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Source and Significance of Raised Serum and Synovial fluid Enzymes in Rheumatoid Arthritis

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Abstract

Fifty four patients with RA were included in the study. Blood samples were obtained from all cases and synovial fluid (SF) samples were obtained from 24 patients presenting with knee effusion. Besides routine tests of rheumatoid activity ESR, CRP, the following enzymes were analysed in serum and SF samples: alkaline phosphatase (ALP), LDH, 5' nucleotidase (5' NT) and adenosine deaminase (ADA), quantitative determination of LDH isoenzymes and qualitative determination of ALP isoenzymes. Cytological examination of SF was also carried out. Results revealed elevated serum levels of ALP, LDH, 5' NT & ADA in 81 %, 27.77%, 23.8% and 50% of cases respectively. Significantly higher SF levels were found for LDH, 5'NT & ADA when compared to serum levels. The values were 240.5 ± 149.6 U/1, 10. 72 ± 9.05 U/1. 52.79 ± 27.78 U/1 for SF and 182.75 ± 97.46 ± 3.57 and 23.6 ± 12.36 for serum enzymes respectively. SF serum ratio demonstrated a value less than one for ALP but greater than one for LDH, 5'NT & ADA, this signifies that ALP originates mainly from the liver and passes to joint fluid due to increased permeability of synovial membrane, while the other three enzymes most probably originate from the joint itself and diffuse to blood stream. Serum LDH isoenzyme pattern was predominantly of LD_1 and LD_1 25.1 ± 5.44% and 33.42 ± 8.72% respectively while SF LDH isoenzyme pattern was predominantly of LD_4 and LD_5 20.95 20.95 ± 5.25 and 35.57 ± 5.78 respectively. This shift of LDH isoenzyme pattern towards the slowly migrating form is probably due to increased demands of the highly cellular SF in RA for anaerobic glycolysis to supply its energy requirements. Serum LDH was significantly elevated in active RA than inactive cases, values being 311.85 ± 97.86 and 208.12 ± 104.53 respectively. A positive correlation was found between SF LDH and PMN cell count indicating the value of LDH as a marker of disease activity better than ESR.

Introduction

THE association of rheumatoid arthritis (RA) and liver disease is poorly understood. This concept was mainly based on abnormal serum activity of alkaline phosphatase (ALP) [1,2], 5' Nucleotidase (5'NT) [3], Lactic dehydrogenase (LDH) [4,5] and γ Glutamyl transpeptidase (γ GT) [6,7].

However, it was reported that in uncomplicated RA there are no clinical manifestations of chronic liver disease with only mild nonspecific changes on liver biopsy [8,9] and persistantly normal serum bilirubin and transaminases [10].

Enzymes are widely distributed in tissues and increased serum levels may occur under quite different conditions. Raised liver transaminases have been reported in cases of RA patients taking salicylates [11] and sulphsalazine [12] suggesting that their measurement may be useful in monitoring for drug hepatotoxicity.

This work was designed to study some enzymological findings in RA patients which include ALP, LDH, 5'HT & adenosine deaminase (ADA) as well as the isoenzyme pattern of LDH & ALP. In patients with joint effusion enzymes were also studied in synovial fluid beside cytological examination in an attempt to clarify the origin of elevated serum enzymes and to correlate these enzymes with disease activity and synovial inflammation.

Material and Methods

The study included 54 patients with RA, all of them satisfied the American Rheumatism association diagnostic criteria for definite RA. History of treatment by non steroidal antiinflammatory drugs (NSAID) and disease modifying drugs were given in all cases.

Patients were classified according to disease activity into inactive (N=34) and active (N=20) groups. Criteria of activity included two of the following: Articular index (AI) at least 30, duration of morning stiffness at least 1 hour and ESR at least 30 mm/hour.

The AI is calculated by couting of clinically active joints defined by the presence of tenderness on firm pressure on joint margins and or/pain on passive movements.

24 patients with RA presented with knee effusion. Blood samples were obtained from all cases and synovial fluid (SF) samples were obtained from 24 patients with knee effusion at the time of examination by vein and knee punctures. SF samples were collected; an aliquot on heparin for cytology and another without anticoagulant, centrifuged, supernatent was separated and stored at - 20 for enzymatic assays, the sediment was spread on 2 slides immediately. One was stained with papanincolau stain and the other slide was air dried and stained by Giemsa

stain for spotting of bacteria and inclusions.

