

THE PROBLEM OF TUMOR FORMATION IN PLANTS

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Plant tumors are unique examples of complex interaction and mutual adaptation between plants and tumor inducing agents. Amongst the various types of abnormal growth, galls (tumors), hypertrophies, malformations and witches-brooms are worth mentioning. However, one feature that is, common to all types of tumors is the transformation of normal healthy cells to those that have revolted against the discipline of the organism and are growing in an uncontrolled and irregular manner at the site of tumor.

In tumor, there is rapid growth of cells. Since this growth is neither required for the development of the body nor it is in coordination with the growth of other adjoining cells, it is abnormal.

Important biological events are involved during the transformation of the normal to abnormal growth. The present article is concerned primarily with tumor growth in plants, yet it deals with the fundamental aspects of normal and diseased cells of all organisms.

Plants offer certain advantages for studies on growth: (i) absence of complex nervous, digestive and circulatory systems, (ii) availability of a large number of genetically uniform samples at a comparatively low cost, (iii) ease of diverse experimental manipulations, (iv) availability of relatively easy methods for inducing diseased growth in them (Riker & Hildebrandt, 1951).

What causes tumorous growth? Perhaps everything under the sun—including the

sun himself. There are over 500 chemical substances that are supposed to be carcinogenic in animals. Some of these are: polycyclic hydrocarbons, aromatic amines, amino-azo dyes and nitroso compounds. The recent addition to this list are the cyclamates, the sweetening agents, commonly used in aerated beverages.

The various agents that have been reported to act as incitants of tumorous growth in plants can be classified as follows:

(i) *Physical agents* like ionising radiations (Hagen et al., 1961), vapour tension, obstruction in the translocation of food material, inadequacy of mechanical or physiological graft union etc.

(ii) *Chemical agents*, in low or higher concentrations, cause injury without killing the host cells (Riker & Berge, 1935). Some of them are carcinogenic hydrocarbons, nitroso compounds, adenine derivatives, indole-3-acetic acid, methylcholanthrene and urethane.

(iii) *Genetic constitution*—A few plants seem to be genetically endowed with the capacity to produce diseased growth of one kind or another. No external agents are concerned with the spontaneous occurrence of diseased growth in these plants. They include the non-parasitic burr-knots on apple trees (Swingle, 1925), spontaneous tumor in B 21 line of sweet clover *Melilotus alba* (Littau & Black, 1952), ovular tumors in interspecific hybrids of *Datura* (Satina, Rappaport &

Blakeslee, 1950), and the tumors in a number of tobacco hybrids (Kupila & Thermann, 1962).

(iv) *Bacteria* induce diseased growth in various plants (Riker et al., 1946). A great deal of work has been done on the crown gall disease and its causal agent *Agrobacterium tumefaciens*. In the bacterial tumors, attempts have been made to elucidate the mechanism of tumor formation. The crown gall has been regarded as the experimental model in the field of plant tumor physiology by a large number of workers (Braun & Stonier, 1958; Gautheret, 1959; Wood & Braun, 1961; Ménagé & Morel, 1965; Lioret, 1966; Braun, 1970).

(v) *Virus*—Abnormal plant growths caused by viruses have been studied extensively (Kunkel, 1924; Samuel et al., 1933; Esau, 1963). Kelly & Black (1949) reported that sweet clover root tumors originate from the pericycle. Lee (1955) made a histological study of the sweet clover (*Melilotus officinalis* and *M. alba*) infected with the wound tumor virus. Lee & Black (1955) investigated the anatomy of *Trifolium* tumors caused by *Aureogenus magniveria*. Treshow (1955) studied the physiology and anatomy of tomato fruit tumor. Braun & Stonier (1958) have reviewed the work done on virus induced tumors.

(vi) *Fungi* also induce galls on plants. Club root of cabbage caused by *Plasmodiophora brassicae*; cedar apple rust; rust gall on red cedar induced by *Gymnosporangium* spp. and the smut galls on corn (maize) produced by *Ustilago maydis* are a few of the examples in which fungi cause diseased growth of various types. Critical reviews on fungal galls have been prepared by Köhler (1931); Cook (1945); Karling (1954); Akai (1951); Caporali (1958); Thind & Sharma (1960);

Singh & Bedi (1966).

