Connective Tissue Activation

II. Abnormalities of Cultured Rheumatoid Synovial Cells

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With the technical assistance of

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Rheumatoid synovial cells grown in vitro demonstrated: a) increased rate of lactate formation, b) increased rate of glucose uptake, c) increased rate of hyaluronate synthesis, d) decreased sensitivity to exogenous activator and e) increased concentrations of an endogenous activator. Exogenous activator was shown to be capable of inducing cortisol unresponsiveness in relation to suppression of hyaluronate synthesis. Evidence that exogenous activator is not long retained by synovial cells suggests that elevated levels in rheumatoid cells are due to accentuated endogenous formation. Elevated activator peptide content of rheumatoid synovial cells provides an explanation for many of the differences between normal and rheumatoid synovial cells in vitro.

Rheumatoid arthritis is a disease of man characterized by chronic joint effusions containing excessive quantities of hyaluronic acid of inferior molecular weight (1), and by destructive, proliferative synovial pannus which exhibits accelerated energy metabolism. Accelerated energy metabolism of rheumatoid synovial tissue was documented by tissue slice studies which revealed increased glucose and oxygen consumption and increased lactate formation (2), and by in vivo studies in rheumatoid joints where severe joint disease was associated with a fall in joint fluid pO_2 and a rise in lactate (3).

While it is reasonably certain that the abnormal biochemical behavior of the rheumatoid synovial membrane may be partially explained by the deviant behavior of synovial intimal (lining) cells themselves, it is by no means clear that a primary defect lies in the rheumatoid synovial intimal cells. It is possible that rheumatoid synovial intimal cells may actually be innocent bystanders, being forced into abnormal functional patterns by continuing extracellular influences. In the context of this question, our earlier reports (4, 5), which first identified quantifiable abnormalities in rheumatoid synovial cells propagated in tissue culture, provide a framework for examining the alternative possibilities. These studies showed that rheumatoid syn-

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ovial cells, cultivated in vitro, produced underpolymerized hyaluronic acid, exhibited an abnormally slow growth rate, and were hyporesponsive to the suppressive regulatory effects of hydrocortisone. The defective response to cortisol shown by rheumatoid cells was manifested by failure to exhibit depressed formation of extracellular products, such as hyaluronic acid and collagen, to the same degree as normal synovial cultures exposed to this hormone. It is noteworthy that these abnormalities were demonstrated in cells which were propagated for months in vitro, suggesting that the cause of the abnormalities was either a persistent nonviable entity, or possibly a replicating infectious agent. It is difficult to be certain that rheumatoid synovial cells do not carry ever-diminishing amounts of a potent trace substance adequate to induce the abnormalities visualized in culture, a trace substance which may have been contributed to the progenitor rheumatoid synovial cells by an external agency.

The demonstration of a connective tissue activating peptide (CTAP), found in most cells, and shown to be capable of stimulating hyaluronic acid and energy metabolism in synovial cells, provides a possible explanation for the self-perpetuating nature of chronic rheumatoid inflammation (6–8). It was clear from our earlier studies that CTAP is present in both lymphocytes and polymorphonuclear leukocytes as well as in cultivated synovial cells of both normal and rheumatoid origin. In a recent communication, we suggested that the activator substance (CTAP) induced normal synovial cells to exhibit behavior characteristic of rheumatoid cells in the culture environment, and provided preliminary evidence indicating that retention of increased amounts of CTAP activity was responsible for many of the abnormalities characteristic of rheumatoid cells (9).

The present report demonstrates other metabolic abnormalities in rheumatoid fibroblasts, shows that such cultures have excessive quantities of CTAP, and finally, provides experimental evidence indicating that increased endogenous formation of CTAP, rather than prolonged storage, is the likely explanation for the high level of activator found in rheumatoid cells.

