ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-THIRD ANNUAL MEETING OF THE INDIAN ACADEMY OF SCIENCES

A. SYMPOSIUM ON CRYSTALLOGRAPHY AND MOLECULAR STRUCTURE

(20–12–1967)

Chairman : PROF. G. N. RAMACHANDRAN

Some Recent Studies on Crystal Structures by X-Ray and Neutron Methods

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Potassium Mercuric Tribromide Monohydrate

The crystal structure of potassium mercuric tribromide monohydrate has been determined by X-ray 3D-data. The structure consists of four molecules linked by hydrogen bonds and van der Waals forces in a unit cell of dimensions $a = 4 \cdot 37$, $b = 16 \cdot 87$, $c = 10 \cdot 14$ Å and the space group $Cmc2_1$. The mercury atom is surrounded by four bromines in an irregular tetrahedron. One of the bromines is shared by two mercury atoms, resulting in a zig zag chain of Hg-Br-Hg along the a-axis. The other bromines are held by hydrogen bonds of the water molecule. Although positions of the hydrogen atoms have not been determined, it is believed that there exists a bifurcated hydrogen bond in the structure.

Cis Cobalt-diazido Bisethylene Diamine Nitrate

This cobalt complex is orthorhombic, space group Pnma, eight-formula units per cell. The structure has been determined from Patterson projections and refined by the least-squares method with two-dimensional intensity data.

The cobalt atom has the usual distorted octahedral co-ordination with four N atoms of the ethylene diamine group and two N atoms from each of the azido group. The bond distances in ethylenediamine molecule are normal and agree with the reported values. The azido group is linear and appears to be unsymmetric. The valence angle Co-N-N is about 120°. The two nitrate groups are stacked one above the other and form a close packing inside the complex ion. So far only one structure of a co-ordination compound bonding an azido group has been reported in the literature.

Potassium Sulphamate

A single crystal neutron diffraction investigation of potassium sulphamate has been made in which the intensities of 501 reflections were measured. The structure was refined by least-squares technique with individual anisotropic temperature factors. The heavy atom positions obtained agree well with those of Jeffrey and Stadler. The hydrogen atom positions differ substantially from those postulated from the X-ray work. The sulphamate ions are packed one above the other with hydrogen bonds linking them in infinite chains parallel to the c-axis.

Triglycine Sulphate

All the hydrogen atom positions have been determined with the help of intensities of 410 reflections in the three prism zones collected by neutron diffraction methods. The positions of these atoms are discussed in relation to the structure of the compound.

On the Periodic Occurrence of Stacking Faults in Certain Close-Packed Crystal Structures

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A number of close-packed crystal structures like ZnS, SiC, CdI_2, PbI_2, etc., are known to display a high concentration of stacking faults in their basal planes. The insertion of stacking faults is to be expected since, thermodynamically, the different manners of close-packing atoms lead to structures with very nearly the same free energy. The stacking faults should, however, be distributed randomly giving rise to a one-dimensionally disordered structure. While this does occur, there is a marked tendency for the stacking faults to occur periodically, often with perfect crystalline regularity, over macroscopic distances, giving rise to a long-period lattice.

Till recently this was believed to be a growth feature associated with spiral growth around screw dislocations of large but non-integral Burgers vectors. This has, however, been disproved by recent experimental observations of various workers, and a fresh explanation is being sought.

It was suggested by Schneer in 1955 that different polytypes of a compound may be related by second or higher-order transformations since they differ little in their internal energies. He assumed that the phase-transformation from the cubic close-packed to the hexagonal close-packed structure proceeds over a temperature range by infinitesimal steps, and the long-period polytypes represent intermediate states in the transition. At any intermediate temperature, assuming a Boltzman distribution of layers in the hexagonal (h) and cubic (k) states, it was shown that polytypic structures will correspond to potential minima if they are characterised by a maximum number of interaction contacts between layers in unlike states.

This paper presents experimental evidence in favour of Schneer's theory. It is shown that nearly all known polytypes of ZnS, SiC, CdI_2 and PbI_2 are characterised by a maximum number of unlike interaction contacts. In ZnS the phenomenon appears to be a sensitive function of the conditions of growth. It is possible that individual polytypes may be associated with specific temperatures within a short transition range.

