KININ* ACTIVITY FROM PLANT EXTRACTS

I. BIOLOGICAL ASSAY AND SOURCES OF ACTIVITY

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Summary

A biological assay for substances inducing cell division is described. Blocks of tobacco stem pith were cultured aseptically on 1 ml of basal medium containing mineral salts, sucrose, and vitamins. The addition of auxin to the basal medium caused cell enlargement, but the addition of kinetin alone produced no reaction. Auxin and kinetin together induced cell division, resulting in nests of cells readily distinguishable visually from the original cells. Callus produced in this way could be subcultured indefinitely on medium containing both 3-indolylacetic acid (IAA) and kinetin.

When tobacco pith was cultured on basal medium plus IAA, cell division was induced by extracts of a number of plant meristematic tissues added as supplements. Auxin was necessary for the reaction. Active extracts were obtained from fruitlet tissues of apple, quince, pear, and plum harvested at various times after pollination.

Extracts of seeds, placenta, and carpel wall of tomato at various stages of development were assayed for ability to induce cell division. Such ability was detected in extracts of cambium from stems of *Pinus radiata*, *Eucalyptus regnans*, and *Nicotiana tabacum*.

The cell-division response of tobacco pith to apple-fruitlet extract was not enhanced by the addition of polyols or casein hydrolysate, alone or in mixtures, to the culture medium. The response to kinetin was not enhanced by the addition of urea or sorbitol alone or in combinations.

I. Introduction

That plant cell growth is controlled by substances or hormones of low molecular weight has become a basic tenet of plant physiology. According to this proposition there are, in plants, substances which specifically initiate and maintain phases of cell growth, while not being substrates for the metabolic processes necessary for the manifestation of these phases. Such substances occur in very low concentrations, and this is taken as an indication of their inability to act otherwise than as regulators.

This proposition has not yet been proven for any phase of cell growth, although the circumstantial evidence is strong for the phase of cell enlargement. Proof requires the elucidation of the mechanism of action of a growth substance found in tissues in which the relevant phase of growth is occurring.

The present investigations are first steps towards testing the hypothesis in connection with the phase of cell division. Since Haberlandt's (1913) pioneer work,

* The word "kinin" has also been used for a class of substances which cause constriction of blood vessels and other physiological reactions in animals. These substances, which have been isolated from animal sources, are polypeptides and are unrelated to plant kinins.

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evidence has slowly accumulated for the existence of substances which together with auxin are necessary for the induction of cell division. So far, such a substance has not been isolated from natural sources, and obviously this is a prerequisite to investigations of a mechanism. We have sought such substances in meristematic zones of plants, where they might be expected to be inducing or maintaining cell division (Goldacre 1959). Substances which induce cell division in plant tissues have been given the generic name of "kinins" (Strong 1958). We have used the term to describe substances which, in the biological assay to be discussed, induce cell division.

Until its chemical nature is known and it is tested on a wide variety of tissues, the physiological definition of any plant growth substance depends on the particular biological assay used. This paper reports the development of a suitable biological assay system and a survey, based on this assay, of kinins in a number of plant extracts.

II. Materials and Methods

(a) *Small-scale Biological Assay for Kinins*

(i) *Tissue.*—The stem pith tissue of *Nicotiana tabacum* cv. Wisconsin Havana 38 was used. Plants were grown in soil in a glasshouse maintained at 30°C by day and 25°C by night. High mineral nutrition and high light intensities were necessary for the production of broad, easily-worked stems. Cloudy weather produced thin, intractable stems. Stems about 24 in. long were harvested and sterile pieces 6 in. long were obtained by soaking in sodium hypochlorite solution for 30 min or by peeling off the outer layers of tissue. The stem pieces were cut transversely into cylinders 10 mm long and then with longitudinal cuts, pith blocks 3 by 5 by 10 mm were obtained. Care was taken not to include internal phloem, which of itself induces cell division in isolated pith tissue. The blocks of tissue were collected into sterile basal medium.

