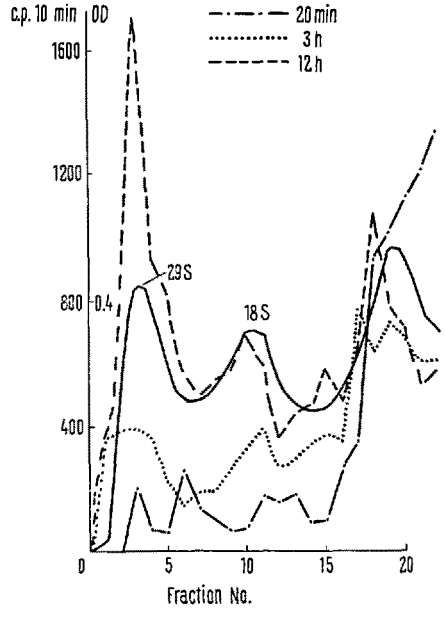
Labelling of Ribosomal RNA Peaks in the Liver of Rats After Administration of Tritiated Fluorouracil

HEIDELBERGER et al.[1] have shown that 5-fluorouracil (FU) is incorporated into liver ribonucleic acid (RNA). Labelled FU, given to mice, was recovered from the nucleic acids as labelled FU and only small amounts in other bases[2,3]. FU is also incorporated into poliovirus RNA[4] and tobacco mosaic virus RNA[5].

We have studied the effect of FU on the labelling of rat liver with cytidine-^{3}H[6,7]. The RNA labelling decreased after moderate doses of FU but was well recognizable in the ribosomal RNA peaks after 3 and 12 h. Very high doses of FU seemed to depress the RNA labelling almost completely, but characteristic ultrastructural alterations were obtained in the liver nucleoli, which also increased in size[8]. As the ribosomal RNA is considered to be elaborated in the nucleolus, the FU may be considered to interfere with the synthesis of ribosomal RNA. It was therefore considered to be of interest to investigate whether labelled FU labels ribosomal RNA.

Experiment. Six male white rats, weighing about 45 g each, were fed on a protein-free diet[9] for 4 days, starved for 24 h and killed at about 10.00 on the fifth day. The animals were given an i.p. injection of 23 μc 5-fluorouracil-6-T (580 mc/m*M*, the Radiochemical Centre, Amersham, England) per g body weight 20 min, 3 h or 12 h prior to sacrifice. RNA was extracted and, after high speed centrifugation, analyzed for UV-absorption and radioactivity as described elsewhere[7]. Due to the low labelling, counts/10 min were recorded.

Results and discussion. The results are given in the Figure. At 20 min there was low labelling without distinct peaks. At 3 and 12 h there was heavy labelling over the ribosomal RNA peaks. Since it has previously been shown (see introduction) that FU is incorporated as such into RNA and not converted into other bases, at least to any appreciable extent, the present results suggest that FU is incorporated into the ribosomal RNA of the liver of rat. Studies are planned to examine whether there are any differences in the half-life of RNA containing FU and its behaviour in different cell components, as compared with normal RNA[10].



Sedimentation patterns of RNA from the livers of rats labelled with 5-fluorouracil-6-T. Faster moving components are to the left. c.p. 10 min, counts per 10 min; OD, optical density at 2537 Å.

Zusammenfassung. Nach i.p. Injektion von 5-Fluorouracil-^{3}H wurde bei Ratten zu verschiedenen Zeiten die RNS-Neubildung der Leber bestimmt. Die Resultate sprechen dafür, dass Fluorouracil in die ribosomale RNA der Leber eingebaut wird.

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Effect of Cortical Spreading Depression on Norepinephrine-H3 Metabolism in Brain Stem

Alterations in the content of the biogenic amines in the cerebral hemispheres have been reported after lesion or stimulation of brain stem structures[1–4]. In contrast, ADLER et al. (1965) report that serotonin and noradrenaline levels in the brain stem were not changed 7 days after the surgical ablation of frontal or posterior cortical lobes[5], but DONOSO and STEFANO found that bilateral spreading depression of the cerebral hemispheres caused a significant lowering of the noradrenaline content of the hypothalamus[6].

Spreading depression may be produced by the local application of various chemical, electrical or mechanical stimuli to the cerebral cortex[7]. It is characterized by the attenuation of normal cortical rhythm which propagates outward from the local site of application but does not pass to the opposite hemisphere[8]. The present paper describes the changes in the metabolism of i.c. administered norepinephrine-H3 in rat brain stem associated with cortical spreading depression induced by the application of potassium chloride.

