to block the effect of a hormone upon one receptive tissue, but has no apparent effect upon its action on a second receptive tissue. We are led to one of two alternatives. Either the actions of this hormone upon these two tissues are fundamentally different, or the hormone exerts the same basic action on both tissues; but because of the biochemical uniqueness of the respective tissues, the ultimate physiological expressions of its action are entirely different in the two tissues. The latter possibility seems more likely in the case of parathyroid hormone.

There is considerable evidence which indicates that the fundamental action of this hormone is upon the translocation of ions across cellular and subcellular membranes (*3*). Kroeger has recently found that changes in gene activity, or at least chromosomal morphology, can be brought about by changes in the ionic environment of cells (*14*). The link between altered ion transport and gene activation in the present circumstance is unknown.

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16 March 1964

## Wheat Gibberellins

Abstract. *Two "gibberellin-like" substances were present in malted wheat, compared to only one in sound, unmalted wheat. Alcoholic extracts of wheat malt fractionated by paper chromatography and bioassayed for gibberellin-like activity by three methods, indicated a new gibberellin in malted wheat. This component increased particularly in the embryo section of the sprouted wheat during the first 3 to 4 days of germination.*

Substances inducing responses in plants similar to gibberellic acid have been isolated from many of the higher plants (*1*) including barley (*2*), wheat (*3*), and other grasses (*4*). While some plants contain more than one gibberellin (*1*), only one has been reported for wheat. We have isolated from malted wheat a second compound with gibberellin-like properties which differs chromatographically from the one previously reported (*3*).

Wheat malt was extracted with 80 percent methanol, 80 percent ethanol, or 50 percent acetone for 24 hours at 23° to 25°C. Extracts were partially purified by the ethyl acetate-buffer procedure (*3*). The concentrated extracts were chromatographed on paper with solvent systems as follows: *n*-amyl alcohol, pyridine, and water, 35:35:30; *n*-butanol and 1.5*N* ammonium hydroxide, 3:1; and *n*-butanol, acetic acid, and water, 95:5:30. The papers were dried, treated with sulfuric acid, and exposed to ultraviolet light to detect the gibberellin (*5*). Other similar papers were cut to appropriate sections at right angles to the flow of the solvents, and the gibberellins were extracted with ethanol.

Bioassays for gibberellin-like activity were of three types: the dwarf pea seedling test, wheat-leaf section test (*3*), and a wheat endosperm test. The wheat endosperm assay was based on the effect of gibberellins on the formation of alpha-amylase in wheat endosperm. Distal halves of wheat were incubated for 2 days between filter paper moistened with aqueous solutions of the eluated materials. Contamination by fungi was prevented by including formaldehyde (0.05 percent) in the liquid. Endosperm pieces were tested for alpha-amylase by placing them on starch-agar gels and incubating them for 18 hours at 30°C (*6*).

The $R_F$ values obtained for gibberellic acid with the three solvent systems employed agreed very closely with those reported previously (*5*). Faint fluorescent spots (at $R_F$ 0.85) were observed on a few chromatograms of ethanolic extracts of malt when the butanol–acetic acid–water solvent system was employed, but could not be detected routinely in agreement with Radley (*3*) and Phinney *et al.* (*5*).

Pea seedlings receiving treatment by the chromatographic fractions having $R_F$ values of 0.2 to 0.3 and 0.8 to 0.9 and gibberellic acid exhibited significantly more growth than the control seedlings or those treated with the other chromatographic fractions. This was emphasized particularly by the 5th day of growth of the pea seedlings. The chromatographic mobility of gibberellic acid was identical to that fraction having $R_F$ of 0.8 to 0.9 and agrees with that previously reported (*3*). The active fraction having $R_F$ of 0.2 to 0.3 has not been previously reported and was found only in malted wheat. However, the amount isolated appeared to be less than that of the fraction having $R_F$ of 0.8 to 0.9.

The presence of two active components in ethanol and methanol extracts was also demonstrated by the wheat-leaf section test. A single gibberellin-like substance, having an $R_F$ equivalent to gibberellic acid was detected in the acetone extract. The

Table 1. Comparison of chromatographic mobility ($R_F$) of biologically active gibberellin-like fractions of wheat malt extracts and gibberellic acid. The solvent systems were: A, *n*-butanol and 1.5*N* ammonium hydroxide, 3:1; B, *n*-amyl alcohol, pyridine, and water, 25:25:30; C, *n*-butanol, *n*-acetic acid, and water, 95:5:30. F, fluorescence; PS, pea seedling; WL, wheat leaf; WE, wheat endosperm; x, slow-moving component; y, fast-moving component.

| Test employed | Solvent system | | | | |
|---|---|---|---|---|---|
| | A | B | | C | |
| | | x | y | x | y |
| *Gibberellic acid* | | | | | |
| F | 0.25 | | 0.60 | | 0.85 |
| *Ethanol extract* | | | | | |
| P S | 0.25 | 0.19 | 0.62 | 0.26 | 0.85 |
| W L | 0.25 | 0.17 | 0.62 | 0.24 | 0.84 |
| W E | 0.27 | 0.17 | 0.62 | 0.24 | 0.86 |
| *Methanol extract* | | | | | |
| P S | 0.24 | 0.17 | 0.63 | 0.24 | 0.83 |
| W L | 0.26 | 0.15 | 0.59 | 0.23 | 0.85 |
| *Acetone extract* | | | | | |
| W E | 0.27 | 0.15 | 0.63 | 0.25 | 0.85 |
| P S | 0.27 | | 0.63 | | 0.86 |
| W E | 0.25 | | 0.61 | | 0.85 |
| W L | 0.27 | | 0.61 | | 0.83 |

amount of the gibberellin having an $R_F$ of 0.8 to 0.9 obtained from the acetone extract was somewhat less than that found in comparable fractions of ethanol and methanol extracts.

