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Adjuvant Disease in Rats: Biochemical Criteria for Distinguishing Several Phases of Inflammation and Arthritis (37863)

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At least three phases have been distinguished in the development of arthritis in rats following inoculation with an adjuvant: these are the prearthritic, arthritic, and osteogenic phases.[3] It is of some importance to distinguish these (1) when evaluating anti-inflammatory compounds administered prophylactically, i.e., before and during arthritis development, or therapeutically, i.e., against established arthritis (2).

The hypoalbuminemia in rats during the prearthritic and early chronic stage of adjuvant arthritis has been well-documented (3–6). This report describes changes in two other biochemical parameters associated with (a) the different stages of arthritis development, (b) the remission of chronic inflammation, and (c) the permanent crippling (osteogenic) phase of adjuvant disease. These parameters are the dysfunction in hepatic drug metabolism (7, 8) and the altered plasma-thiol content (9, 10).

Experimental. *p*-Chloromercuribenzenesulfonic acid (PCMBS)-^{205}Hg was obtained from Amersham-Searle, Chicago, and diluted with nonradioactive PCMBS to a specific activity of 50 μCi/mg.

Male rats (200–250 g) were used throughout; outbred Wistar rats were obtained from Hilltop Lab Animals, Chatsworth, CA; inbred Lewis and Buffalo rats were obtained from Microbiological Associates, Walkersville, MD. Normal arthritogenic adjuvants were constituted with 10 mg dried *Mycobacterium tuberculosis* (human) (Central Vet. Lab, Weybridge, England) or *M. butyricum* (Difco Labs, Detroit, MI) per ml in mineral oil. A super adjuvant was constituted likewise using a strain of *M. tuberculosis* (Froman) isolated from a patient at the Olive View Hospital, Sylmar, CA, and made available through the courtesy of Drs. H. Glenshure and J. Levy (UCLA). This latter adjuvant caused gross arthritis in all extremities of Buffalo rats, a rat strain that is rather resistant to conventional adjuvant preparations and normally manifests only minimal arthritis (11). Adjuvants (50 μl) were inoculated in one rear paw or tail or one ear of an animal on Day 0; arthritis regularly developed by Day 14 and was assessed by foot paw measurements with a micrometer screw gauge. Albumin was determined fluorometrically (12), albumin:total protein (A/P) ratios by determining total plasma protein with a biuret reagent (13), α_2-glycoprotein and fibroginogen together as "plasma inflammation units" (14), and serum thiols by a radiometric procedure (15) as follows. Tail vein plasma (0.1 ml) was diluted with water (0.65 ml) and incubated with 1 mM PCMBS-^{205}Hg (0.2 ml) for 5 min at room temperature, the mixture

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TABLE I. Changes in Plasma Albumin, Plasma Inflammation Units (PIU), and Sleep Times of Rats after Inoculation of Adjuvant in (A) One Ear or (B) One Rear Paw.[a]

Day	Albumin × control	PIU × control	Sleep time × control
(A)			
−1	1.0	1.0	1.0
+2	ND	ND	1.23
3	0.74	3.5	1.05
4	ND	ND	1.17
6	0.76	1.0	0.98
9	0.69	4.0	2.60
12	0.44	8.6	7.9
13	0.61	27.0	5.3
(B)			
−1	1.0	1.0	1.0
+2	0.72	36.0	2.63
3	0.54	24.0	4.45
4	0.68	24.6	4.35
6	0.62	7.1	3.0
9	0.56	3.8	3.3
12	ND	15.8	3.3
14	0.32	26.0	6.4

[a] Groups of four animals. See Table II footnotes for details.

diluted with 1.5 ml of 75% (w/v) trichoracetic acid (TCA), and centrifuged after standing for 15 min. The precipitate was twice washed by resuspension in 2 ml 3.7% TCA, centrifuged again, and then counted in a Nuclear-Chicago gamma radiation counter. After counting, the precipitate was resolubilized by adding water, neutralizing with NaOH, and the total protein content determined with the biuret reagent.

Animals were challenged at 3-day intervals with a sublethal dose of ip sodium hexobarbital (normally 150 mg/kg; reduced to 100 mg/kg in animals with advanced inflammatory arthritis). The duration of narcosis (sleep time) provided a measure of hepatic drug metabolizing activity (DMA).

Results. Distinction of two early phases of systemic disease by route of adjuvant inoculation. Inoculation of an adjuvant in a rear paw or tail-base, as conventionally practiced, induces a rapid impairment in drug metabolizing activity (DMA) that reflects a systemic response to an acute local inflammation (8, 16). This is readily observed by the extended hexobarbital-induced narcosis, apparent within 48 hr of inoculation.

