Biochemical Pharmacology, Vol. 21, pp. 875–886. Pergamon Press, 1972. Printed in Great Britain

CHANGES IN LIVER LYSOSOME FRAGILITY, ERYTHROCYTE MEMBRANE STABILITY, AND LOCAL AND SYSTEMIC LYSOSOMAL ENZYME LEVELS IN ADJUVANT-INDUCED POLYARTHRITIS

LOUIS J. IGNARRO and JOSEPH SLYWKA

Department of Biochemistry, Geigy Pharmaceuticals, Division of CIBA-GEIGY Corporation, Ardsley, N.Y. 10502, U.S.A.

(*Received* 9 *July* 1971; *accepted* 17 *September* 1971)

Abstract—Adjuvant-induced polyarthritis in rats is characterized by the development of liver lysosome fragility, and elevated plasma and local hind paw activities of lysosomal acid hydrolases. During the first few days of the disease, there is a marked increase in liver lysosome fragility and a sharp elevation in plasma β-glucuronidase and acid phosphatase. Commencing on day 4, both liver lysosome fragility and plasma levels of acid hydrolases decrease. Liver lysosome fragility and plasma acid hydrolases again increase on day 9 and continue to rise. Between days 4 and 9, when liver lysosomes appear to be stabilized and plasma levels of acid hydrolases are near control values, the onset of erythrocyte membrane stabilization occurs which endures throughout the disease. Acid phosphatase activity in the injected hind paw increases immediately after induction of polyarthritis as does the swelling, whereas enzyme activity in and swelling of the contralateral hind paw increase 9 or 10 days later. Paramethasone, phenylbutazone and indomethacin, administered orally for various periods of time, attenuated the swelling of primary and secondary lesions and reduced the severity of the biochemical lesions examined.

LYSOSOMAL enzymes as mediators of the inflammatory process have received great attention during the past few years.[1–8] Elevated activities of acid hydrolases in inflamed tissues, including rheumatoid synovial membranes, have been demonstrated.[9–17] The usefulness of many steroidal and non-steroidal anti-inflammatory drugs in the alleviation of swelling and pain associated with rheumatoid arthritis has been attributed to the capacity of such drugs to stabilize lysosomes[4,5,18–23] and to inhibit lysosomal enzymes.[24,25]

Adjuvant polyarthritis in rats has been employed widely as a model of chronic systemic inflammation for the detection and evaluation of new anti-inflammatory drugs.[26–31] Several studies suggest that lysosomal enzymes are intimately associated with the pathogenesis of polyarthritis. Piliero and Colombo[32] showed that serum levels of lysozyme increase in rats induced with polyarthritis. After a preliminary publication of the data presented in this paper,[33] three subsequent reports confirming some of our original findings appeared. Collins and Lewis[34] demonstrated that plasma levels of acid phosphatase, β-glucuronidase and *N*-acetyl-β-D-glucosaminidase were elevated in rats with polyarthritis. Anderson[35] reported increased lysosomal enzyme activity in homogenates of adjuvant-injected hind paws. Walz *et al.*[36] confirmed the elevated plasma levels of lysozyme in polyarthritic rats.

In the studies reported here, emphasis was placed on the precise temporal relationship of inflammation and three biochemical parameters: liver lysosome fragility, plasma levels of acid hydrolases and local hind paw concentrations of acid phosphatase. The association of liver lysosome fragility with elevated plasma levels of acid hydrolases and the correlation between swelling and local concentration of acid phosphatase are discussed. Evidence that lysosome membrane stabilization may be one important mechanism of action of effective anti-inflammatory drugs is presented.

MATERIALS AND METHODS

Induction of adjuvant polyarthritis in rats. Rats (200–250 g, Sprague–Dawley, CFE, Carworth Farms) were injected in the sub-plantar region of the left hind paw with 0·05 ml of a fine suspension of *Mycobacterium tuberculosis* in paraffin oil. The suspension was prepared just prior to use by the following procedure. *M. tuberculosis* (lyophilized mixture of strains C, DT, PN obtained from Central Veterinary Laboratory, Weybridge, England) was finely ground in an agate mortar and transferred to a small tissue grinder (Ten Broeck). Paraffin oil was delivered dropwise into the tissue grinder, the contents of which were continually agitated with a "Vortex" mixer, in sufficient quantity to yield a final suspension of 5 mg of *M. tuberculosis* per ml of paraffin oil. The suspension was ground for an additional 2 or 3 min with a motor-driven ground-glass pestle. Ninety-five to 100 per cent of the rats treated in the above manner developed a polyarthritis which was characterized on days 17–21 by swelling of all four paws, increased vascularity of the ears with the formation of nodules, and nodular formation of the tail. Swelling of the paws was measured at various time intervals by weighing the amputated paws.

