with some ethologists expressing the feeling that the concept was a weak one, circular in definition and *ad hoc* in application. Replies to these arguments did not seem to convince. At times, this discussion seemed to veer from the problem of defining reinforcement to that of the theory and nature of reinforcement, and, at other times, to considering reinforcement as a construct, as an experimental operation, or simply as a mistake! There seemed to be hidden in this theme a number of deep-lying differences in conceptual approach to behavior theory, its structure, function, substance, and ends. What the opposing ethologists were urging as reasons for discarding reinforcement theory, the active Americans took merely as reasons for improving the theory.

A topic historically of high interest to ethology is that of the initiation of behavior. The more frequent focus of attention is on the cue, or trigger, or "releaser," of a behavior pattern or sequence, rather than on why or how a particular response is made to any specific cue (as if in some way, the response is more *given*; as if it were more a forced consequence of the cue than a matter of the cue being a forced antecedent of the response). The well-known use by ethologists of models in studying behavioral releasers has been extended to actually scaling the values or effectiveness of releasers, the general method being that of pitting cues against each other, following procedures akin to those of psychophysics and psychometrics. Ethology regards motivation as closely related to the problem of releasing mechanisms, and deals with motivation more as learning theorists do with "incentives," rather than as a state variable or as a parameter of behavioral functions. Because some of the American members were familiar with the scaling of incentives, general issues about scaling procedures provided a basis for active discussion among a subgroup of conferees.

One full day was devoted to subgroup meetings, at which three ethologists, acting as representatives for their discipline, illustrated their mode of approach to selected empirical and theoretical problems by outlining specific current research in their own laboratories. Three of the American experimental psychologists made similar presentations in subgroups to their European colleagues. The three ethological themes were:

1) studies of interacting and mutually modifying response patterns in the agonistic behavior of certain bird species;

2) an analysis of some functions, including generalization gradients, of releasing stimuli; and,

3) experiments on the relative importance as response determiners of various stimulus parameters in the egg-directed behavior of certain bird species.

The three experimental psychology themes were:

1) the general perspectives that inform most modern learning theories, and the basic categories of observation and fact that those theories try to handle;

2) the place in learning theory of the distinction between learning and performance variables, and the new importance of "incentive" in some current learning theories; and,

3) the historical development of experimental research and theory on anxiety, escape, and avoidance behavior, and a re-examination of the older formulations in the light of recent discoveries.

These presentations, and the discussions they evoked, were regarded by all conferees as having been very stimulating, and it was widely regretted that they came on the last day of the conference when the imminent dispersal of participants prevented more extensive follow-up.

Other topics touched upon during the week's discussions included units of behavior, the nature and definition of "stimulus" and "response," critical periods and imprinting, the relation of classical conditioning to imprinting, and human neonate behavior and early experience.

The organization of the conference was made flexible in order to enable the participants to pursue whatever subjects of interest emerged during the discussions. The smallness of the conference made it possible for conferees to meet in a body whenever the whole group expressed interest in a single topic or to divide into smaller groups to discuss topics of individual interest.

At the close of the conference, all participants agreed that it had been a worthwhile enterprise. In particular, the informality of the conference, the absence of set papers, and the ease of making personal contacts were felt to have been especially valuable. Hopes were expressed that a basis had been laid for future interaction among participants, possibly through prepublication exchanges of papers for comment and criticism and through exchanges of pre- and postdoctoral students.

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Protoplasts and L-Forms

That bacteria undergo changes to L-forms as well as to protoplasts in vivo has stimulated much research. Impetus has been furnished especially by the establishment of similar organisms (Pleuropneumonia-like organisms, PPLO) as the Eaton agent, which causes primary atypical pneumonia in man. Elucidation of the nature of the Eaton agent, now known to be *Mycoplasma pneumoniae* instead of a virus, led to the study of the possible pathogenetic significance of such organisms in a variety of unsolved medical mysteries. Such organisms require special procedures for isolation and identification. L-forms, protoplasts, and Mycoplasma (PPLO) differ from bacteria in that they lack a rigid cell wall and pass through filters. By this latter characteristic they are similar to viruses. However, they are separable from viruses by growth on cell-free media and reproductive cycle. Relationships of L-forms and protoplasts to their parent bacteria in classical forms are becoming understood. Thus "mycoplasmology" is emerging as a specialty in itself; at the meeting of the American Society for Microbiology, 3–7 May 1964, in Washington, D.C., sessions were organized on this subject for the first time.

Protoplasts and L-forms were discussed at an invited session. Assigned papers on current significant contributions were followed by panel discussion.

