EFFECT OF ALKALOIDS ON THE GROWTH OF FUNGI

by

James Charles Bates

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W. H. Horr
Instructor in charge

5/15/35
Date

A. J. Smith
Head or Chairman of Dept.

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INTRODUCTION

In undertaking a study of the effect of alkaloids on the growth of fungi, one naturally wonders as to the origin and role of these substances within the plants which produce them.

The vegetable alkaloids are basic nitrogenous substances which occur in plants usually in combination with organic acids (tannic, citric, tartaric, malic, oxalic, succinic, etc.) and are characterized by powerful physiological activity. They contain the elements C, H, N or C, H, O and N and are complex in constitution, generally containing pyrrole, pyrrolidine, pyridine, quinoline or isoquinoline groups in their structure.

Most of the alkaloids are crystalline (coniine and nicotine are liquids) and most of them are levorotatory. They are insoluble in water, soluble in alcohol, ether, chloroform, etc., to a greater or less extent, form water-soluble salts with acids, have a bitter taste and some are excessively poisonous. Most of the alkaloids are used in biological work in the form of salts, such as hydrochloride, nitrate, sulphate, phosphate, etc.

Alkaloids are not easily identified, but as a class they are precipitated by the "alkaloidal reagents," such as tungstic, phosphomolybdic, tannic and picric acids, potassium-mercuric iodide ($KI + HgI_2$), etc. Many of them are identified by color reactions with sulphuric acid and an

oxidizing agent.

The method of obtaining alkaloids from plants often is by extracting with acidified (HCl or H_2SO_4) water and reprecipitating with bases.

The physiological action of alkaloids on the animal body has been extensively studied and their practical application in medical practice is well known. Their commercial value has led to studies of their localization within the plant, methods of extraction and purification, and horticultural experiments aimed at an increased yield.

The alkaloids are rather limited in their distribution in the plant kingdom, occurring, for the most part, in the Leguminosae, Papaveraceae, Ranunculaceae, Rubiaceae, and Solanaceae. They may occur in solution in the cell sap in the young parenchyma or may be stored in older tissue in the solid state. They are generally found in the seeds and fruits, but some occur in the leaves, stems, and roots (Haas and Hill, 1913).

The origin of the alkaloids in the plant is not definitely known. Some consider that they arise in the formation of proteins, while others believe that they are formed in the disintegration of the proteins. They are thought to be nitrogenous waste products by most investigators.

As to the role of the alkaloids within the plants which produce them, there have been differences in opinion among the investigators. Some have ascribed to these substances a protective function against the attack of animals. The lit-

erature on this subject indicates that the protective function, when performed, is only an incidental one.

Some have ascribed to the alkaloids a nutritive value due especially to the nitrogen contained in their molecules. A part of the evidence bearing on this subject comes from investigations of the localization of alkaloids, their quantitative variations, mode of formation, migration during the course of vegetation and of seed germination, variations provoked under the influence of different external factors, and grafting experiments.

The literature of the above-mentioned investigations has been contributed by numerous investigators and summarized by Goris (1914). From the following brief summary of these investigations, taken from Goris, one may obtain some idea as to the general nature of this work and its bearing on the role of alkaloids.

The localization of alkaloids has been studied by microchemical reactions produced within the tissues of the plant under the influence of various reagents. Some of these reagents form insoluble precipitates with the alkaloids while others give beautiful color reactions which are, in many cases, of but short duration. These tests give but little information concerning the actual amount of alkaloid present.

After reviewing the voluminous literature on the localization of alkaloids, covering investigations in twenty-one families, Goris concluded that there is a very general mode of distribution of these substances within the plant. They

are commonly found in the external parts of the root; in the epidermis, trichomes and subepidermal layers of the stem; in the endodermis of the stem and root; and the peripheral layers of the pith and in the perideame. They are found more variable and in smaller amounts in the parenchyma of the cortex, phloëm, medullary rays and parenchyma of the wood. They have been found in the sieve tubes of the phloëm, but they are more commonly found in the companion cells and phloëm parenchyma. They are sometimes found in the integuments of the seed, sometimes in the endosperm, and occasionally in the embryo. When the tissues of the embryo have developed, they may or may not occur in the growing point.

The alkaloids have been found frequently and in considerable amount in the latex vessels of the Papaveraceae and Fumariaceae, the secreting cells of Conium maculatum L. and associated with raphides in the Amaryllidaceae.

In general, the alkaloids are found in regions where cellular activity is greatest.

From his study of the work on localization, Goris concludes that the alkaloids are translocated, to a great extent, to external tissues of the stem which are later sloughed off. Goris also accepts the presence of alkaloids largely in the secretory tissue as evidence that they are not reserve compounds. Several hypotheses, based on similarities and differences between structural formulae of alkaloid molecules and those of various other compounds found in plants, have been advanced to explain the mode of forma-

tion of alkaloids. The most commonly accepted hypothesis seems to be that of Winterstein and Trier (1910) who consider the alkaloids as waste products arising from the metabolism of albuminous material. The origin of the purine bases from the decomposition of nucleoproteins is commonly accepted. It is interesting to note the frequency with which certain cyclic nitrogenous groups are common to both alkaloids and aminoacids, for example, proline and nicotine both contain the pyrrolidine ring while the indole ring is common to tryptophane and brucine.

Cimician and Ravenna (1912) injected various nitrogen compounds into plants which normally contain alkaloids. The alkaloid content of these plants was determined after a time and compared to controls which had not been injected. The nicotine content of the tobacco plant injected with asparagine was greater than that of the control plant.

The variation in alkaloid content of plants produced under various cultural conditions has been studied by several investigators. Their results indicate that cultivated plants contain larger quantities of alkaloids than wild plants. Moreover, they have showed that dry sunny weather and the application of various fertilizer tend to increase the alkaloid yield.

The variation in alkaloid content of the adult plant during the vegetative period has been studied by a number of investigators. In general, they have found an increase in the alkaloid content of the plant up to the time of flowering

and then a decrease with the aging of the plant.

Studies on the variation in alkaloid content of plants induced by various external factors have been made by Clautriau (1885-86). This investigator found no decrease in the alkaloid content of plants after being kept for several days in the absence of light.

Clautriau also studied the effect of ringing on the caffeine content of branches of the coffee and tea plants. The coffee plant has opposite branches which bear an equal number of leaf pairs, are comparable in size and have an identical alkaloid content. One branch was treated and the opposite branch was used as a control. These experiments were carried out in the presence of light, absence of light, and in light with the absence of CO_2.

In the presence of light the treated branch showed a lower caffeine content than the control; in the absence of light there was no difference in the caffeine content of the two branches; while in the presence of light and absence of CO_2, the treated branch showed a greater caffeine content than the control. In this last experiment, the albumin-nitrogen content of the treated and control branches varied inversely with the caffeine content.

According to Goris, these experiments show that the alkaloids arise as waste products of albumin metabolism when the plant is forced to live on its reserves.

Clautriau (loc. cit.) studied the influence of nitrogen nutrition on the alkaloid content of the tea plant by placing

the stems of cut branches into some nutrient solutions with and some without nitrogen compounds. He found that the presence or absence of nitrates or ammonia in the nutrient solution had no influence on the caffeine content of the branches.

The effect of grafting on the alkaloid content of both stock and scion was studied by several investigators on plants in which the stock belonged to an alkaloid containing plant and the scion to a non-alkaloid containing plant and vice versa. The results showed that the alkaloid content of the same plant was greater when used as stock than when used as scion. This is due to the greater amount of nutrients obtained by the stock than by the scion.

Moreover, the results of these investigations showed some translocation of alkaloids through the graft but the quantities were very small and generally confined within short distances of the joint. Goris found no evidence from these experiments in favor of a nutritive role of the alkaloids.

The migration of alkaloids during the germination of seeds has been studied by several investigators. Clautriau (loc. cit.) germinated seeds of Coffea arabica L. between moistened filter papers. After the radicles had emerged from the seeds, the latter were placed on small screens in such a manner that the radicles extended into a container of distilled water below the screen. After the radicle and caulicle had elongated but before the cotyledons had completely spread apart, the caffeine content of the seedlings

was determined. He found an increase instead of a decrease in the caffeine content of the seedling in comparison with that contained in the seeds before germination. This increase occurred in experiments conducted in the presence and in the absence of light.

The above experiment was repeated with tea seeds which do not contain caffeine, the latter being localized in the pericarp. He found caffeine present in seedlings produced both in the presence and absence of light. Moreover, it has been demonstrated that this alkaloid does not disappear during the later development of the seedling. Suzuki (1901) obtained results concordant with those of Clautriau.

Clautriau (1894) found that seeds of Datura stramonium L., when freed from alkaloid by removing the integuments and washing with distilled water, germinated more rapidly than untreated seeds and produced seedlings which were normal in every respect. These seedlings showed an abundance of alkaloid in the growing point and around the central cylinder. Similar results were obtained by Clautriau with Conium maculatum L. and by Tunmann (1910) with Strychnos nux-vomica L.

