DISCUSSION

The main conclusions are as follows:

Potato because of its importance as an article of food has been investigated to a very considerable extent from different points of view such as, (i) agronomic aspects in relation to tuber raising, (ii) physiological studies in relation to nutritive values and keeping qualities, (iii) raising of disease resistant varieties of tubers. The problem of cultivating and keeping potatoes in a disease free state has still not been investigated to an extent that makes clearer the understanding of factors that are primarily concerned not only in inducing pathogenesis in the tuber but also in the formation of resistance promoting systems incited by the tuber itself. It is known that the loss in potatoes and seed potatoes in particular during storage is as high as 40%-60% due principally to *Fusarium coeruleum* and other fungi inspite of the environmental atmosphere being controlled.

It will be seen from the results that potato varieties differ greatly in their susceptibility to infection by *F. coeruleum* and none of the varieties of tubers show complete immunity. It is also to be noted that soil microflora
of the potato plantations abounding in disease inducing fungi, bacteria and actinomycetes contribute towards inoculum potential of the fungus specifically pathogenic to the tuber. Risbeth (1955) studying the Fusarium wilt of banana in Jamaica found that the infected banana plant is direct source of infection spreading to other plants and that infected soil plays a part in dispersal. Numerical assessment of the resistance of different varieties of potatoes by inoculation experiments showed that Darjeeling White Round and Silbilati varieties of tubers were more susceptible than the other varieties of potatoes. Inoculation experiments of potatoes and storage at different temperatures also demonstrate that maximum infection takes place at 25°C - 30°C, but at temperatures below and above this range there is decrease in the extent of infection. It is thus evident that once infection is established by direct inoculation at wounds in the tubers, the growth of the fungus inside the tissues of the host and disease reactions induced develop in consequence.

The growth of the fungus inside the tissues of the host is influenced by the nutritive composition of the tuber itself as the tuber according to Woodman (1942) and Winton and Winton (1935) is stated to contain carbohydrates, protein, minerals, ash, ascorbic acid and iron (page 48, 49).
Studies in vitro on the growth rate of *Fusarium coeruleum* indicate that the growth is influenced by the concentrations and limits of the different nutritive factors in a way that may parallel the growth of the fungus in vivo. Gregory and Horne (1923) have indicated that the growth rate of the apple attacking fungi in vitro parallels the growth of these fungi in vivo, growth rate in apples being measured in terms of radial advance of the fungi inside the apple tissues. This hypothesis has limited application because the biological systems differ quite considerably from what appears in vitro (Baruah, 1941).

The experimental results with *Fusarium coeruleum* however indicates that the growth rate is influenced by the carbohydrates, nitrogen, salts, trace elements, auxins, vitamin and pH. The fact that the extracts from the core and cortex of potato tubers when added to a synthetic nutrient medium enhanced the growth rate of the fungus to a marked extent and not the extract from the periderm which, on the other hand, is inhibitory to the growth of fungus points to the possibility of significant correlation between the host substrates and the pathogen. It has not however been possible to correlate the growth of the fungus inside the tissues of
the host with the nutritive composition of the tuber itself as there has been no appropriate method unlike that in apples, characterised by soft rot measuring the rate of advance of the hyphae into the tissues. The nature of this aspect of rot in potato caused by Fusarium coeruleum is affirmed by the evidence that this fungus produces pectinolytic enzymes not potent enough to macerate tissues quickly or even cause rapid changes in viscosity of a standard pectin solution during hydrolysis. Potato itself has a total pectin value of only 2%-3%, a factor which may be responsible for not promoting the formation of active pectinolytic enzymes (Baruah, 1941). Indications are, however, given of slight cellulolytic action of F. coeruleum extracts. Further experiments on enzymatic activities on F. coeruleum have also shown that F. coeruleum normally does not exhibit oxidase or peroxidase reaction whereas the fungus grown in a medium containing a phenolic substance (Bavandamm reaction) produces an enzyme capable of oxidising the phenol. Baruah (1959) in studying the phenolic substrates of potatoes isolated and identified, besides tyrosine identified by Isherwood (1937), a number of phenolic compounds such as chlorogenic acid, caffeic acid, scopoletin, ascutelten and quercetin. It is thus probable that a complex mechanism operates itself during pathogenesis in the tuber as evident during the histological changes during infection by the
formation of dark granular substances in the cells adjoining the zone of infection. This has been further strengthened by ultra-violet analysis of infected potatoes showing definitive zones of fluorescence increasing in intensity and specific colouration as infection progresses. Hughes and Swain (1960) state that Phytophthora-infected potatoes showed a large increase in the amount of scopoletin in the tubers and suggest that this substance may prove highly toxic to the pathogen. Further experimental evidence indicates that Fusarium coeruleum not only secretes enzymes of the nature already indicated above but also toxins which may have a significant contributory influence by inhibiting the growth of potato seedlings after sprouting. Inoculation experiments of seed tubers by F. coeruleum and also treatment of sprouted tubers by metabolic extracts of F. coeruleum have shown that growth of the seedlings as compared to that of the controls without any such treatments is inhibited (Plate - XXI & XXII). This result is in agreement with Ludwig’s findings (1957).

