When the isolated hearts of rabbits which had just been treated for 10 days with reserpine (0.1 mg/kg daily) were subjected to anoxia for 4 min, much less noradrenaline was released into the coronary bed than from the hearts of the untreated animals. The amounts released were proportional to the size of the rather severely depleted cardiac noradrenaline stores. No release of noradrenaline whatever was observed in preparations of rabbit hearts which had undergone only moderately severe losses of noradrenaline as a result of prior administration of tyramine (30 mg/kg rabbit given 30 min before excision of the heart). This finding suggests that the anoxia-sensitive fraction of the noradrenaline in the rabbit heart might form part of, or be identical with, the fraction releasable by tyramine.

Further characterization of the material in the perfusates at present determined as noradrenaline is provided by the following observations: (1) The fluorescence excitation and emission spectra of the aluminas eluates of the perfusates treated by the trihydroxyindole procedure used were distinguishable from those of the lutine formed from authentic noradrenaline. (2) The blood-pressure-raising activity of the aluminas eluates, assayed in the cat, corresponded roughly to that of the fluorometrically estimated quantities of noradrenaline in these eluates. (3) In heart preparations loaded with tritium-labelled noradrenaline a 4-min arrest of perfusion was followed by a sharp temporary rise in the rate of outflow of radioactive noradrenaline.

There is thus little doubt that myocardial anoxia can bring about, in the apparent absence of sympathetic nerve activity and with little delay, the release and loss of a good part of the noradrenaline stored in cardiac muscle. The liberation of the adrenergic transmitter may be held largely responsible for the initial outburst of phosphorylase activity and glycolysis in anoxic myocardium, and may conceivably play a part in the genesis of cardiac arrhythmias such as occur in the wake of an occlusion of a coronary vessel.

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**Reserpine in a Tissue Culture of Alstonia constricta F. Muell**

In a tissue culture study of certain members of the Apocynaceae, the plant *Alstonia constricta* F. Muell was investigated. The root and trunk barks of this tree, which is native to Australia, have been known to yield several indole alkaloids including reserpine. The callus tissue cultures were initiated from germinating seeds and maintained on a modified White’s medium which included 2,4-dichlorophenoxyacetic acid, 1 mg/l, and 10 per cent coconut water. The tissue was continuously cultivated for over two years and periodically it was gathered, lyophilized and stored for subsequent chemical analysis.

Recently a preliminary chemical analysis was performed. Several grams of dried callus were reduced to a number 20 powder and the powdered tissue was extracted with alkaloids according to a slight modification of the procedure reported by Svoboda. This procedure involves the use of several solvents, the final one being chloroform. The chloroform extract was subjected to analysis by thin-layer chromatography. In this procedure the Desaga1 apparatus (Brinkman Instrument Co., Westbury, New York) was used, including 200 mm square plates with a matrix of ‘Silica Gel G’ and a solvent system of ethyl acetate: ethanol 3:1. The solvent front was allowed to travel 100 mm. Each chromatogram was examined under ultra-violet light and its fluorescent pattern was recorded. The plates were then sprayed with modified Dragendorff’s reagent. Since reserpine had previously been reported to occur in root and stem bark of *Alstonia*, this compound was chromatographed alongside the callus extracts.

An examination of the chromatograms under ultra-violet light revealed the presence of five fluorescent spots. Four of these gave a blue fluorescence and one a yellow-green fluorescence. The pure reserpine also fluoresced yellow-green and appeared at the same Rf as the yellow-green fluorescent spot from the callus extract. When the plates had been sprayed with modified Dragendorff’s reagent three distinct positive spots appeared with Rf values of 0.19, 0.68 and 0.93. The Rf of reserpine under the same conditions corresponded to the middle value just listed, 0.68.

Further, to check the possibility that one of the unknown alkaloids was reserpine, the compound appearing at Rf 0.68 as well as known reserpine was scraped from the plate before spraying with modified Dragendorff’s reagent. The alkaloid-silica-gel matrix was eluted with chloroform by mixing in a centrifuge tube. The mixture was centrifuged and the supernatant chloroform extract removed. This extract was analysed spectrophotometrically in a Beckman DK-2 recording spectrophotometer.

The ultra-violet spectrum for the alkaloid at Rf 0.68 was the same as for reserpine, namely a shoulder at 290 nm and a maximum at 267 nm. More tissue is being accumulated so that further investigation may be performed. From this preliminary work it appears that our tissue culture of *A. constricta*, which has been maintained for more than two years, has the capacity to biosynthesize reserpine.

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