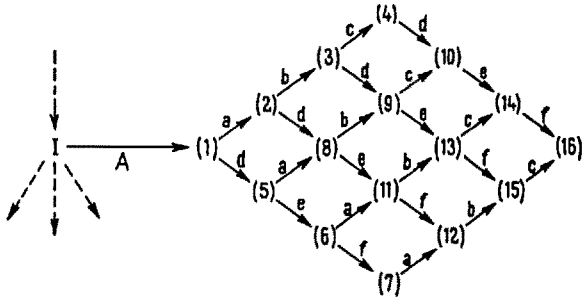
COGITATIONES

Secondary Metabolism: an Explanation in Terms of Induced Enzyme Mechanisms

The phenomena of secondary metabolism are well-known in microorganisms, from bacteria to higher fungi, but less clearly defined in higher plants – with which, therefore, this contribution is not directly concerned. Most of the 'natural products' with which chemists have concerned themselves are secondary metabolites; they include antibiotics and other physiologically active compounds and have considerable technical importance as well as theoretical interest. Secondary metabolites have seldom any direct recognizable function; they exist in enormous variety but have very restricted taxonomic distribution. In recent years the general outlines of their biogenesis has become clear and in many cases our knowledge of this aspect is very detailed; this aspect can be summarized in saying that they arise from key intermediates of general metabolism by a range of unique reactions, the products then being diversified by the interplay of further transformations. This biogenetic picture is generalized and idealized in the Figure.

In general, the secondary metabolites of microorganisms are not produced at all times during the development of the organism; in particular, the processes of growth and of secondary metabolite production can frequently be distinguished. By 'growth' in this context, is meant the balanced replication of cell material, as for example in the multiplication of single cells. The present evidence indicates that these contrasting activities involve physiologically different cell states, and there have been many studies of this 'metabolic phasing', of which those of BECKER are perhaps the most general[1]. The fact that these cellular phases are successive does not mean that in a cell population the transitions are simultaneous, and indeed under practical circumstances there may be considerable overlapping (as shown for typical industrial fermentations by BECKER[1]); however, laboratory conditions can usually be devised in which overlapping is considerably reduced. For filamentous organisms, this usually requires strict control of the culture inoculum and the avoidance of mycelial aggregates in which micro-environment is diversified.



Idealized scheme of biogenesis of secondary metabolites. Broken lines represent general metabolism, in which I is a key intermediate; the special reaction A leads to secondary metabolites, initially to product (1), which is then further metabolized by reactions a-f, interplay of which can lead to a selection of the further products 2-16. Note that the reactions a-f need not have high substrate specificity; if they do, this will somewhat restrict the range of products obtained. All the products belong to one biogenetic class, determined by the reaction A.

When these conditions are fulfilled, and when 'growth' is measured by some more appropriate parameter than the dry weight, which frequently continues to increase when cell replication has stopped, the phases of balanced growth and of secondary metabolism can be investigated. In a wide variety of cases it has been found that the phase of balanced growth is limited by the exhaustion of some particular nutrient component, such as nitrogen or phosphate. The most detailed study so far is that of BORROW et al. on *Gibberella fujikuroi*[2], but other notable cases are alkaloid production by *Claviceps purpurea*[3], anthraquinone formation by *Penicillium islandicum*[4], fat synthesis in yeasts, etc.; mere multiplication of examples is superfluous. The conditions which terminate balanced growth are, of course, significantly similar to those which initiate spore formation and analogous processes, whose relationship to secondary metabolism has frequently been remarked.

Our own work has been mostly concerned with metabolic phasing in *Penicillium urticae*, which in its secondary metabolism produces a range of compounds derived from 6-methylsalicylic acid (6-MSA). We have distinguished here[5] a 'trophophase'[6] with balanced uptake of nitrogen and phosphate, nucleic acid synthesis, etc., and an 'idiophase'[7] marked by carbon assimilation and secondary metabolite production. Respirometric studies[5,8] showed rapid carbon metabolism (glucose breakdown and tricarboxylic acid cycle oxidation) in the trophophase, and markedly slower metabolism in the idiophase. There were also qualitative differences, notably that glucose was mainly oxidized by the hexose monophosphate pathway in the trophophase, and by glycolysis in the idiophase: a similar finding for *P. chrysogenum* is already recorded[9]. The capacity to synthesize 6-MSA, and also to metabolize it, are idiophase characteristics and cannot be demonstrated in the normal trophophase.

The termination of balanced growth is fairly easy to understand; it is less easy to see how this leads to the initiation of secondary metabolic processes. On this question, our work with *P. urticae* gives indications of general significance. The end of the trophophase is marked by a sharp decline in respiration, from its peak value to a temporary minimum, which also coincides with a sharp decline in nitrogen and phosphate uptake. At the same time, the withdrawal of intermediates of the tricarboxylic acid cycle for synthetic reactions, hitherto maximal, falls sharply to a very low level. The respirometric evidence for these events is typified in the Table. Thus the transition from trophophase to idiophase is marked by a

[1] Z. E. BECKER, *Fiziologiya Gribov* (Moskva, 1963), *passim; Mitteilungen der Versuchsstation für das Gärungsgewebe* (Wien) *18*, 1 (1964).

[2] A. BORROW, E. G. JEFFERY, R. H. J. KESSEL, E. C. LLOYD, P. B. LLOYD, and I. S. NIXON, Canad. J. Microbiol. *7*, 227 (1961).

[3] L. C. VINING and W. A. TABER, in *Biochemistry of Industrial Microorganisms* (Ed. C. RAINBOW and A. H. ROSE; London 1963), p. 341.