The above results indicate beyond a doubt that the alkaloids are not utilized during the germination of seeds.

Goris failed to find any evidence, in any of the above mentioned investigations, in support of a nutritive role of the alkaloids.

Additional evidence bearing on the nutritive value of

alkaloids comes from experiments on the effects of these substances on seed germination and the growth of different plants. This problem dealing with the effect of alkaloids on the growth of fungi warrants a brief review of the literature on the previous work on this subject.

LITERATURE CITED

According to Wood, Remington, and Sadtler (1907) strychnine sulphate is used in practical medicine in doses of one fortieth to one twentieth grain (0.0016 to 0.003 gm.). One quarter of a grain has been known to produce death within a few hours.

Quinidine sulphate has a similar physiological action to that of quinine. Antimalarial dose one gram.

Caffeine is a valuable remedy in practical medicine as a cerebral and cardiac stimulant and as a diuretic. Dose, from three to eight grains (0.2 to 0.5 gm.).

Caffeine citrate is possessed of the therapeutic properties of caffeine. Dose, one teaspoonful (3.9 gm.).

Bokorny (1896) found that a 0.02% solution of strychnine nitrate killed Cladophora and Paramecium within six hours. A 0.1% solution stops the movement of infusoria and diatoms almost instantly and kills Cladophora, Vaucheria, Diatoms, Spirogyra, and insect larvae within six hours. A 0.01% solution kills most algae and microscopic animals within forty-eight hours. Strychnine and quinine were found to be more injurious for algae and lower water animals than morphine and nicotine.

The above author states that caffeine is a weak poison for algae and infusoria. Spirogyra is not killed by a 1.3% solution in twenty-four hours. A 0.1% solution is withstood well by Amoeba and does not visibly alter the movement of

Paramecia. He also states that the higher plants are very little susceptible to caffeine.

Sawa (1902) found caffeine and antipyrine in doses of 1-1000 to be toxic for onion and celery plants.

Lutz (1899) stated that caffeine is toxic with respect to the phanerogams and that the chlorohydrate of caffeine is a non-assimilable substance. The chlorohydrates of quinine and cocaine and the sulphate of atropine were likewise non-assimilable.

Stracke (1907) found quinine hydrochloride to be the most toxic alkaloid salt for the higher plants.

Weyl (1881) observed that strychnine caused a rapid yellowing of the leaves of Elodea canadensis.

Overton (1897) observed that caffeine and strychnine quickly penetrated the protoplasm of plant and animal cells. He also observed that the salts of the alkaloids were less toxic than the alkaloid base. This difference he attributes to an alkaline reaction produced by the base which is neutralized when the alkaloid is combined with an acid.

Yasuda (1901) found that a 2.5% solution of strychnine hydrochloride produced no signs of a poisonous action on Penicillium glaucum, Aspergillus niger, Botrytis cinerea, and Mucor stolonifer. He also found that the above fungi grew well in saturated solutions of quinine hydrochloride (slightly above 1%).

Marcet (1825) introduced the roots of young bean plants

into solutions containing 5 grains of the aqueous extracts of opium, nux vomica, belladonna, hemlock, and Cocculus indicus in an ounce of water. All of the plants died within four days. He observed a difference of action following the poisons employed.

Princeps (1828) studied the effect of plant extracts on the plants which produced them. He placed cut branches of Datura stramonium, Hyoscamus niger, and Momordica elaterium in solutions containing from 1-5 grains of the extract of these same plants respectively in an ounce of water. He observed that the leaves withered and the plants soon died.

DeVarigny (1892) gives the following account of Goppert's work as cited by Nobbe (1874). Goppert sprinkled seedlings of wheat, oats, peas, and cress with infusions of belladonna and observed that they developed as well as those sprinkled with pure water.

Bouchardat (1846) found that the injurious action of alkaloids was variable according to the nature of the soil in which they were grown and the nature of the base. The resistance of the plant increased directly with the fertility of the soil. Goris (1914) attributed this increased resistance of the plant in fertile soil to the greater abundance of nitrate which enables the plant to utilize the alkaloid. It seems to the writer that the colloidal action of the soil may also play a part in decreasing the injurious action of the alkaloids.

Wolf and Knop (1865) grew plants in mineral nutrient

solutions to which they added morphine, quinine, cinchonine, and caffeine for study. They observed an injurious action which varied with the alkaloid employed. The plants in the caffeine solution died first and those in the morphine solutions died last; they were unable to identify the alkaloids in the experimental plants.

Goris (1914) cites some work by Reveil (1865) who employed the pure salts of alkaloids in a dilution of 1-1000 of the base (without taking the acid into account) instead of using concentrated solutions. He used soil, calcined sand, or preferably saline solutions for nutritive media. For experimental plants, he used bulbs of hyacinth and crocus, water mints, cuttings of laurel rose, and balsam plants grown from seed in alkaloid solutions. He also germinated barley seeds in alkaloid solutions. The alkaloid was identified in the experimental plants. Reveil concluded from his experiments that atropine is a true fertiliser, since the plants sprinkled with this base grew more vigorously than the controls and that the alkaloid which is assimilated by the plant disappears rapidly during the course of growth. His experiments were later repeated by Marcacci (1887) and DeVarigny (1892) who were unable to confirm them.

Marcacci (1887) studied the influence of the sulphates of morphine, atropine, quinine, cinchonamine, and strychnine and the chlorohydrate of quinine on fermentation and the seeds and roots of plants.

He found that atropine and morphine produced a favorable

action on lactic acid fermentation which was retarded by quinine, veratrine, cinchonamine, and to a still greater extent by strychnine. All of the alkaloids produced a favorable action on alcoholic fermentation except cinchonamine and quinine. It is interesting to note that strychnine produced a favorable action on alcoholic fermentation and a marked inhibiting action on lactic acid fermentation.

With the seeds he used two procedures. In the first method, he soaked the seeds of Indian corn, bean, and lupine in the toxic solutions and planted them afterward. The date and number of germinations were noted. In the second method, the seeds were placed in crushed glass to germinate and sprinkled with the alkaloid solution. The alkaloid concentrations varied from 0.1 to 0.005 centigrams per 10 cc of solution.

In both series of experiments, Marcacci observed a toxic action which varied with the nature of the alkaloids. Quinine and cinchonamine were more toxic than morphine, strychnine and veratrine, while atropine had the weakest action. He observed that the roots suffer most from the action of the poison. They were short, slender, and nearly withered. They tended to grow toward the top or bottom as if trying to escape the poisonous solution. A microscopic examination showed them to be notably altered. The alkaloids are therefore especially fatal poisons for the roots, for even when in contact with the poison, the seeds are able to germinate while the roots are injured from the beginning.

Marcacci also observed that Lemna minor is fatally injured by veratrine, strychnine, quinine, and cinchonamine which cause the plant to rapidly lose its chlorophyll, but is uninjured by morphine and atropine.

DeVarigny (1892) filled small pots to four-fifths their capacity with sand which had been washed and dried at 130 to 150 degrees. To these he added sufficient distilled water or atropine solutions to bring the liquid from 2-5 mm above the level of the sand. The titres of the atropine solutions were 1/100, 1/200, and 1/400. These were planted with lentils or cress. At the end of 15 days the average weight of 20 germinations was one gram for the controls and 0.40 gram for the experimental plants.

In a second experiment DeVarigny grew flowers in vases filled with earth and atropine solutions diluted 1-200 and 1-800. The weight of the plants taken after 10 days was 1.4 grams for those grown in 1-800 atropine solution and 1.25 grams for the controls. When sand was used instead of the soil the weights were 0.80 gram for the controls and 0.70 gram for those grown in 1-800 atropine solution. The weight decreased to 0.35 gram in an atropine solution of 1-100 and root development was completely suppressed.

In a third series of experiments, DeVarigny studied the action of 1-500 and 1-1500 solutions of atropine on the germination of different seeds. According to the results obtained, the seeds could be divided into three categories:

those for which atropine was unfavorable, indifferent, or favorable. In general, atropine did not seem to exert much influence on germination. In general, there was a smaller percentage of germination and the plants were inferior to the controls.

Cornevin (1891) found that the germination of Agrostemma githago seeds was unaffected by saponine and the germination of Cytisis laburnum seeds was unaffected by cytisine. The germination of tobacco seeds was retarded 48 hours by being immersed for 28 hours in a solution of 1-150 of nicotine. He also found that the germination of some seeds was retarded 10 to 23 days when sown in soil and sprinkled with nicotine. With a solution containing an aqueous extract of opium, he was able to advance the germination of poppy seeds 24 hours and with a greater proportion of fertile seeds. The action of narcotine, codeine, and narceine was similar to that of opium; morphine and thebaine exercised no influence; while papaverine retarded germination for 48 hours.