MATERIALS AND METHODS

Synovial membrane for tissue culture studies was obtained at the time of arthrotomy or amputation. A portion of the tissue was divided into small fragments and used to generate cell cultures using techniques reported in detail earlier (4, 10, 11), and another portion was processed for histologic examination. The routine media used in growing cell cultures consisted of 80% medium 1066 with 10% fetal calf serum and 10% heat inactivated human serum, supplemented with L-glutamine, penicillin anl streptomycin. Cells were enumerated and sized electronically with a Coulter Model B cell counter.* Protein was measured by the method of Oyama and Eagle (12), and glucose was estimated with a glucose oxidase method (13). Lactate was measured by the Barker-Summerson method (14) and pyruvate by an enzymatic procedure (15). Hyaluronic acid from serum-containing tissue culture media was measured by a method reported earlier from this laboratory, involving isolation of the polymer (16) and measurement of the uronic acid moiety by a modified carbazole procedure (17). In the assay procedure for CTAP, which was carried out in the absence of serum, hyaluronate was isolated using cetylpyridinium chloride (8) and the uronic acid measured by the carbazole procedure (17). The bioassay procedure for measuring CTAP consists essentially of measuring the capacity of a standard normal synovial cell culture to show stimulation of hyaluronic acid synthesis in the presence of an activating substance. The material to be tested for CTAP activity is added to 2.0 ml of serum-free Eagle's synthetic media and incubated with 1 million normal synovial fibroblasts for 40 hours. At this time the culture is sacrificed, the total culture protein is measured and the total culture hyaluronic acid measured (8). The units of

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CTAP activity may then be estimated from the following relation:

$$\text{CTAP (units/ml)} = \frac{A_2 - A_1}{10 \times V}$$

where A_2 = hyaluronic acid synthesis rate in the stimulated culture corrected to μg HA/mg cell protein/24 hr, and A_1 is this measure in the control flasks. V = the volume of activator material added to the standard test system in milliliters. Statistical comparison of mean values in control and experimental populations was carried out using the Student-Fisher *t* procedure.

RESULTS

Data from characterization† studies. Data collected from routine characterization studies carried out during the past three years with nonrheumatoid and rheumatoid cell strains is displayed in Table 1. In such studies, 1 million synovial fibroblasts are inoculated into a serum dilution bottle nourished by 10 ml of standard serum-containing medium. The medium is changed totally at two days and five days with the culture terminated on the seventh day. The cells are dispersed with trypsin, counted and sized with an electronic cell counter and the media utilized for chemical analysis. The measurements of hyaluronic acid synthesis rate, glucose uptake rate, and lactate output rate are based on the chemical analyses made on the final medium change and the final cell count in the culture flasks. The mean rates of hyaluronic acid synthesis, glucose uptake and lactate formation were considerably increased in the rheumatoid synovial strains, both hyaluronic acid and lactate measurements being statistically significantly different than the controls, and the glucose uptake measurements approaching significant levels. These data from a group of unselected cultures would suggest that the rheumatoid cultures exhibit the characteristics of activation which we have demonstrated can be induced in normal cell strains (6–8).

Responsiveness to exogenous activator. It soon became obvious that rheumatoid cell strains not only behaved as if they were already partially activated but that they responded rather sluggishly when exposed to exogenous CTAP. Table 2 provides a comparison of the basal performance of a group of rheumatoid and nonrheumatoid cell cultures examined under the conditions established for the CTAP bioassay. In the absence of exogenous activator (basal conditions), the rheumatoid strains produced approximately twice as much hyaluronic acid per unit cell of protein per unit time as the nonrheumatoid cultures. When similar amounts of an activator (CTAP) derived from rheumatoid cells was added to the nonrheumatoid cultures, the HA synthesis rate was markedly stimulated while rheumatoid cells under the same circumstances showed only a modest response. The muted response to exogenous activator on the part of rheumatoid cells is shown schematically in Fig 1 where two rheumatoid fibroblast strains are compared with a strain from normal synovium. It is clear that both of the rheumatoid synovial strains show basal levels somewhat higher than the normal culture, but in one case there is virtually no increase in hyaluronate synthesis with increasing amounts of activator, and in the case of DW, the increase is of modest proportions. In other experiments we have shown that adding hyaluronate to the assay cultures, (thus increasing the basal HA level) does not prevent the effects of exogenous activator.

Activator content of synovial cell

†"Characterization" studies denote a standardized set of measurements made on all newly generated cell strains to assess growth rate, HA synthesis rate, HA polymer size, response to cortisol, etc.

