

AD 649214

Neutrophile Alkaline Phosphatase Changes in Tularemia, Sandfly Fever, Q Fever and Noninfectious Fevers

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TWO DECADES AGO, Wachstein¹ called attention to an increase in alkaline phosphatase (AP) activity of human neutrophilic leukocytes during bacterial infections. Enzyme activity within individual cells as well as the number of cells with detectable enzyme were markedly increased, an observation confirmed by others.^{2,7} Infection-induced rise in AP activity was described in neutrophiles of the marrow, peripheral blood, and exudates¹ and was more pronounced in adult cells when compared to band forms.^{1,8} The increase in AP correlated roughly in magnitude and timing with the severity of leukocytosis,^{1,3} although AP has been reported to remain elevated temporarily after leukocytosis subsided.²

A rise in neutrophile AP activity in many "stress" related situations led Valentine and his associates to investigate the possible influence of the pituitary-adrenal axis on this phenomenon.^{9,10} It was shown that glucocorticoids or ACTH (in the presence of a functioning adrenal cortex) could stimulate in vivo a rise in neutrophile AP^{9,10} after an initial lag. A rapid change in the WBC, with epinephrine-induced leukocytosis or bacterial endotoxin-induced leukopenia, was shown by Trubowitz et al.¹¹ to be associated, respectively, with a rapid rise or fall in AP.

Little is known concerning the relationship of a specific etiology of infection to accompanying patterns of neutrophile AP response. During viral illnesses, no rise in AP was detected in some instances,^{6,12} while in others a rise appeared to equal that observed during bacterial infection.⁷ During our extensive investigations of vaccine efficacy it became possible to study prospectively by serial collections the patterns of AP response in normal volunteers exposed to infectious microorganisms. Data were obtained during tularemia and Q fever, neither of which is associated with prominent leukocytosis, and during sandfly fever, a mild viral illness with a characteristic leukopenia. Artificial hyperthermia and endotoxin-induced fever were also studied to compare the responses of neutrophile AP during the stress of these infections to the stress of fever without concomitant infection.

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These studies were conducted in conjunction with a program for development and testing of vaccines for, and therapy of, acute infections. The investigations were supervised by the Commission on Epidemiological Survey of the Armed Forces Epidemiological Board. The cooperation of the War Service Commission of the Seventh Day Adventist Church is gratefully acknowledged.

First submitted April 28, 1966; accepted for publication July 21, 1966.

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1967
FEBRUARY 2 1967

MATERIALS AND METHODS

All volunteers were normal young adult males who ranged in age from 19 to 26 years. They were in excellent health as evidenced by repeatedly normal physical and laboratory examinations. Exposures to *Pasturella tularensis* or *Coxiella burnetii* were accomplished by aerosol; the sandfly fever virus was transmitted by the intravenous injection of 1 ml. of infectious serum. Only subjects who had not been vaccinated against the infecting organism were included in this report.

In other investigations, purified lipopolysaccharide bacterial endotoxin was administered intravenously 3 days in succession in increasing doses of 25, 50, and 100 $\mu\text{g.}$, respectively. Artificial hyperthermia was induced in still other subjects by raising the temperature and humidity of an environmental chamber so as to induce a gradual increase in rectal temperature over an 18-hour period to 102.5 F. and to maintain that level for an additional 6 hours, a pattern which mimicked the first day of fever in typical cases of tularemia.

Methods. Throughout the period of hospitalization of each group, blood films were prepared on microscopic slides at the time of control and postexposure blood counts. Unless otherwise noted, all specimens were obtained at 8 a.m. All slides were air dried and stored without fixation at room temperature to permit all material from a single patient to be stained simultaneously along with known negative and positive controls. The histochemical method of Wiltshaw and Maloney¹³ was used for visualizing the AP, which was then quantitated³ by assigning a rating of 0 to 4+ to each of 100 neutrophils counted consecutively for a possible total "AP index" of 0 to 400. Routine differential percentages were obtained by counting 100 cells and the total WBC was determined by Coulter Counter.

