5. G. I. DRUMMOND, M. W. GILGAN, E. J. REINER and M. SMITH, *J. Am. chem. Soc.* **86**, 1626 (1964).
6. A. R. HANZE, *Biochemistry, N.Y.* **7**, 932 (1968).
7. K. ANZAI and S. MARUMO, *J. Antibiot., Tokyo* **10A**, 20 (1957).
8. C. G. SMITH, G. D. GRAY, R. G. CARLSON and A. R. HANZE, in: *Advances in Enzyme Regulation* (Ed. G. WEBER), Vol. 5, p. 125. Pergamon, New York (1967).
9. G. ACS, E. REICH and M. MORI, *Proc. natn. Acad. Sci. U.S.A.* **52**, 493 (1964).
10. G. I. DRUMMOND and C. A. POWELL, *Molec. Pharmac.* **6**, 24 (1970).
11. B. MOSINGER and M. VAUGHAN, *Biochim. biophys. Acta* **144**, 569 (1967).
12. M. BLECHER, N. S. MERLINO, J. T. RO'ANE and P. D. FLYNN, *J. biol. Chem.* **244**, 3423 (1969).
13. R. W. BUTCHER, R. J. HO, H. C. MENG and E. W. SUTHERLAND, *J. biol. Chem.* **240**, 4515 (1965).
14. M. BLECHER, *Biochem. biophys. Res. Commun.* **17**, 560 (1967).
15. R. W. BUTCHER and E. W. SUTHERLAND, *J. biol. Chem.* **237**, 1244 (1962).
16. M. V. BUELL, O. H. LOWRY, N. R. RONERTS, M. W. CHANG and J. I. KAPPHAHN, *J. biol. Chem.* **232**, 979 (1958).
17. G. I. DRUMMOND, L. DUNCAN and E. HERTZMAN, *J. biol. Chem.* **241**, 5899 (1966).

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Lysosomal enzyme levels in the blood of arthritic rats

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ACID hydrolases have been suggested as possible chemical mediators of inflammation associated with rheumatoid arthritis.[1] Adjuvant induced arthritis in rats is often used as a model for the human disease. In this investigation *N*-acetyl-β-D-glucosaminidase (EC 3.2.1.30), acid phosphatase (EC 3.1.3.2) and β-glucuronidase (EC 3.2.1.3) have been determined in the serum or plasma of rats treated with mycobacterial adjuvant. These enzymes are normally regarded as lysosomal in origin.

Experimental

Arthritis was induced in Wistar strain rats (females, 160–170 g; males, 250–300 g) by methods previously described.[2] In one experiment the adjuvant was introduced into the right hind foot pad. In a second experiment the adjuvant was injected into the tail. Human strains (C, DT and PN) of tubercle bacilli were kindly supplied by the Ministry of Agriculture Veterinary Laboratories, Weybridge, Surrey. Blood was removed from the aorta of rats under deep anaesthia induced by a CO_2–O_2 mixture (1 : 1, v/v) delivered from a Boyle's apparatus. In a second experiment anaesthia was induced by ether. In one experiment the diameter of the ankle joint (right hind foot) was measured in order to assess inflammation. In the second experiment, where the adjuvant was injected into the tail, the mean diameter of the ankle joints of both hind legs was calculated. *N*-acetyl-β-D-glucosaminidase,[3] acid phosphatase[4] and β-glucuronidase[4] were determined in serum or plasma. In the case of β-glucuronidase it was found necessary to incubate the serum with the substrate for 18 hr.

Results and discussion

The results of the two experiments are shown in Figs. 1 and 2. In the first experiment the adjuvant was injected into the feet of female rats. Acid phosphatase and β-glucuronidase activities in the experimental serums are shown in Fig. 1. Control acid phosphatase and β-glucuronidase values (36 rats) were found to lie in the ranges 1·3–1·7 μM *p*-nitrophenol/ml serum/hr for acid phosphatase and 0·2–0·3 μM phenolphthalein/ml serum/hr for β-glucuronidase. Values determined in the experimental serums outside of these figures were regarded as abnormal. The mean diameter for the controls of the right hind ankle joint was found to be 6·8 ± 0·1 mm. The results (Fig. 1) show that there was some elevation

