PLEUROPNEUMONIA-LIKE ORGANISMS AND ARTHRITIS*

BY

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Investigations into rheumatic diseases in man have to some extent been handicapped by the absence of spontaneous and easily reproducible arthropathies in animals. Certain strains of pleuropneumonia-like organisms now provide a means of setting up an infectious arthritis in rodents, which, though possibly unrelated to rheumatic fever and rheumatoid arthritis in man, may well serve to throw fresh light on human joint infections. A brief summary of our present knowledge of pleuropneumonia-like organisms may not be without interest.

The Pleuropneumonia-like Organisms

The first member of this group was described by Nocard and his colleagues as long ago as 1898, when they cultivated but were unable to see the causal agent of bovine pleuropneumonia.

In 1910 Bordet stained the organisms by Giemsa's method and described their pleomorphism, as also did Borrel and his colleagues (1910) who, in view of the morphology which they observed, gave them the name *Asterococcus*. The fact that Berkefeld and Chamberland filtrates were infective gave grounds for believing that these organisms could be classified among the filtrable viruses. The second filtrable organism of the group, derived from sheep suffering from contagious agalactia, was first seen by Celli and de Blasi in 1906: it was later cultivated by Bridré and Donatien (1923 and 1925). Both these organisms can be grown in a variety of media provided that a high concentration of animal protein is present.

When, after the war of 1914-18, a concerted attack was made on the filtrable viruses, attention was naturally focused on those viruses which it appeared could readily be cultivated on artificial media.

In 1934 Shoetensack in Japan isolated from dogs suffering from distemper a filtrable organism which he termed *Asterococcus canis*. Positive growth was obtained from the purulent secretions of the eye and nose, as well as from the lung, liver, and pericardial fluid. Later (Shoetensack, 1936 *a* and *b*), a second type, serologically and biologically different from type 1, was obtained from cases of distemper in dogs. Occasionally the same organisms were isolated from spontaneous pneumonia of dogs. The illustrations provided by Shoetensack, and the subsequent work of Klieneberger (1938 and 1940),

on *Asterococcus canis*, leave no doubt that these organisms from dogs belong to the pleuropneumonia-like group: it is, however, very doubtful whether they play anything more than a secondary rôle in the aetiology of canine distemper.

In 1935 Klieneberger reported that a pleuropneumonia-like organism, now known as L_1 , existed as a symbiont of *Streptobacillus moniliformis* (*Actinomyces muris*), with which it was found growing in culture. Since then a number of these organisms have been isolated from small rodents as well as from man and, more recently, from the developing chick embryo. What appear to be free-living members of the same group have been found by Laidlaw and Elford (1936), and by Seiffert (1937*a* and *b*) in sewage, humus, earth, and similar materials. These free-living organisms show both antigenic and metabolic differences among themselves (Pirie, 1937 and 1938), while their growth does not depend on such high protein concentrations as are necessary for the parasitic members of the group. Further investigations are required to determine the relationship of the free-living to the parasitic members of the pleuropneumonia group of organisms.

Pleuropneumonia-like Organisms from Rodents

Pleuropneumonia-like organisms have now been obtained from rats, mice, and guinea-pigs.

RATS

Many rats normally harbour *Streptobacillus moniliformis* in their nasopharynx (Strangeways, 1933) so that its symbiont L_1 (Klieneberger, 1935 and 1936) can be legitimately regarded as the first member of the pleuropneumonia-like group to be isolated from the rat. The relationship between *Streptobacillus moniliformis* and L_1 was at one period hotly debated, since some observers believed that the pleuropneumonia-like organism was merely a variant of the streptobacillus (Dienes, 1938 to 1940). The isolation of L_1 on at least one occasion from the lungs of a laboratory rat (Klieneberger, 1938) independently of *Streptobacillus moniliformis*, and the cultivation of L_1 for many generations on artificial media with no tendency to reversion to *Streptobacillus moniliformis* (Klieneberger, 1940), have shown conclusively that L_1 is itself a pleuropneumonia-like organism strictly comparable to those other members of the group which have no relation to, or association with, organisms such as *Streptobacillus moniliformis*.

