without BrdU (3×10^{-4} *M*) was added outside the glass ring. The preparations were maintained at room temperature (21–24 °C) for 4 h to allow the passive diffusion of BrdU into embryonic tissues. After 4–5 h of incubation at 37.5 °C, the embryos were washed several times in saline to remove as much unbound BrdU as possible. The embryos were then subcultured for 20 h on thin albumen containing 3×10^{-4} *M* of the following: BrdU (Group I), methionine (Group II), and homocysteine (Group III). Additional embryos (Group IV) were treated the same except that plain nutrient medium was used throughout cultivation. The chemical agents were obtained from K & K Laboratories, Plainview, N.Y. or Sigma Chem. Co., St. Louis, Mo. After the incubation period the embryos were examined to determine developmental defects, fixed in Bouin's fluid, and preserved in 70% ethanol. Morphological features of each embryo were carefully reexamined prior to histological sectioning. Randomly selected embryos were embedded in paraffin, sectioned at 6 μm, and stained with Delafield's hematoxylin and eosin. The results were analyzed statistically using the test for the significance of difference in proportions[11].

Results. Our observations on 144 embryos are summarized in the Table.

In Group I, all 32 embryos showed characteristics of BrdU inhibition: poorly developed brain, incomplete neural tube closure, and few somites. Blastodermal expansion, heart development, and blood island formation were not noticeably affected in this series.

In Group II, 30 (67.7%) embryos were normal and the remainder were abnormal. These results, except for brain development, were comparable with those of untreated control series (Group IV).

The results of Group III were statistically insignificant when compared with those of Group I, i.e., homocysteine could not alleviate the BrdU effect. Homocysteine (3×10^{-4} *M*), when applied alone, was non-toxic because over 80% of the embryos showed normal development.

Discussion. LEE et al.[7] showed in stage 4 chick embryos that 1. 4–5 h of BrdU treatment was sufficient to produce congenital malformations; 2. the inhibitory action of BrdU could be alleviated by subsequent treatment with excess thymidine, suggesting its incorporation into DNA. We have obtained similar results by using methionine in place of thymidine. It is well known that methylating agents are required for the conversion of deoxyuridine 5′-phosphate to thymidine 5′-phosphate. Thus it appears that methionine, by virtue of its labile methyl group, stimulates the synthesis of thymidine 5′-phosphate (and hence its dephosphorylated form, thymidine) and in a roundabout way alleviates the BrdU effect. The failure of homocysteine, a demethylated derivative of methionine, to alleviate the BrdU effect may reflect its inability to directly participate in the transmethylation in the early chick embryo. Whether this interpretation is correct or not requires further investigations. Experiments along this line are presently underway in our laboratory.

Zusammenfassung. Die hemmende Wirkung von 5-Bromodesoxyuridin auf die Entwicklung des Frühstadiums von Hühnerembryonen konnte durch nachfolgende Behandlung mit Methionin in aequimolarer Konzentration, jedoch nicht mit Homocystein, aufgehoben werden.

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Uptake of ^{3}H-Noradrenaline and ^{3}H-5-Hydroxytryptamine in Cultured Rat Brainstem

There is considerable evidence that uptake is an important mechanism for terminating the action of released monoamines at peripheral and central synapses[1]. Specific high affinity uptake systems for noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in various regions of the mammalian CNS have been described previously (for ref. see[2]).

Fluorescence histochemical studies have shown that a great number of monoamine-containing neurones and nerve terminals are located in the brainstem[3,4]. Furthermore, neurones with a specific fluorescence for monoamines were also found in cultures of rat brainstem[5]. Since nervous tissue cultures have proved to be a useful tool to investigate the cellular localization of the uptake of amino acid transmitters[6–8], we were interested to study the uptake pattern of monoamines in cultures of rat brainstem.

Cultures were made from the medulla oblongata and pons of fetal (18 days in utero) and newborn rats. Two explants were placed on collagen-coated coverslips, fed with nutrient medium and sealed into Maximov double coverslip assemblies[9]. The nutrient medium consisted of TC-Minimal Medium Eagle, glutamine, calf serum, bovine serum, glucose and antibiotics (for details see[10,11]). The cultures were kept at 35 °C for 10–28 days in vitro. The outgrowth of the cultures was observed daily with phase contrast optics on a reverse microscope. For the autoradiographic studies, the cultures were incubated in Tyrode solution (37 °C) with or without monoamine-oxidase-inhibitor (pargyline, 0.1 m*M*, kindly provided by