The Structure and Dynamics of the Water Molecule in Crystals

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The programme of the Trombay group on the investigations of hydrated crystals by neutron diffraction is reviewed. The structure of the latest member of the series, $BeSO_4.4H_2O$, has been solved by direct methods using symmetry minimum and minimum function techniques and without making use of the X-ray structure information. The available data on the structure and co-ordination of the water molecule are summarised and the stereochemistry of the O-H . . . O hydrogen bonds in the structure is discussed. An electrostatic model for calculating the potential functions for the librations of the water molecule in crystals is discussed. Some preliminary calculations for $K_2C_2O_4.H_2O$ are also presented.

Fourier Method of Treating Anomalous Dispersion Data

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A Fourier method of treating the anomalous dispersion effect in X-ray intensity data is suggested. It is shown that the anomalous dispersion corrections to the atomic scattering factor leads to a formal representation of 'real' and 'imaginary' components in the electron density distributions in the direct space. The necessary formulas are derived and their possible application to the determination and refinement of the real ($\Delta f'$) and imaginary ($\Delta f''$) correction terms are discussed.

Some results of the recent studies on the theory of the beta synthesis with wrong atoms, are also presented.

X-Ray Crystallographic Investigation of Peptide Crystals

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X-ray crystallographic investigations on simple peptide crystals like *l*-threonyl-*l*-phenylalanine, *p*-nitrobenzyl ester hydrobromide, *l*-prolyl-*l*-phenylalanine-*o*-methoxy hydrobromide, *l*-glutamyl-*l*-methionine, glycyl-*dl*-phenylalanine, etc., are being carried out in this laboratory with the view to determine accurately the bond lengths and bond angles of the various components as well as to gain knowledge regarding the backbone and side group conformations. The stereochemical configurations as derived from least squares refinement using three-dimensional X-ray intensity data are presented. The role of hydrogen bonding in determining the spatial arrangement of the side chains in the crystals is discussed.

B. SYMPOSIUM ON IMPACT OF PHYSIOLOGY ON PLANT PATHOLOGY

(21-12-1967)

Chairman: PROF. T. S. SADASIVAN

Physiology and Phytopathology

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From a descriptive science in the latter part of the last century and well into the early part of this century, plant pathology, in the last four decades has steadily replaced much of the worn-out classical ways of approaching disease etiology and syndrome to a modern streamlined discipline with integrated reasoning into the causes of infection. Indeed, the impact of the more precise physical sciences, particularly biochemistry, has played a significant role in this evolutionary process and with the aid of sophisticated techniques evolved by plant physiologists much is now known of the biochemistry of the infected plants. Pioneering work in these areas were initiated by plant virologists and later by those working in the field of mycopathology. Critical tissue respiration measurements, derangement in carbohydrate and nitrogen metabolisms, transpiratory disturbances bringing in its wake ionic imbalance, exaggerated auxin relationships, isolation and characterization of fungal toxins and antibiotics in the rhizosphere and *in vivo* formation and accumulation of abnormal metabolite(s) (phytoalexins) consequent on pathogenesis, enzymological changes in tissues, availability of energy substances around root systems, cellular changes in RNA and nitrogen synthesis under viral infections are the more important achievements in studying the biochemistry of infected plants.

Quite recently, purification, isolation, characterization and study of the properties of the "satellite" virus and its dependence on tobacco necrosis virus for *in vivo* multiplication and the working out of the amino-acid sequence in virus nucleoproteins and the fascinating work on the complexity of the coding characteristics of this smallest known particulate plant virus are achievements. Of equal significance is the discovery that the derobed tobacco mosaic virus nucleic acid (without its protein shell) is itself infective. The ridding of potato from viruses by tissue culture of the meristematic virus-free zone and heat therapy are exciting fields of control based on sound scientific reasoning and practical achievement. The role of chemotherapy and the immense field of phytotoxicity and the use of nucleic acid analogues in restricting virus multiplication *in vivo* are fields where we could hopefully look for breakthrough in practical pathology.