In 1937 Klieneberger and Steabben obtained a

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pleuropneumonia-like organism, L₃, from the lungs of 17 out of 19 rats suffering from "bronchopneumonia" or "bronchiectasis". Immunologically and in other ways this organism was found to be distinct from L₁ (Klieneberger, 1938). Later, Klieneberger and Steabben (1940) examined the lungs of 251 laboratory rats and 17 wild ones. Lesions were found in the lungs of 108 laboratory rats, but pleuropneumonia-like organisms were isolated from the lungs of 138: altogether 46 out of 139 rats 8 months of age or younger yielded these organisms although their lungs were of normal appearance. Only four of these pleuropneumonia-like organisms were typed, but all were serologically similar. Among the 17 wild rats, one only showed lung lesions and only this one yielded a L₃ type organism. Efforts to produce the lesions of "bronchiectasis" in rats' lungs with cultures of L₃ have proved ineffective. Subcutaneous and intraperitoneal injections of cultures mixed with sterile agar cause abscesses: intravenous and intracerebral inoculations in mice are without effect.

L₄, the third type of pleuropneumonia-like organism from the rat, has been isolated on several occasions under somewhat different circumstances. Early in 1938 Woglom and Warren found that certain suspensions of rat sarcoma 39, when inoculated subcutaneously into rats, caused abscesses which bacteriologically appeared sterile. With Berkefeld filtrates of the pus from the abscesses the condition could be reproduced not only on subcutaneous inoculation but on intravenous injection, when there resulted widespread suppuration about the head and larger joints, the testes, and the extremities. The mouse was even more susceptible than the rat, but guinea-pigs and rabbits were insusceptible. Cytoplasmic inclusions were found in the epithelial cells of the skin in close relation to the abscesses. The causal agent was referred to as "a pyogenic filtrable virus". On culturing some of the infective material from the abscesses, however, Klieneberger (1939) showed that the responsible agent was a pleuropneumonia-like organism which serologically and culturally was identical with the L₄ organism that she had isolated in the previous year from the swollen submaxillary gland of a rat (Klieneberger, 1938). This result was confirmed by Woglom and Warren (1939). In 1939 in the rat colonies of three laboratories in London there was noted a spontaneous polyarthritis, from which a pleuropneumonia-like organism was isolated by Findlay *et al.* It was at first thought that this organism was distinct from other members of the group, but later, observations showed that it was serologically identical with L₄ (Klieneberger, 1940). Somewhat earlier Collier (1938, and 1939, *a, c, d, e, f*), Collier and Esseveld (1938), and Collier and Staverman (1939), working in Java, had reported on a polyarthritis of rats which could be transmitted in series but of which they failed to isolate the causal agent. Later Beeuwkes (1940) and Beeuwkes and Collier (1942) succeeded in culturing a pleuropneumonia-like organism from the spontaneous rat

arthritis, while another similar agent was obtained from rats after inoculation with blood and joint fluid from a patient suffering from acute rheumatic fever. These two strains were biologically similar, but their serological relationship to other L₄ organisms is still uncertain. The same applies to the organisms isolated by Preston (1942) and by Powell and Rice (1944) in America. The first of these American strains was obtained from a rat suffering from purulent knee joint and an ovarian abscess: other strains were obtained from the commonly occurring middle-ear infection of rats. Culturally these strains are very closely related to, if not identical with, L₄. Since a very similar condition was observed by Marshall in Nigeria in 1930 (cf. Findlay, Mackenzie, MacCallum, and Klieneberger, 1939), it is obvious that pleuropneumonia-like organisms are found in rats throughout the world. Their habitat in the normal rat is uncertain.

MICE

Pleuropneumonia-like organisms were first demonstrated in the brain of the mouse by Findlay and his colleagues (1938) in this country, while investigating lymphocytic choriomeningitis, and by Sabin (1938*b*) in America, while studying *Toxoplasma*. A similar condition had been observed some years previously in London while studying the neurotropic strain of yellow fever virus.