(ii) *Culture Tubes.*—Strips of No. 1 Whatman filter paper, 4 by 0·9 cm, were placed in glass tubes, 7·5 by 1·2 cm, fitted with aluminium caps, and 0·5 or 1·0 ml of medium was put into each. When the tubes were placed at 20° to the horizontal in a rack, a layer of medium was held along the length of the filter paper. After sterilization of the tubes at 18 lb/in^2 pressure for 5 min, one pith block was transferred aseptically to each tube so that the block contacted the medium only through the filter paper.

(iii) *Basal Medium.*—Initially the mineral salts of the basal medium described by Goldacre (1959) were used. Part way through the investigation, Dr. C. O. Miller suggested that, as a result of the findings of Murashige (unpublished data), the concentrations of some mineral salts should be increased. Subsequently the mineral salts mixture of Miller (1961) was used in the basal medium. Basal medium contained 2% sucrose.

(iv) *Culture Conditions.*—Treatments were triplicated and tubes were held at high humidity at 25°C in darkness. They were brought into the light for inspection at 14 and 21 days. Inspection was made with the aid of a dissecting microscope at a 10× magnification.

(*b*) *Large-scale Culture of Tobacco Pith*

Under essentially the same conditions as above, tobacco pith blocks were cultured on larger volumes of medium in 6 by 1 in. test tubes. From 10 to 18 ml of solution was used and the pith blocks were supported either on filter paper over stainless steel gauze or on medium solidified with 0·7% agar. In addition blocks were cultured on 40 ml of solidified medium in 150-ml Erlenmeyer flasks.

(*c*) *Measurement of Cell Number*

The method of Brown and Rickless (1949) was used. Pith blocks stood overnight in 2% w/v aqueous chromic oxide solution. They were stained with orsellein BB in 3% acetic acid for 1 hr, then washed and drained. The blocks were macerated in 2·0 ml of water by repeated passage into and out of a pipette. Cell number was counted with a cytometer.

(*d*) *Extraction of Natural Kinins*

(i) *Routine Small-scale Extraction Method.*—Plant tissues were autoclaved at 18 lb/in^2 for 3 min, then macerated and extracted with distilled water. The aqueous extract, usually unpurified, was added to the basal medium before sterilization.

(ii) *Cold Extraction and Sterilization of Apple Fruitlets.*—Apple fruitlets (5 g) were macerated with 20 ml of 70% aqueous ethanol. The solid material was removed by centrifugation. The extract was placed in a sterile flask and the solvents were removed at room temperature under reduced pressure. The residue was dissolved in sterile basal medium containing 10^{-5}M 3-indolylacetic acid (IAA) and transferred aseptically to tubes for assay.

(iii) *Purified Extracts.*—Some experiments reported here were done with purified extracts from a large-scale extraction of apple fruitlets. For details of the extraction and purification methods reference should be made to Part II of this series (Zwar, Bottomley, and Kefford 1963).

(iv) *Expression of Concentration of Plant Extracts.*—For convenience, the concentration of plant extract in a culture medium has been expressed in arbitrary units representing the number of grams of plant tissue which were extracted for the preparation of 1 ml of medium.

III. Results

(*a*) *Growth of Isolated Tobacco Pith*

(i) *Observation of the Growth Phases.*—Two growth reactions occur: cell enlargement and cell division. They can occur together but under optimal conditions for division enlargement is repressed.

At excision, the pith cells are large and regular in dimensions and arrangement (Goldacre and Bottomley 1959) and on basal medium alone the pith blocks remain unchanged. With the addition of auxin, cells enlarge, especially at the end of the block nearest the medium.