[4] S. GATENBECK, Prague Congress on Antibiotics, June (1964), in press; and verbal communication.

[5] J. D. BU'LOCK, *Chemistry of Microbial Products* (Tokyo 1964), p. 152. – J. D. BU'LOCK, D. HAMILTON, and A. J. POWELL, in press.

[6] Nutrient.

[7] Peculiar.

[8] J. D. BU'LOCK, D. HAMILTON, and A. J. POWELL, Biochem. J. *90*, 26 P (1964).

[9] E. C. HEATH and H. KOFFLER, J. Bact. *71*, 174 (1956).

metabolic catastrophe. HAWKER and HEPDEN have observed a very similar situation at the onset of sporulation in *Rhizopus sexualis*[10]. The events observed imply equally sudden accumulations of a variety of metabolic intermediates, which are normally present at low concentrations: it is precisely accumulations of this kind which can lead, by the induced formation of new enzymes, to the opening of new synthetic pathways which will relieve the pressure of key metabolic intermediates. Such new synthetic pathways are in fact what we observe and they constitute the basis of secondary metabolite formation.

In *P. urticae* the new reaction elicited in this response is the synthesis of 6-MSA from acetyl- and malonyl-CoA[11]; the relevant stimulus would be an accumulation of acetyl-CoA or, more probably, of malonyl-CoA. During the trophophase, as an intermediate in fatty acid synthesis, malonyl-CoA is present but probably in limiting amounts; the sudden accumulation of tricarboxylic acid cycle intermediates would markedly stimulate malonyl-CoA synthesis[12] and raise the concentration of this intermediate. Respirometric studies of *P. islandicum*, in which the anthraquinone pigments are again malonyl-CoA derivatives, require a similar interpretation[13]. The case of *Gibberella fujikuroi* is somewhat different; here secondary metabolites in the idiophase are characteristic isoprenoid derivatives, formed from mevalonic acid which during balanced growth is almost certainly the limiting precursor in the synthesis of ergosterol. Addition of extra mevalonate to the culture medium elicits the same response as would be predicted, on the present argument, for an endogenous accumulation of mevalonate at the termination of balanced growth, *viz.* acceleration and earlier initiation of the secondary synthetic pathway[14].

In *P. urticae* the synthesis of 6-MSA corresponds to reaction *A* of the Figure. The reactions corresponding to *a–f* then convert 6-MSA into a variety of other metabolites, as shown for example by BASSETT and TANENBAUM[15]. These further reactions cannot be demonstrated during the normal trophophase and appear to arise as further consequences of enzymic induction. Even in the idiophase, these reactions appear successively, at least in part; in the trophophase, they can be demonstrated prior to endogenous 6-MSA synthesis, but only after a sufficiently prolonged exposure to exogenous 6-MSA. The application of continuous culture methods to *P. urticae* confirms these observations in a significant way. If continuous culture is set up early in the trophophase, at a high dilution rate, trophophase metabolism is maintained indefinitely. With a lower dilution rate, commencing at a later time, typical idiophase production of 6-MSA is observed, with little further metabolism; if dilution is now interrupted for a few hours, the full pattern of 6-MSA metabolites is obtained. These observations strongly suggest that what we have termed the diversifying reactions (*a–f* of the Figure) are mediated, at least in part, by sequentially induced enzymes. This is, in any case, likely on *a priori* grounds; in the particular case of *P. urticae* there is evidence that the synthesis of 6-MSA is inhibited by 6-MSA at concentrations comparable to those normally present in idiophase cultures; any such inhibition would nullify the primary effect of 6-MSA synthesis (that of relieving the 'metabolic pressure') were it not itself relieved by further metabolism of 6-MSA.

We thus arrive at a generalized hypothesis concerning the sequence of events leading to secondary metabolism, *viz.* (1) termination of balanced growth, leading to (2) sudden accumulation of metabolic intermediates, leading to (3) induced synthesis of secondary metabolite, leading to (4) induced further metabolism diversifying the secondary metabolites.

We hope to continue our investigations of *P. urticae* along the lines suggested by this approach, but the generality of this hypothesis obviously requireds substantiation by many other instances.

Typical respirometric results with developing *P. urticae*

	Trophophase	Transition	Idiophase
Total CO_2 output (µl/min/mg)	12–17	3.3–4.3	5.5
$^{14}CO_2$ from U-^{14}C-glucose (units/min/mg)	4.9–9.4	1.4–2.9	1.9–3.7
$^{14}CO_2$ from 1-^{14}C-acetate (units/min/mg)	9.9	1.5	2.6–2.9
Withdrawal of tricarboxylic acid cycle intermediates (%)	85–95	25	45–95

Methods: Total CO_2 from Warburg respirometry; $^{14}CO_2$ from radiorespirometry; percentage dilution of tricarboxylic acid cycle from radiorespirometry with 1-^{14}C- and 2-^{14}C-acetate.

Zusammenfassung. Ist das Wachstum von *Penicillium urticae* und anderer Pilze im Gleichgewicht gestört, so kommt es zu plötzlicher Akkumulierung wichtiger metabolischer Zwischenprodukte. Diese induzieren die Bildung neuer sekundärer Metabolite synthetisierender Enzyme, was Anlass zu weiterer Enzymentstehung ist und die Diversität der Stoffwechselendprodukte bestimmt.

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[13] S. GATENBECK, verbal communication; see also [4].
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[15] E. W. BASSETT and S. W. TANENBAUM, Biochem. biophys. Acta, *28*, 21 (1958).