The L₅ organism isolated in England is closely related serologically to the type A organisms obtained by Sabin in America, although there are certain biological differences. Upon intracerebral injection of material containing the L₅ organism the mice, in from one to ten days but mostly on the second or third day, exhibit a curious rolling or turning on the long axis of the body, with or without other nervous symptoms. Some mice die, others recover in a few days, while still others exhibit a chronic hydrocephalus, with or without choreiform movements, for very long periods.

Sabin (1938*b*) showed that his type A organisms produced a neurolytic exotoxin. This organism has since been found in the brain of a normal mouse and in the conjunctiva and nasal mucosa of apparently healthy mice (Sabin, 1939*b*). Except in very young animals, L₅ organisms would seem to be normal parasites of the mouse (Sabin and Johnson, 1940*a*). Similar organisms have been obtained by Sullivan and Dienes (1939) from the lungs of mice that had had normal mouse lung suspensions instilled intranasally under ether anaesthesia.

L₆, which is distinct serologically and in its colonial growth from L₁, L₃, L₄, and L₅, was obtained from mice by inoculating them intracerebrally with blood from splenectomized mice containing the protozoon *Eperythrozoon coccoides* (Findlay, Klieneberger, MacCallum, and Mackenzie, 1939). Later Klieneberger (1940) reported the isolation by Dr. H. Jahn of a pleuropneumonia-like organism from the swollen joint of a mouse which had been inoculated with *Streptobacillus moniliformis*.

FIG. 1.—Ankle joint of mouse inoculated with L_5 , showing general involvement of joint. Haematoxylin and eosin. $\times 100$.

FIG. 2.—Periarticular tissue of rat inoculated with L_4 , showing infiltration with polymorphonuclear leucocytes. Haematoxylin and eosin. $\times 150$.

The organism, which is not L_1 , was termed M55, but its relation to the other members of the group is uncertain.

Sabin (1938*b*, 1939*a* and *b*) and Sabin and Johnson (1940*b*) isolated a number of other strains termed B, C, D, and E from the brains, noses, and lungs of normal mice or of mice that had received intranasal inoculations of various materials. These strains do not produce an exotoxin but differ from one another serologically.

In this country Edward (1940) obtained strains of pleuropneumonia from the lungs of mice which had received intranasal instillations of mouse lung suspensions. Edward believed that pleuropneumonia-like organisms were the actual cause of the pneumonia, but it was only with the early subcultures that pneumonia was produced, and no tests were made to rule out the presence of a virus. A pleuropneumonia-like organism has been known to contaminate at least one strain of influenza virus, and, in addition, Horsfall and Hahn (1940) have found that while pleuropneumonia-like organisms may be present in the lungs of mice with a spontaneous virus-pneumonia, they are quite distinct from the latent virus responsible for the pneumonia. The writer obtained a pleuropneumonia-like organism from mice suffering from spontaneous arthritis in the Gold Coast. Unfortunately circumstances prohibited its further study. On at least two occasions rat strains have been isolated from mice. Findlay, Klieneberger, MacCallum, and Mackenzie (1939) obtained L_3 from the brain of an apparently normal mouse, while Warren (quoted by Sabin, 1941) isolated a L_4 type of pleuropneumonia-like organism from the brains of mice inoculated intracerebrally with herpes virus.

GUINEA-PIGS

Pleuropneumonia-like organisms have only once been isolated. Klieneberger (1940) obtained a strain in conjunction with *Streptobacillus moniliformis*

from abscesses in the neck, but owing to war-time conditions its further investigation was impossible. Smith (1941) also obtained *Streptobacillus moniliformis* from the same type of abscess but could not separate a pleuropneumonia-like organism from the bacilli.

It is obvious that apparently normal mice and rats and, to a less extent, guinea-pigs harbour pleuropneumonia-like organisms which vary in their serological reactions and in their pathogenicity.

Pleuropneumonia-like Organisms in Man

Dienes and Edsall (1937) were the first to indicate that pleuropneumonia-like organisms might possibly be associated with man when they isolated an organism of this type from the suppurating Bartholin's gland of a woman: she had, however, been working in a laboratory with rats.

