A QUANTITATIVE STUDY OF THE GIBBERELLIN CONTENT OF SEEDS OF *PHASEOLUS VULGARIS* AT DIFFERENT STAGES IN THEIR DEVELOPMENT

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Summary

Extracts were made, using ethyl acetate, from bean seeds at different stages of development. Chromatograms of the extracts were developed using n-butanol–1·5N ammonium hydroxide (3 : 1 v/v) and the chromatograms surveyed by two methods of bioassay for gibberellin activity. Activity was found in two zones, zone 1 at R_F 0·3–0·4, zone 2 at R_F 0·7–0·9. The amount of activity in each zone (compared with that of known amounts of gibberellic acid) changes in step with changes in the growth rate of the seeds. The bean seed has a diauxic pattern of growth. During the first phase of high growth rate, gibberellin activity in both zone 1 and zone 2 rises to a maximum. With the onset of the second (lag) phase of low growth rate, activity in both zones falls to a low level, and at the end of the lag phase zone 2 activity becomes negligible. With the onset of the third phase of high growth rate zone 1 activity rises again and reaches a second maximum, coincident with the maximum growth rate of the seed. Both growth and zone 1 activity then decline.

These data have been interpreted in the light of existing knowledge of gibberellin activity in seeds. It is shown (1) that the fall in gibberellin activity during the first phase of growth cannot be attributed solely to the disappearance of the mucilaginous contents of the embryo sac as the embryo enlarges to fill it; (2) there is probably little connection between the time of cessation of cell division in the seed and the changes in gibberellin activity; (3) that the changes in gibberellin content and in growth rate of the seed coincide so closely that it is not possible to establish the direction of the causal relationship.

I. Introduction

The presence of gibberellins in higher plants has been amply demonstrated in recent years. The level of gibberellin activity in some seeds, both mature (Murakami 1959b) and immature (Phinney *et al.* 1957; Murakami 1959a), is remarkably high. Many different kinds of seeds, but not all of those examined, have been shown to contain gibberellins. As gibberellins may play an important role in the growth and development of the seed and possibly of the fruit, quantitative developmental studies on the gibberellins of seeds were commenced in this Laboratory. Data on the growth of the seeds of *Phaseolus vulgaris* (French beans) have already been presented (Carr and Skene 1961), and in this paper data on the pattern of changes in gibberellins in these seeds during their development are given.

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Despite the many reports of gibberellin activity in seeds there have been few quantitative studies and only one (Ritzel 1957) in which the pattern of changes in gibberellin activity during seed development has been traced quantitatively. It is necessary to bear in mind that there may be changes in the levels of more than one gibberellin during seed maturation and possibly also in the content of antigibberellins (Corcoran and Phinney 1958). Changes of this kind in the content of auxin and "growth inhibitors" have already been studied in considerable detail (Nitsch 1952; Luckwill 1959), but it has also been pointed out (Luckwill 1959) that "until the occurrence of (the gibberellins) has been studied in relation to seed and fruit development . . . speculations as to the possible role of naturally occurring giberellins in the control of fruit growth must of necessity be tentative".

II. Materials and Methods

(a) Bioassay Methods

Two methods of bioassay have been used. The first is essentially the same as that described by McComb and Carr (1958). Dwarf pea plants (cv. Meteor) are grown in perlite under fluorescent lights giving a 12-hr day and after 10 days the interval between the third and fifth nodes, counting the cotyledonary node as node 1, is measured. A 4-μl droplet of absolute ethanol containing the material to be assayed is placed in the axil of the scale leaf subtending the third node. Tests have shown that this amount of ethanol does not affect the growth response of the plants. Six days after treatment the interval between the third and sixth nodes is measured and the percentage increment in growth in excess of that of control plants expressed logarithmically. The relation between log per cent. increment and log weight of gibberellic acid is linear over the range 0·001–5 μg, so by comparing the effects of solutions to be assayed with those of solutions of known gibberellic acid content, activity can be expressed in terms of gibberellic acid. On some occasions when solutions of very low activity were to be assayed, the mode of application to the assay plants was modified to obtain increased sensitivity. Dr. N. J. Scully (personal communication) has found that the entry of gibberellic acid into the plant is facilitated by making a needle puncture through the epidermis. This has been found to increase the sensitivity of the dwarf pea assay by a factor which varies up to about 10. Thus, in order to confirm and measure the low order of activity of some solutions, the stems of the pea plants were punctured at the site of application of the droplet. This modification is referred to in this paper as the "sensitized assay". The linearity of the log response/log dose curve using gibberellic acid does not appear to be affected by the modification. On the occasion of each bioassay, the responses to two levels of gibberellic acid, usually 0·01 and 0·1 μg per plant, were measured and the data used to calibrate the assay to the log response/log dose graph.