Inoculating the same adjuvant in an ear (or forepaw) caused far less extensive local inflammation and little or no alteration in hexobarbital sleep times, but it did induce appreciable hypoalbuminemia during the prearthritic phase of the developing disease (Table I). Extended sleep times were only observed on or after Day 12, i.e., when the signs of arthritis and chronic inflammation in other tissues (distal ear, penis, spleen) developed.

Thus the ear route of adjuvant administration spared the animal one of the early dysfunctions (i.e., in DMA) associated with "normal" adjuvant disease, probably because the initial acute inflammatory response was much more restricted. Nevertheless the liver was not entirely unresponsive to this milder

TABLE II. Changes in Plasma Albumin and Duration of Sleep Times (Hexobarbital) in Three Strains of Rats with a Chronic Arthritis.[a]

Animals	Time[b] (weeks)	Albumin levels × control	Sleep time × control
Without adjuvant	—	1.0	1.0
Lewis	2	0.3	3.5
	4	0.46	2.3
	6	0.68	2.2
	8	0.90	2.0
Wistar	2	0.49	4.0
	3.5	0.58	5.8
	5	0.62	3.6
	6	0.68	1.7
Buffalo[c]	2	ND	4.5
	4	0.40	4.0
	5	0.49	8.0
	6.5	0.69	7.8
	9	0.89	1.4

[a] All values are means from groups of 4 animals. Data compiled from a control group (no adjuvant) on each day was assigned the value of 1.0: Albumin levels (± SD) in this normal groups were 37 ± 3, 41 ± 9, and 35 ± 2 mg/ml for Lewis, Wistar, and Buffalo rats, respectively; normal sleep times (± SD) with ip sodium hexobarbital (150 mg/kg) were 32 ± 3, 28 ± 3, and 24 ± 3 min for Lewis, Wistar, and Buffalo rats, respectively. ND = not determined.

[b] After inoculation with adjuvant in foot or tail.

[c] Inoculated with "super adjuvant" (unresponsive to foot inoculation of normal adjuvant); see *Experimental.*

inflammagenic insult, since the albumin levels were subnormal during the prearthritic period, implying a partial reduction in hepatic albumin synthesis.

Distinction of two later phases of systemic diseases by sleep times and albumin levels. Table II records the change in hexobarbital-induced sleep times and albumin levels over the period when the animals had established distal arthritis, with much tissue swelling at the site of adjuvant inoculation. The data indicate that there are at least two phases of the established arthritis with differing effects on these biochemical parameters. One phase, apparent at 2–4 weeks, is characterized by pliable and edematous paw swelling, perisplenitis and splenomegaly, and accompanied by definite liver dysfunction (lowered DMA and albumin synthesis). This liver response reflects a systemic response to the chronic inflammation of many joints and tissues (including the spleen). An overlapping (and much later) phase is characterized by normalization of sleep times, albumin levels, and spleen size with considerable ankylosis of the extremities and brittleness of swollen feet, reflecting extensive osteogenesis and minimal residual inflammation.

Thus one phase of the arthritic disease, the active inflammatory component, spontaneously remitted over the time period 6–8 weeks postadjuvant but another phase, tissue restructuring and hard-tissue formation, proceeded unchanged. It is evident that measurements of paw swelling alone are insufficient for determining the extent of ongoing (chronic) active inflammation.

Distinction of five phases based on changes in plasma protein thiol levels. Glutathione and other low-molecular-weight thiols are not measured by the procedure used (15). The data presented in Fig. 1 indicate that the depression in macromolecular thiol(s) is closely associated with the development, progress, and remission of chronic inflammation. The total plasma protein content, however, was remarkably constant over the same time period (up to 90 days), as noted by others (3–5). The plasma protein thiol level differs significantly from several other