(vii) *Insects and Mites*—A large number of insects and mites stimulate the tissue of the host plants to initiate galls. These galls may be simple involving a single organ or compound where two or more plant organs are concerned in the production of galls. However, not much is known regarding the precise mechanism by which galls are produced. Parr (1940) induced galls in chestnut oak by injections of an extract of salivary glands of the coccid *Asterolecanium variolosum*. He concluded that enzymes or enzyme-like substance were causative agents. Some success in gall induction by the use of insect parts or their extracts has been achieved by a few workers (Martin, 1942; Arillaga, 1949; Plumb, 1953; Anders, 1958).

In the case of the gall on witch hazel (*Hamamelis virginiana*) produced by *Hormophis hammelidis*, Lewis & Walton (1947) reported that a biologically active Feulgen positive substance was observed in nucleolus of the host cells which was transmitted during mitosis to the nucleoli of the daughter cells. This substance was also found capable of self propagation. Steady growth of gall was dependent on the continued presence of this material. In a study of gall development on beach leaves (*Fagus sylvatica*) induced by larvae of a midge, Boysen-Jensen (1948) concluded that gall formation was caused by growth regulatory substances secreted by the larvae at definite loci. Lewis & Walton (1958) described virus-like substances introduced in the leaves of witch hazel (*Hamamelis* spp) by an aphid. McCalla et al. (1962) showed that unidentified adenine derivatives, glutamic acid and possibly uridine were also present in the salivary glands of *Pontania pacifica* which produced galls in willow (*Salix alba* L). They further stated that these substances