Data obtained on postexposure days of each study were compared to at least 3 pre-exposure control values from the same subjects by t-test to establish an estimate of probability, *p*.

RESULTS

Studies in Tularemia

Following aerosol exposure to *P. tularensis*, the incubation period generally varied from 3 to 5 days and was followed by fever, headache, malaise, and anorexia. The institution of streptomycin therapy at that time reversed the disease process before the development of pulmonary findings. In a number of subjects only mild symptoms and low-grade fever developed. In Table 1 are listed values for the total leukocyte, adult and band neutrophil counts, AP index, and the fever hours (hours per degree of rectal temperature above 100 F.) for 23 subjects with typical early tularemia and for 8 men with only mild illness. These latter were differentiated clinically from the typical group by a failure of their rectal temperatures to exceed 102.5 F. at any time and by a failure of their total fever indexes to reach 60.

The WBC in tularemia rarely exceeded 10,000/mm.³, but coincident with developing illness there did occur a statistically significant rise above control counts of the total WBC, as well as both the percentage and absolute number of adult PMN leukocytes. Band cells also increased significantly and there developed a gradually progressive rise in the AP index. This pattern, though less pronounced, was also evident in those subjects with mild illness (see Table 1).

In an effort to characterize more exactly the timing of leukocyte AP changes with respect to the period of illness itself, the above data were regrouped to

Table 1.—Alterations in Neutrophile AP During Tularemia (Cont.)

	Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
			<i>Typical Group</i>								
Fever, hrs. degree	0	0	12.4	26.3	38.5	26.3	5.8	5.2	3.4	3.2	0.3
WBC (thousands)	6.89	6.70	9.01	8.76	7.94	7.29	7.61	6.68	6.92	6.76	7.43
S.E.	0.22	0.38	0.63	0.35	0.45	0.42	0.42	0.44	0.36	0.44	0.46
p<			.01	.001	.05						
Adult neutrophiles (thousands)	3.60	3.59	6.07	6.01	5.55	4.66	3.76	4.51	3.72	3.61	4.05
S.E.	.15	.28	.69	.31	.46	.32	.30	.22	.20	.19	.21
p<			.001	.0001	.001	.01		.01			
Band neutrophiles (thousands)	.06	.10	.07	.39	.21	.43	.73	.39	.60	.15	.12
S.E.	.02	.05	.03	.12	.10	.10	.18	.11	.27	.04	.05
p<				.01		.001	.001	.01			.05
AP index	17.5	27.6	36.2	61.4	44.8	29.6	46.8	66.7	89.8	63.9	37.7
S.E.	4.2	6.1	9.2	13.5	11.4	6.4	8.2	10.7	21.1	29.4	17.4
p<				.01			.01	.001	.002		
			<i>Mild Group</i>								
Fever, hrs. degree	0	0	4.0	14.5	14.0	11.2	3.9	0.8	0	0	0
WBC (thousands)	6.77	7.00	9.02	7.56	7.28	7.42	6.92	6.06	6.47	7.18	7.00
S.E.	.55	1.35	1.81	1.06	.94	1.18	.76	.76	.48	.78	.57
Adult neutrophiles (thousands)	3.21	3.56	6.10	4.94	4.27	4.14	3.44	2.95	3.17	3.69	3.54
S.E.	.41	.87	1.61	1.02	1.17	.87	.62	.78	.51	.59	.39
Band neutrophiles (thousands)	.04	.14	.10	.14	.30	.47	.35	.04	.05	.14	.15
S.E.	.02	.10	.06	.08	.15	.27	.11	.02	.04	.08	.06
p<							.02				
AP Index	11.8	19.8	27.0	29.2	29.7	42.5	30.0	42.7	48.2	79.2	36.0
S.E.	3.5	5.5	11.1	6.4	9.8	21.4	8.4	15.1	21.2	39.1	11.6
p<				.05							

The average \pm S.E. values for the total WBC, adult and band neutrophiles, and AP index are compared with the febrile response in 23 subjects with typical tularemia and 8 with mild tularemia. Significant differences from control on any postexposure day are expressed as probability, p.

align the day of maximal fever (rather than the day of exposure) of the subjects with typical illness. The onset of typical induced tularemia was associated with a gradual increase in the AP index, which reached a level significantly above control the day prior to maximum fever (Fig. 1). Then, despite therapy and prompt clinical recovery, the AP index slowly mounted further to reach its peak 5 days after the height of illness.

