Biochemical Pharmacology, Vol. 22, pp. 2069–2078. Pergamon Press, 1973. Printed in Great Britain.

THE EFFECT OF ADJUVANT ARTHRITIS AND DRUGS ON THE ABILITY OF RAT PLASMA TO INHIBIT THE TRITON X-100 INDUCED LYSIS OF RABBIT POLYMORPHONUCLEAR LEUCOCYTE GRANULES

JOHN S. LOWE and ERIC H. TURNER

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England

(*Received* 20 *November* 1972; *accepted* 13 *March* 1973)

Abstract—Plasma from normal rats has the ability to inhibit the Triton X-100 lysis of rabbits polymorphonuclear leucocyte granules. During the development of adjuvant-induced arthritis this activity decreases but no change occurs when rats are given injections of *E. coli* in oil into the foot pad. A study has been made of the levels of stabilizing activity present in the plasma of both normal and arthritic rats treated with either anti-inflammatory drugs or drugs alleged to affect the stability of lysosomal membranes. Paramethasone, prednisolone and menadione prevented the fall in levels during the onset of the adjuvant-induced arthritis but only paramethasone caused an increase in the protective effect when given to normal rats. Chloroquine, cortisone (lysosomal stabilizers) testosterone, oestradiol (lysosomal labilizers) progesterone and fenclozic acid failed to correct the fall in stabilizing activity in arthritic rats but progesterone, oestradiol and chloroquine caused a decrease in stabilizing activity when given to normal rats.

THE PRODUCTION of adjuvant-induced arthritis in rats was first described by Stoerck *et al.*[1] and by Pearson.[2] Since this time the syndrome has become widely used as a routine test for potential anti-inflammatory drugs.[3] The changes occurring in the serum proteins during development of the poly-arthritis have also been thoroughly investigated[4,5] and many workers have studied the possible immunological components,[6–8] but despite all this work, very little is known about the fundamental mechanisms which underlie the development of the condition.

During the last decade increasing attention has been focused on the role of lysosomal enzymes in inflammation and rheumatoid arthritis.[9–11] Recently Anderson[12] has made a study of the role of lysosomal enzymes in adjuvant arthritis and demonstrated that the levels of three lysosomal marker enzymes (β-glucuronidase, acid phosphatase and a collagenolytic enzyme) present in homogenates of hind paws from adjuvant-treated rats increased in parallel with the oedema. He made no attempt to distinguish between free and latent enzyme but showed that the enzyme activity in non-injected hind paws remained low until secondary lesions developed and that there was an extensive leucocyte infiltration of the inflamed paws. It has also been shown that human serum has the ability to inhibit the lysis of rabbit polymorphonuclear leucocyte

(PMNL) granules by Triton X-100 and that this stabilizing activity of the serum increased during the last 3 months of pregnancy.[13]

Taking into consideration the fact that rheumatoid arthritis frequently subsides during the last three months of pregnancy[14] and Anderson's[12] results in the adjuvant arthritic rats, we decided to investigate whether rat serum also possesses the ability to inhibit the lysis of rabbit PMNL granules by Triton X-100. Our preliminary experiments showed that this was the case and we wish to report the changes which occur in lysosomal stabilizing activity during development of adjuvant-induced arthritis and also the effects of drugs on these changes.

MATERIALS AND METHODS

Production of arthritis. Male, specific pathogen-free, albino rats, Alderley Park Strain 1, weighing approximately 200 g were used. Adjuvant-induced arthritis was produced by the injection of 0·075 ml of a fine suspension of dead, methanol dried tubercle bacilli in liquid paraffin B.P. (5 mg/ml) intradermally into the right hind foot pad in the manner described by Newbould.[3] This mixture is subsequently referred to as adjuvant. Fourteen days after the injection of tubercle bacilli the degree of the arthritis was assessed by observing the severity of the secondary lesions on the left hind paw, the two front paws, the ears, nose and tail. The secondary lesions were scored as 0, no lesions; 1, mild; 2, moderate; 3, moderately severe and 4, severe. For the experiments with *Escherichia coli* rats were injected with 0·075 ml of a fine suspension of dead, methanol dried *E. coli* in liquid paraffin (5 mg/ml) intradermally into the right hind foot pad.