Measurement of fragility of liver lysosomes. A measure of the relative mechanical and osmotic fragility of liver lysosomes was obtained by determining the degree of redistribution of lysosomal enzymes from the sedimentable fraction to the unsedimentable fraction. Modifications of the methods of Deter and DeDuve,[37] and Guder *et al.*[38] were employed. The amount of lysosomal enzyme activity released, during controlled homogenization in hypotonic sucrose buffer, into the 27,000 *g* supernatant fraction was measured. Rats were sacrificed by decapitation and then exsanguinated. Three separate portions (about 0·7 g) of liver from each rat were excised, weighed, and placed in cold 0·25 M sucrose–0·05 M tris acetate, pH 7·4. After rinsing and mincing, 10 per cent homogenates were prepared in the following manner. One portion of liver was homogenized in 0·09 M sucrose–0·05 M tris acetate, pH 7·4, contained in a Dounce homogenizing vessel (7-ml capacity) by executing exactly 25 up–down strokes with a loose clearance pestle (Dounce pestle A). A second portion of liver was homogenized in 0·06 M sucrose–0·05 M tris acetate, pH 7·4, by the same procedure. A third portion of liver was homogenized in 0·2% (v/v) Triton X-100–0·05 M tris acetate, pH 7·4, contained in a similar Dounce vessel by executing exactly 60 up–down strokes with a tight clearance pestle (Dounce pestle B). Homogenates were maintained on ice for 60 min and then centrifuged at 27,000 *g* for 20 min at 0–4° (Sorvall RC 2-B). Supernatant fractions were removed and assayed for β-glucuronidase or acid phosphatase activity by procedures that have been described previously.[22]

Measurement of lysosomal enzymes in plasma. Rats were anesthetized lightly with Nembutal (50 mg/kg, i.p.), and blood was withdrawn by direct puncture of the left

ventricle. A heparinized 10-ml glass hypodermic syringe fitted with a 19-gauge needle was employed for this procedure. Heparinized blood was centrifuged in a model CL International clinical centrifuge at 1500 *g* for 5 min. The clear plasma layers were removed and aliquots (0·2–0·4 ml) were assayed for acid phosphatase and β-glucuronidase activities.

Measurement of acid phosphatase in hind paws. Rats were sacrificed either by decapitation or exsanguination (cardiac puncture), and both hind paws were amputated just below the tibia. The paws were placed on ice and, working at 0–4°, the skin and claws were removed. Each paw was then weighed and crushed with a 7-in. standard bone crusher, cut into small pieces, and homogenized in sufficient 0·1% (v/v) Triton X-100–0·15 M NaCl–0·05 M tris acetate, pH 7·4, to yield a 20% (w/v) homogenate. Homogenization was conducted for 2 min at full speed (0–4°) with a Virtis "45" homogenizing apparatus equipped with large (1·5 in.) blades. The homogenates were maintained on ice for 60 min and then centrifuged at 27,000 *g* for 20 min at 0–4° (Sorvall RC 2-B). Supernatant fractions (0·2–0·4-ml aliquots) were assayed for acid phosphatase activity.

Measurement of stability of erythrocytes. Blood was withdrawn by cardiac puncture from anesthetized rats as indicated previously. Aliquots of 0·1 ml of whole blood from a given rat were added to seven consecutive glass centrifuge tubes (12-ml capacity) each containing 1·0 ml of one of the following: distilled water, 40 mM NaCl buffer, 50 mM NaCl buffer, 60 mM NaCl buffer, 70 mM NaCl buffer, 80 mM NaCl buffer and 154 mM NaCl (isotonic) buffer. The buffer employed was 0·01 M sodium phosphate, pH 7·0. Following addition of blood, the tubes were mixed gently, incubated for 5 min at 25° and centrifuged at 1500 *g* for 5 min. Aliquots of 0·2 ml of supernatant fractions were added to 4·0 ml of distilled water, and extinctions were measured at 543 mμ in a Bausch & Lomb Spectronic 20 colorimeter.

Administration and testing of drugs. Paramethasone (0·5 mg/kg), indomethacin (1·0 mg/kg), phenylbutazone (25 mg/kg), acetylsalicylic acid (200 mg/kg) and chloroquine phosphate (25 mg/kg) were administered orally to rats daily beginning on day 14 following induction of polyarthritis. Those rats which developed inflammation of both hind paws (95–100 per cent of injected rats) were employed in these studies. Drugs were administered as fine suspensions or as a solution (chloroquine phosphate) in 0·5% carboxymethyl cellulose containing 5% polyethyleneglycol 400, for various periods of time as indicated in the individual experiments (refer to Results). The number of rats per group is also indicated in the individual experiments. In some experiments where drug-treated and control rats were sacrificed by decapitation, livers were excised (for lysosome stability measurements) and paws were amputated, weighed and homogenized as described previously. In other experiments drug-treated and control rats were lightly anesthetized, blood was withdrawn by cardiac puncture and paws were amputated, weighed and homogenized.

RESULTS

Induction of adjuvant polyarthritis. The data in Fig. 1 illustrate the progression in the degree of swelling of both hind paws following a single subplantar injection of adjuvant into the left hind paw. The injected paw begins to swell within several hours and continues for about 3 days. A significant ($P < 0·05$) reduction in the degree of

swelling occurs on days 6 and 7. Beginning at about day 7 swelling of the injected paw increases again and attains maximum intensity on days 17–21. The non-injected paw does not begin to swell until about day 12. Swelling reaches maximum intensity by day 21.