Studies in Sandfly Fever

Symptoms of sandfly fever began abruptly in 7 of 8 men on the second day following exposure. Fever, headache, malaise, and anorexia increased in a typical fashion to maximum intensity the following day and then subsided rapidly. As shown in Figure 2, the characteristic leukocyte changes of sandfly fever were observed in this group: leukopenia and neutropenia with the appearance of many band forms. Bands became numerous during the developing neutropenia, diminished, and then reappeared again several days later during the return of the neutrophile count to normal. Alkaline phosphatase activity

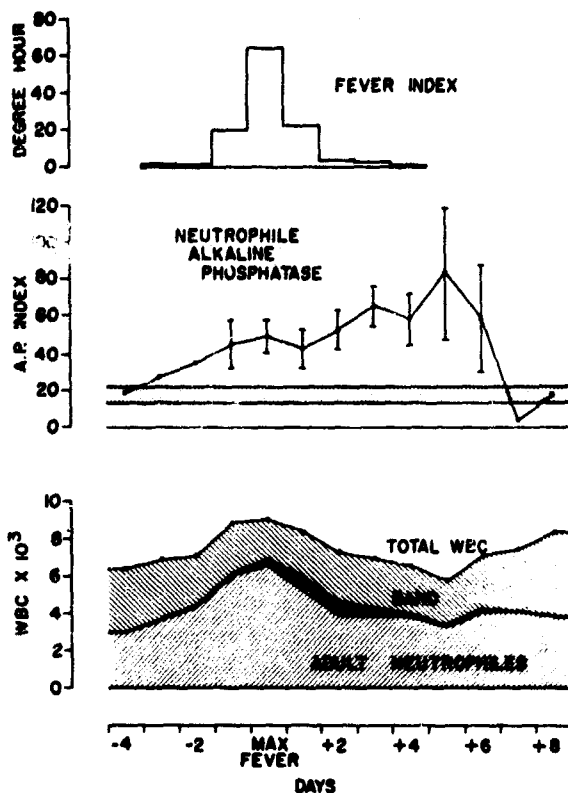


Fig. 1.—Neutrophile AP response in typical tularemia. Average values of 23 subjects are shown, replotted from Table 1 to align the day of maximum fever. Significant elevations of the AP index are plotted as the mean \pm S.E. in comparison to similar values of the control days represented by the horizontal shaded band. The total WBC is subdivided into adult (bottom area) and band (central black area) neutrophile portions, with the remainder (upper area) including all other leukocytes.

rose abruptly on the day of initial symptoms and leukopenia, but then subsided rapidly; it did not rise during the second period of band cell prominence. Although no symptoms developed in the eighth man inoculated with the sandfly virus, he showed patterns of change in his white cell count and AP activity identical to those of the symptomatic group.

Studies in Q Fever

Following exposure to an aerosol of *C. burnetii*, a group of 6 normal subjects remained asymptomatic and afebrile throughout an incubation period of more than one week. On the ninth postexposure day illness began in a typical fashion with malarial fever, headache, malaise, and anorexia (Fig. 3). These manifestations of illness were rapidly reversed by tetracycline therapy.

Although Q fever was not associated with prominent leukocytosis, and maximum elevations of the WBC during the period of illness did not exceed