The second bioassay method is based on the wheat leaf assay of Radley (1958). In brief, 4-mm leaf segments prepared from wheat seedlings (cv. Olympic) are incubated in 5 ml of test solution for 20 hr, with horizontal shaking, at 20°C. Solutions are buffered to pH 5 with 0·015M phosphate–citric acid. Between levels of 0·001 and 0·1 p.p.m. gibberellic acid under the above conditions, final segment length is proportional to the concentration of gibberellic acid applied, giving the assay some

quantitative value. The response to sucrose is small, and confusion of sugar-induced growth with the response to the gibberellin(s) of zone 1 (see below) can be avoided by using ethyl acetate for gibberellin extraction, and also by chromatographic separation of the extract. Auxin-induced growth is very slight and takes place only at relatively high auxin concentrations (10 p.p.m.). There appears to be no gibberellic acid–auxin synergism in the assay. Advantages of this bioassay over the dwarf pea assay are that it is less cumbersome and requires a considerably smaller amount of plant extract. Its disadvantages are that it is not as strictly quantitative or as specific as the dwarf pea assay; however, the latter disadvantage can be minimized, as described above.

The data of the bioassay have been analysed using the t-test and adopting the 5% level of probability to indicate significance.

(b) Extraction Methods

(i) *For Dwarf Pea Assay.*—Beans (*P. vulgaris* cv. Hawkesbury Wonder) were grown in soil in the greenhouse. Pods were harvested when the seeds had attained lengths chosen to represent different stages of maturity. These stages have been described by Carr and Skene (1961). After taking samples for dry weight determination, the seeds were stored in ethyl acetate at $-15°C$ until required. Gibberellin extraction was carried out using ethyl acetate, the actual amount used (300–400 ml) depending on the sample size. Seeds were blended in the extractant, which included that in which they had been stored, for several minutes and the mixture was left overnight at 1°C. After separation of the solid residue, the solution was reduced to a small volume *in vacuo* at a temperature not exceeding 30°C. The concentrate was transferred as a straight line to each of several sheets of Whatman No. 3 chromatography paper, which were then developed simultaneously in a descending system of *n*-butanol–1·5N ammonium hydroxide (3 : 1 v/v). The solvent front was permitted to advance 30 cm down the paper from the start line, after which the papers were removed from the tanks and dried in a stream of cold air. The dried chromatograms were cut into 10 3-cm wide horizontal strips each of which was eluted with acetone. Eluates from corresponding strips were combined and reduced to dryness, the residues being taken up in 0·5 ml ethanol. These solutions were then assayed simultaneously with at least two levels of gibberellic acid.

(ii) *For Wheat Leaf Assay.*—As small seed samples suffice for the wheat leaf assay, pods developed from flowers tagged at anthesis were sampled at a known age. At each sampling *c.* 0·5 g seeds was harvested and immediately extracted at 1°C with 10 ml ethyl acetate for 24 hr. The residue was extracted twice in the same way, the three extracts were combined, and the volume reduced *in vacuo* to a few ml. The extract was transferred to a single piece of Whatman No. 3 chromatography paper and developed as described above. The solvent front was allowed to advance 20 cm from the start line. The paper was then dried thoroughly and cut into 20 strips of equal width covering the whole range of R_F values. The activity on these paper strips was assayed directly by putting each strip into buffer solution containing 10 wheat leaf segments. The responses were compared with those given by two levels of gibberellic acid.

III. RESULTS

(a) Dwarf Pea Assay

Bean seeds were sorted into groups representing five stages of maturity, ranging from about 14 days after anthesis up to about 25 days after anthesis. This period covers most of the first, the whole of the second, and the beginning of the third phase of growth of the bean seed (Carr and Skene 1961). Data on the seed size, seeds per sample, and sample fresh weight are given in Table 1.

The results of the bioassay of the 10 zones of each sample are summarized in Figure 1, in which percentage growth increment in excess of the controls is plotted against R_F value. The histograms marked with an asterisk are those in which the increases in internode lengths of the treated plants were significantly different from those of the controls. There were present two zones of gibberellin activity, the first (zone 1) with a peak at about R_F $0 \cdot 3$–$0 \cdot 4$, the second (zone 2) at R_F $0 \cdot 7$–$0 \cdot 9$. It is

