BACTERIAL INFECTION AND IMMUNITY IN LOWER VERTEBRATES AND INVERTEBRATES

By K. A. BISSET, *From the Bacteriology Department, University of Birmingham*

CONTENTS

1. INTRODUCTION

Since the discovery in the seventies of the last century of the influence of bacteria in the causation of disease, almost the whole of the research on such diseases, the modes of action of pathogenic bacteria and immunological reactions has been carried out on man and other warm-blooded animals. Yet it is recognized that infection and immunity occur in all classes of animals. In fact, bacteria are known to attack almost all living organisms which to some degree all show immunological response, not always in complete accord with the hypotheses derived from observations on warm-blooded animals.

There is some justification for dividing bacterial diseases, for purposes of study, into those that affect animals possessing an effective temperature-regulating mechanism, and those that affect the remainder of the animal kingdom, in which temperature regulation is less exact. The latter group comprises many diseases having certain characters in common, namely, the tendency to be generalized conditions lacking any regional inflammatory reaction (Sweetman, 1936; Bisset, 1946), to be greatly influenced by the external temperature, and often to be caused by common saprophytic or gut- or ecto-parasitic bacteria.

The study of this group of diseases is complicated by the fact that most cold-blooded vertebrates and invertebrates, except insects, are aquatic and usually marine. Consequently many observations have been made on animals in aquaria, where conditions are not normal, and little is known of the diseases affecting the majority of invertebrate groups. On the other hand, information on the diseases of insects is comparatively plentiful. The interest in diseases of insects has been much greater in France and in the United States than in Great Britain, largely due to the greater importance of agricultural pests in those countries, and in France to the economic value of the silk industry. Possibly for the latter reason, almost all the extant work on immunity in insects has been performed upon caterpillars of the Lepidoptera.

Unfortunately, specific names have often been given to bacteria apparently causing disease, without adequate evidence of their pathogenicity or comparison with similar bacteria occurring elsewhere. This practice seems to have led to several widely distributed saprophytic bacteria being each given several specific names, and to mixed being mistaken for pure cultures. A classical case is that of the *Cocco-bacillus acridiorum* of d'Herelle (1911, 1914), which was isolated from an epizootic of locusts in Mexico, and widely distributed for control purposes. The purity of the cultures, and the justification of the specific rank were strongly criticized in many quarters (Paillot, 1933). This did not deter Kufferath (1921) from according the same distinction to the next organism, a *Staphylococcus*, to be isolated from an epizootic of locusts, this time in Greece. Similarly, the bacillus described by Patterson (1903) as the primary infective agent in *Saprolegnia* disease of salmon was discovered to be a mixture of *Proteus* and *Pseudomonas fluorescens* (Bisset, 1946). Such instances could be multiplied indefinitely.

In the group of animals under consideration diseases due to ultra-microscopic viruses, protozoa and fungi appear to be as widely distributed as are those due to bacteria, but are not discussed.

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2. BACTERIAL DISEASES OF COLD-BLOODED VERTEBRATES

(a) *Fish*

Diseases of fish have attracted attention on many occasions in the past. It is recorded that in 1680 fish died in large numbers in a lake in Mansfeld, their bodies being covered with violet patches (Ozanam), and many other instances might be given, but for the fact that from the accounts in the early literature mass mortality from other causes cannot readily be distinguished from mortality due to bacterial disease.

Probably the first disease of fish to be scientifically investigated was an epizootic which occurred among perch, in Lake Geneva, between 1866 and 1868 (Ogle, 1873). It took the form of a generalized bacteraemia (as diseases of fish so often do), and much interest was caused at the time by the fact that bacteria appeared in the blood stream during the life of the fish. Because of the obvious cloacal discharge it was called 'Fish Typhoid'.

