Table 2. Acid unmasked 'hidden' RF in serum and synovial fluid samples and in their IC-enriched fractions of RA patients

		RF in 3% PEG precipitates after acidic dissociation	No RF in 3% PEG precipitates after acidic dissociation	3% PEG insoluble protein (μg/ml) mean ± SE*	3% PEG insoluble C4 (μg/ml) mean ± SE**
Serum	'Hidden' RF positive (n = 21)	17	4	262.4 ± 30.3	4.3 ± 0.9
	'Hidden' RF negative (n = 38)	10	28	229.3 ± 22.4	2.0 ± 0.4
			$p < 0.001$***	p = n.s.****	$p < 0.0025$
SF	'Hidden' RF positive (n = 23)	19	4	297.6 ± 41.9	3.5 ± 0.74
	'Hidden' RF negative (n = 17)	7	10	206.7 ± 49.7	1.35 ± 0.2
			$p < 0.005$	p = n.s.	$p < 0.001$

* Protein content of PEG fractions of 80 healthy persons (mean ± SE) = 105.3 ± 4.5 μg/ml; ** C4 content of PEG fractions of sera of 80 healthy persons (mean ± SE) = 0.34 ± 0.1 μg/ml; *** χ^2-probe (in sera) $\chi^2 = 16.2$, n = 59; in SFs $\chi^2 = 8.0$, n = 40); **** Student's t-test.

taken for the presence of complexes with special size or composition not precipitated by the PEG solution used in our experiments. Anyway, possibly because of the heterogeneity of ICs in samples, different approaches for measuring RF-ICs do not provide completely similar results.

At the same time, we found more than 3% PEG-insoluble protein and significantly higher amounts of complexed C4 in samples with positive 'hidden' RF than in negative ones. The level of complexed complement factors (e.g. C4) seems to provide rather sensitive and specific characterization of ICs[13]. As we found significantly more complexed C4 in ICs of 'hidden' RF positive samples than in those of the 'hidden' RF negative cases, the suggestion can be made that a close relation between the 'hidden' RF and IC-level might be considered.

In these experiments we used Rose-Waaler method at 37 °C, therefore unmasked 'hidden' RF antibodies detected are mainly IgM rheumatoid factors[14]. (Moreover, according to our previous unpublished observations, agglutinating activity of acid-unmasked RFs can be completely abolished by 0.1 M 2-mercapthoethanol.) As neutralization is done only immediately before titration of dissociated RF antibodies, separation from the dissociated ligands (IgG) in most of the cases is not necessary.

The mild acidic treatment to 'split' ICs in sera and SFs seems to offer a simple screening method to detect 'hidden' RFs.

At the same time, results presented here may raise the problems of conventional RF measurements. In certain cases the false-negative of false-low titres of RF antibodies may mean the presence of another pool of circulating RFs, masked in ICs. Therefore a more cautious evaluation of results of conventional laboratory RF tests is needed.

The parallel occurrence of 'hidden' RFs in sera or synovial fluids and in their IC-enriched fractions may suggest that the measurement of 'hidden' RFs in body fluids may permit a very simple estimation of the level of some IC-bound rheumatoid factors providing diagnostic possibility mainly for rheumatoid vasculitis.

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Local adjuvants: The influence of sodium dodecylbenzene sulphonate on immunization with aerosolized antigen[1]

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Summary. Guinea-pig respiratory and serum antibody responses were enhanced following exposure to aerosols of bovine IgG_2 dissolved in solutions of sodium dodecylbenzene sulphate (SDBS). Enhanced response was seen in both primary and secondary immunization. Cell-mediated immune response (indirect macrophage migration influencing test) was not altered by SDBS. Results are discussed with a view to the possible utility of SDBS as adjuvant for prophylactic immunization.

Immunization by application of antigen directly onto mucosal surfaces is the most effective way of stimulating secretory immune responses[2]. Aerosol exposure to antigen can stimulate all levels of the respiratory immune response and in addition can elicit a good systemic response[3].

Despite the potential importance of aerosol immunization in disease prophylaxis, little attention has been given to the development of materials which could enhance the immune response to antigens given by the aerosol route, i.e. local adjuvants. The use of adjuvants systemically is well

documented and there is a broad spectrum of materials with reported adjuvant activity[4]. Recently it has been shown that certain compounds with detergent action can function as systemic adjuvants when linked covalently or simply mixed with antigens[5,6]. We have shown that aerosol administration of an anionic detergent along with proteolytic enzymes from *Bacillus subtilis* enhanced the local and systemic immune response to the protease[7]. To confirm the adjuvant effect of detergent we examined the effect of detergent on the immune response to a bovine serum protein delivered to guinea-pigs by aerosol.