TABLE 1

SIZES AND WEIGHTS OF SEEDS AT EACH SAMPLING INTERVAL

Sample No.	Mean Seed Length (mm)	Mean Seed Weight (mg)	No. of Seeds per Sample	Fresh Weight of Sample (g)
I	5	11	2241	$24 \cdot 65$
II	7	45	823	$37 \cdot 38$
III	10	113	344	$38 \cdot 78$
IV	13	277	186	$51 \cdot 64$
V	15	438	120	$52 \cdot 60$

likely that the gibberellin activity of zone 1 is attributable to the presence of gibberellin A_1. Both gibberellic acid and gibberellin A_1 move at the same R_F as zone 1 in the solvent system used in our experiments, and MacMillan and Suter (1958) have isolated gibberellin A_1 from immature seeds of *P. multiflorus* L. Phinney and Neely (1958) have also identified their "bean factor I", isolated from French bean seed, as gibberellin A_1, but West and Phinney (1959) have since reported that a positive identification has not yet been made. Zone 2 activity is at the same R_F as the activity detected by McComb and Carr (1958) in Telephone pea plants and by McComb (1959) in germinating Telephone pea seeds. It does not appear to be identical with "bean factor II" which, according to West and Phinney (1959), moves with an R_F of $0 \cdot 5$ in the solvent system we have used. According to Lang (1960), bean factor II is probably identical with the gibberellin A_5 isolated by MacMillan, Seaton, and Suter (1959) from *P. multiflorus* seed. As we have not had the opportunity to compare the behaviour of either bean factor II or gibberellin A_5 with the activity on our chromatograms, it must remain open for the present whether such compounds are present in
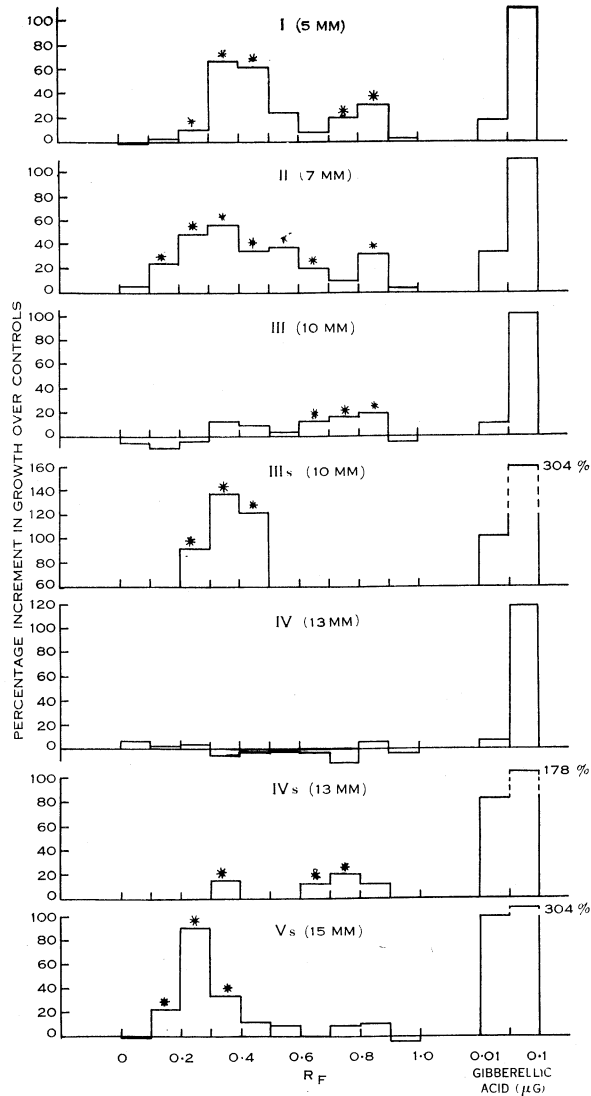
Fig. 1.—Showing gibberellin activities, as measured by responses in the dwarf pea assay, plotted against R_F of the chromatograms of extracts of French beans at different stages of maturity (I–V, see text). The values in parenthesis are the mean lengths of the seeds assayed. Histograms marked with an asterisk are those in which the response is significantly different ($P = 0.05$ or greater) from that of controls. For stages III (10-mm seeds) and IV (13-mm seeds) only, eluates at the R_F values shown in the second diagram were re-assayed using the sensitized assay(s) to confirm activity. However, the levels of zone 1 activity (see text) in 10- and 13-mm seeds have also been confirmed on further seed samples. The histograms at the right-hand side represent responses to the stated amounts of gibberellic acid per plant. By the method of chromatography used, gibberellic acid would run to R_F c. 0.3.

our extracts. However, it does seem probable that there are at least two substances with gibberellin activity present.