Of the bacterial diseases of fish so far described the most economically important is 'Furunculosis' of the Salmonidae (Plehn, 1924; Mackie *et al.* 1930; and others). The name is an unfortunate one, because, although the disease is associated in some cases with boil-like lesions in the surface muscles, it is a generalized bacteraemia characterized by rapid liquefaction of the kidney. The causative organism, usually called *Bacterium salmonicida*, is a small, motile, Gram-negative rod, producing an intense, brown, diffusible pigment in culture.

From an original focus, probably in Denmark or North Germany, this disease has been disseminated by hatchery-bred trout over much of the Northern Hemisphere. The trout, although not highly susceptible as adults, may suffer from the disease when young, and after recovery carry the organism in the kidney whence they excrete it into the water. Salmon, however, are highly susceptible, and in the late nineteen-twenties this disease caused such havoc in Scottish salmon rivers that, in 1937, it became a 'notifiable disease', by Act of Parliament. Certainly the first disease of fish to be accorded this distinction.

One of the most interesting infections of fish to have been investigated bacteriologically occurs in the Mississippi basin and is caused by a *Myxococcus* (Davis, 1921; Garnjobst, 1945). The organism is commonly present in the slime of the fish and is normally harmless. Under conditions of high temperature and low water, however, it may cause an epizootic disease with heavy mortality.

Many other bacteria, of a wide variety of groups, have been isolated from infections in fish; e.g. phosphorescent bacteria (Miura, 1924; Harvey, 1940), acid-fast bacilli (Sutherland, 1922; Aronson, 1926), *Erysipelothrix* (Harkins, 1927; Brunner, 1938; Hettche, 1938), *Proteus* (Wyss, 1898; Babes & Riegler, 1903; Markoff & Jatschewa, 1939), Gram-positive cocci (Anderson, 1909; Bisset, 1946), Bacillaceae (Ceresole, 1900).

(b) *Amphibia and reptiles*

Among cold-blooded vertebrates, other than fish, the disease which has received most attention is that known as 'Red-Leg' in frogs (Sanarelli, 1891; Russell, 1898). The name is derived from the subcutaneous haemorrhages in the belly and thighs which are a constant sign of the disease. The organism is usually described as *Proteus hydrophilus*, but doubt has been cast on the validity of the generic name (Wilson & Miles, 1946). Although originally described in aquaria and frog farms it occurs in nature (Kulp & Borden, 1942). Infections of a similar kind have been reported by Ernst (1890), Venulet & Padlewski (1913) and many others.

References to diseases of reptiles are uncommon. Snakes have been found to be infected with acid-fast bacilli (Sibley, 1889; Griffith, 1941) and possibly with Gram-positive cocci (Cockburn, 1946).

(c) *Immunity in cold-blooded vertebrates*

Since the work of Metschnikoff (1884, 1887), Balbiani (1886), Ruffer (1891) and others, great emphasis has been laid on the importance of phagocytosis in the immunity of lower animals. This question has been so fully studied that a complete review is not attempted here.

Phagocytosis has frequently been demonstrated in cold-blooded vertebrates, usually in the course of experiments designed to test their resistance to pathogens of mammals, and in particular the anthrax bacillus (Mesnil, 1895; Ledingham, 1922). A very full account of phagocytosis in the frog was given by Kanthack & Hardy (1892), but here again the organism studied was not a natural pathogen of frogs but the anthrax bacillus. The complete immunity of cold-blooded animals to this organism seems to have occasioned surprise.

The striking nature of the phenomena of phagocytosis, combined with the unexpected complications in the production of humoral antibodies in cold-blooded animals, appears to have led to a widespread belief that antibodies were completely absent. There is much evidence to the contrary, however. Agglutinins have been produced in both fish and frogs by the inoculation of these animals with bacteria, living or dead (Widal & Sicard, 1897; Pliszka, 1939; Kulp & Borden, 1942; Bisset, 1947), or foreign proteins (Allen & McDaniel, 1937; Cushing, 1942). In almost every case these investigators drew attention to the paramount importance of temperature. Widal & Sicard, who employed living cultures of typhoid bacilli as antigen, found that frogs would produce agglutinins at 37 and 21° C. but not at 12° C. Pliszka, Bisset, Allen & McDaniel discovered that at temperatures between 18 and 25° C. agglutinins were produced, whereas below 10° C. they were entirely absent. Cushing, employing temperatures of 15 and 28° C., was able to produce antibodies in fish at both temperatures, but much more rapidly at the higher one. The paradoxical results of Kuip & Borden (1942), who after repeated failures succeeded in producing agglutinins in a bull-frog, were probably due to the same cause. This almost certainly applies to the numerous failures in the past to demonstrate antibodies in the blood of cold-blooded animals.