Nosso (1894) accurately determined the doses of alkaloid necessary to produce a stimulating action on germination. He prepared solutions with concentrations varying from 2 to 0.0001%. He germinated seeds of Phaseolus multiflorus Willd. on cotton placed in the bottom of a vessel and saturated with the different alkaloid solutions.

Using chlorohydrate of morphine, Nosso found a concentration of 0.001% to be the most favorable. The seedlings

attained a length of 12 centimeters which was double that obtained with seeds germinated on cotton saturated with pure water. All development was checked in solution of 2, 1, and 0.5%. Solutions of 0.1, 0.05, and 0.01% produced seedlings inferior to the controls. The amount of growth produced in the different solutions increased with decreased concentration to 0.001% and then decreased with further decrease in concentration.

The growth obtained in the different nicotine solutions increased uniformly with decreased concentration to 0.01% and then uniformly decreased with further decrease in concentration. There was no development of the caulicle in solutions with concentrations of 2 and 1%.

The amount of growth obtained in the different strychnine solutions varied inversely with the concentration in dilutions ranging from 2 to 0.005%, and varied directly with it in dilutions greater than 0.005%. The maximum length of the caulicle was 11 cm which is approximately double that of the control.

The amount of growth in the different cocaine solutions increased with decreased concentration to 0.01%, remained constant in solutions ranging from 0.01 to 0.001%, and then decreased with further decrease in concentration.

In atropine solutions with concentrations ranging from 0.1 to 0.001% the development of the seedling was about the same as that of the control in pure water. The optimum concentrations were 0.0005 and 0.0001%. In these solutions the

caulicle attained a length approximately double that of the control.

All seedlings developed from seeds which were germinated in caffeine salicylate solutions were inferior to the controls. Some development was permitted by solutions with concentrations ranging from 0.05 to 0.0001%. The negative results obtained in caffeine solutions was attributed to antifermentative actions of salicylic acid.

These experiments show that in high concentrations the alkaloids have a toxic action and in weak doses an exciting action on seed germination. The concentrations which are injurious or beneficial vary with each alkaloid and probably with each plant.

Mirande (1900) obtained good germination of hemp seeds when treated with valerianate of quinine in a dilution of 1-2500 but the seedlings were inferior to the controls.

Otto (1894) found that plants grown on a sandy soil treated with strychnine were pale green and retarded in their development; they flowered but were unable to fruit; cultivated in humus and sprinkled with a solution of strychnine, they developed better and were able to fruit, but were retarded in comparison with the controls. He found that seeds sprinkled with a solution of strychnine phosphate after sowing develop quicker in humus than in a sandy soil but later than controls sown in a non-poisoned soil.

Lutz (1898), working under aseptic conditions, germinated seeds in washed and sterilized sand moistened with a

nutritive solution containing an alkaloid. At the end of the growing period, the plants were pulled up, dried, weighed, and their nitrogen content determined. From the original nitrogen content of the grain he was able to determine the gain or loss of nitrogen. He found that during the development of the seedling there was a loss instead of a gain in nitrogen. The nitrogen escaped from the seedling in the gaseous state after the seeds had germinated. He concluded that the alkaloids when used alone as a source of nitrogen were unassimilable.

DeToni (1905) grew Coix lacryma in nutrient solutions containing 3% of strychnine nitrate as the only nitrogen-containing compound. The plant was nearly equal in development to normal individuals, but seemed to present some differences principally on the absorbing organs. A 3.5% solution killed the plant. After fruiting of the Coix lacryma the solution was set aside and developed a magma of small green algae. He also cultivated this plant in nutrient solutions which contained strychnine sulphate as the only nitrogen-containing compound, but with only partial results.

Comere (1910) grew Ulothrix subtilis Kuts and Spirogyra crassa Kutz in a nutrient solution devoid of nitrogen compounds and to which he progressively added alkaloid salts. Morphine, atropine, and cocaine were directly assimilated in a decreasing order. The salts of quinine were also assimilable while those of strychnine were toxic. It required a concentration of 0.002% to kill Spirogyra and 0.008% to kill

Ulothrix.

Mobecourt (1921) cultivated Botrytis cinerea Pers. on Raulin's liquid containing varying amounts of nicotine, sulphates of atropine and quinine, and aconitine. He found that nicotine sulphate in a concentration of 25/1000 and atropine sulphate in a concentration of 20/1000 did not hinder the growth of this fungus. Growth was not retarded by a concentration of 10/1000 of quinine sulphate; but resulted in little thalli with few conidiophores by a concentration of 20/1000 and completely inhibited by a concentration of 30/1000. Growth was visibly hindered by aconitine in a concentration of 2/1000, and greatly reduced by a concentration of 4/1000. A concentration of 10/1000 did not prevent the germination of spores which was completely inhibited by a concentration of 20/1000.

Yasuda (1901) found that the growth of Penicillium glaucum, Aspergillus niger, Botrytis cinerea and Mucor stolonifer was increased by the addition of the hydrochlorides of cocaine, quinine, cinchonine, morphine, codeine, and strychnine to Richard's nutrient solution. The alkaloids were used in concentrations varying from 0.2 to 2%. As the concentration of alkaloid was increased, the conidiophores and sporangiophores became thinner and shorter. Conidium and sporangium formation was entirely suppressed and replaced by Clamydospores when the optimum concentration for fungus-vegetation was surpassed. The weakest alkaloid for the fungi under consideration was the hydrochloride of morphine, while

the strongest was that of cocaine. The fungi listed in order of their decreasing resistance to alkaloids are: Penicillium glaucum, Aspergillus niger, Botrytis cinerea, and Mucor stolonifer.

Ehrlich (1917) grew different fungi on a mineral nutrient solution to which he added different alkaloids as the only nitrogen-containing compounds and ethyl alcohol or invert sugar as a source of carbon in concentrations of 0.2 and 2% respectively. A control series without the alkaloid was run at the same time.

The fungi used were: Oidium lactis, Aspergillus niger, Penicillium glaucum, Willia anomala, Pichia farinosa, a mixed culture obtained by exposing the culture solution to the air, and an unknown species of wine yeast.

The alkaloids used were: pyridine, piperidine bitartrate, conine, nicotine, cinchonine, quinine, brucine, cocaine and morphine.

Ehrlich determined the amount of fungus dry weight produced and nitrogen content of the fungus substance at the end of a growing period which varied from 3 to 12 months.

He obtained only a small amount of growth, which was least in the yeast cultures and greatest in the mixed cultures. A small amount of nitrogen corresponding to the amount of growth was recovered from the fungus dry substance. He concluded that this nitrogen was obtained by the organism from the decomposition of the alkaloid. This decomposition was greater in the mixed cultures under the united action of

several organisms. He also obtained the aromatic odor of ester compounds in some cases and traces of ammonia with Nessler's reagent in others. The checking of growth which he obtained was attributed to the poisonous action of the decomposition products of the alkaloids. He suggests, that with a better composition of the nutrient solution, a greater amount of growth might be obtained, with a correspondingly greater conversion of the alkaloids. He further suggests, from the results obtained with fungi, that it is conceivable that the higher green plants as well as the fungi can produce enzymes by whose action the alkaloids are constructed, and further use be made of these substances in their metabolic processes.

Lutz (1899) grew Aspergillus niger and Penicillium glaucum on different test solutions and control solutions with the same elemental composition. These solutions were all made according to the formula of Raulin's solution with the following modifications: The nitrate and ammonia nitrogen of Raulin's formula was replaced in the test solutions by a corresponding amount of nitrogen in the form of combinations of the hydrochlorides of amines or alkaloids. The chlorine of the hydrochloride in the test solutions was compensated for in the control solutions by adding a corresponding amount of KCl. The potassium of the KCl added to the control solutions was compensated for in the test solutions by adding a corresponding amount of the neutral tartrate of potassium. This excess tartrate added to the test solutions

was compensated for by decreasing the amount of tartaric acid in Raulin's formula for these solutions. The carbon contained in the amines and alkaloids used in the test solutions was compensated for by decreasing the amount of sugar in Raulin's formula for these solutions.

The relative amounts of fungus growth in grams of dry weight produced on the different culture solutions were used as criteria of the nutritive value of the solutions. Any differences found in nutritive value was attributed to differences in degree of assimilability of nitrogen-containing compounds.

From the result of this investigation Lutz concluded that the alkaloids when used alone as a source of nitrogen were not assimilated by fungi, but were assimilated in considerable quantities in the presence of nitrate or ammonia-nitrogen.

In a later work, Lutz (1903) set up an experiment to determine whether fungi could use alkaloids alone as a source of nitrogen after growth had been started on ammonium nitrate and to make quantitative determinations of the amount of alkaloids utilized in both the presence and absence of ammonium nitrate.

