Enzymatic Activity in Human Synovial Fluid from Rheumatoid and Non-Rheumatoid Patients.* (32590)

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Some investigators have found evidence to suggest that synovial fluid enzymes derive from leukocytes in the fluid(1,2). Other studies have suggested a synovial tissue source as well(3-9). Dabich and Neuhaus(10) showed that the electrophoretic mobility of purified alkaline phosphatase from bovine synovial fluid corresponded to that from cartilage and not from blood.

The present study has explored source of selected enzymes in synovial fluid from rheumatoid (RA) and non-RA patients by comparing levels in suspended cells after centrifugation, wash, resuspension and sonication (I), in supernatant synovial fluid decanted from the cell sediment (II), and in whole sonicated fluid and cells (III). 2 lysosomal enzymes, acid phosphatase (AcP) and lysozyme (LZ), reflect cell breakdown. 2 glycolytic enzymes, lactic dehydrogenase (LDH) and pyruvate kinase (PK), are present in many cells. Adenylic kinase (AK) is of potential muscle origin, although present also in human plasma, erythrocytes(11) and platelets (12,13).

Results suggest a partial derivation of LZ of synovial fluid from connective tissue source. LZ was inhibited partially by RA and non-RA synovial fluid and LDH by RA synovial fluid.

Methods. Synovial fluid was aspirated from large joints (knee or elbow) of RA and non-RA patients and promptly oxalated. Of the non-RA joint effusions, 2 were due to gout, 2 to degenerative arthritis, 1 to trauma, and 4 were idiopathic. The RA patients conformed to accepted diagnostic criteria for rheumatoid arthritis(14). Cell counts were done, and an aliquot of the fluid was centrifuged, usually at 400 *g* for 30 minutes at 5°C; occasionally a very viscous fluid was centrifuged at 4600 *g* for 10 minutes. The supernatant fluid was decanted for use as sample II in enzyme assays. The cellular sediment was washed once in 0.85% NaCl and then resuspended to original synovial fluid volume in a physiologic buffer† at pH 7.4. This suspension was then sonicated in a Raytheon Sonic Oscillator at 9 Kc per second at 1°C for 10 minutes for use in enzyme assays as sample I. Another aliquot of the original whole synovial fluid including suspended cells was sonicated for use as sample III. All 3 samples were refrigerated overnight at 5°C before enzyme assays were done.

LZ was assayed as in the past(15) in a 0.5 ml sample diluted 1:5 in 0.85% saline, using a dried preparation of *M. lysodeikticus* as substrate and translating results into % reduction in light transmission at 540 mμ by 1 ml of undiluted sample. AcP assay was done by the Biochemica-Boehringer modification of the method of Andersch and Szczpinski (16), using p-nitrophenylphosphate as substrate. Results are expressed as μmoles of p-nitrophenol released by 1 ml of undiluted sample in 1 hour. LDH, PK and MK assays all utilized oxidation of reduced diphosphopyridine nucleotide (NADH) as the final step, recorded at 340 mμ, in an incubation mixture containing in excess all necessary factors except the enzyme to be measured in the sample. In LDH assays, 0.5 ml sample (diluted 1:10 with 0.85% saline), 1.9 ml 0.1 M triethanolamine-carbonate buffer, pH 7.6, and added NADH were brought to equilibrium prior to addition of pyruvic acid. In PK assays, a mixture of sample, buffer, 0.02 ml 0.1 M $MgCl_2$, LDH, adenosine diphosphate and NADH were equilibrated prior to addition of phosphoenolpyruvate (PEP). In MK assays, the sample, LDH and NADH were equilibrated prior to addition of pre-mixed PK, LDH, buffer, $MgCl_2$, NADH, adenosine monophosphate, adenosine triphosphate and