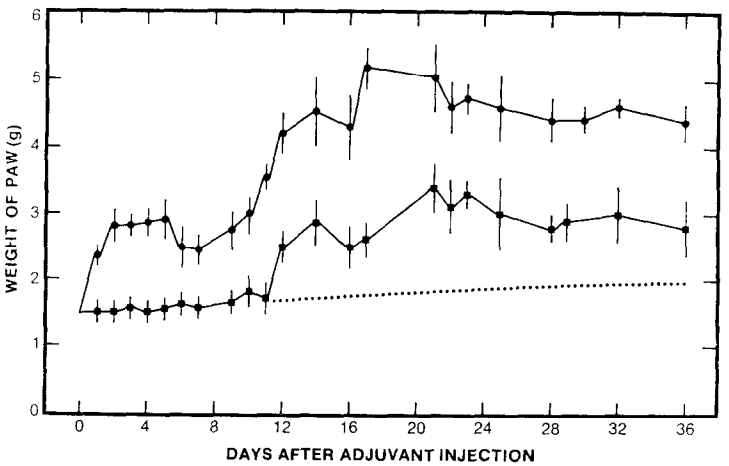

FIG. 1. Increase in weight of hind paws of rats after induction of adjuvant polyarthritis. *Mycobacterium tuberculosis* (0·05 ml of a 5-mg/ml suspension in paraffin oil) was injected into the subplantar region of the left hind paw on day 0. Circles and squares represent the weights of injected (primary lesion) and non-injected (secondary lesion) hind paws, respectively. Each point represents the mean ± S.E.M. obtained from 10 to 16 rats. The dotted line represents the mean (10–16 rats) weights of either hind paw from non-injected control rats; individual values varied by no more than 5 per cent of the corresponding mean. Experiments with control rats were conducted on each of the days that polyarthritic rats were examined.

Effect of adjuvant polyarthritis on liver lysosome fragility. During the first 4 days of the disease, a significant ($P < 0·05$) increase in the fragility of liver lysosomes is evident (Fig. 2), which is characterized by a marked redistribution of lysosomal enzymes from the sedimentable to unsedimentable fraction. Beginning on about day 5, liver lysosome fragility decreases and actually falls below control values on days 7–9. Beginning at about day 11, lysosome fragility again increases and this endures through day 28. Total liver lysosomal enzyme activities do not fluctuate during the course of the disease and are essentially equal to controls.

Effect of adjuvant polyarthritis on plasma levels of lysosomal enzymes. The first 4 days of the disease are characterized by a significant ($P < 0·05$) elevation in plasma acid phosphatase and β-glucuronidase (Fig. 3). A marked decrease in plasma levels of these two enzymes occurs on about day 6 and the levels fall below control values on days 7–11. Beginning on about day 14, acid phosphatase and β-glucuronidase activities in plasma rise sharply and remain elevated through day 28.

Effect of adjuvant polyarthritis on local hind paw activity of acid phosphatase. Local acid phosphatase activity in the injected hind paw (primary lesion) begins to increase 24 hr after induction of polyarthritis and continues to increase through day 5 (Fig. 4). This is followed by a sharp decrease in enzyme activity which lasts through day 11. Subsequently, local acid phosphatase begins to increase at about day 14, attains

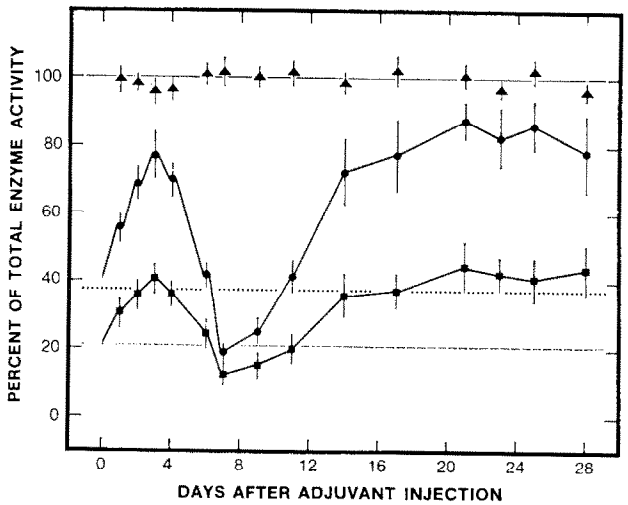

FIG. 2. Effect of adjuvant polyarthritis on liver lysosome fragility in the rat. Triangles represent total unsedimentable β-glucuronidase activity which was determined after disruption of liver lysosomes from polyarthritic rats in Triton X-100. Circles and squares represent unsedimentable β-glucuronidase activity (expressed as per cent of total unsedimentable activity) resulting from homogenization (followed by centrifugation at 27,000 *g*) of liver from polyarthritic rats in 0·06 M sucrose buffer and 0·09 M sucrose buffer, respectively. Each point represents the mean ± S.E.M. obtained from six to ten rats. The solid line signifies the mean (six to ten rats) total unsedimentable β-glucuronidase activity from control rats. The dotted line and dashed line signify the mean (six to ten rats) unsedimentable β-glucuronidase activities (expressed as per cent of total unsedimentable activity) resulting from homogenization and centrifugation of liver from control rats in 0·06 M sucrose buffer and 0·09 M sucrose buffer respectively. The latter experiments were conducted on each of the days that polyarthritic rats were examined; individual values varied by no more than 5 per cent of the corresponding mean. Actual extinction values (540 mμ) for total unsedimentable β-glucuronidase activity were 1·120 ± 0·065 (mean ± S.E.M.) for polyarthritic rats and 1·225 ± 0·088 for control rats. Control values did not change appreciably over a given 30-day period. (One hundred μl of 27,000 *g* supernatant fractions were assayed for enzyme activity in 4 ml of buffer.) Estimations of other extinction values can be obtained directly from the graph.

maximum activity by day 21, and remains elevated through day 36. The activity of acid phosphatase in the non-injected hind paw (secondary lesion) does not begin to increase until day 10. It is of interest that swelling of the non-injected hind paw does not begin until day 12. (Fig. 1). Local enzyme activity in the non-injected paw increases sharply until day 14, after which time the activity remains elevated through day 36.