Blood samples were centrifuged and sera separated for quantitative determination of ALP, LDH, 5'NT & ADA. Total LDH in serum and SF samples was determined by the kinetic method [13].

ALP was determined by colourimetric method at 405 nm by measuring the concentration of p-nitrophenol, liberated from the substrate p-nitrophenol phosphate. 5'NT by a kinetic method based upon measuring nucleoside produced by the hydrolytic action of the enzyme on nucleotide substrate [14]. ADA was determined by a colourimetric method according to Ciusti [15]. LDH isoenzymes were analysed quantitatively by using cellulose acetate electrophoresis and densitometric scanning of stained bands.

ALP isoenzymes were analysed qualitatively on cellulose acetate membranes and compared visually with refernce bands (Helena, Beaumont, TX).

Full blood count, erythrocyte sedimentation rate, serum C-reactive protein, serum rheumatoid factor, serum albumin and serum bilirubin were measured by the routine techniques.

Radiological investigations included plain X ray on both hands and other affected joints and ultrasonography of the abdomen.

Results

Results of this study revealed elevated serum values of ALP, LDH, 5'NT & ADA in 81%, 27.77%, 23,8% & 50% respectively

Table (1) demonstrates the levels of ALP, 5'NT, LDH & ADA in serum and Synovial fluid samples and the difference encountered between the two groups.

Table (2) demonstrates LDH isoenzymes results for both serum and SF.

Table (3) demonstrates the serum values of ALP, 5'NT, LDH and ADA in the active and inactive RA patients.

Table (4) demonstrates correlations between serum enzymes and markers of rheumatoid activity.

Fig. (1) demonstrates comparison between ALP, LDH, ADA & 5'NT in serum & Synovial fluid.

Figs (2,3) demonstrate LDH1 & 5 in serum & Synovial fluid.

Fig (4) demonstrates the cytological findings of SF

Figs. (5) 1, 2, 3, 4, 5, demonstrates cytological findings.

Discussion

The concept of liver involvement in RA was mainly based on abnormal serum activity of ALP [6]. Results of the present study perfectly agree with previous reports where 81% of RA patients demonstrated elevated levels of serum ALP. Comparable works reported 82% ALP elevation in their series of RA patients. Other authors have reported 18,25,

Table (1): ALP, 5'NT LDA in Sera & SF Samples of RA Patients.

	ALP U/L mean ± SD	5' NT U/L	LDH U/L	ADA U/L
Serum	168	5.46	182.75	23.60
n = 24	± 45.41	± 3.57	± 97.46	± 12.36
Synovial fluid	164.79	10.72	240.5	52.79
n = 24	± 65.26	± 9.05	± 149.62	± 27.78
SF / serum	0.98	1.9	1.3	2.2
Comparison between S & SF	NS	S*	S*	S*

* Significant difference between serum and SF.

Table (2): LDH Isoenzymes in Serum & SF Samples.

	LDH_1 U/L	LDH_2 U/L	LDH_3 U/L	LDH_4 U/L	LDH_5 U/L
Serum	* 25.10	* 33.42	22.11	8.9	10.32
	± 5.44	± 8.72	± 8.56	± 3.42	± 3.99
SF	9.44	14.01	19.00	* 20.95	* 35.57
	± 3.66	± 3.16	± 3.33	± 5.25	± 5.78
SF / serum	0.37				

* Significant difference between serum and SF.

Table (3): Comparison between Serum Level of ALP, 5'NT, LDH & ADA in Patients with Inactive & Active RA.

	Inactive	Active	Significance
ALP U/L	184.29 ± 103.11	192.45 ± 55.88	NS
5' NT U/L	4.69 ± 4.15	5.41 ± 4.28	NS
LDH U/L	208.12 ± 104.53	311.85 ± 97.86	S
ADA U/L	19.75 ± 10.75	18.43 ± 10.61	NS

* Significant difference between serum and SF.

Table (4): Serum Enzymes and Markers of Rheumatic Activity.