Undoubtedly, other substances with hormone activity are present on the chromatograms. Eluates from the region R_F 0·7–0·9 showed the unusual property of causing growth of the bud in the leaf axil to which they were applied. Such buds continued to grow until they were about 1·5 cm long. This did not occur in either control plants or those treated with gibberellic acid; nor could it be induced experimentally by applying kinetin to the leaf axil. Whether or not this "bud growth factor" is identical with the gibberellin of zone 2 remains to be investigated.
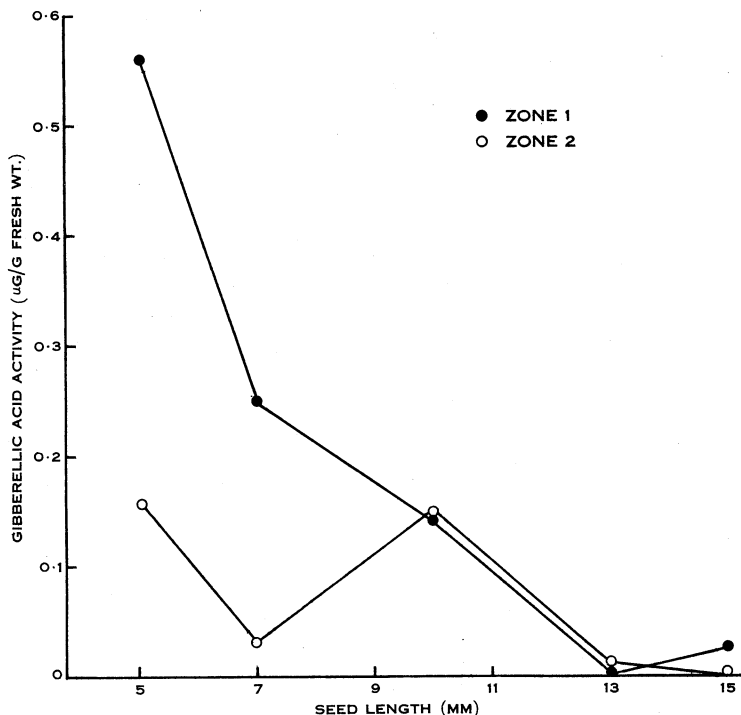

Fig. 2.—Gibberellin activity (μg gibberellic acid) per gram fresh weight of seeds plotted against seed length. Zone 1 refers to activity on the chromatograms in the region R_F 0·3–0·4, zone 2 to activity in the region R_F 0·7–0·9. The seed length is a measure of the stage of development of the bean seed (see Fig. 4). Data from dwarf pea bioassay.

The pattern of change in the concentration of the gibberellins of zones 1 and 2 during the development of the seeds is shown in Figure 2. Activity is plotted as μg gibberellin (as gibberellic acid) per g fresh weight against seed length, representing stages of maturity. It should be remembered that the activities, weight for weight, of zones 1 and 2 are not necessarily identical, using gibberellic acid as a common basis for comparison. In fact, it is unlikely that the substances of zone 1 and zone 2 would have equal growth-promoting activity. Furthermore, there is evidence that the growth-promoting effects of zone 1 are longer lasting in the pea plant than are those

of gibberellic acid. In one experiment, for instance, most of the growth acceleration induced by gibberellic acid occurred before the date at which the routine bioassay measurements were taken, while the growth acceleration induced by treatment with zone 1 eluate continued for at least a further 5 days. This resulted in a significant diminution in the difference in height between the group of plants treated with zone 1 eluate and the group treated with $0\cdot1$ μg of gibberellic acid (Table 2). Radley (1958) had also noted the same phenomenon.

Referring again to Figure 2, it is evident that zone 1 activity is very high in the very immature seed ($0\cdot56$ $\mu g/g$ fresh wt. in seeds 5 mm long) and falls quite rapidly until at the end of the lag phase in seed growth it reaches its lowest level ($0\cdot0024$ $\mu g/g$ in 13-mm seeds). On the other hand, zone 2 activity, on a fresh weight basis, although initially lower than that of zone 1, remains fairly steady during the

TABLE 2

HEIGHT INCREMENT OF METEOR PEA SEEDLINGS IN PERIODS
SUBSEQUENT TO APPLICATION OF ZONE 1 ELUATE AND OF
GIBBERELLIC ACID

Time from Application (days)	Increment in Height over Controls (%)	
	Zone 1 Eluate	$0\cdot1$ μg Gibberellic Acid per Plant
6	$61\cdot9$	$109\cdot0$
11	$74\cdot3$	$81\cdot3$

first exponential phase of seed growth, but it too falls to a low level ($0\cdot0047$ $\mu g/g$) in the 13-mm seed. Even the sensitized assay is too insensitive to measure the still lower level of zone 2 activity in 15-mm seeds, which just fails to attain statistical significance. After the lag phase, zone 1 activity increases again, although in 15-mm seeds it is still relatively low ($0\cdot023$ $\mu g/g$).

