Table 2. Specificity of Synthesis of Different Penicillins by *A. faecalis* Amidase

Substrates	Whole cells	Sonic extract
(III) + Sodium phenylacetate	444	91
(III) + Sodium phenoxyacetate	<10	<10
(III) + Sodium caproate	265	80

Substrate concentrations, 10 mgm./ml. Results expressed in units/ml. in terms of (I).

Table 3. Penicillin *G* Amidase Activity in Re-used *A. faecalis* Cells

Consecutive run No.	Reaction time (hr.)	μgm./ml. (III)
1	5½	2,675
2	18½	4,050
3	4½	3,280
4	18½	lost
5	4½	3,825

times than used in the standard assay system (5 mgm. per ml. final concentration of (I) for a 6-hr. incubation time).

The enzyme activity found in whole cell preparations was extremely stable. Cells have been re-used as much as five times. After centrifugation and further incubation with fresh (I), there was no apparent loss in (III) production (Table 3).

A more detailed account of these experiments will be published elsewhere.

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Production of Lysergic Acid Derivatives by a Strain of *Claviceps paspali* Stevens and Hall in Submerged Culture

A strain of *Claviceps paspali* Stevens and Hall isolated from the grass *Paspalum distichum* L. found growing on a hill in the neighbourhood of Rome was found, after propagation on a rye embryo grown on agar[1], to be capable of producing lysergic acid derivatives in submerged culture.

The culture medium used had the following composition: mannitol, 3 per cent; ammonium succinate, 3 per cent; potassium dihydrogen phosphate, 0·1 per cent; $MgSO_4.7H_2O$, 0·03 per cent; tap water (*p*H 5·2). Experiments were carried out in 500-ml. shake flasks containing 100 ml. of culture fluid and agitated on a rotary shaker, as well as in stirred fermenters up to 500 litres in capacity. The lysergic acid derivatives were almost completely released into the culture medium. Their production, followed colorimetrically by means of the van Urk reaction[2], began on the second day and increased until the ninth day, when concentrations up to 2 mgm. per ml. (calculated as lysergic acid) were reached. An excess of oxygen in the culture fluid was essential for production of alkaloid.

The bases were extracted from the filtered culture media at *p*H 7·2–7·4 with organic solvents and transferred to water (by extraction with diluted acids). The alkaloids were then concentrated by repeating this procedure several times, and finally recovered from the organic solvent phase as the free bases or the maleates.

Four main substances were obtained which were recognized as two pairs of isomers, deriving from lysergic and *iso*lysergic acid respectively.

The members of the first pair of isomers were both obtained in the crystalline state and identified as lysergic acid amide (*iso*ergine[3]) and *iso*lysergic acid amide (ergine[4]); their decomposition points, crystal form, chromatographic behaviour and infra-red spectra were identical with the corresponding properties of authentic specimens. On hydrolysis with *N* sodium hydroxide, lysergic acid amide gave ammonia and lysergic acid in good yield; the lysergic acid thus obtained was identical in all respects with an authentic specimen derived from natural ergot. The mixture of both pairs of alkaloids on hydrolysis with *N* sodium hydroxide gave ammonia and lysergic and *iso*lysergic acid, which were identified chromatographically. On treatment with anhydrous hydrazine or hydrazine hydrate[5], lysergic acid amide as well as *iso*lysergic acid amide gave racemic *iso*-lysergic acid hydrazide in good yield; the latter was identified by its infra-red spectrum.

The members of the second pair of alkaloids yielded readily one molecule of acetaldehyde and one molecule of ammonia on treatment with diluted mineral acids in the boiling water-bath. The lysergic acid derivative was easily transformed into lysergic acid amide, with concomitant evolution of acetaldehyde, at *p*H 8 and 50°. The latter reaction occurs slowly even on standing at room temperature. The isomer derived from lysergic acid was obtained in the crystalline state as the free base from chloroform and as the acid maleate from methanol–ether. The free base was obtained in long solvent-free prisms which started to decompose at 135° C. (Kofler) and showed $[\alpha]_D^{20°} + 29° \pm 2°$ (C = 1 in dimethylformamide) and no appreciable rotation in pyridine. Its ultra-violet spectrum was identical with that of other amide-substituted derivatives of lysergic acid (maxima at 242 and 312 mμ). The infra-red spectrum is shown in Fig. 1.

Analysis (free base): calculated for $C_{16}H_{21}N_3O_2$ (311·37): C, 69·43; H, 6·80; N, 13·50. Found: C, 69·23; 69·30; H, 6·94–7·04; N, 13·69.

Analysis (maleate): calculated for $C_{16}H_{21}N_3O_2.C_4H_4O_4$ (427·44): C, 61·81; H, 5·90; N, 9·83. Found: C, 61·39; H, 6·33; N, 9·37.

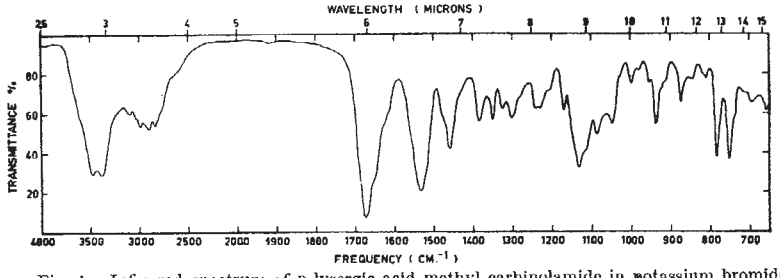

Fig. 1. Infra-red spectrum of D-lysergic acid methyl carbinolamide in potassium bromide (C = 0·3 per cent)

The second member of the pair of isomers, derived from *iso*lysergic acid, was not obtained in the crystalline form; but by isomerization with diluted acetic acid, it was readily transformed into the isomer obtained from lysergic acid described above.

