RÔLE OF NITROGEN IN FUNGOUS THERMOGENESIS[1]

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(WITH TWELVE FIGURES)

Introduction

Thermogenesis by fungi has been definitely proved by MIEHE (**10, 11**), JAMES (**7, 8**), JAMES, RETTGER and THOM (**9**), NORMAN (**12**), GILMAN and BARRON (**3**) and others. The relation of substrate-composition to the thermogenic activity of the fungi involved has, however, received but little attention. Although the data presented by HARRISON (**4**) and those by NORMAN (**13**) clearly indicated the dependence of this process on the carbohydrate content of the substrate; the relationship that the nitrogen content, both as to type and amount, had to this process had not been investigated.

Various investigators have recognized that nitrogen added to substrates high in cellulose, hemicellulose, and similar plant products, bears an important relation to decomposition of these materials by fungi. BARTHEL and BENGTSSON (**1**) found that the favorable influence of stable manure upon cellulose fermentation in the soil was exerted by the nitrogen added with the manure. The soil micro-flora in this case was not limited to fungi. WAKSMAN (**17**) also found that nitrogen additions greatly increased the cellulose-decomposing activity of the soil micro-flora, except under anaerobic conditions. WAKSMAN and DIEHM (**18**), working with pure cultures of various fungi growing in a sand substrate containing additions of hemicellulose compounds and nitrogen, found that the ratio of nitrogen assimilated to hemicellulose decomposed was 1:35.5 for mannan, 1:16.7 for xylan, and approximately 1:35 for galactan. Further, in an experiment with fungous cultures growing in a liquid medium composed of ground corncobs with additional nitrogen, these investigators found that the ratio of nitrogen assimilated to corncob-xylan decomposed was 1:34.5. NORMAN (**13**) grew a number of fungi on oat straw with added ammonium carbonate and found that the "nitrogen factor" ranged from 0.50 for a species of *Phoma* to 0.83 for *Aspergillus terreus* Thom. He found, further, that the "nitrogen equivalent" was 3.33 for *A. terreus*—about an average for the fungi studied. This figure corresponds to a ratio of 1 part nitrogen to 30 parts organic matter and is quite similar to that determined by WAKSMAN and DIEHM for hemicellulose.

Although considerable work has been done on the relation of nitrogen to fungous decomposition of cellulose and related materials, comparatively few

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investigations have been made with reference to its rôle in thermogenesis. JAMES, RETTGER and THOM (9) found that, in the process of microbial heating in unsterilized cracked corn, total nitrogen remained practically constant, but that ammonia nitrogen very markedly increased, indicating a considerable transformation of organic nitrogen to the ammonia form, which, however, was not removed as such from the flask. NORMAN (12) working on thermogenesis of several fungi in cultures of oat straw, added nitrogen to the substrate in the form of ammonium nitrate, but did not test the influence of different amounts of nitrogen.

The problem was investigated in two of its aspects: first, the influence of various kinds and amounts of nitrogen upon temperatures attained in insulated cultures; and second, the influence of these compounds upon the general activity of the fungi, as measured by the loss in dry weight effected by them in substrates of similar composition.

Material and methods

CULTURES AND SUBSTRATE EMPLOYED IN THE EXPERIMENTS

One culture each of *Aspergillus flavus* Link, *A. terreus* Thom, *Penicillium oxalicum* Currie and Thom, and *Rhizopus tritici* Saito was obtained from J. W. HARRISON of the Botany Department of Iowa State College in September, 1931. These fungi had been isolated from alfalfa hay in the summer of 1930, and were chosen because of their thermogenic capacity (4).

In order to study the effect of nitrogen upon the thermogenic activity of these organisms, a substrate low in nitrogen was required, yet one suitable in other respects for their rapid development, and sufficiently porous to permit aeration in thermos-flask cultures. Corncob meal was found to be satisfactory. Well-dried corncobs of the 1930 crop were obtained from the Agronomy Farm of the Iowa Agricultural Experiment Station and ground in a Wiley mill to pass a sieve with 2-mm. apertures. The meal was then carefully mixed and stored in earthenware jars in the laboratory.

Corncobs are composed primarily of cellulose, pentosans, and lignin. Representative samples of the meal, prepared as described above, were analyzed for moisture, total nitrogen and ash content, with the following results:

Moisture (varying during the year)	7.10–9.43 per cent. of total weight
Total nitrogen (Kjeldahl method)	0.40 per cent. of dry weight
Ash	1.24 per cent. of dry weight

Moisture content is expressed as per cent. of the total weight, but because of the variation in moisture during the year and since all figures relating to nitrogen in the following experiments are referred to moisture-free meal, the percentages of the other two components are based on dry weight. The

percentage of total nitrogen, as here determined, corresponds rather closely to that given by HENRY and MORRISON (5) as an average figure. They state that elemental nitrogen comprises approximately 16 per cent. of crude protein, and thus the figure which they give is equivalent to 0.36 per cent. total nitrogen (on the dry weight basis), as compared with 0.40 per cent. obtained from the meal used in these experiments.

For the experiments with thermogenesis, 70-gm. samples of corncob meal were placed in 500-ml. Erlenmeyer flasks, which were plugged with cotton and sterilized by autoclaving one hour at 15 pounds pressure on each of four consecutive days. The samples were then poured into one-pint thermos flasks which had been autoclaved one and one-fourth hours at 15 pounds. For experiments with loss in dry weight, 5-gm. samples were placed in 50-ml. Erlenmeyer flasks, which were plugged with cotton, and autoclaved 30 minutes at 15 pounds on each of four consecutive days.

To provide the desired moisture content and to add the proper amount of nutrients, solutions of the necessary ingredients were prepared, placed in containers of convenient size, and autoclaved 25 minutes at 15 pounds. These were later mixed into the samples of meal. In the thermogenesis experiments, however, this step was delayed, as described later in connection with the inoculation process. The moisture content was brought to approximately 70 per cent. in all cases, since this percentage of moisture had been found in preliminary trials to be satisfactory for both the thermogenesis and the loss-in-dry-weight experiments.

INOCULATION AND GROWTH OF THE CORNCOB-MEAL CULTURES

To prepare inoculum, cultures of the organisms were grown at laboratory temperature on potato-dextrose-agar slants for a sufficient length of time to permit abundant sporulation. In the thermogenesis experiments, inoculation was performed in the following manner: single tubes of inoculum were used for each sample of meal, spore suspensions were prepared in the nutrient solutions mentioned above and poured into the thermos flasks containing the sterile meal. The flasks were temporarily closed with rubber stoppers, which had been washed in 50-per cent. alcohol, and were thoroughly shaken to mix the spores and medium and to provide for better aeration. The flasks were allowed to remain in a horizontal position for approximately one-half hour, and were rotated from time to time to facilitate absorption of the solution. Each flask was then fitted with a cotton plug, a thermometer, and an aeration tube consisting of a section of capillary glass tubing of suitable length, with the lower tip completely recurved to prevent stoppage by particles of meal. Both the thermometer and the aeration tube were washed by immersion in 50-per cent. alcohol before insertion into the flask. The bulb of the thermometer and the curved end of the aeration tube were lowered to

a point near the bottom of the flask. Duplicate platings were made from each culture at the close of all experiments to demonstrate the presence or absence of contaminants.