Table 1. Evidence for Residual Activation in Some Rheumatoid Synovial Strains

Patient	Sex	Age	Diagnosis	HA synthesis rate ($\mu\mu$g/cell/24 hr)	Glucose uptake (μM/10^6 cells/24 hr)	Lactate output (μM/10^6 cells/24 hr)
BS	F	46	DJD	6.3	0.39	—
KP	M	18	Rheumatoid Spondylitis	9.9	1.78	—
CaW	M	75	Normal	11.2	1.86	2.62
FS	M	81	Normal	12.4	1.73	3.37
RN	M	26	TA	14.4	1.17	2.33
TH	M	15	? TA	14.5	2.04	1.62
HH	F	29	Normal	15.3	1.69	2.66
WaK	M	71	Normal	17.7	1.24	2.66
FC	M	54	DJD	17.9	2.80	2.60
DM	F	13	Normal	23.3	0.99	—
CV	F	91	Normal	35.3	2.72	—
Mean				16.2 ± 2.3	1.67 ± 0.21	2.55 ± 0.20
PG	F	35	RA	9.7	1.36	—
OD	F	43	RA	15.7	1.33	4.32
GR	M	46	RA	17.8	2.56	4.53
ES	F	60	RA	21.7	1.30	4.09
EF	F	51	RA	29.7	1.59	—
DW	F	22	RA	43.5	3.70	5.44
RL	M	24	RA	49.9	4.43	—
JJ	F	17	RA	80.5	4.00	7.33
Mean				33.6 ± 8.3	2.53 ± 0.47	5.14 ± 0.59
Statistical information		N		17	17	10
		t		2.280	2.035	4.658
		P		$P < 0.05$	$P < 0.10$	$P < 0.01$

* DJD = degenerative joint disease; TA = traumatic arthritis; RA = rheumatoid arthritis.

† The nonrheumatoid and rheumatoid groups are compared for each parameter in terms of the mean ± the standard error of the mean. The significance of differences between means was evaluated by the Student-Fisher *t* method.

strains. In view of the fact that rheumatoid synovial cells frequently behaved as if they were activated and showed a diminished capacity to respond to exogenous "activator," whether of synovial, splenic, or HEp-2 cell origin, it seemed possible that these cells contained excessive amounts of an endogenous activator. To seek direct confirmation of this possibility we prepared saline extracts of four cell strains after disruption by freeze-thawing. The nonrheumatoid cell strains and two rheumatoid cell strains were subjected to the extraction procedure, the extracts dialyzed against water, clarified by centrifugation, and sterilized by filtration through millipore filters. The crude sterile extracts were placed on normal synovial cultures and the resulting data is recorded in Table 3. It was clear from this preliminary experiment that extracts from the two rheumatoid cell strains were more potent than the extracts of cell

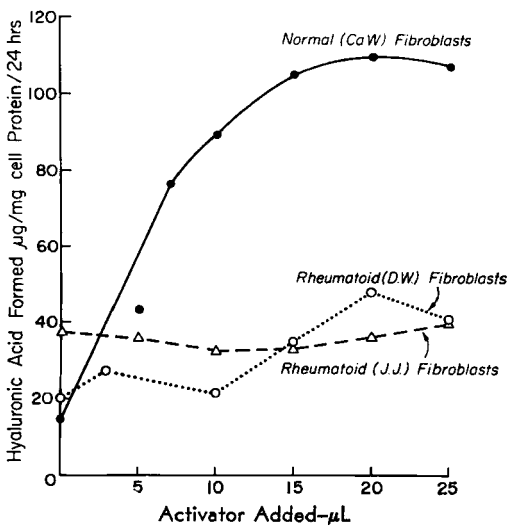

Fig 1. The activator (CTAP) added was derived from rheumatoid cell strain DW. 10 µl of the activator extract represents 10^6 DW cells.

Table 2. Comparative Activation of Nonrheumatoid and Rheumatoid Cells by Exogenous Activators*

Hyaluronate, (HA) synthesis (µg HA/mg cell protein/24 hr)	Nonrheumatoid (30)†	Rheumatoid (12)	Statistical parameters N	t	P
Basal	13.8 ± 1.9	27.0 ± 3.8	40	3.451	0.01
Activated	65.5 ± 4.6	44.6 ± 4.8	40	2.718	0.01
Activated/Basal Ratio,	6.9 ± 0.8	1.9 ± 0.2	40	4.051	0.01

* The experiments summarized in this table were carried out with 1.0×10^6 synovial cells per T-15 flask (or 2.0×10^6/T-30 flask), in the absence of serum. Data are presented as means ± standard errors of the means.

