has no significant effect on subsequent tumor growth. About 35% of the EAC cells are lost during removal of carbonyl iron at the end of incubation. This loss, however, does not affect the size of inoculum because it was performed by injecting in all animals the same number of EAC cells at viability over 85%.

One hypothesis would be that carbonyl iron treatment exerts a delayed cytotoxic effect on the cells. However, it would be necessary to suppose a very drastic decrease in cell number following inoculation, because we have found that the tumor growth rate is independent of inoculum size, at least over the range 1×10^6 to 6×10^6 EAC cells injected (unpublished data). This result indicates that simple reduction in cell survival after inoculation does not explain the observed alteration in the growth curve, so that more specific effects of carbonyl iron on the cells may be involved. One such possible effect would be interaction of the carbonyl iron with the cell surface, resulting in altered antigenic properties and increased immunogenicity of the inoculated cells. The greater reduction in mortality in C57BL/6 mice than in Swiss mice correlates well with the observation that the EAC tumor is more immunogenic in C57BL/6 than in Swiss mice[11]. This increased immunogenicity may be caused by high concentration of Fe^{+++} ions released in the EAC suspension during incubation. In fact, about 1300 µg/100 ml of Fe^{+++}ions are detected in the supernatant of carbonyl iron-treated group-20 mg/ml; while very low amounts are detected in the supernatant of carbonyl iron-treated group 1 mg/ml (82 µg/100 ml) and in supernatant of control group without carbonyl iron (17 µg/100 ml). If carbonyl iron (20 mg/ml) is mixed with EAC cells and immediately removed, the tumor growth does not differ from the EAC growth of the control group. After removal Fe^{+++}ions detected were 97 µg/100 ml. (Determinations performed by: tripyridyltriazine (TPTZ)-test, set-64331, Harleco, Philadelphia, USA.)

The presence of Fe^{+++}ions in the supernatant after incubation of EAC cells with carbonyl iron, could also inhibit the cells replication. This hypothesis is supported by observations from various authors who found that multivalent cations interact with membrane functions of EAC cells[12]. Whatever the mechanism, high concentrations of carbonyl iron are by no means biologically inert, and this fact must be considered when using this method to obtain populations of nonadherent cells[4,8].

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2 The abbreviations used are: EAC (Ehrlich ascites carcinoma); PBS (Dulbecco's phosphate buffered saline); MEM (Eagle's minimum essential medium).
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Autoradiographic localization of the uptake of ^{3}H-β-alanine in rat nervous tissue cultures

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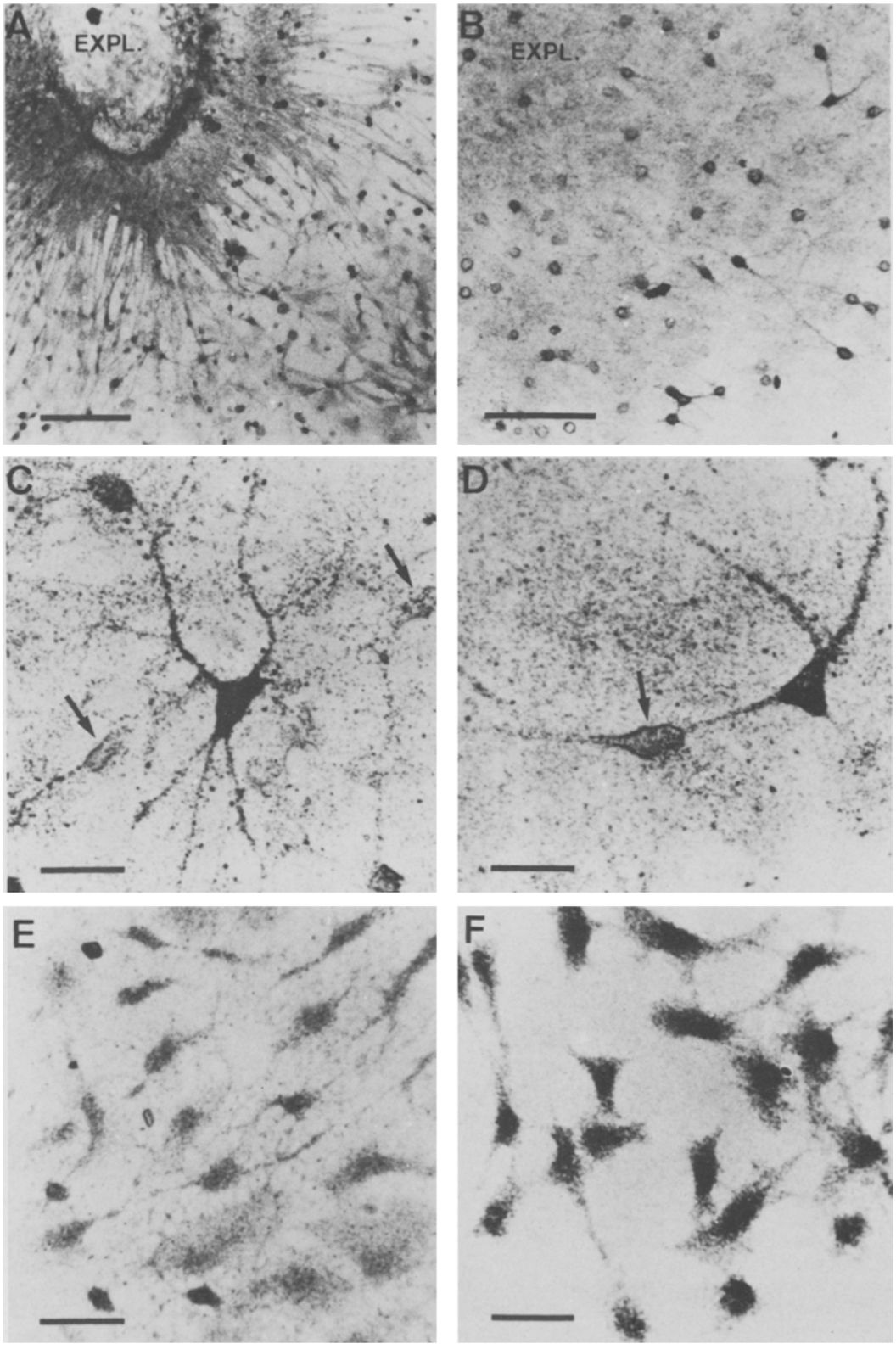
Summary. Autoradiographic studies on the uptake of ^{3}H-β-alanine have shown that, in spinal cord and brain stem cultures, both neurones and glial cells have accumulated the amino acid. In contrast, in cultures of cerebellum and dorsal root ganglia, ^{3}H-β-alanine was only taken up by glial elements.

