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edited by

P. S. NUTMAN & BARBARA MOSSE

SYMBIOTIC
ASSOCIATIONS



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EDITORS' PREFACE

The pressing problems of co-existence in world affairs may have influenced the Committee in their choice of subject for this years' Symposium. If so, it is to be hoped that the more bizarre examples of symbiosis illustrated in this volume will not be followed in the world at large; there are many other ways of escaping the Hobbesian predicament that without 'commonwealth' life must be 'nasty, brutish and short'.

It has often been maintained that the subject of symbiosis has no independent standing, and that its name tends to disguise a fundamentally parasitic relationship—surrounding its admitted complexity with an unscientific aura of teleology. A study of the pathological aspects alone however would be incomplete at best, and it is questionable whether such an approach would contribute much to the understanding of those associations which have no obvious pathological symptoms, but only beneficial effects. Indeed it could more cogently be urged that the study of symbiosis may illuminate some facets of the host-parasite relationship.

In this volume the origin, organization and functioning of symbiotic systems are discussed. In the field of microbiology these include associations between bacteria and their temperate phages, between bacteria, fungi and higher plants, and between micro-organisms and animals. Principally considered are ecology and taxonomy, methods of transmission and metabolic interactions between symbionts, especially at the nutritional level. The difficulty and frequent failure to grow many of the micro- and some of the macro-symbionts in pure culture are measures of our present incomplete understanding of symbiosis. There are also many non-microbial symbiotic associations, some comprising several components, and of even greater complexity in structure and behaviour than the microbial systems discussed in this symposium.

Unfortunately some of the papers originally invited were withdrawn at a late stage and substitutes could not easily be found. It is for this reason that Dr Nüesch's article, which was invited late, appears at the end of the volume.

The editors would like to thank the contributors and the Cambridge University Press for their help and co-operation which have made possible publication well in advance of the meeting at which the papers are to be discussed.

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INTEGRATIVE AND DISINTEGRATIVE FACTORS IN SYMBIOTIC ASSOCIATIONS

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While other contributors in this symposium deal with the description, evolutionary history and mechanisms of particular cases of 'mutualistic associations', we shall attempt in contrast to present some problems which pertain to symbiosis considered as a general biological phenomenon. Three aspects will be considered here: the biological specificity of symbiotic associations, the dependence of symbiotic processes on environmental factors, and the creative manifestations of symbiosis.

SPECIFICITY IN SYMBIOTIC RELATIONSHIPS

The organizing committee of this symposium have directed us to focus attention on those '*symbiotic relations involving micro-organisms in which both members derive ecological advantage*'. It is obvious, however, that there occur frequently in nature various types of biological associations from which the partners derive ecological advantage, but which do not come within the purview of this symposium because they are accidental and transient. As the word symbiosis has come to be used, it generally implies a certain degree of specificity and of permanency. Specificity refers to that 'property of two interacting systems which permits the one to interact with the other with some degree of selectivity' (Weiss, 1955). The concept is most easily visualized in the biochemical fields where, for example, antigen-antibody interactions, and substrate-enzyme systems can be formulated on a molecular basis in stereo-chemical terms.

In a more purely biological context, specificity denotes the pattern of attributes of the two organisms constituting a symbiotic association which allows them to interact with some degree of selectivity—the interaction taking the form of a relationship where each organism forms a more or less critical portion of the environment of its partner. In other words, specificity in our discussion is concerned with the overall pattern of adjustment between two components of a symbiotic pair, it is an expression of the complementariness of all their dynamically interacting attributes. The greater the number of factors which interact and the more selective any one of them becomes, the smaller is the probability of

achieving the required combination for complementariness and hence the higher the degree of symbiotic specificity. Symbiotic specificity is therefore an expression of the mechanisms which determine why and how certain organisms are frequently found in association with others, and why they are restricted in their use of living environment to these organisms—even to particular regions of the latter. A converse aspect of this problem is the knowledge of the factors that can break down established symbiotic associations.

