tion, particularly the former. The effect was blocked by previous atropinization, but not by antihistaminics.

Of the various fractions, the essential oil was comparatively more potent and produced significant action even in dose of 1.5×10^{-3} to 3×10^{-3} mg./Kg. See Fig. 4.

Isolated Tracheal Chain.—The different fractions relaxed the tracheal chain and antagonized acetylcholine and histamine induced bronchospasm as shown in Fig. 5 and Table II.

Lung Perfusion.—As in the case of tracheal chain studies, various fractions of *S. lappa* produced bronchodilatation and antagonized the action of histamine and acetylcholine, as shown in Table III.

SUMMARY AND CONCLUSIONS

1. The chemical analysis of *S. lappa* revealed the presence of reducing sugars, tannins, resins, 1.39 per cent of essential oil, and 0.05 per cent of alkaloids.

2. Small doses of the aqueous and alcoholic extracts raised blood pressure in normotensive dogs and stimulated dog heart *in situ*.

3. Essential oil stimulated the heart *in situ* in low concentrations but depressed it in higher doses.

4. Relaxation of plain muscles of rat's intestine and uterus with antagonism to the spasmogenic action of acetylcholine and histamine was observed with all the fractions.

5. Marked bronchodilator action in isolated tracheal chain and guinea pig lung perfusion experiments was elicited with all the fractions, which also effectively antagonized the experimentally induced bronchoconstriction.

6. The maximum response was obtained with the alkaloid, though compared to epinephrine, the effect was much inferior.

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Biogenesis of the Clavine-Type Ergot Alkaloids

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Mevalonic acid-2-C^{14} fed to saprophytic cultures of ergot, strain 47 A, gave rise, within two weeks, to radioactive penniclavine, elymoclavine, setoclavine, and agroclavine. The radiolable also entered lipidic materials and a pigment, but not the amino acids. The specific activities of elymoclavine and agroclavine were identical for each pair isolated from different cultures, a fact that might be interpreted to mean that they have a common biogenetic pathway, at least to the stage of the final carbon skeleton.

IN ANOTHER REPORT (1) we have demonstrated that saprophytic cultures of *Claviceps purpurea*, strain PRL 1578, uses mevalonic acid as a precursor in the formation of its alkaloids. Mevalonic acid-2-C^{14} fed to the growing fungus renders the alkaloids strongly radioactive. Upon alkaline hydrolysis of the peptidic alkaloids, the resulting amino acids are devoid of radioactivity, all of which resides in the lysergic acid portion.

Having shown that mevalonic acid serves as a precursor for lysergic acid, we considered it important to investigate also if this metabolite is involved in the formation of the clavine-type ergot alkaloids. This became possible when we received from Varro E. Tyler, University of Washington, ergot strain 47 A, which produces abundant quantities of clavine-type alkaloids in saprophytic cultures.

Suggestions that mevalonic acids might be a precursor of ergot alkaloids have already appeared in the literature (2–4). An experiment by Gröger (5), who added mevalonic acid to saprophytic cultures of ergot, failed to settle the question as the increase in yield of alkaloids was too small to allow a conclusion for or against.

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As our investigation was completed, Birch, *et al.* (6), and Gröger, *et al.* (7), published the results of their investigations, which had been carried out independently and simultaneously with ours. We had used a different strain of ergot and also taken a somewhat different experimental approach, but, as this paper shows, our results corroborate their works.

EXPERIMENTAL

Materials.—Ergot, strain 47 A; originally isolated from ergot grown on *Pennisetum typhoideum* and obtained from French Equatorial Africa by A. E. Schwarting, University of Connecticut (8). It produces elymoclavine and agroclavine as its principal alkaloids, but penniclavine and setoclavine also occurred in our cultures.

dl-Mevalonic acid-2-C^{14}, in the form of phenylethylenediamine salt, from Tracerlab Inc.; specific activity: 2.45 mc./mM; radiochemically pure.

Whatman chromatographic paper No. 1.

Kodak no-screen X-ray film.

Beckman spectrophotometer.

Packard automatic Tri-Carb liquid scintillation spectrometer (9).

Authentic clavine alkaloids from A. Hofmann, Sandoz Ltd., Basel.

Cultivation.—Growth flasks of 125-ml. capacity were seeded with a constant quantity of one and the same fungus suspension. Each flask contained 40 ml. of the following sterile medium: glucose 1%, yeast extract 1%, L-tryptophan 0.05%, dissolved in water. Although the yeast extract contains an abundant quantity of a complex mixture of inorganic ions (total 15%), tap water was used in accordance with the procedures of Abe (10) and Taber and Vining (11), who both recorded better yields of alkaloids under these conditions. The seeded flasks were left stationary in the dark at 25°. After a growth period of sixteen days, 2 to 4 μc. of *dl*-mevalonic acid-2-C^{14}, dissolved in 1 ml. of distilled water, was added to the nutrient medium and the fungus allowed to grow for two weeks more.

Extraction, Separation, and Identification of the Clavine Alkaloids.—The medium was separated from the fungal mat by filtration, made alkaline with 3N ammonium hydroxide, and extracted several times in a separatory funnel with a mixture of chloroform and isopropanol (3:1). The mycelium was dried at 35° and defatted by extraction with petroleum ether. The alkaloids were extracted with ammoniacal diethyl ether. The alkaloidal extracts were evaporated and the individual residues from each flask dissolved in chloroform plus isopropanol (3:1) in volumetric flasks.

For purposes of identification, spottings were made from these solutions on chromatographic paper and the strips chromatographed descendingly: (I) nontreated paper, in butanol–acetic acid–water (4:1:1); (II) McIlvain's buffered paper (8), in water-saturated butanol; and (III) formamide-impregnated paper (12), in benzene-pyridine (6:1).