The first visible signs of cell division are opaque clusters of cells. These clusters gradually extend in area and may cover the surface of the pith block (Goldacre and Bottomley 1959). The new cells are always smaller than the cells of the parent pith. The two reactions of pith cells, division and enlargement, may be readily recognized on visual inspection. The enlargement reaction is complete after 4 days, but cell division is usually detected only after 7 days of treatment and thereafter increases. Since cultures are inspected on more than one occasion, doubtful or marginal division reactions may be checked for an increase in reaction area.

The criterion for the detection of kinin activity in our assay has been the observation of clusters of new cells. A semi-quantitative estimate of the kinin concentration may be made by assessing the area of the block covered by new cells or by determining the highest dilution of kinin solution producing perceptible cell division in a given time.

(ii) *Induction of Cell Enlargement.*—The following auxins at a concentration of 10^{-5}M have been found effective in inducing cell enlargement: IAA, 1-naphthylacetic acid, 2,4-dichlorophenoxyacetic acid, 2-benzthiazoleoxyacetic acid, and 5-carboxymethyl-*N*-dimethyl dithiocarbamate.

(iii) *Induction and Maintenance of Cell Division with Kinetin.*—Kinetin, 6-(furfurylamino)-purine (from Californian Foundation for Biochemical Research), which can induce cell division in plant tissues (Miller *et al.* 1955), was used as a standard for investigation of the induction of this process in tobacco pith.

(1) *Requirement of auxin for cell division induction by kinetin.*—The effect of auxin upon tobacco stem pith was investigated with or without 10^{-6}M kinetin in the basal medium. IAA was used at 0, 10^{-7}, 10^{-6}, 10^{-5}, 3×10^{-5}, and 10^{-4}M. Three pith blocks were incubated for 21 days on 40 ml of medium in a 150-ml Erlenmeyer flask, and changes in both fresh weight and cell number were measured. The treatments were triplicated.

The results in Figure 1 show that auxin was required for the increase in fresh weight in the absence and presence of kinetin and for the increase in cell number in the presence of kinetin. In all cases the higher concentrations of auxin produced no increase above the controls.

The auxins listed above as effective in cell enlargement in tobacco pith were also effective in inducing cell division in the presence of kinetin.

The time which could elapse between the addition of auxin and an addition of kinetin at a concentration which was effective in inducing cell division was investigated. Pith blocks were placed in 6 by 1 in. tubes containing 10 ml of basal medium plus 10^{-5}M IAA. After 0, 2, 4, 7, and 14 days, sterile kinetin solution was added to some tubes to give a concentration of 10^{-6}M. Four weeks after kinetin treatment, cell division was observed in the blocks that received kinetin at 0, 2, 4, and 7 days, but not in those treated 14 days after the beginning of culture.

(2) *Kinetin and the maintenance of cell division.*—An experiment was designed to find if kinetin is necessary for the continuance of cell division once it has been initiated in tobacco pith. Large pith blocks were cultured in 6 by 1 in. tubes containing 18 ml of medium solidified with 0·7% agar. The basal medium contained 10^{-6}M

kinetin and 10^{-5}M IAA. There were 10 replicates of each treatment. After 22 days of incubation the blocks were divided into four equal pieces. Two of these were subcultured onto basal medium plus IAA, the other two onto basal medium plus kinetin and IAA as before. After 21 days further incubation, one piece from each of these treatments was weighed, the other piece was divided into two, and each piece was placed on the medium upon which it had last grown.

