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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Pharmacy Department, University of Sydney (Australia), June 22, 1962.

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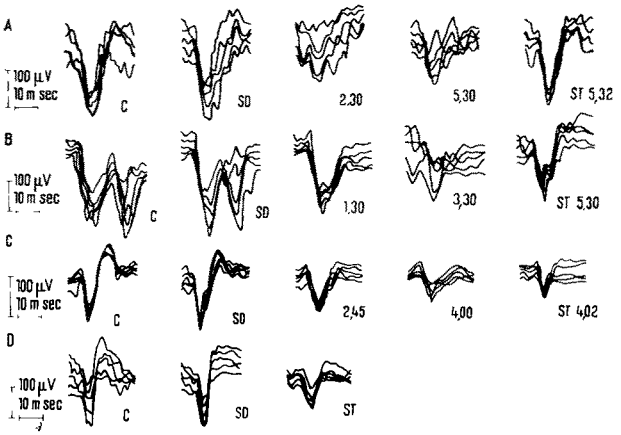
Acoustic Collicular Evoked Responses during Cortical Spreading Depression in Freely Moving Unanesthetized Rats

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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facilitatory effect of the elimination of reticular influences (cerveau isolé) on this phenomenon (MANCIA et al.[6]). It is possible that descendent cortical (and may be also reticular) influences are not only inhibitory but also activating, acting against the intrinsic tendency of specific systems to 'habituation' – reduction of the reaction to stimuli monotonously repeated for a long time.

Zusammenfassung. Bei Ratten wurde mittels «Spreading Depression» eine funktionelle Dekortikation erzeugt. Habituation und Deshabituation akustischer fortgeleiteter Potentiale (evoked responses) im Colliculus caudalis bleiben erhalten. Auch die reversible Abnahme der «evoked responses» während der Orientierungsreaktion bleibt erhalten.

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In vitro Release of Free Fatty Acids by Adipose Tissue in Young and Old Nephrotic Rats[1]

Following the demonstration of *in vitro* free fatty acid (FFA) release by the epididymal fat pad of the rat[2,3], this preparation has been used extensively to study some of the factors influencing fatty acid release from the adipose tissue. In a previous communication it was reported that the release of FFA from adipose tissue is more active in young rats than in older ones[4]). The present paper reports the results of similar studies performed on nephrotic rats and compares the findings with those obtained in normal animals.

Materials and Methods. As in the previous study[4], the male, albino rats of the Wistar strain used in these investigations, were divided into three groups. Young, rapidly growing animals weighing less than 100 g with an approximate age of 30 days (Group 1), nature rats weighing 200–300 g, with an approximate age of 3–4 months (Group 2), and older animals, weighing 350–475 g with an age-range of 4–6 months (Group 3). The experimental procedure, incubation and chemical determinations were the same as previously reported[4].

Nephrosis was produced by intraperitoneal administration of 0.5 ml (in Group 1 animals) or 0.1 ml (in Group 2 or 3 animals) of anti-kidney serum produced in rabbits. The animals were sacrificed 2 weeks after the administration of anti-kidney serum. In case of Groups 2 and 3 about half of the animals were sacrificed 8 and 13 weeks, respectively after the initial injection. No difference could be observed between the animals that were sacrificed two weeks after anti-kidney serum and the ones used several weeks after the injection. In the case of Group 1 rats no more than 2 weeks could elapse between injection and the performance of the experiments, since more time would have placed them into the next group. The presence of proteinuria and hematuria were used as criteria for nephrosis.

Results. Figure 1 compares release of FFA from the epididymal fat pad of nephrotic rats in the different groups. If release of FFA per weight of adipose tissue is considered, Group 2 animals released less FFA than did young rats. This difference is statistically highly signi-

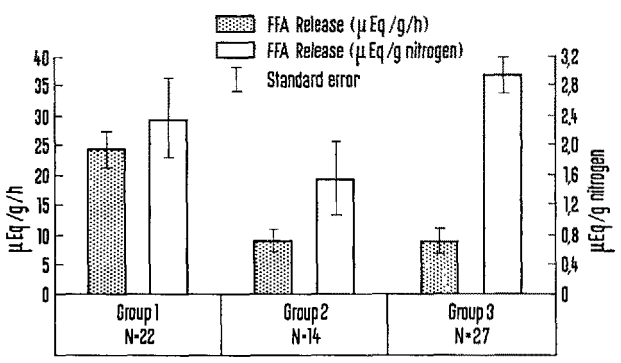

Fig. 1. FFA Release by epididymal fat pads of nephrotic rats.

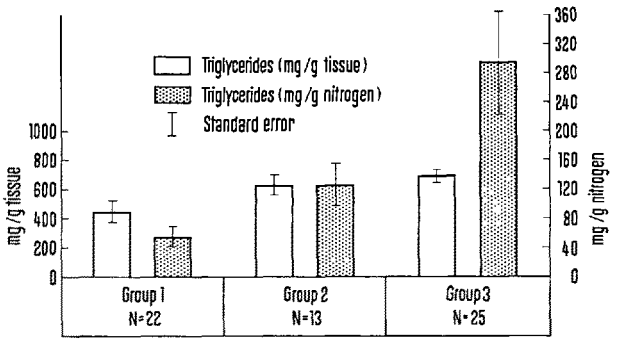

Fig. 2. Triglyceride content of epididymal fat pads in nephrotic rats.

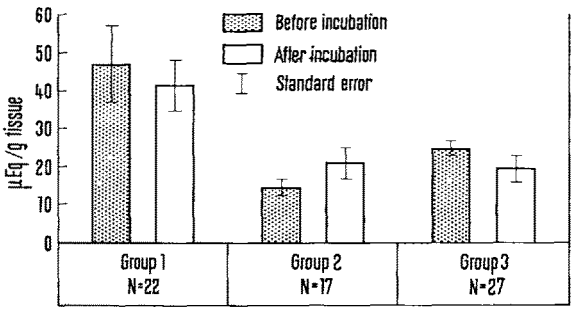

Fig. 3. FFA Content of epididymal fat pads of nephrotic rats.

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