Drugs. Progesterone, testosterone and oestradiol were purchased from G. D. Searle & Co. Ltd., prednisolone from Steraloids Ltd., paramethasone from Syntex, cortisone (acetate) and menadione from B.D.H. Chemicals Ltd. Fenclozic acid (I.C.I. 54,450) was synthesized in these laboratories.[15] Solutions for dosing of all drugs were prepared in Dispersol which is a mixture of Lissapol NX (1 ml/l.) Lissapol C (1 g/l.) and a 30% (w/v) solution of Dispersol OG (3·3 ml/l.). Lissapol NX, Lissapol C and Dispersol OG were obtained from I.C.I. Organics Division, Blackley, Manchester.

Rabbit polymorphonuclear leucocyte granules. Rabbit PMNL's were collected from glycogen-induced peritoneal exudates and the granules isolated using the method of Cohn and Hirsch.[16] Batches of granules were made by pooling the PMNL's from several rabbits. The granules from one batch were divided into aliquots (10 ml) and stored at −20°. A separate aliquot was used for each day's work and once a preparation had been thawed it was never re-frozen for further use. One pooled preparation of granules was used for each experiment and each batch of granules was used within 3 days of isolation.

Lysis of polymorphonuclear leucocyte granule membranes. The principle of the method depends upon the scattering of light by the granules (i.e. lysosomes). When the granules are lysed by the addition of Triton X-100 the amount of light scattered decreases proportionally to the number of granules lysed. The lysosomes preparation (500 μl) and plasma under investigation (50 μl) were placed in a semi-micro quartz cuvette (10 mm path length), the contents mixed on a Rotomixer for approximately 5 sec and the optical density of the mixture recorded at 520 nm for 30 sec. After this time the cell was taken out of the spectrophotometer and Triton X-100 (10 μl) added,

the contents of the cell again mixed on a Rotomixer for exactly 5 sec (stop-watch), replaced in the spectrophotometer, allowed to settle for 10 sec and the optical density at 520 nm again recorded for a further 3 min. Blank determinations (i.e. no protection or complete lysis of the granules) were carried out using 0·34 M sucrose in place of the plasma. The optical densities measured at 15 sec after the addition of Triton X-100 were used to calculate the degree of protection afforded by the plasma. All measurements were made with a Cary model 15 recording spectrophotometer using 3 × 3 mm slits; water was used in the blank cell. The concentration of each aliquot of granules was adjusted by dilution with sucrose (0·34 M) so that on addition of sucrose solution (50 μl) and Triton X-100 (10 μl) to the preparation (500 μl) the same fall in optical density always resulted.

The percentage protection was calculated from the formula $100 - 100\,(A/B)$ where A represents the fall in optical density of granules + plasma + Triton X-100 and B the fall in optical density of granules + sucrose + Triton X-100.

Collection of plasma and serum. Blood was withdrawn from the tail vein of each rat into either heparinized tubes or tubes containing no anticoagulant and the plasma or serum separated by centrifugation. Plasma samples were used for most studies and the samples from individual rats obtained at different times during the experiments were stored at −20° until each experiment was completed. The samples were then examined for stabilizing activity on the same batch of freshly isolated granules.

RESULTS

Presence of polymorphonuclear leucocyte granule stabilizing activity in rat plasma. In preliminary experiments plasma and serum from six normal male rats were used to investigate whether normal rat blood possessed the ability to protect rabbit PMNL granules from lysis by Triton X-100. Both plasma and serum gave the same amount of protection (28·67 ± 5·16 per cent and 28·61 ± 5·90 per cent respectively).

Effect of adjuvant-induced arthritis on the polymorphonuclear leucocyte granule stabilizing activity present in rat plasma and the effect of drugs on this activity. Following the demonstration that normal rat plasma could exert some measure of protection for rabbit PMNL granules from lysis by Triton X-100, a preliminary experiment was designed to see whether the degree of this protection altered during the development of adjuvant-induced arthritis. Blood samples were taken from six rats (day −1), they were then injected with adjuvant and samples of blood withdrawn from the tail vein 1, 3, 6, 10 and 14 days after the injection. The protection afforded by each of these samples was then measured. Each rat had marked arthritic lesions (severity 4). The results are summarized in Table 1; it can be seen that as the arthritis develops there is a decrease in the protective effect of the plasma.