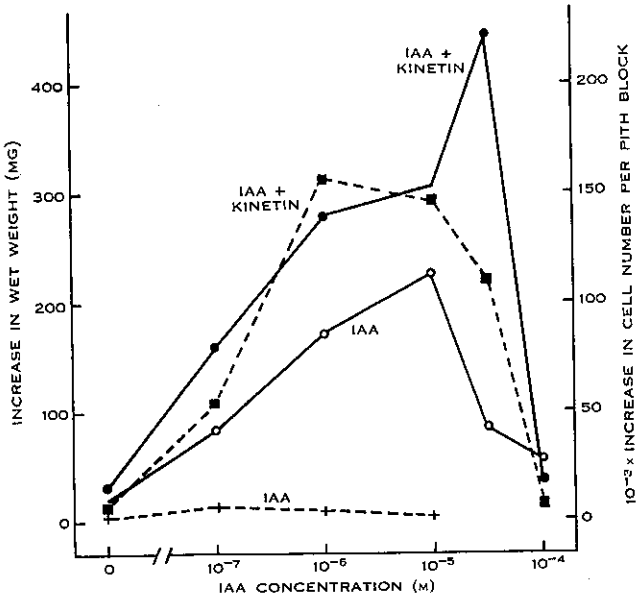

Fig. 1.—Increases in wet weight (———) and cell numbers (— — —) for tobacco pith blocks cultured on media containing the concentrations of IAA shown, with or without 10^{-6}M kinetin.

This procedure of weighing one piece of callus and subdividing the other was continued through a number of subcultures. The results presented in Figure 2 show that callus obtained by culture on IAA plus kinetin grew equally well on the second growth period whether provided with kinetin or not. However, while subculture on IAA plus kinetin may be made many times (in the present experiment 19 subcultures were made), in the absence of kinetin after the first growth period, growth decreased and the blocks were dead after the fourth subculturing (Fig. 2).

(3) *Effect of addition of sorbitol and urea to basal medium.*—The response of tobacco pith blocks to kinetin in the presence of IAA was not enhanced by the addition to the basal medium of urea (10 or 30 mg/l) or sorbitol (10 or 30 mg/l), alone or in combination.

(iv) *Activity of Chemically Defined Substances in the Small-scale Assay.*—In addition to kinetin, *sym*-diphenylurea (Shantz and Steward 1955; Strong 1958; Miller 1960) has been found to stimulate cell division in some tissues and adenine has similar effects to kinetin in bud induction (Skoog and Miller 1956). These substances were tested under the conditions of the small-scale assay in which basal medium

plus IAA was used. So that maximum effects could be observed, incubation was continued for at least 30 days.

Kinetin and diphenylurea were tested without and with the addition of 4 mg/l enzymatic casein hydrolysate (Nutritional Biochemicals Co.) to the basal medium containing 10^{-5}M IAA, but the addition of casein hydrolysate did not influence the results. Kinetin induced cell division at concentrations from 2×10^{-7}M to 2×10^{-5}M. Diphenylurea induced cell division at 2×10^{-5}M to 6×10^{-5}M, but its effects were weak and erratic between individual blocks.

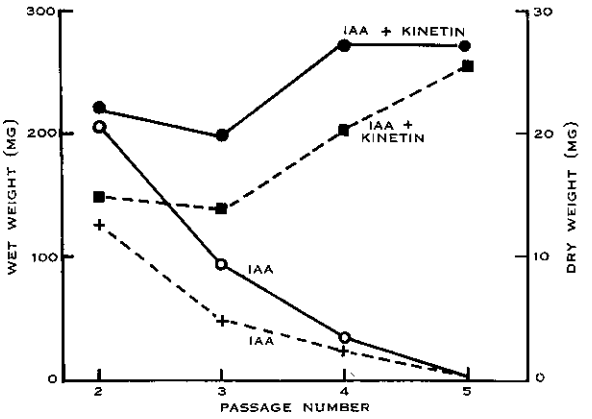

Fig. 2.—Wet weights (———) and dry weights (— — —) of tobacco pith callus cultured on media containing 10^{-5}M IAA plus 10^{-6}M kinetin for the first passage then IAA plus kinetin or IAA alone for subsequent passages. Passage time approximately 22 days.

