Percentage of prohaemocytes in different stages of mitosis after injection of phytohemagglutinin-P

Stage of examination	Total prohaemocyte percentage undergoing mitosis	Prophase	Metaphase	Anaphase	Early telophase	Late telophase
Early 3rd instar before treatment	20.0	-	-	-	-	-
4 h after treatment	84.1	78.9	-	-	-	5.2
8 h after treatment	78.4	67.8	7.1	-	-	3.5
22 h after treatment	100.0	80.0	20.0	-	-	-
26 h after treatment	100.0	83.3	6.1	8.8	-	1.8

The table shows that in all the stages examined, the predominantly large number of prohaemocytes undergoing division are encountered in the prophase stage and none in the early telophase stage. The small percentage of cells in the late telophase stage in the smear made 4 h after the treatment apparently represents some of the cells starting division before the treatment. In the metaphase stage, 7.1, 20 and 6.1% prohaemocytes are observed 8, 22 and 26 h after the injection while 8.8% prohaemocytes are seen in the anaphase stage only 26 h after the treatment. The obvious inference is that prophase is the longest lasting stage in the mitotic cycle of prohaemocytes of *S. ruficornis* and the anaphase and early telophase stages are the most transient. Injection of 0.02 ml of 1% colchicine in the larvae 1 h before collection of haemolymph at each one of the stages mentioned confirmed the results already cited.

We have concluded that in *S. ruficornis*, mitosis among the haemocytes is confined to the prohaemocytes, that the mitotic stimulator phytohaemagglutinin-P effectively induces mitosis in these cells and that in their mitotic cycles, stages other than the prophase are short lived and therefore observed relatively rarely.

1 Acknowledgment. We express our gratitude to the State Council of Scientific and Industrial Research, U.P. for the grant of financial assistance to U.S.S.

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4,5,6,7-Tetrahydro-7-oxobenzo[b]thien-4-ylurea; Sulbenox[1], a novel animal growth stimulant

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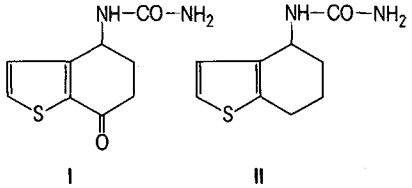
Summary. The title compound, a metabolite of 4,5,6,7-tetrahydrobenzo[b]thien-4-ylurea, was synthesized and found to be an effective growth promoter in sheep, mice, and rats. In sheep it gave over a 6-week growth period at 15 and 60 ppm in the diet an economically and statistically significant growth response.

The increasing cost of livestock production[3] and concern for the continued use of hormones, especially the synthetic estrogen diethylstilbestrol[4], the most commonly used growth stimulant in cattle and sheep, has prompted the search for a safe drug which can improve the efficiency of raising farm animals.

We have synthesized a compound, 4,5,6,7-tetrahydro-7-oxobenzo[b]thien-4-ylurea (**I**), which when administered to animals increases their rate of growth. Furthermore, it appears to have a number of characteristics desirable in a compound to be used as a growth stimulant for meat-producing animals. Thus, it is not estrogenic, androgenic, or goitrogenic in standard rat tests. It is non-mutagenic in the Ames test, its LD_{50} in rats (single oral dose) is greater than 5000 mg/kg b.wt, and at effective growth-stimulating levels the body compositions of experimental animals are normal.



I II

Early investigations with 4,5,6,7-tetrahydrobenzo[b]thien-4-ylurea (**II**) indicated that **II** was a member of a new class of growth stimulants in sheep. Metabolism studies showed that **II** when administered orally to rats, sheep, and cattle was rapidly converted to 2 major metabolites, probably a ketonic and a hydroxylated material[5], which were isolated from urine. Since **I** was a possible metabolite based on spectral data (NMR and CIMS), and could thereby be the active drug, synthesis and structural elucidation work was initiated.

Compound **I** was prepared in 37% yield by ceric ammonium nitrate oxidation of **II** which was obtained by allowing 4,5,6,7-tetrahydrobenzo[b]thiophen-4-amine hydrochloride to react with KOCN. It was recrystallized from methanol to give crystals melting at 245–246 °C; microanalysis for C, H, N was satisfactory and spectral data (NMR, IR, CIMS) were consistent for the strucutre represented by **I**. Comparison of spectral data and R_f values of **I** with those of the ketonic metabolite showed they were identical[5].