TABLE 1. CHANGES IN THE STABILIZING EFFECT OF RAT PLASMA ON RABBIT POLYMORPHONUCLEAR LEUCOCYTE GRANULES AGAINST LYSIS BY TRITON X-100 DURING THE DEVELOPMENT OF ADJUVANT-INDUCED ARTHRITIS

	Time (days after injection of adjuvant)					
	−1	+1	+3	+6	+10	+14
Per cent protection (mean of six rats)	39	27	29	12	12	17

To study this effect in more detail thirty male rats were divided into six groups. Group 1 (six rats) received no treatment and are referred to as normals; group 2 (six rats) were given an injection of adjuvant and are referred to as arthritic; group 3 (six rats) were given an injection of adjuvant and dosed orally with fenclozic acid 20 mg/kg/day; group 4 (six rats) were given an injection of adjuvant and dosed orally with paramethasone 0·5 mg/kg/day; group 5 (three rats) were dosed orally with fenclozic acid 20 mg/kg/day and group 6 (three rats) were dosed orally with paramethasone 0·5 mg/kg/day. All drugs were given for 6 days per week. Blood samples were taken from every rat on the day before the animals of groups 2, 3 and 4 received their injections of adjuvant (day − 1) and subsequently on days 1, 3, 5, 7, 10 and 14 after the injection. (Day of injection referred to as day 0.) Fourteen days after the intradermal injection of adjuvant into the foot pad the severity of the arthritis present in rats of groups 2, 3 and 4 were assessed. Those animals given adjuvant alone (group 2) had scores of 4, 2, 1, 3, 4, 2; those given adjuvant and fenclozic acid (group 3) 2, 2, 0, 0, 0, 3, while those given adjuvant and paramethasone (group 4) failed to develop any secondary lesions. Blood samples were withdrawn from the tail vein and the protection afforded by plasma from each rat in each group against the lysis of PMNL granules by Triton X-100 was determined. The results obtained are summarized in Table 2. With the exception of day +1, the protection afforded by the plasma against the lysis of the granules decreased. This data was subjected to statistical analysis (Student's *t*-test) and the levels of significance are indicated. Paramethasone prevented the fall in plasma levels of stabilizing activity occurring during the development of arthritis and caused an increase in these levels when given to normal rats. In contrast fenclozic acid did not correct the fall in stabilizing activity occurring in the plasma of the arthritic rats, indeed there were occasions (days 1, 3, 7) when the plasma levels were significantly lower than those in the untreated arthritic rats.

Since injection of dead tubercle bacilli in oil into rat foot pads induced both an arthritis and a fall in the plasma lysosomal stabilizing activity, our next experiments were designed to investigate whether the intradermal injection of other bacteria ground in liquid paraffin would produce a similar change in plasma stabilizing action without causing an arthritis. For this purpose six rats were injected with adjuvant in the normal way, six rats with *E. coli* in oil and three rats were retained as untreated controls. Blood samples were collected from each rat on the day before the intradermal injections and subsequently 1, 3, 7 and 14 days after. The rats given the *E. coli* in oil showed no evidence of increase in foot thickness and no secondary lesions developed. In contrast the rats given the adjuvant each developed typical secondary lesions which, on assessment at day 14 proved to be of category 4 severity. The results of the studies on the levels of stabilizing activity present in the plasma are given in Fig. 1; it is clear that the fall in plasma stabilizing activity occurs only in the adjuvant-induced arthritic animals.

