more unique forms could be discerned with any degree of certainty. The same structures were found free and in association with cells in films of the serous surfaces of the liver, spleen, omentum, lungs and heart of mice succumbing after intraperitoneal inoculation while films prepared in the customary way of the transverse sections of these organs usually revealed nothing. They are difficult to find or identify in brain films.

(9) The neurolytic agent has a lower thermal death point (42–45° C. for 15 minutes) than is known for either pathogenic bacteria or mammalian viruses. Toxoplasma, under similar conditions, were not all killed at 45° C. for one half hour.

Tests with several thousand mice (inoculated with brain, blood or viscera or with normal broth) failed to reveal the presence of the neurolytic agent in the normal stock at the time that it was being isolated from the toxoplasma-infected tissues, nor has it been encountered among thousands of other mice of the same stock used in other experiments in the past three years. Furthermore, the properties of the agent are such that one can not at present conceive how it could possibly be transmitted naturally from mouse to mouse either by itself or through any insect vector. Experiments are still in progress on the possibility of a relationship between the neurolytic agent and toxoplasma, but any ultimate decision on its genesis, if that should ever be possible, must be postponed until further opportunity is given other investigators as well as ourselves to determine whether or not it may occur spontaneously in mice under conditions other than infection with toxoplasma.

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## INTERCELLULAR WOUND HORMONES PRODUCED BY HETEROAUXIN

WE have published investigations indicating that yeast[1] and animal tissue cells[2] injured by ultra-violet light and other means produce wound hormones which stimulate cellular proliferation. In the case of the wound hormone from yeast, we have been able to show that it probably contains adenine, guanine, pentose, phosphoric acid and possibly nicotinic acid, but not proteins or sulfur, and that it is thus similar to, but not identical with, coenzyme.[3]

[1] Fardon, Norris, Loofbourow and Ruddy, *Nature*, 139: 589, 1937; Sperti, Loofbourow and Dwyer, *Studies Inst. Divi Thomae*, 1: 163, 1937; Sperti, Loofbourow and Dwyer, *Nature*, 140: 643, 1937.

[2] Sperti, Loofbourow and Lane, SCIENCE, 83: 611, 1937; Loofbourow, Cueto and Lane, in publication.

[3] Cook, Loofbourow and Stimson, Tenth International Congress of Chemistry, Rome, Italy, May, 1938.

Leonian and Lilly[4] concluded from their investigations of the action of heteroauxin on various fungi, algae, etc., that it is a growth inhibitor rather than a growth promoter and that in instances in which it stimulates growth, its action is that of an irritant, leading to the increased production of growth substances by the plant cells.

We have studied the effect of heteroauxin on yeast, using the techniques employed in our wound-hormone experiments.[5] It was found to be toxic throughout a wide range of concentrations, and when yeast was subjected to it in toxic concentrations, wound hormones were produced.

In the toxicity determinations, methylene blue staining was used as a criterion of cell injury. *S. cerevisiae* standing in isotonic salt solutions containing heteroauxin in concentrations from 1:1000 to 1:100,000 showed an increasingly greater percentage of stained cells throughout the period of standing as compared with controls.

In the wound hormone experiments, suspensions of yeast at a concentration of 50 g per L. (wet weight) in isotonic salt solution were divided into two portions, to one of which heteroauxin was added in a concentration of 1:1000. After the suspensions had stood for 24 hours, heteroauxin was added in like concentration to the control suspension, both suspensions were centrifuged and the cell-free supernatant fluids were decanted and taken to dryness. These materials were made up in distilled water to approximately ten times their original concentration in the supernatant fluids, and tested for growth-promoting effect on yeast grown in rocker tubes for 24 hours.[5] The population densities at the end of this period were determined by a photoelectric method.[6] Heteroauxin alone, in the same range of concentrations in which it occurred in the yeast fluids, was added to a portion of the rocker tubes in each experiment.

The tubes to which heteroauxin alone was added showed less growth than controls. Those to which fluid from yeast which had stood with heteroauxin were added showed marked stimulation of growth, while those to which fluid from yeast standing in salt solution only was added showed little or no stimulation, depending upon the concentration of heteroauxin introduced with the fluid.

Separate experiments in which heteroauxin was added to rocker tubes in concentrations from 1:10 to 1:1,000,000 showed depression of growth except for some slight evidence of stimulation in the range near

[4] Leonian and Lilly, *American Jour. Botany*, 24: 135, 1937.

[5] Loofbourow, Dwyer and Morgan, *Studies Inst. Divi Thomae*, in publication.

[6] Loofbourow and Dwyer, *Studies Inst. Divi Thomae*, in publication.

1: 10,000. Since the rocker tube concentrations in the wound-hormone experiments were all above this range, and showed stimulation of a higher order, the stimulation obtained in these experiments can not be accounted for by the direct effect of heteroauxin on the yeast in the rocker tubes.