It is taken for granted that the dynamic equilibrium that exists between two members of any symbiotic pair is the result of the evolutionary development of both. But while any one or several of the characters of each symbiont may play a role in this process, and direct its partner along certain lines of development, few are the cases in which the relevant genetic changes have been recognized. The relation of orchids to their mycorrhizal fungi constitutes one of the first examples studied in some detail. In the primitive orchid *Bletilla hyacinthina*, the seeds can germinate aseptically and develop into slender seedlings with distinct leaves, but fail to develop a protocorm; in this species, therefore, the effect of the fungus is morphogenetic. Moreover, the rhizome of the infected *Bletilla* is free from mycorrhizal fungi; the new roots must be infected every year. In the less primitive orchids, such as *Cattleya*, *Cypripedium* and *Ophridium*, aseptical germination can take place, but the embryo does not develop at all although it becomes green. Here also, symbiosis is transient with infection taking place anew each year as the new roots develop. In the Sarcanthinae, which are more specialized, germination occurs only in association with *Rhizoctonia mucoroides*, and the fungus persists throughout the year in the plant. The most complete type of association has been observed in *Neottia nidus-avis* in which the same fungal infection persists throughout the life of the plant extending from the protocorm via the rhizome into the roots and into the base of the inflorescence instead of being limited to the roots (see review, Caullery, 1952).

These examples illustrate that selective processes can progressively increase the complexity of structural and physiological adaptation of each symbiont to its new habitat, finally reaching complete integration. The specializations evolved by each symbiont are generally related to the particular functions of environment performed for it by its partner—whether it be providing for space, protection, food or reproduction. It is the evolution of such specializations by symbiotic organisms within the living environment which lays the groundwork for symbiotic specificity. Such specialization may revolve around the function of surface to surface adhesion which permits the initiation of the partnership; increasing adjust-

ment to that surface will then serve to restrict adaptability of this function to other environments, i.e. adhesion to other surfaces. In other cases highly selective factors of the internal environment are involved—including nutritional complementariness, ionic and gaseous composition, resistance to cellular defence mechanisms, etc. The increased degree of specificity of a single function may help to restrict a particular organism to a single or a few closely related partners.

Specialization within a living framework may have additional influences on the subsequent evolution of the organisms concerned, for example by tending to encourage retrogressive evolution. The interaction of two organisms may also involve an increasing number of activities. What starts out as a place to hold can become a protective site as well, and eventually may become a source of readily available food provided by the partner—either by its food-getting activities, or more frequently in the form of its own tissues. Each specialized adjustment evolved for each added activity performed by the partner entails decreasing adaptability to different conditions, i.e. raises the degree of specificity. As more and more of a symbiont's needs are satisfied by its partner, selective pressure on it decreases. Structures and functions no longer required are lost or degenerate, since natural selection does not operate against genetic changes which damage useless somatic attributes.

In general, the less self-sufficient a symbiont becomes, the more it depends on its partner and the less adaptable it becomes to other partners. Increasing mutual dependence leads to increasingly complex forms of integration between the two symbionts. Such integration results in decreasing flexibility, or conversely, in increasing fastidiousness or specificity for either or both partners.

All of these evolutionary processes may of course, and usually do, affect the two symbionts differentially. Some symbionts are less and others more dependent on their partners. There are thus degrees of self-sufficiency, from the symbiont which unconcernedly 'hangs his hat' on its equally unconcerned partner, to the totally interdependent symbiotic pair forming an integrated mechanism, little different from that which binds together the different parts of a single organism.

The fact that profound morphological, physiological and even behavioral differences can result from the interaction of a symbiont with different species or even with different individuals is well exemplified by the so-called insect parasitoids (Salt, 1941). In a given insect parasitoid the gross size, the proportion of body parts, the presence and absence of wings, the length and nature of the developmental period, the fecundity and the vigour, may be markedly influenced by the different species or

individuals of hosts in which they develop. Even the behaviour is differentially affected, different hosts influencing the behaviour of the larvae, or of the emergent adults, or of the free adult's later activities. Although parasitoids represent a very specialized type of symbiosis, the same kind of phenomenon has been described for entirely different organisms such as trematodes. The polymorphism displayed by trypanosomes depending on the tissues or fluid within which they develop represents basically the same thing. This dynamic aspect of symbiosis is underscored by repeated observations of the effect of a change in one symbiont altering the specificity of the entire association; this has been noted for changes involving age, sex, hormonal state and nutritional needs as well as social behaviour.

ENVIRONMENT AND SYMBIOTIC RELATIONSHIPS

The manifestations of the interplay between two components of any symbiotic pair are of course conditioned by the factors of the environment which operate at the time of observation. For this reason, the terms most commonly used to denote biological relationships—such as symbiotic, commensal, mutualistic, parasitic or even pathogenic—are rather misleading if they imply a state of permanency of the relationship which does not take into account its dynamic character. Many of yesterday's parasites have now become mutualistic symbionts, and perhaps more importantly today's commensals and symbionts can behave as destructive parasites under certain conditions.

