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GIBBERELLINS AND α-AMYLASE FORMATION IN GERMINATING BARLEY

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Abstract—Gibberellin-like material was estimated in barley corns "malted" at 14·4° or germinated on moistened filter paper at 25°, using a lettuce hypocotyl elongation assay. α-Amylase was measured (*a*) in these samples, (*b*) in grains (endosperms) deembryonated after various malting times then incubated further, and (*c*) in endosperms dosed individually with known amounts of GA_3 (gibberellic acid), and incubated at 14·4°. The dosing technique provides the basis for a method of detecting and estimating 0·05 mμg or less GA_3. A relationship existed between the "gibberellin" content and the rate of α-amylase synthesis in malting grain. Time lags were detected between the appearance of "gibberellin" in the whole grain, the arrival of the "gibberellin" in the endosperm and the appearance of α-amylase. For the first time the quantitative relations between GA_3 added to individual endosperms and consequent α-amylase production were investigated. These quantitative results strongly supported the concept that "gibberellin", originating from the embryo, is of overwhelming importance in regulating the synthesis of α-amylase in the aleurone layer in malting grain, at least when the endosperm has been hydrated and sensitized by the postulated "embryo factor". The endogenous gibberellin behaved more like GA_3 than GA_1, or a mixture of varying proportions. Grain germinated on moistened filter papers at 25° gave apparently anomalous results in that the maximum value of α-amylase occurred before the greatest level of "gibberellin".

INTRODUCTION

THERE is a great deal of evidence that gibberellin-like materials are important in regulating the production of enzymes in germinating grain. They are thought to be released by the barley embryo during early germination and to move to the aleurone where they trigger the *de novo* synthesis of a variety of hydrolytic enzymes including α-amylase. In turn these enzymes are released into the starchy endosperm where they catalyse the hydrolysis of reserve substances, forming materials that can diffuse to the embryo and act as its nutrients. Much of the evidence for this view has been summarized.[1–3] Certainly the embryo produces a diffusible substance that triggers enzyme synthesis in the aleurone,[4–7] and it has frequently been shown that the effect of this material on the aleurone may be augmented or replaced by many gibberellins.[1–3] GA_3 (gibberellic acid)[8–10] and GA_1[11,12] have been found in immature barley grains and in exudates from isolated barley embryos. Two other unidentified gibberellins have been detected in immature grain.[12,13]

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However, it is possible that gibberellins are not the only factors responsible for controlling the synthesis of, for example, α-amylase. Thus ethylene,[14] which is produced by plants, and cyclic 3′,5′-AMP,[15] a natural constituent of many tissues, have been shown to enhance enzyme production in aleurone tissue dosed with GA_3. Further, there are reports that materials other than gibberellins can induce enzyme synthesis in barley or rice endosperms including (−)-kaurene,[16] sclerin,[17] helminthosporol,[17–20] helminthosporic acid,[17–20] dihydrohelminthosporic acid,[21] mevalonic acid,[22] fumaric acid,[23] and chemically uncharacterized solvent residues.[24] Other workers have failed to find this type of biological activity with (−)-kaurene,[24] (−)-kaurenol,[24] (−)-kaurenoic acid,[25] mevalonic acid or its lactone,[24, 25] or fumaric acid,[7] so adding to the uncertainty. With the exception of one report, on tests with wheat endosperm,[26] other plant growth regulators such as indoleacetic acid,[24, 27, 28] benzylaminopurine,[24] kinetin,[24, 27] and numerous other substances[24] have been found to lack a stimulating action on the aleurone layer. Abscisic acid,[29, 30] acetic acid,[31] and many metabolic poisons[1–3] inhibit the response of barley aleurone layers to added GA_3.

Quantitative measurements were made of the changes in the levels of gibberellin-like material and of α-amylase during the germination of barley. It had been reported that gibberellins are continuously required to maintain α-amylase synthesis[30] and so a relationship was anticipated between these two variants. As grains that have been deembryonated after an initial period of germination can continue to malt,[32] to produce α-amylase[33] and protease[34] a lag could be expected between an increase in the level of gibberellin-like material and the resulting α-amylase synthesis. We planned to use deembryonation techniques to investigate this lag period.

Enzyme production, sugar release or other changes in barley endosperms have been shown to vary with the gibberellin content of bathing culture media.[35–37] Such data cannot be used to decide whether the amount of endogenous gibberellin found in grain is more or less than that required to account for the observed amount of enzyme synthesis. For this it is necessary to measure enzyme production by hydrated, deembryonated grains dosed individually with known amounts of gibberellin. This we have achieved, we believe for the first time.

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The results of the experiments provide strong evidence that gibberellins are the most important, if not the only activators of the synthesis of α-amylase in barley aleurone tissue.

