cells bodies develop and intense yellow fluorescence with similar but less pronounced fluorescence lability (Figure 2). Examination of the corresponding regions in the medulla of rats treated with Ro 4-4602 and L-DOPA reveals a general mild increased fluorescence in the neuropil, however, the neuron cell bodies of the nucleus raphe/pallidus and those in the ventral arcuate nucleus exhibit a distinct green fluorescence with mild fluorescence lability (Figure 3). Rats administered L-DOPA or Ro 4-4602 alone do not reveal significant observable differences from the control group upon examination of the same brain regions. Comparable results were seen in all of the experimental animals.

Discussion. The present histochemical studies show that the administration of a peripheral decarboxylase inhibitor in dosages which exhibit minimal central nervous system activity[1,3] followed by large dosages of L-DOPA results in a change in the fluorescence characteristics of some cell bodies in the brain stem. The change in the fluorescence characteristics from yellow, UV labile to green UV stable indicates that these nerve cell bodies which normally contain 5 HT can contain a catecholamine under the above experimental conditions. These observations are compatible with the suggestion by BARTHOLINI et al.[3] that displacement of endogenous 5 HT may occur as a result of cerebral accumulation of catecholamines. The observations by BARTHOLINI et al.[3] of increased 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5 HT, in brain during a 90 min period following the administration of Ro 4-4602 and L-DOPA further strengthen this possibility. EVERETT and BORSCHERDENG[5] confirmed this by showing a two-fold increase of 5-HIAA levels in mouse brain 1 h following the administration of L-DOPA alone. Recent in vitro observations by NG et al.[8] strongly support the displacement hypothesis.

The present study indicates that serotonergic nerve cell bodies have the capacity under certain conditions to take up and concentrate exogenous catecholamines. That the nerve terminals arising from serotonergic neurons have the same uptake capacity for catecholamines and their precursors has not been demonstrated, however, this possibility seems quite likely on the basis of the observations of BARTHOLINI et al.[3] and NG et al.[8] and has been suggested by BUTCHER et al.[7]. Although the accumulated evidence supports displacement as the mechanism for depletion of cerebral 5 HT following L-DOPA administration, the possibility of blood-brain transport competition of L-DOPA with 5 HT precursors may also be responsible, in part, for some of the biochemical observations.

The implication of these observations is that they may form a basis to understand some of the clinical phenomena observed in patients with Parkinson's disease to whom massive doses of L-DOPA are continuously administered. Patients with this disease commonly experience the onset of choreo-athetoid activity during treatment with L-DOPA[11]. These symptoms are related both to duration of treatment and total daily dosage of L-DOPA. Since the major biochemical deficit in this disease is depletion of brain monoamines (both dopamine and serotonin)[12,13], the susceptibility for either serotonin displacement or occupancy of serotonin binding sites by L-DOPA may be quite pronounced and allow L-DOPA (decarboxylated to dopamine) to act as a false transmitter in such circumstances.

Zusammenfassung. Die histochemische Fluoreszenzmethode wird zum Studium der Nervenzellen des Gehirnstammes der Ratte angewandt. Die erhebliche Zunahme des Dopamins, hervorgerufen durch die Injektion eines peripher wirkenden Dekarboxylase-Hemmstoffes zusammen mit L-DOPA, wird im Gehirn verfolgt und gefunden, dass serotonergische Neuronen imstande sind, Catecholamine aufzunehmen.

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Effect of Polyalkyl Polyphosphonates on Bone Development in Young Rats

Phosphate ions are one of the main constituents of the mineral of bone tissue. These ions are not only incorporated into bone, but also chemisorbed by it, as demonstrated by P^{32} tracer experiments (BAUER et al.[1]). A molecule carrying two PO_x^{n-} groups (e.g., a pyrophosphate ion, $[HOPOPOH]^{2-}$ with O O O O) will show a higher affinity for bone mineral since it is a bidentate complexing agent, capable of being attached to calcium ions through 2 oxygen atoms[2]. The absorption of pyrophosphate onto hydroxyapatite, the mineral form of calcium phosphate in bone, inhibits its crystal growth and dissolution[2,3]. Similar results have been observed with organic diphosphonates, $R_1R_2C(PO_3H)_2^{2-}$ (R = H, OH, or alkyl group), both in vivo and in vitro[4,5]. The latter findings recently encouraged a clinical application of ethane-1-hydroxy 1,1-diphosphonate in the treatment of myositis ossificans[6].

Polymeric alkyl phosphonates, e.g., polyethylene polyphosphonate, which carry many phosphonate groups on

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an alkyl backbone have been shown to adsorb onto enamel and dentin of teeth in vitro[7,8]. Using P^{32}-labeled polyethylene polyphosphonate, it was shown that these compounds are taken up by bone similarly to phosphate ions[9]. Further, it has been shown that the uptake of both calcium and phosphate ions by bone is inhibited in polyethylene polyphosphonate treated rats[10]. It was of interest therefore to investigate whether the effect of polyethylene phosphonate on bone metabolism results in inhibition of bone growth to an extent demonstrable by histological examination. It was also of interest to explore to what extent are polyalkyl polyphosphonates toxic to mammals; in other words, do they have physiological effects other than on the bone system.