Later, in America, Dienes (1940*a*) reported the isolation of five strains from the cervixes of women. Dienes and Smith (1942) published reports of the isolation of these organisms from the cervical, vaginal, and urethral secretions of women and from the urethral and prostatic secretions of man: organisms were found in 22 per cent. of 129 unselected patients. Some of the women were suffering from arthritis, some from gonorrhoea, and some from non-specific inflammatory lesions of the genito-urinary tract, while all the men were suffering from chronic prostatitis. In Australia, Beveridge (1943), Johnston and McEwin (1945), and Beveridge *et al.* (1946) have confirmed these results since they have found the organisms in both males and females with non-specific urethritis. Of 70 men with non-specific urethritis examined by Beveridge and his colleagues, 14 yielded positive results. Of 11 women who had had connexion with these positive men, only 3 yielded pleuropneumonia-like organisms. Of 101 normal women, 3 gave positive results, while 67 healthy male

medical students were all negative. Klieneberger-Nobel (1945), working at the London Hospital and the Whitechapel Clinic, obtained pleuropneumonia-like organisms from 40 per cent. of women attending the venereal disease clinic and 33 per cent. of those attending the gynaecological department for a variety of presumably non-venereal conditions: only 14 per cent. of women attending the antenatal clinic were infected. Salaman *et al.* (1946), in military personnel, found that, of gonorrhoea patients, 34 per cent. of men and 60 per cent. of women harboured pleuropneumonia-like organisms. The same organisms were common in women suffering from vaginitis due to *Trichomonas* and from non-specific cervicitis. The organisms were less common in non-specific urethritis in men, and are uncommon in prostatitis: they were found in the urethra of approximately 17 per cent. of normal men and 6 per cent. of normal women. The frequency with which pleuropneumonia-like organisms are associated with cultures of the gonococcus was originally noted by Dienes (1940*b*), and later by Brown and Hayes (1942) and Salaman (1946).

The relationship of these human strains to those of rodents has received very little attention. One unequivocal human strain studied by Warren and Sabin (1942) was not pathogenic for mice, while no evidence of toxin production was obtained. Antisera against rat and mouse strains failed to agglutinate the human organism.

These observations show that pleuropneumonia-like organisms are by no means rare parasites of the genital tracts of both men and women. Although more commonly associated with inflammatory conditions, such as non-specific urethritis and cervicitis, they were also present in apparently healthy individuals. Their isolation from human beings suffering from a particular disease does not, therefore, necessarily indicate that they are casually related to that disease.

Pleuropneumonia-like Organisms in Chick Embryos

The occurrence of a pleuropneumonia-like organism in association with a strain of influenza virus A has already been noted. More recently van Herick and Eaton (1945), in the course of passing a strain of the virus of primary atypical pneumonia amniotically in the developing chick embryo (Eaton, Meicklejohn, and van Herick, 1944), obtained a pleuropneumonia-like organism from the egg. This agent caused a sudden increase in the number of deaths of the chick embryos and was characterized by the appearance of small, opaque areas as well as by small white granular thickenings on the amniotic membrane, in which there was an abundance of organisms. The amniotic fluid was cloudy and the embryos often stunted. Woglom and Warren (1938), and Findlay, Mackenzie, MacCallum, and Klieneberger (1939) had already shown that *L*₄ would readily grow in the developing chick embryo.

The egg-passage pleuropneumonia-like organism

when injected into chickens caused a transient weakness of the legs 26 days after inoculation with, in the case of one of the chickens inoculated intranasally, tremors and ataxia. Cotton rats (*Sigmodon hispidus* sp.), when injected intranasally, showed a red oedematous consolidation of the lungs. Filtrates which were sterile when inoculated into serum broth produced similar lesions. The toxin causing pulmonary haemorrhage and oedema in cotton rats was thermostable and, as indicated by preliminary experiments, non-antigenic.

Sabin (1938*b*) had found that his type A organism from mice produced a true neurotropic exotoxin which gave rise to choreic movements. This toxin was thermolabile, antigenic, and neutralizable with an immune serum: even earlier Nocard *et al.* (1898) had obtained evidence of the production by the organism of bovine pleuropneumonia of a soluble substance toxic for rabbits.