† Numbers in parentheses indicate the number of measurements. Nonrheumatoid strains used were CaW, WaK, and RN. Rheumatoid cell strains included: OD, DW, GR, and JJ.

strains from patients with osteoarthritis or traumatic arthritis. This preliminary assessment of the increased CTAP content of rheumatoid cell strains led us subsequently to make quantitative measurements of this material in a larger series of nonrheumatoid and rheumatoid cell strains. Extracts of cells were prepared by freeze-thawing with a phosphate saline buffer containing 0.1% β-mercaptoethanol, followed by centrifugation and then dialysis and filtration of the supernatant fluid. Both the nonrheumatoid and the rheumatoid cell extracts were assayed simultaneously on two occasions, once against normal cell strain HH and a second time against nonrheumatoid strain TH. In each bioassay, the rheumatoid cell strains had about four times as much CTAP activity as the normal cell strains, as can be seen from Table 4. Table 4 also provides an opportunity to see the sort of variation that may occur when a particular activator preparation is assayed against different cell strains. It is for this reason that we have advocated that quantitative comparisons be carried out by simultaneous assay of different preparations against the same indicator cell strain.

Chronic activation of normal synovial connective tissue cells. In order to determine whether or not repetitive exposure of a normal cell strain to exogenous activator would induce permanent behavioral changes, we undertook a seven-week experiment with normal cell strain HH wherein three cultures received phosphate buffer and three received a potent activator (partially purified by gel filtration) derived from the HEp-2 cell strain. The activator (11.5 µg protein) or vehicle (0.2 ml) was added at the time of the initial inoculation of 1 million cells into the culture vessels and at each medium change. On Day 7 the cultures were trypsinized, the cells counted,

Table 3. Activation of Normal Fibroblasts by Rheumatoid and Nonrheumatoid Cells*

Source of cell extracts	Amount of extract added (cell equivalent)	Hyaluronate/flask (μg)	HA increment/ flask/10^6 cell equivalents of extract (μg)
None	0	213	0
Osteoarthritis (BS)	3.2×10^6	384	54
Traumatic arthritis (MB)	3.6×10^6	489	77
Rheumatoid arthritis (DW)	2.6×10^6	812	230
Rheumatoid arthritis (EF)	5.5×10^6	925	130

* Normal WaK synovial cells served as the indicator strain in this experiment, carried out during the final 3 days of a 7-day growth cycle in serum dilution bottles nourished by our standard serum-containing media. Similar numbers of normal WaK synovial cells were in the various flasks during the experiment, and duplicate flasks were in each group.

Table 4. The Increased Activator (CTAP) Content of Rheumatoid Cell Strains

Non-rheumatoid cell strains	CTAP (units/10^7 cells)		Rheumatoid cell strains	CTAP (units/10^7 cells)	
	Measured with strain HH	Measured with strain TH		Measured with strain HH	Measured with strain TH
RN	1.7	0.9	PG	8.5	3.1
FC	2.0	0.8	ES	12.1	—
TH	2.7	2.6	DW	16.2	10.0
CaW	2.8	4.0	GR	20.4	9.0
FS	3.5	0.4	JJ	22.0	14.4
WaK	4.2	0.8	OD	24.2	14.4
HH	13.1	6.5	RL	27.4	12.2
Mean	4.3 ± 1.5	2.3 ± 0.9		18.7 ± 2.6	10.5 ± 1.7
	Statistical information		N	12	11
			t	4.861	4.411
			P	< 0.01	< 0.01

sized, and the media stored for chemical analysis. Three million cells from the activated cultures were used to inoculate three new daughter subcultures and the control flasks were subcultured in the same manner. The excess cells from both the control and activated cultures not used for subcultures were frozen for subsequent examination. This process was repeated at weekly intervals for seven weeks. At the end of the fifth week a third group of cultures was established, consisting of cells which *had been exposed to continuous activation for five weeks, but to which no further "activator" material was added.* This third group was the "omit activator" group.

As shown in Fig 2, the hyaluronic acid synthesis rate of the nonactivated cultures remained essentially constant over the period of observation, while those cultures receiving activator substance as a medium component exhibited a hyaluronic acid synthesis rate approximately double that of

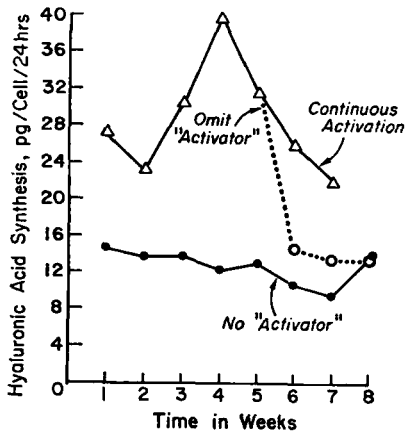

Fig 2. Hyaluronate synthesis remained elevated in the presence of exogenous activator, but returned to control levels by three weeks after discontinuation of this additive.