Materials and methods. Two polyalkyl polyphosphonates have been synthesized from polyethylene by phosphonation with phosphorus trichloride in the presence of oxygen according to SCHROEDER and SAPCHAK[11]. The polymers prepared were: PP-1: Mol wt. 7000, 13% P, i.e., a phosphonate group on every 5th carbon, 46 phosphonate groups per molecule (average values); and PP-2: Mol wt. 2000, 18% P, i.e., a phosphonate group on every 4th carbon, 15 phosphonate groups per molecule. These polyalkyl polyphosphonates were dissolved in neutral normal saline solutions adjusted to pH 7.1–7.3. The solutions administered to rats were: 1.7 mg/ml, i.e., $1.1 \times 10^{-2} N$ (based on HPO_4^{2-} equivalents), $3.3 \times 10^{-2} N$, and $1.0 \times 10^{-1} N$ PP-1; 1.5 mg/ml, i.e., $1.1 \times 10^{-2} N$, $3.3 \times 10^{-2} N$, and $1 \times 10^{-1} N$ PP-2. Rats (Sprague Dawley), 4 weeks old, average weight 100 ± 5 g, were injected i.p. with daily doses of 1 ml of these solutions (6 experimental groups) for periods up to 50 days. Two other groups were injected daily with the highest doses of PP-1 and PP-2, followed 2 h later by 20 microequivalents of Ca^{++}. The control groups included rats treated with daily injections of saline, with phosphate or calcium, 15 and 20 microequivalents, respectively, or with EDTA (1.7 microequivalents). Each of the experimental groups consisted of 5 rats. The rats were fed standard Purina rat food and water ad libitum. At predetermined intervals (12, 20, 30, 40, and 50 days following the beginning of treatment), rats were sacrificed, blood was taken for analysis of sodium, potassium, calcium, inorganic phosphate, glucose, uric acid, serum glutamic oxalacetic transaminase, cholesterol, albumin, total protein, alkaline phosphatase, lactic dehydrogenase and bilirubin. The following organs were taken for histological examination: liver, kidney, lung, small intestine, femur, incisor tooth and vertebra. Representative sections were fixed in 10% formalin and stained with hematoxylin and eosin. Sections of the bones were stained with hematoxylin and eosin. The weight of the animals was monitored and the calcium content of bone (femur) was determined in the later periods of the experiment.

Results. The rats of all groups, starting with an average weight of 100 ± 5 g gained weight until the 4th period when their weight leveled off at 269 ± 10 g for rats of all groups except for the group administered the highest dose of PP-2. The rats of this group gained weight slower beginning at the second period and leveled off at 225 ± 5 g.

When the wet weight of the whole femur after 50 days of treatment was compared with the weight of the whole rat, it was found that the femur/whole body ratios of the groups receiving EDTA as well as the highest doses of PP-1 and PP-2 were smaller than the corresponding ratios of the other groups. These ratios were 0.134, 0.129, and 0.137×10^{-3}, respectively, compared with an average of $1.59 \pm 0.16 \times 10^{-3}$ found in the other groups. However, only the EDTA treated rats showed a significantly lower calcium content in femur bone (total Ca in bone/weight of bone = 16.9% in the EDTA treated rats compared with an average of 20.8 ± 1.2% for all other groups).

No significant change could be detected in any of the parameters of blood chemistry investigated throughout the whole experiment. None of the viscera including lung, kidney, intestine, and liver showed any gross or microscopic pathological changes in any group at any time during the experiment. Thus, none of the injected substances caused visceral damage or metastatic calcification.

Examination of the bone sections showed significant deviations from normal bone growth in all the polyphosphonate treated animals. These bones which were less calcified showed excessive osteoid tissue. Sections of femur, vertebra, and tooth from rats injected with saline demonstrated normal calcification and bone growth; osteoid tissue and cartilage were unremarkable. Sections of bones and cartilage from the animals receiving only phosphate and calcium were similar to those of the controls. Rats treated with EDTA showed less bone calcification beginning at the third period.

These changes were different from those observed in the groups receiving polyphosphonates. In these groups inhibition of bone formation was observed in all bones and cartilage. This effect was more conspicuous at the higher doses and at longer exposures to the polyalkyl polyphosphonates. These effects were not negated by combining the compounds with equivalent amounts of Ca^{++}.

Discussion. There is little doubt that alkylpolyphosphonates affect bone metabolism as has been demonstrated in this study by the gross development and histologic examination of bone. Although these compounds show no visceral toxic effect even at relatively high doses and prolonged treatment, they undoubtedly inhibit bone formation in the young animals. This inhibition of bone growth is not directly related to lowering of Ca level in the plasma, as much lower doses of EDTA were shown to have far more adverse effects on bone development. Furthermore, the effect of polyalkyl polyphosphonates was not negated by the simultaneous administration of twice equivalent amount of Ca^{++} ions[12].

Zusammenfassung. Es wird gezeigt, dass Knochenentwicklung junger Ratten durch Behandlung mit Polyethylen-Polyphosphonat ohne toxische Wirkung verhindert wird. Die Polyphosphonate wirken direkt auf den Knochen und können eventuell verschiedene klinische Anwendungen finden.

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