Since each loss-in-dry-weight experiment required the inoculation of a relatively large number of samples, the needle method was employed in preference to the use of spore suspensions. After the nutrient solutions had been introduced into the samples of sterile cob meal, the flasks were shaken sufficiently to loosen the meal and to facilitate aeration; a mass of spores and hyphae of convenient size was then introduced into the center of the substrate by a single stab. All cultures for loss-in-dry-weight determinations were made in duplicate.

The term "check" was used in the results from all experiments to indicate comparable uninoculated samples. A single check was used in each thermogenesis test, except where otherwise indicated in the results; in the experiments on loss in dry weight all checks were in duplicate.

After inoculation, the thermos flasks were placed on a laboratory table and subjected to the influence of varying room temperatures; however, since all cultures of any given experiment were handled at the same time, the results obtained within each experiment are comparable. In addition to the aeration by diffusion through the cotton plugs, a slow current of air was drawn through the cultures for one-half hour each day by means of the vacuum pump. The rate of aeration was controlled by bubbling the air from each aeration tube through dilute sulphuric acid. The amount of air drawn through the cultures in this manner was approximately 1.8 liters per day. Temperatures were recorded from two to four times each day, depending upon the rapidity of the temperature change, and charts were prepared from these detailed data. The figures, however, indicate only significant points and general trends.

All cultures in the loss-in-dry-weight experiments, except in preliminary trials, were incubated at room temperature for a period of 28 days. Dry weights were determined by oven-drying at 100° C. for 48 hours, and the actual loss in dry weight was calculated by direct comparison with checks handled in a similar manner and oven-dried at the same time. Percentage loss in dry weight was computed on the basis of original dry weight of the meal in each culture.

Experimental results

Comparison of the influence of additions of a single nitrogenous compound and a full-nutrient solution upon thermogenesis

In order to study the comparative effects of additions of a full-nutrient combination and of a single nitrogenous compound, alone, two nutrient solutions were prepared as follows:

Solution (1)	
$Ca(NO_3)_2 \cdot 4H_2O$	9.0 gm.
H_2O (distilled) to make	1000.0 ml.

Solution (2)	
$Ca(NO_3)_2$	9.0 gm.
KH_2PO_4	3.0 gm.
KCl	1.5 gm.
$MgSO_4 \cdot 7H_2O$	1.5 gm.
$FeSO_4$	0.03 gm.
Dextrose ($C_6H_{12}O_6$)	45.0 gm.
H_2O (distilled) to make	1000.0 ml.

These solutions were sterilized by autoclaving in the usual manner. Two samples of meal received, respectively, 50-ml. lots of solution (1) and (2), together with sufficient sterile water to give the moisture content of 70 per cent. and were inoculated with *Aspergillus flavus*. Calcium nitrate was the only source of nitrogen in each solution, and the rate of addition was 0.08 gm. elemental nitrogen per 100 gm. dry meal.

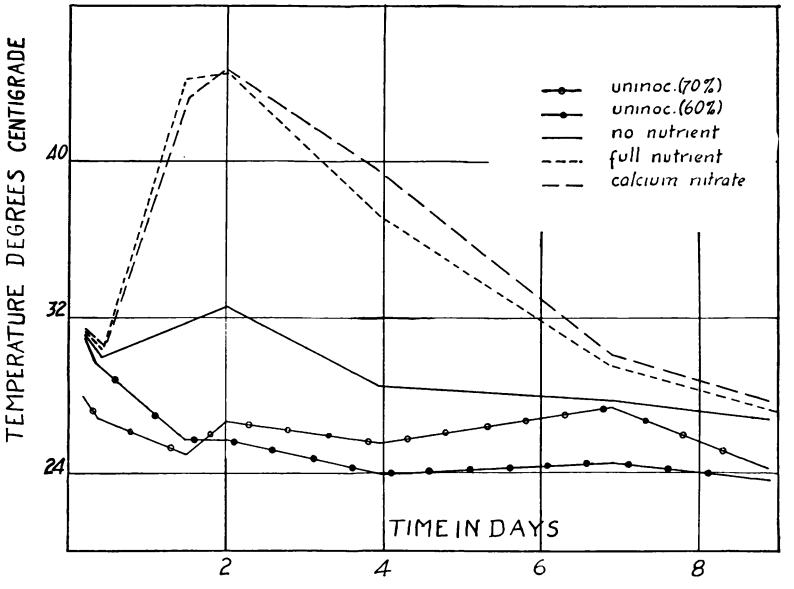

FIG. 1. Influence of different nutrient additions on temperatures in corncob meal cultures of *Aspergillus flavus*.

The results are presented in figure 1. The check, with moisture content equivalent to that in the two cultures, showed considerable fluctuation, which was found later to be caused by a faulty thermos flask. Another part of this experiment, dealing with the influence of moisture content upon thermogenesis, was being conducted at the same time; temperatures recorded for the check with 60 per cent. moisture are shown merely as indicative of the trend which temperatures in the 70 per cent. check normally would have taken. The results obtained from the inoculated cultures indicated that,

in this experiment, nitrogen, or at most calcium nitrate, alone, was the limiting factor in thermogenesis of *A. flavus,* since the addition of other nutrients resulted in but little difference in the temperatures reached. The culture containing the additional calcium nitrate without other nutrient additions reached its peak temperature of 44.75° C. at the end of the second day—18.25° above that of the check and 12.25° higher than the peak reached by the culture without added nutrient. The fact that nitrogen, and not calcium alone, was responsible for this pronounced stimulation of thermogenesis was demonstrated in subsequent experiments.

Influence of varying additions of nitrogen

When it was shown that the addition of a single nitrogenous compound to the meal greatly increased thermogenesis of *A. flavus,* experiments were made to test the effects of adding nitrogen in varying amounts. Three general types of nitrogenous compounds were separately employed, namely, organic-, ammonium- and nitrate-forms.

Thermogenesis.—Since the number of thermos flasks available was limited, only one organism, *A. flavus,* was used for experiments dealing with the influence of varying additions of nitrogen upon thermogenesis. Three experiments were conducted, in each of which the three compounds—asparagine, mono-ammonium phosphate ($NH_4H_2PO_4$), and calcium nitrate ($Ca(NO_3)_2 \cdot 4\,H_2O$)—were tested.