The data are plotted as activity (in terms of gibberellic acid) per 1000 seeds against seed length in Figure 3. On a per seed basis, the gibberellin activity in both zones 1 and 2 rises during the first phase of seed growth and reaches its maximum just before the onset of the lag phase in growth of the seed. When activity per seed is at its maximum (in seeds about 9–10 mm long) the activity is about equally distributed in zones 1 and 2. During the lag phase there is a marked fall in activity per seed. In 13-mm seeds, zone 2 activity per seed is approximately at the same level as zone 1 activity but with the resumption of active growth following the lag phase the zone 2 activity declines still further and is inappreciable in 15-mm seeds, while the zone 1 activity increases concomitantly with the increase in the growth rate.

(b) Wheat Leaf Assay

One of the difficulties with the dwarf pea assay is the relatively large quantity of plant extract required. Using this assay, it was necessary to make mass collections of beans and to divide the collections into size classes, representing different stages of maturity. With the wheat leaf assay, on the other hand, the numbers of seeds required for extraction was relatively small. This meant that flowers could be tagged on the day of anthesis and the pods which developed used for both growth studies and gibberellin assays. As the wheat leaf assay appears to be not very sensitive to the
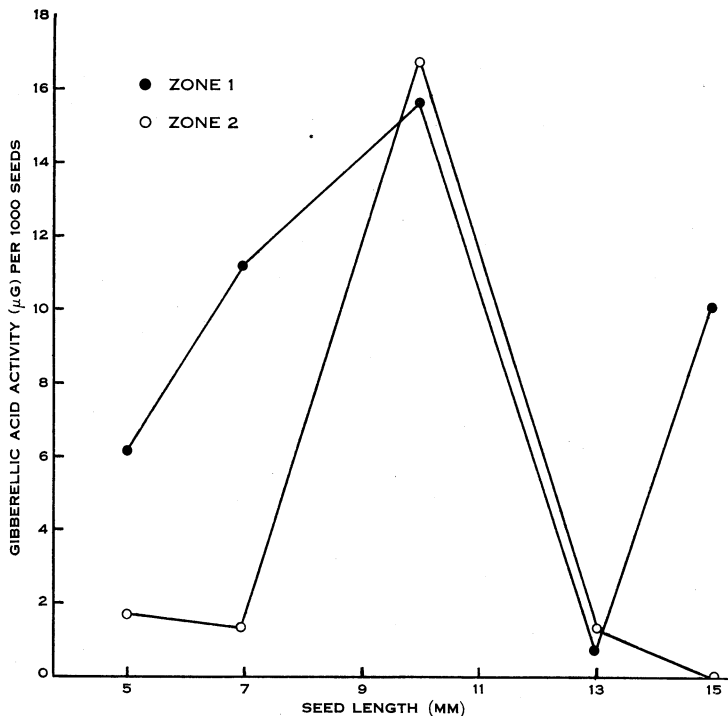

Fig. 3.—Gibberellin activity (μg gibberellic acid) per 1000 seeds, plotted against seed length. The lag phase in seed growth begins when the seed is about 9–10 mm long. Data from dwarf pea bioassay.

gibberellin of zone 2, only the activity of zone 1 has been followed by means of this assay. Its use, however, has enabled us to fill in a considerable amount of the detail of the pattern of changes in gibberellin activity, particularly in very young seeds (of low weight but high activity) and almost mature seeds (of high weight but low activity). We agree with Lang (1960) that it is essential to assay extracts containing natural gibberellins on more than one type of test plant and it is encouraging that the pattern of changes revealed by the wheat leaf assay (Fig. 4) is both qualitatively and quantitatively in full agreement with that revealed by the dwarf pea bioassay.

The activity per seed is shown to fall rapidly during the later stages of the first phase of seed growth, conforming to the pattern shown in Figure 3, having risen during the earlier, exponential stage. Zone 1 activity remains low during the lag

phase then rises very rapidly as exponential growth begins again at the onset of the third phase of growth. The maximum level per seed is attained at 26 days after flowering when the seeds are 18–20 mm long. After this, zone 1 activity declines rather slowly until, in the mature dry seed, little or no activity can be detected by the wheat leaf assay. There are changes in the distribution of activity along the chromatogram during the later stages of seed maturation, with the appearance of new zones of activity, but these have not yet been investigated in detail.
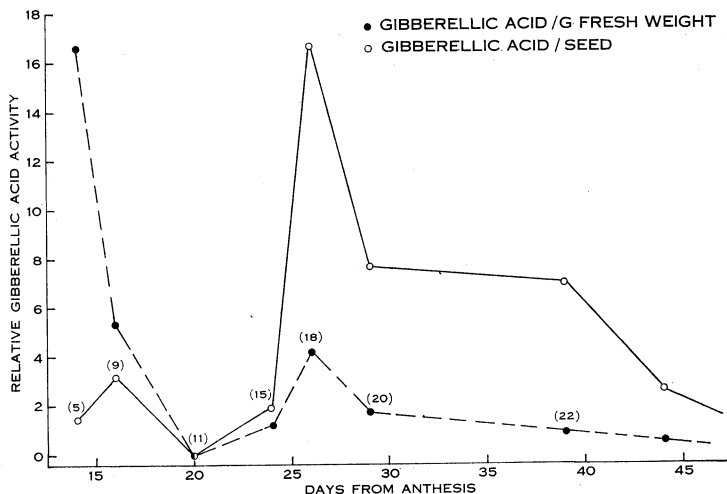