It has been proposed that β-alanine may function as an inhibitory transmitter substance in various regions of the central nervous system (CNS)[1]. Although the concentration of β-alanine in the mammalian CNS is quite low, the regional distribution of the amino acid varies considerably, being similar to that of GABA[2]. Microelectrophoretically administered β-alanine has a depressant action on neurones of the mammalian CNS which is blocked by strychnine[3-6]. Furthermore, β-alanine was found to be taken up by high and low affinity transport mechanisms in the frog spinal cord[7] and in brain slices of the rat[8,9]. Autoradiographic studies of the uptake of β-alanine in isolated rat sensory ganglia, and in small slices of cortex and cerebellum, have shown that the amino acid was exclusively accumulated by glial cells[10,11], whereas in the retina both amacrine neurones and glial cells were labelled[12]. The present study is concerned with the cellular localization of the uptake of ^{3}H-β-alanine in cultures of spinal cord, brain stem, cerebellum and dorsal root ganglia (DRG) of the rat using autoradiography.

The cultures were prepared from the spinal cord, medulla oblongata-pons, cerebellum and dorsal root ganglia of fetal and newborn rats and grown in the Maximov double coverslip assemblies for 8-35 days[13]. For the autoradiographic studies, the cultures were incubated at 37 °C in Hank's solution containing ^{3}H-β-alanine (NEN, specific activity 31.5 Ci/mM) in a concentration of 5×10^{-7} and 10^{-6} M for 1-15 min. Some experiments were performed at 0 °C or in Na^+-free solution[13]. After the incubation, the cultures were rinsed in Tyrode solution, fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, dehydrated and air-dried. Ilford L4 emulsion was placed over the cultures by the loop technique[14]. The autoradiograms were developed after 2 weeks with a Kodak D 19 developer.

After incubation of spinal cord and brain stem cultures with ^{3}H-β-alanine, it was observed that both neurones and glial cells have accumulated the amino acid. There was a difference in the number of labelled neurones between spinal cord and brain stem. In cultured brain stem, a relatively great number of neurones have taken up the



A, B Autoradiographs of brain stem and spinal cord cultures (23 days in vitro) after incubation with ^{3}H-β-alanine, 10^{-6} M for 3 min. Both neurones and glial cells have accumulated the amino acid. In the brain stem culture a greater number of neurones is labelled (A) than in the spinal cord culture (B). EXPL. = explant. Bars: A: 150 µm, B: 100 µm. *C, D* Cultured brain stem (C) and spinal neurone (D) being intensely labelled over the cell body and processes, whereas other neurones (arrows) have not accumulated ^{3}H-β-alanine (10^{-6} M for 3 min). Bars: 30 µm. *E, F* Labelled glial cells in the outgrowth zone of brain stem cultures (23 days in vitro) after incubation with ^{3}H-β-alanine, 10^{-6} M. Incubation time is 5 min for E and 10 min for F. Note the difference in the intensity of labelling between E and F. Bars: 30 µm.