Five concentrations of adenine between $1\cdot5\times10^{-3}$ and $1\cdot5\times10^{-6}$M, of adenine sulphate between $1\cdot5\times10^{-2}$ and $1\cdot5\times10^{-5}$M, and of 6-methyladenine between $2\cdot5\times10^{-5}$ and 10^{-6}M were tested separately. In addition adenine and adenine sulphate were also each mixed with 6-methyladenine in all possible concentration pairs and tested. Alone, the substances were inactive and the only combinations to induce cell division were $1\cdot5\times10^{-6}$M adenine with $2\cdot5\times10^{-5}$M 6-methyladenine, and $1\cdot5\times10^{-3}$M adenine sulphate with 3×10^{-6}M 6-methyladenine. These effects were very slight. Miller (1962), using a soybean callus assay, has demonstrated the weak kinin activity of mixtures of adenine and 6-methyladenine.

(*b*) *Survey of Natural Sources of Kinin Activity*

(i) *Detection of Activity in Extracts of Fruitlets.*—Kinin activity has been detected in unpurified aqueous extracts of the following fruits harvested between 7 and 21 days after pollination:

(1) Apple (*Pyrus malus* L.): cv. Delicious, Dougherty, Golden Delicious, Granny Smith, Gravenstein, Jonathan, Red Statesman, Twenty Ounce, Willy Sharp, Worcester Pearmain.

(2) Quince (*Cydonia oblonga* Mill.): unknown variety.

(3) Pear (*Pyrus communis* L.): two varieties, both unknown.

(4) Plum (*Prunus cerasifera* Ehrh.): cv. Nigra.

(ii) *Kinins in Apple Fruit Tissues of Varying Age.*—The flowers and fruits of Granny Smith apple were separated into component tissues at a number of stages of development and unpurified aqueous extracts were tested for kinin activity. Results were as follows:

(1) Bursting flower buds, 21 days before pollination, were divided into receptacle and other flower parts. Activity was detected in extracts of both tissues.

(2) Fruitlets 5 mm in diameter, harvested 14 days after pollination, were divided into ovules, cortex, and furry epidermis. Extracts of all tissues induced cell division and the relative activities per fruitlet were assessed as: cortex > ovules > epidermis.

(3) Fruitlets of two sizes (6–7 mm diameter, 0·2 g average fresh weight, and 15 mm diameter, 2·5 g average fresh weight) were extracted whole and the crude aqueous extracts assayed. Activity was detected in both extracts. It was estimated that there was greater activity on a fresh weight basis in the small fruit but, per fruitlet, the extract of the larger ones was more active.

(4) In extracts of a whole fruit 7 cm in diameter and approaching maturity, and of seeds from a similar fruit, weak activity was detected.

(iii) *Kinins in Tomato Fruit Tissues of Varying Age.*—Kinins were detected in unpurified aqueous extracts of tomato (*Lycopersicon esculentum* cv. Grosse Lisse) ovaries and fruits varying in diameter from 0·5 to 6 cm and harvested before and after pollination. Fruits of varying size were divided into three fractions: (1) seeds, (2) placenta, and (3) carpel wall and partitions. For fruits 0·5–1·0 cm diameter, extracts of all tissues were active. For fruits 1·25–2·5 cm in diameter extracts of the placenta and the seed were active, but the carpel wall extract was inactive. For fruits 6 cm in diameter and larger, whether they were green or red, only the seed extract was active.

(iv) *Kinins in Extracts of Stem Cambium.*—Tissues exterior to the cambium were peeled from stems of *Pinus radiata*, *Eucalyptus regnans*, and *Nicotiana tabacum* cv. Wisconsin Havana 38. The exposed cambium was then scraped off, including some outer layers of xylem. Water extracts of these tissues induced cell division.

(c) *Cold Extraction of Apple Kinin*

When apple fruitlets were extracted at room temperature and the extract was sterilized by filtration, its kinin activity was the same as that of an extract obtained by hot extraction and subsequent heat sterilization.

