marily eosinotactic(12-13) apart from function as an immune reactant.

Attempts to modify this inflammatory cellular reaction with antihistamines, chemical inhibitors of histamine-liberation, and anti-serotonin and anti-heparin agents have been without effect. Identification of a possible physico-chemical factor responsible for eosinotactic responses to antigen-antibody union is the subject of our continued investigation.

Summary. Eosinophilic granular cell infiltrations into sinuses of regional popliteal lymph nodes of the rabbit were demonstrated. These followed reversed passive sensitization experiments at foot pad sites in response to antigen-antibody union. Systems of varying character found effective were: bovine serum albumin and bovine serum gamma globulin with respective corresponding antisera of rabbit, guinea pig, and chicken origins; diphtheria toxoid with corresponding rabbit, guinea pig, and horse antitoxin; and tetanus toxoid-horse antitoxin.

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Size of Minimal Reproductive Units of Bacterial L Forms. (28267)

CLAES WEIBULL
Central Bacteriological Laboratory of Stockholm City, Stockholm, Sweden

The size of the minimal reproductive units of the stable Proteus L form L9 was determined by Weibull and Lundin(1). Samples of liquid cultures of this L form were spread on agar plates. Blocks of agar were transferred to slides and covered with cover slips. The slide cultures thus obtained were sealed with paraffin and incubated for 24 hours or longer. The size of the individual L elements in the slide cultures was determined photographically before and after incubation. It was thus found that only L elements having a diameter > 0.6 to $0.7\ \mu$ enlarged measurably during growth. Similar experiments carried out on 3 other Proteus L forms and L forms of a staphylococcus and a diphtheroid will be described in this report.

Materials and methods. A. *Organisms.* The Proteus L forms used (strain L VI, L 18, and L D52) have been described(2). Stock cultures were grown in the serum-free medium described by Abrams(3). L forms derived from a staphylococcus and a diphtheroid were provided by Dr. L. Dienes, Massachusetts General Hospital, Boston, Mass.(4). Stock cultures were grown on plates containing meat broth supplemented with 10% inactivated horse serum, 3% NaCl, 1,000 units of penicillin g/ml, and 1% agar.

B. *Photomicrography.* A Leitz 90 X phase contrast objective and a 8 X compensating ocular was used. L forms moving freely in liquid medium between slide and cover slip were photographed with a microcamera equipped with 35 mm Gevaert Duplo Ortho film, and with the Leitz Multiblitz-Mikro 300 W electronic flash as the light source. Photographs of L forms in slide cultures were taken

with a box camera. Gevaert Ortho 05 plates were used. Negatives obtained on films and plates were enlarged when printed to give a final magnification of 2,000 X.

Results. A. *Preparation of liquid serum-free cultures of L forms.* Previous investigations (5) have shown that Proteus L forms grow mainly in the form of large aggregates of more or less pleomorphic elements in serum-containing media. Such cultures were rather unsatisfactory for growth experiments. However, it was found that these L forms could be grown in Abrams' serum-free medium(2,5). In this medium the growth primarily consisted of spherical elements of various sizes.

Liquid cultures of the L forms of the staphylococcus and the diphtheroid were obtained by transferring an agar block with several L colonies on its surface to a flask containing meat broth supplemented with 10% horse serum, 3% NaCl, and 1,000 units of penicillin/ml. By gradually diminishing the serum content of the broth (concentrations used were 10%, 3%, and 0.5% of serum), and by using large inocula, serum-free cultures of the L forms were obtained. These cultures could be agitated on a rotary shaker (100 rpm) without a marked inhibition of bacterial growth. It was noted that the L forms grew more vigorously on meat broth prepared from fresh meat than on broth obtained by redissolving dehydrated, commercial media.

In media containing a high serum concentration, the L forms of the staphylococcus and the diphtheroid mainly occurred in the form of large aggregates of more or less pleomorphic elements, as did the Proteus L forms. In serum-free media, on the other hand, aggregates were seldom seen. Most of the L elements in such cultures were spherical in shape, the sizes of the spheres varying within wide limits. Fig. 1 to 6 show L bodies moving freely in the liquid, serum-free medium. As can be seen, some of the L bodies consisted of envelopes partly filled with granular elements. The granules moved freely within the envelopes.