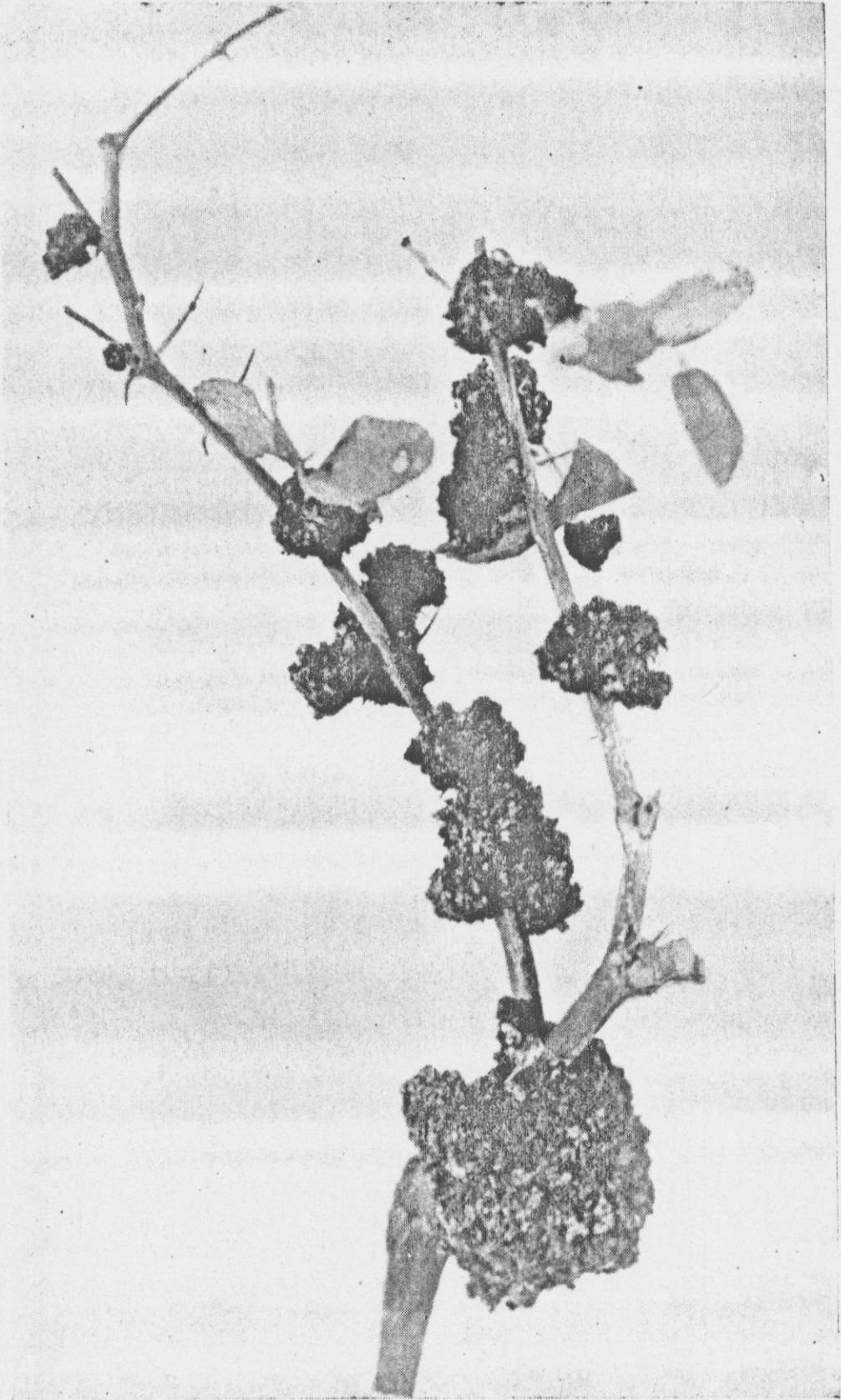


FIG. 1—Portion of *Zizyphus jujuba* plant showing cauliflower-like appearance of the galls.

study of normal and crown gall habituated grape vine (*Vitis vinifera*) tissue. He showed higher protein content in normal tissue than in the corresponding tumor tissue. In *Parthenocissus* and the common beet tissue, however, an opposite picture was obtained (Klein, 1952; Scott et al., 1962).

Isolated tissues growing in vitro appeared important in the study of insect induced plant gall tissues especially from woody plants (Hildebrandt, 1956). A large number of single cell clones have been isolated from established callus cultures that originated from normal, crown gall, insect gall and virus infected tissues. Single cells were isolated from complex crown gall tissues to examine the recovery of gall cells from disease (Braun, 1959). Single cell clones of tobacco tissues from *Nicotiana tabacum*, *N. tabacum* X *N. glutinosa* and *N. glutinosa* with tobacco mosaic virus have been shown to vary in their growth rates, texture and amount of virus they contain after several months in culture (Hildebrandt, 1958).

Effects of carbohydrates nitrogenous compounds, auxins, vitamins and nucleic acids on *Phylloxera* gall, and on normal grape stem were studied (Arya, 1965). Arya concluded that certain differences were manifested in responses of diseased and normal tissue clones to various metabolites incorporated in the medium. Warick & Hildebrandt (1966) observed no qualitative differences in the amino acid contents of gall and normal tissue clones. Quantitative differences in the amount of total and individual sugars were observed between the two sets of tissues (Warick & Hildebrandt, 1967). Differential response to gamma radiations from Cobalt 60 source has been reported (Arya & Hildebrandt, 1969).

Kant (1967) studied the anatomy of

insect galls of Rajasthan area. A brief account of the *Zizyphus* stem gall is given below. *Zizyphus jujuba* Lamk. stem gall is induced by *Eriophyes cernuus* Masee, a mite belonging to the family Eriophyidae. The gall induced by this mite was described by Houard (1912) from Senegal but the causal agent remained unidentified till Masee (1927) described it from the Blue Nile province of Sudan. This is one of the most common galls occurring throughout India (Mani, 1959).

The *Zizyphus* galls are (Fig. 1) irregular, globose, lobed, rugose or tuberculated, and hard. These galls represent axillary branches and have the appearance of the curd of cauliflower, but are reddish brown in colour and 23mm to 50mm in diameter.

The entire gall mass is composed of undifferentiated parenchyma except the basal region where vascular strands are present. Numerous individual mites feed and spend a part of their life cycle lying in the crevices of the gall, which are directly connected with the outside. The cells lining the crevices form a sort of nutritive layer and they are characterised by the presence of dense cytoplasm with one or more nuclei. Gall formation is initiated in axillary buds when they are at the primordial stage. A mite lays its eggs on a very young axillary bud which develops abnormally. Hypertrophy or hyperplasia of the cells of the axillary bud plays an important role in the development of gall. The gall of *Zizyphus jujuba* is a remarkable acroecidium in which the cecidozoa occur externally between the naked tuberculated parenchyma cells on the surface of the gall.

The problem of gall development in *Zizyphus* is being studied by using isolated normal and gall tissues in culture (Kant & Arya, 1969). The normal tissue was planted on 'D' medium of Hildebrandt (1963)

and subsequently transferred to 'C' medium of Hildebrandt. Of the several media tried, Murashige & Skoog's medium (modified, 1962) proved best for its growth (Vyas, 1971).