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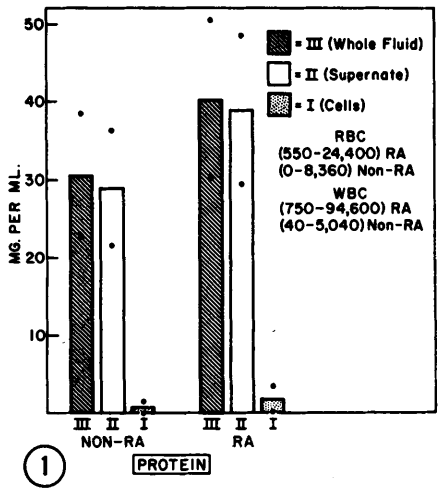
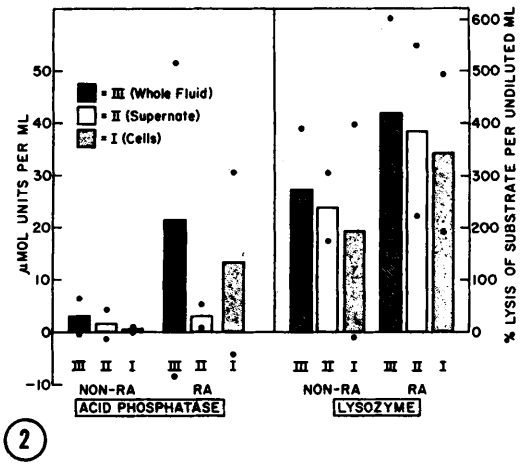
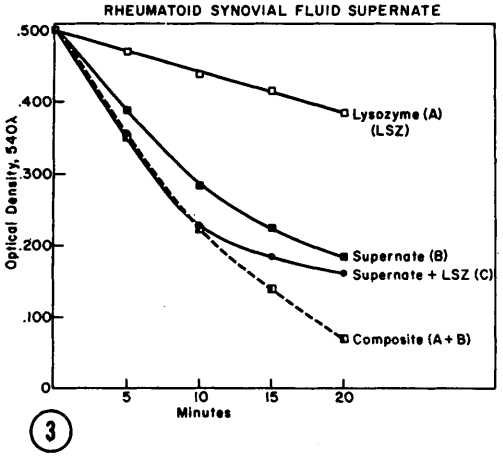
† 10-ml stock solution (7.5% NaCl, 0.75% KCl, 0.1% Na_2HPO_4, 0.12% KH_2PO_4, 0.5% K_2HPO_4) and 7 ml 1% Na_2HPO_4 per 100 ml, aqueous.







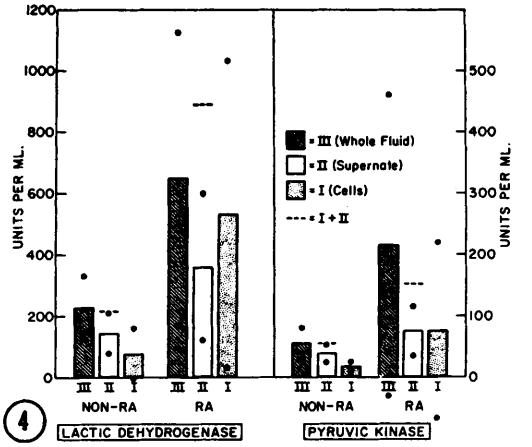


FIG. 1. Protein content of synovial fluid samples. Dots indicate ± 1 S.D. The RBC and WBC tabulation (see text) shows the range of blood cell counts in the whole synovial fluids.

FIG. 2. Levels of enzyme activity in synovial fluid samples. Dots indicate ± 1 S.D. (see text).

FIG. 3. Lysozyme assays on synovial fluid (supernate, B), commercial egg-white lysozyme (A), and a mixture of the two, assayed after 10 minutes at room temperature. (C) shows inhibition of lysozyme by supernate after the first 10 minutes of the assay at 37°C.

FIG. 4. Levels of enzyme activity in synovial fluid samples. Dots indicate ± 1 S.D. (see text).

PEP. All results for LDH, PK and MK assays are expressed in units of activity per 1 ml of undiluted sample causing a decrease in optical density (at 340 mμ) of 0.001 per minute. Protein content of synovial fluid was measured by biuret reaction(17), using synovial fluid diluted 1:5 in 0.85% NaCl, and expressing results here as mg equivalence (per undiluted ml of synovial fluid) to a human serum albumin standard.

Results. Fig. 1 demonstrates that the protein of Sample I (sonicated cells) plus II (supernatant synovial fluid) roughly equaled III (sonicated whole synovial fluid plus cells). The protein content of RA synovial fluid exceeded that of non-RA fluid, particularly in the supernatant fluid (II). Fig. 1 shows also the wide range of erythrocyte and leukocyte counts in the fluids studied, reflecting in Fig. 2 and 4 in the very wide standard in the deviations for AcP, LDH and PK activity in cell-containing fractions, I and III. The highest activity of AcP, LDH and PK was encountered in those fluids with very high WBC counts, although correlation did not achieve statistical significance.