Materials and methods. Bovine IgG_2 was prepared as described by Duncan et al.[8]. A preparation containing 40% sodium dodecylbenzene sulphonate (SDBS) was obtained from BDH Laboratories, Toronto, Ontario. Aqueous solutions of bovine IgG_2 (1.0 mg/ml) or bovine IgG_2 (1.0 mg/ml) dissolved in 1.0% SDBS were aerosolized with an ultrasonic nebulizer (DeVilbiss, Model 880) as described previously[7]. Aerosols so generated are known to contain droplets of the size which could reach all levels of the respiratory tract[9].

Female Hartley strain guinea-pigs weighing 350–400 g were exposed for 30 min to aerosolized antigen or antigen + detergent on days 0 and 3. At weekly intervals 5 animals were removed from each group. These animals were killed and serum, bronchoalveolar washings (BAW) and peritoneal washings were obtained. The remaining guinea-pigs were reexposed to the appropriate aerosol on day 51, and on days 55 and 61, 5 animals from each group were killed and serum[7] BAW and peritoneal washings collected.

Serum and supernatants from BAW (dialysed, lyophilized and reconstituted to 10 mg/ml) were titrated for IgG_2-specific antibody using an indirect (antiglobulin) haemagglutination technique with antigen adsorbed to ECDI (Sigma) activated sheep erythrocytes[7]. Cells were obtained from BAW or peritoneal lavage by centrifugation and were cultured in the presence or absence of bovine IgG_2 and culture supernatants used in an indirect macrophage migration influencing test[7]. The Student t-test and Mann-Whitney Rank test were used to statistically evaluate the data.

Results. No signs of altered or impaired respiratory function were observed in animals exposed to aerosolized bovine IgG_2 or IgG_2 + detergent and previous experiments had demonstrated that aerosolized detergent alone did not induce significant acute or chronic inflammatory lesions in guinea-pig lungs[10].

Antibody titers for both BAW and serum are given in figure 1. Mean titers of BAW supernatants from animals exposed to IgG_2 plus detergent were significantly ($p < 0.025$) higher on day 14 when compared to those from animals exposed to IgG_2 alone. Serum antibody titers were significantly higher ($p < 0.05$) for IgG_2 + detergent on days 28, 42, 49, 55 and 62. Supernatants from cultures of broncho-alveolar washing cells obtained from animals exposed to bovine IgG_2 caused migration stimulation on day 28 while addition of detergent to aerosols of bovine IgG_2

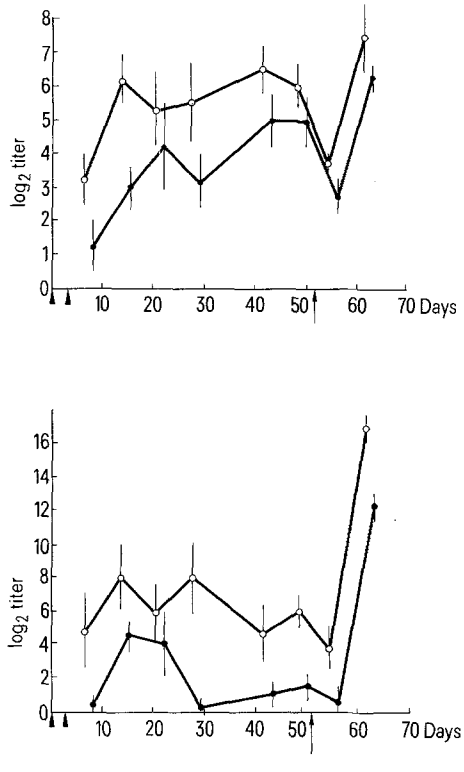

Fig. 1. Mean indirect haemagglutination anti bovine IgG_2 titres in broncho-alveolar washing supernatants (top) or serum (bottom) after aerosol exposure of guinea-pigs to bovine IgG_2 with or without detergent. O—O bovine IgG_2 + detergent; ●—● bovine IgG_2 alone; ► primary immunization; → secondary immunization. Vertical lines represent SEM.