It has been found that the transformation of normal cell to that of a gall involves altered synthesis of carbohydrates, proteins, hormones and nucleic acids.

Auxin-cytokinin metabolism—In general the tumor tissues can be grown indefinitely *in vitro* without exogenous supply of auxin (De Ropp, 1947; Gautheret, 1947; Kulescha & Gautheret, 1948; Braun & Stonier, 1958; Arya, 1963) whereas the normal tissues require auxin.

The difference in auxin requirement for tumor and normal tissue may be because: (i) only the tumor tissue has the capacity to synthesize auxin *in vitro* state, (ii) tumor tissue synthesizes more auxin than the normal tissue, (iii) less destruction of auxin takes place in tumor tissue due to the

presence of auxin protectors or because of the absence of auxin inhibitors. Earlier findings (Braun, 1947; Gautheret, 1947) led to the obvious suggestion that crown gall tissue synthesizes higher amounts of auxin. Stonier (1969) showed the presence of very high molecular weight auxin protectors in crown gall tumor tissue which function by inhibiting the peroxidase catalysed oxidation of IAA. Thus the high level of auxin in tumor tissue may be explained on the basis of lack of destruction of auxin.

The *Zizyphus* gall tissue, in contrast to the normal tissue, was able to grow on auxin-free medium (Fig. 2). With the increase in concentration of kinetin in the medium (up to 2.5 mg/l), the growth of gall tissue was enhanced, whereas no response was shown by the normal tissue. When the concentration of NAA in the minus kinetin medium was increased the gall tissue grew profusely whereas the

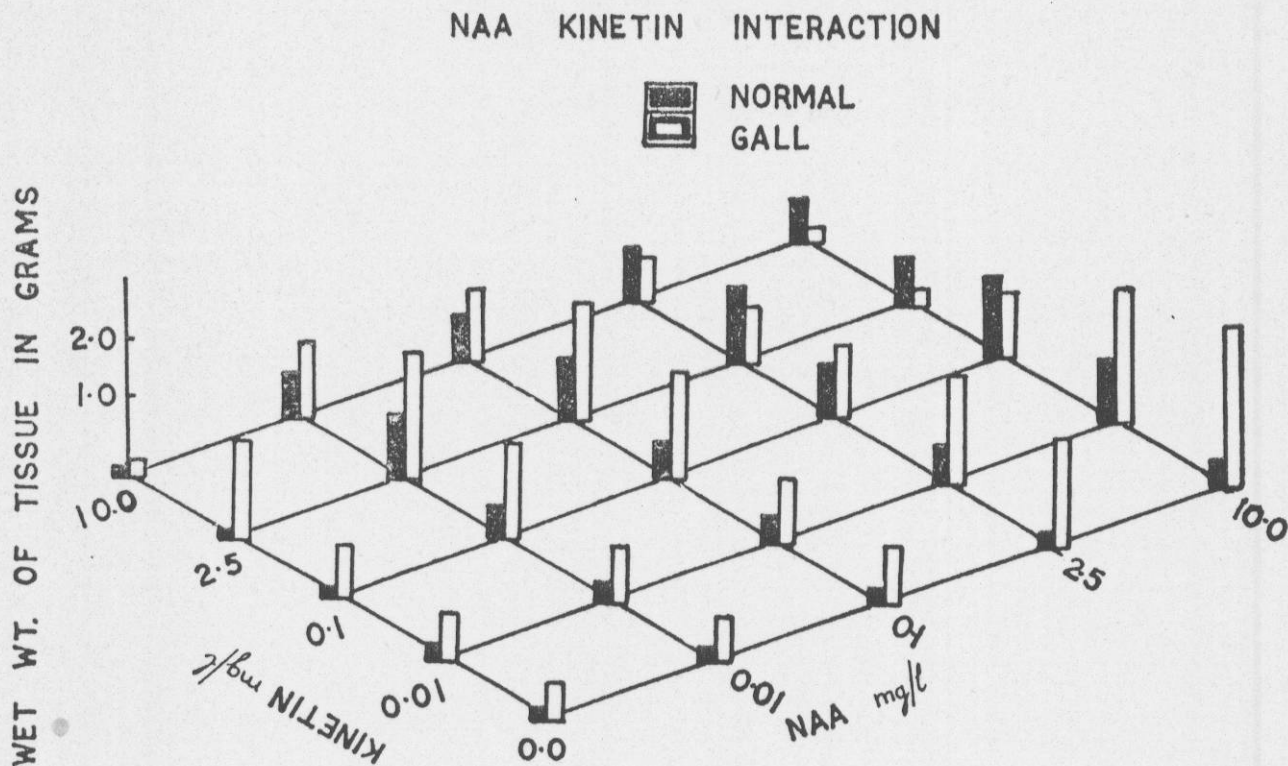


FIG. 2—Comparative growth of normal and gall tissues on Murashige & Skoog medium supplemented with various combinations of NAA and kinetin.