Fig. 4.—Gibberellin activity (relative values) per gram fresh weight and per seed plotted against days from anthesis. These data were obtained by

CORRIGENDUM

In Figure 4 the ordinate scale for gibberellic acid per gram fresh weight is absent; the scale given refers only to the gibberellic acid per seed graph. One unit of this scale is equivalent to five units of gibberellic acid per gram fresh weight.

commences to grow rapidly again up to about the 26th day after anthesis and then growth gradually declines, eventually to zero.

There are thus two phases of rapid growth separated by a brief phase of very slow growth. The gibberellin activity correlates remarkably well with this diauxic pattern of seed growth. The gibberellin activity per seed in zone 1 has been plotted together with the increment curve of fresh weight per 3 days in Figure 5. It is apparent that when the seeds are growing rapidly the gibberellin content is high; when growth is declining the gibberellin content is falling. This synchronization of gibberellin activity with growth rate is so close that it raises the problem of which is cause and which effect.

On closer analysis it becomes clear that the situation is much more complex than the comparisons in Figure 5 would suggest. The end of the first phase of growth coincides with the filling-out of the embryo sac by the seed and the disappearance of the last remnants of the "non-cellular endosperm" (or mucilaginous contents of the embryo sac). Since the endosperm of some seeds, e.g. *Echinocystis* (Phinney *et al.* 1957), is known to be a rich source of gibberellin activity it might be suggested that the fall in gibberellin activity of the whole seed at the end of phase 1 is due merely to the disappearance of the mucilaginous contents of the embryo sac. However, there is some evidence that this cannot account entirely for the changes in level of activity
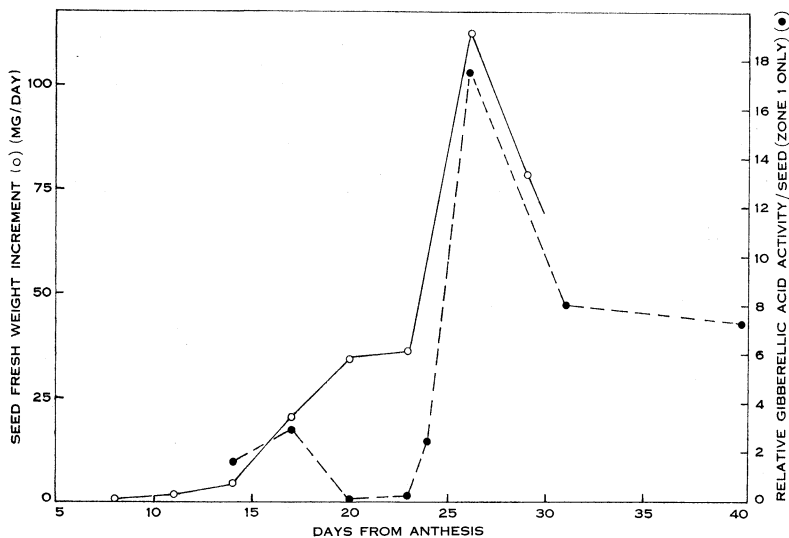

Fig. 5.—Increment in fresh weight (mg/day) and relative gibberellin activity per seed, plotted against days from anthesis, to show the close correspondence between the growth rate and gibberellin activity in developing bean seeds. The data for gibberellin activity are for zone 1 only, and where they are available the data from the dwarf pea bioassay have been given preference over those from the wheat leaf bioassay. Nevertheless, the data from the two methods of bioassay are not noticeably discordant (see Figs. 2, 3, and 4).