Sprague-Dawley rats weighing 200 g were surgically prepared for application of potassium chloride to the cerebral cortex according to the method of BUREŠ et al.[9]. 24 h later, the rats were injected i.c. with 0.14 (5.88 μc) norepinephrine-H3[10]. 2 h after the i.c. injection, 25% potassium chloride was applied to 1 cerebral hemisphere (ipsilateral side) according to the method of BUREŠ et al.[9] and 25% sodium chloride was applied to the opposite cerebral hemisphere (contralateral side). The animals were killed 3 h after the i.c. injection and the brains removed. The cerebral cortex and subcortical white matter were dissected away, and the cerebellum was removed by transection of the cerebellar peduncles. The remainder of the brain, to be referred to as the brain stem[11], was used in the biochemical analyses.

Norepinephrine-H3 levels in the brain stem on the side to which potassium chloride was applied were lower than levels in the corresponding region of the contralateral side. Similar changes in normetanephrine-H3 levels were also observed (Table). To determine whether these differences reflected a decrease in norepinephrine-H3 on the ipsilateral side or from an increase in norepinephrine-H3 on the contralateral side, animals in which sodium chloride was applied to both hemispheres were also examined in the second experiment. As would be expected under these conditions, no differences in norepinephrine levels were observed between the 2 sides (Table B). The norepinephrine-H3 values, however, were intermediate to those observed for the ipsilateral and contralateral brain stem regions under the previous conditions, suggesting that spreading depression may cause an increase in norepinephrine turnover (decreased levels of norepinephrine-H3) in the ipsilateral brain stem and a simultaneous decrease in norepinephrine turnover (increased levels of norepinephrine-H3) in the contralateral brain stem.

Antipyrine-C14 administered i.v. in the brain was not altered in animals with unilateral spreading depression, suggesting that changes in norepinephrine-H3 metabolism in the brain stem were not due to hemodynamic alterations. The observed decrease of norepinephrine-H3 level on the ipsilateral side could have resulted from either the sudden discharge of norepinephrine immediately after application of potassium chloride or from a more sustained increase in norepinephrine turnover throughout the period of cortical depression. It is unlikely that potassium chloride applied locally to the cortex may diffuse into the brain stem in sufficient concentration to exert a direct action on these structures[12,13].

The mechanism by which cortical spreading depression may alter norepinephrine metabolism in the brain stem remains obscure. The present findings, however, do suggest that changes in cerebral cortical activity may cause alterations in the metabolism of norepinephrine in brain stem structures.

Zusammenfassung. Die durch 25prozentiges Kaliumchlorid induzierte, verbreitende Hemmung der Rinde wurde mit einer vermehrten Auslösung von Noradrenalin-H3 in dem gleichseitigen Hirnstamm und einer verwandten Abnahme an der gegenüberliegenden Seite verbunden, ein Hinweis, dass der Brenzkatechinäthanolaminumsatz ipsilateral erhöht und kontralateral vermindert wurde.

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Effect of cortical spreading depression on norepinephrine-H3 Metabolism in the rat brain stem

(A) Experiment 1

Condition	No.	Norepinephrine-H3 mμc/g	Normetanephrine-H3 mμc/g
Potassium chloride	6	439 ± 46^{a}	19.8 ± 0.9^{b}
Sodium chloride	6	666 ± 52^{a}	23.0 ± 1.9^{b}

(B) Experiment 2

Condition	No.	Norepinephrine-H3 mμc/g	Normetanephrine-H3 mμc/g
Potassium chloride	9	420 ± 37^{c}	18.2 ± 1.3^{d}
Sodium chloride	9	578 ± 71^{c}	21.2 ± 1.9^{d}
Sodium chloride	4	500 ± 35	20.0 ± 0.8
Sodium chloride	4	481 ± 11	20.6 ± 0.9

Norepinephrine was injected i.c.. 2 h later, 25% potassium chloride or 25% sodium was applied to the cerebral hemispheres as described in the text. Animals were killed 3 h after the i.c. injection. The brain was hemisected along its longitudinal axis, and the cerebral hemispheres and cerebellum were removed. Norepinephrine-H3 and normetanephrine-H3 levels in each half of the brain stem were determined by methods previously described[12]. Data are expressed as mμc/g of brain stem $\pm$ S.E.M. The *t*-test for paired data was used in the statistical analysis. [a] $p < 0.01$ for paired differences (two-tailed), [b] $p < 0.05$ for paired differences (two-tailed), [c] $p < 0.03$ for paired differences (one-tailed), [d] $p < 0.05$ for paired differences (one-tailed).

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