RESULTS AND DISCUSSION

Experiments with Whole and Deembryonated Grains

Decorticated barley (250 g) was germinated under malting conditions at 14·4°. Samples were taken at intervals and extracts were assayed for α-amylase activity and for gibberellin-like material (Fig. 1). The extraction sequence and the paper chromatographic system were selected such that they separated the gibberellin-like material from substances that inhibited the lettuce hypocotyl bioassay used to measure the activity of this material in terms of GA_3 equivalents. All the gibberellin-like material detected was in the acidic fraction soluble in ethyl acetate and had the same R_f as GA_1 and GA_3 (0·4–0·6); inhibitory material was detected

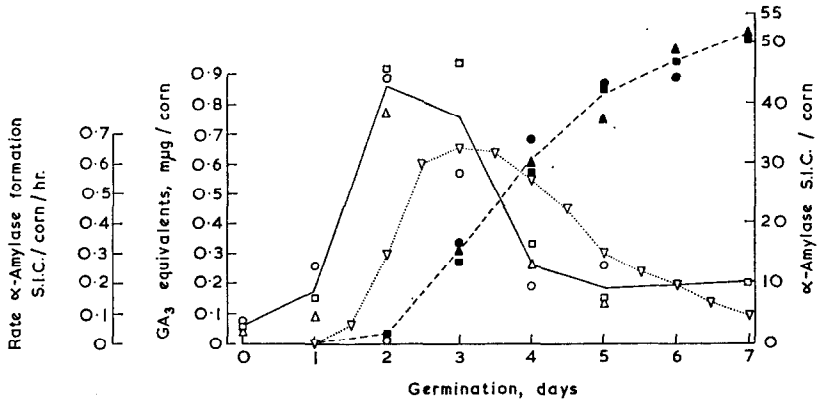

FIG. 1. CHANGES IN LEVELS OF α-AMYLASE AND GIBBERELLIN-LIKE MATERIALS IN GRAIN MALTED AT 14·4°.

Gibberellin-like material, —o—□—△—; α-amylase, --●--■--▲--.
Rates of formation of α-amylase (calculated), ---▽---.
Symbols represent average values from three separate experiments.

in this fraction (R_f 0·8–1·0). The continuous increase in α-amylase activity during the germination period is similar to changes observed in commercial malting and contrasts with the rise and fall in enzyme activity observed in grain germinating in contact with moistened filter paper[1–3] (Fig. 12).

As expected, under malting conditions, α-amylase appears to be stable. Comparison of the changes in the rate of α-amylase synthesis with the levels of gibberellin-like material at various times (Fig. 1), shows that in each case the quantities increase to a maximum value, and then decline. However the rate of synthesis of α-amylase reached a maximum value approximately 20 hr after the "gibberellin". This lag period probably encompasses not only the time take for the passage of "gibberellin" from its site of formation in the embryo to its site of action in the aleurone layer, but also the period of time needed for the aleurone tissue to respond and produce measurable quantities of enzyme.

Experiments involving the removal of embryos, the known sources of the "gibberellins", were used to obtain a better understanding of this lag. Embryos and scutella were removed from "malting" grains after several periods of germination, and the deembryonated grains,

"endosperms", were incubated for a further period before their α-amylase contents were measured. At intervals whole grains were also collected and their α-amylase contents were determined (Fig. 2). Deembryonated grains had the ability to form α-amylase during subsequent incubation if embryos were removed after at least 10–18 hr germination. The further incubation period of 3 or 4½ days allowed the aleurone to produce α-amylase in response to "gibberellin" derived from the embryo before its removal. In the whole grain α-amylase could not be detected until about 27 hr after the onset of germination. These results were

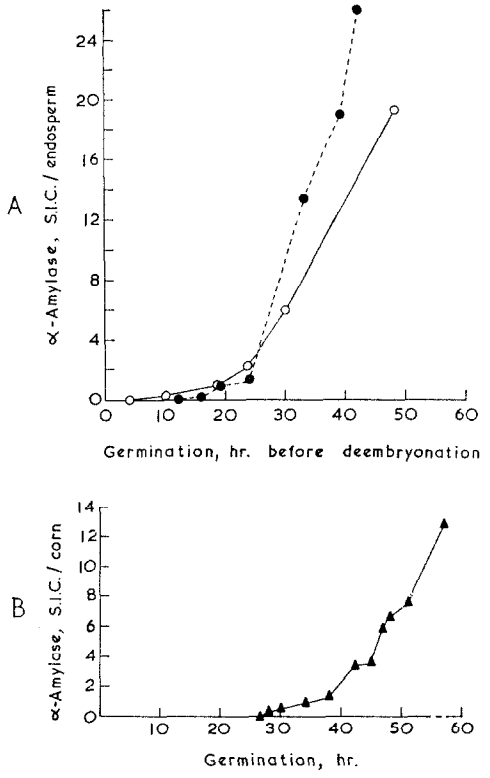

FIG. 2. A COMPARISON OF THE α-AMYLASE DEVELOPED IN WHOLE GRAIN MALTED AT 14·4° AND IN GRAIN DEEMBRYONATED AT VARIOUS TIMES THEN INCUBATED FOR A FURTHER PERIOD.