The two lysergic acid derivatives yielding acetaldehyde are thought to have the acetaldehyde moiety linked to the lysergic acid moiety in the form of the carbinolamide linkage:

$$\begin{array}{c} CH_3 \\ | \\ -CONH.C.OH \\ | \\ H \end{array}$$

Neither the two isomers of lysergic acid amide nor those of lysergic acid methyl carbinolamide have previously been reported to occur in Nature.

Full details of this work will be published elsewhere.

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Microbial Synthesis of Protein in Relation to the Biogenesis of Nucleic Acids with *Azotobacter vinelandii* as the Test Organism

Caspersson[1] and Brachet[2] first directed attention to the possible role of ribonucleic acid in protein synthesis. This role has been re-evaluated by Brachet and Six[3]. Ribonucleic acid is supposed to serve as a template for protein synthesis. Evidence in favour of and against this hypothesis has been documented by Chantrenne[4], Meister[5], Simkin[6] and Hartman and Buchanan[7].

One of the most useful tools in demonstrating the relationship between protein and nucleic acid syntheses is to use antibiotics and antimetabolites which interfere with either one or both the processes[1-6]. The mechanism of incorporation of radioactive amino-acids into protein of particulate preparations of *Azotobacter vinelandii* is being investigated in detail in this laboratory. As a prelude to that work, the effect of some antibiotics and antimetabolites on the synthesis of nucleic acid and of protein in whole cells of *A. vinelandii* has been studied. Similar effects have been observed in cell-free preparations as well.

Disodium hydrogen phosphate (^{32}P) and sodium sulphate (^{35}S) were found to be rapidly incorporated into the growing cells of *A. vinelandii*. The fraction soluble in trichloracetic acid attained saturation with either tracer, phosphorus-32 or sulphur-35, within about an hour, whereas incorporation into the fractions insoluble in trichloracetic acid maintained a steady rate of incorporation in parallel with the growth of the cells. The resting cells of *A. vinelandii* similarly incorporated phosphorus-32 and sulphur-35 at a comparatively good rate. As in the case of growing cells, a source of energy, for example sucrose, was needed for the incorporation of both the tracers into the resting cells. Analyses of the fractions insoluble in trichloracetic acid showed that 90 per cent of the phosphorus-32 was incorporated into nucleic acids, whereas 97 per cent of the sulphur-35 was incorporated into protein. Thus the incorporation of phosphorus-32 and sulphur-35 into the fractions insoluble in trichloracetic acid of the resting cells can be looked upon as reflecting protein and nucleic acid syntheses respectively.

The results of inhibition studies of the incorporations of phosphorus-32 and sulphur-35 into resting cells of *A. vinelandii* in the presence of antibiotics and antimetabolites are presented in Table 1. The detailed kinetic results will be presented elsewhere. The concentrations of the inhibitors chosen are three times those required for inhibition of growth.

Table 1. Effect of Antibiotics and Antimetabolites on the Incorporation of Phosphorus-32 and Sulphur-35 into the Nucleic Acids and Protein of the Resting Cells of *A. vinelandii*

Antibiotic or antimetabolite used	Inhibition of incorporation (per cent)*	
	Phosphorus-32	Sulphur-35
Chloramphenicol (600 μgm./ml.)	9	90
Streptomycin (400 μgm./ml.)	45	90
Penicillin (114 μgm./ml.)	19	54
p-Fluorophenylalanine (720 μgm./ml.)	17	36
8-Azaguanine (600 μgm./ml.)	18	71

* Percentage inhibitions have been calculated with reference to the incorporation of the tracers into the fractions of the control insoluble in trichloracetic acid containing no antibiotic or antimetabolite, which was 400 counts/min. in the case of phosphorus-32 and 375 counts/min. in the case of sulphur-35.

In experiments on the incorporation of phosphorus-32, incubation was at 30° C. for 2 hr. with constant shaking in a total volume of 10 ml. containing 610 μmoles of sucrose, 42 μmoles of radioactive potassium phosphate, *p*H 7·0 ($2·4 \times 10^6$ counts/min.), 150 mgm. (on dry weight basis) of cells of *A. vinelandii* and a suitable amount of growth inhibitor or antimetabolite (see Table 1). For the incorporation of sulphur-35, conditions were exactly the same with the exception that 23 μmoles of radioactive sodium sulphate (2×10^6 counts/min.) were added instead of phosphate. Non-radioactive phosphate buffer (42 μmoles) was, however, included. Control incubations without any antibiotic or antimetabolite were always run. Incorporation was stopped by quick centrifugation at 20,000*g* followed by washings; the cells were washed four times, each time with 10 ml. of 0·05 *M* phosphate or 0·05 *M* sulphate respectively. Washed cells were treated with 1 ml. of cold 10 per cent trichloracetic acid for 30 min. The precipitate collected by centrifugation was re-treated with 1 ml. of cold 5 per cent trichloracetic acid. Finally, the precipitate was washed twice in each case with 10 ml. of 0·05 *M* phosphate (or sulphate) and suspended in 10 ml. of water. Aliquots were plated, dried and counted in a thin-window Geiger counter (Nuclear Chicago Co.). Since the plating was done at infinite thinness, no self-absorption correction was needed.

Incorporation of phosphorus-32 into nucleic acids is affected only to a small extent in the presence of chloramphenicol. Protein synthesis as reflected by incorporation of sulphur-35 is, however, inhibited to a great extent as expected. Streptomycin similarly interferes with incorporation of sulphur-35. Incorporation of phosphorus-32 is also inhibited to a considerable extent. Penicillin, which is known to