Effect of adjuvant polyarthritis on erythrocyte membrane stability. The data illustrated in Fig. 5 reveal the progressive decrease in osmotic fragility of erythrocytes during the course of development of the disease. The decrease in osmotic fragility, or increase in erythrocyte membrane stability, begins on day 4 and progresses through day 35. The extent of total hemolysis (in water) at any given time during the development of the disease does not differ from controls.

Effect of anti-inflammatory drugs on paw swelling and biochemical lesions associated with adjuvant polyarthritis. Paramethasone, phenylbutazone and indomethacin were effective in attenuating the swelling of both hind paws (Table 1). Acetylsalicylic acid

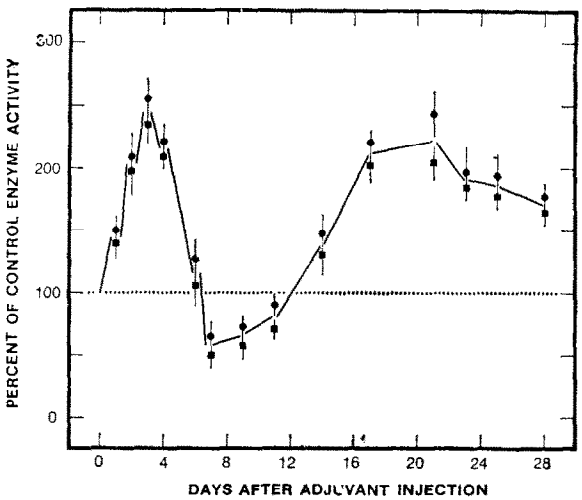

FIG. 3. Effect of adjuvant polyarthritis on plasma levels of acid phosphatase and β-glucuronidase in the rat. Circles and squares represent the activities (expressed as per cent of activities in plasma from controls) of acid phosphatase and β-glucuronidase, respectively, in plasma from polyarthritic rats. Each point represents the mean ± S.E.M. obtained from four to eight rats. The mean activities (four to eight rats) of both enzymes in plasma from control rats were set at 100 per cent as indicated by the dotted line. Experiments were conducted on each of the days that polyarthritic rats were examined. Actual extinction values (mean ± S.E.M.) for acid phosphatase (405 mμ) and β-glucuronidase (540 mμ) activities from control plasma (0·2–0·4-ml aliquots) were 0·231 ± 0·018 and 0·115 ± 0·010 respectively. These control values did not change appreciably over a given 30-day period.

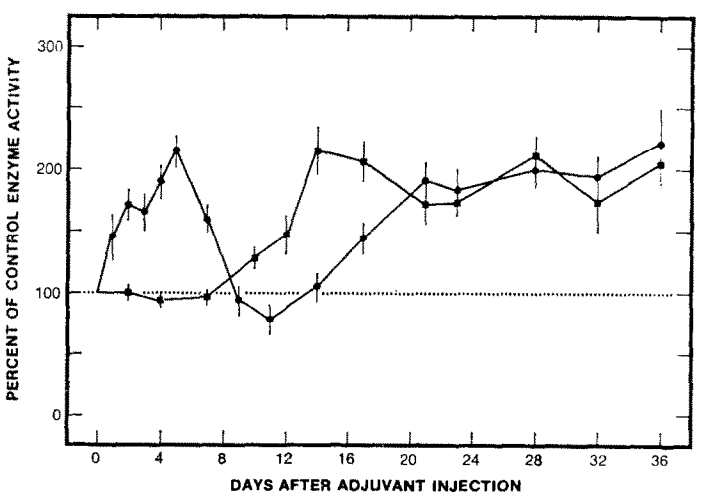

FIG. 4. Effect of adjuvant polyarthritis on local acid phosphatase activity in hind paws of rats. Circles and squares represent the activity (expressed as per cent of activity in paws from controls) of acid phosphatase in injected and non-injected hind paws respectively. Each point represents the mean ± S.E.M. obtained from four to eight rats. The dotted line signifies the mean (four to eight rats) activity of acid phosphatase in either hind paw from control rats; experiments were conducted on each of the days that polyarthritic rats were examined. Actual extinction values (405 mμ) for acid phosphatase activity from control paws (0·2–0·4-ml aliquots of 27,000 *g* supernatant fractions from 20%, w/v, homogenates) were 0·510 ± 0·038 (mean ± S.E.M.). These control values did not change appreciably over a given 30-day period.