The chemical and physical basis of

stability for L-forms was discussed by O. E. Landman (Georgetown University) who, with Antoinette Ryter (Institut Pasteur, Paris) and R. E. Knott, discovered that mesosomes were absent from L-forms studied by electronmicrographs. The mesosomes were lost by eversion during lysozyme treatment when the bacteria (*Bacillus subtilis*) were converted to protoplasts. Earlier electronmicroscopic studies from several laboratories had indicated that mesosomes play an important role in septum-forming capacity and wall formation. Since L-forms and protoplasts have lost both septum-forming ability and wall-forming ability and now have been shown to lack mesosomes, it was postulated that the cellular functions lost during mass conversion to the stable L-forms (or protoplast state) were associated with the mesosomes. Landman and Halle (*J. Mol. Biol.* 1963) observed that when 15 to 30 percent gelatin was substituted for soft agar each L-body had reverted overnight. This followed plating of lysozyme protoplasts (of *B. subtilis*) on certain defined soft agar media with each protoplast giving rise to an L-colony. The L-forms were propagated indefinitely with the aid of the reversion inhibitor D-methionine.

The topic then shifted to models of growth and reversion of L-forms of Gram negative bacteria as exemplified by *Proteus* organisms. W. van Iterson (University of Amsterdam) presented work on the fine structure of *Proteus* L-form in exponential phase of growth, based upon studies with A. C. Ruys and M. J. Botman. Their electronmicrographs of L-9 strain of stable *Proteus* L-forms of Klieneberger (grown in brain-heart broth) showed very large vacuolar regions and a peculiar pattern in which small bodies separated themselves from other protoplasm through the formation of thin cytoplasmic bridges. Sequential sections revealed the highly vacuolated cytoplasm bounded by a thin "unit type" membrane with no evidence of cell wall material. Within the vacuoles there sometimes could be detected small elements which were not attached to the remaining cytoplasm and which may have represented basic propagating elements. Some of the elements contained no apparent nuclear material and probably were incapable of propagation. Most of the L-bodies possessed ribosomal-like particles in varying numbers. No other distinctive cytological features were observed. Van Iterson concluded that it was not yet possible to decide whether the propagation of such L-forms takes place by means of small elements, by further partition of the vacuolated large bodies, or by both. Her studies were performed on stable L-forms which grew well in *agitated* brain-heart broth cultures.

The characteristics of liquid growth and reversion of *Proteus* L-forms were discussed by R. A. Altenbern (U.S. Army Biological Laboratories, Fort Detrick, Maryland). A mutant strain of *Proteus* (3B type L-form), in which the division mechanism was resistant to penicillin while the system forming the cell wall was penicillin sensitive, grew in liquid medium containing penicillin. The propagating L-forms varied from rhizoid (at low penicillin concentrations) to spherical, budding forms (at 300 units of penicillin per milliliter), and reverted *en masse* to normal bacteria upon withdrawal of penicillin; the reversion was accompanied by pronounced deposition of diaminopimelic acid into the wall of the reverting element. Chloramphenicol completely prevented reversion although no protein synthesis could be detected during reversion. Reversion of the 3A type granular L-form of *Proteus* was rare and occurred with definite probability which was altered by the temperature of incubation. Formation and reversion of L-forms of the 3A type were nonmutational and probably did not involve any infectious-type cytoplasmic particles.

Parts of streptococcal protoplast populations have been stabilized by spermine (a polyamine) in the absence of osmotic stabilizing agents, as reported by H. Gooder (University of North Carolina, Chapel Hill) who discussed the L-type growth of streptococcal protoplasts, particularly in relation to his joint work with J. R. King. Suspensions of protoplasts were prepared by enzymatic removal of cell wall by lysozyme for these group D streptococci (an appropriate muralytic lysin for group A streptococci having been reported by Gooder with W. R. Maxted, of London). Samples of protoplast suspensions were plated on media suitable for development of L-colonies, but no antibiotic was necessary, and growth in liquid medium was only possible in either system after prolonged successive subculture in lowered concentrations of agar. Tending to confirm observations by Landman and Halle (1962) was the finding that, in this *S. faecalis* system, agar concentration of 2.5 percent favored reversion of protoplast to the streptococcus but that neither freshly induced growth nor stable cultures of L-forms showed this effect.

E. H. Freimer (Rockefeller Institute, New York) discussed streptococcal L-forms and protoplasts and the immunochemical nature of their cytoplasmic membranes, citing material from a report (with J. B. Zabrinskie and B. Seegal) on the immunological relation between streptococcal membranes and human heart tissue. The membrane, chemically distinct from the cell wall, was composed of 72 percent protein, 26 percent lipid, and 2 percent carbohydrate. It has been shown by capillary precipitin tests and analysis by microdiffusion that the membrane contains antigens distinct from those of the cell wall and from those of the cytoplasm which it envelops. This antigenic material, common to the membranes of group A streptococci, can be distinguished by immunodiffusion from related antigenic substances in membranes of several other serological groups of hemolytic streptococci. In addition, this antigenic material did not cross-react with the membrane antigens of other Gram-positive cocci. Immunological studies of streptococcal protoplast membranes revealed that they cross-reacted with human cardiac tissue. This property was common to all group A strains tested as well as to some group C strains. Other streptococci and Gram-positive bacteria were nonreactive. This reactivity could be localized to the myofibers and vascular smooth muscle of both normal and rheumatic *hearts* by fluorescent antibody technique. The immunologically active material in the streptococcal cell was demonstrated, by fractionation experiments, to reside in the cell membrane.