In all the experiments just mentioned controls were used; unimmunized animals produced no antibodies at either temperature, except in the case of carp, used by Cushing, which showed natural agglutinins in low titre at both temperatures for the sperms of the sea-urchin. Schwarzmann (1927) stated, however, that normal frog serum contains haemolysins and haemagglutinins for the erythrocytes of various mammals, and that these vary in titre according to the season of the year. It may be assumed that here also temperature is the important factor.

This effect is further discussed in a later section.

3. BACTERIAL DISEASES OF INVERTEBRATES

(*a*) *Protozoa*

Bacterial infections of Protozoa have been studied. Bourne (1891) observed what he believed to be infecting bacteria in a large *Amoeba*, and Petschenko (1910) observed the course of a bacterial infection in *Paramecium*, which resulted in the destruction of the nucleus. Petschenko showed that, in culture, a strain of *Paramecium* was rapidly evolved, which was much more resistant to the disease.

The question of immunity in Protozoa was discussed by Métalnikov (1926), who quoted evidence that an individual might be trained greatly to increase the rapidity of its elimination of harmful particles. Although this may be regarded as an immunological reaction its connexion with food-selection mechanisms is apparent.

(*b*) *Insects*

It is not surprising that the diseases of insects of which we have the most information are those affecting the insects domesticated by man, the silkworm and the honey-bee. In both it is the larva which most usually suffers from bacterial infection.

The various 'foul-broods' of the bee (Tarr, 1937, 1938 *a*, *b*; and others) are exceptional among diseases of insects in that they are usually caused by Gram-positive bacteria, the celebrated *Bacillus larvae* of American Foul Brood being probably the best known of all insect pathogens; Gram-positive cocci have also been incriminated in the production of 'foul-brood' (Davis & Tarr, 1936). The spores of *B. larvae* are carried about the hive by adult worker bees, the larvae are infected in the brood cells and rapidly reduced to glutinous, brown masses. Thereafter the bacteria sporulate until redisseminated by workers cleaning the cells. The adult bees appear to be totally immune. The infection is probably carried from hive to hive by the theft of honey from weak, infected colonies by strong, uninfected ones, which thus infect themselves. Other bacterial brood diseases of the bee are similar but have been less fully worked out.

Gram-positive cocci have been isolated from a small number of other infections of insects; e.g. a *Streptococcus* from a disease of caterpillars of the gipsy-moth (Glaser, 1918*b*), which was also exceptional in being, experimentally, fairly specific for its host; a *Staphylococcus* from locusts (Kufferath, 1921); and a *Staphylococcus* in one of the rare examples of a disease of adult house-flies (Glaser, 1924), producing, after a lengthy period of incubation, distension of the abdomen and death.

The majority of recorded bacterial infections of insects have been caused by small, Gram-negative cocco-bacilli (Paillot, 1933), and the best known diseases are those collectively entitled 'flâcherie' in silkworms. The literature on the subject is extensive and not very enlightening. It was reviewed by Glaser (1914) and Paillot (1927, 1933).

These infections are generalized, bacteraemic conditions, usually commencing in the gut by the ingestion of potentially pathogenic bacteria. Indeed, as in the case of cold-blooded vertebrates, almost all bacterial infections of insects appear to be generalized (Sweetman, 1936).