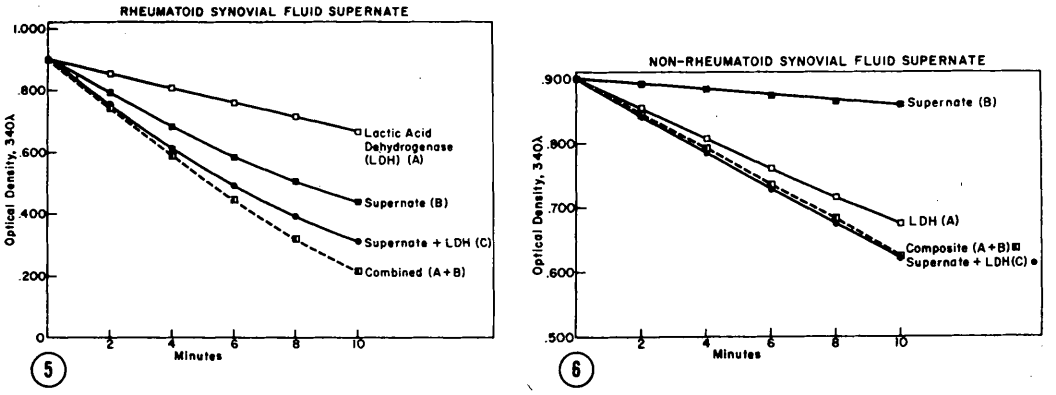


FIG. 5. Lactic dehydrogenase (LDH) assays on rheumatoid synovial fluid (supernate, B), commercial LDH (A), and a mixture of the two, assayed after 10 min at room temperature. (C) shows inhibition of LDH by supernate.

FIG. 6. Lactic dehydrogenase (LDH) assays on non-rheumatoid synovial fluid (supernate, B), commercial LDH (A), and a mixture of the two, assayed after 10 min at room temperature. (C) shows no evidence of inhibition of LDH by supernate.

Fig. 2 (left) shows markedly increased AcP activity in RA whole synovial fluid (III) as compared to non-RA fluid, particularly in the cellular fraction (I). Samples I plus II again roughly equaled III. Fig. 2 (right), shows that Sample II of RA fluids was rather markedly increased in its LZ activity over non-RA fluids and that LZ activity of Samples I plus II was obviously far in excess of III, unlike AcP and protein. Fig. 3 presents data testing the possibility of an inhibitor for LZ in the fluid. Commercial LZ (Armour; crystallized from egg white) was added (1 μg) to supernatant synovial fluid and assayed after 10 min at room temperature. LZ and supernatant fluid were assayed also separately as shown. During 20 min assay, no inhibition was evident for the first 10 min; then definite inhibition of LZ activity by the supernatant synovial fluid became evident.

On comparing RA and non-RA data of Fig. 4 for degree of increased enzyme activity in RA over non-RA samples, the increase in LDH and PK activity was of approximately equal degree in supernatant synovial fluid samples (II). In the RA group but not in the non-RA group, the sum of LDH activity of Samples I and II considerably exceeded III. This was noted also in the 2 gouty fluids of the non-RA group. Evidence for an inhibitor was sought as before, adding 0.1 μg of LDH (Biochemica-Boehringer) to RA and non-RA supernatant synovial fluid, 10 min prior to assay. Some degree of inhibition was noted with RA fluid (Fig. 5), none with non-RA fluid (Fig. 6).

Fig. 7 presents the results of AK assays. The increase in activity in RA versus non-RA fluids (III) was again related chiefly to the cells (I). There was no suggestion of significant inhibition of the enzyme by the fluids.

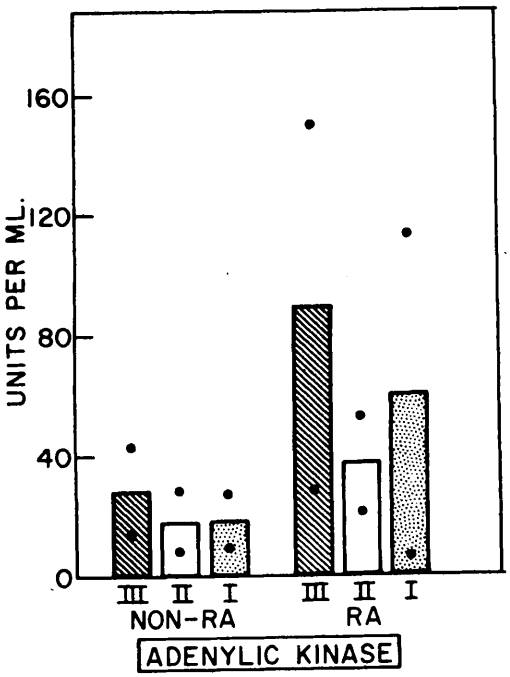


FIG. 7. Levels of enzyme activity in synovial fluid samples. Dots indicate ± 1 S.D. (see text).