The results are presented in figure 2. Additions of asparagine ranged

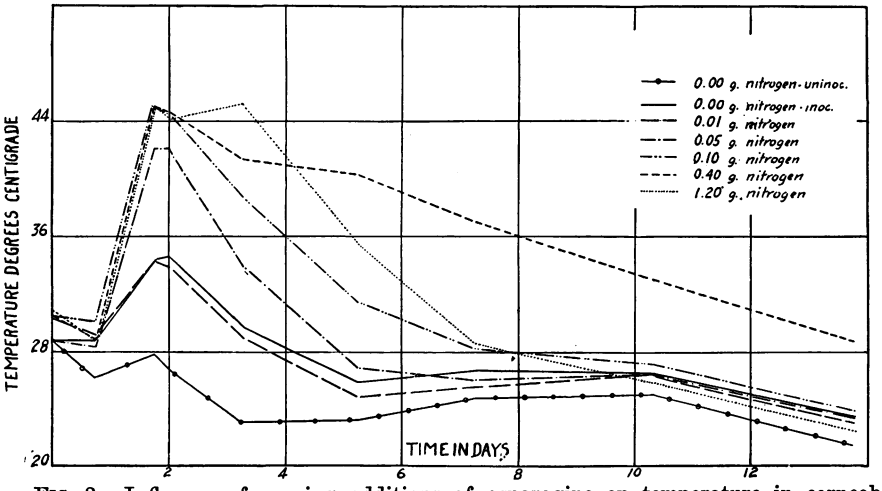

Fig. 2. Influence of varying additions of asparagine on temperature in corncob meal cultures of *Aspergillus flavus.*

from none to 1.20 gm. nitrogen per 100 gm. of dry meal, the upper limit being imposed by its low solubility. The pronounced increase in thermogenesis

with the additions of a single nitrogenous compound is again shown. All cultures containing asparagine additions of 0.10 gm. or more per 100 gm. of dry meal reached temperatures of 44.75° C. or higher—more than 10° above the peak reached by the culture without added asparagine and 17.5° or more above the check. Additions of asparagine in excess of 0.10 gm. nitrogen did not materially alter the rapidity of the response or the temperature peak, although a considerable prolongation of the period of heat production occurred in the culture containing the 0.40-gm. addition. The second peak and subsequent rapid fall in temperature of the culture receiving 1.20 gm. nitrogen may have been caused by a bacterial contaminant which was found at the close of the experiment. At this time the originally introduced organism seemed to be very largely destroyed, as evidenced by platings. Whether or not the contaminant could have had any appreciable influence upon the first peak in temperature is a matter of conjecture, but beginning with only a small amount of inoculum, it seems doubtful that it should have had any appreciable effect before the end of the second day.

The experiment with varying additions of mono-ammonium phosphate was similar to that in which asparagine was employed, except that additions of the nutrient were made over a much wider range—from none to 3.20 gm. nitrogen per 100 gm. of dry meal. The results are shown in figure 3. The differences observed between cultures receiving from 0.10 to 1.60 gm. nitrogen may be attributed to experimental error, such as that resulting from

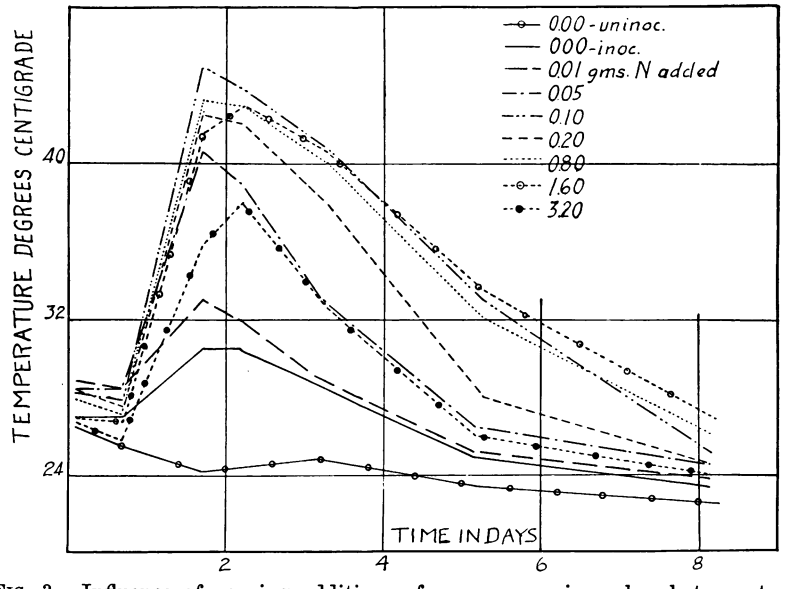

FIG. 3. Influence of varying additions of mono-ammonium phosphate on temperatures in corncob meal cultures of *Aspergillus flavus*.

slight differences in thermos flasks, in distribution of inoculum, and in conditions for aeration within the cultures. It is obvious, however, that 3.20 gm. nitrogen is well beyond the optimum. In this experiment, an addition of nitrogen as low as 0.01 gm. apparently resulted in a slight increase in thermogenesis, contrary to the experiment with asparagine.

In the third experiment of this series, varying additions of calcium nitrate were employed throughout the same range as with ammonium phosphate; and the results obtained (fig. 4) were quite similar. In these two experi-

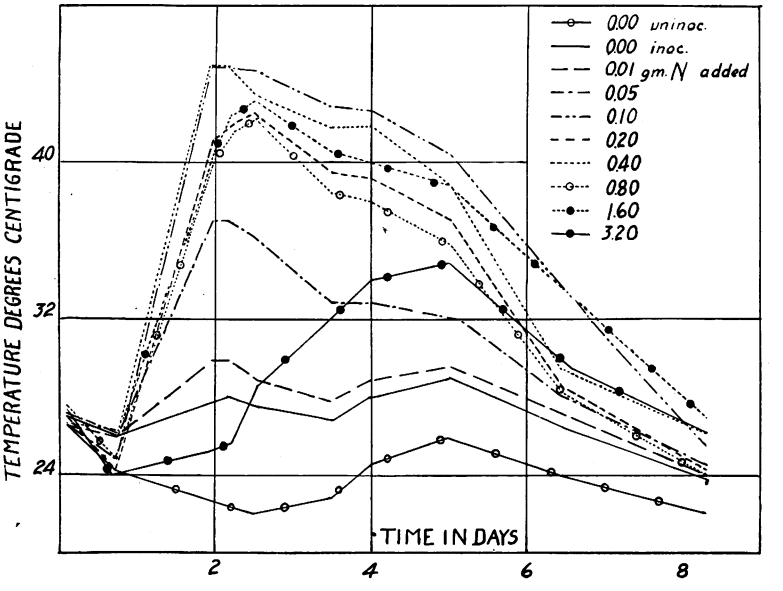

FIG. 4. Influence of varying additions of calcium nitrate on temperatures in corncob meal cultures of *Aspergillus flavus*.

ments, in which inorganic nitrogenous compounds were used, additions ranging from 0.10 to 1.60 gm. nitrogen per 100 gm. of meal produced only slightly differing results. Additions of 3.20 gm. and of 0.05 gm. nitrogen and below were decidedly less effective, although the amount of heating was markedly higher than where no nitrogen was added. With asparagine as with the inorganic compounds, additions ranging from 0.10 to 1.20 gm. nitrogen resulted in but slightly differing maximum temperatures. A decided prolongation of the period of heating occurred, however, in the one experiment where 0.40 gm. of nitrogen was added, as compared with 0.10 gm. The fact that the culture containing asparagine in the amount of 0.01 gm. nitrogen reached a maximum temperature slightly lower than the culture with no added nitrogen is probably not significant.

