arrows) and the cell membranes (Figure 4, single ringed arrow) together with extrusion of their cytoplasmic contents.

Discussion. The present ultrastructural study of the effects of an alcoholic extract of the sponge, *Suberites inconstans*, has confirmed some of the findings obtained by means of phase contrast microscopy. The earliest effect of the extract appeared to be exerted on mitochondria. Since mitochondria are very sensitive cell organelles which succumb rapidly to various forms of adverse conditions, e.g. anoxia and cytotoxic chemicals, such changes in the ultrastructure of the mitochondria would be expected to be the first observable effect of cytotoxicity.

The reaction of HeLa cells to colloidal gold[4], hydroxyurea[5], and several antibiotics, notably actinomycin D[6,7], toyocamycin[8,9], daunomycin[10], and phleomycin[11–13], have been reported. The effect of most of these substances on the viability of HeLa cells treated with them appear to be related to their ability to inhibit nucleic acid synthesis. The effect of the sponge extract on HeLa cell growth has been shown by our previous study[2] to be related to inhibition of DNA synthesis.

The present ultrastructural study has suggested that the extract may also have a profound effect on cell membrane permeability. The appearance of a clear zone beneath the cell membrane (Figure 3, CZ) would suggest an intracellular accumulation of fluid which had leaked into the cell. This could possibly account for the cytoplasmic contents being pushed into a perinuclear position. The mechanism of rupture of both nuclear and cell membranes is at present unknown.

Résumé. Des observations ultrastructurales ont été faites sur les cellules HeLa de l'éponge *Suberites inconstans* extraites par une solution alcoholisée. Aux degrés toxiques de concentration, les mitochondries furent les premières à présenter des vacuoles et à perdre leurs cristae. Il s'en suivit une rupture de la membrane nucléaire, l'apparition de véhicules cytoplasmiques, une accumulation de fluide dans le cytoplasme et finalement une rupture de la membrane cellulaire due à la perte du contenu cytoplasmique.

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Alleviation of Inhibitory Action of 5-Bromode-oxyuridine by Methionine in Early Chick Embryos

Numerous reports have shown that the thymidine analogue, 5-bromodeoxyuridine (BrdU), can suppress the synthesis of cell-specific macromolecules, alter the cell surface of differentiating cells[1–4], and interfere with embryonic development[5–8]. Lee et al.[7] reported that the inhibitory action of BrdU could be alleviated by subsequent treatment with excess thymidine in explanted streak stage chick embryos. We now report that the BrdU effect can also be alleviated by methionine, a methylating agent known to occur in animal cells.

Materials and methods. Fresh and fertile White Leghorn eggs were incubated at 37.5 °C for 17–19 h to obtain stage 4 embryos[9]. The embryos were explanted by New's[10] technique. Nutrient medium (thin albumen) with or

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The development of stage 4 chick embryos, subcultured for 20 h on 4 different media following pretreatment with BrdU (3×10^{-4} *M*) for 4–5 h (Groups I–III).The embryos of Group IV were treated the same except that plain nutrient medium was used throughout cultivation

Group No.	No. of embryos at subculturing	Subculture medium	Embryos (%) showing abnormalities in			
			Brain	Neural tube	Heart	Somites
I	32	BrdU (3×10^{-4} *M*)	100.0	84.4	21.9	100.0
II	42	Methionine (3×10^{-4} *M*)	33.3[a]	16.7[a]	14.3	19.1[a]
III	36	Homocysteine (3×10^{-4} *M*)	86.1	77.8	27.8	91.7
IV	34	Nutrient medium	14.7[a]	11.8[a]	11.8[a]	14.7[a]

[a] Statistically significant at the 0.01 level with the same structure in Group I.

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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