte lysosomes are clearly distinguishable from their actions on lysosomes from the heavy fraction of rabbit liver. Nonsteroidal anti-inflammatory drugs appear to stabilize lysosomes from this fraction of rabbit liver. These drugs were also reported to stabilize lysosomes from a similar fraction of rat liver.[1,9]

A possible explanation for these differences can be advanced in the case of lysosomes obtained from a mixture of leukocytes from rat blood. Lysosomes from this latter source were found to be more stable *in vitro* than lysosomes from the 3500 *g* fraction of rat liver.[1] As the apparent stability *in vitro* of lysosomes increases, a more vigorous incubation procedure is required to induce quantitatively meaningful increments in lysosome labilization. Under these artificial conditions of enhanced membrane fragility, drugs no longer stabilize but actually labilize such lysosomes. These findings are similar to those reported earlier[1] where drugs were found to potentiate the labilization of liver lysosomes which were made more fragile by sodium ion or high incubation temperatures. Such a hypothesis may at least partially explain the labilizing action of drugs on rat blood leukocyte lysosomes, but would not appear to be an adequate explanation in the case of lysosomes from rabbit peritoneal PMN leukocytes, since lysosomes from the latter source do not appear to be any less fragile than those from rat blood leukocytes.

Experiments are in progress to study the effects of environmental conditions *in vitro* on the capacity of drugs to alter membrane stability of lysosomes from rabbit peritoneal PMN leukocytes. Further, in order to gain a batter understanding of the comparative effects of drugs on lysosomes from various sources, it will be necessary to examine in greater detail liver lysosomes that sediment at 3500 *g*.

Acknowledgements—The excellent technical assistance of Miss Natalie Krassikoff is gratefully acknowledged. The author wishes to express his sincere appreciation to Dr. William D. Cash for his valuable assistance and criticism in the preparation of this manuscript.

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