LOSS IN DRY WEIGHT.—In connection with those experiments in which varying nitrogen additions were tested for influence upon thermogenesis by

A. flavus, another series of experiments was conducted dealing with the effects upon fungous activity of varying additions of the same nitrogenous compounds, as shown by loss in dry weight. In these experiments relatively large numbers of cultures could be handled at one time, and thus all four of the fungi were employed in each test.

Before beginning with this series, however, a single experiment was carried out with *A. flavus* to determine the length of the growth period required for satisfactory comparisons in dry-weight loss. Four series of cultures—16 in each series—to which ammonium carbonate had been added at the respective estimated rates of 0.17, 0.22, 0.28, and 0.35 gm. of nitrogen per 100 gm. dry meal, and one series receiving no added nitrogen, were grown in comparison with uninoculated checks. Because of loss of ammonium carbonate in autoclaving, the amounts of nitrogen added to the cultures in the different series were estimated from total-nitrogen analyses made on ammonium-carbonate solutions prepared and autoclaved in exactly the same manner as those added to the cob-meal samples. At weekly intervals for a period of eight weeks, moisture-content and dry-weight determinations were made for one pair of cultures from each of the five series. At the end of eight weeks the rate of dry-weight loss in each series had apparently become almost nil and no loss whatever had occurred in the checks. By the end of four weeks dry-weight loss in every case was considerably greater than half the total loss reached in eight weeks and the relative position of each series, with respect to dry-weight loss, was very nearly the same throughout the period from the end of the third to the end of the eighth week. It seemed safe to conclude that a four-weeks' growth period should be sufficient for similar comparative tests, and consequently this period of growth was used in all subsequent experiments dealing with loss in dry weight.[2]

The influence of varying additions of asparagine upon dry-weight-loss effected by the four fungi—*A. flavus, A. terreus, Penicillium oxalicum,* and *Rhizopus tritici*—is shown in figure 5. In this experiment, as with that pertaining to the effects of asparagine upon thermogenesis, the low solubility of asparagine prevented any addition of this nutrient in a quantity greater than 1.2 gm. of nitrogen per 100 gm. dry meal. In every case progressive increases in dry-weight loss with larger additions of asparagine, up to 0.8 or 1.2 gm. nitrogen per 100 gm. of meal, occurred.

Figure 6 shows the results obtained in an experiment to determine loss in dry weight of cultures supplied with varying additions of mono-ammonium phosphate. The test was similar to that dealing with the effect of aspara-

[2] The standard error of a mean of two determinations, ± 0.3002, calculated on the basis of ten cultures of *A. flavus* grown under the conditions of these experiments showed that the inoculating technique employed was sufficiently accurate, considering the magnitude of dry-weight losses. It is also evident that, under similar conditions, only relatively small differences between pairs of cultures would be required to be significant.

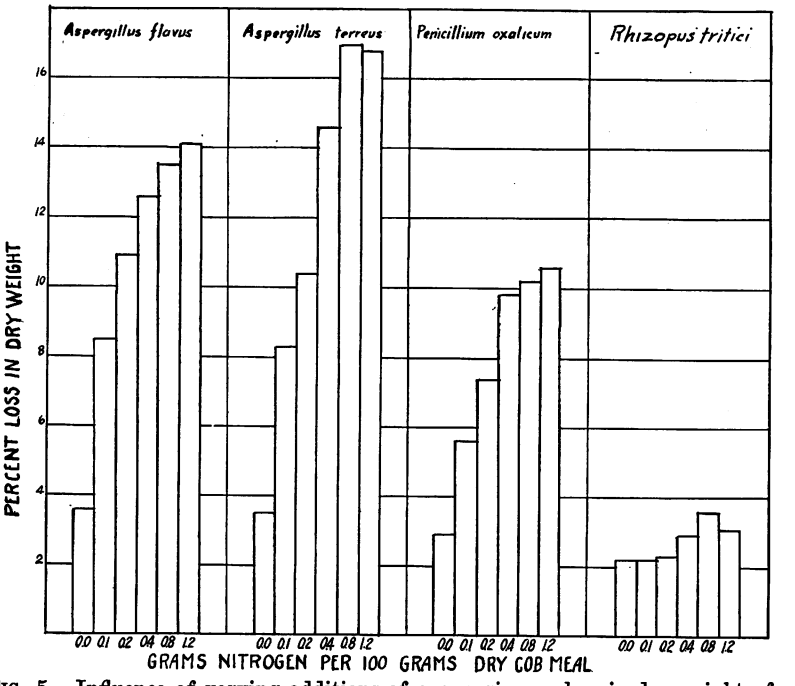

FIG. 5. Influence of varying additions of asparagine on loss in dry weight of corncob meal cultures of *Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

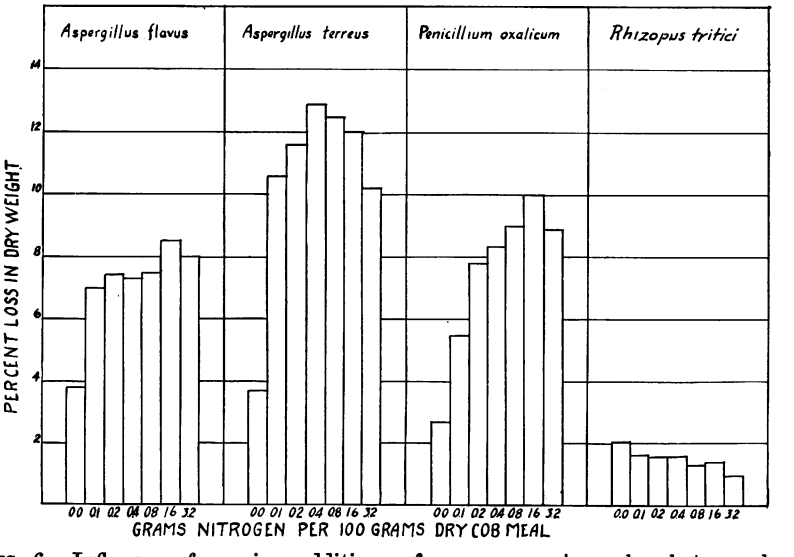

FIG. 6. Influence of varying additions of mono-ammonium phosphate on loss in dry weight of corncob meal cultures of *Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

gine, but additions of the nutrient covered a wider range—from none to 3.2 gm. nitrogen per 100 gm. of dry meal. The response of *Penicillium oxalicum* to ammonium phosphate was almost identical with its response to asparagine, and the optimum appeared to be near the same concentration of nitrogen. The two species of *Aspergillus* responded less vigorously to ammonium phosphate. With *A. flavus,* the optimum nitrogen addition, using the latter compound, appeared to approximate that for asparagine, but with *A. terreus* the optimum was apparently lower, about 0.4 or 0.4 to 0.8 gm. of nitrogen. With *Rhizopus tritici* a progressive decrease in dry-weight loss with the greater additions of nitrogen was found. This seems somewhat peculiar in view of the fact that with moderate additions of ammonium phosphate this fungus produced much more aerial mycelium in the early part of the growth period than where no nitrogen was added. The difference was still evident at the end of the period, though only very slightly. With the 3.2-gm. addition of nitrogen, no mycelial growth was apparent.