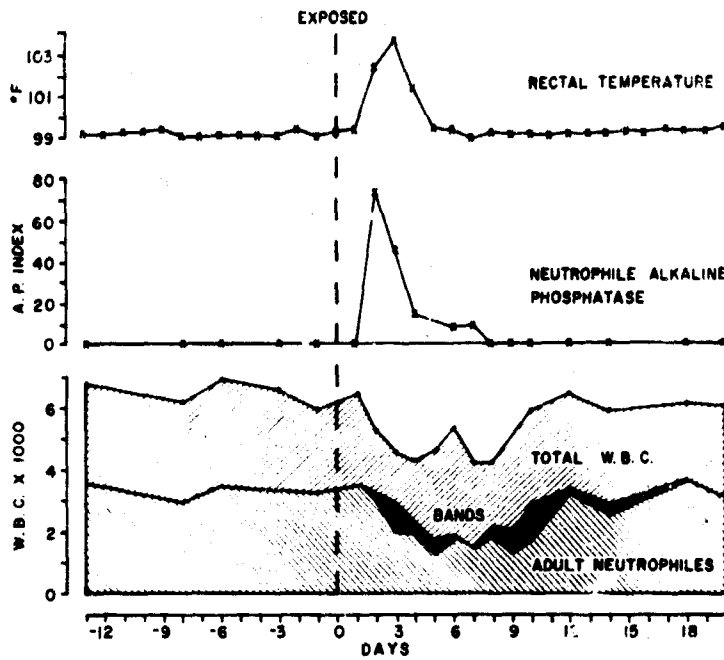


Fig. 2.—Neutrophile AP response in sandfly fever. Average values for 6 subjects are shown pictured as in Fig. 1. The day of exposure is indicated by a vertical dashed line.

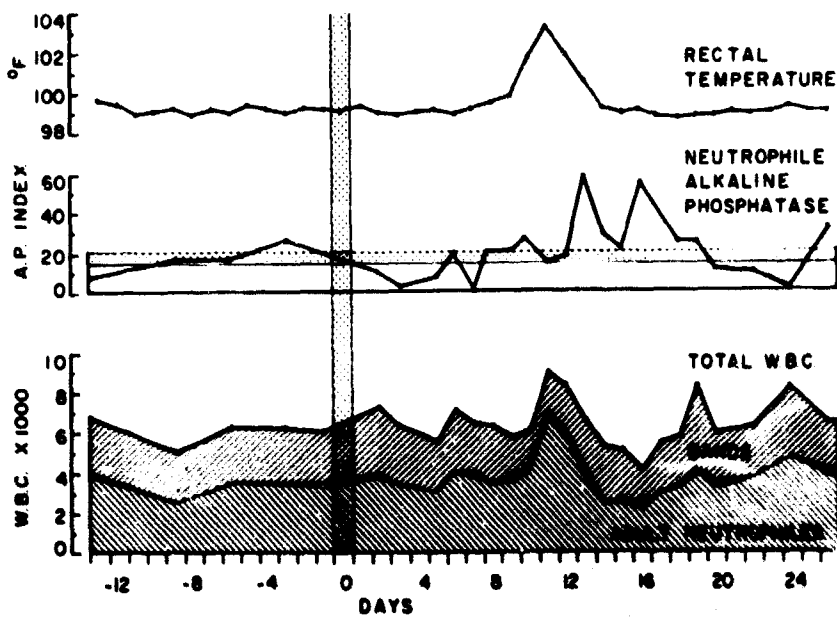


Fig. 3.—Neutrophile AP response in Q fever. Average values for 6 subjects are shown, with the day of exposure shown with a vertical shaded band.