This dependence of the performance of biological associations upon environmental factors certainly accounts in part for the many controversies concerning the nature of symbiosis. Whereas the word symbiosis usually connotes an association from which both partners derive benefit, many authors emphasize instead the fact that a struggle is constantly going on between the two members of the association. In this respect, it seems appropriate to quote here the statement made by Caullery (1952) in his book on *Parasitism and Symbiosis*. Speaking of the presence of the mycorrhizal fungus in orchids he wrote: 'It is a phenomenon of parasitism, an infection, a state of disease which has become essential to the development of the plant, but can also stunt and even kill the plant.' Or again speaking of lichens: 'It is necessary to abandon the idea of a purely mutualistic association with equivalent reciprocal benefits. It is a conflict between alga and fungus.'

There is no doubt that the mycorrhizal fungi can on the one hand invade their hosts and kill them, while on the other hand much of the mycorrhizal mycelium is destroyed within the phagocytic cells of all orchids.

Even in the case of lichens—for which de Bary (1879) invented the word symbiosis to symbolize his belief that the association was beneficial to both the alga and the fungus—there are many biologists who hold the view that one of the organisms of the pair in reality behaves as a parasite. Consistent with this view is the well-known fact that the association between alga and fungus can be readily disturbed by changing the composition of the atmosphere or the supporting medium. It is for this reason that lichens disappear from the trees and rocks of urban areas where the air is polluted, or when abundant nutrients are added to the medium. Conversely, it is because many ill-defined factors are involved in achieving and maintaining the proper equilibrium between alga and fungus that it has proved so difficult to synthesize lichens from their two separate constituents. Interestingly enough, the synthesis of lichens can be realized by placing the fungus and the alga in a medium so deficient that it is unable to support the growth of either one of them separately (Ahmadjian, 1962)!

The change from mutually beneficial to pathological relationships can be illustrated also by examples taken from the behaviour of the intestinal flora in mammals. There is no doubt that certain members of the intestinal flora which are usually regarded as innocuous can exert a pathological role under certain circumstances—for example, following total body radiation. On the other hand, it has long been thought that the intestinal flora plays a useful role in animal economy by synthesizing certain growth factors and perhaps also by stimulating certain natural defence mechanisms—a view which has recently received support from observations on germ-free animals. These animals have proved to be more exacting in their nutritional requirements than are conventional animals, and furthermore they differ from the latter in certain physiological and anatomical features, as will be reported on p. 8 in this chapter.

We shall now deal with other aspects of the complex interplay between intestinal flora and host, quoting briefly some results obtained with a new colony of mice developed four years ago at the Rockefeller Institute (Schaedler & Dubos, 1962; Dubos & Schaedler, 1962*a*, *b*; Dubos, Schaedler & Costello, 1963; Dubos, Schaedler & Stephens, 1963).

This colony, designated NCS, has been derived from the standard colony of so-called Swiss mice (SS) and is maintained in such a manner that it is now free of many of the common mouse pathogens. Of special interest is the fact that no *Escherichia coli*, *Proteus*, or *Pseudomonas* can be isolated from the stools of NCS mice as long as they are maintained under proper conditions. In contrast, the faecal flora of NCS mice

contains very large numbers of viable lactobacilli, belonging to at least three different morphological types.

It must be emphasized at this point that the diet fed the mice has a profound effect on the composition of their faecal flora. The lactobacilli decrease in numbers (100-fold), and one type (Rhizoid) disappears completely within a few days after the animals have been placed on a semi-synthetic diet containing purified casein as sole source of protein—this despite the fact that the casein diet contains all the known growth factors and supports rapid weight gain. In contrast, a very high lactobacillus population including the Rhizoid type is found in the stools of mice fed diets containing certain unidentified natural substances. A particular brand of commercial pellets (D & G) proved very effective in enriching the lactobacillus population, both quantitatively and qualitatively.

When penicillin or tetracycline is administered to the mice in their drinking-water, all viable lactobacilli immediately disappear from the faecal flora. Within one or a very few days the stools are then found to contain enormous numbers of enterococci and Gram-negative bacilli (including *Escherichia coli*). Concomitant with this change in intestinal flora there occurs a marked loss of body weight, amounting to 10 % or more of the initial weight of the animal.

