radioactivity in which the major portion was present as a single peak in the area (25–36 cm) corresponding to albumin. However, in both of these cases a small amount of radioactivity (ca 11%) was detected in the region (37–48 cm) corresponding to the second peak of Figure 2. Finally, it is apparent from the Table that the radioactivity in the second region is greatly increased when the incubation was performed with S_2. This result must be related, directly or indirectly, to the paucity of microsomes in this fraction since, on recombination of this fraction with microsomes ($S_2 + M_2$) the second peak of radioactivity is again low.

Efforts are now being directed toward the identificatino of the metabolite(s) in each of the areas of radioactivity[8].

Zusammenfassung. Die Inkubation von 16-C^{14}-Oestron und 4-C^{14}-Oestradiol-β-acetat in Puffer (pH 7.4) bei Gegenwart von Rattenleber ergibt elektrophoretisch zwei deutliche radioaktive Bänder. Das erste Band entsteht im Albuminbereich und stimmt mit den früheren Beobachtungen von SZEGO und ROBERTS[2] überein. Das zweite, früher nicht beschriebene Band entsteht deutlich ausserhalb des Albuminbereiches. Beide Kurvengipfel werden mit wenig homogenisierter Rattenleber, die möglichst frei von Mikrosomen ist, gewonnen. Der zweite Gipfelpunkt wird in diesem Fall der obenauf schwimmenden Phase zugeschrieben.

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The Rollin H. Stevens Memorial Laboratory of the Detroit Institute of Cancer Research, Detroit (Michigan U.S.A.), May 30, 1962.

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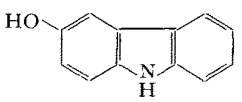
Metabolism of Carbazole[1]

Previous studies[2,3] in these laboratories of the metabolism of ergometrine and lysergic acid diethylamide indicated that these indole derivatives were metabolised in the rat by hydroxylation in the aromatic ring of the indole skeleton. Paper chromatographic evidence showed that the major metabolite of ergometrine was the glucuronide of 12-hydroxyergometrine.

To ascertain the position of hydroxylation in the aromatic ring of indole derivatives the metabolic fate of carbazole has been investigated in the rat and rabbit.

After an intraperitoneal dose of 4 mg/kg of carbazole in propylene glycol, the urine of rats contained a conjugated hydroxycarbazole as the major metabolite. This metabolite was purified by paper chromatography using several systems and then hydrolysed with 0.5*N* hydrochloric acid. Comparative chromatography of the phenol so obtained with the four possible monohydroxycarbazoles on paper and thin-layer chromatograms (Table) indicated it to be 3-hydroxycarbazole (I).



(I)

Compound	Rf, system 1[a]	Rf, system 2[b]	Colour[c]
1-Hydroxycarbazole	0.62	0.57	red
2-Hydroxycarbazole	0.39	0.26	orange
3-Hydroxycarbazole	0.57	0.31	purple
4-Hydroxycarbazole	0.81	0.45	pink
Hydrolysed metabolite	0.57	0.31	purple

[a] Chloroform, benzene, ethyl acetate, water (6, 2, 2, 5) on Silica-gel thin-layer chromatograms.
[b] Toluene, iso-octane, methanol, water (15, 5, 16, 4) on Whatman No. 4 paper.
[c] Reagent: Diazotised sulphanilamide.

A two-fold increase in urinary glucuronide content, as determined by the method of FISHMAN and GREEN[4] was observed after oral dosing of carbazole (1 g) in acacia suspension to a rabbit. The glucuronide was separated by the method of SMITH and WILLIAMS[5] as a colourless gum which after hydrolysis with a β-glucuronidase preparation[6] at 36° in acetate buffer[7] afforded a phenolic product identical on both paper and thin-layer chromatograms, with the hydrolysed metabolite from rat urine and with 3-hydroxycarbazole.

Carbazole-C^{14} (6.0×10^5 d.p.m./mg) was prepared from aniline-C^{14} sulphate (Radiochemical Centre, Amersham) by diazotisation and reduction to phenylhydrazine-C^{14} hydrochloride, condensation with cyclohexanone to 1,2,3,4-tetrahydrocarbazole-C^{14} and dehydrogenation with palladium charcoal (10%) in mesitylene. After intraperitoneal injection of carbazole-C^{14} (2.48×10^6 d.p.m.) to rats the 48-h urine contained 1.61×10^6 d.p.m. (65% of original dose). Hydrolysis of the urine with 0.5*N* hydrochloric acid and ether extraction gave an extract containing 1.36×10^6 d.p.m. (55% of original dose). The ether extract was chromatographed on paper using solvent system 2 and the 3-hydroxycarbazole (detected by both diazotised sulphanilamide reagent and by radioactive scan) after elution with methanol and purification possessed 0.82×10^6 d.p.m. (33% of original counts). The remaining counts (0.50×10^6 d.p.m.) in the ether extract were present in a more polar band which remained on the starting line of the chromatogram. This more polar band was separated using the solvent system ethyl acetate, pyridine, water (3:1:1) into two phenolic bands which were possibly di- or polyhydroxylated carbazoles.