The principal features associated with Fusarium infection of potatoes are most marked, in the case of changes in the carbohydrate content, nitrogen content, ascorbic acid, iron and phenols. These substances constitute
the preponderant nutritive factors in the healthy tissues as well.

Suzuki (1957) studying the Murasaki-mompa caused by *Helicobasidium mompa* in sweet potatoes distinguished four types of infection, and also defence reactions against fungal attack. He finds "at the first stage of infection, the middle lamellae of cork layer cells are penetrated by the fungus, as has been shown before. Pectic material in the middle lamellae swells when the hyphae come in contact with it and the pH value is decreased. After passing through the cork layers the fungus comes into contact with the starchy parenchyma. In susceptible varieties these tissues are then macerated by the action of fungal enzymes. Itaconic acid is isolated from such tissues (Araki et al., 1957). The decrease of pH in these tissues is mainly due to the accumulation of chlorogenic acid and caffeic acid and to itaconic acid produced by the fungus. When treated with ruthenium red in the early stage, the pectic substance of the invaded tissues—in contact with the hyphae—is stained yellow, and the tissues beneath the phellogen are stained carmine red. Ferric chloride-potassium ferricyanide solution stains pectic materials in the cork layers blue. No colour reaction occurs in pectic materials produced post-infectionally."
The appreciable decrease in the total sugars in the tuber during infection may be due to (i) the direct utilization by the fungus for its growth, (ii) an increased rate of respiration of the host tissues during infection causing the oxidation of larger quantities of carbohydrates (Horsfall and Dimond, 1960; Cochrane, 1958). In the same way there is a marked decrease in the nitrogen content of the tubers during infection. This is attributable directly to fungal metabolism. The ascorbic acid content in potatoes decreases during storage but the decrease is more marked in the infected tubers. In the healthy tubers it has been shown (Smith and Gillies, 1940) that ascorbic acid decreases during storage to half its value in a month, and DHA increases though not in proportion to AA loss. It is suggested that this may be due to the conversion of DHA to some other oxidised form which is irreversible. But the increased rate of disappearance during infection may be due to the breakdown of tissues of the host and the increase in the oxygen content of the tissues causing autoxidation of the ascorbic acid. The high iron value in potatoes is also a possible source of an oxidising agent of ascorbic acid (Kallie and Zilva, 1955) which may account for a rapid decrease in the ascorbic acid content in the tubers during infection.
In assessing the metal ion content of potato tubers it becomes evident, that the disappearance of ascorbic acid during infection and the parallel decrease in the iron content, that the tissue iron while catalysing the oxidation of ascorbic acid may also be catalysed in its turn by ascorbic acid in forming metal - protein complexes in the tuber thereby reducing the free iron and ascorbic acid values during infection. It has been shown (Gaumann and Kern, 1955) that iron-protein chelated compounds are produced during infection of plant tissues causing toxicity and resistance in plants. This is also possible in the case of infected tubers or the iron molecules may be utilised in the formation of enzyme proteins by the fungus or the host tissues with iron as prosthetic group.

The exact mechanism by which the biochemical reaction takes place in browning of tissues, which is a characteristic feature of hypersensitivity in the host, is not clearly known. Horsfall and Dimond (1960) after histological observations of necrosis (Browning and Drying) in the injured tissues of infected plants, drew the general assumption that "(i) polyphenolic compounds are oxidised by polyphenol oxidase which may exist in a latent state in the intact plant tissue and be activated on exposure to pathogenic infection;
(ii) the oxidised polyphenols, now quinones, are condensed to form polyquinoid structures or sometimes react with amino acids or proteins to form melanin-like substances. The net effect of these reactions may constitute a defense mechanism of the host by forming a barrier. Potatoes infected with Phytophthora infestans show a considerable increase in the activity of polyphenol oxidase and the amount of polyphenols (Rubin et al., 1947; Rubin and Axenova, 1957). Gaumann et al., (1953) isolated a browning factor from culture filtrates and called it vasinfuscarin and suggested that it was enzymatic in character. In this investigation, significantly enough, tests carried out on tubers using phenol reagents and ultra-violet fluorescence showed differences in the phenolic concentration not only in the various zones of tuber but from variety to variety. There was also a definite increase in concentration of certain fluorescent compounds adjacent to the site of infection. The results of inoculation tests on different potato varieties and the phenolic composition confirmed the previous findings that there is a positive relationship between susceptibility to Fusarium infection and a low phenol content. The concentration of total phenols is high in the periderm but normally low in the cortex showing a slight increase in the medulla; hence the pathogen, once it has forced an entry through periderm lesions
production of a strong phenol oxidase system by the pathogen is also a clear indication of the mechanisms of attack and defense in diseased systems.

It would be of interest to carry out further researches into the qualitative and quantitative changes in the primary metabolic compounds such as amino acids, carbohydrates, organic acids and secondary components such as phenols and alkaloids and a graphic representation of this quantitative changes during incubation of a parasite should help in the better understanding of resistance or susceptibility of a crop to a specific disease.