filtration was carried out. The 3 fractions from the hydroxylapatite column having the highest inhibitor activity were combined, and EDTA was added to give a final concentration of 0.001 M. This fraction was concentrated to about 1.5 ml in Visking tube using Sephadex G-25, and fed into the Sephadex G-100 column (1 · 60 cm). The column was developed with the phosphate buffer (NaCl 0.15 M, EDTA 0.001 M, and potassium phosphate 0.01 M pH 6.4). The rate of elution was about 15 ml/h. The elution was collected in 3 ml fraction and assayed.

An analysis of the results of the purification procedures of RNase inhibitor from pig cerebral cortex is shown in the Table. Specific activity of the most active fraction from Sephadex G-100 gel filtration was purified about 6500 fold and purer than the sample from liver by SHORTMAN. Some properties of the most active RNase inhibitor fraction are as follows. This fraction was colourless and clear. The protein concentration was about 100 μg/1.0 ml, and RNA concentration was lower than 1 μg/ml. No carbohydrate was found by the Molisch reaction. No hexosamine was detected by Ehrlich test. No heparin was detected by the toluidin blue test. Inhibitor was labil for heating and not dialyzable. The UV-absorption spectrum of this active inhibitor fraction showed maximum absorption peak at 280 nm. Figure 1 shows Disc electrophoretic pattern of the most purified RNase inhibitor fraction. Densitometric estimation of the electrophoretic pattern taken with Joyce double-beam recording microdensitometer is also shown (Figure 2). The major band represented about 80–85% of total protein. The preincubation of this inhibitor with streptomyces protease and trypsin reduced the activity of inhibitor. No loss of activity was found by freezing (– 20 °C) of this purified inhibitor for more than 2 months. Also no loss of activity was observed by storing for 2 weeks at 0–4 °C. The low concentration of PCMB ($1 \cdot 10^{-6} M$) inactivated the inhibitor and was largely reversed by cysteine ($1 \cdot 10^{-3} M$) suggesting the role of SH group in RNase inhibitor. This RNase inhibitor did not have any effect on the RNase T_1[9]. Such properties suggested that this RNase inhibitor might be an acidic protein having the molecular weight of about 60,000, in consideration of the elution rate from Sephadex G-100.

The possible physiological function of this RNase inhibitor in the protein biosynthesis of the brain tissue was reported in a separate paper[5]. The authors intend to try further purification. A detailed account will be published later[10].

Zusammenfassung. Der natürliche Ribonuclease-Hemmstoff aus Schweinsgrosshirnrindengewebe konnte mit DEAE-Cellulose-Chromatographie, Hydroxylapatit-Chromatographie und Sephadex G-100 Gel-Filtration ca. 6500fach gereinigt werden. Der gereinigte RNase-Hemmstoff zeigte eine Hauptbande (etwa 80–85% des Totaleiweisses) und einige kleinere Bande bei der Polyacrylamid-Gel-Electrophorese. Untersuchungen über die Natur dieses Hemmstoffs werden weitergeführt.

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[10] Authors would like to thank Dr. H. SUGANO (Department of Chemistry, Niigata University School of Science) and Dr. K. TANIMURA (Brain Research Institute, Niigata University School of Medicine) for valuable help and also thank Dr. T. KURIHARA (Department of Chemistry, Tokyo University School of Science) for the gift of RNase T_1.