The pleuropneumonia-like organism isolated by van Herick and Eaton was found to agglutinate chick red cells, as do influenza, Newcastle disease virus, vaccinia, and fowl plague virus: in addition, sera from year-old fowls from the hatchery which had supplied the eggs used in the virus investigation showed the presence of appreciable amounts of antibody against the pleuropneumonia-like organism. There is, therefore, a strong presumption that pleuropneumonia-like organisms may exist in the normal fowl. In view of the large number of different vaccines now prepared in the developing hen's egg, the isolation from eggs of pleuropneumonia-like organisms which are filtrable is a matter requiring further attention.

Characteristics of the Pleuropneumonia-like Organisms

The pleuropneumonia-like organisms form a very definite group, but their exact systematic position is still somewhat uncertain. Borrel and his associates (1910), using dark-field examinations and Loeffler's flagellar stain, saw in the organism of bovine pleuropneumonia not only granules and round forms, but long branching filaments, extending in some cases in many directions from the round forms. The name *Asterococcus* appeared to be appropriate. Turner (1935) in dark-field preparations observed the multipolar germination of the round forms. The majority of observers, including Klieneberger and Smiles (1942), believed that the pleomorphism, the growth in branching filaments, the particular softness, and the special manner of reproduction, differentiate these organisms from the bacteria. Turner (1935) in fact placed them in a new order, *Borrelomycetales*, Sabin in a new class, *Paramycetes*. The suggestion by Ledingham (1933) that they should be classified with the *Actinomycetaceae* was not considered by later observers, nor was the still earlier suggestion by Bordet (1910) that the pleomorphism of bovine pleuropneumonia might be analogous to the pleomorphism of certain bacterial cultures.

It is now generally agreed not only that the

morphology of all the pleuropneumonia-like organisms is very similar, whether they are parasitic or free-living, but that they cannot be regarded as growth forms of *Streptobacillus moniliformis* or of the other organisms with which they are often found in artificial culture media. The most recent observations by Weiss (1944), Dienes (1945 and 1946) and Smith *et al.* (1946), employing both stained preparations and the electron microscope, show that in young colonies pleuropneumonia-like organisms, whether isolated from rats, mice, or man, appear as small bacilli with bipolar staining. The bacillary forms round up and may swell to large size. These large round forms either develop vacuoles and degenerate into empty blebs, or reproduce small bacillary forms inside their membranes. Occasionally freshly isolated strains of certain common bacteria, such as *Haemophilus*, are known to swell into round forms of variable size, reproducing the small forms again, as do rickettsiae and certain viruses, such as those of lymphogranuloma venereum and psittacosis. The pleuropneumonia-like organisms are, it is suggested, intermediate between small Gram-positive organisms, such as *Pasteurella* and *Haemophilus*, and these viruses. Smith *et al.* (1946), with the aid of the electron microscope, distinguished spherical forms, short filaments, and many rod-shaped forms which show the structure characteristic of bacilli in that they are provided with a cell wall and intracellular granules lying within the cytoplasm. These observations tend to suggest that the branching of the filaments stressed by earlier observers such as Turner (1935) is an artefact. Klieneberger and Smiles (1942) and Dienes (1945) believe that the filaments are due to the stretching of the round forms. The morphology of the pleuropneumonia-like organisms is thus comparatively simple, since small, "elementary" corpuscles either multiply as such or swell up to large forms which again break up into elementary bodies.

Pleuropneumonia-like organisms do not take Gram's stain but are readily coloured by Giemsa's method. They give a positive reaction for nucleic acid by the Feulgen technique. Many, but by no means all, strains of pleuropneumonia-like organisms require an adjuvant such as sterile agar or a suspension of cells in order to set up infection when injected into animals. In size, as shown by filtration through gradocol membranes, pleuropneumonia-like organisms, or rather the granular forms, vary from 125 to 250 m μ . They are non-motile, are readily destroyed by heat, and have no specific resistance to glycerol.

