as high as 1.1 (see Fig. 1), which is comparable to the ratio of $E^{\text{reduced}}_{550\,\text{m}\mu}/E^{\text{oxidized}}_{280\,\text{m}\mu}$ obtained for a typical cytochrome *c**.

The *b*-type cytochrome was reduced by lactate in the presence of baker's yeast lactic dehydrogenase[6–9] under anaerobic conditions, but not under aerobic conditions. The enzymic reduction of the cytochrome was measured spectrophotometrically *in vacuo* in a Thunberg tube modified for spectrophotometry (Fig. 3). When air was introduced into the tube, the reduced cytochrome was immediately oxidized. The autoxidation of the cytochrome was not inhibited by 10^{-2} *M* KCN. Neither KCN nor CO caused any modification in the absorption bands as judged from the observation made with a microspectroscope. The biological function of the cytochrome, for which we propose the name "cytochrome *b*-561, 554 (*Sclerotinia libertiana*)"[10], is a matter for further investigation.

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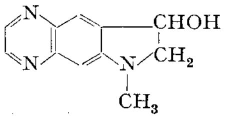
*It should be remarked that no *c*-type cytochrome has ever been extracted from this fungus.

The condensation of catechols with ethylenediamine

It has been shown by HARLEY-MASON AND LAIRD[1] that the main product of the reaction of ethylenediamine with adrenaline is dihydro-3-hydroxy-1-methylpyrrolo-(4,5-*g*)-quinoxaline (I), while the reaction with catechol yields 1,2,3,4-tetrahydro-1,4,5,8-tetraaza-anthracene (II). HARLEY-MASON (personal communication) further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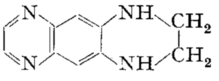
II

found that the main product formed from noradrenaline is identical with that formed from catechol and that the elimination of the β-ethanolamine side chain of noradrenaline occurring during this reaction depends on the presence of the β-hydroxyl group.

Several paper-chromatographic studies of the reaction products obtained from adrenaline and noradrenaline have been reported. They indicated the presence of a variety of compounds. Thus BURN AND FIELD[2] found two fluorescent derivatives of adrenaline and two of noradrenaline, while YOUNG AND FISCHER[3] described the formation of three derivatives from adrenaline and two from noradrenaline. According to YAGI AND NAGATSU[4] the condensation product of adrenaline separates into two spots of which the fainter one is identical with the main product of noradrenaline. NADEAU AND JOLY[5], finally, detected no less than ten different components derived from adrenaline and about the same number derived from noradrenaline.

In at least some of these experiments concentrations of catecholamines and ethylenediamine were employed which diverged greatly from those specified for the fluorimetric estimation[6] and which might therefore have been more favourable for the occurrence of side reactions. Moreover, the possibility cannot be excluded that the primary condensation product underwent decomposition either during the preliminary processing or during chromatography itself.

On the basis of the reaction mechanisms elucidated by HARLEY-MASON it is to be expected that the condensation product of 3,4-dihydroxymandelic acid with ethylenediamine would be identical with those formed from catechol and noradrenaline, but different from those of 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylalanine which do not possess the β-hydroxyl group in the side chain. This assumption is supported by the partition of the condensation products between an aqueous and an isobutanol phase: whereas the product from 3,4-dihydroxymandelic acid, like that from catechol or noradrenaline, is almost quantitatively extracted into isobutanol, those from 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylalanine remain largely in the aqueous layer[7].

The possible identity of the condensation products of catechol, noradrenaline and 3,4-dihydroxymandelic acid has now been further investigated by a comparison of their fluorescence spectra and by paper chromatography.

For the study of the fluorescence spectra 0.35 μg catechol or equivalent quantities of noradrenaline or 3,4-dihydroxymandelic acid, in 10 ml aqueous solution, were condensed with ethylenediamine under the conditions described by WEIL-MALHERBE AND BONE[6]. The resulting isobutanol extract was examined in the Aminco-Bowman Spectrophotofluorometer. The three extracts had identical maxima of activation and fluorescence at 420 and 485 mμ, respectively. The fluorescence curves rose sharply from zero at 450 mμ to the maximum and returned more gradually to zero at 590 mμ. After correction for slight differences of intensity the three curves were completely superimposable. In contrast to the slight asymmetry of these fluorescence curves, the fluorescence curve of the adrenaline derivative (peak at 525 mμ) was perfectly symmetrical, which is in favour of the assumption that it is caused by a single fluorescent product.

For the paper-chromatographic experiments the condensation was carried out under conditions close to those chosen for the fluorimetric assay: an aqueous solution containing 20 μg in 50 ml was incubated with 2.5 ml 2 *M* ethylenediamine dihydrochloride and 3.5 ml ethylenediamine at 55° for 30 min. After cooling the mixture was saturated with NaCl and extracted with 30 ml isobutanol. The isobutanol extract was evaporated *in vacuo* to dryness at 30° in a rotary "flash"-evaporator, the residue dissolved in small portions of methanol and concentrated in a test tube *in vacuo* to about 0.3 ml. In view of the reported photosensitivity of some of the condensation products[8], amber or red glassware was used throughout. The application of the solution (0.05 ml) to the paper (Whatman No. 3) was carried out in red light and the chromatography jars were kept under a darkened hood. Out of ten different solvent systems the best results were obtained with a mixture of isobutanol–ethylenediamine–water (50:3:20; organic phase) used in the descending direction. As shown in Table I the condensation product of adrenaline gave a single spot with intense yellow fluorescence. Each of the products formed from catechol, noradrenaline and 3,4-dihydroxymandelic acid yielded two indentical spots; a third faint spot with low R_F value was observed among the noradrenaline derivatives.

TABLE I

PAPER CHROMATOGRAPHY OF ETHYLENEDIAMINE CONDENSATION PRODUCTS

Solvent: isobutanol–ethylenediamine–water (50:3:20), descending technique.

Substance	*Number of spots*	R_F	*Fluorescence*
Adrenaline	1	0.30–0.34	Yellow
Noradrenaline	3	(1) 0.07	Green-yellow
		(2) 0.15–0.22	Light green
		(3) 0.55–0.59	Blue-green
Catechol	2	(1) 0.15–0.22	Light green
		(2) 0.55–0.59	Blue-green
Dihydroxymandelic acid	2	(1) 0.15–0.22	Light green
		(2) 0.55–0.59	Blue-green

These results suggest that the condensation of ethylenediamine with adrenaline leads to a single fluorescent product and that side reactions are negligible. In the case of catechol, noradrenaline and 3,4-dihydroxymandelic acid two main products which appear to be identical for the three compounds are formed.

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