Working under aseptic conditions, Lutz grew Aspergillus niger, Aspergillus repens, and Penicillium glaucum on Raulin's solution for a two day period. Dry weight determinations were then made on one third of the cultures. Raulin's solution was removed from the remaining cultures and replaced,

after being washed free from nitrogen compounds, by Raulin's solution containing nitrogen only in the form of alkaloid-nitrogen in one half the cultures and by Raulin's solution containing alkaloids to which ammonium nitrate was progressively added in the other half. All of the above solutions contained an equivalent amount of nitrogen.

Dry weight determinations were made on the above solutions at the end of a 26 day growing period. The alkaloid remaining in the culture solution was precipitated by adding an excess of potassium mercuric iodide. This precipitate was washed with distilled water, dried, and weighed. From the weight of the precipitate and the original known amount of alkaloid added to the culture solution, he calculated the amount of alkaloid utilized by the fungus.

The alkaloids used were the hydrochlorides of cocaine, morphine, and quinine.

He found some utilization of alkaloids from solutions whose nitrogen was only in the form of alkaloid-nitrogen. This he attributes to traces of ammonium nitrate absorbed from Raulin's solution before replacement by the alkaloid solution and which could not be washed from the mycelium. The amount of growth and of alkaloid utilized were considerably greater in the alkaloid solutions containing ammonium nitrate. He reports an absorption of 0.947 gm of chlorohydrate of cocaine and 0.400 gm of chlorohydrate of quinine from 50 cc of culture solution in 26 days.

Lutz suggests that the alkaloids are transformed into

albumins in the presence of ammonium nitrate. He does not consider them as either reserve compounds or waste products in the strict sense of the word, but as intermediate compounds between the albumins and mineral nitrogen compounds.

From the literature reviewed on the alkaloids it appears that these compounds when used in high dilution have an accelerating action on growth and when used in high concentrations a toxic action. The salts of the alkaloids seem to be less toxic than the pure alkaloids. The intensity of action varies with different alkaloids and with different experimental plants. In the higher plants the roots and root hairs seem to be the most sensitive part of the plant to the toxic action. The fungi seem to be least susceptible to the toxic action of the alkaloids. In many cases the fungi seem to be benefited by high concentrations of these substances while the algae and higher plants are killed by very dilute solutions. It also appears from the work of Ehrlich (1917) that some of the fungi are able to assimilate small quantities of alkaloids in the absence of other nitrogen containing compounds. According to Lutz (l. c.) the alkaloids may be assimilated in large quantities in the presence of nitrate or ammonia nitrogen. Lutz's method is open to objection on the ground that he used the amount of dry weight obtained as the sole criterion of the amount of nitrogen assimilated. Klotz (1923) has shown that the nitrogen content of the fungus dry substance varies with the nitrogen and carbon sources of the medium, the length of

incubation, rate of growth, and the H-ion concentration of the medium. A more acid medium prevents autolysis and thereby tends to increase the nitrogen content of the fungus. A rapid growth is accompanied by a lower per cent of nitrogen in the fungus. It follows from this, that any substance which accelerates the rate of growth, if only by a purely stimulating action, would effect a more efficient utilization of the nitrogen compounds in the production of fungus dry weight.

With these results of previous investigators in mind the writer has undertaken a study on the effect of caffeine, quinidine, and strychnine on the growth of Rhizopus nigricans and Aspergillus niger.

EXPERIMENTAL

A. EFFECT OF ALKALOIDS ON GROWTH AND SPORULATION

MATERIAL AND METHOD

The effect on growth was determined by growing a fungus on 100 cc portions of a nutrient medium containing varying amounts of the alkaloid under investigation and making dry weight determinations at regular intervals following inoculation. The relative time required from inoculation until the first appearance of visible mycelium and sporangia was used as a measure of the effect of the alkaloid on germination and sporulation.

The fungi used, Rhizopus nigricans Ehrhart and Aspergillus niger van Tieghem, were obtained from the stock cultures of the Department of Botany. These organisms show considerable difference in their ability to use nitrate-nitrogen; it being readily assimilated by Aspergillus niger but only slightly available to Rhizopus nigricans.

Coon's solution, as described by Young and Bennett (1922), was modified by increasing the sugar content to 2 per cent, and used for a culture medium to avoid difficulties of chemical analysis and interpretation of results which would arise from using a culture medium containing complex organic-nitrogen compounds.

Nutrient solution

$MgSO_4$----------	1.23	grams
KH_2PO_4----------	2.72	grams
KNO_3----------	2.02	grams
dextrose---------	20.00	grams
distilled water--	1000	cc

The above nutrient solution yields a good growth of Aspergillus niger, but only a very scant growth of Rhizopus nigricans is obtained unless the solution is reinforced with a more available form of nitrogen.

The following alkaloids were used: sulphates of strychnine and quinidine, caffeine citrate, and caffeine base. The alkaloids were obtained from the Merk Chemical Company and weighed out in the proper amounts and used without previous drying or purification. Alkaloid solutions of varying concentrations were prepared by adding the alkaloid in different amounts to 100 cc portions of the nutrient solution. These 100 cc portions were placed in 125 cc Erlenmeyer flasks, plugged with cotton, and sterilized in an Arnold steam sterilizer.

A heavy suspension of the spores was obtained by growing the fungus on potato dextrose agar and suspending the spores in distilled water. A drop of the heavy spore suspension was added to each flask with a looped inoculating needle or dropped from a burette. All flasks were inoculated at the same time and from the same spore suspension.

Dry weight determinations were made at the end of a 23 day growing period in the experiments with Rhizopus nigricans and at regular 7 day intervals following inoculation in the

experiments with Aspergillus niger. The mycelium was obtained by filtering the solutions through Gooch crucibles which previously had been partially filled with asbestos fibers, dried to constant weight in a dessicator, and weighed. The mycelium was washed by running distilled water through the filter. The filtering was hastened by using a filter flask connected to a vacuum pump. The mycelium obtained from five culture flasks was used for each dry weight determination in the experiments with Rhizopus nigricans. In the experiments with Aspergillus niger the mycelium from two culture flasks was used for each determination. The crucibles with mycelium and spores were dried and reweighed as described above. The increase in weight represents the amount of fungus growth.

RESULTS

Table I shows the effect of different concentrations of strychnine sulphate, caffeine, caffeine citrate, and quinidine sulphate on the growth of Rhizopus nigricans at room temperature for a 23 day growing period. In column 1 is given the different amounts of alkaloid in milligrams added to 100 cc of nutrient solution. Column II shows the amount of growth in grams of dry weight in the various strychnine solutions whose concentrations are given in column 1. Columns III, IV, and V show the amount of growth obtained in the caffeine, caffeine citrate, and quinidine sulphate solutions respectively. The small amount of growth obtained was due to the inability of Rhizopus nigricans to assimilate

either the alkaloid or nitrate nitrogen as was later demonstrated by adding peptone to the culture solution, in which case, a vigorous growth was obtained.

The table shows that the amount of growth was greater in the nutrient solution to which strychnine sulphate was added than in the nutrient solution alone; the amount of growth varying directly with the concentration of strychnine sulphate and inversely with the concentration of caffeine, caffeine citrate, and quinidine sulphate. Sporulation failed to occur in all of the above solutions.

Table II shows the effect of different concentrations of strychnine sulphate on the growth of Aspergillus niger at room temperature. Column 1 shows the different amounts of strychnine sulphate in milligrams added to 100 cc portions of the nutrient solution. The amount of growth obtained in each solution after 7, 14, 21, 28, and 35 day periods are given in columns 2, 3, 4, 5, and 6 respectively. Tables III, IV, and V show the effects of varying concentrations of quinidine sulphate, caffeine citrate, and caffeine respectively on the growth of Aspergillus niger. These results were obtained by the same method of experimentation and the data treated the same as those in table II.

The amount of growth varied directly with the concentration of strychnine and quinidine sulphates as shown in tables II and III respectively. The action of quinidine was different from that of strychnine in that in the former there was a considerable initial lag in the rate of growth

in solutions with concentrations above 150 milligrams per 100 cc. The length of the initial lag which varied directly with the alkaloid concentration, reached a maximum value of 49 days in the highest concentration used.

Table IV shows that caffeine citrate in concentrations of 50 milligrams or less per 100 cc of culture solution may cause a slight increase in growth over the control solution, but in higher concentrations it seemed to have a rather marked inhibiting effect. Caffeine produces a depressing action on growth in concentrations of 50 to 1000 milligrams per 100 cc of culture solution which increases in direct proportion to the concentration as is shown in table V.

Table VI shows the effect of varying concentrations of different alkaloids on the time interval in days required between inoculation and the first appearance of growth and sporulation in cultures of Aspergillus niger. Column 1 gives the concentration of alkaloid in milligrams per 100 cc of nutrient solution. Column 2 shows the number of days required for visible growth to appear in solutions with different concentrations of strychnine sulphate. Columns 3, 4, and 5 show the same for solutions of quinidine sulphate, caffeine citrate, and caffeine respectively. The time required for sporulation in strychnine sulphate, quinidine sulphate, caffeine citrate, and caffeine solutions of varying concentrations are shown in columns 6, 7, 8 and 9 respectively.