Strips were cut from the chromatograms, and inspected under ultraviolet light for presence of fluorescent areas. These areas were marked off with pencil and the strip then dipped for identification in dimethylaminobenzaldehyde reagent, Ehrlich's reagent for indole compounds (13). In addition, the identity of the alkaloids were revealed also by the nature of the color given with the Ehrlich reagent.

Another strip from each chromatogram was placed on X-ray film for two weeks. The locations of the fluorescent zones and the Ehrlich-positive zones of each chromatogram were then compared with the locations of the dark areas on the autoradiograms.

Estimation of Alkaloids and Determination of Specific Activity.—Aliquots of the total crude-alkaloid fractions were used for determination of alkaloid content according to the procedure of Taber and Vining (11) by dissolving the alkaloids, evaporated under vacuum, in 3 ml. of 0.2 N sulfuric acid, adding 6 ml. of dimethylaminobenzaldehyde T.S. (B. P.), allowing one hour for color development, and, then, measuring absorbance at 550 mμ. Ergometrine was used as the reference standard for crude alkaloid mixtures.

In order to determine specific activity, of the alkaloids, aliquots of the eluted spots or streaks of agroclavine were assayed as in the determination of total alkaloid using pure agroclavine, as a standard. In the same way elymoclavine was determined using pure elymoclavine as a standard. Other aliquots, amounting to 2,000 to 7,000 c. p. m., were evaporated and dissolved in toluene and counted in a scintillation counter. Penniclavine and setoclavine cannot be determined by this method.

Specific Activities of Alkaloids from a Single Culture

	Alkaloid	c.p.m./mM
From medium:	Agroclavine	2.94×10^6
	Elymoclavine	2.94×10^6
From fungal mat:	Agroclavine	2.47×10^6
	Elymoclavine	2.47×10^6

Amino Acids.—The defatted mycelium was extracted with 60% alcohol, the extract concentrated by evaporation, freed of alkaloids, then passed through a column of Amberlite IR-120 (H). The column was washed with distilled water ("washings"), then eluted with 2 N ammonium hydroxide. The eluate was evaporated. The residue had no radioactivity. It was chromatographed on paper with solvent system 1, and 20 amino acids detected by spraying with ninhydrin spray reagent.

Pigments.—The radioactive "washings" above were concentrated, spotted on paper and chromatographed, and the chromatographs put on X-ray film. The darkened areas on the autoradiograms corresponded in location exactly to that of the water-soluble brown pigment and to that of nonconverted mevalonic acid on the chromatograms.

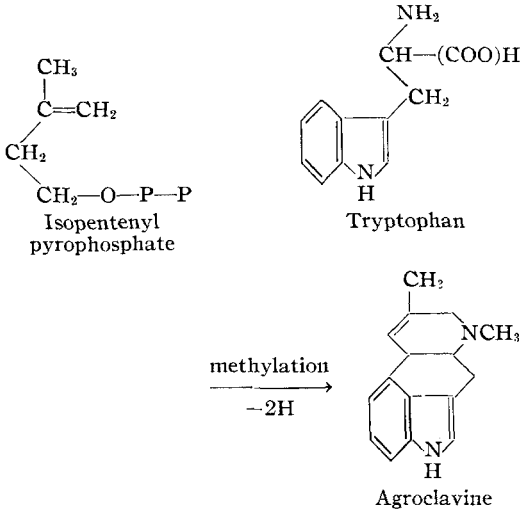
Alkaloid	R_f Values I	II	III	U. V. Color in Light	Color with Ehrlich Reagent
Penniclavine	0.41	0.22	0.10	Brilliant blue	Purple-blue, turning green
Elymoclavine	0.54	0.31	0.16	None	Purple-blue
Setoclavine	0.61	0.43	0.48	Brilliant blue	Purple-blue, turning yellow-brown
Agroclavine	0.72	0.54	0.57	None	Purple-blue

DISCUSSION AND SUMMARY

In our experiments, from 0.51 to 2.18% of the radioactivity of mevalonic acid-2-C^{14} entered the alkaloid fraction of ergot strain 47 A when it was cultivated in stationary saprophytic cultures. Stig Agurell in this laboratory has since obtained incorporations amounting to 6–7% of added radioactivity (private communication). The specific activities of agroclavine and of elymoclavine, from any one culture, were always identical and of the order of 1.7×10^6 to 11.4×10^6 c.p.m./m*M*.

Also penniclavine and setoclavine were strongly radioactive, but their specific activities were not determined.

Without an accurate determination of the distribution of the radioactivity within the molecules of the alkaloids, it cannot be concluded with absolute certainty that the isoprenoid portion of mevalonic acid is incorporated *in toto* into the ergot alkaloids. There are, however, indirect evidences in support of the hypothesis. Thus, we found no indication of the label from mevalonic acid having entered respiratory pathways. One would have expected to find the label in metabolites of these pathways had mevalonic acid broken down metabolically. Nor could any radioactivity be found in the amino acids. The only labeled materials besides alkaloids and nontransformed mevalonic acid was a pigment and the lipidic fraction. The latter is rich in ergosterol, which is known to form from mevalonic acid (14).



The mechanism of the condensation leading from tryptophan and mevalonic acid to ergot alkaloids can only be guessed at. One would expect that a similar mechanism is operating as demonstrated by Lynen, *et al.* (15), for the biogenesis of farnesol, the precursor of sterols; that is, di-phosphorylation of mevalonic acid, decarboxylation, and dehydration, to form isopentenyl pyrophosphate, which, in turn, would condense with tryptophan or a derivative of it. Baxter, *et al.* (16), have shown that 5-hydroxytryptophan is an unlikely intermediate in the process.

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