Effects of β-Internal (P^{32}) Irradiation on the 5-HT Content of CNS Levels[1]

It is known that the CNS is a highly radiosensitive tissue, as proved not only from the histopathological and neurophysiological but also neurochemical standpoint. As to the last, one of the most important aspects is the one concerning the central neurotransmitters, e.g. ACh (EGAÑA[2,3]), GABA, L-noradrenalin and 5-HT. In recent years several authors have reported effects of irradiation on the 5-HT content of the brain: ERSHOFF et al.[4] do not find significant 5-HT variations after X- and γ-whole body exposure as compared to untreated pair-fed control; however, the values of these groups are lower than those of untreated ad libitum fed controls. RANSON et al.[5] communicate a significant descent of 5-HT of rat hypothalamus after X-irradiation. SPECK[6] verifies a decrease of brain 5-HT, following high dose exposure with a subsequent recovery at 48 h. RANDIĆ et al.[7,8] found that a 900–4000 r whole body irradiation produces so significant changes; higher doses increase 5-HT concentration of rat brain. All the aforementioned results refer to whole brain (RENSON et al.[5] excepted) and use whole body exposure. We are not acquainted with publications on results of β-internal irradiation and the effects at diverse CNS levels. In addition, the β-internal exposure constitutes a distinct radio-energy absorption compared with the X- or γ-whole body one. So the analysis of the exposure effects at different CNS levels allows us to examine this problem in more specific brain areas in which 5-HT has a particular neurochemical significance, e.g. hypothalamus, mesencephalon etc.

[1] This work has been carried out under a grant from the International Atomic Energy Agency, Vienna; contract No. 95 RB/1-RB/2.

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5-HT content of CNS levels, normals and β-internally irradiated μg/(g CNS level wet weight)

	Brain cortex	Diencephalon	Hypothalamus	Olfactory bulbs	Mesencephalon (midbrain)	Spinal cord
Normal	0.130 (± 0.01)	0.240 (± 0.03)	0.440 (± 0.03)	0.290 (± 0.06)	0.210 (± 0.02)	0.110 (± 0.001)
Irradiated						
'1 single dose' 2 h	0.120 (± 0.03)	0.250 (± 0.03)	0.490 (± 0.12)	0.280 (± 0.06)	0.230 (± 0.02)	0.140 (± 0.02)
6 h	0.160 (± 0.02)	0.340 (± 0.06)	0.550 (± 0.09)	0.310 (± 0.05)	0.300 (± 0.04)	0.140 (± 0.01)
12 h	0.140 (± 0.001)	0.280 (± 0.03)	0.520 (± 0.04)	0.280 (± 0.03)	0.220 (± 0.01)	0.120 (± 0.01)
24 h	0.140 (± 0.01)	0.230 (± 0.02)	0.490 (± 0.01)	0.310 (± 0.03)	0.270 (± 0.03)	0.130 (± 0.01)
48 h	0.140 (± 0.01)	0.270 (± 0.006)	0.530 (± 0.08)	0.300 (± 0.05)	0.260 (± 0.04)	0.150 (± 0.01)
72 h	0.130 (± 0.02)	0.310 (± 0.04)	0.400 (± 0.04)	0.270 (± 0.03)	0.230 (± 0.02)	0.140 (± 0.01)
90–100 days	0.120 (± 0.01)	0.200 (± 0.03)	0.350 (± 0.03)	0.210 (± 0.02)	0.210 (± 0.02)	0.110 (± 0.001)
'1 month'	0.100 (± 0.009)	0.180 (± 0.004)	0.380 (± 0.03)	0.220 (± 0.09)	0.200 (± 0.02)	0.110 (± 0.001)
'4 doses'	0.100 (± 0.02)	0.200 (± 0.02)	0.380 (± 0.05)	0.220 (± 0.05)	0.190 (± 0.03)	0.090 (± 0.001)

The 5-HT content was estimated by VANE's technique[11]. For the meaning of 'diencephalon' see the text. All the values are corrected by a factor of 20% (80% recovery factor), and are given as the mean ± respective S.D. Significant differences regarding t were calculated. The values of normal and irradiated CNS levels have been compared and also the irradiation ones among themselves. There are significant differences between normal and irradiated ($P > 0.01$): brain cortex 6 h, '1 month' and '4 doses'; 'diencephalon' 6, 12, 17 h, '1 month' and '4 doses'; hypothalamus 6, 12, 24, 48 h, 90–100 days, '1 month' and '4 doses'; olfactory bulbs 90–100 days and '4 doses'; mesencephalon 6 and 24 h; spinal cord 2, 6, 24, 48, 72 h and '4 doses'. Exposure see [9].