	Correlation Coeffecient					
	A.I.	ESR	CRP	5'N	ALP	LDH
5'N	-0.1297	-0.1327	-0.0785		-0.1025	0.11892
DAD	-0.0113	-0.2199	-0.0304	0.0944	-0.2175	-0.1395
ALP	0.16819	-0.0092	0.19187			
LDH	0.34929*	0.2326*	0.0856		-0.0466	

35,46 & 51 percent of patients with raised serum ALP [1, 3, 16, 17]. It was reported that early studies of ALP using King Armstrong units (KAU) yieled a smaller percentage of patients with elevated levels than later studies that used Scandinavian methods [18].

Nevertheless, different studies are in general agreement that ALP is raised in RA which may be the only abnormality detected. One explanation for isolated (apart from ALT, AST & 5'NT) elevation of ALP in most studies is the selective induction of ALP by inflammatory mediators such as interleukin-1 [19] known to circulate in active RA. However, this is argued in the present study by the lack of difference between serum ALP in cases of active and inactive RA and also by the absence of correlation between serum ALP and markers of RA activity as AI and ESR (Table 4).

On electrophoretic separation of ALP isoenzymes our results revealed that 64.8% had liver isoenzyme as the sole or major component even in the presence of normal serum ALP level, while 35.2% had both liver and bone isoenzymes.

Comparable findings were reported by Thompson et al [2] who suggested that serum ALP was predominantly of bone and / or liver type and are in contrast to Fernandes et al [1] who detected an intestinal band in 6 out of 15 RA patients.

Synovial permeability can be assessed by the ratio $SF/_{serum}$ concentrations of molecules that are not produced or destroyed in joints. In the present study $SF_{/S}$ ALP is less than 1, a comparable ratio of 0.8 was reported by Thompson & coworkers [2] and was explained by diffusion of ALP from serum to synovial fluid as inflammation increased permeability of synovial membrane [20]. This may be also supported by the negative correlation encountered between S & SF ALP in the present work. Our results are in contrast to those reported by Cimmino et al [21] who found no such correlation