There are reports that some drugs stabilize membranes while others cause a labilization.[17] For this reason it was decided to investigate the action of progesterone, testosterone, oestradiol (lysosomal labilizers) cortisone, prednisolone, menadione and chloroquine (lysosomal stabilizers) on the levels of plasma lysosomal stabilizing activity present in both normal rats and rats with developing adjuvant-induced arthritis. Seventy-two male rats were divided into sixteen groups as detailed in Table 3. Blood was withdrawn from the tail vein of each rat in every group on the day before

TABLE 2. VARIATION IN THE STABILIZING EFFECT OF RAT PLASMA ON RABBIT POLYMORPHONUCLEAR LEUCOCYTE GRANULES AGAINST LYSIS BY TRITON X-100 DURING THE DEVELOPMENT OF ADJUVANT-INDUCED ARTHRITIS

Group	Number of rats	Per cent protection 15 sec after adding Triton X-100 on days													
		−1		+1		+3		+5		+7		+10		+14	
		Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
1. Normal	6	36·33	2·10	36·22	1·48	39·33	1·78	36·30	2·99	43·38	0·82	37·68	2·75	43·08	1·44
2. Arthritic	6	37·78	1·98	48·82*	1·93	32·83*	0·94	29·23*	1·01	37·05*	1·87	30·62	2·18	26·23*	2·02
3. Arthritic + fenclozic acid	6	35·75	2·02	36·20†	2·89	24·47*†	1·46	25·50*	1·23	29·40*†	1·96	26·25*	2·10	25·72*	2·83
4. Arthritic + paramethasone	6	36·85	3·59	58·83*†	2·71	67·65*†	1·99	41·32†	3·58	54·55*†	3·39	58·87*†	4·07	42·03†	3·54
5. Normal + fenclozic acid	3	28·70	5·72	23·13	7·86	31·17	3·23	27·23	1·89	29·70*	4·40	26·23	1·88	31·13*	0·73
6. Normal + paramethasone	3	30·90	3·79	57·13*	5·15	55·43	9·22	40·80	2·37	63·13*	1·43	65·23*	1·43	41·43	1·65

* Indicates that when compared with values for normal plasma the P value is 0·005.

† Indicates that when compared with values for arthritic plasma the P value is 0·005.

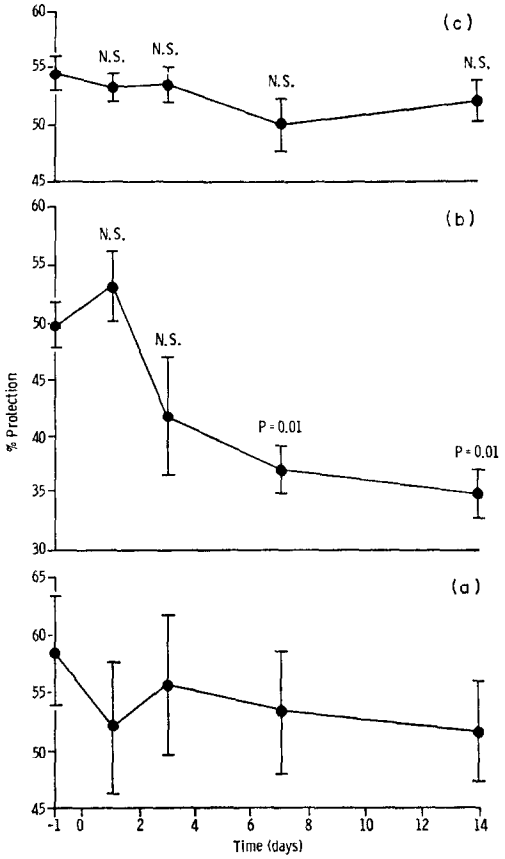

FIG. 1. Variation in the plasma lysosomal stabilizing activity of (a) normal rats (3), (b) rats given an injection of dead tubercle bacilli in oil intradermally into the right hind foot pad (6) and (c) rats given an injection of dead *E. coli* in oil into the right hind foot pad (6). Each point represents the means ± S.E.M. for the numbers of rats indicated. The levels of significant difference from normal values are indicated (*t*-test).