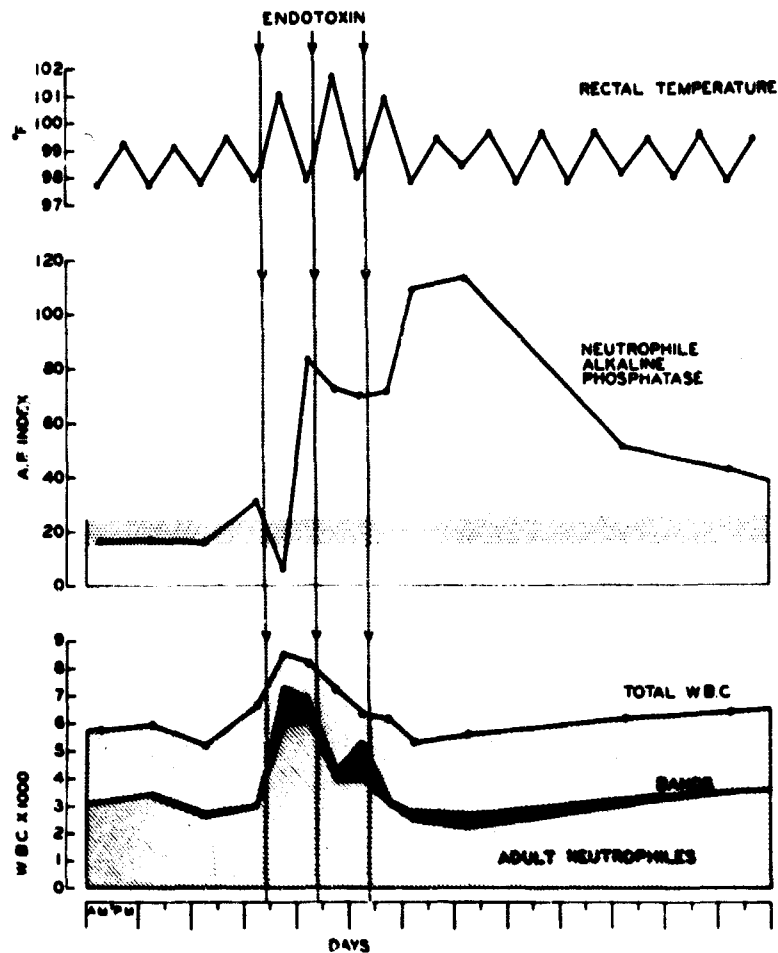


Fig. 4.—Neutrophile AP responses after bacterial endotoxin. Endotoxin was administered at the times indicated by the 3 vertical dotted lines. Average febrile responses of 6 subjects are shown with respect to their diurnal rectal temperature changes of control and postfever days.

10,000/mm.³, there was an increase in the adult neutrophile count which raised the total WBC above control and incubation period counts. Band forms increased minimally. During the incubation period and throughout early illness, the AP index remained within or slightly below the control values. Only after recovery did the AP index increase in a slight and irregular fashion.

Studies in Endotoxin-Induced Fever

The intravenous administration of bacterial endotoxin is followed by an initial period of leukopenia and then, as fever mounts, by leukocytosis. By serial blood counts which encompassed the early leukopenic response to endotoxin, Trubowitz, Moschides, and Feldman¹¹ detected an early fall in neutrophile AP which reached a minimum of 30 per cent of control 30 minutes after toxin had been given. In the present study (Fig. 4) of 6 subjects, the

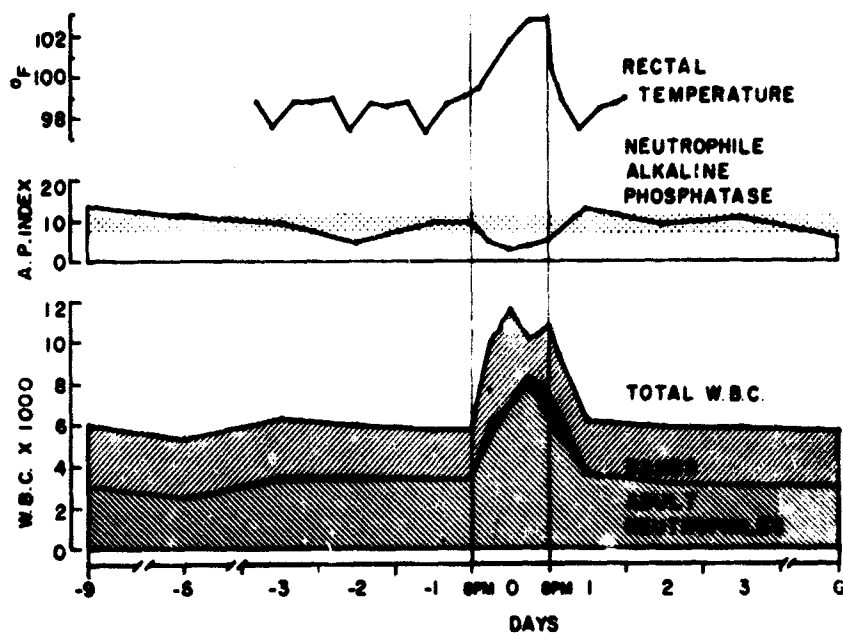


Fig. 5.—Neutrophile AP response to artificial hyperthermia. This was induced as described in the text by changing the heat and humidity of an environmental chamber during the times between the vertical lines on day 0. Average values for 8 men are shown.