Disease has long been recognized as important in checking the ravages of insects among crops, especially in warm climates. Krasilshtshik (1893) described two infections among swarms of grass-hoppers in Russia. One of them, because of the blackening which it caused in the body of the insect, he termed 'graphitose'. Destructive diseases of larvae of agricultural importance have often been reported in America (White, 1923 *a*, *b*), including the infamous Colorado beetle (White, 1928, 1935). Paillot (1917, 1919*c*) described an epizootic disease of cockchafers caused by a bacillus of remarkable morphology, resembling *Pseudomonas malvacearum* (Stoughton, 1929) and other plant pathogens in its chromatinic structures.

The lethal effect of a disease of locusts, observed in Mexico by d'Herelle (1911, 1914), led to attempts to utilize the infecting organism for control purposes. Some success was achieved (Sergent & l'Héritier, 1914), but not sufficient to warrant continuation of the method. Biological control of insects by means of bacterial disease has been widely attempted, particularly in the case of the beetle larva known as the European corn-borer (Chorine, 1931), and certain caterpillars (King & Atkinson, 1928), but the results have not been uniformly encouraging. Much of the information on this subject is to be found in the works of Paillot (1933), Sweetman (1936) and Fernald & Shephard (1942).

(c) *Other invertebrates*

Information on diseases of invertebrates other than insects is very scanty. ZoBell (1946) gives a short summary of some recorded instances in marine animals, but it is the author's opinion that some of the examples quoted as bacterial infection are exceedingly doubtful. Among the best documented of such diseases are a general infection of 'sand-fleas' (the amphipod Crustacea, *Orchestia* and *Talorchestia*) by a luminous bacterium, causing the infected animals to appear luminous (Inman, 1927), and a shell disease of lobsters (Hess, 1937) caused by chitinoclastic bacteria.

(d) *Immunity in invertebrates*

It is well known that phagocytosis was first observed in an invertebrate, *Daphnia* (Metschnikoff, 1884), and, as has been remarked in the case of cold-blooded vertebrates, much weight, perhaps overmuch, has since been placed upon it. A great deal of evidence exists however, to demonstrate the presence of humoral antibodies in the blood and body fluids of invertebrates. It is rather surprising to find that our knowledge of immunological reactions in invertebrates, especially marine forms, greatly exceeds our meagre information of their actual diseases. Some of this immunological work has been reviewed by Cantacuzène (1919*b*), and more recently by Huff (1940). The question of the protective function of insect blood is also referred to by Mellanby (1939). Cantacuzène demonstrated agglutinins, lysins and complement-fixation in several species of crab (1912*a*, 1913 *a*, *b*, *c*), agglutinins in a snail (1916), an ascidian (1919*a*), *Sipunculus* (1922 *a*, *b*, *c*), *Maia* (1923) and *Phascolosoma* (1925). His attempts to demonstrate the presence of complement in marine invertebrates were, however, unsuccessful (1912*b*), as were those of Hollande (1919) and Paillot (1921*a*) with insects. Despite the absence of complement, haemolysis and bacteriolysis have frequently been described in insects (Paillot, 1919*b*, 1920*a*, *b*, 1921*a*, *c*; Métalnikov & Gaschen, 1922; Métalnikov, 1923; Couvreur & Chahovitch, 1921 *a*, *b*). Agglutinins in insect blood have been demonstrated *in vitro* by Glaser (1918*a*). Passive immunity has been conferred on insects by the inoculation of immune serum of the same species (Zernoff, 1928; Hollande & Vicher, 1928). Métalnikov believed natural immunity to be exclusively phagocytic (1925), although he showed that some insects could acquire powerful antibodies by immunization, within a few hours (1920*d*; Métalnikov & Gaschen, 1922). This phenomenon was also demonstrated by Paillot (1920 *a*, *b*) who emphasized the importance of humoral antibodies. In the earthworm phagocytosis has been studied by Lim Boon Keng (1895) and Cameron (1932), and in insects by Cameron (1934) and others. Particular attention has been paid to the destruction of the tubercle bacillus (Hollande, 1920; Hollande & Aghar, 1928; Hollande & Gély, 1929). Fiessinger observed (1920) that this organism, after ingestion by the phagocytes of the bee-moth, retained its infectiveness for guinea-pigs. Métalnikov (1920*c*) believed that this was due to the survival of occasional bacilli for exceptional periods. Zernoff (1928) showed that passive immunity could be conferred on the larva of the bee-moth by the injection either of washed leucocytes or of cell-free serum from an immune larva.