the control flasks. The hyaluronic acid synthesis rate of cultures that had been continuously activated for five weeks, and from which the activator was withdrawn, exhibited a prompt fall in hyaluronic acid synthesis rate. The effects of activation on the hyaluronic acid synthesis rate could no longer be distinguished three weeks after withdrawal of the peptide material. Fig 3 presents the details of the effect of chronic activation on several parameters of carbohydrate metabolism. It is clear from Fig 3 that with repetitive addition of activator substance to the cultures, increased glucose uptake, increased elaboration of lactate and maintenance of a very high lactate to pyruvate ratio was observed, *as long as exogenous activator was added to the cultures.* All of the parameters of carbohydrate metabolism returned rapidly to the control levels when the activator substance was omitted, as indicated in Fig 3. Measures of cellular energy metabolism seemed to return to control values after omission of CTAP somewhat faster than did the rate of hyaluronic acid synthesis. The effect of chronic activation on the proliferative capacity of normal synovial cells appears complex, and may involve dominance of the activation process over factors controlling "strain aging." It is clear from Fig 4 that culture growth was similar in control and activated cultures during the first three weeks, and then control cultures showed a decreased growth rate (a function of strain aging) which we have noted previously (18). Activated cultures did not show the same degree of slowing of the growth rate, and in a sense, the activator may be regarded as responsible for increased proliferation relative to their age matched (in vitro age) controls.

Activator content of synovial cells during chronic activation. Counted aliquots of cells were extracted with saline buffer (pH 7.0) containing β-mercaptoethanol (0.1%) after repetitive freeze-thawing and were processed as indicated above to be assayed for their CTAP content. Fig 5 illustrates the relative CTAP concentration in cells from control and activated cultures during the time course of the chronic activation experiment. The HH control cultures maintained a relatively constant level of CTAP in the range of six to seven units per 10^7 cells while the cultures receiving activator at each medium change very rapidly manifested extremely high levels of CTAP activity. It was interesting to note that the very high levels of CTAP activity did not occur until the end of the second week of activation. Perhaps the most important point to be extracted from this experiment is that *in those cultures from which the activator was omitted, there was a prompt fall in the cellular level of CTAP activity back to control levels.* We take this experiment to suggest that normal synovial fibroblasts are not permanently altered by repetitive exposure to an exogenous activator and that

their abnormal biochemical behavior and elevated content of activator return relatively rapidly to normal when the external source of stimulation is removed.

Activation as a possible cause for relative unresponsiveness to glucocorticoids. It is clear from Table 5 that both activator and lactate effectively oppose that action of cortisol, which depresses hyaluronate formation, unlike the situation reported earlier for ascorbic acid (19). The

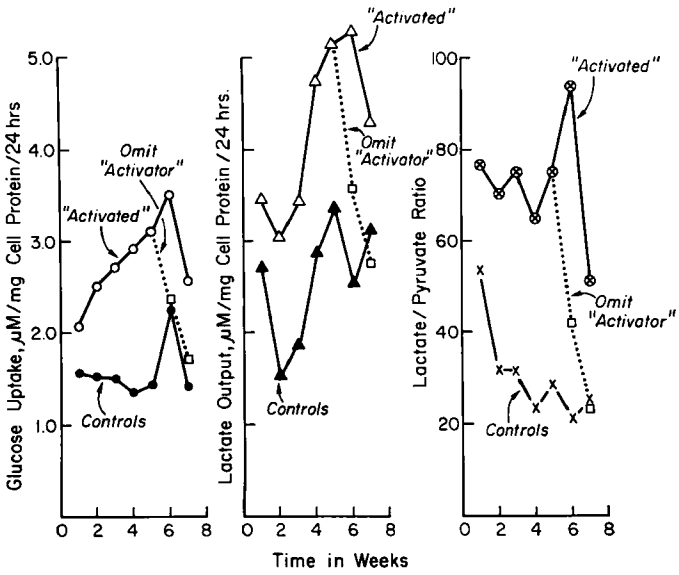

Fig 3. Glucose uptake and lactate formation were greatly stimulated by activator, but rapidly returned to normal levels on omission of the polypeptide. The markedly elevated lactate:pyruvate ratio reflects moderate depression of medium pyruvate as well as elevated lactate.

