Table 1. CATECHOLAMINES IN THE BRAIN STEM OF THE RABBIT

	Adrenaline (μgm./kgm.)	Noradrenaline (μgm./kgm.)	Hydroxytyramine (μgm./kgm.)	Dihydroxyphenylalanine (μgm./kgm.)
Homogenate, acidified immediately	89	320	776	0 (< 15)
Homogenate, acidified later*	85	306	824	
Coarse fraction	8·2	28·5	74	
Mitochondria	35·8	144	305	
Microsomes	4·5	16·6	42	
Supernatant	36·6	114	394	

*Homogenate acidified later: a sample of the sucrose homogenate was kept at 0° until the fractionating operations were completed and was then acidified.

tetraacetate (*p*H 7), was separated into four fractions at 0°: (1) a coarse fraction, obtained by centrifuging in an angle head at 300*g* for 5 min.; (2) a mitochondrial fraction, obtained by centrifuging at 10,000*g* for 20 min.; (3) a microsome fraction, obtained by adjusting the supernatant to *p*H 5·5 and afterwards centrifuging at 10,000*g* for 15 min.; and (4) the final supernatant fraction. All fractions were extracted with 0·1 *N* hydrochloric acid (final concentration). From these extracts catechols were isolated by adsorption on columns of alumina, further fractionated by chromatography on cation exchange resins and estimated fluorimetrically, as outlined in previous publications[1,2].

A representative result is shown in Table 1. Catecholamines were about equally divided between mitochondrial and supernatant fractions; the smaller amounts found in the coarse and microsome fractions were probably due to their contamination with the other two fractions. The total of the four intracellular fractions tallies well with the amounts found in the unfractionated homogenate. It is interesting to note that the three catecholamines occur in similar proportions in the different fractions. Whereas Montagu[1] found 3,4-dihydroxyphenylalanine in human brain, measurable amounts were not encountered in our analyses.

In the experiments described the rabbits were injected with iproniazid 1 hr. before death (100 mgm./kgm., intraperitoneally). The enzymic breakdown of catecholamines during fractionation, which is considerable otherwise, is thereby effectively inhibited.

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Sodium Salt of the Directly Reacting Bile Pigment

THE composition of the bile pigment which shows direct van den Bergh reaction has been elucidated in recent studies[1–8]. This pigment is considered to be essentially a mixture of soluble salts of bilirubinyl-*bis*(β-D-glucosiduronic) and bilirubinyl-mono(β-D-glucosiduronic) acids. The preparation of the insoluble lead salt of the first of these acids has been reported in previous communications[3]. This precipitation renders possible a concentration of the pigment from aqueous alcoholic solutions of rough material.

Without decomposition, the lead salt of this pigment is insoluble in all solvents. For the present work a soluble pigment was obtained from its lead salt by conversion with cation exchanger (formaldehydic resin from phenolsulphonic and β-naphthalenesulphonic acids 'Katex *FN*') in Na^+ cycle. This conversion was carried out without considerable decomposition, provided that no stronger acid, and especially no traces of alkalinity[4], are released from the resin. Such a conversion of salts with low solubility to soluble ones by means of cation exchanger has already been described[5]. In this case it was carried out by shaking a thick suspension of lead salt with a slurry of cation exchanger for about ½ hr. in an evacuated centrifuge tube. Usually about 1 ml. (= 0·4 gm.) of the resin is sufficient for 20 mgm. of bilirubin contained in the suspension. After removing the resin by centrifugation, the clear supernatant is quickly dried *in vacuo*.

The resulting amorphous material is collected in the form of dark-brown lamellæ, freely soluble in water, relatively soluble in methanol, less soluble in ethanol, insoluble in chloroform, ether and acetone. Colloidal particles of the lead salt which escaped conversion often become suspended owing to the high peptization properties of the pigment solution. These residues can be removed by precipitation or re-extraction with methanol. The resulting material gave by analysis 62 per cent pigments (bilirubin + biliverdin—55 per cent bilirubin), 28 per cent glucuronic acid, calculated on the weight basis as glucurone. Theoretically, the disodium salt of diglucuronide should yield 59·5 per cent pigment and 35·9 per cent glucuronic acid (on the above-mentioned basis)[4].

This product can be purified further by reversed phase chromatography as designed by Cole and coworkers[6] on columns arranged according to Billing[7] but on a larger scale (silicone-treated kieselguhr was generously given by Dr. G. H. Lathe of London). Instead of phosphate buffer in the system *B*[6] a 0·02-*M* acetate buffer of *p*H 5·6 was used in order to allow further precipitation of the lead salt from the methanolic eluate of a corresponding segment. Glucuronic acid was always detected although in quantities not identical with theoretical ones.

The assumption of a glucuronide character of this pigment is now supported by the recent biosynthetic experiments of Carbone and Grodsky[8], Lathe and Walker[9] and Schmid[10].

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