The extreme rapidity with which certain insects acquire immunity, apparently both humoral and phagocytic, after injection with heat-killed vaccines, enabled Métalnikov (1926, 1932) to perform a number of remarkable experiments. The most interesting were those in which he showed that in a caterpillar, tightly ligatured about the middle, immunization of the posterior half conferred immunity upon that half alone; while immunization of the anterior half produced immunity in the entire animal, apparently transferred by the nervous system.

It should be noted that in the majority of cases where phagocytosis has been observed, and has been claimed to be the main if not the sole defensive mechanism, no attempt whatever has been made to discover whether humoral antibodies exist. Where they have been sought they have usually been found.

An exactly similar observation to that made by several workers (p. 129) on the influence of temperature on the production of antibodies in cold-blooded vertebrates was made by Paillot (1921*b*) on caterpillars of the bee-moth. Paillot claimed, however, that a reduction in temperature from 20 to 10° C. inhibited, not only the production of antibodies, but also phagocytosis.

It is not intended to discuss here the question of bacterial 'symbionts' in insects. Insects like other animals carry bacteria in the gut, these appear to be mainly the species which occur commonly in their food (Cao, 1906; Muzzarelli, 1925). There is some evidence that these bacteria are beneficial or even necessary to the host, in order to break down otherwise unassimilable foodstuffs (Leach, 1931, 1940; Steinhaus, 1940; Blewett & Fraenkel, 1944). Bacteria thus acquired by the larva seem to be able to survive pupation and reappear in the imago (Petri, 1910; Glaser, 1923; Leach, 1926, 1934; Johnson, 1930), although the degree of infection may diminish considerably on emergence (Bacot, 1911 *a*, *b*; Ledingham, 1911). In some genera (e.g. *Culex*) this phenomenon is not found, and the imagines emerge sterile although the larvae may have been heavily infected (Violle & Sautet, 1937). Some work has been done on the comparative resistance to bacterial infection of the various stages in the life of an insect. Chigasaki (1925 *a*, *b*) found the larva of *Galleria* more resistant than the imago. The pupa had less resistance than either. Their capacity to acquire immunity by 'vaccination' was in like proportion. Métalnikov (1925) found little difference between the stages, while Li Hao (1937) reported that phagocytosis by wandering haematocytes was much less marked in the imago. It is worthy of notice that the great majority of recorded diseases of insects affect the larva alone, although in the case of a fatal infection of house-flies the bacterium, which is acquired during the larval stage, produces the disease only at or after metamorphosis (Roubaud & Déscazeaux, 1923).