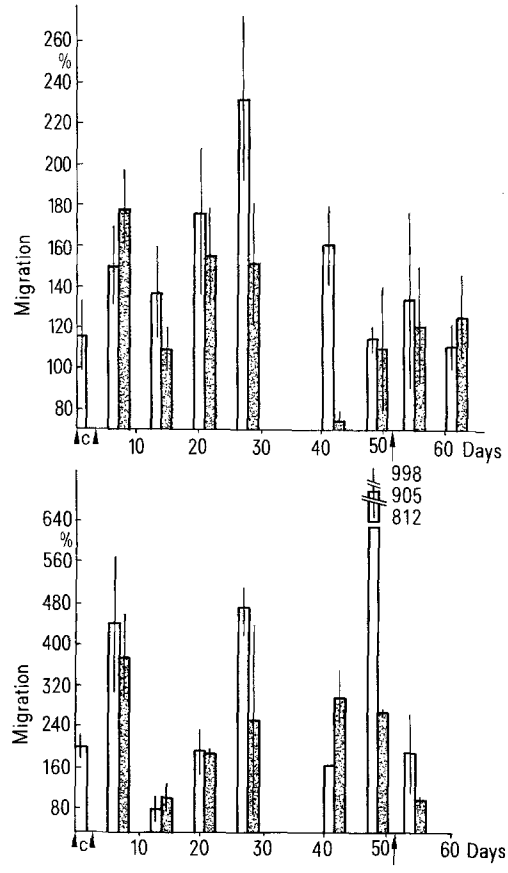

Fig. 2. Mean macrophage migration under the influence of supernatants from guinea-pig broncho-alveolar cells (top) or peritoneal exudate cells (bottom) cultured with bovine IgG_2 after aerosol exposure of animals to bovine IgG_2 with or without detergent. ▨ IgG_2 + detergent; ▭ IgG_2 alone; c unexposed, control animals; ► primary immunization; → secondary immunization. Vertical lines represent SEM.

induced supernatants which caused stimulation on day 7 and inhibition on day 42 (figure 2). Supernatants of peritoneal washing cell cultures gave the following pattern of migration. Supernatants from animals exposed to bovine IgG_2 caused stimulation on day 28 and 49 and inhibition on day 14 while supernatants from animals exposed to bovine IgG_2 + detergent caused stimulation on day 7 and day 49 and inhibition on day 14 (figure 2).

Discussion. SDBS exerted an adjuvant effect on the immune response to aerosolized bovine IgG_2. In animals exposed to IgG + detergent titers of specific antibodies in BAW supernatants were significantly higher shortly after primary immunization and in serum, antibodies persisted at higher levels for several weeks and remained higher after secondary immunization. Observed alterations in macrophage migration did not seem to be influenced by exposure to detergent at time of immunization. Taken with a previous report of adjuvanticity of SDBS in solution with *Bacillus subtilis* protease[7] the present results may indicate a general adjuvanicity of SDBS given with aerosolized antigen. This may suggest a rather universal utility of SDBS as aerosol adjuvant although it is likely that its function should be confirmed with specific antigens.

The mechanisms of adjuvant activity of detergent are speculative but may include alteration of membrane permeability, interaction and alteration of antigen, or stimulation of cells responding to antigen. Dioctyl sodium sulphosuccinate increases absorption of poorly absorbable drugs across gut epithelium[11] and a similar phenomenon may occur in the respiratory tract. Detergents bind avidly to protein molecule which could effect antigenicity[12] and conjugation of lipids to protein antigens has been shown to alter the immune response to those materials[13]. In each case, detergent may enhance immune response. Surface active materials can stimulate cellular metabolism and differentiation[14] and release of lysolecithin-like materials from macrophages has been implicated in the adjuvant activity of a variety of materials[15]. SDBS, therefore acting upon lymphocytes or macrophages may enhance antibody synthesis.

It is thought that live, replicating bacterial vaccines stimulate a better immune response at mucosal sites than do antigenically identical killed vaccines[2]. This may be due to the ability of live vaccines to provide both a critical antigen mass and penetration of innate mucosal barriers. However the convenience of using killed organisms or their components may be better exploited of adjuvants of the type described here improve the immune response to the level seen with live agents. In that SDBS enhanced both the serum and BAW antibody response it may be useful when aerosol immunization is more practical or associated with fewer side effects than is parenteral administration[16,17].

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Circadian variation in urinary melatonin in clinically healthy women in Japan and the United States of America[1]

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Summary. Urinary melatonin excretion is lower in East-Asian (Japanese) than in North-American (whites of mixed ethnic origin) women. Moreover, a statistically significant circadian rhythm is demonstrated by population-mean cosinor in the data pool from both groups of women. Furthermore, statistical significance characterizes interactions of effects from geographic differences (between ethnic groups) with temporal factors. Such spatio-temporal interactions await further scrutiny with a view inter alia of carcinogenesis as it is influenced by a spectrum of intermodulating rhythms.

Circadian rhythms have been reported for urinary excretion and blood levels of melatonin in human beings, rats and calves[2-6], along with changes in serum melatonin during the menstrual cycle[7]. Apart from basic physiologic and pharmacologic aspects beyond our scope, interest in this variable stems from the question whether melatonin excretion may vary in populations with different breast cancer risk. To study this possibility, adult female subjects, some menstrually cycling, others post-menopausal (table 1) were admitted to the Clinical Research Center at the University of Minnesota in Minneapolis between 08.00 h and 09.00 h, for a stay of about 28 h between mid-July and