products showing the following absorption bands (wavelengths in $m\mu$):

	Ultra-violet	SbCl ₃
1	390, 370, 350	620
2	368, 350, 330	620
3	368, 350, 330	614
4	304	614 and faint band at 575

The spectroscopic characteristics of the material from zone 3 are those of 'cyclized' subvitamin A, and zone 2 appears to be the 'cyclized' isovitamin A of Embree & Shantz (1943).

Oxidation was continued with oxygen at 70° for 110 hr. and samples were removed at intervals, 'cyclized', and chromatographed. In each case, except towards the end of the oxidation, a zone was obtained which showed ultra-violet absorption maxima at 370, 350 and 330 $m\mu$ (the band at 350 $m\mu$ being the highest maximum) and a band at 614 $m\mu$ in the antimony trichloride reaction.

SUMMARY

1. β -Apo-2-carotenol, β -apo-2-carotenol, axerophthylideneacetone, the C₂₀ aldehyde obtained by oxidation of vitamin-A alcohol, and the alcohol obtained by Ponderff reduction of the latter, all fail to undergo 'cyclization' on treatment with alcoholic HCl, whereas axerophthylideneisopropyl alcohol gives a compound which shows the characteristic fine structure of a 'cyclized' product in the ultra-violet region.

2. The bearing of these and other observations on the structure of 'cyclized' vitamin A, vitamin A₂, and subvitamin A is discussed.

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The Influence of Sodium Nitrate Concentration on the Metabolism of *Penicillium sclerotiorum* van Beyma

By D. REILLY, T. LONG AND T. P. CURTIN, *The National University of Ireland (University College, Cork)*

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Penicillium sclerotiorum produces two metabolic products: sclerotiorine, a chlorinated pigment (Curtin & Reilly, 1940), and sclerotiose, a poly-glucose (Albericci, Curtin & Reilly, 1943). The present paper records the effect of different concentrations of nitrate in the culture medium on the utilization of chloride and on the production of sclerotiorine and sclerotiose. The results support the earlier conclusion that sclerotiorine production is not directly connected with chloride utilization, and further show that sclerotiose production is affected by the nitrate concentration of the medium.

METHODS

Acid Czapek-Dox medium, in which the NaNO₃ content ranged from 0 to 0.4% by increments of 0.05%, was used: glucose, 50 g.; KCl, 0.5 g.; MgSO₄, 7H₂O, 0.5 g.; KH₂PO₄, 1.0 g.; FeSO₄, 7H₂O, 0.01 g.; NaNO₃, 0.4–0 g.; distilled water to 1000 ml. Nine 8 l. batches were distributed in 100 ml. amounts in 20 oz. medical flat bottles and sterilized by steaming for 1 hr. on each of three consecutive days. The batches were then inoculated with a spore suspension of *P. sclerotiorum* van Beyma (2 ml./bottle) taken from a 14-day-old culture, remotely transferred from that obtained in March 1940, from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

The cultures were incubated in the dark over a period of 24 days at the optimum temperature of growth, 25°. Every 3 days the mixed filtrates from three bottles of uniform appearance were used for pH, glucose and chloride determinations.

Determination of chloride. Direct titration of residual chloride was not possible as the substrate reduces both AgNO_3 and KMnO_4 . Oxidation and subsequent titration were therefore performed after a modification of the method used by Raistrick & Smith (1936). The filtered substrates were first adjusted to 300 ml. by the addition of distilled water, so compensating for losses in volume by evaporation; 50 ml. were then shaken for 10 min. with 2.5 g. norite charcoal and filtered at the pump. To 25 ml. of this filtrate, excess $\text{N}/100 \text{ AgNO}_3$ and 5 ml. 6N-HNO_3 (free from HNO_2) were added. The temperature was raised to boiling-point and the precipitated AgCl was filtered off, and washed with a little dilute HNO_3 . When cold, this new filtrate was back-titrated with $\text{N}/100 \text{ NH}_4\text{CNS}$, with 1 ml. of standard ferric alum solution as an indicator. It was found that the medium extracted chlorides from the norite to a value of 0.00097 g. $\text{KCl}/2.5 \text{ g. charcoal}$. This figure was deducted in all cases.

Determination of growth, pigment and polysaccharide. At the end of the 24-day growth period, the remaining bottles of each batch were filtered, and the mycelium washed, dried *in vacuo* at 55–60° and weighed. Each sample was then Soxhlet-extracted with light petroleum and its yield of pigment weighed on the second recrystallization. All traces of solvent were removed *in vacuo*, and the mycelium was then extracted with boiling water to isolate the polysaccharide sclerotiose.

The red aqueous liquors were boiled with norite (2 g./l.), cooled, and the precipitated polyglucose filtered off. This compound was twice reprecipitated from distilled water, filtered and macerated with absolute ethanol. On further filtration, it was triturated with absolute diethyl ether, which was then removed *in vacuo*. The white amorphous solid was dried at 50° for some hours and weighed.