The faecal flora, and the body weight, progressively return to their original level after discontinuance of the drug. However, while this reversal is relatively rapid in animals fed the D & G pellets, it takes place much more slowly with the semi-synthetic casein diet. The differences in rate are illustrated in Fig. 1.

Mice fed the D & G pellets are also more resistant to certain experimental bacterial infections than are those fed the casein diet. Thus it appears as if some nutritional factors can favour the establishment of the lactobacillus flora while at the same time increasing the ability of the animal to overcome other bacterial species (in the intestinal tract as well as in the inner organs).

Ever since Metchnikoff and Tissier, it has been considered that lactobacilli constitute the dominant microbial species in breast-fed human infants as well as in other animals. As suggested by the findings with mice, such lactobacilli may play a useful role with regard to weight gain and resistance to infection. Other findings, not to be discussed here, also suggest that the presence of these organisms in large numbers is correlated with higher resistance to certain toxic agencies—see also the experience with specific pathogen free (SPF) mice recently reported by Paget (1962).

It must be acknowledged that the information on the relation of intestinal flora to biological characteristics is far too incomplete to warrant

definite conclusions. It is obvious, for example, that penicillin, tetracycline and diet affect not only the lactobacillus population but also other components of the digestive flora (such as the diphtheroid and bacteroides types) and influence certain physiological activities of the animal. Nevertheless, granted our present lack of knowledge of the interplay between the host and its digestive flora, it is probable that disturbance of their

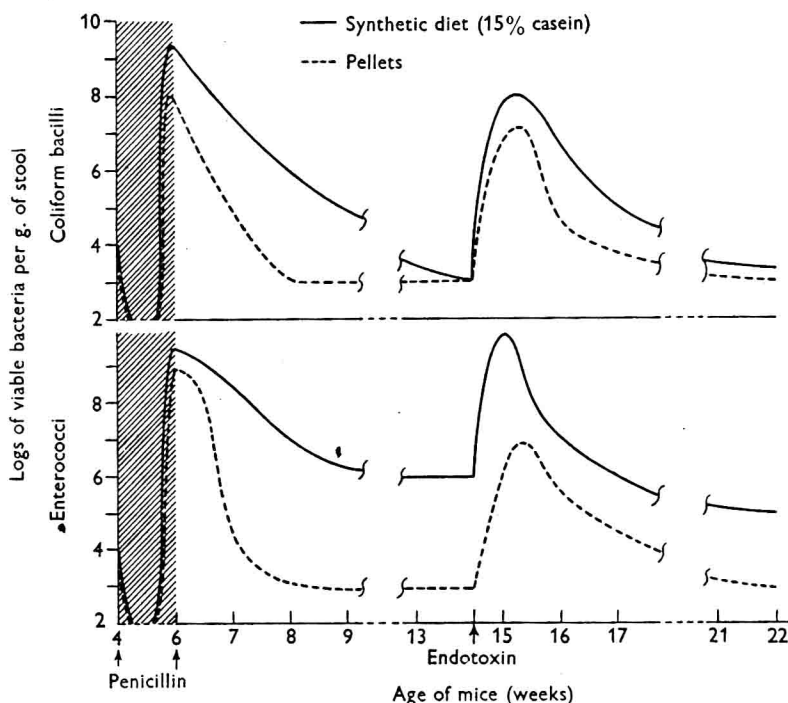


Fig. 1. The numbers of enterococci and coliform bacteria in NCS mice which received penicillin in the drinking-water for 1 week, and were fed pellets, or a synthetic casein diet, and were later given a single injection of endotoxin (0.34 mg).

equilibrium has unfavourable effects on the host, the lactobacilli or most commonly both. We have emphasized above the role of the nutritional state in the maintenance of this equilibrium. Had space permitted we could have mentioned other factors which may be of equal importance—ranging from the weather to the type of litter on which the animals are kept and the effect of crowding in the mouse colony.

Thus it is clear that in symbiotic associations between micro-organisms and animals, just as in other symbioses, the details of the interrelationship depend not only upon the intrinsic properties of the symbionts but also upon their joint environment.