4. INFECTIONS BY BACTERIA COMMONLY REGARDED AS NON-PATHOGENIC

Many workers have expressed the opinion that saprophytic bacteria, or normally non-pathogenic gut- or ecto-parasites, may upon occasion cause disease and death of cold-blooded animals. The distinction between saprophytes and gut-parasites of such animals is not a clear one. As noted on p. 131, the gut-parasites of insects are usually those bacteria occurring commonly in their food. In the case of cold-blooded vertebrates also, the bacteria present in their food and in the surrounding water are usually to be found in their gut (Houston, 1905; Bettencourt & Borges, 1909; Browne, 1917). With animals possessing a moist integument bacteria may invade the tissues through this surface. This has been shown to occur in earthworms (Cameron, 1932) and goldfish (Bisset, 1946), and the author has also demonstrated it in slugs by the method employed by Cameron on earthworms, which consists simply in placing the outside of the animal in contact with a culture of an easily recognized, pigmented organism, which can afterwards be recovered from the body cavity of experimental animals but not of controls. Normally the defences of the host appear to be sufficient to keep in check the potential pathogenicity of the bacterium, but occasionally disease may result. Balbiani (1886) first observed this phenomenon in a variety of species of Arthropoda, Krasilshtshik (1893) described it in grasshoppers, Sawamura (1906) in silkworms, Chatton (1913) in cockchafers and silkworms, Glaser (1925) in tent-caterpillars, Pospelov (1926) in locusts, Inman (1927) in sand-fleas, Cameron (1934) in caterpillars of the bee-moth, and Davis & Tarr (1936) in bees. While, experimentally, Toumanoff (1925) found the stick insect to be highly susceptible to infection with *B. tumefaciens*, a plant pathogen, Métalnikov (1920*a*, *b*) showed that insects are highly susceptible to inoculation with many common saprophytes. Cases have also been reported where a specific disease appears to be associated regularly with the occurrence of more than one bacterium, although only one of these is capable of causing the initial infection (Paillot, 1919*a*; Trensz, 1933).

Among the factors determining the appearance of pathological effects in a previously harmless infestation, temperature and humidity appear to be specially important. Pospelov (1926) found that a temperature of 20° C., with a high humidity, caused a normal gut-parasite of locusts to produce disease and death. A fall in humidity and a rise in temperature to 28° C. cured most of the survivors of infection. King & Atkinson (1928), working with a disease of *Euxoa* caterpillars, found that the bacterium was normally present in many of the insects, but that epizootics started only in hot, humid weather. Chorine (1933) found that when infected caterpillars were brought from the extreme heat of the desert into the laboratory, they were frequently cured by the fall in temperature.

In insects, the introduction of gut-parasites into the haemocoel will frequently cause disease (Sawamura, 1906; Chatton, 1913). On the other hand, diseases caused by bacteria that are normally gut-parasites may be confined to the alimentary tract (Picard & Blanc, 1913; Paillot, 1927).

In both the cases recorded on p. 131, of disease in marine invertebrates, the organism incriminated was indistinguishable from non-pathogenic forms. The luminous bacterium causing disease of Amphipod Crustacea appeared to be a normal gut-parasite, causing disease only in hot August weather (Inman, 1927). Similarly, chitinoclastic bacteria, apparently identical with those causing disease in lobsters (Hess, 1937), are common, sea-water dwelling saprophytes. It was observed that lobsters from lobster-pounds were often more seriously diseased than were 'wild' lobsters caught in the same area.

Among the cold-blooded vertebrates a very similar state of affairs appears to exist. In addition to the wide variety of infecting organisms recorded already, Nobécourt (1923) produced a fatal infection in frogs, with a plant pathogen, *B. carotovorus*, while Calmette (1923), Williamson (1929) and Bisset (1946) believed that fish and frogs might become infected with saprophytic water bacteria. Here also temperature appears to be an important factor, and in many cases infections of cold-blooded vertebrates are completely controlled by it (Ernst, 1890;

Emerson & Norris, 1905; Markoff & Jatschewa, 1939; Bisset, 1946, 1947).

Some of the apparent anomalies with respect to the effect of temperature are discussed in the next section.

Damage to tissues caused by handling may be sufficient to permit the entrance of otherwise harmless bacteria, and so cause disease in fish (Davis, 1921); and a mixed infection with what appeared to be water bacteria has been observed in the swim-bladder of trout suffering primarily from a helminthic disease (Drew, 1909). Similarly, Ravitch-Stcherbo (1936) described a pigmented bacillus, normally confined to the gut of *Amphioxus*, which under aquarium conditions was liable to cause a generalized infection.