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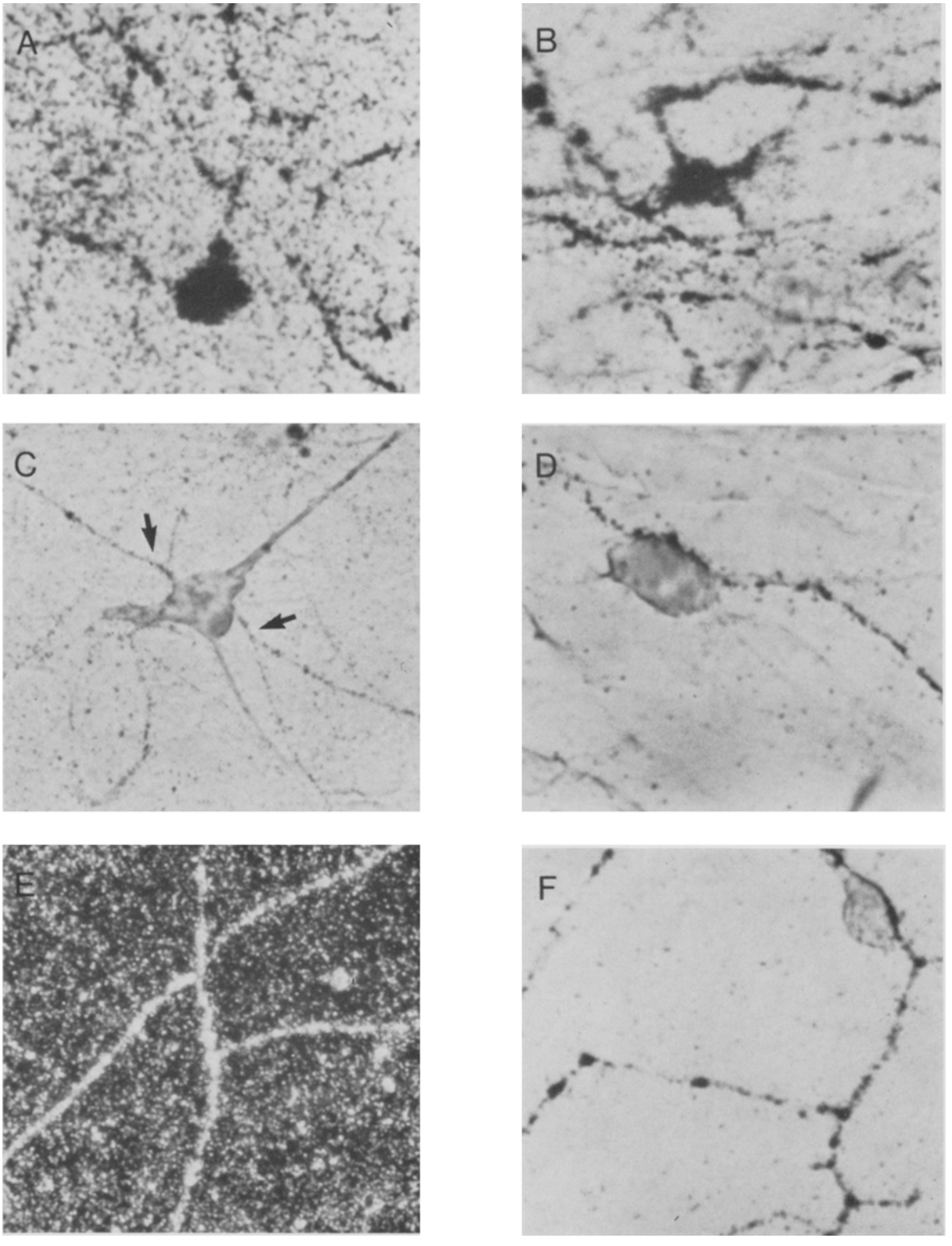

Light microscopic autoradiographs of rat brainstem cultures after incubation with ^{3}H-NA or ^{3}H-5-HT. A) Neurone showing a strong autoradiographic reaction of the cell body and processes after incubation with ^{3}H-NA, 10^{-6} *M* for 2 min (culture, 22 days in vitro). B) Intensively labelled neurone of a 16-day-old brainstem culture, (incubation with ^{3}H-5-HT, 10^{-6} *M*, for 5 min). C) Brainstem culture, (22 days in vitro) after incubation with ^{3}H-NA (10^{-6}*M* for 2 min). The cell body and processes of this neurone appear to contain no silver grains. The heavily labelled processes (arrows), probably axons from other neurones, seem to make contacts with this cell. D) An intensely labelled nerve fibre appears to form contacts with the cell body of an unlabelled neurone. Brainstem culture, 16 days in vitro, after incubation with ^{3}H-5-HT, 10^{-6} *M* for 2 min. E) and F) Labelled nerve fibres in the outgrowth zone of brainstem cultures. The monoamines are concentrated in small dots giving the appearance of varicosities. E) ^{3}H-NA, 10^{-6} *M* for 2 min, dark field illumination, culture, 22 days in vitro. F) ^{3}H-5-HT, 10^{-6} *M* for 5 min, bright field illumination, culture, 16 days in vitro. Bar: 20 μm.