normal tissue showed very little growth.

L-tryptophan is known to be a precursor of IAA (Thimann & Grochowska, 1958; Sherwin & Purves, 1959). Effect of l-tryptophan on the gall and normal tissues grown on auxin free medium showed that both the tissues utilized this compound at an optimum concentration of 5.0 mg/l but the growth response of gall tissue was much higher as compared to that of the normal tissue (Fig. 3). The results obtained suggested that l-tryptophan could be substituted for auxin. From cucumber hypocotyls, Sherwin & Purves (1969) were able to isolate tryptophan decarboxylase, which is responsible for converting tryptophan to tryptamine. The higher growth response of gall tissue with l-tryptophan may be explained on the basis of the findings of Stehse & Wildman (1950) that l-tryptophan is preferentially used for protein synthesis.

In *Zizyphus* tissues, the gall tissue contained more auxin than the normal tissue. Auxin prototrophy was induced in normal tissue and was enhanced in the gall tissue supplied with l-tryptophan and zinc sulphate. This shows the possibility of the existence of an enzyme system involved in the synthesis of l-tryptophan from substrates like serine and indole and the subsequent conversion of l-tryptophan into IAA in both the tissues. A comparative study of the enzyme system which controls the synthesis of l-tryptophan and IAA in gall and normal tissues may be rewarding in understanding auxin metabolism.

Inhibitory effects of kinetin injections on tumor formation were recorded by Cziharz & Brucker (1951) in *Bryophyllum*. According to them kinetin stimulates the synthesis of normal DNA which inhibits the formation of tumor inducing principle (abnormal DNA). The growth of *Zizy-*

phus gall tissue is also inhibited by kinetin, and this substantiates the contention of Cziharz & Brucker (1961).

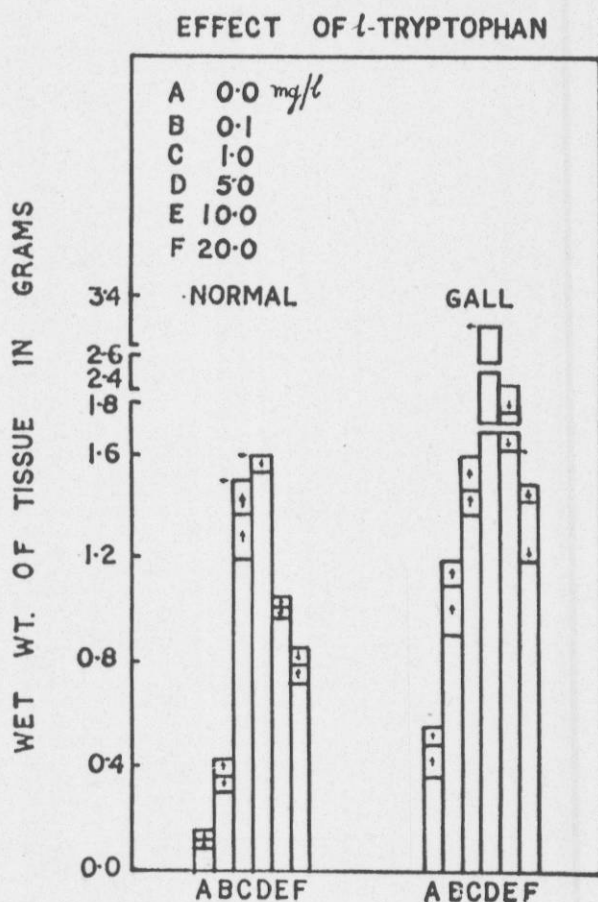


FIG. 3—Comparative growth of normal and gall tissues on Murashige & Skoog medium (minus auxin) supplemented with different concentrations of l-tryptophan.