Possible pathogenic implications for L-forms and transitional forms were presented by Ruth G. Wittler (Veterans Administration and Walter Reed Army Institute Research, Washington, D.C.). Whereas stable L-forms of bacteria have been described as laboratory-made phenomena not known to occur in vivo, the unstable L-forms did arise in vivo. Investigation into their pathogenicity has been thwarted, however, by the inherent tendency to change their morphology. Thus, to assess pathogenicity of an unstable L-form, not only must the transitory entity called "L-form" be considered, but also the larger number of transi-

tional forms must be taken into account. The transitional forms are nonbacterial phases, including the L-phase, in which the organism preserves its generic identity, survives, and multiplies.

Data suggesting the possible pathogenic activity of transitional forms have been gathered from cases of subacute bacterial endocarditis, rheumatic fever, Reiter's syndrome, rheumatoid arthritis, staphylococcal asteomyelitis, and other chronic or recurrent diseases. Transitional forms isolated from patients have multiplied in tissue culture and occasionally exhibited cytopathogenicity. In cell-free culture media, these forms could revert to the parent bacteria. As a model, in a case of subacute bacterial endocarditis, transitional forms persisted for 5 years, were resistant to all antibiotics, and remained capable of reversion to bacterial form in vivo. Changes in morphological form of the organism could be correlated with changes in the clinical symptoms of the patient. The capacity of transitional forms to survive, multiply, and revert in vivo would suggest intrinsic pathogenic potential.

Discussion was enthusiastic and lengthy. Concerning the formation of protoplasts in vivo, L. Muschel (University of Minnesota) commented on early contributions of his group with Gram-negative bacteria, such as *E. coli*. E. A. Mortimer (Western Reserve University) presented preliminary results from experiments in mice inoculated intraperitoneally with several strains of group A streptococci. The L-forms were recovered from heart blood in about half of the instances and from peritoneal exudate in the remainder; L-forms and streptococci both were isolated at death of mice. By reversion, as well as other techniques, the L-forms have been shown to be derived from the group A streptococci. A reverse relationship may exist between virulence for mice and the production of L-forms. Although it was believed that the L-forms were produced in the mice, one could not be absolutely certain that they were not produced in vitro on L-form medium. In any event, this appeared to be an exciting way to produce these L-forms of group A streptococci, and another facet of host-parasite relationships may be observed.

Louis Dienes (Massachusetts General Hospital, Boston) closed the meeting with observations suggesting that L-forms may take part in some pathological processes. T. M. Brown (George Washington University) had expressed similar views in comments on antibody studies of human material (presented with H. W. Clark and J. S. Bailey) which indicate that the rheumatoid factor is associated with *Mycoplasma* immunologic complex. Antibody studies by Y. Crawford (Naval Medical Research Unit No. 4, Great Lakes, Illinois) have suggested L-forms in streptococcosis.

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Marine Microorganisms

Many disciplines of science—chemistry, geology, physics, biology—are involved in marine research. One rapidly developing field is marine microbiology. In order to introduce other workers to this field, a conference on biology of marine microorganisms was held in Berkeley, California, 21–23 December 1964. Approximately 70 participants from all parts of the United States attended.

The conference opened with a discussion of microbial environments in the sea. After introductory remarks by M. B. Allen (Kaiser Foundation Research Institute) two extremes of environment were described—the deep sea, by C. E. ZoBell (Scripps Institution of Oceanography), and the surface film, or neuston, by R. E. Norris (University of Washington). Techniques used in the study of marine microorganisms were also described—methods for the collection and study of bacteria (C. E. ZoBell), chemical methods (J. D. H. Strickland, Scripps Institution of Oceanography), collection and preservation of phytoplankton (R. W. Holmes, Scripps Institution of Oceanography), and the collection and cultivation of living phytoplankton (M. B. Allen).

The principal types of marine microorganisms were described and discussed. These included marine bacteria (J. Liston, University of Washington), marine fungi, including those living as endosymbionts with invertebrates (H. Whisler, University of Washington), and diatoms and dinoflagellates (R. W. Holmes). R. E. Norris discussed the still little-known nannoplankton which he suggested might better be called cryptoplankton because it includes all