WBC obtained at the height of fever after the initial administration of endotoxin was elevated, with an increased number of adult neutrophils and many band forms. In support of the findings of Trubowitz et al.¹¹ the AP index was found to be below control values at that time, despite the presence of leukocytosis. By the following morning, however, neutrophilia was observed to persist and there had developed a sharp rise in the AP index. With the administration of endotoxin in increased amounts on each of the next 2 days, the absolute adult neutrophile and band counts did not remain as high as when measured at the height of fever following the initial dose. In contrast, the elevated AP index continued to rise, reaching its maximum value 2 days after the final dose of endotoxin.

Studies in Artificial Hyperthermia

Leukocytosis with a shift to the left is widely recognized as an accompanying feature of hyperthermia produced artificially by a high ambient temperature. Leukocytosis was observed (Fig. 5) when fever was induced in a group of 8 men in a manner designed to mimic the pattern of fever during the onset day of acute tularemia. Control values at 8 p.m., when subjects entered the environmental chamber, were similar to other control values obtained at 8 a.m. There then occurred an increase in adult neutrophiles and band forms during fever; control counts were reestablished within 12 hours after subjects left the hot environment.

Despite this leukocytosis and the accompanying environmental stress, AP activity showed no detectable changes. Because this finding was not expected, additional blood films collected throughout the entire study were stained on 3 separate occasions, and showed a consistent failure of AP to increase during or after fever.

DISCUSSION

Several different patterns were observed in the sequential responses of neutrophilic AP after challenge by a variety of infectious microorganisms or etiologies of fever. Our inability to explain these patterns readily on the basis of current theories is indicative of the continuing lack of insight concerning the exact function of the enzyme and the mechanism of its induction within leukocytes. The alkaline phosphatases of bacteria have been studied extensively; in contrast, investigations concerning changes during infection of these enzymes in various tissues of animal hosts are few,^{14,15} and serve to illustrate the complexity of the problem. An understanding of the basic mechanisms which might bring about the variety of changes observed in the present study would appear to require knowledge concerning cofactor requirements of the enzyme, concentrations of specific substrates, a number of physiologic or hormonal influences, cell age, factors which control the mobilization and removal of neutrophils, as well as an appreciation of the genetic potential for AP induction in cells of differing types and species.

In hypophosphatasia, a genetic deficiency of tissue AP, the enzyme is lacking in neutrophils and cannot be activated by glucocorticoids¹⁶ or ACTH.⁶ Neutrophils of several species lack this enzyme.¹ Based upon evidence of significant increases of neutrophilic AP in subjects with mongolism and neutrophilic AP depression in certain types of chronic granulocytic leukemia, Alter and associates¹⁷ suggested that a short acrocentric chromosome of the human might serve as a genetic locus for leukocyte AP.

Valentine, Tanaka, and Fredericks¹⁸ reviewed evidence that zinc and/or magnesium were important cofactors of alkaline phosphatases from various tissue sources; they demonstrated that 0.0001 M zinc protected against AP loss or restored AP activity in stored neutrophils and blocked its inactivation by ethylene-diamine-tetra-acetate. Magnesium was ineffective in comparison.¹⁸

Several physiologic, biochemical, or hormonal changes appear to influence neutrophilic AP activity. Vaccari et al.¹⁹ reported that increased neutrophilic AP in rabbits during formalin shock or humans in surgical shock could be correlated with elevated rates of whole blood glycolysis. In infancy, leukocyte AP activity is moderately elevated and falls to adult values over a period of several years. Pregnancy^{20,21} and estrogen administration to males²² are associated with increased leukocyte AP. A prompt rise in the peripheral WBC after epinephrine administration was accompanied by increased neutrophilic AP,¹¹ it has not been determined if this rise was mediated directly by epinephrine, through pituitary-adrenal stimulation, or represented merely a mobilization of stored cells.