TABLE 3. THE EFFECTS OF VARIOUS DRUGS ON RAT ARTHRITIS

Treatment	Number of rats	Mean weight change (g)	Severity of secondary lesions
Arthritic + progesterone 1 mg/kg/day	6	+16·2	2, 3, 3, 3, 3, 0
Normal + progesterone 1 mg/kg/day	3	+32·0	—
Arthritic + testosterone 1 mg/kg/day	6	+16·3	2, 1, 3, 3, 3, 4
Normal + testosterone 1 mg/kg/day	3	+60·4	—
Arthritic + oestradiol 1 mg/kg/day	6	−12·0	3, 3, 2, 3, 3, 3
Normal + oestradiol 1 mg/kg/day	3	+19·0	—
Arthritic + cortisone 100 mg/kg/day	6	+5·0	2, 0, 1, 2, 1, 2
Normal + cortisone 100 mg/kg/day	3	+26·0	—
Arthritic + prednisolone 20 mg/kg/day	6	+3·0	1, 0, 0, 0, 1, 0
Normal + prednisolone 20 mg/kg/day	3	+17·0	—
Arthritic + menadione 100 mg/kg/day	6	+2·0	0, 0, 1, 0, 2, 3
Normal + menadione 100 mg/kg/day	3	+35·0	—
Arthritic + chloroquine 25 mg/kg/day	6	+12·2	2, 3, 1, 2, 3, 2
Normal + chloroquine 25 mg/kg/day	3	+28·7	—
Arthritic	6	+32·8	2, 0, 0, 4, 3, 3
Normal	3	+57·3	—

TABLE 4. THE STABILIZING EFFECT OF RAT PLASMA ON RABBIT POLYMORPHONUCLEAR LEUCOCYTE GRANULES AGAINST LYSIS BY TRITON X-100 % PROTECTION MEASURED 15 SEC AFTER ADDITION OF TRITON X-100

No. of rats	Treatment	Per cent protection on day							
		−1		+3		+7		+14	
		Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
6	Arthritic + progesterone	39·32	3·69	20·60	2·99	18·72	2·82	16·03	2·66
3	Normal + progesterone	34·30	3·55	28·60	1·37	20·47	1·91	18·27	1·27
6	Arthritic + testosterone	40·60	1·76	28·48	2·38	27·58	2·90	25·92	1·33
3	Normal + testosterone	46·07	6·16	33·93	1·61	31·50	2·16	38·43†	0·81
6	Arthritic + oestradiol	39·00	1·64	13·30*	1·80	16·95	3·18	16·10	2·71
3	Normal + oestradiol	37·30	4·50	29·93	0·57	19·20	3·68	24·30	5·55
6	Arthritic + cortisone	43·52	1·48	29·52	3·67	19·33	2·61	24·58	3·70
3	Normal + cortisone	33·90	3·40	32·20	3·54	31·07	3·16	36·73	3·00
6	Arthritic + prednisolone	51·07	1·15	40·83*	2·72	43·58*	2·20	41·46†	5·34
3	Normal + prednisolone	48·63	2·82	54·13*	1·07	49·23*	1·62	52·33*	1·83
6	Arthritic + menadione	51·70	4·47	41·27*	1·05	39·57†	3·56	34·47	3·39
3	Normal + menadione	50·83	3·51	46·93*	2·27	46·37*	2·83	53·10*	3·68
6	Arthritic + chloroquine	37·43	2·98	17·62†	3·19	13·85*	1·99	10·07	4·32
3	Normal + chloroquine	39·60	5·75	28·30	4·97	18·23	3·51	19·80	2·80
6	Arthritic	47·08	2·33	27·05	1·88	26·05	2·80	22·82	4·05
3	Normal	56·30	6·80	49·17*	8·7	45·30*	5·09	37·2	2·82

* Indicates that when compared with values for arthritic plasma P value is 0·005.
† Indicates that when compared with values for arthritic plasma P value is 0·0025.