in the first phase of growth. For instance, supposing that in the above case the concentration of gibberellin in the embryo sac mucilage remained constant, the amount of gibberellin activity in the embryo sac would increase only up to the stage when the seeds are about 5 mm long, because after that stage the growth in volume of the embryo is greater than the growth in volume of the embryo sac (cf. Carr and Skene 1961, Fig. 9). The data of Figure 3 show that activity in both zone 1 and zone 2 is increasing even in seeds 7 mm long, that is, when the contents of the embryo sac are decreasing in volume. Also, examination of the gibberellin content of the immature embryos excised from the seeds has provided evidence that a considerable fraction of the activity in the seed before the onset of the lag phase in growth is in the embryo, although the contents of the embryo sac appear to contain activity as well (Skene, unpublished data). Radley (1958) claims that gibberellin activity in immature

(2–6 mm long) runner bean seeds is approximately equally distributed between the "embryos", cotyledons, and testas, on the basis of the extracted weights of these parts. It is difficult, however, to obtain very small embryos free from all traces of the mucilaginous contents of the embryo sac and it would be preferable to assay these contents separately.

Although the fall in zone 2 activity may be connected with the disappearance of the "endosperm" towards the end of the first phase of growth, the fact that the zone 1 activity rises to a high level in the third phase of growth shows that the presence of the endosperm is at least not essential for continued gibberellin production, and could indicate that the endosperm is not the sole source of activity during the first phase of growth. Moreover, the fact that a single zone of activity, at the same R_F as our zone 2, was found by McComb (1959) in extracts of imbibed mature pea seeds of a tall variety and in extracts of growing plants of the same variety (McComb and Carr 1958) is evidence that the gibberellin(s) of zone 2 are not formed exclusively, if at all, in the endosperm.

The slowing down of growth during the lag phase presents an interesting problem, since it is contemporaneous with (1) the filling of the embryo sac and (2) a rapid fall in gibberellin activity. At least two hypotheses may be put forward to account for the coincidence of these phenomena. According to the first, the mechanical hindrance imposed by the testa might slow down the growth of the embryo and the slowing down might result in metabolic changes, including the production of less extractable gibberellin. This would imply that gibberellin production would be an effect rather than a cause of seed growth. As a corollary to this, some form of plasticization of the testa might be set in train during the lag phase, allowing growth and gibberellin production to proceed during the third phase. The second hypothesis would state that since very many changes in the metabolic and biochemical activities of the seed and fruit appear to coincide with the onset of the lag phase, the fall in growth rate of the whole seed and its parts might be the resultant of a drastic revision of the pattern of metabolism of the seed, one aspect of which could be a reduced output of gibberellins. On this viewpoint the fall in growth rate could be an effect of the reduced content of extractable gibberellin, i.e. growth might be regulated by the level of gibberellin activity. This hypothesis would involve the premise that it is either the extractable giberellin itself which is effective in regulating growth, or that this is directly proportional to that fraction of the total gibberellin content which is effective. On a per seed basis, the activity of both zones 1 and 2 rises during the first exponential phase of growth and reach a maximum at the end of it. It can be ascertained from the work of McKee, Robertson, and Lee (1955) on peas that cell number in the seed ceases to increase long before the end of the exponential phase of dry weight increase. If this is also true of beans, there is likely to be little correlation between the amount of activity of either zone 1 or zone 2 and the intensity of cell division in the developing bean seed.

A considerable interest attaches to the question of the locus of synthesis of the gibberellins of the seed. According to Radley (1958), all parts of the bean seed —the cotyledons, "embryo", and testa—contain approximately equal amounts of gibberellin activity, on a basis of the weights of these parts after extraction. Since the

total weight after extraction was about half of the fresh weight of the seeds before extraction, it is difficult to estimate whether Radley's statement can be applied to the fresh weights of the parts before extraction. In any case, from a consideration of the data of Figures 2 and 3 it seems unlikely that the gibberellin activity of French bean seeds is uniformly distributed in all parts of the seed and it certainly does not remain a constant fraction of the fresh weight of the seed. The fact that the activity per seed is rising during the first phase of growth while the activity per gram fresh weight is declining rules out the possibility that the seed commences growing with a fixed "capital" of gibberellin which is merely diluted by increase in size of the seed. There must be further production of gibberellin during growth; it is more probable that only a limited number of production loci are involved and that these constitute a diminishing fraction of the total weight than that the decline in gibberellin per gram fresh weight is due to a uniform dilution throughout the mass of the seed. The embryonic axis (embryo minus cotyledons) probably contains gibberellin (Radley 1958) and the rapid rise and eventual fall in the growth rate of the embryonic axis during the third phase of seed growth correspond very closely in time with the second rise and eventual decline in zone 1 activity. However, the embryonic axis is not the only part of the seed which is growing rapidly during the early part of phase 3 and on a per seed basis much, if not all, of the gibberellin activity in 20-mm seeds is in the cotyledons (Skene, unpublished data).