A. α-Amylase content of grains deembryonated then incubated for 3 days, —o—, or 4½ days, —●—.

B. α-Amylase content of whole grain.

interpreted as showing that sufficient gibberellin to induce enzyme formation did not reach the endosperm for 10–18 hr, and then the aleurone did not respond until several more hours had passed.

The potential of endosperms to form α-amylase after the removal of the embryos was related to the amount of "gibberellin" in the grain in the following way. The α-amylase contents of whole grains and embryos dissected from whole grains were followed during a 7-day malting period. In addition embryos were removed from samples of grain at intervals and the endosperms were "malted" for the remainder of the 7 day period. Their α-amylase contents were then determined (Fig. 3). From these results the α-amylase formed in the endosperms after

deembryonation during the remainder of the 7 day incubation period, was calculated. The levels of "gibberellin" in the grain and the capacity of endosperms to continue to form α-amylase were closely related (Fig. 4), suggesting that the level of the "gibberellin" was

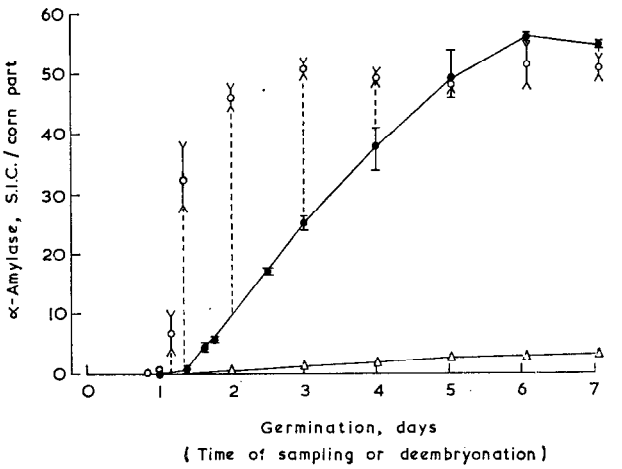

FIG. 3. THE DEVELOPMENT OF α-AMYLASE IN WHOLE GRAINS AND EMBRYOS OF WHOLE GRAINS GROWING UNDER MALTING CONDITIONS, 14·4° COMPARED WITH THE α-AMYLASE FORMED IN GRAINS DEEMBRYONATED AT VARIOUS TIMES AND INCUBATED FOR A FURTHER PERIOD.

α-Amylase in samples taken at times shown; whole grains, —●—; embryos, —△—.
α-Amylase in endosperms prepared by deembryonating grains at time shown, then incubating to a total time of 170 hr, o. Solid vertical lines show maximum variations of results. Vertical dashed lines indicate the approximate "enzyme-forming potential" of the endosperms before allowing for the α-amylase of the embryos (see text).

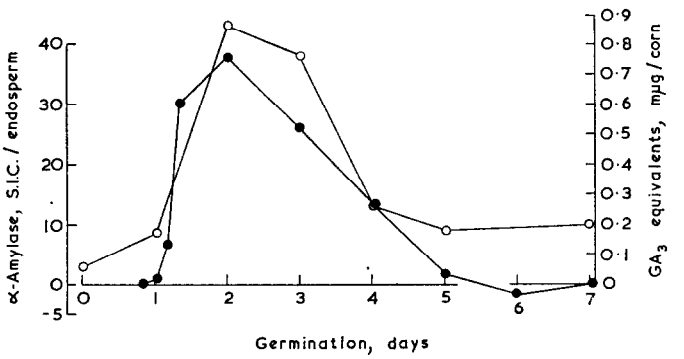

FIG. 4. COMPARISON OF THE LEVELS OF GIBBERELLIN-LIKE MATERIAL IN WHOLE GRAIN MALTED FOR VARIOUS TIMES, 14·4°, WITH THE ABILITY OF ENDOSPERMS PREPARED FROM EQUIVALENT GRAINS TO FORM ADDITIONAL α-AMYLASE WHEN INCUBATED FURTHER.

Gibberellin-like material (Fig. 1), —o—; "Enzyme-forming potential" (Fig. 3), —●—.

the major factor in controlling the production of the enzyme. Furthermore the maximum level of enzyme formed in deembryonated grains after 7 days equalled the quantity in the whole grain less the quantity found in the embryo after 7 days (Fig. 3), so that, as expected, the removal of the embryo at any time after 3 days' germination had no effect on the quantity of enzyme ultimately produced in the endosperm.