the injection of adjuvant and, on the same day, rats in the appropriate groups were dosed orally with the drugs listed in Table 3. Further blood samples were taken from each rat three and seven days after the injection of adjuvant. The animals were weighed throughout the experimental period; after 14 days the severity of the arthritis was assessed (Table 3), blood removed from each rat by cardiac puncture and the rats killed. Each plasma sample was then assayed for its ability to protect rabbit PMNL granules from lysis by Triton X-100; the results obtained are presented in Table 4. Only prednisolone and menadione had any significant action on the severity of secondary lesions and these were the only two drugs which prevented the fall in plasma stabilizing activity. One rat from the six dosed with cortisone had no secondary lesions, two others had mild lesions and the remaining three had lesions of moderate severity. In this group the stabilizing activity of the plasma decreased. Chloroquine did not affect the severity of the arthritis a finding in agreement with previous work.[9] The plasma stabilizing activity fell to very low levels in this group, indeed normal rats dosed with chloroquine also had low plasma levels of stabilizing activity. Essentially similar results were obtained with progesterone and oestradiol. Testosterone did not prevent the fall in plasma stabilizing activity occurring during the onset of arthritis but, unlike chloroquine, oestradiol and progesterone, it did not depress the level when administered to normal rats.

DISCUSSION

Our results show that plasma taken from rats with developing adjuvant-induced arthritis has a decreased ability to protect PMNL granules from lysis by Triton X-100. In contrast, rats given an injection of *E. coli* in oil into the foot pad did not develop arthritis and no alteration in the level of plasma lysosomal stabilizing activity occurred.

Anti-inflammatory drugs can stabilize the membranes of liver lysosomes[17–19] and PMNL granules.[20] We considered it worthwhile investigating whether a range of compounds claimed to either labilize or stabilize lysosomal membranes had any influence on the level of plasma lysosomal stabilizing activity and, if so, whether this action correlated with the ability to ameliorate the severity of the arthritis.

Fenclozic acid, a compound of similar potency to aspirin in the treatment of rheumatoid arthritis[21] failed to correct the fall in plasma stabilizing activity. Since three animals in this group failed to develop secondary lesions it is unlikely that this drug manifests its anti-inflammatory activity directly via lysosomal stability. The most pronounced effects were seen with paramethasone (Table 2) which not only raised levels of stabilizing activity in plasma from normal rats but also in plasma from arthritic animals, none of which developed secondary lesions. Cortisone, prednisolone, chloroquine[17] and menadione[22] are stabilizers of lysosomal membranes. Only prednisolone and menadione prevented the fall in lysosomal stabilizing activity and ameliorated the severity of the arthritis (Tables 3 and 4). Cortisone produced some effect on the arthritis but only one rat failed to develop secondary lesions and levels of lysosomal stabilizing activity were not significantly different from those present in untreated arthritic rats. Rats dosed with either chloroquine or the lysosomal labilizers testosterone and oestradiol[17] had the most consistent secondary lesions; possibly rats treated with progesterone come into this category but one animal in this group failed to develop secondary lesions. Progesterone, chloroquine and oestradiol lowered the stabilizing activity present in plasma of normal rats.

In correlating these results with published work it must be appreciated that practically all the investigations on stabilizing or labilizing effects of drugs on lysosomal membranes has been carried out *in vitro*. We have investigated something entirely different, viz. the ability of an orally administered compound to affect levels of a factor normally present in plasma. There is no *a priori* reason why these two properties should be correlated although with paramethasone, progesterone, testosterone, prednisolone and possibly menadione such might be the case.

Mast cells, lymphocytes and PMNL's accumulate in the non-injected hind feet of rats at the time of the onset of secondary lesions,[23] i.e. when levels of stabilizing activity is low. Such conditions may facilitate the escape of lysosomal enzymes from PMNL granules. If this process occurs then it provides a possible explanation for the mode of action of paramethasone and prednisolone. Alternatively, the results may have little bearing on either mode of action of anti-inflammatory drugs or the mechanism of tissue damage since the lytic agent used is artificial. It has been suggested that phospholipases are freed from leucocyte lysosomes during carageenin-induced inflammation in rats.[24] The accumulation of leucocytes[23] and lysosomal enzymes[12] in the non-injected hind feet of arthritic rats supports the idea that such a process also occurs in this condition. If so, the lytic agents could be phospholipases and lysolecithins which they produce.