(d) *Growth Response of Tobacco Pith to the Apple Kinins*

(i) *Auxin Requirements for Cell-division Induction.*—The necessity of auxin for the induction of cell division in tobacco pith blocks is illustrated in Figure 3. In

that experiment, apple fruitlet extract, which had been extracted with ethyl acetate at pH 4·5 (this treatment should remove native auxins) and purified with polyamide resin (Zwar, Bottomley, and Kefford 1963), was tested at 0, 0·1, 0·25, or 0·5 g/ml (arbitrary units described in Section II(*d*)(iv)) in the absence of auxin or in the presence of 10^{-7}M or 10^{-5}M IAA. In the absence of auxin no growth occurred; in the presence of auxin alone cell enlargement occurred. Both auxin and apple kinins were required for the induction of cell division.

In an experiment in which was used the same partly purified apple extract at 0·5, 0·25, or 0·1 g/ml, IAA, 1-naphthylacetic acid, and 2,4-dichlorophenoxyacetic acid were compared as auxins in the induction of cell division. All auxins were effective at 10^{-6}M concentration, and had their optimal effect at about 2×10^{-5}M. It was estimated that IAA produced the greatest quantitative response.

IAA (M)	+ Apple Extract (g/ml)	IAA (M)	+ Apple Extract (g/ml)	IAA (M)	+ Apple Extract (g/ml)	IAA (M)	+ Apple Extract (g/ml)
0	0	0	0·1	0	0·25	0	0·5
10^{-7}	0	10^{-7}	0·1	10^{-7}	0·25	10^{-7}	0·5
10^{-5}	0	10^{-5}	0·1	10^{-5}	0·25	10^{-5}	0·5

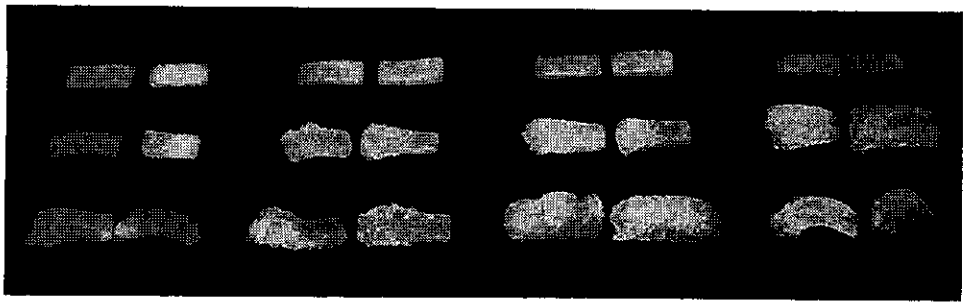

Fig. 3.—Effect of increasing concentrations of IAA and of apple fruitlet extract, applied either singly or in combination, on the growth of tobacco stem pith blocks. Two representative blocks for each of the 12 treatments listed above the figure are shown.

(ii) *Effect of Casein Hydrolysate and Polyols upon Response to Apple Extract.*— The response of tobacco pith to apple extract was examined in the presence of basal medium plus IAA and the following addenda: 0·5 g/l enzymatic casein hydrolysate alone or mixed with 100 mg/l myoinositol and 100 mg/l sorbitol. The apple extract had been purified with polyamide, then by displacement with ammonia from the ion-exchange resin "Dowex 50"-H^+ (Zwar, Bottomley, and Kefford 1963). It was used at 0·05, 0·25, and 2·5 g/ml. In no case did 0·05 g/ml of apple extract induce cell division, nor did any addition increase the amount of cell division observed at the higher concentrations.

A similar conclusion was reached in an experiment involving 100 mg/l each of sorbitol, myoinositol, and scylloinositol and 0·05, 0·15, 0·5, 0·75 g/ml of apple extract purified with polyamide and by subsequent elution from carbon with pyridine

(Zwar, Bottomley, and Kefford 1963). In this case cell division was detected at 0·15 g/ml of apple extract.