The relationships of vectors (whether they be insects, nematodes or fungi) in virus multiplication and transmission and host physiology which they alter are significantly complex to warrant intensive studies.

Physiology of Virus-Infected Plants

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The physiology of virus-infected plants has been ably reviewed recently by Diener (1963). Much of the information has emanated from work on mosaic type diseases. Information available indicates derangements in all major metabolic activities such as photosynthesis, respiration and carbohydrate metabolism, organic acid metabolism and nitrogen metabolism. By and large, however, it may be stated that information has been gathered from studies on fully virus diseased plants. Little information is available on the sequence of physiological changes from inoculation to full development of virus disease symptoms. In this paper, therefore, it is proposed to discuss such changes in pigeon-pea plants affected by the sterility mosaic disease and cassava plants affected by the cassava mosaic disease.

Though the major facets of metabolic activities have been studied we would like to present here only a few of the most interesting results obtained. These concern: (1) activity of chlorophyllase, (2) ferrous and ferric iron changes, (3) inorganic and organic phosphorus and (4) respiration.

In both healthy and diseased plants chlorophyll A and B increased with age. However, there were significantly lower amounts of these pigments in diseased plants from onset of the disease. Chlorophyllase activity increased with age in healthy and diseased plants, the increase in diseased plants being very much steeper. Disease did not result in a significant reduction in total iron. However, there appeared to be a progressive significant conversion of ferrous iron to ferric iron in diseased leaves. Diseased leaves at all ages contained higher quantities of total phosphorus. Much of this phosphorus was in the organic form in diseased leaves suggesting a greater conversion of inorganic phosphorus to the organic form as the disease progressed. Respiratory rates increased with progress of disease up to the production of obvious symptoms. Thereafter respiratory rates dropped and even reached levels lower than in healthy leaves. In pigeon-pea leaves cytochrome oxidase activity increased with progress of disease and age and was much higher in diseased leaves than in healthy leaves. Mitochondrial nitrogen was much higher in the diseased than in healthy leaves. There was also an increase in activity of succinic oxidase in diseased leaves.

Trace Metals in Plant Diseases

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Trace metals—iron, manganese, boron, copper, zinc and molybdenum—although required in small quantities by plants play a very important role in health and disease of plants. They function, mainly, as constituents of enzymes of the plants as well as the pathogenic micro-organisms. The essentiality of all trace elements required by plants, with the exception of boron, has been demonstrated for plant pathogens. Growth, sporulation and physiological functions like toxin production in fungi are profoundly influenced by the availability of these elements. Among the various elements there is considerable interaction leading to synergistic and antagonistic effects on the hosts and the pathogens.

Apart from their direct effect on plants and pathogens, trace metals are involved in host-pathogen interactions leading to pathogenesis. The influence of certain elements notably zinc and manganese, through their effects on sporulation and survival of the pathogens and rhizophere microflora, on the development of soil-borne fusarial wilts is now well known. The wilt toxins produced by these fungi are potentiated by iron by forming toxin-metal chelates causing local deficiencies and excesses within the plant.

The effects of trace metals on plants when they are in excess and deficiency are relatively better known than those involving host pathogen interactions. The limited experimental evidence reveals that trace metals are closely concerned with pathogenesis and there is scope and need for further work with the ultimate aim of controlling plant diseases by manipulation of metal availability through soil amendments.

Phsiology of Disease Resistance in Sugarcane with Particular Reference to Red Rot

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Resistance to red rot, wilt and bacterial red stripe is a hypersensitive reaction (HR). A detoxification mechanism possibly functions against eye spot and leaf scald. Against smut a hypersensitive anti-infectional defence operates in highly resistant varieties but in varieties with moderate resistance there is suppression of tillering and a linear elongation of the stem. Resistance to *Pythium* root rot is an exclusion phenomenon depending on the antagonistic character of the rhizosphere microflora.