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The hydrolysed metabolite was unambiguously shown to be 3-hydroxycarbazole by dilution of an aliquot with an authentic sample and recrystallisation to constant m.p. and radioactive count (m.p. 260–261°, 8530 ± 160 d.p.m.). A second sample was methylated with diazomethane, diluted with authentic 3-methoxycarbazole and recrystallised to constant m.p. and radioactive count (m.p. 147–149°, 6224 ± 180 d.p.m.).

The *in vivo* hydroxylation at position 3 of the carbazole nucleus is in agreement with a mechanism requiring hydroxylation at the position of the highest electron density[8]. The increase in glucuronide content and the hydrolysis of the metabolite to a phenol with β-glucuronidase indicates the phenol is conjugated with glucuronic acid. This position of attack supports the evidence for 12-hydroxylation of ergometrine as suggested by SLAYTOR and WRIGHT[3].

Zusammenfassung. Es wurde mit Hilfe der Papier- und Dünnschichtchromatographie und der Isotopen-Verdünnungstechnik des C^{14}-markierten Materials gezeigt, dass die *in vivo*-Hydroxylierung von Carbazol sowohl im Ratten- als auch im Kaninchenorganismus am Ort der höchsten Elektronenstauung stattfindet und zur Bildung von 3-Hydroxycarbazol führt.

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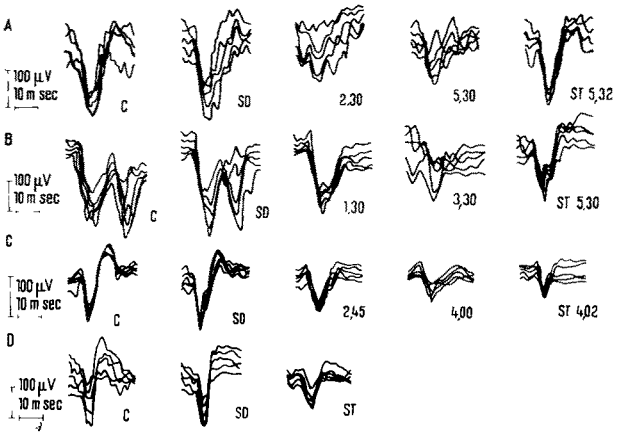
Acoustic Collicular Evoked Responses during Cortical Spreading Depression in Freely Moving Unanesthetized Rats

Some data indicate that probably also cortical mechanisms participate in the central regulation of transfer of information along specific afferent pathways (see WEISS and FIFKOVÁ[1], ALTMAN[2], HARMONY et al.[3]. In this paper we attempted to ascertain whether the phenomenon of «habituation» and changes in primary responses during the orienting reaction can be reproduced during bilateral cortical spreading depression of Leão (SD) eliminating reversibly the function of the cortex BUREŠ and BUREŠOVÁ[4].

Methods. Bipolar silver electrodes were implanted stereotaxically under oscilloscope control in the inferior colliculus on one side in 10 anesthetized (Allobarbital 40 mg/kg) white Wistar rats. Some days later responses to click stimuli (at the rate 1 per 2 sec) were recorded by photographing the screen of the cathode ray oscilloscope. Unanesthetized, freely moving animals were placed in a little cage (5 × 20 × 20 cm) situated at a constant distance (10–20 cm) from the loudspeaker. SD was evoked by applying filter papers (2 × 2 mm) soaked in 25% KCl to the dura mater via trephine openings, prepared some hours before the experiment proper, under ether anesthesia.



A, B, C—Three experiments with 'habituation' to click stimuli during SD (each record from five superposed traces). C, before SD; SD, after eliciting of bilateral SD; in various time intervals after beginning of stimulation, see number; ST, attempt to 'dishabituate' the response with external stimuli (successful only in case A, B); D, (C; SD, see above); ST, decrease of amplitude during orienting reaction.

Results. It was found (in accordance with our earlier experiments on curarized animals, WEISS and FIFKOVA[1]) that SD itself does not influence the amplitude of collicular primary responses. Despite the functional elimination of both cortices in some experiments (10 times in 14 trials) a decrease of the amplitude of the reponses was seen after long lasting (4–5 h) repetition of the clicks during bilateral SD (illustrative examples see Figure A, B, C). In the control experiments carried out on the same animals on the day before or after, a lowering of amplitude was observed in one experiment only (from 12 trials). The last negative result is in agreement with the findings on other animals (MARSH et al.[5]). In some cases (not always) after a strong external stimulus (for instance, opening of the screened box, etc.) a «dishabituation» increase of the amplitude of the acoustic response, before habituated, was seen during bilateral SD. When a strong external stimulus (eliciting orienting reaction) evokes, before habituation, a transient decrease of the acoustic potential, this phenomenon persists also during bilateral SD. A more detailed and careful quantitative analysis of these preliminary results will be made.

Discussion. Our results indicate that, in contradiction to conditioned reflexes interfering with reversible functional elimination of the cortex BUREŠ and BUREŠOVÁ[4], the phenomenon of 'habituation', 'dishabituation' and decrease of primary responses during orienting reaction on collicular level can be realized also without a functioning cortex. The fact (needing more detailed confirmation) that 'habituation' was more regularly observed during SD than without it, may be connected with the

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