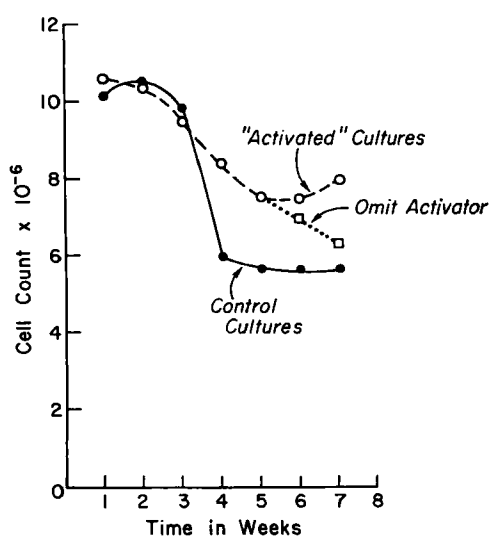

Fig 4. Chronic activation appears to impede the diminishing growth rate that accompanies strain aging.

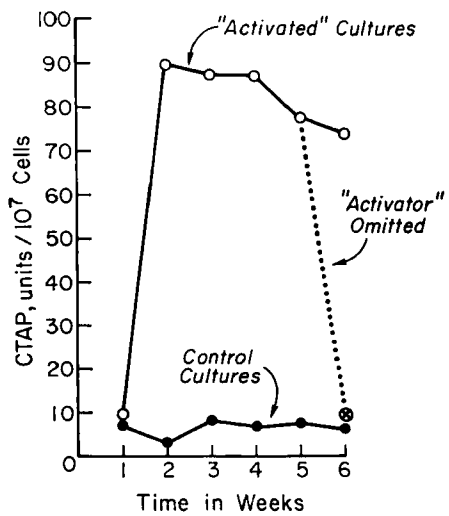

Fig 5. High levels of activator are recovered from cells receiving exogenous activator, but only during the period when it was being administered to the cultures.

nonrheumatoid strains, stimulated by activator were not depressed to the level induced by cortisol alone. While it is obvious that cortisol has a major suppressive effect on activator-induced hyperproduction of hyaluronate, (two right hand columns) as we reported earlier (20), it is clear that populations of activated cells would be hyporesponsive to cortisol suppression when compared to nonactivated cell populations. Since activation increases lactate in the cellular environment, we have been interested in the possibility that this metabolic alteration was involved in the resistance to glucocorticoid hormone. As shown in Table 5, lactate concentrations of 22 μM/ml (WaK) partially antagonized the steroid effect, while 11.0 μM/ml had an equivocal effect. Under our experimental procedure we seldom develop lactate concentrations over 9.0 μM/ml, even in highly activated cultures, suggesting that lactate accumulation is probably not the major explanation for steroid resistance seen in this in vitro system.

Table 5. Activation and Relative Unresponsiveness to Glucocorticoid*

	Hyaluronate synthesis ($\mu\mu$g/cell/day)			
	No additive	+ Cortisol	+ Activator	Activator + cortisol
NonRA strain (RN)	11.1	6.1	34.4	12.8
NonRA strain (CaW)	10.0	7.2	57.6	24.7
RA strain (DW)	59.5	38.5	89.4	51.4
	No Additive	+ Cortisol	+ Lactate	Lactate + Cortisol
NonRA strain (WaK)	18.8	9.8	28.5	21.5
NonRA strain (RN)	11.1	6.1	13.9†	7.7

* These experiments were carried out in serum dilution bottles, inoculated with 10^6 cells, nourished with standard serum-containing medium changed on Day 2 and 5 and terminated on Day 7. Test substances were added for the last 2 days of the culture period. Cortisol was added at a concentration of 1.0 μg/ml medium. lactate at 2.0 mg/ml medium and activator sufficient to generate a major response.

† Lactate was 1.0 mg/ml of medium. All values are the mean of triplicate determinations.