The wheat endosperm test was the simplest procedure for detecting gibberellin-like activity and was used routinely during the current investigation. Results obtained through the use of this assay confirmed those obtained by pea-seedling and wheat-leaf assays. Two gibberellins were detected when ethanolic and methanolic extracts were examined for gibberellin-like activity.

A summary of the results obtained by the use of the various extraction, chromatographic, and assay procedures are given in Table 1. The $R_F$ value for the component having the major share of the gibberellin-like activity was similar to that for gibberellic acid, regardless of the chromatographic solvent system employed. The second gibberellin-like fraction was separated by the amyl alcohol–pyridine–water and butanol–acetic acid–water solvent systems but could not be separated by the butanol and ammonium hydroxide solvent. The "second" gibberellin was not extractable with 50-percent acetone.

The wheat gibberellin that resembles gibberellic acid in its chromatographic mobility was found in wheat and malted wheat. The slower-moving gibberellin was found only in malted wheat and in larger amounts in the embryo end of the sprouted seed and in the outer layers of the seed, presumably in the aleurone. Both gibberellins appear to increase in quantity during the first 3 or 4 days of germination.

According to Harris (*7*) one of the principal roles of the embryo during the early stages of germination is secretion of a gibberellin-like hormone into the endosperm and aleurone portions of the seed. The apparently greater amounts of gibberellin, particularly of the new substance, in the embryo and adjacent areas at the start of germination in malted wheat would support Harris's hypothesis.

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24 February 1964

## Interferon Production in Chickens Injected with *Brucella abortus*

Abstract. *Intravenous injection of large numbers of live, virulent* Brucella abortus *in chickens resulted in the appearance in the serum of a viral inhibitor indistinguishable from interferon. Inhibitor was detected as early as 3 hours after inoculation of brucellae and reached a peak between 6 and 12 hours.*

The antiviral substance, interferon, is produced by vertebrate cells in response to infection with different viruses (*1*). The nature of the essential stimulus that induces cells to make interferon is still unknown. To test the possibility that intracellular infectious agents other than viruses may induce the production of interferon, experiments were designed in which *Brucella abortus* was used instead of virus.

A modification of the procedure for the production of circulating interferon in intact animals by the intravenous injection of viruses was used (*2*). White Leghorn roosters about 1 year old were injected in the cubital vein with $10^{10}$ live *Brucella abortus* organisms in a 2-ml volume.

The virulent, $CO_2$-requiring ES strain (*3*) was grown on tryptose agar for 48 hours and suspended to the desired concentration in nonpyrogenic physiological saline solution. Blood samples were obtained from individual chickens by cardiac puncture at different times after inoculation with bacteria, and serums were assayed for inhibitor activity by the plaque-reduction method (*4*) in primary chick embryo cultures. Cultures in 60-mm petri dishes were exposed for 20 hours to 3 ml of twofold serial dilutions of serum and then challenged with 40 to 60 plaque-forming units of vesicular stomatitis virus. The titers of inhibiting substances were calculated from the dilution of the serum which reduced the plaque count to 50 percent of that of the controls; the titers were expressed as inhibitor units per 3 ml of serum.

Figure 1 shows that antiviral activity was detected as early as 3 hours after inoculation of brucellae and rose steadily, reaching a peak at about 12 hours; by 24 hours the inhibitor activity was much reduced, and no activity was detectable 48 hours after inoculation. The properties of this inhibitor were compared to those of an interferon in allantoic fluid from chick embryos infected with the WS strain of type A influenza virus (*4*). The properties of the brucellae-induced inhibitor in chicken serum were as follows. The inhibitor was nondialyzable, stable at *p*H 2.6 for at least 24 hours, and stable to heating at 56°C for 1 hour; it did not sediment at 105,000*g* for 1 hour, and it was inactivated by incubation for 3 hours at 37°C with crystalline trypsin (50 $\mu$g/ml). Serum containing inhibitor activity was incubated with $10^5$ plaque-forming units of vesicular stomatitis virus for 1 hour at 37°C and then diluted to test for infectivity. No inactivation of virus was detected, indicating that the action of the inhibitor is not on the virus directly but on the cells. These properties of the serum inhibitor are identical to those of interferon produced in chick embryo cells upon stimulation by virus.

In order to determine the minimum number of brucellae required to produce detectable inhibitor in the circulation, varying numbers of bacteria were injected intravenously and serums were obtained at 6 to 8 hours after inoculation. In addition, the capacity of heat-killed brucellae to induce inhibitor production was also tested. While $10^{10}$ live brucellae injected intravenously produced significant amounts of inhibitor in the circulation, $10^8$ or fewer bacteria failed to produce a detectable response. Heat-killed brucellae, even when $10^{10}$ organisms were inoculated, failed to elicit any response. The