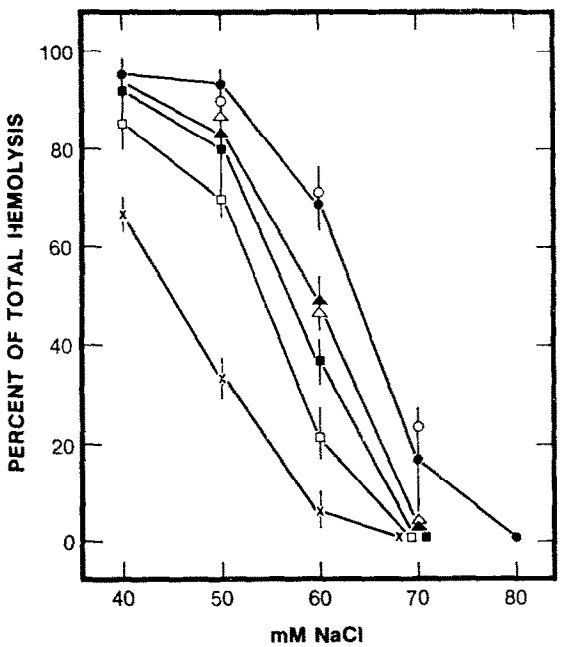

FIG. 5. Effect of adjuvant polyarthritis on erythrocyte membrane stability in the rat. The data represent extent of hemolysis expressed as per cent of total hemolysis in water. Each point represents the mean ± S.E.M. obtained from 3 to 6 rats. Symbols: control, ●; day 1 (disease induced on day 0), ○; day 4, ▲; day 8, △; day 16, ■; day 25, □; day 35, ×. Actual extinction values (543 mμ) for total hemoglobin release (100% hemolysis of 0·1 ml of whole blood in 1·0 ml of distilled water, followed by dilution of 0·2 ml aliquot of supernatant fraction to 4 ml) for control and diseased rats, respectively, were 0·465 ± 0·022 and 0·430 ± 0·021 (mean ± S.E.M.).

and chloroquine, at the doses employed, were ineffective. Administration of paramethasone was terminated on day 21 but significant anti-inflammatory activity remained through day 33. Phenylbutazone and indomethacin were given through day 26; significant anti-inflammatory activity was still apparent for both drugs through day 33.

Paramethasone, phenylbutazone and indomethacin significantly reduced the liver lysosome fragility associated with adjuvant polyarthritis (Table 2). These data are interpreted as a direct stabilizing effect on lysosomes since the drugs reduced the extent of redistribution of acid phosphatase from sedimentable to unsedimentable form. Acetylsalicylic acid and chloroquine were ineffective at the doses employed. Similar results were obtained when β-glucuronidase (phenolphthalein glucuronide as substrate) and aryl sulfatase (*p*-nitrocatechol sulfate as substrate) were measured as marker enzymes. No direct inhibition of these three lysosomal enzymes by any of the drugs tested was observed.[22] For example, total lysosomal enzyme activities of liver from drug-treated polyarthritic rats were not appeciably different than those of liver from control polyarthritic rats.

The elevated plasma levels of acid phosphatase were significantly reduced during the course of treatment with paramethasone, phenylbutazone and indomethacin (Table 3). Significant effects were observed as early as day 21 (paramethasone and

indomethacin) and as late as day 33 (indomethacin). Acetylsalicylic acid showed a very weak effect only after administration of high doses. Chloroquine showed no effect at the dose used in this study.

The elevated local activity of acid phosphatase in the injected hind paws of rats induced with polyarthritis was significantly reduced after treatment with parametha-

TABLE 1. EFFECT OF ANTI-INFLAMMATORY DRUGS ON PAW SWELLING IN RATS WITH ADJUVANT POLYARTHRITIS

Drug (dose)*	Lesion†	Per cent inhibition of swelling			
		Day 15	Day 21	Day 26	Day 33
Paramethasone (0·5 mg/kg)	1°	0	64 ± 11‡	51 ± 20‡	29 ± 3‡
	2°	0	73 ± 17‡	44 ± 8‡	31 ± 6‡
Indomethacin (1·0 mg/kg)	1°	4 ± 1§	32 ± 9‡	61 ± 16‡	39 ± 8‡
	2°	16 ± 10	53 ± 15‡	69 ± 18‡	31 ± 6‡
Phenylbutazone (25 mg/kg)	1°	0	16 ± 12	52 ± 12‡	45 ± 20‡
	2°	0	20 ± 5‡	44 ± 14‡	23 ± 18
Acetylsalicylic acid	1°	0	5 ± 5	15 ± 5	
(200 mg/kg)	2°	0	0	14 ± 8	
Chloroquine (25 mg/kg)	1°	0	0	0	
	2°	0	0	3 ± 4	

* Administration of all drugs (p.o.) was begun on day 14 after induction of polyarthritis. Paramethasone was given once daily for 8 days; all other drugs were given once daily for 13 days.

† Injected and non-injected hind paws, are signified by 1° and 2° respectively.

‡ $P < 0.05$.

§ Each value represents the mean ± S.E.M. obtained from six to ten rats.

TABLE 2. EFFECT OF ANTI-INFLAMMATORY DRUGS ON LIVER LYSOSOME FRAGILITY IN RATS WITH ADJUVANT POLYARTHRITIS

Drug (dose)*	Release of acid phosphatase in 0·06 M sucrose as per cent of total release†‡			
	Day 15	Day 21	Day 26	Day 33
Adjuvant control	74 ± 5§	90 ± 6	74 ± 3	80 ± 4
Paramethasone (0·5 mg/kg)	75 ± 5	38 ± 8‖	60 ± 4‖	73 ± 10
Indomethacin (1·0 mg/kg)	71 ± 6	61 ± 14‖	34 ± 8‖	66 ± 4‖
Phenylbutazone (25 mg/kg)	78 ± 13	74 ± 7‖	46 ± 9‖	70 ± 12
Acetylsalicylic acid				
(200 mg/kg)	79 ± 9	83 ± 8	82 ± 15	
Chloroquine (25 mg/kg)	83 ± 10	82 ± 9	91 ± 11	

* Refer to Table 1 legend for details regarding drug administration.