The effect of varying additions of calcium nitrate upon loss in dry weight is shown in figure 7. The experiment was similar to the preceding test with ammonium phosphate, except that additions of calcium nitrate ranged from none to 1.6 gm. nitrogen per 100 gm. of dry meal. As with ammonium phosphate, *R. tritici* showed decidedly negative results. In this case, however, the early mycelial growth, where only small amounts of nitrogen had been

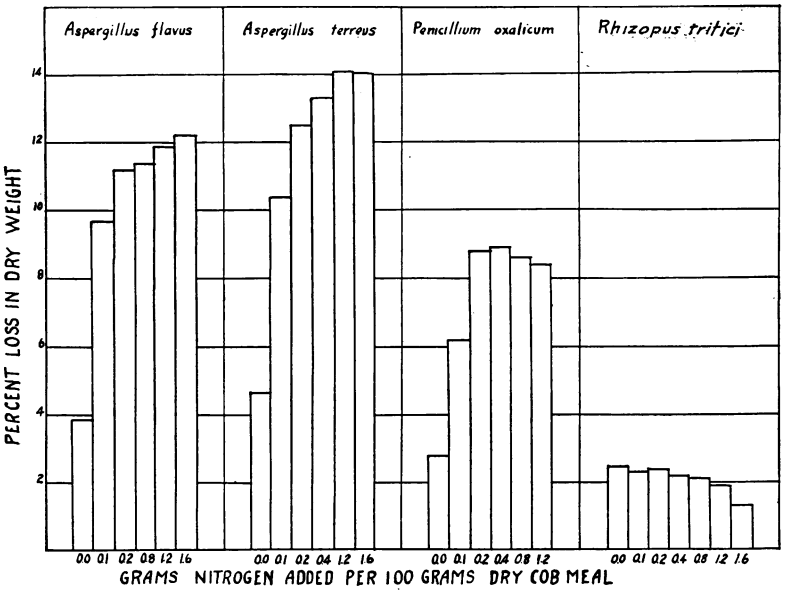

FIG. 7. Influence of varying additions of calcium nitrate on loss in dry weight of corncob meal cultures of *Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

added, showed but little, if any, increase in vigor, as compared with cultures with no added nitrogen; and, with the higher concentrations of nitrogen, growth was very weak throughout the experiment. *Aspergillus flavus* showed a response intermediate between that resulting from asparagine and from ammonium phosphate and here the optimum nitrogen addition was rather high, being about 1.6 gm. or above. *A. terreus* likewise showed an intermediate response, and the optimum nitrogen addition was approximately 1.2 to 1.6 gm. The response of *Penicillium oxalicum* was somewhat less than with either of the other two forms of nitrogen, and the optimum addition appeared to lie approximately between 0.2 and 0.8 gm. of nitrogen.

In comparing the effects of varying nitrogen additions upon thermogenesis and loss in dry weight of *Aspergillus flavus* cultures, it is notable that, whereas high additions of nitrogen—such as 1.2 to 1.6 gm. per 100 gm. of dry meal—gave greatest losses in dry weight where the fungus was allowed to grow for 28 days, considerably lower additions of nitrogen were sufficient to induce as rapid thermogenesis, during the peak of activity, as that obtained with larger quantities.

Influence of different forms of nitrogen added in equal quantities

In the work thus far reported only one form of nitrogen was used in each experiment. In order to compare the influence of different forms of nitrogen when added in equal quantities, two series of experiments were conducted, including four tests dealing with thermogenesis and one with loss in dry weight, in which all four organisms were employed. The following five compounds were compared:

Asparagine
Ammonium chloride (NH_4Cl)
Mono-ammonium phosphate ($NH_4H_2PO_4$)
Ammonium sulphate ($(NH_4)_2SO_4$)
Calcium nitrate ($Ca(NO_3)_2 \cdot 4\,H_2O$)

Each was used at the rate of 0.50 gm. of nitrogen per 100 gm. of dry meal.

Thermogenesis experiments.—In each of the four tests conducted relative to the influence of different forms of nitrogen upon thermogenesis, a different one of the four fungi was employed. Each experiment included one check and six cultures, one of which contained no added nitrogen. Each of the other five cultures was given an addition of one of the five compounds named above.

Figure 8 indicates the temperatures recorded in thermos-flask cultures of *A. flavus* throughout a period of 11 days. The striking feature of this experiment is the unusually high temperature of 49.25° C. reached by the

asparagine-containing cultures. Second to asparagine was calcium nitrate. The other cultures containing added nitrogen showed but slight differences, except for the ammonium-sulphate culture, which was noticeably lower. All

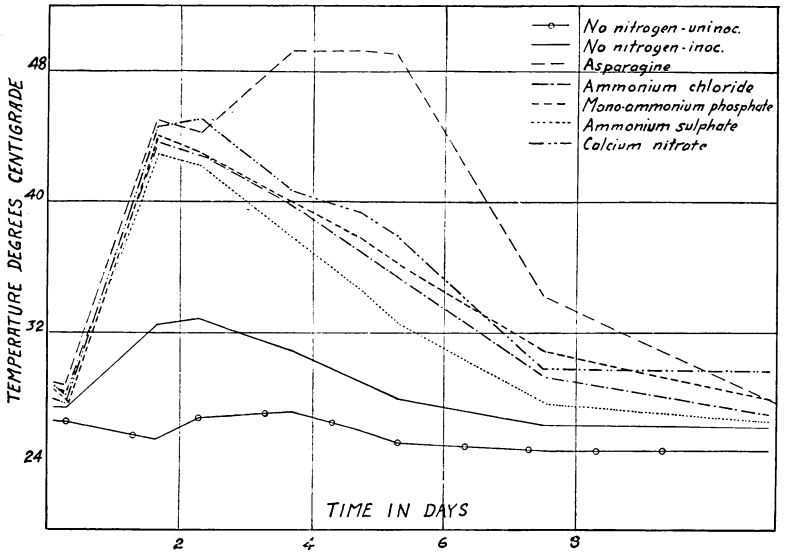

FIG. 8. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Aspergillus flavus*.

cultures supplied with nitrogen, in any form, showed temperatures far above those receiving no nitrogen addition.

The influence of different forms of nitrogen upon thermogenesis in cultures of *A. terreus* is shown in figure 9. Here, again, asparagine appeared to be superior to the other compounds, although the difference was much smaller than in the preceding experiment. The highest temperature re-

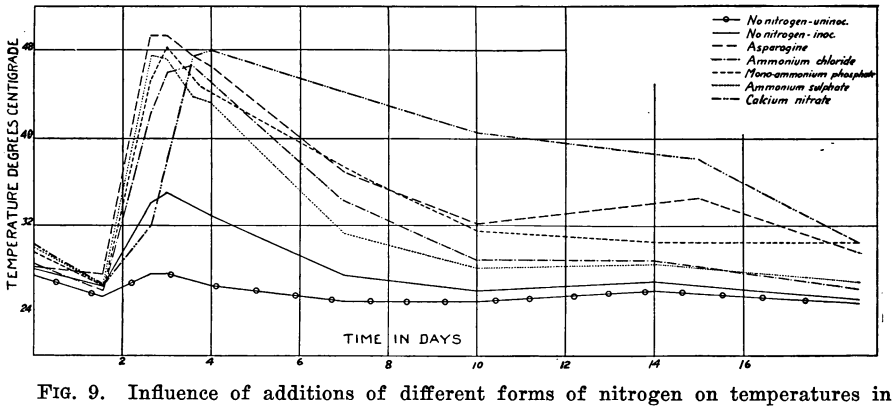

FIG. 9. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Aspergillus terreus*.

corded was 49.25° C., which was held for a period of at least six hours. Calcium nitrate, as with *A. flavus*, seemed to rank second, although this culture did not quite reach the temperature attained by the ammonium phosphate culture. Somewhat lower in thermogenesis, but still far above the no-nitrogen culture, were those supplied with ammonium chloride and ammonium sulphate. In general, then, the response of *A. terreus* to additions of different forms of nitrogen was much the same as that of *A. flavus*.

