Generally, very high recoveries have been obtained, amounting to more than 90 per cent at 10–20-fold concentration. In connexion with experiments involving some proteins of low molecular weight (cytochrome *c* and a phosphatase from *E. coli*), some complications have appeared. A considerable part of the activity remained in the gel and could be recovered only after extensive washing of the filter cake. Difficulties will probably arise also in the concentration of very viscous solutions.

It is of practical importance that the 'Sephadex' could be regenerated. This was done by first washing with water, followed by two suspensions of the material in ethanol, and drying at 80° C. The dried substance could then be used for new concentration experiments without any detectable difference in its properties.

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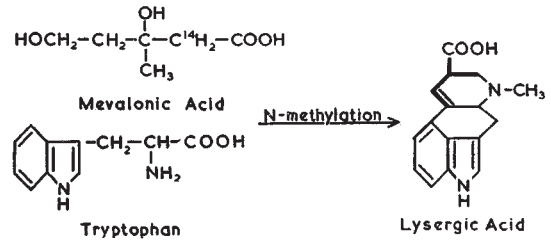
Biogenesis of Lysergic Acid in Ergot

Several schemes have been proposed for the biogenesis of the medicinally valuable lysergic acid moiety of the ergot alkaloids[1–5]. Recently, it was shown by Taber and Vining[6], and later verified by Baxter *et al.*[7], that tryptophan (β-^{14}C) serves as a precursor for lysergic acid. Gröger and co-workers[8] demonstrated that tryptophan (β-^{14}C) is also a precursor for the chemically related clavine alkaloids, which are produced by certain strains of ergot; but the carboxyl group of tryptophan does not enter the molecule. The nature of the precursor of the remaining portions of lysergic acid and of the clavine alkaloids has remained a puzzle. Gröger[2] demonstrated that, when acetate (1-^{14}C) was fed to saprophytic cultures of ergot, the radioactivity was incorporated into the clavine alkaloids. Since the alkaloids were not degraded, the biogenetic significance of this work is not clear.

Mevalonic acid has been recognized as a key compound in the biogenesis of isoprenoids, terpenoids, and steroids. In 1959, Gröger[2] added mevalonic acid to the saprophytic culture medium, but found no significant increases in total production of clavine alkaloids. We have found that mevalonic acid serves as the precursor for the *iso*pentenyl portion of lysergic acid:



So far as we are aware, this is the first demonstration of mevalonic acid serving as a biogenetic precursor for an alkaloid. (Since this communication was written, clavine alkaloids have been derived from mevalonic acid by Birch, A. J., *et al.*, *Z. Naturforsch.*, **15**, b, 141; 1960.)

A series of cultures of *Claviceps purpurea* (*PRL* 1578) were grown in batches of 40 ml. of liquid nutrient medium in 125-ml. flasks. After 15–30 days, 1 μc. of radiologically pure mevalonic acid-2-^{14}C (21·9 mgm. = 1 mc.) was added to each flask. Two weeks later the medium and the mycelium were separated by filtration, the medium made alkaline with ammonia, and the alkaloids extracted with chloroform. The mycelium was dried, finely powdered, and defatted with petroleum ether. The alkaloids were extracted with ammoniacal ether. The two alkaloidal extracts were evaporated to dryness, and the residues were dissolved in a mixture of chloroform and *iso*propanol (3 : 1); a portion was spotted on formamide-impregnated paper[9] and chromatographed (descending) with carbon tetrachloride/chloroform/benzene (7 : 2 : 1). In this solvent system the ergot alkaloids are separated well and in order of increasing R_F values: ergotamine, ergosine, ergotaminine, ergosinine, ergocornine plus ergocristine, ergocryptine, ergocorninine plus ergocristinine, and ergocryptinine; the water-soluble ergometrine and ergometrinine remain at the origin.

Ergotamine and ergosine were eluted separately from the carbon tetrachloride–chloroform–benzene–chromatogram with 50 per cent ethanol and hydrolysed according to the method of Jacobs and Craig[10]. The hydrolysate was chromatographed with butanol/acetic acid/water (4 : 1 : 1) on Whatman No. 1 paper. Autoradiograms showed the radioactivity to coincide exactly with the location of lysergic acid (R_F 0·40) as established from its fluorescence under ultra-violet light and the blue colour given by this indolic compound with Ehrlich's reagent. The behaviour of the compound was identical to that of authentic lysergic acid and to the behaviour of lysergic acid in the hydrolysate of pure ergotamine.

For the separation, identification, and isolation of ergometrine a second portion of the alkaloid mixture was chromatographed (descending) in butanol–acetic acid–water on Whatman No. 1 paper. In this solvent system ergometrine has an R_F of 0·60 while the peptide-type alkaloids have an R_F of 0·85. Both these fluorescent radioactive areas were eluted separately with 50 per cent ethanol, the eluate hydrolysed and chromatographed (descending) with butanol–acetic acid–water on Whatman No. 1 paper. In both cases the lysergic acid was found to be radioactive as revealed by autoradiograms. All the radioactivity of the alkaloids resided in the lysergic acid.