5. THE INFLUENCE OF TEMPERATURE ON BACTERIAL DISEASES OF COLD-BLOODED ANIMALS

The importance of the external temperature has already been remarked, but its effects are frequently anomalous; for instance, Emerson & Norris (1905) reported that, in frogs infected with 'Red-Leg', the course of the disease might be arrested by placing them at a temperature a little above freezing-point, but it would run its usual course when they were restored to room temperature. While Ernst (1890), working with frogs suffering from a similar disease, reported that their resistance to initial infection was greater at higher temperatures, Davis (1921) found that injured fish readily became infected at high water temperatures, and Mackie *et al.* (1930) found that outbreaks of 'Furunculosis' among salmon and trout coincided with warm weather and low river levels. The same anomalies have been reported among the invertebrates. Several workers have observed the increased incidence of epizootics among insects in hot, humid weather (Fernald & Shephard, 1942), whereas Pospelov (1926) found that a rise in temperature would cure locusts suffering from a gut infection.

Bisset (1946) reported that when fish, parasitized by what appeared to be saprophytic water bacteria, were placed at a temperature of 20° C. the degree of infection increased and some of the fish died, but the survivors cleared themselves completely of infection. In groups of fish kept constantly at 10° C. they appeared incapable of clearing themselves and a condition of symptomless parasitism persisted. Uninfected fish were much more resistant to initial infection at 20 than at 10° C.

It has already been recorded that in both insects and lower vertebrates a reduction in temperature from 20 to 10° C. results in an almost complete cessation of production of humoral antibodies. It has also been shown (Bisset, 1947) that whereas frogs, infected with *Pseudomonas fluorescens*, eliminate it from their bodies much more rapidly at 20 than at 8° C., the bacterium, although capable of growing well at both temperatures, is more greatly enhanced in virulence by passage through a series of frogs at 20 than at 8° C.

It is perhaps in the interplay between the effect of temperature upon the invasiveness of bacteria and upon the defences of cold-blooded animals that the explanation of some of these anomalies is to be found.

6. SUMMARY AND DISCUSSION

A review is given of some of the literature concerned with bacterial infections and immunity in invertebrates and cold-blooded vertebrates. Among the former, insects have been most fully studied, although a certain amount is known of the immunological reactions of marine invertebrates. Diseases of insects are mainly generalized, bacteraemic conditions, and the great majority are caused by Gram-negative cocco-bacilli. Diseases of cold-blooded vertebrates also are usually generalized, but bacteria from a very wide variety of groups have been isolated from them.

It is probable that this resemblance in the infections of these widely separated groups of animals is due to the fact that, unlike mammals and birds, with whose reactions we are more familiar, the regulation of their temperature and the constitution of their body fluids is not exact. This toleration of wide variations of their own physical and chemical constitution must reduce their sensitivity to the changes produced by infection, and hence decrease the likelihood of a local reaction, designed to confine the invading organism to the immediate region of its point of entry, i.e. a local inflammation.

The production of humoral antibodies appears to be almost universal in the animal kingdom, although greatly affected by changes in temperature.

The effect of temperature upon the balance between host and parasite is also discussed. This question bears upon the problem of occasional pathogenesis by saprophytes. Even among mammalian pathogens the borderline between parasite and saprophyte is an indistinct one, especially in such cases as *Proteus* and *Pseudomonas pyocyanea*, and even those species which are usually regarded as exclusively parasitic may readily be constrained to adopt a saprophytic existence on artificial culture. Where cold-blooded animals are concerned the boundary is even more difficult to draw, and it is possible that under suitable conditions, bacteria which are normally saprophytes may be capable of causing infection. This point will be impossible of proof until more sensitive means of definition of bacterial species are discovered.

7. CONCLUSION

Although the study of disease of lower animals was initiated in the early days of bacteriology, and a considerable amount of work has from time to time been performed, it has not received wide attention, either from medical bacteriologists or from biologists, and research has been sporadic and disconnected. This has resulted in an exceedingly one-sided knowledge of the relationship between animal host and bacterial parasite.

Even the brief mention which has been possible in this review of the unexpected phenomena of immunity in some of the neglected 99 % of animal species may serve to indicate how much light may yet be thrown upon the general nature of infection and immunity by a wider extension of the field of inquiry.

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