Pathological Changes

There is still much to be learnt about the pathogenicity of organisms of the pleuropneumonia group. While the organisms of bovine pleuropneumonia and contagious agalactia are definitely pathogenic for certain ruminants, the organisms isolated from rats have been found pathogenic only for rats and mice, while those obtained from

mice are either non-pathogenic or pathogenic only for the same species. Apart from one strain which, as previously mentioned, was not pathogenic for rodents, the pathogenicity of human strains either for man or for rodents is unknown. Much further work requires to be done on the possible pathogenicity of human strains for rodents.

The three free-living strains, A, B, and C, isolated by Laidlaw and Elford (1936) showed no pathogenicity for rats, mice, or rabbits, and serologically were distinct from available parasitic strains (Klieneberger, 1940).

In the case of bovine pleuropneumonia, it is well known that in the later stages of the disease inflammatory arthritic changes may occur, while injections into the root of the tail of sucking calves or of reindeer causes an inflammation of the joints. Intracerebral injection into cows is also said to cause invariably an inflammation of the joints. Meyer (1910) believes that in adult cows the tendency to produce arthritic changes after prophylactic tail inoculations was not due to individual predisposition but to the particular strain of organism. The power to produce arthritic changes was lost by continued passage either in animals or in artificial media, and no secondary joint affections could be produced subsequently by inoculation of cultures.

In contagious agalactia of sheep, in addition to a mastitis and a conjunctivitis, an arthritis is by no means uncommon. According to Pigoury (1938), arthritis occurs in 10 to 20 per cent. of goats, coming on usually about a week after the beginning of the disease. One joint alone is usually affected, generally that between the radius and the carpus, more rarely the tibio-femoral, the tibio-astragaloid or the metacarpals. The symptoms vary from simple stiffness to acute arthritis with complete functional incapacity. Spontaneous cure generally takes place after one or two months, but in some cases ankylosis is complete, with abscess formation involving the articular surfaces. In the chronic form of the disease there may be remissions and exacerbations of the arthritis without ultimate deformity. Some generalized wasting of the muscles is common. The process in the mammary gland is that of an interstitial mastitis with disappearance of glandular tissue: in the eye there is an interstitial parenchymatous keratitis with infiltration of small cells and vascular proliferation. In the rat, L₄ strains cause abscess formation when inoculated subcutaneously. After intravenous injection death may occur in a few days without any joint involvement, but if the animal lives one or more joints become affected. The first sign in an affected joint—usually the tibio-tarsal, radio-carpal, intertarsal, or phalangeal—is swelling with a glistening, smooth, pearl-coloured appearance of the skin. Later, the whole region of the joint takes on a pink or even purplish tint and is slightly oedematous. This condition may eventually subside leaving only some slight thickening, or, while the joint is still swollen, the skin may ulcerate: gangrene of the limb may then result. Histologically the inflammatory lesions are at first periarticular

with an infiltration of polymorphonuclear leucocytes: abscess formation occurs, involving the capsule, synovial membrane, and, at a later stage, bone and cartilage. A considerable exudate may be found within the joint capsule, while the cartilage is uninvolved. As the disease progresses, cartilage is sooner or later destroyed by a rapid necrotizing process: osteomyelitis may occur.

In the affected joints there is considerable cellular infiltration and thickening of the periarticular connective tissue but no involvement of cartilage or synovial membranes. Sometimes, however, there is erosion of the articular cartilage or the formation of polypoid growths of newly formed connective tissue. Endarteritis is described in the joint tissues by Carré (1912).

In mice infected with organisms of the L_5 type, the arthritic changes are very similar in character to those produced by L_4 but involvement of the articular cartilage is very rare and complete recovery occurs without ankylosis. With organisms of his type B group, Sabin (1940) found that the articular changes are more widespread and involve all the joint structures. The changes are essentially proliferative in character and come on within 24 hours of inoculation. The capsular tissues, synovial membranes, and perichondrium are later involved, so that obliteration of the free joint space is common. Destruction of the cartilage is seen later, but not until at least four weeks after intravenous injection: the normal articulating cartilage may be replaced by immature chondroblasts or osteoblasts, and in an ankylosed joint there may be considerable ossification in the distorted articulating surfaces, which are joined by dense fibrous tissue.