The time required for the first appearance of growth

and sporulation in strychnine sulphate solutions of all concentrations was the same as that for the control solution. In the solutions of quinidine sulphate, caffeine citrate and caffeine with concentrations above 100 milligrams per 100 cc, a longer time was required than in the control, the length of time varying directly with the alkaloid concentration. In the two highest concentrations of caffeine sporulation was completely suppressed.

Figure 1 was taken from the results given in tables II, III, IV, and V and shows the maximum dry weight produced with each alkaloid in each of the concentrations used. The alkaloid concentrations are given in units of 100 milligrams per 100 cc of solution. The amounts of dry weight produced are given in milligrams. This figure shows at a glance that strychnine and quinidine sulphates increased, while caffeine citrate and caffeine decreased dry weight production. In the strychnine sulphate solutions the amount of growth increased with increased concentration of strychnine sulphate up to 1800 milligrams per 100 cc which was the highest concentration used. In the quinidine sulphate solutions the amount of growth likewise varied directly with the alkaloid concentration up to 1000 milligrams per 100 cc and then abruptly decreased. This abrupt decrease in amount of growth may be traced to the exceedingly long initial lag in the growth rate which is produced by high concentrations of quinidine. From these results one may conclude that strychnine and quinidine sulphates increase, while caffeine citrate and

caffeine decrease the amount of growth. The action of strychnine and quinidine may be that of a stimulus or nutrient or both. If these alkaloids have a nutritive value, it may be either the nitrogen or carbon or both that is assimilated.

TABLE I

EFFECT OF DIFFERENT ALKALOIDS ON THE GROWTH OF RHIZOPUS NIGRICANS AT ROOM TEMPERATURE

I	II	III	IV	V
	Strychnine sulphate	Caffeine	Caffeine citrate	Quinidine sulphate
Alkaloid concentration mg./100cc.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.
0	.0112	.0112	.0112	.0112
50	.0129	.0127	.0104	.0095
100	.0152	.0106	.0101	.0068
200	.0176	.0092	.0096	.0059
300	.0194	.0086	.0068	-----
400	.0186	-----	.0021	-----
500	.0256	-----	-----	-----
750	.0358	-----	-----	-----

TABLE II

EFFECT OF STRYCHNINE SULPHATE ON THE GROWTH OF ASPERGILLUS NIGER AT ROOM TEMPERATURE

I	II	III	IV	V	VI
Days	7	14	21	28	35
Alkaloid concentration mg./100cc.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.
0	.1342	.3855	.5637	.5710	.5934
50	.1634	.3832	.5603	.5728	.6162
150	.1596	.3922	.5866	.5935	.5945
300	.1685	.3907	.6043	.5983	.6176
500	.2220	.4738	.6725	.6111	.6483
750	.1933	.4851	.6616	.6715	.6810
1050	.1844	.5046	.7158	.7060	.7023
1400	.2612	.4876	.7410	.7667	.7890
1800	.1517	.5484	.7552	.8017	.7274

TABLE III

EFFECT OF VARYING CONCENTRATIONS OF QUINIDINE SULPHATE ON THE GROWTH OF ASPERGILLUS NIGER AT ROOM TEMPERATURE

I	II	III	IV	V	VI	VII
Days	7	14	21	28	35	42
Alkaloid concentration mg./100cc.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.
0	.1342	.3855	.5637	.5710	.5984	.5883
50	.1686	.2320	.5677	.6093	.6816	-----
150	.1536	.2876	.5667	.6044	.6717	-----
300	.1124	.2747	.5704	.6434	.6864	-----
500	.0674	.3135	.5573	.7011	.7334	-----
750	-----	.2004	.5624	.5717	.7824	.7416
1000	-----	-----	.1686	.5953	.7005	.8616
1250	-----	-----	-----	.1290	.1712	.6102
1500	First appearance of growth 49 days after inoculation.					

TABLE IV

EFFECT OF VARYING CONCENTRATIONS OF CAFFEINE CITRATE ON THE GROWTH OF ASPERGILLUS NIGER AT ROOM TEMPERATURE

I	II	III	IV	V	VI	VII
Days	7	14	21	28	35	42
Alkaloid concentration mg./100cc.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.
0	.1342	.3855	.5637	.5710	.5984	.5883
50	.1153	.3984	.5310	.5802	.6055	-----
150	.1210	.4126	.5399	.5290	.5143	-----
300	.1672	.4314	.5248	.4977	.4621	-----
500	.0920	.4100	.4889	.4754	.4675	-----
750	.0772	.3850	.5011	.5130	.4928	-----
1050	.0589	.2667	.5246	.5321	.5257	-----
1400	-----	.1012	.4356	.4924	.4410	.4985
1800	-----	.0364	.3092	.3991	.4205	.4736

TABLE V

EFFECT OF VARYING CONCENTRATIONS OF CAFFEINE ON THE GROWTH OF ASPERGILLUS NIGER AT ROOM TEMPERATURE

I	II	III	IV	V	VI	VII
Days	7	14	21	28	35	42
Alkaloid concentration mg./100cc.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.	Growth in gm. dry wt.
0	.1342	.3855	.5637	.5710	.5984	.5883
50	.1396	.3092	.5386	.5662	.5783	-----
100	.1262	.3152	.5330	.5479	.5240	-----
250	.1456	.2791	.3958	.3963	.3938	-----
400	.1708	.2432	.3843	.4199	.4046	-----
550	.0960	.2308	.3670	.4198	.3719	-----
700	.0558	.1875	.3210	.3607	.3464	-----
850	.0346	.1258	.2642	.3422	.3314	-----
1000	-----	.1239	.2280	.2410	.2744	.2917

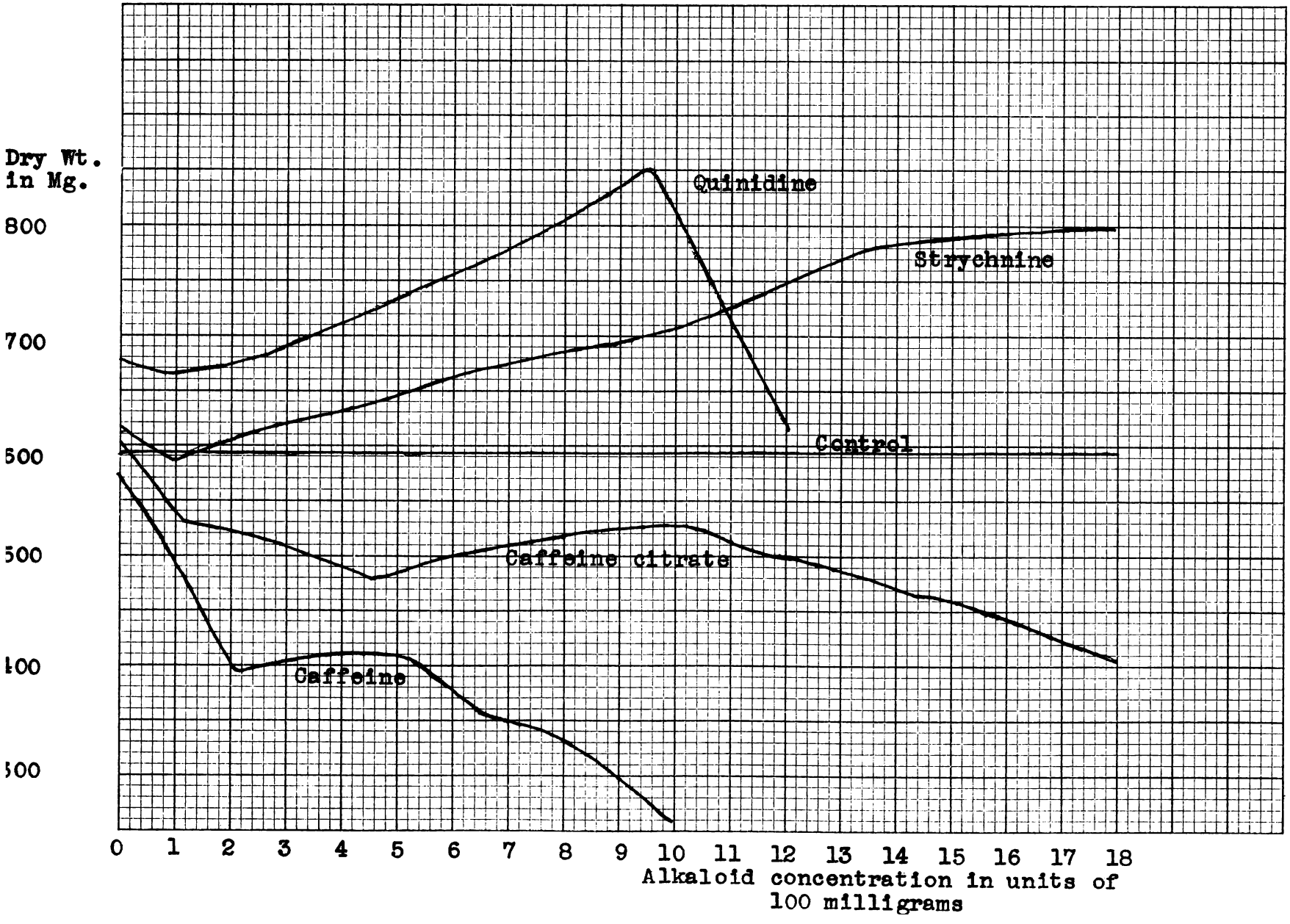
TABLE VI

TIME REQUIRED IN DAYS FOR THE FIRST APPEARANCE OF GROWTH AND SPORULATION OF ASPERGILLUS NIGER AT ROOM TEMPERATURE ON DIFFERENT ALKALOID SOLUTIONS OF VARYING CONCENTRATIONS