† Total release of acid phosphatase activity was determined after disruption of liver lysosomes in Triton X-100 as described in the text.

‡ All values represent the release of acid phosphatase activity (expressed as per cent of total release) after homogenization of liver in 0·06 M sucrose buffer as described in the text.

§ Each value represents the mean ± S.E.M. obtained from four to six rats.

‖ $P < 0.05$.

TABLE 3. EFFECT OF ANTI-INFLAMMATORY DRUGS ON PLASMA ACID PHOSPHATASE IN RATS WITH ADJUVANT POLYARTHRITIS

Drug (dosc)*	Acid phosphatase activity as per cent of control†			
	Day 15	Day 21	Day 26	Day 33
Paramethasone (0·5 mg/kg)	102 ± 14‡	68 ± 8§	66 ± 8§	91 ± 10
Indomethacin (1·0 mg/kg)	104 ± 6	79 ± 7§	60 ± 7§	79 ± 8§
Phenylbutazone (25 mg/kg)	94 ± 15	89 ± 9	63 ± 10§	80 ± 10
Acetylsalicylic acid (200 mg/kg)	102 ± 10	97 ± 10	75 ± 12§	
Chloroquine (25 mg/kg)	104 ± 11	108 ± 11	113 ± 11	

* Refer to Table 1 legend for details regarding drug administration.
† Acid phosphatase activity in plasma was measured according to procedures outlined in the text.
‡ Each value represents the mean ± S.E.M. obtained from four to six rats.
§ $P < 0.05$.

sone, phenylbutazone or indomethacin (Table 4). Following the termination of drug administration, individual drug effects began to diminish. However, phenylbutazone still showed significant activity 7 days after the drug was withdrawn. It is of interest that significant anti-inflammatory activity (primary lesion, Table 1) was still evident 7 days after phenylbutazone was withdrawn. Acetylsalicylic acid showed only weak activity after 13 days of administration. Chloroquine exhibited negligible effects at the dose employed.

TABLE 4. EFFECT OF ANTI-INFLAMMATORY DRUGS ON LOCAL HIND PAW ACTIVITY OF ACID PHOSPHATASE IN RATS WITH ADJUVANT POLYARTHRITIS

Drug (dose)*	Acid phosphatase activity as per cent of control†			
	Day 15	Day 21	Day 26	Day 33
Paramethasone (0·5 mg/kg)	95 ± 8‡	54 ± 6§	73 ± 10§	96 ± 11
Indomethacin (1·0 mg/kg)	102 ± 10	58 ± 8§	53 ± 9§	93 ± 6
Phenylbutazone (25 mg/kg)	107 ± 10	64 ± 10§	59 ± 14§	76 ± 8§
Acetylsalicylic acid (200 mg/kg)	96 ± 9	76 ± 18	80 ± 7§	
Chloroquine (25 mg/kg)	104 ± 16	125 ± 14	122 ± 12	

* Refer to Table 1 legend for details regarding drug administration.
† Acid phosphatase activity in injected hind paws of polyarthritic rats and hind paws of control rats was measured according to procedures outlined in the text.
‡ Each value represents the mean ± S.E.M. obtained from four to six rats.
§ $P < 0.05$.

DISCUSSION

The entrance of immune complexes into phagocytic cells by the process of endocytosis is now well documented.[39–41] Movat, Lovett, Taichman *et al.*[42–45] showed that

immune complexes are endocytosed by leucocytes into phagocytic vacuoles (phagosomes) which then unite with primary lysosomes to form more fragile secondary lysosomes. This series of events is followed by extrusion of lysosomal enzymes into the extracellular environment. These investigators also demonstrated that elevation in plasma of cathepsins, β-glucuronidase and acid phosphatase paralleled the endocytosis of immune complexes. Similar findings were reported by Treadwell[46] who examined the role of liver Kupffer cells in systemic anaphylaxis. Shortly after challenge of sensitized animals with antigen or immune complexes, liver lysosomes extruded acid hydrolases thereby elevating plasma levels of these enzymes 6 to 7-fold. The findings in the present report appear to be similar to those just described. Adjuvant polyarthritis in rats is characterized by the development of more fragile liver lysosomes. The close temporal relationship between liver lysosome fragility and elevations in plasma of β-glucuronidase and acid phosphatase suggests that leakage or extrusion of lysosomal enzymes is a direct consequence of enhanced endocytic activity. The demonstration that certain anti-inflammatory drugs, which are known to stabilize lysosomes, reduce the liver lysosome fragility associated with polyarthritis supports this interpretation.