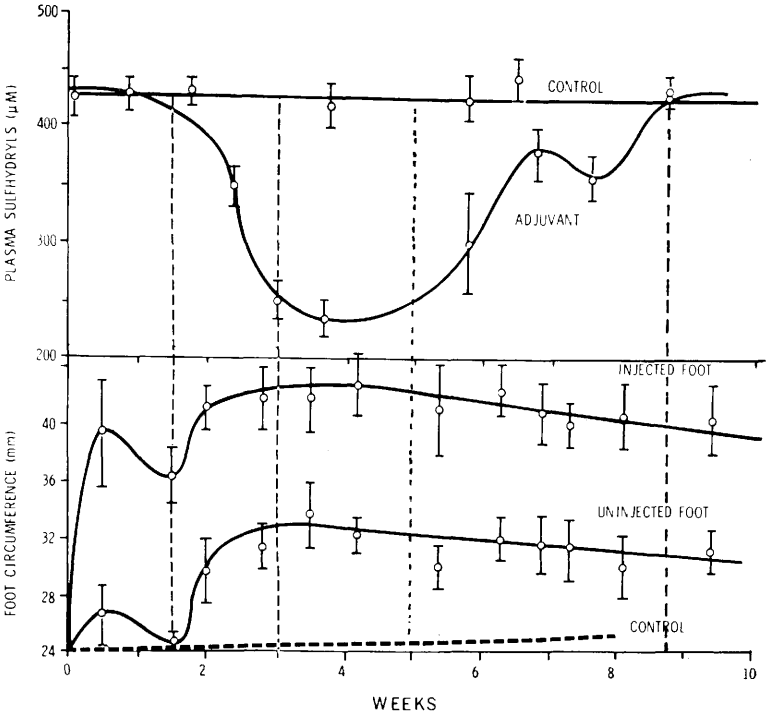

FIG. 1. Upper curve: Changes in the plasma thiol levels during the development of adjuvant disease, indicated by the foot swelling (lower curves).

TABLE III. Some Features Distinguishing Distinct Phases of Adjuvant Disease.[a]

Phase	Approximate time (days after adjuvant inoculation)	General characteristics	Local inflammation[b]	Splenomegaly	Impaired DMA	Distal foot swelling	Hypoalbuminemia	Thiol depression[c]
(a)	1–4	Acute local inflammation; systemic effect (liver)	++	0	+ (0)	0	+ (0)	(±)
(b)	7–12	Remission of acute inflammation; prearthritis	+	0	+	0	++ (±)	0
(c)	12–28	Chronic inflammation with periarthritis	+++	+	++	+	++	++
(d)	21–	Residual systemic inflammation; osteogenic activity	+	+	+	++	+	++
(e)	35 onwards (indefinitely)	Permanent articular deformity, minimal (burnt-out) inflammation	0	0	↓0	+ (+)	0	↓0

[a] Characteristic responses are those following tail/rear paw injection of an arthritogenic adjuvant. Alternate responses following ear inoculation of adjuvant are shown in parentheses.

[b] At site of adjuvant inoculation.

[c] Thiol titer of plasma proteins.

biochemical parameters associated with adjuvant disease (e.g., albumin/globulin ratios, inflammation units) in that it changed very little during the initial acute, nonimmune, inflammatory phase.

At least five phases of the adjuvant disease can be recognized on the basis of changes in macromolecular thiol values (Figure 1). They are: (a) A nonimmune inflammatory phase characterized by a small (if any) transient depression in the thiol titer (0–4 days); (b) a developing phase of chronic inflammation and arthritis associated with a rapid drop in thiol titer (approximately 14 days onwards); (c) a period of established chronic inflammation associated with stable but depressed thiol levels (extending up to Day 50); (d) remission of chronic inflammation accompanied by fluctuating but increasing plasma thiol content (Days 35–60); (e) permanent articular deformity associated with normal thiol values (after Day 60). The durations of each period, indicated in parentheses, are only approximate values. The actual time of incidence and length of the various phases are influenced by several different factors, e.g., the type and quantity of adjuvant, the site of injection, the strain of rat, etc.

Discussion. Previous studies have validated that the altered sleep times following ip hexobarbital reflect significant changes in hepatic drug metabolizing activity in animals with both acute (16) and chronic (17) inflammation. Table III summarizes some evidence for the existence of at least five different phases in the response of a susceptible strain of rats to an arthritogenic adjuvant. Nonarthritogenic "adjuvants" (16, 17) only induce a response corresponding to phase (a) (Table III).

Plasma protein thiol levels are characteristically depressed in different connective-tissue diseases (18). This depression is particularly severe in patients with associated vasculitis and, furthermore, appears to be unrelated to changes in the albumin/globulin ratio. The animal studies suggest that the thiol reduction is associated with the phase of gross tissue injury (and restructuring) rather than with edemic inflammation *per se* (e.g., acute phase).

Animals developing arthritis reduced their food consumption, the minimum occurring about Day 15 when food intake was about 50% that of the controls. Food consumption was almost normal again by Day 30 when the animals had largely recovered from the gross systemic inflammatory phase. Phase (e) has therefore many characteristics of almost normal "well-being" though many of the joints are permanently deformed and the active inflammation is "burnt-out."

Spontaneous remission of the splenomegaly has been observed 2 weeks or more after onset of arthritis (19, 20). The spleen: body weight ratio may provide a more realistic guide to the virulence of the inflammatory disease from Day 18 onwards than measurements of foot swelling. Normalization of albumin levels was observed by Day 39 in a previous study (5).

Summary. (1) The impaired drug metabolism, associated with adjuvant arthritis, spontaneously returns towards normal after 6 weeks. (2) The plasma protein thiol titer falls precipitiously with onset of arthritis but eventually returns to normal. (3) Based on these and other criteria, at least five phases of the disease can be distinguished which are summarized in Table III. (4) The Buffalo rat may develop massive arthritis, though relatively resistant to conventional adjuvants.

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