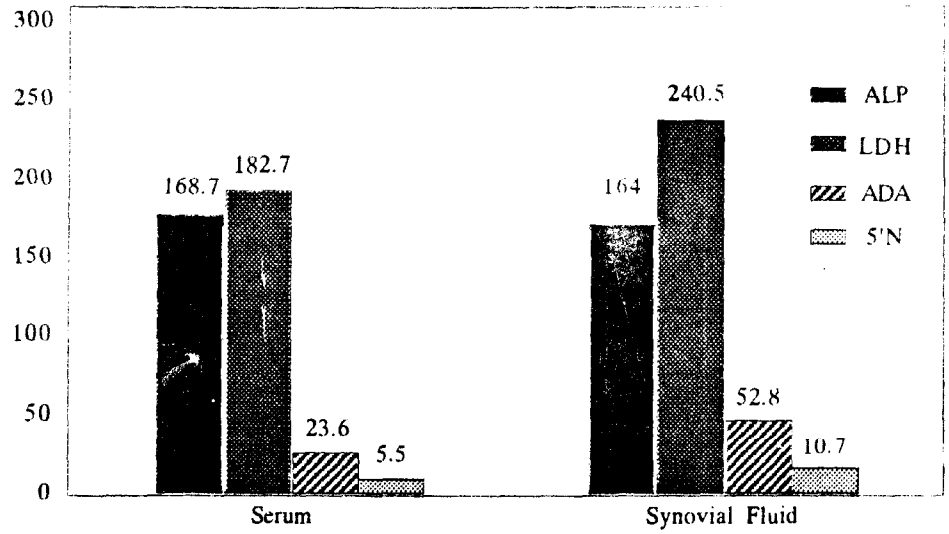
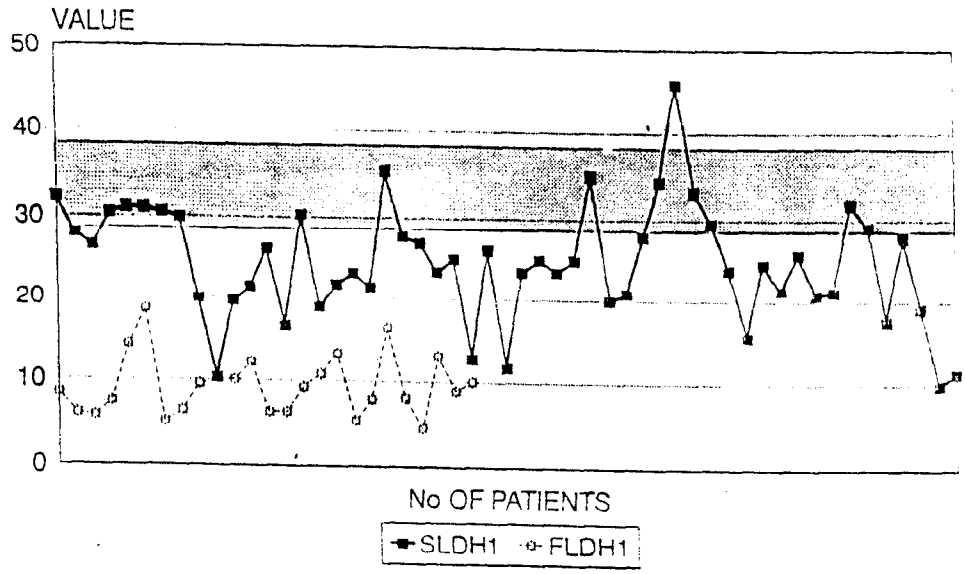
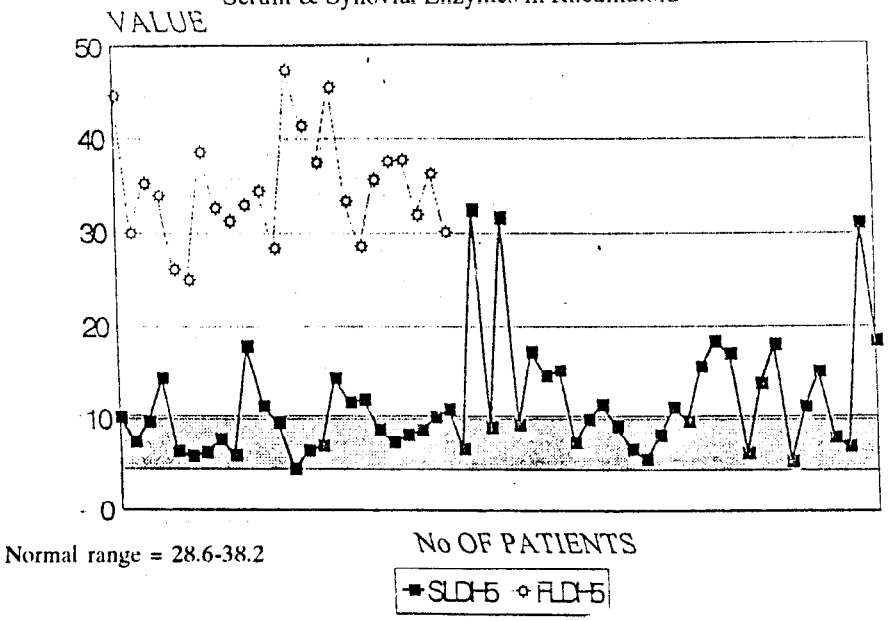


Fig. (1): Comparison between serum and synovial fluid enzymes (mean).



Normal range = 28.6-38.2

Fig. (2): Distribution of LDH 1 in serum and synovial fluid in relation to normal value.



Normal range = 28.6-38.2

Fig. (3): Distribution of LDH 5 in serum and synovial fluid in relation to normal value.