Nucleic acid metabolism—Nucleic acid biosynthesis by gall and normal tissues of *Zizyphus*, treated with different growth regulators was studied. RNA content of the normal tissue increased with increase in concentration of NAA (10.0 mg/l) in the medium while no appreciable change in the RNA content of gall tissue was observed. This indicated that added concentration of NAA in the medium was concerned with increased synthesis of RNA in the normal tissue. L-tryptophan in the medium induced increased synthesis of RNA in the gall tissue alone. In what manner is NAA concerned with the

increased synthesis of RNA in gall tissue cannot be explained at present. The amount of RNA in the normal and gall tissue was directly proportional to the growth rate of the tissues.

The DNA content of the normal tissue remained steady but that of the gall tissue declined gradually during 20 days growth after an initial rise. The difference in cell composition of the two tissues could be responsible for the above finding. The percent ratio of relatively large cells increased in the gall tissue mass during the late phase of the growth of the gall. The larger cells were less active than the smaller cells. Gall tissue contained initially more DNA than the normal tissue. In a study of crown gall and normal tissue of *Parthenocissus tricuspidata* Robson et al. (1959) concluded that tumor cells contained more DNA than normal cells, and believed that the excess of DNA might be of non-chromosomal origin. Arya et al. (1962) obtained similar results in *Phylloxera* gall and normal grape stem tissues.

Gall induction—The general problem of gall induction involves certain interrelated aspects such as the initial stimulus, gall development, effect of stimulus, categories of the tissues affected, in the gall tissue and extent and period for which stimulus remains operative, (Hough, 1953). The possible nature of initial stimulus has evaded understanding so far (McCalla et al., 1962).

The nature of the tumor-inducing principle in (TIP) crown gall has been a subject of study by a number of workers. Brown & Gardner (1936) induced galls in decapitated leaves using heteroauxin. Klein & Link (1952) believed that bacterial auxin was required as a carcinogen to complete the transformation process and to assist the rapid multiplication of tumor cells.

Klein et al. (1953) claimed that a specific polymer of DNA may be the tumor inducing principle.

In the case of insect galls it was believed that the inciting insect contained a specific poison. The variation in the chemical nature of the poison was responsible for differences in the nature of the galls. Rahn (1936) advocated the hypothesis that mitogenetic rays given by the larvae caused abnormal proliferation. The presence of bacteria in the vicinity of larva was also considered as a factor in the formation of galls (Köstoff & Kendall, 1929).

That insect galls may be formed by certain viruses associated with the insect larvae gained strength with the discovery of the production of plant tumors by an insect-transmitted virus, *Aureogenus magnevena* Black, (Black, 1945). Parr (1940) stated that "Whatever may be the nature of the substance or substances reacting, the opinion is now almost universal that the stimulus for gall production by insect is chemical". Boysen-Jensen (1948) concluded that the gall was a type of callus produced by larval secretions. Anders (1960, 1961) has presented evidence about the presence of proteolytic enzymes like protease and peptidase in aphid saliva and has emphasized their role in cecidogenetic changes in host tissue.

The problem of chemical nature of the gall inducing principle can be tackled by artificially inducing galls and by carrying out biochemical analysis of the affected tissue. Several attempts to induce gall formation by injecting extracts of macerated larvae, gall tissue or certain chemical substances have failed.

Plant hormones known to play an important role in abnormal growth. Galls have been induced on *Populus deltoides* Bartr. (La Rue, 1935), *Ricinus communis*

L. and *Phaseolus vulgaris* L. (Solacolu & Constantinesco, 1936) using heteroauxin. A direct correlation between auxin concentration, ascorbic acid oxidase activity and pronounced cell enlargement was established in the case of *Phylloxera* gall (Newcomb, 1951).

Attempts were made by the present authors to induce gall formation using gall and normal callus tissue grafts on sterile stem segment of *Zizyphus* grown in vitro, and also by means of gall tissue extract and NAA separately added to the culture medium. Gall development in the

sterile stem segment was induced by the gall callus tissue graft, gall extract and NAA. This suggests that an auxin-like factor is involved in gall production. However, the fact that auxin concentration of gall callus or gall extract is much below the concentration of NAA which can produce such galls suggests the complex interplay of certain other factors present in gall callus or gall extract besides auxin. How far the galls induced under these different sets of experimental conditions are morphologically, histologically and physiologically similar needs further study.

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