(e) *Some Properties of Kinins in Crude Apple Extracts*

(i) *Insolubility in Organic Solvents.*—The kinins were not extracted by diethyl ether from aqueous solutions at pH 3, 4·5, or 9·0. This meant that the activity could be separated from kinetin, which is extractable by ether (Miller *et al.* 1955). Subsequent extraction experiments were done on an aqueous apple extract that had been extracted with ether. Samples of this solution were extracted six times with equal volumes of the following solvents: n-amyl alcohol, ethyl acetate, chloroform, isopropyl ether, benzyl alcohol, cyclohexanone, and n-butanol. Because preliminary experiments had shown that toxic substances were extracted by organic solvents, the six fractions of each solvent phase were combined and extracted eight times with water. These water extracts were combined and assayed for kinins together with the aqueous phases which remained. It was concluded that none of the above solvents had extracted detectable amounts of kinins.

(ii) *Stability to Heat.*—An aqueous extract of apple fruitlets which had been purified by dialysis and extraction with ethyl acetate retained its kinin activity when heated to 100°C for 1 hr in aqueous solutions of pH 1·5–10·5.

(iii) *Miscellaneous Properties.*—The kinins extracted from apple fruitlets passed a dialysis membrane, were inactivated by boiling with 1N hydrochloric acid for 1 hr, but were unaffected by hydrochloric acid at room temperature. The kinins of crude extract were not precipitated by silver or lead salts but were precipitated by mercury salts. They were unaffected by treatment with emulsin, were inactivated by treatment with diazomethane, and were not adsorbed by alumina gel, alumina, or titanium oxide.

IV. Discussion

The biological assay is of central importance to any attempt to detect and concentrate cell division inducing substances in plant extracts. Therefore its features require detailed discussion.

On the basal medium with IAA added, the cells of tobacco pith were found to expand but not to divide. Thus the detection of any cell division on a medium containing plant extract was sufficient to denote the presence of a kinin. The absence of an endogenous cell division rate reduced the possibility of the activity of a crude extract being due to the promotion of the endogenous rate by supplementation of the nutrients of a suboptimal basal medium. The absence of an endogenous cell division rate also made the reading of the assay much easier.

That the kinin activity detected in crude plant extracts was not due to supplementation of nutrients was further supported by experiments with known substances. The present experiments, and those at the University of Wisconsin with a similar assay system with tobacco callus, have shown that there is no cell division following additions of extra mineral salts, mixtures of purified amino acids, or polyols to the basal medium with IAA. The only chemically known substances which are consistently active are kinetin and its derivatives. It is therefore assumed that an active

extract contains at least one substance which has a biological activity equivalent (at least in this test) to kinetin and its derivatives.

The bioassay system developed has the advantage that the volume of test solution required is small; at times it has been reduced to 0·3 ml with no obvious change in the reaction of the pith blocks.

Although this assay is more rapid than most assays for cell division, it still requires at least 7 days to obtain a result. The assay moreover is not quantitative, although it can be made roughly so by estimation of the amount of division or by a dilution series. In our view the strong evidence available for the specificity of the assay more than outweighs its disadvantages.

In this paper, the active material is called a cell-division inducer or kinin. This is an accurate description of the phenomenon observed in the assay, because an active extract converts the otherwise inadequate medium into one competent to induce cell division. However, it could be argued that the composition of the basal medium decides the component identified as the inducer, because, for example, the omission of IAA from an otherwise complete medium would produce a situation in which IAA would be identified as a cell-division inducer. The same could apply for sucrose or mineral salts. It is true that the assay tells nothing about the mechanism of the reaction induced by active material, or how closely any one of the components of the medium is related to cell division. However, in any medium which supports growth, the requirements for an energy source and for mineral salts are widely recognized. IAA and the active material are distinguished from the remaining components by being organic substances required in very low concentrations (Zwar, Bottomley, and Kefford 1963). The unknown material is thus, like IAA, a plant growth regulator. Its presence (or that of another kinin) is certainly obligatory for cell division, but rigorous proof that it is a specific cell-division inducer must await elucidation of its mechanism of action.