Little is known about the nature of the stabilizing activity but it may be associated with a large molecule since activity is retained when serum is dialysed. During development of adjuvant-induced arthritis no significant changes occur in total protein, free fatty acids, lactic acid and serum cholesterol[25] but significant changes are seen in the the serum protein pattern, e.g. a marked fall in albumin, elevation of α_1-globulin, α_2-globulin and seromucoid together with the appearance of an α_2-acute phase protein.[4,5] Stabilizing activity may be associated with these changes. This suggestion has recently been made by Persellin[26] who has shown that rat serum contains a factor which stabilizes rat liver lysosomes from thermal lysis. The amounts of this factor increased during the development of arthritis and decreased as the condition diminished. Serum protein changes and the duration of arthritis vary with the strain of rat* and this may account for the difference between Persellin's results and ours. The significance of these differences is indicated by the fact that in the Holtzman Farm rats used by Persellin the arthritis had subsided 40 days after the injection of adjuvant but in the Alderley Park strain the arthritis is extremely severe at this time.[3] Work in progress is designed to resolve these problems.

Acknowledgements—We are grateful for the skilled technical assistance of Miss J. Lee and Miss H. J. Welford.

REFERENCES

1. H. C. Stoerck, T. C. Bielinski and T. Budzilovich, *Am. J. Path.* **30,** 616 (1954).
2. C. M. Pearson, *Proc. Soc. exp. Biol. Med.* **91,** 95 (1956).
3. B. B. Newbould, *Br. J. Pharmac.* **21,** 127 (1963).
4. J. S. Lowe, *Biochem. Pharmac.* **13,** 633 (1964).
5. H. E. Weimar, F. D. Wood and C. M. Pearson, *Can. J. Biochem.* **46,** 743 (1968).
6. J. R. Ward, R. S. Cloud, E. L. Krawitt and R. S. Jones, *Arthritis Rheum.* **7,** 654 (1964).
7. I. Gery and B. H. Waksman, *Int. Archs. Allergy appl. Immun.* **31,** 57 (1967).
8. C. M. Pearson and F. D. Wood, *J. exp. Med.* **120,** 547 (1964).

* M. E. J. Billingham, personal communication.

9. D. P. Page Thomas, in *Lysosomes in Biology and Pathology* (Eds. J. T. Dingle and H. B. Fell), Vol. 2, p. 89. North-Holland, Amsterdam (1969).
10. G. Weissmann, *A. rev. Med.* **18,** 97 (1967).
11. J. Chayen and L. Bitensky, *Ann. rheum. Dis.* **30,** 522 (1971).
12. A. J. Anderson, *Ann. rheum. Dis.* **29,** 307 (1970).
13. K. H. Hempel, L. A. Fernandez and R. H. Persellin, *Nature, Lond.* **225,** 955 (1970).
14. P. S. Hench, E. C. Kendall, C. H. Slocumb and H. F. Polley, *Ann. rheum. Dis.* **8,** 97 (1949).
15. W. Hepworth, B. B. Newbould, D. S. Platt and G. J. Stacey, *Nature, Lond.* **221,** 582 (1969).
16. Z. A. Cohn and J. G. Hirsch, *J. exp. Med.* **112,** 983 (1960).
17. A. C. Allison, in *Advances in Chemotherapy* (Eds. A. Golden, F. Hawking and R. J. Schnitzer), p. 253. Academic Press, New York (1968).
18. G. Weissmann and J. T. Dingle, *Expl Cell Res.* **25,** 207 (1961).
19. G. Weissmann and L. Thomas, *J. exp. Med.* **116,** 433 (1962).
20. L. J. Ignarro, *Biochem. Pharmac.* **20,** 2861 (1971).
21. T. M. Chalmers, J. H. Kellgren and D. S. Platt, *Ann. rheum. Dis.* **28,** 595 (1969).
22. J. Chayen, L. Bitensky, R. G. Butcher and L. W. Poulter, *Nature, Lond.* **222,** 281 (1969).
23. A. Gryfe, P. M. Sanders and D. L. Gardner, *Ann. rheum. Dis.* **30,** 24 (1971).
24. A. J. Anderson, W. E. Brocklehurst and A. L. Willis, *Pharmac. Res. Commun.* **3,** 13 (1971).
25. E. Myles Glenn, J. Gray and W. Kooyers, *Am. J. vet. Res.* **26,** 1195 (1965).
26. R. H. Persellin, *Arthritis Rheum.* **15,** 144 (1972).