HR in red rot operates against infection and against enlargement of the disease lesion. It is related to the speed of activation of polyphenol oxidase (PPO). In an incompatible host-parasite (H/P) combination at 30° C, PPO activity reaches a peak between 6 and 10 days after infection and falls off steeply thereafter and is strictly localised. In a compatible H/P combination it is relatively weak to begin with, reaches a peak 3 weeks after infection considerably behind the advancing margin of the lesion and falls gradually. At 35° C all H/P combinations are incompatible though the temperature itself is not lethal either to the fungus or to the host tissue. Between 25 and 32° C differences between resistant and susceptible varieties are clearly and rapidly brought out and at 16° C the reaction is slow to develop.

On juvenile leaves following germination of spores of an avirulent race, leachates contain a factor inhibiting germination of spores including those of the more virulent races. This would indicate the presence of a phytoalexin.

A second aspect of resistance, important in the epidemiology of red rot, is the development of dormant infections in bud scales and leaf scar tissue. Penetration of host cells is followed by the formation of thick-walled chlamydospores. This happens when resistance is partial. In active lesions in the stem, chlamydospores are formed in the periphery of the lesion in a partially incompatible H/P reaction. Aqueous extracts of tissues adjacent to a resistant type of lesion induce formation of abundant chlamydospores in culture. It appears possible that increase of phenols to sublethal concentrations in host cells as a response to infection triggers the development of dormant bodies of the parasite in intermediate H/P combinations.

A third feature of H/P interactions in red rot is the rate of development of degenerate adaptations in the pathogen in host tissues. This is characteristic of several host varieties. At $> 32°$ C the majority of reisolates from infected tissues are of the avirulent type while reisolations made from infected host tissues maintained at 20° C and less yield mostly the parental virulent type. This possibly has a bearing on the prevalence of degenerate types of the pathogen in tropical areas where red rot is no problem and the persistence of virulent types in sub-tropical and temperate areas where red rot is a major hazard to sugarcane.

Enzymes in Pathogenesis

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The events leading to the development of the functional condition which we call as "Disease" are included in the term Pathogenesis and this represents the dynamic aspect of Plant pathology. Disease is caused not merely by the association of a parasite and a host but by their interaction. The early steps in this process start when the pathogen breaks its way through the host tissues and cells. At this stage, the ability of the pathogen to degrade the constituents of cell walls such as pectin, cellulose, hemicelluloses and possibly proteins by secreting the appropriate hydrolytic enzymes would seem important. Although evidence for such enzymatic breakdown of cell wall material has been obtained in a large number of diseases such as soft-rots, damping-off of seedlings, foot-rot of cereals and root rots and possibly in vascular wilts, it still remains equivocal. This, however, is only due to the absence of the technique for purifying and identifying the different enzymes from sources *in vivo*, namely the enzymes secreted by the pathogen and those produced by the host itself. This is important because, many of these enzymes are of common occurrence in both the host plants and the pathogens. A large body of evidence that has been presented so far makes it clear that these extra-cellular hydrolytic enzymes are important biochemical weapons the pathogens employ in mounting the attack on their hosts.

Another facet of this problem concerns the reaction of the host tissues to this attack. Enzymes such as Polyphenol oxidase, Peroxidase are stimulated in the affected tissues. Their increased activity could initiate a chain of events at the cellular and tissue levels. The actual mode by which this observed increase of oxidases is brought about is not clear. Their increased activity could reflect their increased synthesis by the host *de novo* in response to the attack by the pathogen. The possibility of their release from the sub-cellular particles and consequent activation cannot also be ruled out.

Be that as it may, the stimulated activity of these oxidases appears as the most important change in the course of host-parasite interaction. Activity of polyphenol oxidases lead to the formation of quinones which on polymerisation give rise to melanin-like pigments imparting a brown colouration to the diseased tissues. The intermediary production of quinones itself can affect the activity of various enzymes as they will act as non-specific protein precipitants. Peroxidase, likewise, can bring about the oxidation of a number of substances such as phenols, auxin, etc., in addition to its role in detoxication of H_2O_2 arising during altered metabolism. The need for an increased peroxidase action implying an increased production of H_2O_2 is significant. In cotton plants infected by *Fusarium vasinfectum* a powerful oxidative deaminase activity has been recorded which would in turn lead to the formation of increased levels of H_2O_2.