Discussion. The data show that there was little leakage of AcP from cell lysosomes prior to aspiration of fluid from the joint. This is in accord with the observations of others(8). Therefore the increased LZ in rheumatoid fluid may well reflect a connective tissue source, being too great to be due to plasma contamination alone. An inhibitor for LZ was present in both RA and non-RA synovial fluid and for LDH in RA fluid and in 2 gouty fluids. Such an inhibitor might derive from connective tissue source or from plasma. Appropriate studies are in progress. Whether an LDH inhibitor in synovial fluid might affect some homeostatic mechanism for controlling the inflammatory response in joints is a matter for speculation only. There is no demonstration thus far that the inhibition noted here *in vitro* is of physiologic significance.

Summary. Enzyme assays have been done on sonicated cells after separation from synovial fluid (I), supernatant synovial fluid (II), and sonicated whole synovial fluid including cells (III). 2 lysosomal enzymes (acid phosphatase and lysozyme), 2 glycolytic enzymes (lactic dehydrogenase and pyruvate kinase), and adenylate kinase levels were measured in each of the 3 samples from each synovial fluid. Rheumatoid and non-rheumatoid fluids were studied.

Results suggested that lysozyme derived at least in part from connective tissue. Lysozyme released from suspended cells by sonication was partially inhibited by supernatant synovial fluid (both rheumatoid and non-rheumatoid), as was commercial egg-white lysozyme added to synovial fluid. Lactic dehydrogenase was inhibited somewhat by rheumatoid synovial fluid and by 2 gouty synovial fluids studied. Commercial lactic dehydrogenase added to rheumatoid and non-rheumatoid synovial fluid was partially inhibited by the rheumatoid and not by the non-rheumatoid fluid.

1. West, M., Poske, R. M., Black, A. B., Pilz, C. G., Zimmerman, H. J., J. Lab. Clin. Med., 1963, v62, 175.

2. Caygill, J. C., Jevons, F. R,, Clin. Chim. Acta, 1966, v13, 61.

3. Ziff, M., Simson, J., Scull, E., Smith, A., Shatton, J., Mainland, D., J. Clin., Invest., 1955, v34, 27.

4. Jacox, R. F., Feldmahn, A., ibid., 1955, v34, 263.

5. von Greiling, H., Schuler, B., Med. et Hyg., 1964, v22, 1141.

6. Smith, C., Hamerman, D., Arthr. & Rheum., 1962, v5, 410.

7. Vesell, E. S., Osterland, K. C., Bearn, A. G., Kunkel, H. G., J. Clin. Invest., 1962, v41, 2012.

8. Weissmann, G., Ann. Rev. Med., 1967, v18, 97.

9. Jasani, M. K., Katori, M., Lewis, G. P., J. Physiol., 1967, v190, 26P.

10. Dabich, D., Neuhaus, O. W., Proc. Soc. Exp. Biol. & Med., 1966, v123, 584.

11. Kashket, S., Denstedt, O. F., Canad. J. Bioch. & Physiol., 1958, v36, 1057.

12. Kerby, G. P., Taylor, S. M., Thromb. et Diath. Hem., 1964, v12, 510.

13. Haslam, R. J., Mills, D. C. B., Bioch. J., 1967, v103, 773.

14. Ropes, M. W., Bennet, G. A., Cobb, S., Jacox, R., Jessar, R. A., Arthr. & Rheum., 1959, v2, 16.

15. Kerby, G. P., Proc. Soc. Exp. Biol. & Med.. 1952, v81, 129.

16. Andersch, M. A., Szczypinski, A. J., Amer. J. Clin. Path., 1947, v17, 571.

17. Kerby, G. P., Taylor, S. M., Langley, N. M., J. Clin. Invest., 1961, v40, 1900.

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