amino acid (figure A), whereas in spinal cord cultures only few neurones were labelled (figure B). Figures C and D illustrate a neurone of a brain stem and a spinal cord culture with an intense labelling over the cell body and processes. Other neurones (arrows) in the same cultures were almost free of label. In contrast, in cultures of cerebellum and DRG, ^{3}H-β-alanine was only taken up by glial cells. Similar observations have been made in slices of cerebellum and in isolated DRG of the rat, demonstrating that β-alanine was exclusively accumulated by glial cells[10,11]. The time course of the uptake of ^{3}H-β-alanine into glial cells was slower than that of other amino acid transmitters such as glycine, GABA, glutamate and aspartate[15]. After an incubation time of 5 min, glial cells were only weakly labelled with ^{3}H-β-alanine (figure E), whereas after 10 min there was a heavy accumulation of the amino acid in all glial cells (figure F). These observations are consistent with biochemical studies in the frog spinal cord[7] and in brain slices of the rat[8], demonstrating a slower rate of uptake of β-alanine in comparison to the rapid time course of GABA. The uptake of ^{3}H-β-alanine was temperature- and sodium-dependent being considerably reduced or abolished after incubation at 0 °C or in sodium-free incubation medium. Our results, in demonstrating that in cultured spinal cord and brain stem ^{3}H-β-alanine is taken up by neurones and not only by glial cells, provide further evidence for a transmitter role of β-alanine in these regions[1].

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Exogenous melatonin and melanophore development in *Xenopus*

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Summary. *Xenopus* larvae raised from stage 21 in melatonin solution and upon a dark background had fewer head melanophores at stage 48 than control animals not exposed to melatonin. Rearing larvae in melatonin solution seems to mimic rearing larvae on a light background.

The ability to mimic the body blanching response of *Xenopus* larvae with the pineal substance melatonin provides not only an extremely specific bioassay method[1]; but serves as the basis for a well defined theory of pineal control of body blanching in this species[2]. Placing larvae on a white background will also produce blanching[3], and rearing *Xenopus* embryos on a white background will even reduce the number of melanophores present in the larvae[4,5] We have reared *Xenopus* embryos in melatonin solution to see whether melanophore number is changed and we find that it is, although exposure to melatonin must occur early in development.

Methods. *Xenopus laevis* embryos were obtained by artificial ovulation and breeding, and staged using the normal table of Nieuwhoop and Faber[6]. At stage 20 embryos were removed from their investing membranes with sharpened jeweler's forceps and transferred to appropriate dishes. Embryos were cultured in groups of 10 in covered finger bowls on a black background containing 50 ml spring water or 50 ml spring water with a melatonin concentration of 2 µg/ml. During development the water was changed periodically. Embryos were introduced to the melatonin solution at stages 21, 26, 35/36 and 40. Embryos were killed at stage 48 by transferring to neutral buffered formalin solution and melanophores on the right side of the head were counted[5].

Results. Only the embryos introduced to the melatonin solution at stage 21 showed any significant difference from spring water control larvae. Their melanophore number was only 58% of control with a SEM of 4.9% and a $p < 0.001$ derived from Student's t-test.

Discussion. The significant difference related to melanophores between stage 21 embryos and later stages tested is the migration of neural crest tissue, although definitive differentiation into melanophores is not manifested until stage 33/34[6]. Previous measurement of light and dark background reared embryos showed a melanophore reduction in light background animals that averaged about 59% of dark background animals[5]. The involvement of endogenous melatonin in melanophore development is difficult however to ascertain. Melatonin level is not in any way outstanding at stage 20[7], nor is its formative enzyme hydroxindole-O-methyltransferase (HIOMT)[8]. In animals reared on differing backgrounds and then measured for melatonin and HIOMT at stage 48 there is a reduction of melatonin levels in light background reared larvae of about one half[5] and an increase in whole embryo HIOMT of about 40%[9]. The HIOMT increase is centered in the brain where a 9fold elevation of light background over dark background is found[9]. Although melatonin has not been implicated in developmental processes as has its precursor 5-hydroxytryptamine (5-HT)[10], it has been suggested as a possible agent in cellular contractile phenomena[11]. While there is no suggestable mechanism of action for the effect of exogenous melatonin upon melanophore development, it is of interest to note that it can be used to mimic light background rearing just as it can be used to mimic body blanching.