It is of interest to compare the level of gibberellin activity of French bean seeds with the data of the many published reports of gibberellin activity in seeds. However, in the majority of these the level of activity is not stated quantitatively. In lupins and beans, to quote Ritzel (1957), "both activity per seed and activity per gram of seed increased with seed age and there was evidence of loss of activity only after the seed approached final size". No values are given by Ritzel, but Murakami (1959b) states that the concentration of gibberellins in mature, dry *Lupinus luteus* seeds is approximately equivalent to $0 \cdot 1$ μg/g of "gibberellin A". As he points out, this is a relatively high value and it may be in accord with Ritzel's data. Radley (1958) states that the high level of activity in immature runner bean seeds falls in the mature seed to a level comparable with that in the growing plant. In seeds 2–6 mm long, activity was equivalent to $0 \cdot 25$ μg of gibberellic acid per gram fresh weight of seed. This agrees well with the range $0 \cdot 25$–$0 \cdot 56$ μg/g fresh weight reported above for zone 1 activity. In none of these cases have the changes in activity demonstrated above been recorded, but it is particularly interesting to recall that Mitchell, Skaggs, and Anderson (1951) found that the amount of ether-extractable hormones increased in Black Valentine bean seeds up to about 8 days after pollination and then decreased rapidly to an immeasurable amount (when assayed on the same variety of bean plant) on about the 15th day. These early data are in close accordance with the pattern of changes in gibberellin content of French bean seed during the first and second growth phases.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

CARR, D. J., and SKENE, K. G. M. (1961).—Diauxic growth curves of seeds, with special reference to French beans (*Phaseolus vulgaris* L.). *Aust. J. Biol. Sci.* **14**: 1–12.

CORCORAN, M. R., and PHINNEY, B. O. (1958).—An inhibitor of gibberellin-induced growth. *Plant Physiol.* **33**(suppl.): 40.

LANG, A. (1960).—Gibberellin-like substances in photo-induced and vegetative *Hyoscyamus* plants. *Planta* **54**: 498–504.

LUCKWILL, L. C. (1959).—Factors controlling the growth and form of fruits. *J. Linn. Soc. (Bot.)* **56**: 294–302.

MACMILLAN, J., SEATON, J. C., and SUTER, P. J. (1959).—A new plant growth-promoting acid— gibberellin A₅—from the seed of *Phaseolus multiflorus*. *Proc. Chem. Soc. Lond.* **1959**: 325–6.

MACMILLAN, J., and SUTER, P. J. (1958).—The occurrence of gibberellin A₁ in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). *Naturwissenschaften* **45**: 46.

MCCOMB, A. J. (1959).—Studies on the gibberellins in relation to higher plants. M.Sc. Thesis, University of Melbourne.

MCCOMB, A. J., and CARR, D. J. (1958).—Evidence from a dwarf pea bioassay for naturally occurring gibberellins in the growing plant. *Nature* **181**: 1548.

MCKEE, H. S., ROBERTSON, R. N., and LEE, J. B. (1955).—Physiology of pea fruits. I. The developing fruit. *Aust. J. Biol. Sci.* **8**: 137–63.

MITCHELL, J. W., SKAGGS, D. P., and ANDERSON, W. P. (1951).—Plant growth-stimulating hormones in immature bean seeds. *Science* **114**: 159–61.

MURAKAMI, Y. (1959a).—A paper chromatographic survey of gibberellins and auxins in immature seeds of leguminous plants. *Bot. Mag., Tokyo* **72**: 36–43.

MURAKAMI, Y. (1959b).—The occurrence of gibberellins in mature dry seeds. *Bot. Mag., Tokyo* **72**: 438–42.

NITSCH, J. P. (1952).—Plant hormones in the development of fruits. *Quart. Rev. Biol.* **27**: 33–57.

PHINNEY, B. O., and NEELY, P. M. (1958).—Differential biological properties of gibberellin-like factors isolated from beans and peas. *Plant Physiol.* **33** (suppl.): 38.

PHINNEY, B. O., WEST, C. A., RITZEL, M., and NEELY, P. M. (1957).—Evidence for "gibberellin-like" substances from flowering plants. *Proc. Nat. Acad. Sci., Wash.* **43**: 398–404.

RADLEY, M. (1958).—The distribution of substances similar to gibberellic acid in higher plants. *Ann. Bot., Lond.* (N.S.) **22**: 297–307.

RITZEL, M. B. (1957).—The distribution and time of occurrence of gibberellin-like substances from plants. *Plant Physiol.* **32** (suppl.): 31–2.

WEST, C. A., and PHINNEY, B. O. (1959).—Gibberellins from flowering plants. I. Isolation and properties of a gibberellin from *Phaseolus vulgaris* L. *J. Amer. Chem. Soc.* **81**: 2424–7