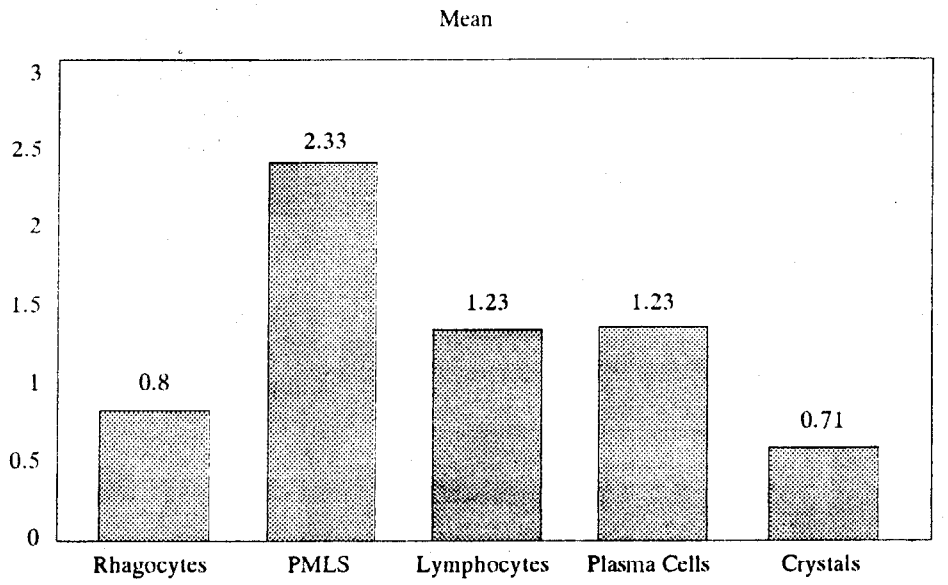


Fig. (4); Synovial fluid cytology results.

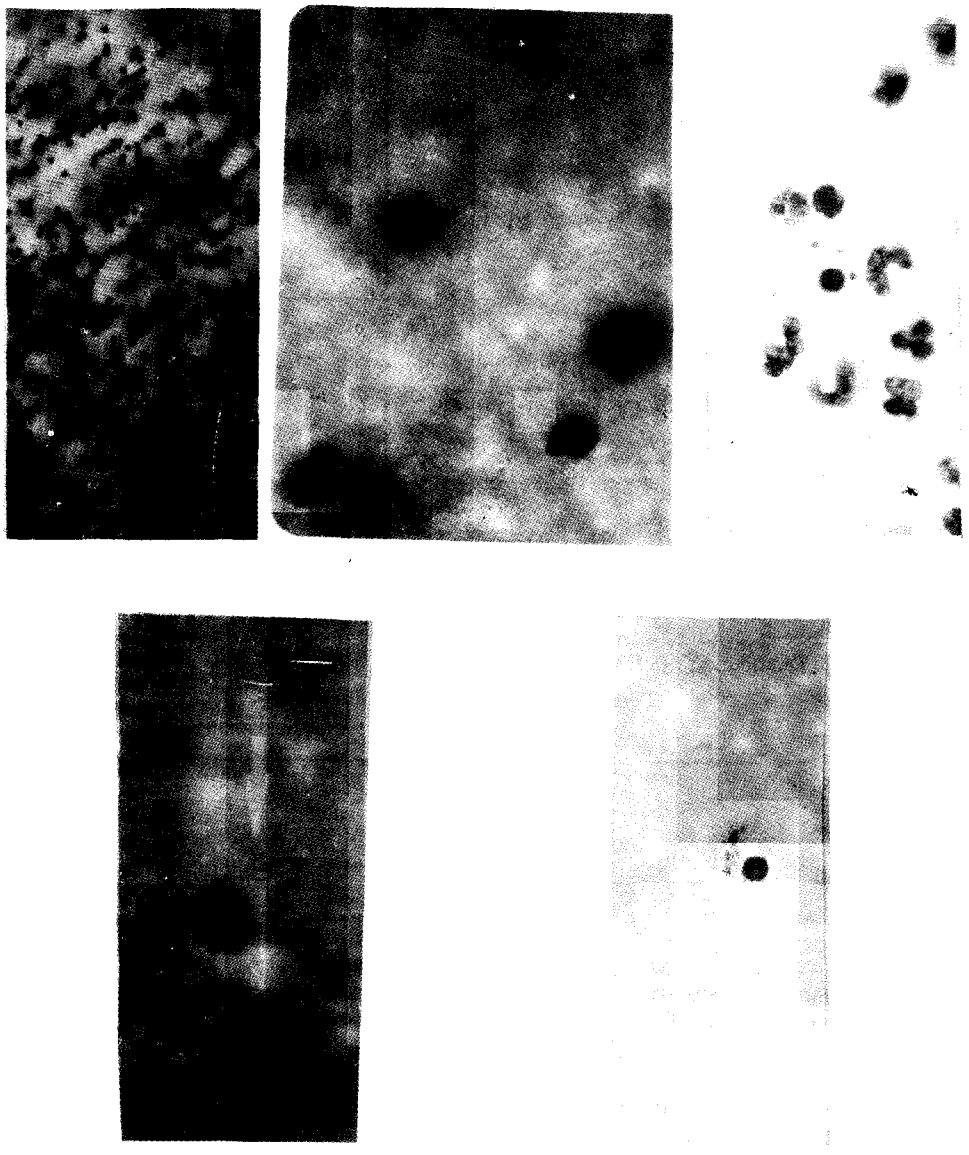


Fig. (5): Cytology of synovial fluid effusion:

(a) Smear pattern of St. showing marked cellularity with PNLs (PAP x 100).