The close coincidence of the two graphs in Fig. 4 provides indirect evidence that the "gibberellin" is either GA_1 or GA_3 or a constant ratio of these substances. It is known that barley endosperms respond equally well to GA_1 or GA_3,[12, 36, 37] whereas GA_3 is about twenty-five times as active on a weight basis as GA_1 in the lettuce hypocotyl bioassay.[38] Therefore, if GA_1 had predominated during the early stages and GA_3 later, as might have been expected from reports by Radley,[11, 12] the two graphs (Fig. 4) would not have been so nearly coincident. Paper chromatography of extracts from barley malted for $2\frac{1}{2}$ days showed the presence of material moving like GA_3, but not GA_1, in the system of Bird and Pugh.[39]

The lack of synthesis of α-amylase in endosperms after 4 days (Fig. 4) may have been caused in part by a shortage of water or of "gibberellin" or a change in the sensitivity of the system to gibberellin.[40]

Experiments with Endosperms Individually Dosed with GA_3

Grains were sterilized and the embryos and scutella were removed. The endosperms were hydrated for 18 hr, at 14·4°, in a succinate buffer containing calcium ions, in an attempt to stabilize the α-amylase formed later, and then drained. A microdrop of buffer, usually 1 μl, with or without GA_3, was placed in the depression formerly occupied by the scutellum and the time course of α-amylase formation was determined. α-Amylase was not formed in detectable quantities until after 19–26 hr in grain incubated at 14·4° (Fig. 5), or 15–17 hr in

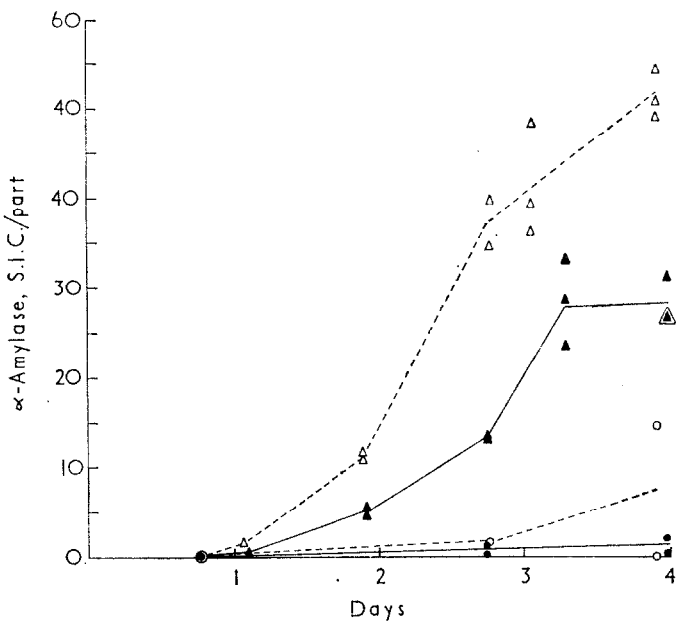

FIG. 5. TIME COURSE OF α-AMYLASE FORMATION IN ENDOSPERMS PREPARED FROM "TOP CUT" GRAIN HYDRATED WITH BUFFER THEN DOSED WITH GA_3 AND INCUBATED AT 14·4°.

Experiment (a): dosing with buffer, --o--; or GA_3 in buffer (60 mμg/endosperm),--△--.
Experiment (b): Dosing with buffer, —●—; or GA_3 in buffer (60 mμg/endosperm), —▲—.

[38] P. W. BRIAN, H. G. HEMMING and D. LOWE, *Nature* **193**, 946 (1962).
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grain incubated at 25° (Fig. 6). These times should be compared with the results obtained with grain hydrated by steeping before the removal of embryos, and the application of GA_3 (Fig. 11).

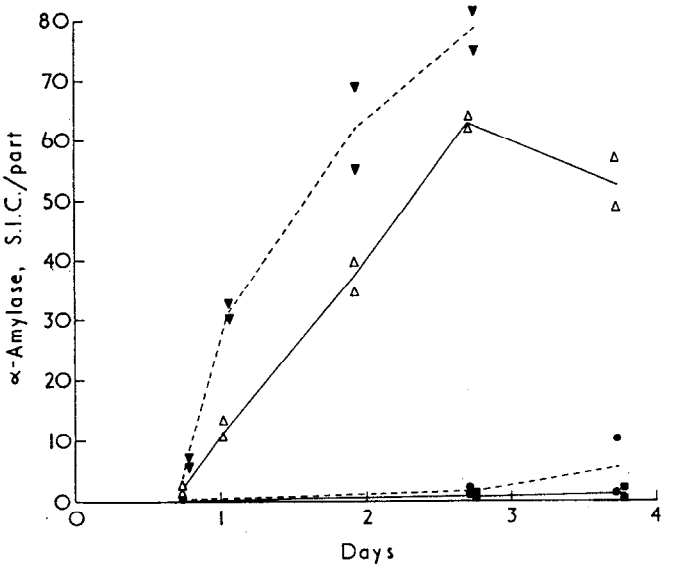

FIG. 6. EXPERIMENT AS IN FIG. 5, BUT INCUBATING AT 25°.

Experiment (a): buffer, - - -●- - -, GA_3 in buffer (60 mμg/endosperm), --▼--. Experiment (b): buffer, —■—, GA_3 in buffer (60 mμg/endosperm), —△—.