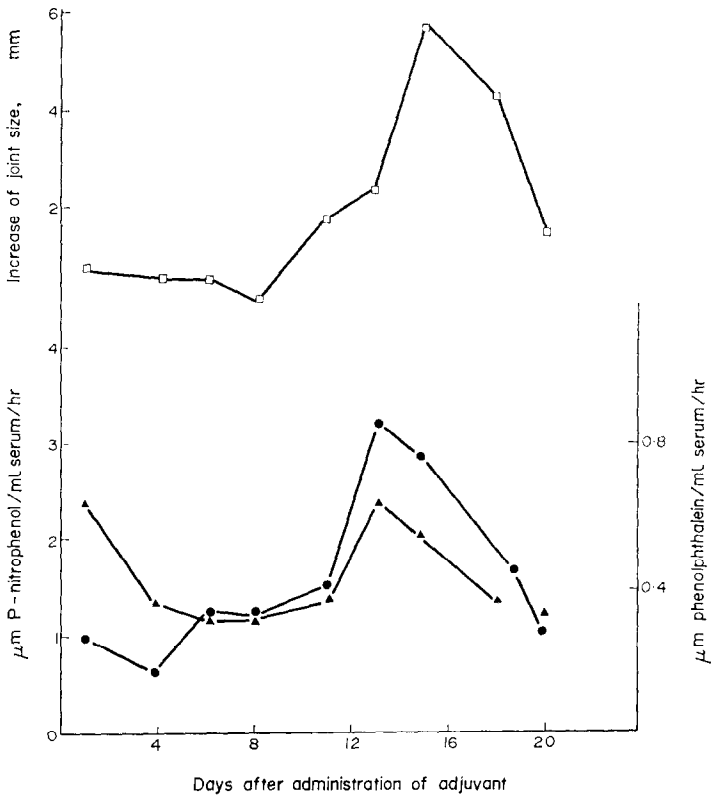

FIG. 1. Serum acid hydrolase levels and joint sizes of female rats treated with mycobacterial adjuvants. ●—●, acid phosphatase; ▲—▲, β-glucuronidase. Inflammation was assessed as the increase of joint size over the control value (see text). □—□, each result represents the mean value of duplicate determinations from four animals.

of the acid phosphatase level in the serum 24 hr after the injection. This high level was probably associated with the initial inflammation caused by the injection. The enzyme value was normal in the next serum sample, taken 4 days after the initial injection. Inflammation started 8 days after the administration of adjuvant and reached maximum values on day 15. The acid hydrolase values rose sharply to reach maximum values on day 13 but by day 15 the acid hydrolase values, although still abnormally high, had started to fall. Twenty days after the administration of adjuvant the acid hydrolase values were normal and the inflammation subsiding.

This experiment clearly showed that acid hydrolase values in serum were elevated in association with the development of arthritis in the rats. The experiment also suggested the possibility that high acid hydrolase values may precede the development of inflammation since the inflammation continued to increase despite falling enzyme values. This possibility was examined in the second experiment. Here, the adjuvant was administered into the tails of male rats in order to avoid the initial inflammation of the ankle joints due to the injection. In addition *N*-acetyl-β-D-glucosaminidase was determined instead of β-glucuronidase since preliminary experiments had shown that the level of this enzyme in plasma was higher than that of β-glucuronidase. The results of this experiment are shown in Fig. 2. They show clearly that the rise in acid hydrolyse values associated with the onset of arthritis occurred before the full development of inflammation. This suggests that lysosomal damage must precede the inflammatory response and is consistent with the hypothesis that lysosomal damage is a contributory factor to the development of inflammation. However, it appears probable that lysosomal damage is only part of a sequence of biochemical events since inflammation continues to develop despite falling enzyme levels in blood.

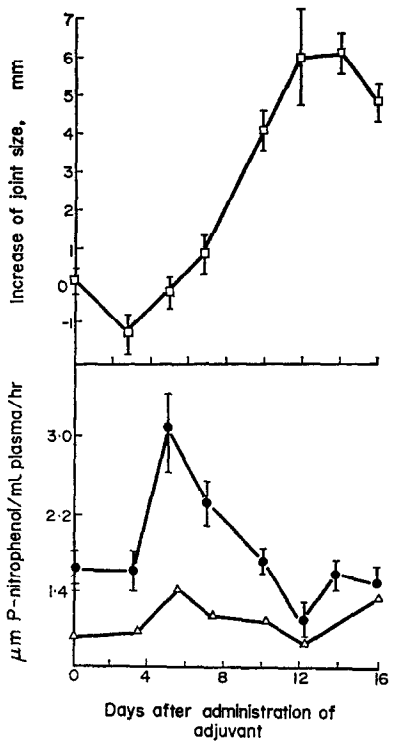

FIG. 2. Plasma acid hydrolase levels and joint sizes of 60 male rats treated with mycobacterial adjuvants. △—△, *N*-acetyl-β-D-glucosaminidase; ●—●, acid phosphatase; □—□, increase in joint size (see text). Vertical lines indicate S.E.M.

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REFERENCES

1. C. H. LACK, in: *Lysosomes in Biology and Pathology* (Eds. J. T. DINGLE and H. B. FELL), Vol. 1, p. 493. North Holland, Amsterdam (1969).
2. J. S. LOWE, *Biochem. Pharmac.* **13**, 633 (1964).
3. J. FINDLAY, G. A. LEVVY and C. A. MARSH, *Biochem. J.* **69**, 467 (1958).
4. A. M. SYMONS, D. A. LEWIS and R. J. ANCILL, *Biochem. Pharmac.* **18**, 2581 (1969).