DISCUSSION

These data show that rheumatoid synovial cells frequently exhibit increased utilization of glucose, increased formation of lactate and increased production of hyaluronic acid. These phenomena have been previously shown to be the expected response of normal synovial cells to exposure to an activator substance. The failure of rheumatoid cells to show the same vigorous response to exogenous CTAP that may be demonstrated with nonrheumatoid synovial cells also suggested that the *rheumatoid cells might be different by virtue of an increased content of endogenous activator.* Experimental evidence indicated that the concentration of CTAP activity in rheumatoid synovial cells in culture was approximately four times that found in nonrheumatoid cells, possibly providing an explanation for their abnormal metabolic activity. The chronic activation experiment indicated that exogenous activator added to normal cells on a repetitive basis would induce a state of hyperfunction with respect to hyaluronic acid formation and energy metabolism which faded soon after the external source of stimulation was removed. In addition, the measurable content of activator in the cells maintained by external stimulation remained markedly elevated only so long as the external source of CTAP was provided. In view of this

finding, it seems unlikely that the demonstration of increased amounts of CTAP in rheumatoid synovial cells after several months in tissue culture represents retained activator derived from lymphoid cells or polymorphonuclear leukocytes in the original donor tissue milieu. It seems most reasonable to conclude that the rheumatoid synovial cells have been altered so that they form the connective tissue activating peptide either in excessive quantities, or possessing unusual activity properties.

Present data indicate that addition of exogenous activator (CTAP) to cultures renders them hyporesponsive to cortisol with respect to suppression of hyaluronate formation. Since rheumatoid cultures, which are usually hyporesponsive to cortisol in this regard, have excessive endogenous activator, it appears likely that defective cortisol regulation of HA formation is related to the excessive CTAP content of rheumatoid synovial cells. Accelerated utilization of glucose and formation of lactate seen in rheumatoid synovial cells in vitro may be explained on the same basis. Increased synovial activator may also explain the increased energy metabolism of rheumatoid synovium and the net increase in hyaluronate formed by this tissue in rheumatoid arthritis. Other abnormal characteristics of rheumatoid cells in vitro, such as inferior growth rate, tendency to form low molecular weight hyaluronate, and defective cortisol regulation of collagen synthesis are currently being studied to evaluate their relationship to the elevated CTAP content of rheumatoid synovial cells.

We interpret the data showing a constellation of propagable abnormalities in rheumatoid synovial cells as suggesting that a "primary defect" resides in the synovial cells themselves. The present data indicate that one propagable abnormality, excess CTAP, causes some of the others, including increased HA formation and increased glucose metabolism. The term primary defect is used here to indicate that rheumatoid synovial cells are essentially permanently altered in a way that leads to abnormal cell function, in contradistinction to the "innocent bystander" hypothesis which would picture the rheumatoid synovial cells as basically normal cells reacting to the continuing influence of CTAP formed by infiltrating leukocytes.

The mechanism by which such a primary defect might arise in synovial cells is not elucidated by the present study. Cellular alteration by physical, chemical, immune or infectious modalities would be among the many possibilities to be considered. Reports of possible differences between nonrheumatoid and rheumatoid synovial cultures with respect to viral susceptibility are of interest in the light of the present work. In an extensive study, Ford and Oh found no spontaneous degeneration of rheumatoid cultures suggesting latent viral infection (21). Further, they could not demonstrate a significant difference between rheumatoid and nonrheumatoid cultures with respect to susceptibility to Newcastle disease virus (NDV), nor did they find evidence of virus-inactivating or virus-interfering activity in fluid from rheumatoid cultures. On the other hand, Smith and Hamerman reported preliminary studies (22) to the effect that about 85% of nonrheumatoid cells were susceptible to NDV, while 30 to 60% of rheumatoid cells were susceptible, a difference which they felt was significant (23). More recently, Grayzel has reported that nonrheumatoid cells were killed by rubella virus (strain F-8) while rheumatoid strains were resistant (24). Rheumatoid cell resistance to rubella was not believed to be mediated by interferon. Interpretation of these data is

difficult, for one may argue: a) that viral resistance reflects a latent or defective virus within rheumatoid cells which in turn induces the spectrum of metabolic abnormalities which we have identified, or b) that activator-induced metabolic abnormalities of the rheumatoid cell cause it to behave differently towards viral agents than do normal synovial cells. In support of the first position it is important to recall the possibly analogous situation where chicken fibroblasts infected with Rous sarcoma virus show accelerated acid mucopolysaccharide synthesis and an increase in hyaluronic acid synthetase (25, 26). On the other hand, it would not be surprising if the relatively major physiological differences (eg, growth rate, HA and glucose metabolism) between normal and rheumatoid cells lead to different viral susceptibility, especially in view of evidence that even passage status and rate of cell replication have a major effect on cell-virus-relationships (27).

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