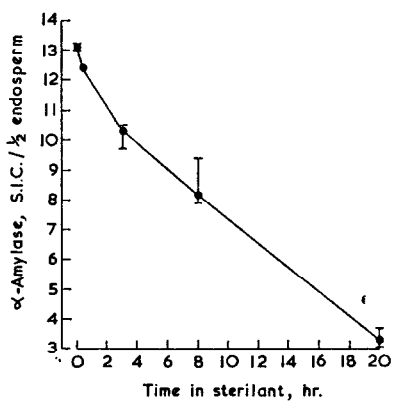

FIG. 7. EFFECT OF EXPOSING GRAINS TO SODIUM HYPOCHLORITE SOLUTION ON THE RESPONSE OF DERIVED ½ ENDOSPERMS TO GA_3.

Grains were immersed in 10% commercial NaClO for the time shown, washed then steeped at 14·4° for the remainder of a 20 hr period. Half endosperms were then prepared and groups of five were shaken in succinate buffer, 2 ml, containing $CaSO_4$ and GA_3, 50 μg/ml, pH 6·0, for 48 hr at 25°. α-Amylase was assayed in the incubation mixture. Each point is the mean of three determinations; scatter of results shown.

In both sets of results (Figs. 5 and 6), it is noticeable that the quantity of α-amylase produced was different for each of two experiments, and that in one "control run" α-amylase was

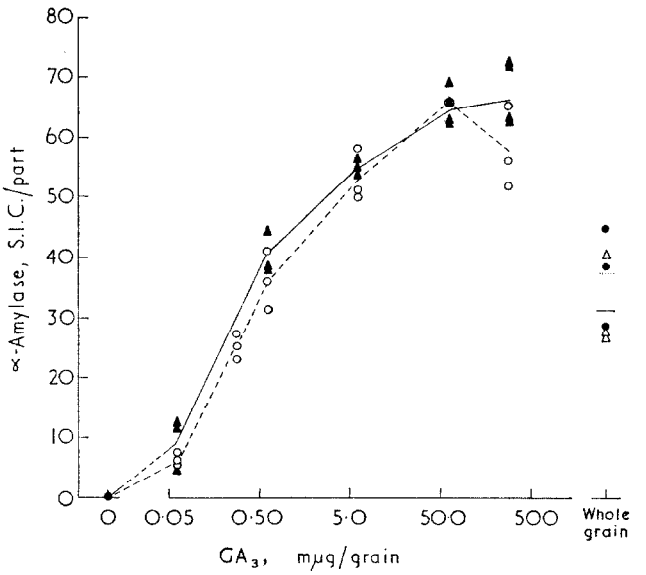

FIG. 8. α-AMYLASE PRODUCED BY ENDOSPERMS, EITHER PREPARED FROM STEEPED GRAIN, IN RESPONSE TO VARIOUS DOSES OF GA_3, AT 14·4°, OR PREPARED FROM WHOLE GRAINS AT THE END OF THE GERMINATING PERIOD.

Grain was steeped as in "malting" then a sample was deembryonated after the final draining. These endosperms were incubated for 24 hr at 14·4°, then dosed with GA_3 and incubated for a further 72 hr before assaying the α-amylase content. Another sample of the whole grain was malted for the full 96 hr the embryos were removed and the α-amylase contents of the endosperms were measured.

Experiment (a): dosed endosperms, —▲—; endosperms from whole, germinated grains, △—.
Experiment (b): dosed endosperms, --o--; endosperms from whole, germinated grains, ●--.

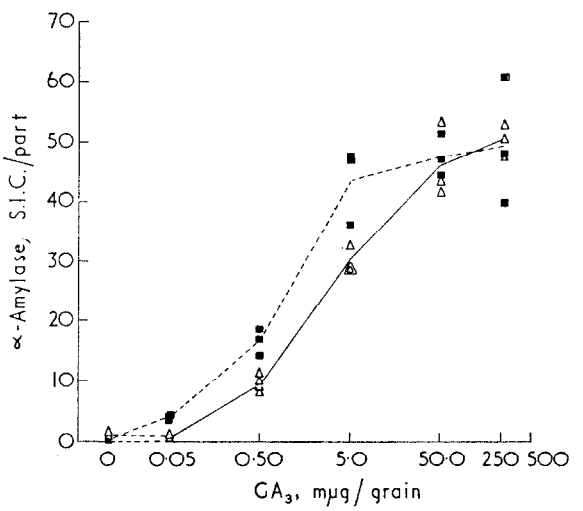

FIG. 9. α-AMYLASE PRODUCED BY ENDOSPERMS, DEEMBRYONATED BEFORE HYDRATING IN BUFFER, IN RESPONSE TO VARIOUS DOSES OF GA_3, AT 14·4°.

"Top cut" grains were deembryonated, hydrated in succinate buffer, 18 hr, 14·4°, then dosed with GA_3 and incubated, 72 hr, 14·4°.