Katz and Piliero[47] showed that the number of polymorphonuclear (PMN) leucocytes in the circulation of rats with polyarthritis increases with the development of secondary lesions. Walz *et al.*[36] reported that elevation in serum lysozyme in polyarthritis[32] is not necessarily a reflection of the number of circulating PMN leucocytes. Serum lysozyme is elevated during days 2–6 of the disease while peripheral blood PMN leucocytes do not increase in number until day 8. These data are similar to our finding of elevated plasma β-glucuronidase and acid phosphatase during days 2–6 of the disease. The concomitant presence of more fragile lysosomes in liver argues in favor of liver rather than peripheral blood leucocytes as the major source of plasma acid hydrolases during the early stages of adjuvant polyarthritis.

During the preparation of this manuscript, Collins and Lewis[34] reported that plasma β-glucuronidase, acid phosphatase and *N*-acetyl-β-D-glucosaminidase were elevated during the early and late stages of adjuvant polyarthritis in rats. The authors suggested that, in view of the elevation of plasma enzymes prior to the development of primary and secondary lesions, damage to lysosomes might precede the inflammatory response. This interpretation is in accord with our demonstration that liver lysosome fragility, elevated plasma acid hydrolases and elevated hind paw acid phosphatase all appear well in advance of the onset of secondary lesions.

Recently, Anderson[35] demonstrated a direct correlation between hind paw edema and lysosomal enzyme activities in paw homogenates from polyarthritic rats. Histological examination of inflamed paws after the onset of secondary lesions revealed extensive leucocyte infiltration. Our experiments were designed to study closely in both hind paws the temporal relationship of swelling and local activity of acid phosphatase. Acid phosphatase activity in the injected hind paw (primary lesion) increased immediately after induction of polyarthritis as did the swelling, while enzyme activity in the non-injected hind paw (a secondary lesion) increased 9 or 10 days later.

Adjuvant polyarthritis in the rat is also distinguished by the development of a marked decrease in osmotic fragility of erythrocytes which begins on day 4 and endures throughout the disease. To our knowledge this is the first time such a phenomenon has been described for this disease model. This enhanced membrane stability

may be related in some manner to the well known increase in erythrocyte sedimentation rate which occurs in polyarthritis and rheumatoid arthritis. The finding reported by Piliero and Colombo,[48] that adjuvant polyarthritis brings forth a stabilizing effect on heat-induced protein coagulation, suggests that changes in the properties of proteins occur during the disease. Similar changes in protein or lipoprotein structure might account for the development of erythrocyte membrane stabilization in polyarthritis.

Paramethasone, phenylbutazone and indomethacin were effective while acetylsalicylic acid and chloroquine were ineffective in alleviating the swelling of both primary and secondary lesions associated with adjuvant polyarthritis. Such findings have been reported previously by other investigators.[27–31] Generally, the capacity of the drugs to attenuate swelling was associated with a similar capacity to reduce liver lysosome fragility, and reduce plasma and hind paw activities of lysosomal enzymes. Paramethasone, phenylbutazone and indomethacin were very effective in reducing the severity of the three biochemical lesions studied.

In a recent report,[35] phenylbutazone and hydrocortisone were shown to reduce the swelling and elevated lysosomal enzyme activities of hind paws in polyarthritic rats. The author concluded that lysosomal enzymes constitute the "common final pathway" of inflammation[19] and that the beneficial action of anti-inflammatory drugs may result from lysosome membrane stabilization, lysosomal enzyme inhibition or decreased infiltration of leucocytes. Evidence for these latter mechanisms of drug action exists. Phenylbutazone,[20–22] paramethasone[22] and indomethacin[22] were shown to stabilize rat liver lysosomes in *vitro*. These three drugs were also reported to stabilize rat liver lysosomes *in vivo*.[23] Phenylbutazone was reported to inhibit directly certain lysosomal acid hydrolases.[24] Inhibition of migration of PMN leucocytes by indomethacin in a model of crystal-induced inflammation in canine joints was demonstrated by Phelps and McCarty.[49] Other mechanisms such as inhibition of merging of membranes of endocytic vacuoles with primary lysosomes[50,51] might also explain partially the actions of these anti-inflammatory drugs.

In summary, the precise temporal relationship of physiological and biochemical lesions in polyarthritis suggests that lysosomal enzymes are intimately involved in the mechanisms associated with the development of inflammatory reactions. Further evidence in support of this view derives from the marked capacity of certain anti-inflammatory drugs to stabilize liver lysosomes, reduce plasma and hind paw activities of lysosomal enzymes towards normal values and attenuate the local swelling associated with polyarthritis.

Acknowledgements—The authors wish to express their sincere appreciation to Dr. William D. Cash for his valuable assistance and criticism in the preparation of this manuscript.

REFERENCES

1. J. T. Dingle, *Biochem. J.* **79,** 509 (1961).
2. J. T. Dingle, J. A. Lucy and H. B. Fell, *Biochem. J.* **79,** 497 (1961).
3. H. B. Fell and L. Thomas, *J. exp. Med.* **114,** 343 (1961).
4. G. Weissmann and J. T. Dingle, *Expl Cell Res.* **25,** 207 (1961).
5. G. Weissmann and L. Thomas, *J. exp. Med.* **116,** 433 (1962).
6. A. Janoff and B. W. Zweifach, *J. exp. Med.* **120,** 747 (1964).
7. C. DeDuve and R. Wattiaux, *A. Rev. Physiol.* **28,** 435 (1966).
8. R. R. A. Coombs and H. B. Fell, in *Lysosomes in Biology and Pathology* (Eds. J. T. Dingle and H. B. Fell), Vol. 2, p. 3. North-Holland, Amsterdam (1969).