The effects of additions of different forms of nitrogen upon thermogenesis in cultures of *Penicillium oxalicum* are presented in figure 10. As usual, asparagine produced the most rapid response, and the temperature reached was higher than in all other cultures except one. The striking feature of

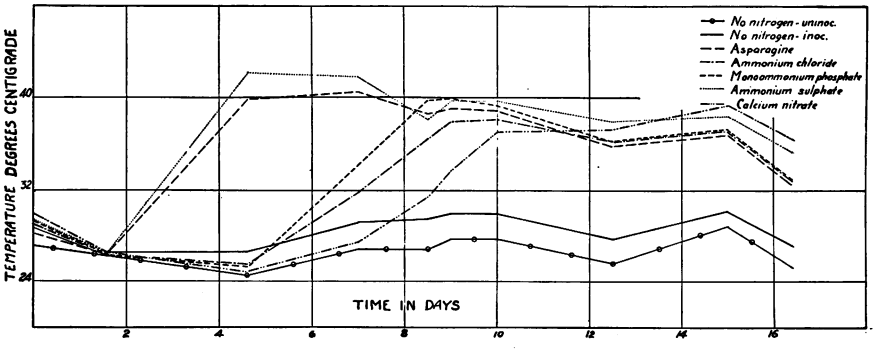

FIG. 10. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Penicillium oxalicum*.

this experiment is the marked superiority of ammonium sulphate, which produced a temperature of 42.25° C.—1.75° higher than that attained by the asparagine culture. It should be pointed out here that the delay of other cultures in beginning thermogenesis may not be significant in this experiment, from the standpoint of nutritional value of the nitrogen compounds to the growing cultures, since the beginning of apparent growth of *P. oxalicum* in loss-in-dry-weight experiments was frequently delayed several days, and in some instances did not occur at all. In this experiment, consequently, thermogenesis in certain cultures may have been brought about by the effects of the added salts in decreasing spore germination, rather than by any toxic effect upon, or by unavailability to, the fungous mycelia. If it so happened that initial growth was largely inhibited in parts of any cultures, then a slow rise in temperature might be attributed to heating in only a part of the culture at any one time. In such cultures the temperature peak would necessarily have been somewhat lower, though a longer period of thermogenesis could have been expected. This may have been so with calcium nitrate, for example, as shown in figure 10. It seems probable that, if the suitability of

the several compounds from the start were considered, asparagine and ammonium sulphate were the most favorable for thermogenesis of *P. oxalicum* in this experiment. As in the two preceding experiments, all forms of nitrogen resulted in very pronounced increases in thermogenesis.

The effects of additions of different forms of nitrogen upon thermogenesis in cultures of *Rhizopus tritici* are given in figure 11. In this experiment, contaminants were found in four cultures at the end of the ninth day, which cast some doubt upon the validity of the results. In three of the cultures, however, those receiving ammonium-chloride, -phosphate, and -sulphate, re-

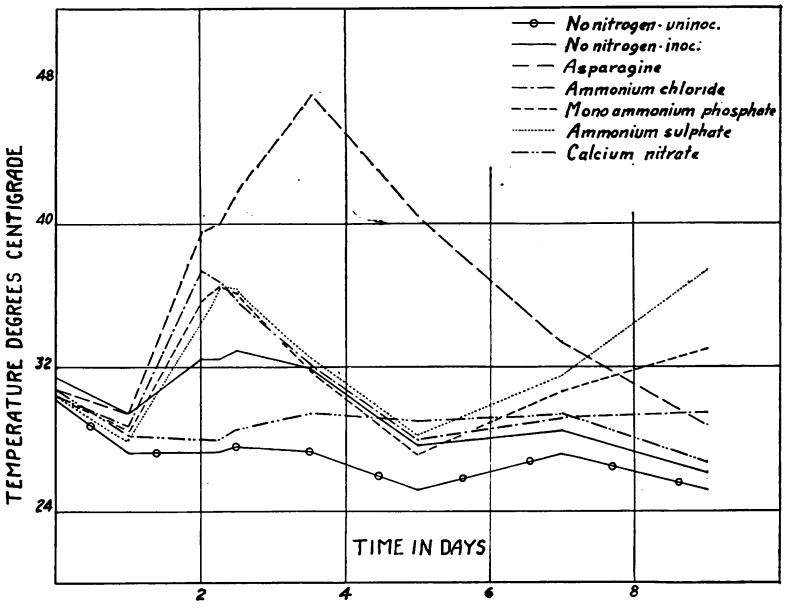

FIG. 11. Influence of additions of different forms of nitrogen on temperatures in corncob meal cultures of *Rhizopus tritici*.

spectively, it seemed quite probable that no appreciable effect could have resulted from the presence of the contaminants before the third or fourth day at the earliest. This assumption is based upon the fact that the contaminants were fungi and were apparent in only a part of each of the cultures; further, it appears that the period of secondary heating by these organisms began somewhere between the fifth and sixth days. It is a matter of conjecture whether or not the bacterial contaminant in the asparagine culture had any appreciable influence upon the peak temperature which was reached during the fourth day. It is doubtful, however, if its effect was of any importance during the period when the other cultures were at their peak temperatures—about the end of, or shortly after, the second day.

If it may be assumed that the effect of contaminants was absent or negligible until after the first two and one-half days, then asparagine was apparently the most suitable source of nitrogen, with ammonium chloride ranking next. The effect of calcium nitrate was decidedly inhibitory, since the maximum rise in temperature—above the check—was 1.75° less in the calcium nitrate culture than in the culture containing no added nitrogen. The latter reached a temperature of 33.0° C.—5.25° above the check. This is the only case, in this series of four experiments in which the addition of a nitrogenous compound failed to result in a temperature considerably higher than that reached in the comparable no-nitrogen culture. It may be mentioned that, in the next experiment (fig. 12), dealing with loss in dry weight of cultures of these four fungi and comparing the same five compounds, the only culture which did not respond to nitrogen by producing more abundant apparent mycelial growth during the early part of the 28-day period was *R. tritici*, supplied with calcium nitrate.