Pleuropneumonia-like organisms of the L_5 type when injected intracerebrally cause an intense inflammatory reaction in the brain, together with neurolysis of many cells. When injected into the pleura or peritoneum there are no local lesions, but an exotoxin is produced which causes both a disintegration of the posterior pole of the cerebellum and degenerative non-inflammatory changes in the cerebral cortex, basal ganglia, and nervous part of the retina (Sabin, 1938b). True cytoplasmic inclusion bodies do not appear to be formed by pleuropneumonia-like organisms but the presence of the organisms in the cytoplasm of macrophage cells may sometimes be demonstrated. In mice inoculated with some strains a conjunctivitis is not infrequent.

It will be seen that certain strains of the pleuropneumonia-like organisms have a predilection for joint structures: they may cause an acute suppurative arthritis or a more chronic proliferative change somewhat akin to that of rheumatoid arthritis in man. Much further work requires to be carried out on the pathogenicity of these organisms.

Chemotherapy

Earlier investigators apparently obtained beneficial action in contagious agalactia by the injection of the sodium salt of acetarsol (3-acetyl-amino-4-hydroxy-

phenylarsonic acid) which, according to Bridré *et al.* (1928), exerts a preventive and curative action in the disease both as it occurs naturally and as it is experimentally produced in sheep or goats. Pigoury (1938) found that a single dose of 0.75 gr. had a therapeutic effect on the course of the mastitis and arthritis. Of 27 treated animals, all showed improvement, while in 30 controls either untreated or merely given urotropine the disease ran its normal course. A single dose of acetarsol protected an animal against infection for a week. Witt (1925) observed some beneficial action of arsenicals in bovine pleuropneumonia. Arsenicals have not, however, come into general use for the treatment of these infections in domestic animals. With the spontaneous rat arthritis due to L_4 , Collier (1939b and g), who was then unaware of the causal agent, found that aurothioglyucose (Solganal B) was capable of preventing the development of the arthritis. Later, working with known strains of L_4 , Findlay, Mackenzie, MacCallum, and Klieneberger (1939) and Findlay, Mackenzie, and MacCallum (1940) confirmed the preventive action of sodium aurothioglyucose (Solganal B), and found that other organic gold compounds, such as sodium aurothiomalate, lophon, parmanil, and neosolganal, were also effective. If treatment with 10 mg. of sodium aurothioglyucose per 20 gr. of body weight was delayed till 48 hours after inoculation of the organism there was evidence of curative action in mice, none of the treated mice being dead in 96 hours after infection while in the untreated series 6 out of 24 were already dead. When the administration of aurothioglyucose was delayed till four or five days after infection, there appeared to be some slowing up of the infective process. In mice which had been successfully treated with gold compounds, no pleuropneumonia-like organisms could be isolated subsequently from the affected joints. The same was true when organic gold compounds were used to treat an infection due to *Streptobacillus moniliformis* associated with a pleuropneumonia-like organism obtained from guinea-pigs.

Apart from organic gold salts, neoarsphenamine was found to have a slight action in preventing the development of arthritis in mice due to L_4 while neoarsphenamine, acetarsol, and, to a less extent, tryparsamide had also a slight action in preventing the nervous symptoms in mice due to L_5 organisms. No action could be detected on rodent pleuropneumonia-like organisms of sulphanilamide, sulphapyridine, sodium salicylate, and lithium antimonyl tartrate. Sabin and Warren (1940a, b) tested the action of organic gold salts on the arthritis caused in mice by their type B organism: here the curative action was more in evidence. A new compound, calcium aurothiomalate, was claimed by them to be practically non-toxic in mice and yet to produce a curative action even after a single dose of 1 mg. With type B organisms Sabin and Warren failed to find any bactericidal action by gold salts *in vitro*. With L_4 cultures there appeared to be a zone effect, the bactericidal action depending on the

concentration of sodium aurothioglucose (Findlay *et al.*, 1940). A similar zone phenomenon has been found by Courmont *et al.* (1932) and by Pichat (1939) in connexion with the bactericidal action of gold salts on tubercle bacilli. Preston (1942), and Powell and Rice (1944) have also found that sodium aurothiomalate was quite effective in rat arthritis due to L_4 , although owing to its toxicity its administration was hazardous. The latter observers found that penicillin was ineffective: similar results have been obtained in this country.