9. D. A. KALBHEN, *Archs int. Pharmacodyn. Thér.* **143,** 337 (1963).
10. R. DOMENJOZ and K. MORSDORF, in *International Symposium on Non-steroidal Anti-inflammatory Drugs* (Eds. S. GARATTINI and M. N. G. DUKES), p. 162. Excerpta Medica Foundation, Amsterdam (1965).
11. G. WEISSMANN, *A. Rev. Med.* **18,** 97 (1967).
12. G. COPPI and G. BONARDI, *J. Pharm. Pharmac.* **20,** 661 (1968).
13. C. SMITH and D. HAMERMAN, *Arthritis Rheum.* **5,** 411 (1962).
14. M. LUSCOMBE, *Nature, Lond.* **197,** 1010 (1963).
15. P. BARLAND, A. B. NOVIKOFF and D. HAMERMAN, *Trans. Ass. Am. Phycns* **77,** 239 (1964).
16. G. P. KERBY and S. M. TAYLOR, *Proc. Soc. exp. Biol. Med.* **126,** 865 (1967).
17. G. WEISSMANN, I. SPILBERG and K. KRAKAUER, *Arthritis Rheum.* **12,** 103 (1969).
18. G. WEISSMANN, *Fedn Proc.* **23,** 1038 (1964).
19. G. WEISSMANN, *Arthritis Rheum.* **9,** 834 (1966).
20. K. TANAKA and Y. IIZUKA, *Biochem. Pharmac.* **17,** 2023 (1968).
21. L. J. IGNARRO, *Fedn Proc.* **29,** 784 (1970).
22. L. J. IGNARRO, *Biochem. Pharmac.* **20,** 2847 (1971).
23. L. J. IGNARRO, *Pharmacologist* **13,** 237 (1971).
24. A. J. ANDERSON, *Biochem. Pharmac.* **17,** 2253 (1968).
25. A. J. ANDERSON, *Biochem. J.* **113,** 457 (1969).
26. C. M. PEARSON, *Proc. Soc. exp. Biol. Med.* **91,** 95 (1956).
27. B. B. NEWBOULD, *Br. J. Pharmac. Chemother.* **21,** 127 (1963).
28. M. L. GRAEME, E. FABRY and E. B. SIGG, *J. Pharmac. exp. Ther.* **153,** 373 (1966).
29. C. A. WINTER and G. W. NUSS, *Arthritis Rheum.* **9,** 394 (1966).
30. S. J. PILIERO, M. L. GRAEME, E. B. SIGG, G. CHINEA and C. COLOMBO, *Life Sci.* **5,** 1057 (1966).
31. C. V. WINDER, M. A. LEMBKE and M. D. STEPHENS, *Arthritis Rheum.* **12,** 472 (1969).
32. S. J. PILIERO and C. COLOMBO, *J. Pharmac. exp. Ther.* **165,** 294 (1969).
33. L. J. IGNARRO and J. SLYWKA, *Pharmacologist* **12,** 202 (1970).
34. A. J. COLLINS and D. A. LEWIS, *Biochem. Pharmac.* **20,** 251 (1971).
35. A. J. ANDERSON, *Ann. rheum. Dis.* **29,** 307 (1970).
36. D. T. WALZ, M. J. DIMARTINO, J. H. KUCH and W. ZUCCARELLO, *Proc. Soc. exp. Biol. Med.* **136,** 907 (1971).
37. R. L. DETER and C. DEDUVE, *J. Cell Biol.* **33,** 437 (1967).
38. W. GUDER, K. D. HEPP and O. WIELAND, *Biochim. biophys. Acta* **222,** 593 (1970).
39. R. M. FENNEL and A. SANTAMARIA, *Am. J. Path.* **41,** 521 (1962).
40. F. J. DIXON, *Harvey Lect.* **58,** 21 (1963).
41. L. E. GRANT, M. H. ROSS, J. MOSES, P. PROSE, B. W. ZWEIFACH and R. H. EBERT, *Z. Zellforsch. mikrosk. anat.* **77,** 554 (1967).
42. H. Z. MOVAT, T. URIUHARA, D. L. MACMORINE and J. S. BURKE, *Life Sci.* **3,** 1025 (1964).
43. C. A. LOVETT and H. Z. MOVAT, *Proc. Soc. exp. Biol. Med.* **122,** 991 (1966).
44. N. S. TAICHMAN and H. Z. MOVAT, *Int. Archs Allergy appl. Immun.* **30,** 97 (1966).
45. H. Z. MOVAT, T. URIUHARA, N. S. TAICHMAN, H. C. ROWSELL and J. F. MUSTARD, *Immunology* **14,** 637 (1968).
46. P. E. TREADWELL, *J. Immun.* **94,** 692 (1965).
47. L. KATZ and S. J. PILIERO, *Ann. N.Y. Acad. Sci.* **147,** 537 (1969).
48. S. J. PILIERO and C. COLOMBO, *J. clin. Pharmac.* **7,** 198 (1967).
49. P. PHELPS and D. J. MCCARTY, JR., *Arthritis Rheum.* **9,** 532 (1966).
50. S. E. MALAWISTA and P. BODEL, *J. clin. Invest.* **45,** 1044 (1966).
51. K. T. RAJAN, *Nature, Lond.* **210,** 959 (1966).