Experiment (a): —△—; (b): --■--.

detected after 3 days' incubation. The high blanks were attributed to the production of α-amylase by contaminating micro-organisms and so in the repeat trials exposure to the sterilant, sodium hypochlorite, was prolonged; the blanks remained low, but the quantity of enzyme formed in the presence of GA_3 was depressed. Exposure of grain to hypochlorite increasingly reduced the ability of the endosperm to respond subsequently to GA_3 (Fig. 7) and so the grains in the previous trials were probably "over-sterilized" (Figs. 5 and 6).

It has been reported that for up to about 24 hr the longer the endosperms have been hydrated the more rapidly they form enzymes in response to GA_3.[41–43] Further, if the endosperm is hydrated before deembryonation rather than afterwards, the response to GA_3 is even more marked.[41,42] This second effect has been attributed to a factor diffusing from the embryo which can potentiate enzyme synthesis. As this postulated "embryo factor" would be involved in sensitizing the enzyme-producing systems of the endosperms of malting grain,

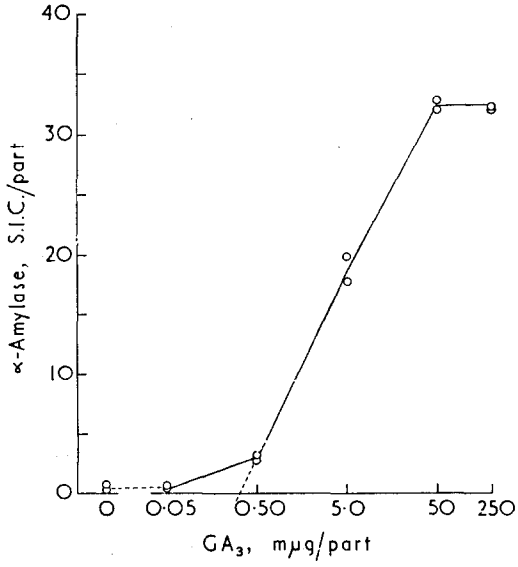

FIG. 10. EXPERIMENT AS IN FIG. 9, BUT INCUBATING FOR 42 hr AT 25°.

but would not have influenced the results shown in Figs. 5 and 6, experiments were performed to determine the effects of doses of GA_3 on endosperms in a physiological state closer to that of the malting grain when the endogenous "gibberellin" first reaches the endosperm. Grain was sterilized and steeped as in malting, then the embryos were removed after the second 1-hr draining period. After incubation for 24 hr at 14·4° the endosperms were dosed with known amounts of GA_3 in measured microdrops of buffer; this dosing time corresponded approximately with the time of the rapid increase in the "enzyme-forming potential" in malting grain (Fig. 3). The endosperms were incubated for a further 72 hr at 14·4° before the α-amylase content was assayed. These samples showed a greater response to given doses of GA_3 (Fig. 8) than did those deembryonated before steeping (Figs. 9 and 10). As an appreciable response was found to 0·05 mμg GA_3 (Fig. 8), this method could be the basis of a sensitive technique

[41] C. PETRIDIS, R. VERBEEK and L. MASSART, *J. Inst. Brewing* **71**, 469 (1965).
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for detecting and measuring gibberellins. Whole grains were malted for the entire 96 hr period and the α-amylase content of the endosperms was measured (Fig. 8). The average values obtained were equal to the amount of α-amylase induced in endosperms dosed with 0·25 mμg GA_3 in one experiment and 0·6 mμg GA_3 in another. This latter value is close to the quantity of "gibberellin" detected (by the lettuce bioassay) in the endosperms of grains malted for 2 days (Table 1), that is when the level of "gibberellin" is near a maximum (Fig. 1). In view of the differing sensitivities of lettuce hypocotyls and barley endosperms to GA_1 and GA_3 this agreement is consistent with the suggestions (*a*) that "gibberellin" is predominantly GA_3, and (*b*) that the maximum quantity of "gibberellin" that occurs in the endosperm may be approximately bioassayed by this technique.

Time course experiments with grains that were deembryonated before hydration showed a lag time of 19–26 hr between the application of the GA_3 and the production of a measurable amount of α-amylase at 14·4° (Fig. 8), while grains that were steeped before being deembryonated, and incubated 24 hr before applying the GA_3, showed a shorter lag time (14–18 hr) and a greater enzyme production in a fixed time (Fig. 11). This more rapid response to GA_3

TABLE 1. DISTRIBUTION OF GIBBERELLIN-LIKE MATERIAL IN BARLEY MALTED FOR DIFFERENT PERIODS

Days germination, after steep	"Gibberellin" content (GA_3 equivalents, mμg/part)		
	Embryo	Endosperm	Whole grain
0	0·11	0·08	0·19
1	0·09	0·11	0·20
2	0·29	0·62	0·91

Each value is the average of two estimations using the lettuce hypocotyl bioassay. Grain was germinated as for Fig. 1.