Loss in dry weight.—As mentioned in the preceding paragraph, an experiment was conducted in which the influence, upon loss in dry weight, of additions of the five different forms of nitrogen previously tested was com-

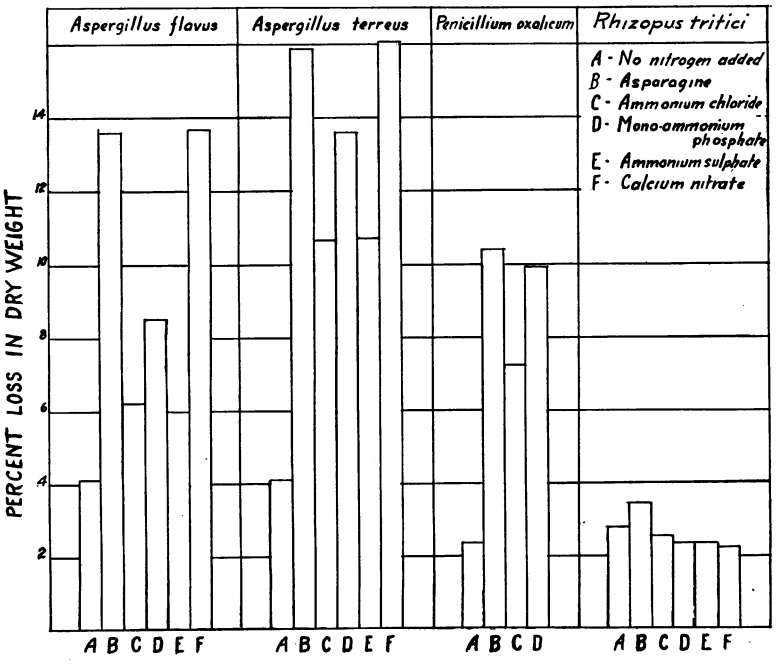

Fig. 12. Influence of additions of different forms of nitrogen on loss in dry weight of corncob meal cultures of *Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici.*

pared for the four fungi used in other experiments. The results are presented in figure 12.

The correlation, generally, between the results obtained in this and the four preceding experiments was noteworthy. For *Aspergillus flavus* and *A. terreus,* asparagine and calcium nitrate were distinctly superior to all other compounds used as sources of nitrogen, both for thermogenesis and loss-in-dry-weight cultures, and ammonium phosphate ranked next in both types of experiments.

Penicillium oxalicum produced no apparent growth in the samples of meal containing ammonium sulphate and calcium nitrate in the loss-in-dry-weight experiment (fig. 12). However, the distinct response to additions of asparagine and ammonium phosphate agreed with the results obtained in the thermogenesis experiment with this organism (fig. 10).

With *Rhizopus tritici,* the superiority of asparagine was quite distinct in both types of experiments. With the three ammonium salts, the effect upon dry-weight loss was inhibitory, whereas these compounds considerably increased thermogenesis. In the experiment with loss in dry weight, the apparent mycelial development was much greater during the early part of the 28-day growth period in cultures containing additions of these salts than in no-nitrogen cultures. At the end of the period, however, this difference had disappeared. It seems probable, then, that the presence of these compounds made possible more rapid development, as well as thermogenesis, during the first few days after inoculation, but that products resulting from their decomposition inhibited fungous activity after a longer period of growth, thus actually decreasing the total loss in dry weight in 28 days. Calcium nitrate resulted in the greatest reduction in dry-weight loss. As previously pointed out, it did not stimulate apparent mycelial growth even during the first part of the 28-day period, and correlated with this fact was its inhibiting effect upon thermogenesis.

Discussion

From the data presented it is apparent that, under the conditions of the experiments, nitrogen was an important factor in limiting both thermogenesis and loss in dry weight in cultures of the four fungi studied. Table I summarizes the results obtained from additions of five different compounds, respectively, to the corncob-meal substrate.

Examination of this table shows a rather close correlation between thermogenesis and loss in dry weight. For both species of *Aspergillus,* asparagine and calcium nitrate were outstanding in producing the highest thermogenesis as well as the largest losses in dry weight; ammonium sulphate and ammonium chloride were least effective in both types of tests; and monoammonium phosphate was intermediate.

TABLE I

SUMMARY OF THE RESULTS ON THERMOGENESIS AND LOSS-IN-DRY-WEIGHT OBTAINED IN CULTURES OF FOUR FUNGI GROWING ON VARIOUS SUBSTRATES

FORM OF NITROGEN ADDED	MAXIMUM RISE IN TEMPERATURE AND LOSS IN DRY WEIGHT							
	A. flavus		*A. terreus*		*P. oxalicum*		*R. tritici*	
	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.
	°C.	%	°C.	%	°C.	%	°C.	%
No nitrogen	7.00	4.01	7.25	4.01	3.00	2.38	5.25	2.82
Asparagine	23.50	13.59	21.75	15.91	15.25	10.39	19.75†	3.52
Ammonium chloride	18.25	6.22	19.50	10.66	10.50	7.25	10.00	2.49
Mono-ammonium phosphate	18.50	8.55	20.50	13.64	13.00	9.96	9.00	2.38
Ammonium sulphate	17.50	5.95	20.00	10.72	17.75	.....	9.00	2.38
Calcium nitrate	19.00	13.69	21.50	16.07	11.75	.....	3.50	2.27

* Influence of different forms of nitrogen on thermogenesis was tested in a separate experiment for each organism. The figures shown indicate maximum differences between checks and cultures.

† Maximum temperature may have been affected by a bacterial contaminant.

With *Penicillium oxalicum,* also, a correlation is shown by the partial data. The greatest amount of thermogenesis occurred in the culture containing ammonium sulphate, but unfortunately no loss-in-dry-weight data were obtained for this compound. Next in order in thermogenesis were the cultures containing additions of asparagine and mono-ammonium phosphate, respectively; and, as with the two species of *Aspergillus,* ammonium chloride was distinctly less effective. Likewise, among those loss-in-dry-weight cultures in which growth took place, asparagine gave the greatest response, mono-ammonium phosphate was second, and ammonium chloride third.

The results shown for *Rhizopus tritici* are of especial interest, in that only asparagine increased the loss in dry weight, whereas all forms of nitrogen used, except calcium nitrate, distinctly increased thermogenesis. An explanation of this seemingly peculiar occurrence has been proposed in connection with the presentation of the original data (figs. 11 and 12). It is sufficient to repeat here that all forms of nitrogen except the nitrate obviously stimulated early mycelial growth in the loss-in-dry-weight cultures, and it would seem that such a stimulation in the thermos-flask cultures was probably accompanied by more rapid decomposition of the substrate for a short period, and thus was responsible for the greater thermogenesis during this time. The accumulation of decomposition products of these compounds, however, was probably the cause for the decrease in the final loss in dry weight in 28 days—an effect not entering into the results appreciably during the short period in which the thermogenesis experiments were conducted. The dis-

tinct superiority of asparagine, as a source of nitrogen for *R. tritici,* is quite evident in its influence upon thermogenesis as well as upon loss in dry weight. As might be expected from the classification of molds by ROBBINS (**15**) calcium nitrate was not only unsuitable for this organism, but was decidedly inhibitory in its action. These results indicate an extension of the findings of ROBBINS (**15**) that *Aspergillus niger* has a higher apparent reducing intensity than *Rhizopus nigricans.* The data indicate that the same relation holds between *Rhizopus tritici* and *Aspergillus flavus.*

From the results set forth above it seems probable that the specific effect of each form of nitrogen in promoting thermogenesis depends directly upon the concomitant intensity of fungus decomposition of the substrate.