THE CREATIVE MANIFESTATIONS OF SYMBIOSIS

The most extensively studied, because most obvious, manifestations of symbiosis are those concerned with nutrition. In lichens, the alga supplies carbohydrates obtained by photosynthesis while the fungus secures moisture and minerals. In the digestive tract of mammals, various types of bacteria synthesize certain vitamins and probably amino acids from materials present in the excretions of the animal. Such examples of advantageous nutritional relationship have been or could be worked out for most symbiotic systems. But important as they are, the nutritional effects of symbiosis are not its most interesting manifestation. More remarkable is the fact that many symbiotic systems produce substances and structures that neither one of the two components produces when growing alone. These creative effects are so numerous and so varied that it would be impossible to catalogue them here, and a few examples will have to suffice, namely: the production of peculiar organic acids, pigments, and complex reproductive bodies by lichens; the production of haemoglobin, and of special structures in the bacterial root nodules of leguminous plants; the morphogenetic effects associated with the presence of unidentified micro-organisms in the leaf nodules of certain tropical plants; the synthesis of diphtheria toxin or of new somatic antigens by bacteria when they are lysogenized by the proper strains of bacteriophage, etc. Description of these and other examples will be found in Allen & Allen (1954), Humm (1944), Zinder (1959), Jacob & Wollman (1961) and Dubos (1960). To this list we should like to add the very recent finding that the intestinal flora also exerts morphogenetic effects.

Germ-free animals present anatomical features which differentiate them from conventional animals, particularly in the poor development of the lymphatic system and the large size of the caecum. In general such abnormalities are rapidly corrected when germ-free animals are recontaminated. Recently it has been found in guinea-pigs that the germ-free state results in an incomplete histological development of the intestinal mucosa (Sprinz *et al.* 1961; Sprinz, 1962). The intestinal mucosa of the germ-free guinea-pig resembles that of the prenatal pig but differs from that of the conventionally reared animal by a near absence of inflammatory cells in the lamina propria, distinctly shallow crypt glands lined by numerous markedly distended goblet cells, absence of degenerative changes in the epithelium lining the villi, taller and more delicately shaped villi in the small intestine, and a villous pattern in the caecum. Oral introduction of bacteria rapidly brings about a change in the architecture and

histology of the bowel; within a matter of a few weeks the pattern approaches that of conventionally raised animals.*

There is of course no reason to assume that a common mechanism will be found for such varied effects. They are probably unrelated and each particular case will be found to involve highly specialized reactions. Nevertheless, a few general remarks seem in order to suggest that some of these phenomena may be related more closely than appears at first sight.

There now exists convincing evidence that single enzymes can catalyse several types of reactions; one of the examples most carefully studied is that of crystalline glutamic dehydrogenase which can not only oxidize glutamic acid but can also catalyse the oxidative deamination of L-alanine (Tomkins & Yielding, 1961; Tomkins, Yielding & Curran, 1961). This is relevant to our discussion in that certain steroid hormones, 1:10 phenanthroline and alkaline pH, all promote disaggregation of the enzyme protein into subunits, stimulate alanine deamination and inhibit glutamic dehydrogenase. In contrast, ADP, DPN and TPN, which cause association of the enzyme protein, inhibit alanine deamination and stimulate glutamic dehydrogenase. These findings suggest a means by which steroid hormones and other biologically active substances and conditions can alter both the kinetic properties and the substrate specificity of an enzyme by changing its physical structure. It is known, furthermore, that the affinity of antigen for antibody, and more generally of one large molecule for another, is determined not only by molecular configuration, but also by the environment in which the two come into contact. Taken together, these facts may help in interpreting some of the problems posed by the specificity and creative effects of symbiotic associations.

In the first section of this chapter we have considered specificity as operating at the level of the interacting organisms. In reality, however, the mechanisms of specificity may not operate through the organism as a whole, but rather through particular structures and processes. As in the interaction of molecules, the interactions between organisms may take place between functional groups. We have seen, moreover, that the reactivity of biologically active molecules can be modified by the environment. In the light of these facts it becomes clear that specificity cannot be defined completely on the basis of chemical structures at a given time because living things change when their environment changes. In other words, the concept of biological specificity involves factors other than those considered in orthodox chemistry, because it must include the total environment.

* It is of interest that SPF rats, which can be assumed to have an intestinal flora somewhat different from that of conventional animals, also exhibit differences in the structure of their intestinal mucosa; particularly the intestinal villi are more 'delicate' structures than in conventional animals (Paget, 1962).