of endosperms prepared from grain hydrated with the embryo still attached, might be attributed to the action of the hypothetical "embryo factor".[41, 42] This 14–18 hr lag agrees with that found between the peak "gibberellin" content and the maximal rate of α-amylase production (Fig. 1) and also with the difference in time between the first appearance of α-amylase in the whole grains and the "enzyme-forming potential" of samples of grains deembryonated at intervals and incubated for a further period (Fig. 2). The times cannot be determined precisely from Fig. 2 as the initial levels of α-amylase are very small; however, a difference of 14 hr was found between the earliest detection of α-amylase in the whole grain and the average of the times "α-amylase-forming potential", which we ascribe to the arrival of "gibberellin", was first detected in the incubated endosperm (Fig. 2). The doses of GA_3 used in the various time course experiments, 0·6 mμg or 60·0 mμg per endosperm, approximated to the level of "gibberellin" found in the endosperm in malted grain after 2 days' germination (Table 1), and to a dose that caused a maximum production of α-amylase (Fig. 8).

Thus it seems that the synthesis of α-amylase in "malting" barley is controlled in the main by the gibberellin-like material. This "gibberellin" appears to be predominantly GA_3 throughout the germination period.

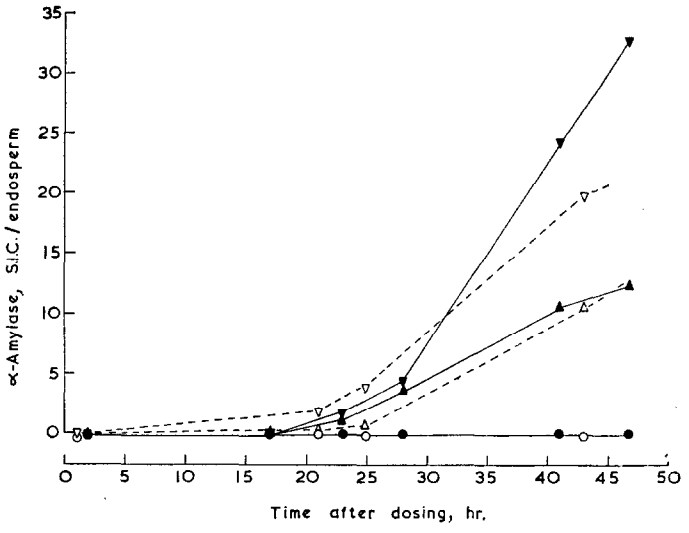

FIG. 11. TIME COURSE OF α-AMYLASE PRODUCTION BY ENDOSPERMS, PREPARED FROM STEEPED GRAIN, IN RESPONSE TO GA_3, 14·4°.

Endosperms were prepared as for Fig. 8 then dosed with buffer or GA_3 in buffer; samples were taken at intervals and assayed.

Experiment (a): buffer, —o—; GA_3 (0·6 mμg/endosperm), --△--; GA_3 (60·0 mμg/endosperm), --▽--. Experiment (b): buffer, —●—; GA_3 (0·6 mμg/endosperm), —▲—; GA_3 (60·0 mμg/endosperm), —▼—.

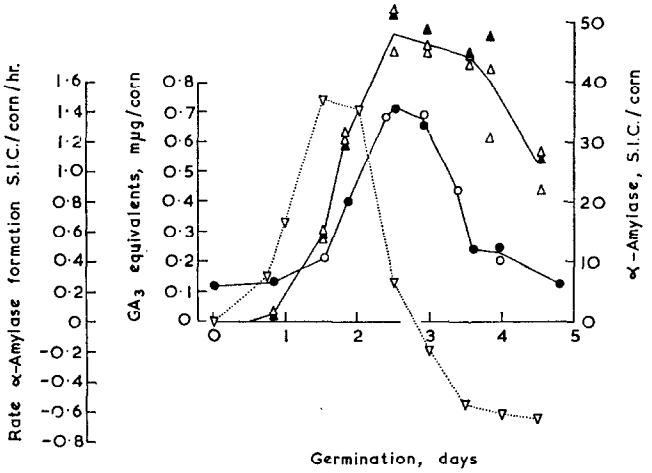

FIG. 12. CHANGES IN α-AMYLASE AND GIBBERELLIN-LIKE MATERIAL IN GRAIN GERMINATED ON MOISTENED FILTER PAPER, AT 25°.

Gibberellin-like material (two experiments), —o—●— α-amylase (two experiments),—△—▲—. Rate of α-amylase formation (calculated), ...▽.....

Results with Grain Germinated on Moistened Filter Paper

To throw light on previous experiments[1–3] "gibberellin" and α-amylase were followed in barley germinated on moistened filter paper at 25° (Fig. 12). The rate of α-amylase synthesis

rose to a maximum, fell rapidly, and was succeeded by a period of destruction, as expected (Fig. 12). However, the occurrence of the maximal value of α-amylase before the greatest level of "gibberellin" was totally unexpected; work is in progress to try and provide an explanation for this apparent anomaly.