It is concluded that the effect of heteroauxin on yeast is consistent with the mode of action suggested by Leonian and Lilly for its effect on plant tissues.

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## CHANGES IN WHEAT METABOLISM CAUSED BY POWDERY MILDEW

INFECTION of wheat with the obligate parasite *Erysiphe graminis tritici* results in a gradual decline in vigor and growth, culminating, in cases of severe infection, in the death of the host. This effect of the mildew is accomplished in spite of the fact that the parasite does not penetrate beyond the epidermal cells, in which large-lobed haustoria are formed. Up to the present time it has not been possible to measure separately the respiration and fermentation of an obligate parasite and that of its host. We have been able to accomplish this, however, in the case of *E. graminis tritici*. By carefully scraping away all the wheat tissue except for the lower epidermis, the latter may be obtained as a strip a single cell thick, with the uninjured mildew attached. Measurements on 5 cm.$^2$ of normal epidermis give no respiration within the limits of the apparatus ($\pm$ 0.05 c. mm $0_2$/cm$^2$ surface per hour), while with a similar area of mildewed epidermis the oxygen consumption is 1.3 c. mm per cm$^2$ surface per hour. On removal of the mildew from the epidermis with a camel's hair brush the oxygen consumption disappears. Thus the oxygen consumption of mildewed epidermis is, within the limits of experimental error, a measure of the mildew respiration. In this way it is possible to distinguish changes which the mildew induces in the host respiration from gross changes in the host-mildew association.

Our experiments were carried out using a Fenn[1] volumetric micro-respirometer, at 22.0° C. Only the first leaf of normal wheat 15 to 25 days old was used. Within these age limits, respiration and fermentation of wheat are fairly constant. Normal wheat was heavily inoculated about 10 days after planting, and the first leaf used for experimental work 7 to 8 days later. The infected wheat was therefore 17 to 18 days old.

Pratt has shown in an article appearing recently[2] that in wheat inoculated with *Erysiphe graminis tritici* there is a rapid rise in the respiration to a value two to three times that of normal wheat of similar age. We have obtained similar results, as may be seen from the data in Table I. In these experiments the respiration of infected wheat was increased 250 per cent. above that of normal wheat. In addition, we have found that if the mildew is removed from the wheat with a camel's hair brush the respiration does not return to the level of uninfected wheat. This conclusion is borne out by measurements of the respiration of mildewed epidermis. One cm.$^2$ of infected epidermis consumes only 1.3 c. mm of $0_2$ per hour, which is much less than the increase in respiration produced in diseased wheat by the mildew (Table I).

TABLE I

| Tissue | c. mm $O_2$ consumed/cm$^2$ leaf surface/hour | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | Average |
| Normal epidermis | ±.05 | ±.05 | ... | .. |
| Mildewed epidermis | 1.17 | 1.61 | 1.21 | 1.3 |
| Infected epidermis Mildew removed | ±.05 | ±.05 | ... | .. |
| Normal wheat | 1.81 | 1.67 | 1.68 | 1.7 |
| Mildewed wheat | 6.33 | 5.22 | 6.46 | 6.0 |
| Mildewed wheat | 10.7 | 6.6 | ... | .. |
| Mildew removed | 7.4 | 5.4 | ... | .. |

Further confirmation of this conclusion was obtained by the use of sodium azide as a poison to differentiate between the respiration of mildew and wheat. A 0.001 molar solution of sodium azide has no appreciable effect on the respiration of normal wheat, but nearly eliminates the respiration of the mildew (Table II).

TABLE II

| Tissue | c. mm $0_2$ consumed/cm$^2$/hour | | | | Decrease from control value |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | Ave. | |
| Mildew | 1.61 | 1.08 | 1.21 | 1.30 | |
| Mildew $NaN_3$ | .18 | .13 | ... | 0.15 | |
| Normal wheat | 1.81 | 1.68 | 1.67 | 1.72* | |
| Mildewed wheat | 6.33 | 5.34 | 6.46 | 6.04 | |
| Mildewed wheat $10^{-3}$ M $NaN_3$ | 4.45 | 3.50 | ... | 4.45 | 1.39 |

* In this group of experiments no measurements were made of the respiration of normal wheat in azide. In another set, however, the decrease in azide was only 8 per cent.

If the increased respiration due to infection is largely an increase in host respiration, sodium azide should cause a decrease in the oxygen consumption of mildewed wheat which is not greater than that of the mildewed epidermis. As may be seen from Table II, this is actually the case. Although cyanide is just as effective a poison for the respiration of mildew, it is unsatisfactory as a differential poison, since it stimulates wheat respiration.

The fermentation of normal wheat can readily be compared with that of the infected wheat tissue, as

[1] W. O. Fenn, *Amer. Jour. Physiol.*, 84: 110, 1928.
[2] R. Pratt, SCIENCE, 88: 62, 1938.