	Time required in days for first appearance of growth				Time required in days for sporulation			
I	II	III	IV	V	VI	VII	VIII	IX
Alkaloid used	SS	QS	CC	C	SS	QS	CC	C
Alk. Conc. Mg./100 cc.	Days				Days			
0 control	1	1	1	1	3	3	3	3
100	1	1	1	1	3	3	3	3
625	1	2	2	2	3	5	11	25
1175	1	5	5	2	3	8	15	--
1750	1	180	12	5	3	180	34	--

SS--strychnine sulphate
QS--quinidine sulphate
CC--caffeine citrate
C--caffeine

FIGURE I.


Dry Wt. in Mg.
800
700
600
500
400
300
Quinidine
Strychnine
Control
Caffeine citrate
Caffeine
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Alkaloid concentration in units of 100 milligrams

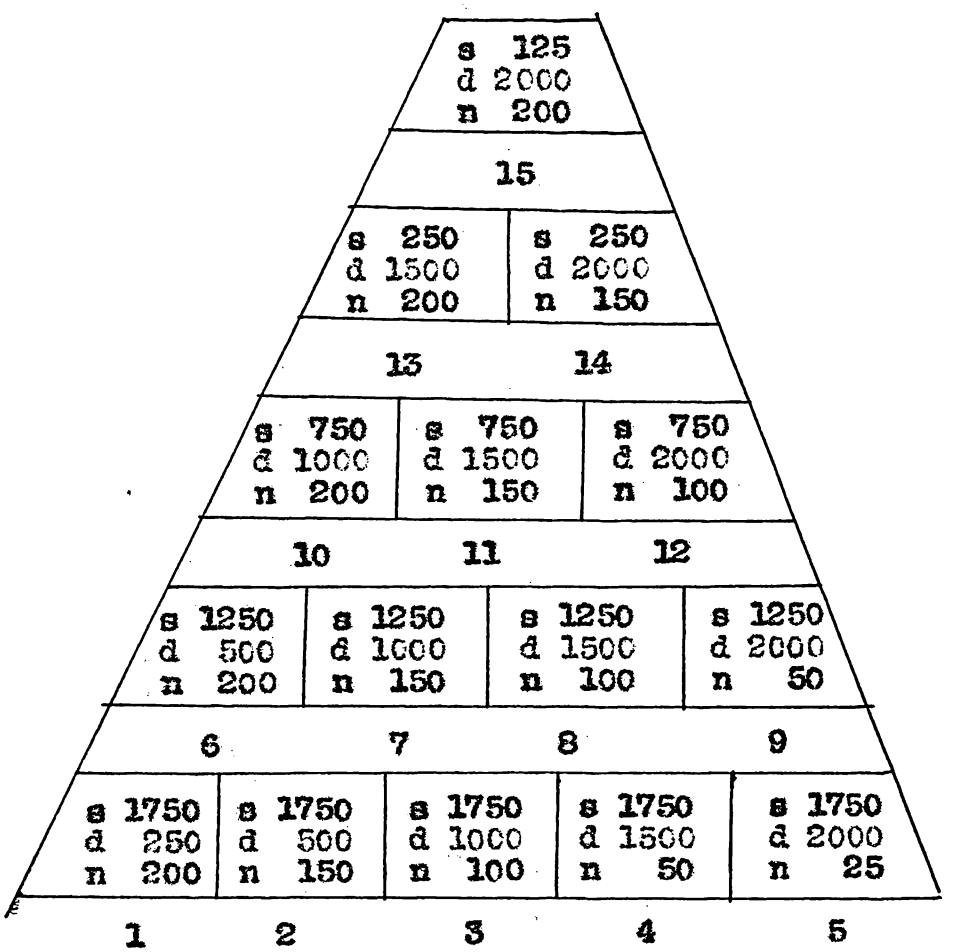
B. NUTRITIVE VALUE OF STRYCHNINE

MATERIAL AND METHOD

In the previous experiment it was found that strychnine and quinidine sulphates accelerated and increased the growth of Aspergillus niger. This experiment was undertaken to find out whether strychnine sulphate has a nutritive value or merely a stimulating action or perhaps both, and in case strychnine is assimilated, to determine whether the carbon or nitrogen or both are utilized.

A triangle experiment was set up using fifteen different culture solutions. Each of the fifteen different media contained 1.23 grams of magnesium sulphate and 2.72 grams of potassium dihydrogen phosphate per liter. In addition the solutions contained varying amounts of the following com - pounds: dextrose, an available form of carbon; potassium nitrate, an available form of nitrogen; and strychnine sulphate which contains both carbon and nitrogen whose availability was to be studied. These three compounds varied in amount in such a way that each might be a limiting factor in growth. The ratio of dextrose to potassium nitrate varied in the different solutions from 1.25 to 80. Figure 2 shows the dextrose, potassium nitrate, and strychnine sulphate content of each of the fifteen different culture solutions in milligrams per 100 cc of solution. The different culture solutions are referred to by numbers given below the squares. 1125 cc amounts of each of the fifteen different culture solutions were made up in 2-liter flasks and measured out in

STRYCHNINE SULPHATE, DEXTROSE, AND POTASSIUM NITRATE CONTENT OF THE 15 CULTURE SOLUTIONS IN Mgs PER 100 CC.



s, strychnine sulphate. d, dextrose. n, potassium nitrate. Each of the above solutions contain 123 milligrams of magnesium sulphate and 272 milligrams of potassium dihydrogen phosphate per 100 cc.

FIGURE II

75 cc portions into 125 cc Erylenmeyer flasks. After 30 minutes sterilization in the Arnold steam sterilizer on three successive days the H-ion was adjusted to Ph 4.8.

The procedure for preparing the spore suspension and inoculating was the same as that used in the previous experiment.

Dry weight determinations were made with the procedure used in the previous experiment at 11, 28, 41, and 54 day intervals following inoculation. The culture solution was removed from the filter flask and the latter rinsed with sufficient distilled water to bring the solution up to its original volume. The culture solution was then divided into aliquots which were used for H-ion and quantitative sugar determinations, and a qualitative test for the presence of nitrates.

Ph readings were made with a Sargent quinhydrone acid Ph meter after heating the solution to expel any carbonic acid present. Quantitative sugar determinations were made by Benedict's method as given by Mathews (1920). The presence of nitrates was indicated by adding a few drops of a solution of diphenylamine to a few cc of the culture solution in a test tube and running chemically pure, concentrated sulphuric acid down the side of the tube. A deep blue ring at the juncture of the two liquids indicated the presence of nitrate.

RESULTS

Table VII shows the amount of growth obtained with each of the 15 different culture solutions at the end of 11, 28, 41, and 54 day growing periods. Each weight listed represents the total mycelium obtained from two culture flasks. The time intervals during which sugar and nitrates were still present in the different culture solutions are indicated by straight and wavy underlines respectively.

The results indicate that there was practically no further increase in growth after the dextrose was exhausted in culture solutions No. 1, 2, 6, 7, and 10 (also 3 and 14 as indicated by their Ph values and dextrose/KNO_3 ratios) in which the dextrose disappeared before the nitrate. There was a gradual but slow increase in dry weight production after the nitrate was exhausted in cultures No. 4, 5, 9, and 12 (also No. 8 as indicated by its Ph value and dextrose/KNO_3 ratio) in which the nitrate disappeared before the dextrose. This would indicate that strychnine-nitrogen may be slowly assimilated after the nitrate-nitrogen is exhausted and does not preclude the possibility of its being assimilated along with the nitrate. On the other hand, the strychnine-carbon could only be assimilated along with the dextrose for there is no further increase in growth after the sugar has been exhausted.

Table VIII shows that there is a considerable increase in the acidity of the solution when the nitrate is exhausted

before the dextrose in cultures No. 4, 5, 9, and 12 (also No. 8 as indicated by its Ph value and dextrose/KNO_3 ratio). This may be due to the conversion of the surplus sugar to organic acids (Klotz, 1923). The Ph value of the solution remains higher when the dextrose is exhausted before the nitrate in cultures No. 1, 2, 6, 7, and 10 (also Nos. 3 and 14 as indicated by their Ph values and dextrose/KNO_3 ratios).

