STUDIES ON HORMONES

I. Effect of Hormone Injections of the Spikes of Wheat on the Metabolic Activities of the Leaves

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PREFACE
THE broad title of the paper "Studies on Hormones" under which it is proposed to publish a series of papers from this laboratory, signifies the use of substances both of plant origin as well as chemical substances which effect plant metabolism in very minute amounts. The present paper, first of the series, deals with the effect of the hormones of the spikes of wheat on the metabolic activities of the plant. The work was carried out at four different reproductive phases of the plant, namely, (a) young spikes prior to emerging out of the leaf-sheath, (b) pre-anthesis, (c) anthesis, (d) post-anthesis, i.e., 15 days after pollination. The following metabolic studies of the leaves at these four stages were studied, namely (1) Respiration, (2) Total nitrogen, (3) Amino nitrogen, (4) Sugars.

MATERIAL AND METHOD
Vijaya (Ranjan) wheats were used throughout the experiments. These plants were grown in the fields of the Botanical Garden.

Respiratory studies were made by bubbling air, issuing from the respiratory chamber, into petten-kofer tubes previously filled with baryta. The respiratory chamber was in its turn kept in a Hearson's electric incubator maintained at a temperature of ±35°. The current of air was automatically switched, after every 3 hours, into the next petten-kofer tube by the help of Blackman's air current commutator. Each tube was then lifted from its frame, washed and titrated against standard HCl. The amount of CO₂ given out by the leaves was then calculated.

Hormone extraction
About 20 gm. of spikes, each lot of a particular age group as mentioned before, were brought from the experimental field and were kept in the freezing chamber of the frigidaire overnight. They were then crushed to a moderate fineness. Ether was then added equal to 20 times the weight of the crushed material. The material was allowed to remain in ether overnight. The
following day the material was filtered and the filtrate was distilled off under reduced pressure. When all the ether was thus removed, 150 c.c. of distilled water was added to the residue and shaken thoroughly. This solution contained the crude hormone.

_Injection_

Leaves approximately of the same age were brought from the field with their excised bases dipping in a beaker of water. The control set and the experimental sets were then separately weighed and then injected. The method of injection was to dip the leaves in distilled water or the hormone solution, as the case may be, kept in a tube. The air then was removed by connecting it with a hand worked Garyk Vacuum Pump. When most of the air was withdrawn the negative pressure was slowly released to allow the liquid to get injected in the inter-cellular spaces. It was noticed by the senior author in Cambridge that about 13% of oxygen enters the injected leaves. Thus, though there is undoubted restriction in the flow of gases, there is a sufficient supply of oxygen to the cells to maintain them above the anaerobic level.

_Total Nitrogen Estimation_

The estimations of total nitrogen of the leaves, at four different stages, were done by Kjeldahl’s method. Amino-nitrogen was estimated by the help of Vanslykes apparatus—with minor modifications.

_Sugars_

Sugar estimation was done by Somogyi’s method with minor modifications. The following sugars were estimated: Mono and Di-saccharides, and total sugars after hydrolysis.

_Observations_

_Respiration._—The first respiratory studies were undertaken on the leaves of the first stage, which is graphically depicted in Fig. 1. The respiration starts at 30 mg. CO₂ and drops off to about 18 mg. in 24 hours. This is the usual type of floating respiration. Fig. 2 gives the respiratory rate of leaves, at the second stage, _i.e._, the pre-anthesis stage of the spikes. The curve of the continuous line shows the respiratory rate of leaves injected with hormone while the dotted line depicts the control. The difference in the respiratory rate is noticeable only during the first 9 hours and that too is not very pronounced. On the other hand, the respiratory rate at the 3rd stage (see Fig. 3) shows a significant difference between the experimental and control sets. For, whereas in the experimental set the respiration
Studies on Hormones—I

Fig. 1. Stage I: before flowering (leaves injected with distilled water)