Mevalonic acid did not enter appreciably the general metabolism of the mould. More than 90 per cent of the radioactivity of converted mevalonic acid-2-^{14}C was found incorporated into the lysergic acid portion of the ergot alkaloids. The petroleum ether fraction of the mycelium contained less than 10 per cent of the total added radioactivity. The amino-acids which were extracted from the mycelium showed no radioactivity.

A more detailed and extended account of this work will be published elsewhere.

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PHYSIOLOGY

Rectifying Properties of Heart Muscle

A FACTOR of likely importance in the genesis of the long-lasting action potentials of vertebrate heart muscle is the influence of membrane potential on the potassium permeability of the fibres. That a difference here exists between cardiac muscle and nerve is evident from Weidmann's[1] demonstration of a low slope conductance during the plateau of the action potential. But little information is so far available on the voltage dependence of the membrane conductance under conditions designed to avoid the generation of action potentials.

To study this point, so far as the technical limitations imposed by the structure of cardiac muscle at present allow, excised Purkinje fibres from sheep ventricles were used. While sharing the essential electrical properties of myocardial tissue, these fibres do not produce a mechanical response on depolarization; they are also insensitive to parasympathomimetic substances so that choline chloride or sucrose may be used as a substitute for extracellular sodium chloride to abolish excitability.

Fig. 1, *A* and *B*, shows how the membrane conductance of a Purkinje fibre in sodium-deficient

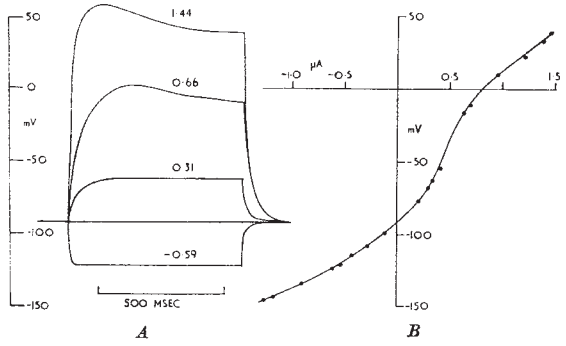

Fig. 1 *A*. Superimposed tracings of electrotonic potentials from a sheep's heart Purkinje fibre. Sodium ions in Ringer's solution replaced by choline. Depolarization is shown in an upward deflexion from a resting potential of −92 mV. Two closely spaced KCl-filled intracellular electrodes were used to record the membrane potential and to pass practically rectangular current pulses of a strength indicated by the figures on each record. *B*, Current-voltage relation for the same preparation. Ordinate, membrane potential at end of current pulse lasting 700 m.sec. Abscissa, total membrane current

solution depends on the direction and magnitude of the polarizing current. An outward current of 0·31 μamp., for example, produced the same voltage deflexion as is caused by nearly twice as great an inward current. Allowing for the cable properties of the fibre, this means that the conductance to a depolarizing current may be about four times less than to a hyperpolarizing current. Since the contribution of chloride ions to the membrane conductance of cardiac muscle is small[2], a decrease in the potassium conductance presumably occurs on depolarization. The direction of the potassium conductance change in Purkinje fibres is thus opposite to the predominant change in nerve[3], but has a parallel in skeletal muscle[4].

With strong depolarizing currents a decline in the electrotonic potentials during the pulse may be observed, suggesting a gradual increase in conductance. This second effect probably accounts for the S-shaped relation between the amplitude of the electrotonic potential at the end of a long pulse and the polarizing current (Fig. 1 *B*), but over the physiological range the conductance remains below its value at the resting potential.

The slope conductance decreases appreciably over the range occupied by the slow diastolic depolarization in spontaneously beating preparations[1]. This alinearity may require consideration in interpreting the resistance changes observed during the pacemaker potential[5].

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Cardiac Action and Pacemaker Potentials based on the Hodgkin-Huxley Equations

SINCE the equations describing the nerve action potential were formulated by Hodgkin and Huxley[1], the range of phenomena to which they have been shown to apply has been greatly extended. Huxley[2] has applied them to the influence of temperature on the propagated response and to the repetitive firing observed in low calcium concentrations. More recently, Fitzhugh[3] has shown that the long action potentials induced by tetraethylammonium ions in squid nerve may also be reproduced.

The computations described in this communication were carried out with the aim of reconstructing the long-lasting action potential and pacemaker potential of cardiac muscle. Although this work was done independently, the results agree with those of Fitzhugh in showing that action potentials of long duration may be accounted for by Hodgkin and Huxley's formulation of the membrane properties. The description of the potassium current, however, differs from that used by Fitzhugh and provides a better description of the conductance changes.

The equations I have used to describe the sodium current are very similar to Hodgkin and Huxley's,