Respiration Under Pathogenesis

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An augmentation of respiration almost invariably follows pathogenic attack of plant tissues and is one of the physiological manifestations of disease. Aided by a knowledge of the physiology of respiration, plant pathologists have been investigating this alteration in the respiratory pattern following infection. Quantitative and qualitative changes have been recorded. The quantitative changes could be due to an increased availability of substrates for respiration or of phosphate acceptor adenosine diphosphate (ADP). Increased availability of ADP can be the result of an uncoupling of electron transport from phosphorylation of ADP or of an increased utilization of adenosine triphosphate (ATP) in synthetic processes. The possibility of production by the pathogen of an uncoupling toxin has been examined. Acceleration of synthetic processes has been recorded particularly in infection by obligate parasites. Increased oxygen uptake could also be due to increase in activity of terminal oxidases like polyphenol oxidase and ascorbic acid oxidase.

The causes of qualitative changes (alteration in respiratory pathways) are not easily explained. The possibility that such changes are actually due to the contribution by the pathogen cannot be ignored.

All these phenomena associated with respiration under pathogenesis are discussed with reference to the *Cercospora*-disease of groundnut and other host-parasite combinations.

Stress Physiology in Plant Bacterial Diseases

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It is when a bacterial lesion is first visible that the bacterium would be multiplying most rapidly. Further expansion of lesion size suggests continuing interaction between the pathogen and host. Study of the infected host tissue prior to the lesion formation and during its expansion indicates changes in respiratory rate, growth-regulating substances, soluble organic compounds, phenols, proteins and enzymes. Toxins may be produced as in the case of wild fire of tobacco.

Interaction between host and pathogen involves production of some compounds and/ or utilization of soluble organic compounds of the host cells by the pathogen, and a host reaction by producing compounds antagonistic to bacterial compounds and/or increased metabolic activity. Pathogen's multiplication necessarily involves increased synthesis of some compounds in the host cells, and less of others.

The visible after-effects of this interaction are retardation of growth rate of the host, premature senescence and death of the infected plant. Decreased photosynthesis, increased host catabolic activity, proteolysis, decreased carbohydrate content, and imbalanced enzymic activity contribute to these effects. Specific identification of the crucial changes in the affected host cell, like activity of some lipases, transamination and polymerisation of amino-acids or inhibition of some oxidases, would point to possibilities of controlling these abnormal physiological events and so control the disease.

Applications of Plant Tissue and Cell Culture in the Study of Physiology of Parasitism

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The technique of growing tissues and cells isolated from plants on nutrient media offers several advantages over intact plants for experimental studies. Precise control of chemical and physical regimen enable the study of the effects of a number of interacting factors on growth of cells; the latter conveniently measured in terms of increase in weight. Suspension cultures of cells make possible their use in certain studies hitherto possible only with micro-organisms. Clones and plantlets may be produced by the growth and differentiation of single cells *in vitro*. Microchambers permit continuous microscopic observations of various cellular features in single isolated living cells.

Comparative studies on tissue cultures derived from healthy and diseased tissue are useful in the study of the nature of self-limiting and non-self-limiting overgrowths. One classic example showing the utility of the *in vitro* study in the physiology of diseased tissue is crown-gall. Tissue culture methods unequivocally established the autonomous nature of tumor cells. They also revealed differences in the biosynthetic pathways and membrane properties between normal and malignant cells. By isolation of single cell clones it was proved that teratoma tissue is composed entirely of tumor cells. It was also possible to reverse the teratoma state to a normal condition. The latter suggested that abnormal growth is possibly due to a change in the expression of genes rather than gene mutation.

The growth of obligate parasites on host tissue cultures has been attempted, aimed at their eventual axenic culture. Although their routine cultivation on tissue cultures has not yet been possible, nevertheles this approach has provided insights into certain factors determining penetration, infection, and multiplication of pathogen. The properties of host surface and the presence of vascular tissue appear to be important in certain host-parasite relationships. The effect of metabolites and analogs on viruses in tissue cultures should prove useful in studies of synthesis and inhibition of infectious nucleic acid.