Given orally or parenterally, **I** was effective in stimulating growth in female, male, and castrate male rats. When compound **I** was administered orally in the diet of lambs over a 6-week growing period, economically and statistically significant improvements in average daily gain and feed efficiency were elicited. These results are summarized in

Performance of lambs fed experimental compounds*

Treatment	Level	Average daily gain, kg (%)++	Feed/gain (%)+
Control	–	0.215[a]	6.212[a]
II	15 ppm	0.227 (+5.6)[ab]	6.062 (+2.4)[a]
II	60 ppm	0.238 (+10.7)[b]	5.811 (+6.5)[b]
I	15 ppm	0.243 (+13.0)[c]	5.808 (+6.5)[b]
I	60 ppm	0.239 (+11.2)[b]	5.756 (+7.3)[b]

* Values represent data pooled from 2 42-day lamb-feeding trials conducted with western crossbred wether lambs with an average initial weight of 28.9 kg. All lambs were pretreated with sulfamethazine, chlortetracycline, 2 enterotoxemia injections and levamisole for prophylactic disease control. Lambs were allotted to 5 pens of 6 lambs each per treatment in each experiment. The basal diet contained 48% ground corn, 10% soybean meal, 15% dehydrated alfalfa meal, 15% ground corn cobs, 10% molasses, 0.5% iodized salt, 1.0% dicalcium phosphate and 0.5% trace mineral and vitamin mix. + Values in ( ) represent percent improvement over controls. + Values followed by one or more common superscripts were judged not significantly different from each other ($p = 0.05$).

the table. Evaluation of **I** as a cattle growth stimulant is still in progress. This growth-promoting effect is apparently not attributable to an antibacterial effect, since **I** and **II** are inactive against selected microorganisms, in vitro and in vivo. Investigation of the mode of action of **I** is now being conducted, especially with regard to its effect on the endocrine system.

1 United States adopted name (USAN).
2 We thank Dr T.J. Bentley, D.J. France, and Dr L.D. Spicer for synthesis and structural elucidation work, Dr M.W. Bullock and G.W. Cox for metabolism studies, Drs J. Harter, D. Ingle, J.M. Pensack and L. Wozniak and their associates for biological data, and Drs D.J. Thoennes and M.L. Thomson for spectral data and interpretations.
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On the significance of the rostral process of bipolar neurosecretory cells in the caudal neurosecretory system of certain catfishes

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Summary. Neurosecretory substance has been identified in the rostral process of bipolar neurosecretory cells of the caudal neurosecretory system in 4 Indian catfishes, using acid violet stain. The role of the rostral process in the transmission of the neurosecretory substance is discussed.

The occurrence of bipolar neurosecretory cells alongside ordinary (monopolar) neurosecretory cells in the caudal neurosecretory system of the teleosts has been reported in the common carp and the roach[2]. These bipolar cells are situated anterior to the urophysial swelling, and each possesses 2 principal processes – the rostral process directed away from the swelling, and the caudal process directed towards the swelling. The caudal and rostral processes of these cells do not differ from each other in size or morphology, but whereas the caudal process contains neurosecretory substance, the rostral process is devoid of it. It is believed that the caudal process is associated with the transmission of the neurosecretory substance to the urophysial swelling which acts as a neurohaemal organ. The present investigation aims at throwing light on the possible significance of the bipolar neurosecretory cells, with particular reference to the rostral process that distinguishes them.

Materials and methods. The present study was carried out on 4 locally available catfishes, *Clarias batrachus, Heteropneustes fossilis, Rita rita* and *Mystus vittatus.* The fishes were anesthetized with MS222 (Sandoz) and a portion of the caudal region containing about 10–12 vertebrae from the end was removed. The spinal cord, along with the urophysial swelling and the filum terminale was exposed, fixed in situ in aqueous Bouin's fluid for 18 h, and then finally removed. Paraffin sections were cut 6–8 µm thick, and staining was done with acid violet, which is considered specific for neurosecretory material[3].

Results and discussion. In all the 4 catfishes examined, caudal neurosecretory cells are distributed in the spinal cord in the region of the last 3–10 vertebrae, in front of the urophysial swelling. Some of these cells are relatively very large (about 100 µm × 40 µm) and distinctly bipolar, and in these neurosecretory granules, staining with acid violet[3], are discernible not only in the soma and the caudal processes but also in the rostral processes (figures 1–3). It is evident that neurosecretory material produced in the cells passes into the 2 processes. Further, endothelial capillaries are found to make close contact with all parts of the cells viz. the soma, the proximal part of the caudal process, and the rostral process (figure 2). These capillaries arise from blood vessels which supply the posterior part of the spinal cord independently of the blood supply to the urophysial swelling. We are thus led to believe that, unlike those of the

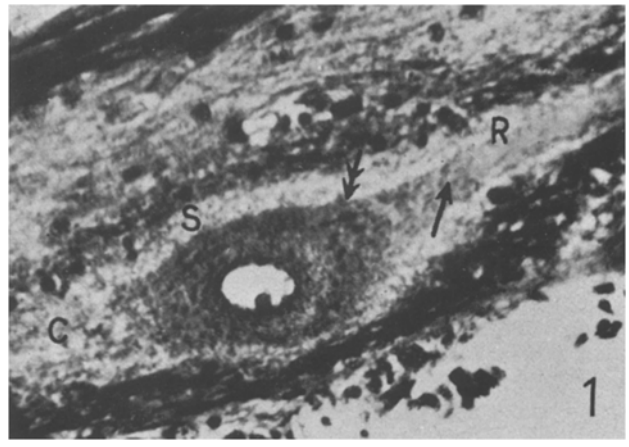

Fig. 1. A typical bipolar cell of *Heteropneustes fossilis* showing the caudal (C) and the rostral (R) process. Note the neurosecretory grains in the soma (double head arrow) and the rostral process (single arrow). × 600.