Table XIX shows, for each culture solution, the amount of sugar consumed in grams per gram of fungus dry weight produced at the end of 11, 28, 41, and 54 day growing periods. From the variation in amount of sugar consumed per gram of fungus dry weight produced in the different culture solutions it appears that either the carbon of the strychnine is assimilated along with the dextrose to a greater extent in some solutions than in others or that the sugar is more efficiently utilized in the production of fungus dry weight in some cases than in others.

Table 10 shows the amount of potassium nitrate consumed in the production of one gram of fungus dry weight for several culture solutions. The variation in amount would indicate that the nitrate was reinforced to a greater extent with strychnine-nitrogen in some solutions than in others or that it was more efficiently utilized in the production of dry weight in some cases than in others.

Figure 3 shows in graphical form: the relative amounts of potassium nitrate, dextrose, and strychnine sulphate; and the maximum amount of fungus dry weight produced in each of

the fifteen different culture solutions. The range in amount of these substances shown in the graph are as follows: KNO_3, from 25 to 200 mg.; dextrose, from 250 to 2000 mg.; strychnine sulphate, from 125 to 1750 mg.; and fungus dry weight from 124.8 to 793.6 mg. The numbers symbolizing the different culture solutions are the same as those used in the previous figure and tables, and are given in the lower row of figures below the graph. The ratios of dextrose to potassium nitrate in the different culture solutions are given in the upper row of figures below the graph. The numbers above the graph show the length of growing period in days during which the maximum growth was produced in each of the fifteen different culture solutions.

The graph shows at a glance that there is but little if any correlation between the amount of growth and the concentration of strychnine sulphate. The amount of growth shows a close correlation with the dextrose concentration when the dextrose/KNO_3 ratio is below 15 and a close correlation with the KNO_3 concentration when the dextrose/KNO_3 ratio is above 13. In the latter case, the resulting H-ion concentration of the culture solution is comparatively high as compared with the former, Table VIII. This acidity may be due to the conversion of the surplus sugar to organic acids (Klotz, l. c.).

A more careful analysis of the graph shows that equal quantities of dextrose in cultures No. 6 and 2 did not produce equal amounts of fungus dry weight although dextrose was a limiting factor in growth. This was also true of cul-

tures no. 10, 7 and 3 which contained equal amounts of dextrose. The greatest growth occurred in the solution with a dextrose/KNO_3 ratio nearer the optimum value of 13.3. This is also true of potassium nitrate when it is a limiting factor: cultures no. 9 and 4; and 8 and 12. In the latter two solutions the smaller amount of growth was obtained in solution no. 8 whose dextrose/KNO_3 ratio was closer to the optimum value (13.3). This may be due to two limiting factors in solution no. 8, KNO_3 then dextrose, and only one limiting factor, KNO_3, in solution No. 12. Analysis of solution no. 8 showed that the KNO_3 and dextrose disappeared at approximately the same time, Table VII. That the KNO_3 disappeared before the dextrose was shown by the resulting H-ion concentration of the culture solution, Table VIII. Solution No. 12 had a greater amount of dextrose than solution no. 8. After the disappearance of the KNO_3, the fungus may have utilized the strychnine-nitrogen and continued to assimilate the surplus dextrose as was shown by a gradual increase in dry weight, Table VII and Figure III.

TABLE VII

AMOUNT OF GROWTH IN GRAMS OF DRY WEIGHT PRODUCED IN EACH OF THE 15 DIFFERENT CULTURE SOLUTIONS

days	11	28	41	54
Culture number	weight in grams	weight in grams	weight in grams	weight in grams
1	.1062	.1248	.1092	-----
2	.1602	.2278	.1866	.1919
3	.0962	.5370	.4786	.3928
4	.1838	.3020	.3556	.3010
5	.1228	.1432	.1508	.1648
6	.1232	.2080	.1863	.1734
7	.1290	.3656	.5245	.4294
8	.2320	.5018	.6318	.5567
9	.2956	.2952	.3095	.3057
10	.1539	.3828	.4440	.3790
11	.3175	.4308	.4780	.4669
12	.1918	.6230	.6316	.6476
13	.2566	.4490	.4972	.5206
14	.5082	.7938	.7207	.7423
15	.3968	.4688	.7146	.7345

TABLE VIII

HYDROGEN ION CONCENTRATION OF CULTURE SOLUTIONS TAKEN AT THE END OF THE GROWING PERIOD

days	11	28	41	54
Culture number	Ph value	Ph value	Ph value	Ph value
1	2.95	2.90	2.87	----
2	2.75	2.35	2.30	2.70
3	2.60	2.20	2.00	2.40
4	2.50	1.90	*	*
5	2.45	1.90	*	*
6	2.50	2.37	2.28	2.30
7	2.64	2.20	2.28	2.38
8	2.45	1.90	*	*
9	2.20	*	*	*
10	2.55	2.37	2.63	2.68
11	2.40	2.37	2.55	2.50
12	2.65	2.20	*	*
13	2.40	2.30	2.64	2.60
14	2.20	2.20	2.00	2.00
15	2.40	2.48	2.00	2.20

* Ph too low to measure with potentiometer employed.

TABLE IX

DEXTROSE CONSUMED IN GRAMS PER GRAM OF FUNGUS DRY WEIGHT PRODUCED

days	11	28	41	54
Culture number	grams of dextrose	grams of dextrose	grams of dextrose	grams of dextrose
1	3.53	3.02	3.43	----
2	2.72	3.25	4.02	3.91
3	2.90	2.48	3.13	3.82
4	3.07	3.82	3.93	4.74
5	0.99	5.27	5.44	6.83
6	2.87	3.61	4.03	4.33
7	2.41	2.99	2.86	3.49
8	2.79	3.50	3.56	4.04
9	2.64	4.32	4.28	5.13
10	2.80	3.00	3.38	3.96
11	2.82	3.20	3.11	4.11
12	2.67	3.03	3.84	4.38
13	2.83	3.23	3.38	4.32
14	2.74	3.44	4.16	4.04
15	2.51	2.94	3.63	3.84