Fig. 2. Stage II: Pre-anthesis

Fig. 3. Stage III: Anthesis.
starts at 28 mg. CO₂ per 30 gr. hours in the control set it starts at 16. This difference, however, narrows down with time. There is a significant change in the shape of the respiratory curve at the 4th, i.e., final stage. Unlike the

![Diagram 4](image)

**FIG. 4.** Stage IV: Post anthesis

![Diagram 5](image)

**FIG. 5.** Injection of leaf extract
previous case where the respiration of the experimental set started high, the respiration in this case started near about the level of the control set. However, the respiration gradually mounted up to reach a peak value at the end of the 12th hour. Thereafter it slowly dropped off (see Fig. 4). Fig. 5 shows the respiratory rate of leaves injected with no hormones from the spike but with an extract of the crushed leaves. The extraction was done in a manner similar to the extraction of the spike hormone. This was done only to find out whether any extract injected into the leaves effected its respiration. No noticeable effect could be recorded.

**Total Nitrogen.**—The study of total nitrogen reveals that during the first stage, that is before flowering the total nitrogen of the leaves is high as shown by Fig. 6. Thereafter there is a rapid fall in the two subsequent stages while at the 4th and final stage it again rises. On the contrary the sugar fractions at different stages of the wheat plant reveals (Fig. 7) that whereas the hexoses increase in the third and fourth stages, there is a definite fall in the sucrose and the total content in the second and fourth stage. While this fall is not noticeable at the third stage.

The analysis of the sugars in relation to hormone injection (Fig. 8) shows that, inversely, as the hexose increases 24 hours after injection, there is a fall of the sucrose and total sugars,
A very noticeable feature of hormone injection is the variation in the amino N₂ as is shown in Fig. 9. The amino nitrogen before injection is slightly less than 0·07 gm. per 100 gm. While 24 hours after injection of the hormone it increases to 0·11 gm.
DISCUSSION

According to Sande Bakhuysen⁴ fertilization in wheat is a critical stage in the life of the plant. At gametic union growth hormones, produced in the inflorescence, no longer leave the flowers. He further goes to say that this is responsible for the catabolic impulse which depresses the rate of respiration and moisture content, hastens senescence and final death of the plant. But this somehow appears to be a contradictory statement. Because if the hormones of the spikes are unable to pass downwards to the leaves how is it that they control the catabolic impulses. Murneek¹ has also demonstrated that developing flowers and fruits have a marked effect on the metabolism of the plant and that they exert a certain amount of physiological control. The point, which so far is not very clear is, whether such control is evident only after reproduction has sufficiently progressed towards the point of completion, that is seed formation, or whether the effect is noticed at the earlier stages of reproduction. Again whether the effect is of one type at the earlier stages and a reverse type at the later stages of seed formation.

Soding⁵ believes that it is the young inflorescence which controls the growth of the flower stem.

In a more recent work Wittwer⁶ is of opinion that intervals of maximal increase in periods of greatest vegetative extension, accumulation of dry matter, etc., follows closely the beginning of syngamy in the flower bud and
embryo initiation in the young fruit. While agreeing generally with Wittwer's findings, in as much as that syngamy is an important stage, the injection of spike hormones in the leaves, leaves no room for doubt that the reactions are of a more complicated nature. On the one hand it is very clear from Figs. 2 and 3 that the production of the hormone is almost negligible upto the pre-anthesis stage; on the other at the mature stage or the stage when pollination has just taken place (Fig. 3), there is copious production of the hormone. It is not very clear that at this stage the abscission layer, at the base of the spike, is formed (anatomical studies will be necessary) for if it were so then neither water nor food material would travel from the leaves towards the spike. It should be remembered that the spikes are still green and the development of the young embryo is still to take place. Then how is it that the respiration rate of the leaves at this stage keeps at a low level. It is but logical to conclude that if no abscission layer is formed at this stage then the spike hormones should travel down and by the increase of hydrolysis facility, augment the respiration rate. Yet this does not happen. It is possible that the young spikes at this stage are having intense meristematic activity and all the hormones that are produced are utilized to this end in the spike itself. Pal's work at Rothamsted has shown that the green spikes also manufacture most of their carbohydrates. This will indirectly confirm the view that the hormones are so utilized in the spikes, and none become available for the leaves; and, therefore, the respiration keeps low (see Fig. 3). Further evidence is shown by the injection of the spike hormone to the leaf which causes rapid increase of the respiration rate, so that, if the hormone had travelled down, increased rate of respiration would have taken place. Another significant fact that emerges out is, that with the lapse of time, the effect of hormones wears out and at the end of 24 hours the respiration almost comes down to the level of the control set. This proves that to keep the respiration rate high, there must be a continuous production of the hormones. At the late mature stage, that is the fourth stage, the action of the hormones seems to be different. Here the effect is noticeable sometime after the injection, and, with time, there is a progressively increasing effect; the peak value being reached after a lapse of 9 hours. After this period the curve again falls off. This delayed action may be due to increased difficulty in the entry of the hormone extract, from the inter-cellular spaces into the paranchymatous cells within.