Dr. L. MAÎTRE, Ciba-Geigy Ltd. Basel) for 15 min. Afterwards they were transfered to Hank's solution containing either ^{3}H-L-noradrenaline (New England Nuclear Corp., specific activity: 6.41 Ci/m*M*) or ^{3}H-5-hydroxytryptamine (The Radiochemical Center Amersham, specific activity: 9.5 Ci/m*M*) in a concentration of 10^{-6} *M*. Approximately half of the cultures were incubated in the solution containing the isotopes to which pargyline was added. After the incubation (30 sec to 15 min at 37 °C) the cultures were rinsed 3 times for 5 min in Tyrode solution, fixed with 3% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.3), dehydrated and mounted on object slides. The air-dried cultures were covered with Ilford L4 emulsion (Ilford Ltd. Essex) using the loop technique described by JENKINS[12]. Development and fixation of the autoradiograms were performed after 2–3 weeks with Kodak D19 developer and 25–30% sodium thriosulphate respectively[6–8, 11]. The autoradiograms were examined and photographed on a Wild epi-illumination microscope.

^{3}H-NA and ^{3}H-5-HT were found to be taken up by the majority of brainstem cultures. A strong accumulation of monoamines was mainly observed in nerve fibres growing out from the explant into the outgrowth zones. As shown in the Figure, ^{3}H-NA (E) and ^{3}H-5-HT (F) are concentrated in small dots in these fibres giving the appearance of varicosities described in fluorescence microscopic studies[4]. The monoamines were also found to be taken up by a small proportion of neurones. Figure A and B illustrate neurones with a strong accumulation of grains over the cell bodies and processes after incubation with NA and 5-HT respectively. A great number of neurones appeared to be free of label. It was frequently observed that labelled fibres, probably axons from monoamine-containing neurones approached the cell body and processes of these unlabelled neurones and seemed to form contacts with these cells. An example of fibres labelled with ^{3}H-NA making contact with a neurone which did not accumulate the monoamine is illustrated in Figure C (arrows). Figure D shows a nerve fibre with an intense autoradiographic reaction after incubation with ^{3}H-5-HT passing an unlabelled neurone. It appears that this fibre forms contacts with the neuronal cell body by 'en passant' synapses. Electronmicroscopic studies have revealed that axo-somatic synapses as well as synapses 'en passant' frequently occur in spinal cord and brainstem cultures[11].

No difference was observed between cultures which were treated with MAO-inhibitor and untreated ones. Preliminary studies have shown that ^{3}H-dopamine reveals a similar uptake pattern to that of ^{3}H-NA and ^{3}H-5-HT.

Previous investigations on the cellular localization of the uptake of amino acid transmitters into spinal cord and brainstem cultures have revealed that glycine, GABA and glutamate are not only taken up by neurones but to a great extent also by glial cells[6–8, 11]. In contrast to these observations uptake of ^{3}H-NA and ^{3}H-5-HT was exclusively found to be localized in neurones and neuronal processes. These results are consistent with studies on the uptake of labelled monoamines in CNS tissue in situ[13–16] and in slices[14, 17], demonstrating that ^{3}H-NA and ^{3}H-5-HT are predominantly localized in nerve endings and unmyelinated axons and to a smaller extent also in nerve cell bodies.

From these results it is concluded that there is a difference between the cellular localization of the uptake of monoamines and that of amino acid transmitters where glial cells seem to play a role in their inactivation[6, 7, 11, 18–20].

Zusammenfassung. Die zelluläre Lokalisation der Aufnahme von ^{3}H-NA und ^{3}H-5-HT in den Hirnstamm von Ratten wurde am Modell der Nervengewebskultur untersucht. Im Gegensatz zu den Aminosäure-Transmittern, welche in Neurone und Gliazellen aufgenommen werden, zeigen Monoamine nur eine Aufnahme in Zellkörper und Fortsätze von Neuronen, nicht jedoch in Gliazellen. Es besteht eine Korrelation zwischen den Ergebnissen von fluoreszenzmikroskopischen Versuchen und unseren autoradiographischen Untersuchungen.

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Virusähnlicher Kerneinschluss beim Basaliom

Virus-Like Inclusion in Basal Cell Carcinoma

Gebilde, die ultrastrukturell den Paramyxoviren ähneln, wurden auch in den Zellen mesenchymaler Tumoren beschrieben. So konnten STEWART et al.[1] im Kern und im Cytoplasma von Lymphoblasten bei M. Hodgkin derartige Strukturen darstellen. JENSON et al.[2] fanden beim Osteosarkom sowohl intranucleär (Lungenmetastasen) als auch intracytoplasmatisch (Primärtumor) situierte Paramyxovirus-ähnliche Gebilde. Solche tubulären bzw. filamentösen Aggregate sind im Material von GYÖRKEY et al.[3] nur intracytoplasmatisch lokalisiert. Beim M. Kaposi fanden diese Autoren allerdings solche Strukturen zwischen den beiden Lamellen der Kernmembran (ähnlich GYÖRKEY et al.[4] bei Ery-

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