A total of 562 albino rats of both sexes and weighing 200–250 g, were divided into 2 large groups: normal and β-internally irradiated. P^{32} as $K_2HP^{32}O_4$ carrier free was given. 3 irradiated subgroups were studied: '1 single dose' of 125 μc P^{32}/100 g rat with the animals being sacrificed at 2, 6, 12, 24, 48, 72 h and 90–100 days; '1 month' treatment given in a twice a week course totalling 320–350 μc/100 g; and '4 doses' totalling 500 μc/100 g. The dose-rate, total-dose and full dosimetric studies have been published elsewhere[9]. The extracts were prepared from the following CNS levels: brain cortex, 'diencephalon' (excepting hypothalamus), hypothalamus, olfactory bulbs, mesencephalon and spinal cord. 2 or 3 animals were used for each level determination. The TWAROG et al.[10] technique with minor modifications was used, paying particular attention to the psychic state of the animal previous to the sacrifice. The extracts were diluted in Ringer-Krebs-PO_4-glucose, pH 7.4 in a constant ratio of mg tissue-ml fluid particular to each CNS level. The 5-HT content was measured with VANE's[11] technique of the gastric male fundus (it is 10–15% more sensitive than female). The routine sensitivity of the preparation, made more specific with hyosine-hydrobromide 10^{-7}, is in the order 100–200 ng/l 5-HT base (used as creatine-sulphate salt, 'Sigma'). The determinations were made mostly in triplicate and several times in quadruplicate and sextuplicate. The % of the recovery factor was carefully studied; 80% (± 5) was obtained, so a correction factor of 20% was added in the final tabulation of the results.

The Table indicates the general results, which show, in general terms, a prompt increase of 5-HT in the '1 single dose' experiments at 2 or 6 h, remaining in such state up to 72 h with particular fluctuations according to the CNS level. The higher exposures show, in general, the lowest contents ('1 single dose' 90–100 days, '1 month' and '4 doses'). The results deserve 2 general comments: (1) in 'normals' it confirms the uneven 5-HT distribution in rat neuro-axis, with the hypothalamus and mid-brain as the areas of high content, this finding is similar to those found for other mammalians (GARATTINI et al.[12]); (2) the exposure induces definitive changes in the 5-HT content: (a) In the low total-dose and high dose-rate experiments ('1 single dose' 2, 6, 12, 24, 48 and 72 h) there is a tendency to increase in different %, this fact being particularly noticeable in 'diencephalon', hypothalamus, and midbrain. In these short-term experiments it would seem reasonable to relate these variations to the high dose-rate as compared to the total-dose which is the lowest among all the experiments. This postulate is supported by the fact that the higher increase of 5-HT content appears in hypothalamus and mesencephalon, the least total-irradiated CNS levels. (b) Regarding the long-term and high-dose experiments, the most acceptable conclusion should be that the total-dose is responsible for the 5-HT decrease in all the CNS levels analysed. The present results do not enable us to pinpoint the specific physicochemical and biochemical processes involved either in the 5-HT increment (permeability phenomena, binding processes, enhanced synthesis or alteration of the 5-HT degradation) which we suppose dose-rate linked, or in the lowering content which should be total-dose dependent (inhibition of the synthesis associated or not with an enhanced MAO activity)[13].

Zusammenfassung. Die interne β-Bestrahlung in kleinen Dosen vergrössert den 5-Hydroxytryptamin-Inhalt des zentralen Nervensystems der Ratte: Gehirnrinde, Dyencephalus, Hypothalamus, Geruchbulbe, Mittelhirn und Rückenmark. Bei Experimenten mit höheren Dosen (über 500 rad) sinkt der 5-Hydroxytryptamin-Gehalt.

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