Valentine and associates^{18,22} postulated that neutrophilic AP was elevated in a variety of circumstances associated with "stress." A rise in leukocyte AP was

observed after the administration of ACTH, cortisone, or cortisol.⁹ Such an ACTH response required the presence of functioning adrenocortical tissue,¹⁰ and appeared to be mediated through glucocorticoid action but not by DOCA,²² Compound S, or ascorbic acid.¹⁹ This observation was substantiated more recently in cultured HeLa cells treated with a large variety of steroids.²³ In human: given ACTH, 40 units every 8 hours for 3 days, the rise in neutrophilic AP progressed gradually to a peak 3 to 4 times greater than control.^{9, 22} Cortisol, in a dose of 60-70 mg. administered orally throughout an identical schedule, produced only a twofold rise¹⁰ after an initial lag with a prompt return to control values when steroids were discontinued. Pienert²⁴ observed that large doses of cortisone or its derivatives inhibited neutrophilic AP, whereas small doses were stimulatory. A transient fall in leukocyte AP has also been reported after intravenous injections of prednisolone.¹¹

In subjects with tularemia, Q fever, and endotoxin fever, the greatest elevation of neutrophilic AP was delayed until several days after maximal fever and illness. This delayed peak seemed similar to that reported after surgery⁹ in which the maximal rise in neutrophilic AP did not occur until 72 hours post-operatively, and the peak confirmed an earlier report² of a persisting AP elevation after infection-related neutrophilia had subsided. The timing of the peak rise in neutrophilic AP, as observed in tularemia and Q fever, seemed incompatible with a response due solely to adrenal stimulation. Plasma and urinary steroid responses were measured during the studies reported herein.²⁵ In each of these infections, urinary 17-OHCS excretion rose with developing symptoms and fever to a maximum approximately double control values which occurred in coincidence with the peak of fever.²² With the institution of specific antibiotic therapy, the 17-OHCS excretion consistently returned to baseline control levels within one day, although fever took several days to disappear completely. During the fever of these acute infections, the 8 a.m. plasma 17-OHCS concentrations did not differ from controls, however, the usual diurnal afternoon and evening fall in plasma cortisol failed to occur.²² Thus, the adrenal response in these brief, promptly-treated infections did not seem to achieve the magnitude or duration of corticoid excess required experimentally to stimulate human leukocyte AP.

As emphasized by Valentine et al.,¹⁰ the demonstration of an adrenocortical-mediated increase in unit-cell AP activity did not preclude existence of other important mechanisms which might initiate a similar response. The relatively small increase in adrenal activity and its early termination in these infections gave indirect evidence against adrenocortical mediation of the delayed and prolonged increases in leukocyte AP which were observed. This also raised the possibility that enzyme induction was related to some other factor associated with infection, *per se*. Such a distinction, based upon the timing of sequential changes, seemed possible only during infections such as those studied herein, in which the lack of an explosive leukocytosis permitted patterns to appear as if in "slow motion."

The men subjected to artificial hyperthermia experienced an adrenal response equal to, or slightly greater, than the response observed during infection.^{21, 22} The lack of any detectable increase in neutrophilic AP during or after

the stress and leukocytosis of artificial hyperthermia is further evidence that the measured increase of adrenocortical activity was inadequate to induce activation of neutrophile AP. However, other interpretations may also be possible. Hyperthermia induced by a high environmental temperature is accompanied consistently by respiratory alkalosis. In addition to an elevated serum pH, we observed a fall in serum inorganic phosphorus and magnesium, and an acutely negative magnesium balance.²⁶ These elements are both important for bacterial AP induction.²⁷ Perhaps the changes in these ions, alone or in combination, or a change in some other key trace metal ion, such as zinc, might have inhibited any rise in neutrophile AP anticipated during stress of the degree observed.