RESULTS

Generally speaking, the mycelial felt thinned out with increasing nitrate concentration, while the coloured medium changed from initial red to the normal yellow, and finally was almost colourless. In all cases, except on the nitrate-free medium on which no growth was apparent, the pH value reached a minimum in 6–9 days. The final pH values, which in the lower concentrations of nitrate were not much higher than the minimum, gradually approached neutrality with increasing nitrate concentration (up to 0.25%) and after this point remained unaltered by further change. Similarly, the initial slow rate of glucose consumption reached a maximum on the 0.25% nitrate medium, and was uninfluenced by greater nitrate concentration. The values of the residual KCl content on the different media are shown in Fig. 1.

It is concluded that utilization of KCl is inversely proportional to the nitrate concentration of the medium.

The yields of mycelium, pigment and polyglucose, calculated in g./l., are shown in Fig. 2. It

Fig. 1. Residual KCl content of media of varying nitrate content over period of growth of *Penicillium sclerotiorum*. Curves are labelled with the respective percentage of sodium nitrate in the medium used. Ordinates: residual KCl (g./l.). Abscissa: time of incubation in days.

Fig. 2. Yields of mycelium, pigment and polyglucose from *Penicillium sclerotiorum* grown on media of varying nitrate content. Ordinates: polyglucose produced (g./l.) ———; pigment produced (g./l.) - - - - -; mycelium produced (g./l.) ..... Abscissa: percentage sodium nitrate in medium.

is apparent that optimum production of mycelium, pigment and sclerotiose is obtained on the Czapek-

Dox medium which contains 1.0 g. NaNO_3 /l. (0.1 %).

As the nitrate concentration is further increased, the growth, indicated by the mycelium yield and the production of sclerotiose, is progressively reduced.

While Fig. 1 indicates that maximum KCl consumption is obtained on media of low nitrate content, Fig. 2 shows a parallelism of sclerotiorine production with growth, in nitrate concentrations up to 0.2 %, and hence independence of the KCl consumption. Above 0.2 % sodium nitrate, it would appear that pigment production is inhibited by the nitrate. As this is also true of KCl utilization, it may be asserted that the production of sclerotiorine is responsive to nitrate concentration only when this factor greatly reduces the amount of KCl metabolized by the organism. The facts justify the previous assertion that pigment production is merely a by-product of the chloride consumption.

SUMMARY

1. The KCl metabolism of *Penicillium sclerotium* is an inverse function of the nitrate concentration of the culture medium, and the pigment yield is unaffected until such time as the chloride consumption is much reduced below normal.

2. The production of sclerotiose is almost inversely proportional to the nitrate content of the medium, yields in excess of 10 % being obtained on a substrate in which the nitrate concentration is half that usually used, as compared with 3 % for the standard (0.2 % NaNO_3) medium.

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Tissue Nucleic Acids

1. RIBONUCLEIC ACIDS AND NUCLEOTIDES IN EMBRYONIC AND ADULT TISSUE

By J. N. DAVIDSON AND CHARITY WAYMOUTH, *Biochemical Laboratory,
Physiology Department, University of Aberdeen*

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Desoxyribonucleic acid (thymonucleic acid, chromonucleic acid), known for a long time to be a constituent of the nuclei of animal cells, together with histones and the recently discovered protein chromosomin (Stedman & Stedman, 1943*a*), has been found also in plant tissues (Feulgen & Rossenbeck, 1924), in bean seedlings (Kiesel & Belozerski, 1934; Belozerski, 1936), in onion bulbs (Belozerski, 1939), in the rye grain (Behrens, 1938) and in yeast cells (Delaporte & Roukhlman, 1938). At the same time evidence has accumulated that pentose nucleic acid (ribonucleic acid, yeast nucleic acid, zymonucleic acid, plasmonucleic acid, phytonucleic acid), which differs from desoxyribonucleic acid in containing *d*-ribofuranose in place of *d*-2-desoxyribofuranose and uracil in place of thymine (methyl uracil), and which was originally thought to be solely a plant constituent (cf. Jones, 1920), is present in animal tissues.

Pentose derivatives have long been known to be present in animal tissues (Blumenthal, 1898; Grund, 1902; Wohlge-

mut, 1903; Neuberg, 1904; Mendel & Leavenworth, 1908). As well as the mono- and dinucleotide coenzymes containing adenine, e.g. adenosine triphosphate, the nicotinamide nucleotides and riboflavin-adenine-dinucleotide, pentose nucleotides of other bases have been found, e.g. uridylic acid (Levene & Mandel, 1907) in shell-fish eggs, and all four nucleotides of ribonucleic acid (Calvery, 1928) in hydrolysates of chick embryo pulp. Ribopolynucleotides may also occur. The so-called β -nucleoprotein of the pancreas, originally prepared by O. Hammarsten (1894) and subsequently investigated by Bang (1898-9, 1900-1), Feulgen (1919-20), E. Hammarsten (1920), Jones & Perkins (1924-5) and Steudel (1936), was shown by Jorpes (1924, 1928, 1934) to consist essentially of a ribopolynucleotide—probably a pentanucleotide and almost certainly similar to but not identical with the ribonucleic acid of yeast. It may form as much as 10 % of the weight of the pancreas, and, like yeast nucleic acid, contains uracil (Jorpes, 1924). The presence of a ribonucleic acid containing thymine in the mammary gland is suggested by Mandel & Levene (1905). As a result of this work, and of the work of Jones & Perkins (1924-5), who found that a ribonucleotide resembling the guanylic acid of the pancreas was also present in spleen and liver, and of similar observations by Thomas & Berariu