The study of the interaction of the host and the parasite at cellular level is possible by growing both in microchamber. Details of infection process can be watched. The dynamic effects of toxin on plant cells can also be evaluated. Calluses derived from single cell bearing inclusion bodies in microchambers have been induced to differentiate *in vitro* demonstrating the totipotency of virus-infected cell.

Callus tissue is a favourable substrate for the aseptic cultivation of many plant-parasitic nematodes. Nematodes thus reared are useful in fulfilment of Koch's postulates. Tissue cultures are also useful in defining factors influencing nematode multiplication.

Tissue culture systems have been used as bioassays for growth regulators. Cytokinins produced by a plant-pathogenic bacterium and a *mycorrhizal* fungus have been recently identified. The role of cytokinins in other diseases is being investigated by using such bioassays.

Plant tissue and cell culture if used in conjunction with histo-chemical and biochemical techniques should greatly aid in our understanding of the physiology of parasitism.

Tracer Techniques in Plant Pathology

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As in other fields of biology, application of tracer techniques to problems of plant infection has led to many significant contributions of fundamental importance. Particularly, the study of metabolic pathways in obligate parasites like rust has been considerably facilitated by the use of stable and radioactive isotopes. The ability of wheat stem rust uredospores to utilize simple inorganic compounds like NH_4Cl has been demonstrated by feeding the spores with the N^{15} enriched compound and subsequent analysis of the products by mass spectrometry. The radioactive Carbon-14 has been widely used in many studies connected with uredospore metabolism. For instance, the relative contribution of the EMP and Pentose phosphate pathway (PP) in the production of respiratory CO_2 could be assessed, though not unequivocally, by studying the relative rates of release of $C^{14}O_2$ from glucose specifically labelled in position 1 and 6. Such studies indicate a low value of the C_6/C_1 ratio for uredospores suggesting that a high proportion of the respiratory CO_2 is released through the PP pathway. Low C_6/C_1 ratio is also characteristic of rust-infected tissue. Again, specifically labelled fatty acids (C_2-C_9) have been useful in studying the functional role of the TCA and glyoxylate cycles in uredospores. Evaluation of the distribution of radioactivity between the various carbons of the glutamic acid skeleton has been a useful index in the assessment of the TCA and glyoxylate cycles.

Autoradiography is another technique which has been successfully employed in the elucidation of certain metabolic changes in the infected plant. Using P^{32} and C^{14} the mobilization of materials to the infection court has been demonstrated in the case of diseases caused by obligate parasites. Microautoradiography with tritium labelled compounds has thrown considerable light on the host-parasite relationships in rust diseases. Qualitative and quantitative changes in RNA and protein are characteristic of parasitised plant tissues. *De novo* synthesis of proteins, especially isoenzymes, occurs as a consequence of infection. However, information is lacking on the changes in messenger, transfer or ribosomal RNA in host-parasite associations. Hopefully these cell biological aspects of host-parasite relations could be solved by diligent use of tracer techniques and cell-free systems.

Immunoserology in the Study of Plant Pathogens

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The parasitic attributes of the pathogen correspond—like lock and key—to similar or opposite qualities of the host. They are usually complementary pairs. Under pathogenesis the host is diseased, but from the standpoint of the pathogen the host provides the congenial environment. In evolving itself to a parasitic mode of life the pathogen must have adjusted its synthetic mechanism to be in tune with that of the host. The above hypothesis could be verified if one analyses the nucleoprotein of the host and parasite.

Plants unlike animals do not completely recover from infection and display the typical immunity. However, the plant pathogens, fungi or bacteria, do possess antigenic constituents. It has been possible to produce antibodies against plant pathogenic bacteria and fungi in experimental animals like rabbits and guinea-pigs, and demonstrate them using the common antigen-antibody reaction. In fact using immunoserology it is possible to compare and differentiate strains and species of pathogenic fungi and bacteria. Data to substantiate this are presented.

Furthermore, it is now believed that a strong common antigen relationship between a host and parasite might account for the host specificity exhibited by many plant pathogenic fungi and bacteria. Data in support of this view are also presented.