In contrast to the consistent elevations of neutrophile AP reported during pyogenic bacterial infection, results obtained during viral illness have varied.^{6,7,12} Lack of AP change in 55 patients with viral illness^{6,12} contrasted with Plenert's statement⁷ that an increased AP index was one of the most indicative signs of viral illness in children. It is possible that these divergent findings were the result of age differences alone. However, our serial observations indicated that an AP rise during the benign viral illness of sandfly fever did occur in adults. This rise, small in magnitude and brief in duration, developed during early symptoms, in conjunction with an abrupt fall in the neutrophile count. Perhaps the observed change in the AP index was merely a secondary phenomenon associated with rapid mobilization and subsequent disappearance of stored leukocytes.¹¹ Because of the unusually marked neutropenia of sandfly fever, the early rise in AP described herein may not be typical of other viral illnesses; its timing and course was certainly different from the AP increase observed with tularemia and Q fever.

Earlier reports^{4,8} that AP activity was less apparent in young neutrophiles were supported by these studies. Band forms appeared at two distinct periods of sandfly fever, during the development and recovery from leukopenia, but a rise in neutrophile AP accompanied only the first appearance of bands. In artificial hyperthermia, leukocytosis with many band forms was noted in the absence of increased AP activity.

SUMMARY

Responses of neutrophile AP were studied in volunteers during induced tularemia, Q fever, sandfly fever, and during artificial hyperthermia and endotoxin fever. Symptoms of typical early tularemia were accompanied by a slight increase in the neutrophile count and AP index. The latter continued to rise slowly and eventually became maximal 5 days after the height of clinical illness, long after fever and leukocytosis had disappeared. Similar but less obvious changes occurred after mild tularemia and after typical early Q fever. A transient rise of neutrophile AP occurred only at the onset of fever and neutropenia in sandfly fever. An early fall in neutrophile AP after endotoxin administration was confirmed; this was followed in turn by a prolonged rise not described heretofore. Despite the stress and leukocytosis of environmentally induced artificial hyperthermia, leukocyte AP did not increase.

Based upon these observations it seemed evident that increased leukocyte

AP activity could not be correlated in all circumstances with fever per se, the appearance of band cells, or the magnitude or timing of "stress" as inferred from increased plasma and urinary steroids. Increased neutrophile AP activity in these infections did not seem to be related directly to glucocorticoid excess. Possible factors involved in the induction of neutrophile AP may include the genetic potential of various cell types, coenzyme requirements, hormonal responses, as well as other yet unknown factors associated with acute infection.

SUMMARIO IN INTERLINGUA

Le responsas del phosphatase alcalin (PA) de neutrophilos esseva studiate in voluntarios durante inducite episodios de tularemia, de febre Q, de febre Pappataci, de hyperthermia artificial, e de febre a endotoxina. Le symptomas de typic tularemia precoce esseva accompagnate de un leve augmento in le numeration neutrophilic e le indice de PA. Iste ultimo continuava su augmento lentamente attingente su maximo 5 dies post le culmine del morbo clinic, i.e., longemente post que le febre e le leucocytos habeva disparite. Simile sed minus obvie alterationes occurreva post leve formas de tularemia e post typic febre Q precoce. Un augmento transiente del PA neutrophilic occurreva soimente al tempore del declaration de febre e de neutropenia in febre Pappataci. Un precoce declino in le PA neutrophilic post le administration de endotoxina esseva confirmate, e isto esseva sequite per un prolongate augmento non previemente describite. In despecto del stress e del leucocytosis de artificialmente inducite hyperthermia ambiental, le PA del leucocytos non montava.

A base de iste observationes il pare evidente que un augmentate activitate de PA leucocytic non poteva esser correlationate in omne casos con febre per se, con le apparition de cellulas bandate, o con le augmento de steroides plasmatic e urinari. Le augmentate activitate de PA neutrophilic in le infectiones studiate non pareva esser relationate directemente con un excesso de glucocorticoide. Factores possibilmente participante in le induction de PA neutrophilic include forsan le potential genetic de varie typos cellular, le requirimentos de coenzyma, le responsas hormonal, e etiam altere non ancora cognoscite factores associate con infection acute.

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