B. *Size of the minimal reproductive units of L forms.* The solid media used in the slide cultures contained, in addition to the

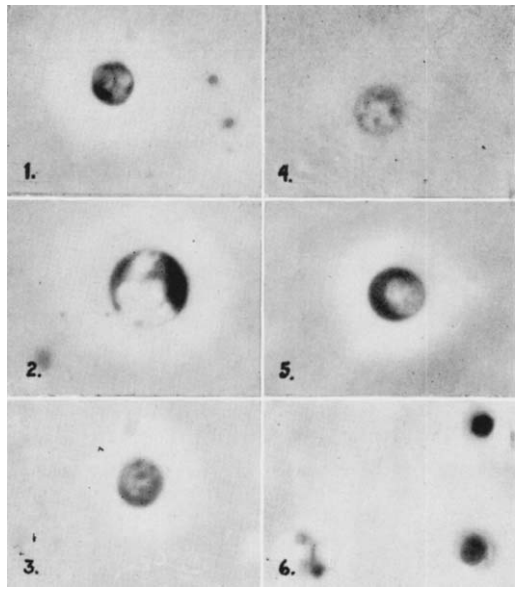

FIG. 1-6. L forms of a staphylococcus (1-3) and of a diphtheroid (4-6) moving freely in a liquid, serum-free medium.

ingredients of the liquid, serum-free media described above, 10% inactivated horse serum and 0.8% Difco agar. In some slide cultures a solid medium was used, which had been obtained by centrifuging an overnight culture of the L form under study, killing the remaining viable L elements by boiling this fluid, and adding serum and agar. This medium is designated as "old" medium in Table I. In previous experiments(1) this "old" medium had a favorable effect on the growth of Proteus L forms.

Fig. 7 shows a slide culture of staphylococcal L forms before and after incubation at 37°. It can be seen that only large L bodies (diameter $>$ 0.9-1.0 μ) grew during incubation.

The results of all the slide culture experiments are summarized in Table I. It can be seen that the majority of the particles present in the cultures did not grow. Most of these non-viable particles had a diameter $<$0.6 μ. The size of the minimal reproductive units of the L forms studied varied between 0.6 and 1.0 μ, *i.e.*, these elements had a size similar to that of normal bacteria and considerably larger than the minimal reproductive units of Mycoplasma spp. as determined from slide

TABLE I. Size of Minimal Reproductive Units of Various L Forms as Determined by Slide Culture Experiments.

Organism	No. of particles measured	No. of growing particles	Diameter of minimal reproductive units, μ	Medium
Diphtheroid	64	15	.9	Fresh
"	53	19	.9	Old*
Staphylococcus	244	16	1.0	Fresh
"	93	17	.9	Old
Proteus L VI	322	77	.8	Fresh
"	428	73	.6	Old
Proteus L 18	60	18	.7	Fresh, Old
" L D52	113	28	.7	Fresh, Old

* For explanation see text.

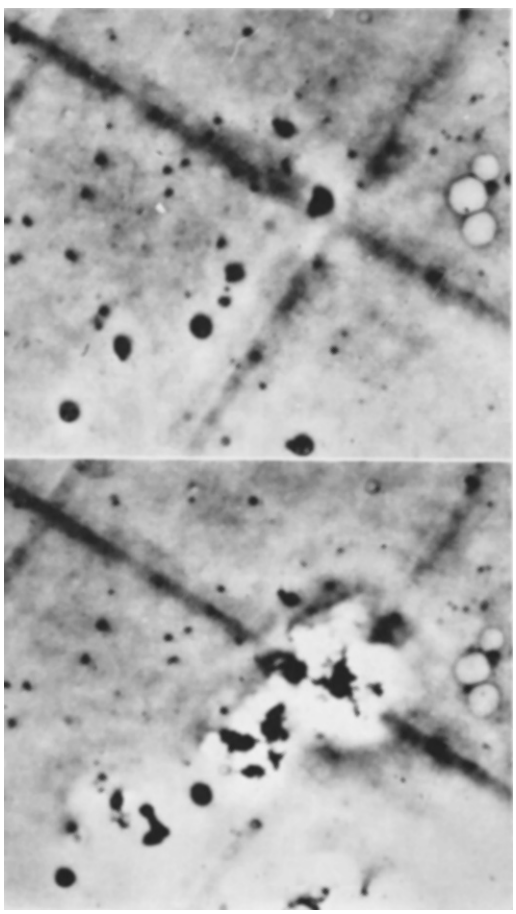

FIG. 7. A slide culture of staphylococcal L forms before (top) and after (bottom) 18 hr incubation at 37°. L bodies were grown in liquid medium before being transferred to the agar block of slide culture. Dark lines represent rulings in a formvar film attached to the cover slip(1). Distance between 2 parallel lines is 25 μ.

culture experiments(6). According to Panos, Barkulis, and Hayashi(7) the minimal reproductive units of streptococcal L forms have a diameter of 0.3 μ. It should be emphasized, however, that the size of the streptococcal reproductive units was determined by means of filtration experiments and electron microscopy. The merits and drawbacks of the slide culture and filtration methods have been discussed(1).

Our results are in agreement with previous investigations on Proteus L forms(1,8) and L forms of *Vibrio cholerae*(8).

Summary. L forms of *Proteus mirabilis*, a staphylococcus and a diphtheroid were grown in serum-free media. Slide culture experiments indicated that the diameter of the minimal reproductive units varied between 0.6 and 1.0 μ.

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