The active material of apple extract is, unlike kinetin, insoluble in ether and most other organic solvents. It therefore seems most unlikely that the activity is due to kinetin. Moreover it is unlikely that the activity is an artefact caused by heating, for extracts prepared and sterilized at room temperature were equal in activity to those which were autoclaved.

The requirement for auxin in cell-division induction in tobacco pith (Miller *et al.* 1955) was confirmed and several auxins were effective. In the absence of a cell-division inducer, all auxins produced cell enlargement in tobacco pith. The induction of enlargement did not preclude the induction of cell division when kinetin was added not more than 7 days after the commencement of incubation. Kinetin was required for the maintenance as well as the initiation of cell division, which does not support the proposal of Goldacre (1959) that dividing cells produce division-inducing substances to continue the meristematic activity.

Steward and his colleagues (Steward and Shantz 1959; Pollard, Shantz, and Steward 1961; Steward 1961) have investigated the impressive activity of coconut milk in stimulating the growth of isolated carrot root phloem tissues. The total activity of coconut milk is considered to be due to a reduced nitrogen component,

a polyol component, and growth regulator components. To obtain maximum activity, approaching that of the original coconut milk, all components must be present, but each component appears capable of stimulating growth in some samples of carrot tissue. Cell division in tobacco pith is unaffected by the addition of casein hydrolysate, which can substitute for the reduced nitrogen component of coconut milk. Nor is it stimulated by additions of sorbitol or inositol which are major constituents of the polyol component of coconut milk (Pollard, Shantz, and Steward 1961). These substances are therefore not necessary components of the basal medium for the exhibition of maximum activity in tobacco pith, nor can they induce or stimulate cell division when present in a plant extract.

Steward and co-workers (Pollard, Shantz, and Steward 1961) now recognize an auxin requirement for the growth of carrot tissue, and IAA, 2,4-dichlorophenoxyacetic acid, 2-benzthiazoleoxyacetic acid, and a compound of IAA and arabinose have, at different times, been shown to stimulate such growth. Some naturally occurring polyphenols such as chlorogenic acid and leucoanthocyanin-like compounds (Steward and Shantz 1959) stimulate growth of the carrot tissue, and substances in this class have recently been proposed as co-factors in the control of auxin levels in tissues (Wada 1961; Furuya, Galston, and Stowe 1962; Mumford, Smith, and Castle 1962). In the tobacco pith assay, auxin is provided in the medium.

It has been claimed that adenine and other purines cause slight growth stimulation in carrot tissue (Steward and Shantz 1959), but neither adenine nor 6-methyladenine had any effect when tested alone and they had only a slight effect when tested together on tobacco pith.

The portion of the growth regulator component of coconut milk which is not auxin (Pollard, Shantz, and Steward 1961) corresponds most closely with kinins in apple extracts; its nature is unknown. Kinin activity apparently similar to that detected in apple extracts has been found by other workers. The sources of plant extract and the biological assay tissues used were as follows: germinating peas with tobacco stem callus (Zwar and Skoog 1963); immature maize endosperm with tobacco stem pith (Beauchesne 1962); immature maize seed with soybean stem callus (Miller 1961); and apple, plum, and other fruits with carrot root phloem (Letham and Bollard 1961).

In the present investigation, kinins have been detected in a variety of meristematic zones. These zones were chosen not because it was thought that kinin activity would be exclusive to them, but because they might have the highest kinin concentration (Goldacre 1959). It was also possible that little activity could be extracted from them, because the active substances were being consumed by cell division. However, the distribution of activity within the apple and tomato fruits was found to follow the intensity of cell division in the component tissues (Houghtaling 1935; Bain and Robertson 1951). That is, there was a correlation between extractable kinins and the intensity of cell division occurring in a tissue. It should be remembered, however, that the extracts were unpurified, so that differences in assayed activity may have been due to factors other than differences in concentration of kinin.

V. Acknowledgments

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