More recently Powell *et al.* (1946) have shown that streptomycin is highly effective if started shortly after infection. For rats of 100 g. body weight, 3,000 units a day for three days was preventive, the daily dose of 3,000 units being given in three doses each of 1,000 units. In another experiment, with a total of 36,000 units per rat spread over four days in three doses a day, 8 out of 10 rats were entirely free from infection while 2 others showed a questionable and transitory trace of swelling in one toe for two days. The 10 control rats were all dead in three weeks.

It is perhaps of interest to note that bile salts have some action on the development of experimental arthritis in rats. Snow and Hines (1941) found that ligation of the common bile duct of rats before or 20 hours after the injection of L_4 organisms delayed the onset and decreased the degree of arthritis. No similar effect was produced when an arthritis was set up with streptococci.

The Relationship of Pleuropneumonia-like Organisms to Human Diseases

With the discovery that pleuropneumonia-like organisms are the cause of arthritic conditions in cows, sheep, goats, rats, and mice, the question naturally arose whether these same organisms might not be responsible for arthropathies in man. Swift and Brown (1939) in fact claimed to have isolated pleuropneumonia-like organisms from patients with rheumatic fever. They subsequently agreed, however, that their direct "cultures" had been misinterpreted while the strains obtained after passage through mice were biologically and immunologically identical with those normally carried by these animals (Sabin 1939a).

Subsequent attempts by a number of investigators to cultivate pleuropneumonia-like organisms from joint exudates and from the tissues of patients with rheumatic fever or rheumatoid arthritis have all failed (Sabin, 1939a; Sullivan and Dienes, 1939; Findlay *et al.*, 1940; Sabin and Johnson, 1940a; and Preston, 1942). At present, therefore, the direct relationship of pleuropneumonia-like organisms to rheumatic fever and rheumatoid arthritis must remain non-proven. Further investigations might, however, be undertaken to determine whether streptococci isolated from rheumatic joints are ever contaminated with the pleuropneumonia-like organisms: in addition the effects of infecting streptococci into joints already infected by pleuropneumonia-like organisms appears worthy of study.

Apart from the possible relationship of pleuropneumonia-like organisms to non-specific urethritis, there appear to be two human diseases where further investigation of the rôle played by pleuropneumonia-like organisms might be worth while. These diseases are Haverhill fever and Reiter's syndrome. Haverhill fever is one of the two forms of rat-bite fever: it is characterized by fever and polyarthritis and is usually thought to be due to *Streptobacillus moniliformis*, which has been cultivated from the blood (cf. Farrell *et al.*, 1939). In view of the frequency with which pleuropneumonia-like organisms are associated with this bacillus, the question arises how far the symptomatology in man is due to an associated pleuropneumonia-like organism and how far to the *Streptobacillus*. All cases of rat-bite fever should, when possible, be investigated for pleuropneumonia-like organisms. *Streptobacillus moniliformis*, however, is very susceptible to penicillin, while the evidence at present suggests that pleuropneumonia-like organisms are not.

The second human infection for which there is reason to think a pleuropneumonia-like organism may be responsible is Reiter's syndrome, originally described in Germany in 1916, and characterized by the triad of polyarthritis, urethritis, and conjunctivitis. Efforts should be made to culture pleuropneumonia-like organisms from the conjunctiva, joints, and urethral discharge of all patients suffering from Reiter's disease. Unfortunately, complement fixation tests with cultures of human pleuropneumonia-like organisms have not, up to the present, proved highly specific: however, there is some evidence that skin sensitivity tests may be of greater value in diagnosing those who are suffering from an active infection due to pleuropneumonia-like organisms.

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