(b) Hypersegmented PNLs and histiocytes (PAP x 1000).

(c) Rhagocyte with prominant cytoplasmic inclusions (M. 66 x 1000) arrow.

(d) Rhagocyte cells with dense nuclear fragments (arrow) (PAP x 100).

(e) Mononuclear rhagocyte with fine cytoplasmic inclusions (M 66 x 1000).

between SF & serum ALP and in addition reported a $SF_{/S}$ ratio of more than 1 and claimed that ALP is locally produced in RA joints.

Results of the present work demonstrated elevated 5'NT in 23.8% of RA patients, whith concomitant elevation of ALP in 60% of them. Although elevated levels of 5'NT are usually indicative of liver disease, yet clinical evidence of liver affection in RA is limited. The present study demonstrated 13.3% of patients with homogenously enlarged liver by ultrasonography. On the other hand routine analysis of ALT & AST revealed almost invariably normal results. Hepatomegaly was reported in 10.6% of 216 patients with RA, and 22% of patients with RA had hepatomegaly as shown by liver scintigraphy in another study [16]. Thompson & cowrkers [2] claimed that although RA patients take a number of potentially hepatotoxic drugs, yet no relationship between altrations in hepatic function and use of NSAID, disease modifying drugs or immunosuppressive agents has been demonstrated except for occasional isolated cases of hepatotoxic reactions leading to elevated serum aminotransferases.

The clinical evidence of liver affection in RA is limited [16]. A review of liver biopsy in RA does not reveal any consistent abnormality. In unselected series of RA patients, liver histology was normal in 35%, showed mononuclear portal infiltrate in 43% and fatty change in 22%. However, when liver biopsy was done in patients with biochemical evidence of liver dysfunction, only 1% were normal, while chronic liver disease including primary biliary cirrhosis, chronic active hepatitis and amyloidosis were detected in 13% of cases [10].

The ratio $SF/_{serum}$ 5` NY was found to be greater than one (Table 1), a finding which agrees with previous reports [2]. This may signify that this enzyme may originate from inflamed joints and drain (presumably via lymphatics) in to the blood. this is supported by the positive correlation encountered between serum and SF level of 5'NT in contrast to previous reports [3] finding no such correlation in their patients. The present study demonstrated elevated levels of serum LDH in 27.77% of RA patients. LDH was found to be significantly elevated in active RA patients when compared to inactive ones (Table 3).

A positive correlation was demonstrated between markers of rheumatoid activity namely ESR & AI & LDH in serum & SD (table 4). Similar correlation between SF LDH was demonstrated [22] but not with serum LDH. Synovial LDH levels were found to be significantly higher than serum levels with $SF/_{serum}$ LDH greater than one.

A finding which indicates that it originates from inflamed joints and drain into the blood. A positive correlation was also

demonstrated between SF LDH & polymorph count. These data suggest that SF polymorphs are the source of raised serum LDH which is probably released from dead and damaged cells in SF and diffuses via lymphatics from all inflamed joints to the blood. Serum levels of LDH were reported to reflect overall polymorph turnover and offer a marker of inflammation that may have advantage over non specific measures such as ESR [23]. Similar findings were previously reported [5,2].

LDH activity in rheumatoid synovia and SF is of fundamental importance since by continuous reoxidation of reduced NAD it allows glycolysis to take place under existing hypoxic conditions [24]. A significant decrease in partial pressure of O_2 (PO_2) and pH together with increased PCO_2 and lactate concentrations are often found in RA SF [1, 2, 25, 26]. These changes presumably stem from increased cellularity of RA tissue and fluid.