With regard to the formation of the abscission layer it will be more logical to assume that this layer is formed not at the 3rd but at the 4th stage, when the development of the embryo has gone far ahead and food and water are no longer required, at the region of the spike to the same extent. The
respiration rate of the leaves, at this stage, is the lowest. The total sugar is also less (see Fig. 7) though total N₂ is high.

That the hormones of the spikes have a profound effect in augmenting the metabolic rate is seen from Fig. 10. Here the continuous line represents the respiration rate during the first three hours of the 4 different stages, and the dotted line represents the rate at the end of 24 hours of these stages. Here at the pre-anthesis stage the respiration is lowest. It rises at anthesis period, which synchronises within maximum hormone production. Here too the total sugar is high (see Fig. 7). Due to senescence and the consequent fibrosis of the cells, the entry of hormones is delayed and only after 24 hours, indicated by dotted line in Fig. 10, its action is felt and the respiration rises.

Fig. 10. Continuous line represents the initial respiration of four different stages
Dotted line represents respiration rate of four stages at the end of 24 hours
(Values taken from Figs. 1-4)

The effect of the injection of hormones upon the amine acid and sugar contents of the leaves also supports this. Fig. 8 shows that the amino-acids rapidly rises in 24 hours. We may, therefore, assume that the hormones increase hydrolysis facility of the proteins. This will explain the low values of total N₂ at the anthesis and post-anthesis stages; for these stages synchronise with the greatest hormone activity, which travelling downwards, towards the leaves, increase the hydrolysis of the proteins. The amino-acids so formed quickly get translocated to the growing spikes. Consequently the total N₂ of the leaves will fall. On the other hand, at the 4th stage, when the abscission layer has been formed translocation of the proteins are stopped, causing their accumulation in the leaves.
Further, these hormones not only increase hydrolysis facility of the proteins, but also of the carbohydrates (see Figs. 8 and 9). For, although the total sugars decrease, the hexoses increase. This is understandable in view of the increased respiratory activity, consequent upon hormone injection.

**SUMMARY**

1. Hormones of the spikes of wheat extracted at four different stages, *viz.*, *(a)* Young spikes enclosed within the leaf-sheath, *(b)* Pre-anthesis *(c)* Anthesis, *(d)* Post-anthesis, *i.e.*, 15 days after pollination, were injected into the leaves, of ages synchronising with the 4 stages of the plant.

2. The injections of the extracts at the first two stages did not bring about any significant difference in their respiratory activity. But the extracts at the two subsequent stages, *viz.*, Anthesis and Post-anthesis had a marked effect; with this difference that the extract at stage 3 brought an immediate enhancement of the respiratory activity while at the 4th stage it reached its maximal at the end of 24 hours.

3. The hormone extract increases the hydrolysis facility of both the proteins and carbohydrates.

**BIBLIOGRAPHY**