EXPERIMENTAL

Preparation of Grain

Two-row barley, *Hordeum distichon* L., var. Proctor, was graded for width on slotted sieves. Unless otherwise stated "middle cut" barley was used, i.e. grain passed by 2·8 mm slots but excluded by 2·5 mm slots; occasionally "top cut" barley, i.e. grain excluded by the 2·8 mm slots, was used as indicated in the text. Grain was decorticated with 50% H_2SO_4.[1] In all experiments decorticated grain was sterilized by immersion for 10 min, usually with reductions in pressure to displace air bubbles, in 10% commercial NaClO solution (10–14% available Cl) in water, then rinsed at least ten times with sterile distilled water. Faulty corns were detected by brief immersion in I_2/KI solution between the first two water washes, in all small-scale experiments.[1] All manipulations were made under aseptic conditions.

Culture Conditions

For germination on moist filter papers at 25°, 600 corns were placed in each of six plastic bowls containing three Whatman No. 1, 27 cm filter paper discs moistened with sterile water, 100 ml, and covered with aluminium foil. "Malting" involved "steeping", i.e. soaking sterile corns twice for 24 hr at 14·4° in an excess of distilled water, with a drain of 1 hr after each steep. The water had been autoclaved, and it covered the grain to a depth of 5–8 cm, so access of oxygen was restricted. The steeped corns were germinated in a layer 3–5 cm deep in a covered glass vessel at 14·4°—"malting conditions"—and were frequently stirred to prevent the roots matting.

Endosperms were usually incubated on racks, i.e. microscope slides or drilled pieces of PTFE sheet, held in Petri dishes lined with wet filter paper. In one set of experiments, Fig. 3, the endosperms were kept with malting grain. All germinations and incubations were carried out in atmospheres of high humidity.

Dosing Individual Barley Endosperms

Endosperms, i.e. starchy endosperms, aleurone layers and testa, were prepared by lifting the embryo and scutellum from sterilized grain under aseptic conditions. GA_3 was dissolved in cold 20-mM sodium succinate buffer, pH 6·0, containing 20-mM $CaSO_4$. This buffer was also used to hydrate endosperms in some experiments. The gibberellin solutions were sterilized by passage from syringes through Millipore filters, supported in Swinnex holders, via hypodermic needles into sterile "bijou" bottles, closed with rubber septa.

To place microdrops of gibberellin solutions, 1 μl or another known volume, onto the "scuttellar depression" of the endosperm a microsyringe, 5 μl capacity, was used. A sterile, disposable length of Portex PP30 tubing was fitted tightly on the needle of the syringe so that the gibberellin solutions did not enter the microsyringe. The volumes delivered were checked by weighing.

Extraction and Chromatography of Gibberellin-like Material from Barley

The method selected was as follows: A sample of 250 or 350 corns was homogenized with methanol (80 ml), allowed to stand 4 hr then filtered. The residue was re-extracted and the combined methanolic extracts were concentrated by rotary evaporation to 15–20 ml at 25°. Water (25 ml) was added and concentration was continued to 25 ml. This aqueous solution was adjusted to pH 7·5 ($NaHCO_3$ saturated solution), and extracted with ethyl acetate (4 × 25 ml) to separate the basic and neutral materials. The aqueous phase was adjusted to pH 2·5 (2 N HCl) and extracted with ethyl acetate (6 × 30 ml). This ethyl acetate was pooled, washed once (H_2SO_4, 1%) and concentrated to 10 ml, giving a solution of acidic materials. Aliquot portions were fractionated by descending chromatography using Whatman No. 1 paper and *n*-butanol:ethanol:0·880 ammonia:water, 40:11:2:7 (v/v).

Bioassay of Gibberellins

The lettuce hypocotyl bioassay was based on the method of Frankland and Wareing.[44] Seeds of *Lactuca sativa* var. Arctic King were germinated in the dark at room temperature on moistened filter paper. Seeds, usually twenty, showing root tips of uniform length were spread evenly on segments of chromatograms, or on chromatography paper moistened with distilled water or standard solutions of GA_3 (3·0 ml; 0·01; 0·10; and 1·00 μg/ml), in crystallizing dishes, 6·5 cm dia. Germination continued for 3 or 3½ days in a moist atmosphere

[44] B. FRANKLAND and P. F. WAREING, *Nature* **185**, 255 (1960).

at 27°, 35 cm below a light source of three 40-W daylight fluorescent tubes and six 15-W pearl tungsten bulbs arranged to give uniform illumination. At the end of the incubation period the length of the hypocotyls were measured to the mearest millimetre. It was found that 0·01 μg GA_3/ml gave a response that was significantly different from the water blank.

Estimation of α-Amylase

Our standard method of enzyme extraction and assay was used.[45] Grain samples were sometimes stored frozen at −18° before the enzyme was extracted.

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[45] D. E. Briggs, *J. Inst. Brewing* **73**, 361 (1967).