Under such hypoxic conditions RA synovia must catabolize many more molecules of glucose to meet energy requirement [27]. NADH reoxidation by LDH may explain to a certian extent the increased LDH activity in RA joints.

Although T LDH was found to be significantly elevated in active RA yet LDH isoenzymes demonstrated no difference between active and inactive groups (table 2 & Fig 2,3). In the sera of RA patients the LDH isoenzyme profile appears to demonstrate a shift towards the slowly migrating forms because of relative increase in LDH 4 and 5 and decrease in LDH 1 & 2. Similarly RA SF LDH isoenzyme distribution indicates a significant decrease in LDH 1 & 2 and significant increase in LDH4 & 5. These findings perfectly agree with previous reports [22]. These authors claimed that LDH_{5/LDH_1} ratio is a good indicator of isoenzymatic shift towards the slowly migrating forms. A partial explanation for an almost inverse relationship between LDH_1 & LDH_5 activities in RA SF is their affinity to pyruvate [27]. LDH_1 affinity for pyruvate is significantly greater than LDH_1. Pyruvate shifts towards the aerobic Krebs cycle in the presence of LDH_1 and towards glycolysis in the presence of LDH_5 [27, 28]. In addition, a decrease in RA SF pH reduces the affinity of LDH_5 for pyruvate and increases the susceptibility of LDH_1 to inhibition by the same substrate, thus stimulating the metabolism towards more glycolysis [25, 29]. As for ADA, results of the present work revealed 50% increased serum ADA levels. Interest in ADA has been concentrated upon discovery of a relationship between ADA deficiency and immunologic dysfunction [30]. ADA is an enzyme of purine catabolism which catalyzes the pathway from adenosine to inosine and deoxyadenosine to deoxyinosine [31]. Essentially, ADA is widely distributed in human tissues and

shows the highest activity in lymphoid cells [32]. Results of the present study revealed significantly higher SF ADA than one, in addition, a positive correlation was demonstrated between SF ADA and serum ADA. These data signify that the enzyme propably originates mainly in the joints and diffuses to the blood stream. Several hypotheses can be put forward to explain the elevated ADA levels. First, endothelial cells of synovial capillaries have increased expression of intracellular adhesion molecule (ICAM-1) which acts as site of attachment for circulating mononuclear cells. ICAM-1 expression is enhanced by interleukin 1 a mediator of inflammation known to increase in RA synovia [19]. Secondly, there are more T-lymphocytes than B lymphocytes to produce antibodies including rheumatoid factor and antibodies against cartilage collagen [33]. ADA was positively correlated with T helper cells.

Thirdly, the increased SF PMN leucocyte encountered in this study correlates with ADA may signify increased catabolism of their nuclei with ultimate increase in ADA, since elevated ADA levels were reported to occur in empyema [36] and correlated with PMN cells. The principal chemoattractants for neutrophils are within the joint space rather than the synovial membrane [37] and hence the greater number of polymorphs in synovial fluid.

Cytological examination of synovial fluid in the present study revealed marked increase in PMN leucocyte (Fig. 1) with significantly elevated counts in active cases. Besides polymorphs a number of other cells could be detected including histiocytes, small lymphocytes and large mononuclear cells. The latter probably include some transformed lymphocytes and synovial living cells (Fig. 2,3). Although classification of individual large mononuclear cells may be difficult, yet it may be worth attempting since transformed lymphocytes tend to occur in RA and not in acute gout or pseudogout [34]. It was reported that once within the joint fluid, neutrophils probably are rapidly activated by phagocytosis of cellular debris and aggregates of immune complexes and become known as RA cells or rhagocytes. Rhagocytes have multiple pyknotic dence nuclei and contain cytoplasmic inclusion bodies (Fig 5).

In conclusion, our results suggest that in RA, only serum ALP originates from the liver, while serum LDH, 5'NT and ADA enzymes originate from inflamed joints. Serum LDH is a measure of polymorph lysis in all joints, and it offers a marker of joint inflammation more specific than other measures such as ESR. Because hepatic necrosis does not occur in RA, the serum transaminases may be used to monitor drug toxicity. Marked synovial fluid neutrophilia with rhagocytes is a useful marker of joint inflammation.

Felson et al [35] recently recommended that clinical evidence of rheumatoid

activity can never be exchanged by laboratory findings and both should go hand in hand supplementing each other.

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