The influence of varying additions of three forms of nitrogen upon thermogenesis and loss in dry weight in cultures of *Aspergillus flavus* may be summarized as follows (table II).

TABLE II

THE INFLUENCE OF VARYING ADDITIONS OF THREE FORMS OF NITROGEN UPON THERMOGENESIS AND LOSS IN DRY WEIGHT IN CULTURES OF *Aspergillus flavus*

Nitrogen added per 100 gm. cob meal	Maximum rise in temperature and loss in dry weight					
	Asparagine*		Mono-ammonium phosphate*		Calcium nitrate*	
	Temp. rise†	Loss in dry wt.	Temp. rise†	Loss in dry wt.	Temp. rise†	Loss in dry wt.
gm.	°C.	%	°C.	%	°C.	%
0.00	8.00	3.59	6.50	3.80	5.50	3.89
0.01	7.00	.....	8.75	.....	7.25	.....
0.05	15.00	.....	16.25	.....	14.50	.....
0.10	17.75	8.43	20.50	7.00	22.50	9.72
0.20	.....	10.87	18.25	7.38	20.50	11.18
0.40	18.75	12.61	.....	7.32	22.25	11.02
0.80	.....	13.54	19.25	7.54	20.25	11.39
1.20	17.50‡	14.13	.....	.....	.....	11.93
1.60	.....	.....	18.75	8.52	21.25	12.21
3.20	.....	.....	13.25	8.08	9.50	.....

* Each form of nitrogen was tested in a separate experiment.
† Figures indicate maximum differences between checks and cultures.
‡ First peak in temperature is shown since second peak was probably affected by a bacterial contaminant.

In examining table II, the average maximum rise in temperature above the check for the no-nitrogen cultures was 6.67° C.; and for those cultures containing 0.01-, 0.05-, and 0.10-gm. additions of nitrogen it amounted to 7.67°, 15.25°, and 20.25° C., respectively. Above this point, increased additions gave results quite similar to that of 0.10 gm., except for the extremely large amount of 3.20 gm., which was obviously well beyond the optimum. With dry-weight loss, however, the results were somewhat different. With

each of the three forms of nitrogen, a progressive increase in percentage of loss in dry weight resulted from all the larger additions, except the extremely large amount of 3.20 gm.

Since the use of nitrogen in excess of 0.10 gm. per 100 gm. of meal did not materially increase thermogenesis—either in maximum temperature rise or in prolongation of heating—but did substantially increase loss in dry weight throughout a longer period (28 days), it might seem that small amounts of nitrogen were utilized by the fungus in the process of decomposition of the more readily available constituents of the corncob meal, during which time the most rapid development and respiration, together with greatest thermogenesis, took place. Following this period, with the aid of more nitrogen, the fungus was probably able to continue growth and respiration at a much less rapid rate, utilizing some of the more resistant carbohydrates present, and producing heat much more slowly. The more readily available food materials probably consisted principally of certain of the hemicelluloses, which comprise, according to BURKE (**2**) about 37 per cent. of corncobs, and the more resistant materials possibly included some of the true celluloses. This explanation is based largely upon the results of NORMAN (**12**), who found that the hemicelluloses were the constituents most rapidly lost in the early stages of decomposition of unsterilized oat straw, and who noted that this period corresponded with that of maximum thermogenesis in a vacuum flask containing similar material. The fact should not be overlooked that conditions for decomposition inside a mass of heating straw are decidedly different from those in straw which is not heating, though it seems doubtful whether this should greatly alter the sequence of decomposition.

The fact that the addition of a full nutrient combination was no more effective in stimulating thermogenesis of *A. flavus* than was an addition of the nitrogenous compound (calcium nitrate) alone, is of special interest, since it indicated that, so far as the food-nutrient composition of the substrate was concerned, nitrogen, or at most calcium nitrate, was the most important factor in thermogenesis in corncob-meal cultures of this fungus. Experiments in which other forms of nitrogen were employed demonstrated the fact that nitrogen, and not calcium, was the limiting factor.

Summary

1. The influence of various nitrogen additions, and certain other factors, upon thermogenesis and loss in dry weight of corncob-meal cultures of four species of fungi was studied.

2. The organisms employed—*Aspergillus flavus, A. terreus, Penicillium oxalicum* and *Rhizopus tritici*—had previously been isolated from alfalfa hay, and found to be capable of considerable heat production in moist hay cultures.

3. Five forms of nitrogen—asparagine, ammonium chloride, mono-ammonium phosphate, ammonium sulphate and calcium nitrate — each greatly stimulated both thermogenesis and loss in dry weight when added to the substrate in cultures of *Aspergillus flavus, A. terreus,* and *Penicillium oxalicum.* With *Rhizopus tritici,* all forms of nitrogen markedly increased thermogenesis, with the exception of the nitrate form which showed a distinct inhibiting effect. Only asparagine increased dry-weight loss in cultures of this organism, all other compounds showing a depressing influence, with the nitrate being most effective in this regard.

4. Asparagine was found to be most generally suitable for the four fungi employed, both in its effect upon thermogenesis and upon loss in dry weight. The highest temperature recorded throughout the experiments—49.25° C.—was reached in cultures of *Aspergillus flavus* and *A. terreus,* each of which had been supplied with asparagine. The significance of these figures is shown by the fact that the maximum temperature above the check in both instances exceeded 21.50° C., as compared with cultures containing no added nitrogen, in which the temperatures reached were not more than 7.25° C. above the check with either fungus. The greatest loss in dry weight, in 28 days, occurring throughout the course of the experiments, was 16.95 per cent., which was found in cultures of *A. terreus* supplied with asparagine, and may be compared with 3.54 per cent. loss occurring at the same time in cultures of this organism containing no added nitrogen.

5. Nitrogen additions ranging from 0.01 to 0.10 gm. per 100 gm. of dry cob-meal generally resulted in progressive increases in thermogenesis in cultures of *A. flavus.* The average maximum rise in temperature above the check, for additions of three forms of nitrogen—asparagine, mono-ammonium phosphate, and calcium nitrate—at the rate of 0.00, 0.01, 0.05, and 0.10 gm. per 100 gm. of meal, were 6.67°, 7.67°, 15.25° and 20.25° C., respectively. Greater additions, ranging from 0.20 to 1.60 gm., inclusive, generally gave results similar to that shown for the 0.10-gm. addition, with an average of 19.64° maximum rise in temperature; the use of 3.20 gm. nitrogen, as mono-ammonium phosphate and calcium nitrate, resulted in an average maximum rise of only 11.38°.

6. Progressive increases in dry-weight loss in cultures of *A. flavus* followed the larger additions of asparagine, mono-ammonium phosphate and calcium nitrate, respectively, up to 1.2 and 1.6 gm. nitrogen per 100 gm. of dry cob-meal. The other three organisms differed considerably in response to various amounts of these compounds.

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