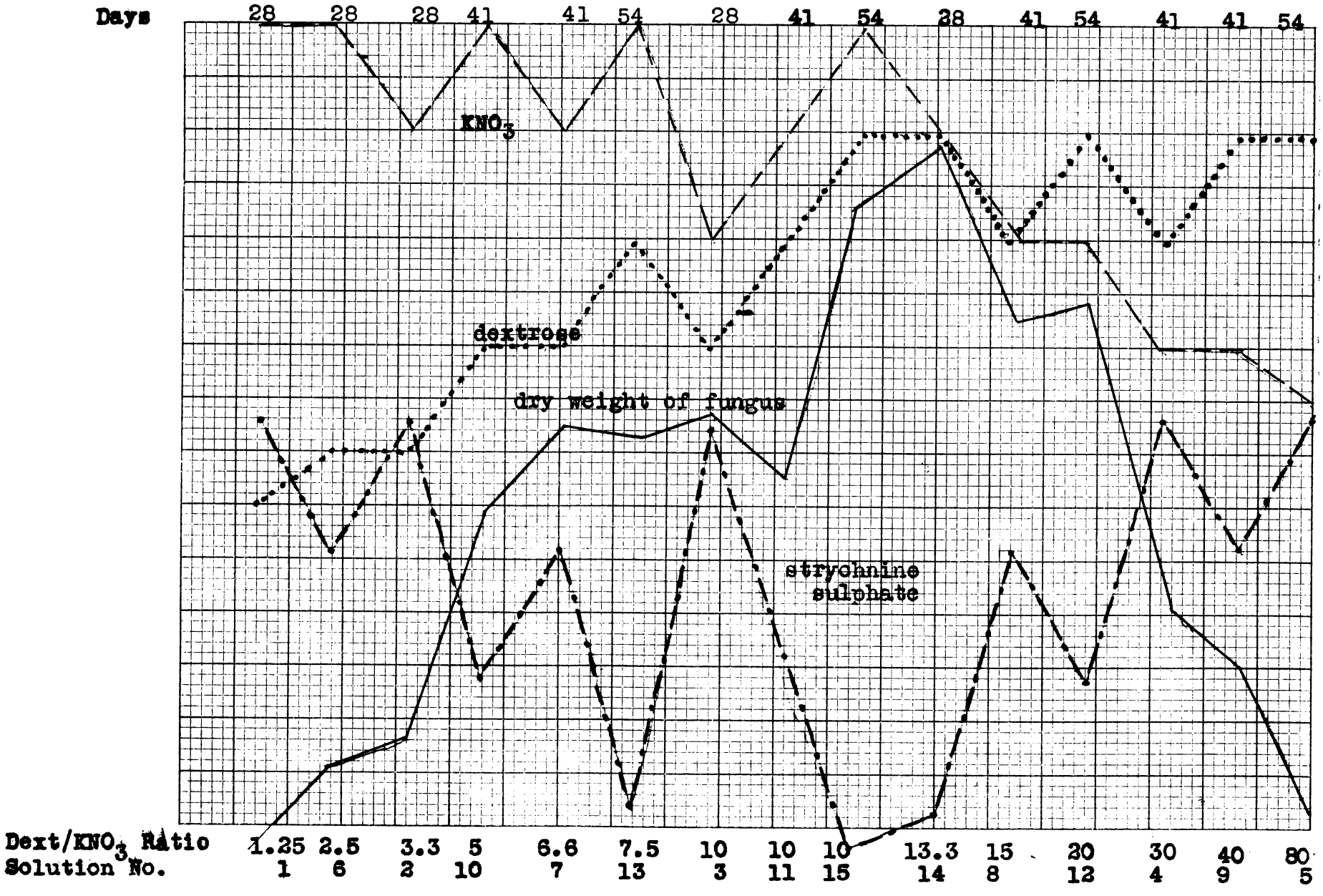
TABLE X

POTASSIUM NITRATE CONSUMED IN GRAMS

PER GRAM OF FUNGUS DRY WEIGHT PRODUCED

CULTURE SOLUTION NUMBER	GRAMS OF KNO_3
3	.279
4	.210
5	.227
8	.237
9	.242
12	.231
14	.283

FIGURE III


Days
28 28 28 41 41 54 28 41 54 28 41 54 41 41 54
KNO3
dextrose
dry weight of fungus
strychnine sulphate
Dext/KNO3 Ratio
1.25 2.5 3.3 5 6.6 7.5 10 10 10 13.3 15 20 30 40 80
Solution No.
1 6 2 10 7 13 3 11 15 14 8 12 4 9 5

C. QUANTITATIVE DETERMINATION OF STRYCHNINE ASSIMILATED

MATERIAL AND METHOD

This experiment was undertaken to determine the amount of strychnine removed by Aspergillus niger from culture solutions with varying dextrose/potassium nitrate ratios.

The composition of the different culture solutions in milligrams per 100 cc of solution and their respective dextrose/potassium nitrate ratios are as follows:

Solution No.		4	9	14	19	24
sugar/KNO_3 ratio		4	9	14	19	24
dextrose	mgm.	572	1287	2002	2002	2002
KNO_3	mgm.	143	143	143	105.37	83.42
strychnine	mgm.	1047	1047	1047	1047	1047
$MgSO_4$	mgm.	123	123	123	123	123
KH_2PO_4	mgm.	272	272	272	272	272

A control series with the above composition minus the strychnine was run at the same time.

The culture solutions were made up in 1800 cc amounts from stock solutions.

All of the culture solutions showed a H-ion concentration of Ph 4.33.

The culture solutions were measured out in 75 cc portions into 125 cc Erylenmeyer flasks and plugged with cotton. The solutions were then sterilized and inoculated by the proced-

ure used in the previous experiment.

A few cultures were subjected, from time to time, to Fehling's test for the presence of sugar and the test for the presence of nitrates described in the previous experiment. These cultures were later discarded. When the above tests indicated that either the dextrose or nitrate had completely disappeared from the culture solutions, the mycelium was removed, dried to constant weight, and weighed as described in the previous experiment. Three cultures were used for each determination. The strychnine content of the culture solutions was then determined by the following procedure: the solution was made alkaline by the addition of ammonia which precipitated the strychnine base in the form of crystals. This also precipitated a small amount of other substance from which the strychnine was separated by its solubility in chloroform. After standing over night, the solutions were filtered through No. 589 filter paper manufactured by Carl Schleicher and Schüll, the crystals dried in a vacuum oven at 40 degrees centigrade, and weighed. The strychnine portion of the precipitate was then removed by running chloroform through the filter paper. The filter paper and insoluble residue was dried to constant weight and reweighed. The loss in weight represents the amount of strychnine.

The accuracy of the above method for the quantitative determination of strychnine was found by adding equal amounts of strychnine sulphate to 100 cc amounts of distilled water

and to 100 cc amounts of different culture solutions in which the fungus had been growing. The amounts of strychnine recovered from the above solutions were as follows:

from distilled water	from culture solutions
.7846 grams	.7731 grams
.7809 grams	.7825 grams
.7851 grams	.7737 grams
.7827 grams	.7845 grams

This shows a maximum experimental error of less than 2 per cent.

RESULT

Qualitative tests for the presence of sugar and nitrate showed that the dextrose disappeared before the nitrate in solutions Nos. 4 and 9 and that the nitrate disappeared before the dextrose in the remainder. The relative time required in days for the complete disappearance of dextrose in solutions Nos. 4 and 9 and the disappearance of nitrate in solutions Nos. 14, 19, and 24 are as follows:

solution number	strychnine solutions	control solutions
4	16 days	22 days
9	18 days	23 days
14	16 days	22 days
19	12 days	18 days
24	12 days	16 days

The above data indicates that the disappearance of dextrose and nitrates from the culture solution is hastened by the presence of strychnine.

The amount of dry weight produced and strychnine assimilated in grams for each of the five different culture solutions are as follows:

solution number	4	9	14	19	24
(growth) strychnine solution	.3866	.6856	.8822	.8649	.7095
(growth) control solution	.2817	.5573	.8011	.7403	.6477
strychnine assimilated	.0000	.0000	.0360	.0036	.0000

The above data indicates a slight assimilation of strychnine in solution No. 14 which has an optimum dextrose/KNO_3 ratio, while no change could be detected in the strychnine content of the remaining solutions. This indicates only a very low nutritive value for strychnine, if any.

SUMMARY

1. The growth of Rhizopus nigricans on Coon's nutrient solution is slightly increased by the addition of strychnine sulphate to the culture solution; the amount of growth varying directly with the alkaloid concentration.

2. The growth of Rhizopus nigricans on Coon's nutrient solution is decreased by the addition of quinidine sulphate, caffeine, or caffeine citrate to the nutrient solution; the amount of growth varying inversely with the alkaloid concentration.

3. The growth of Aspergillus niger on Coon's nutrient solution is increased by the addition of strychnine or quinidine sulphates to the nutrient solution; the amount of growth varying directly with the alkaloid concentration.

4. There is a pronounced initial lag in the growth rate produced by the higher concentrations of quinidine sulphate which varies directly with the alkaloid concentration.

5. The growth of Aspergillus niger on Coon's nutrient solution is decreased by the addition of caffeine or caffeine citrate to the nutrient solution; the amount of growth varying inversely with the alkaloid concentration.

6. The time required for germination and sporulation of Aspergillus niger on Coon's nutrient solution is unaffected by the addition of strychnine sulphate to the nutrient solution. Quinidine sulphate, caffeine, and caffeine citrate in concentrations above 100 milligrams per 100 cc of nutrient

solution increases the time required for germination and sporulation; the length of time varying directly with the alkaloid concentration.

7. There is no increase in amount of growth of Aspergillus niger on Coon's nutrient solution containing strychnine after the dextrose is exhausted, which indicates that strychnine alone cannot serve as a source of carbon for this fungus. Therefore, if strychnine is utilized it must be assimilated along with the dextrose.

8. The amount of growth of Aspergillus niger on Coon's solution containing strychnine continues increasing slowly after the nitrate is exhausted, this indicating that strychnine-nitrogen may be assimilated to a slight extent in the absence of nitrate-nitrogen and does not preclude the possibility of its being assimilated in larger quantities along with the nitrate.

9. When the ratio of dextrose to KNO_3 is above 13.3, the nitrate disappears from the culture solution before the dextrose and the surplus sugar is probably converted to organic acids which increase the H-ion concentration of the culture solution.

10. Equal amounts of dextrose, in nutrient solutions in which dextrose and strychnine are the only source of carbon and the dextrose is all consumed, do not always produce equal amounts of fungus dry weight. This indicates that the dextrose is either reinforced by the strychnine-carbon to a greater extent in some solutions than in others or that the

dextrose is more efficiently utilized in the production of dry weight in some cases than in others. The greater amount of growth is obtained in the solution whose dextrose/KNO_3 ratio is nearer the optimum value of 13.3. Similar results are obtained with equal amounts of KNO_3 which would lead to the same conclusion about nitrogen assimilation.

11. Quantitative determinations showed an assimilation of 36 milligrams of strychnine from 225 cc of a culture solution with an optimum dextrose/KNO_3 ratio. This does not agree with the conclusion of Luttz (l. c.), that the alkaloids are assimilated in considerable quantities in the presence of nitrate nitrogen. It seems more probable that the increased amount of growth obtained in strychnine solutions is due more to a stimulating action of the alkaloid, which effects a more efficient utilization of the carbon and nitrogen compounds in the production of fungus dry weight, than to a nutritive value.

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