blockers[18]. It is not clear how physostigmine or d-tubocurarine induced neuromuscular blockade could influence muscle carnosine levels after such a short period of drug treatment. The half life of carnosine in rat skeletal muscle has been reported to be greater than 29 days[19]. Our data does not indicate any change in anserine levels with these treatments. The exact significance of our observations is unclear, and additional experiments are needed to determine if they may support the premise that carnosine may be involved with the maintenance of normal electrochemical coupling in mammalian skeletal muscle.

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H_2 Receptor antagonists and human granulopoiesis[1]

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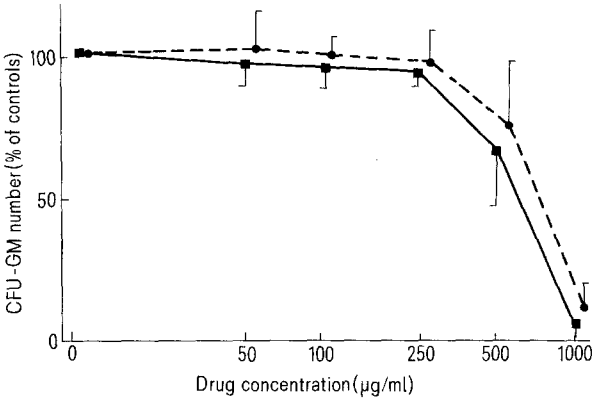
Summary. The effect of 2 H_2 receptor antagonists (ranitidine and cimetidine) on the in vitro growth of human granulomonopoietic precursors (CFU-GM) was studied. Ranitidine, although having an anti H_2 receptor activity much greater than that of cimetidine, displays the same toxicity for CFU-GM.
Key words. Granulopoiesis, human; H_2 receptor antagonists; ranitidine; cimetidine; granulomonopoietic precursors, inhibition of.

A certain degree of hematopoietic toxicity has been observed with the H_2 receptor antagonists used in the treatment of peptic ulcer. Metiamide, indeed, has been responsible for several cases of marrow aplasia and has, therefore, been withdrawn. Its substitute, cimetidine, has a cyanoguanidine group instead of the thiourea group thought to be the cause of this toxicity[2], but has nevertheless been incriminated in numerous cases of neutropenia and some instances of aplasia[3-5]. It has also been regarded as potentiating the myelosuppression induced by cytostatic treatment[6]. Interaction with the H_2 receptors on hemopoietic stem cells has been put forward as the explanation of this marrow toxicity[5]. Both metiamide and cimetidine, in fact, block the entry of resting pluripotent murine stem cells (CFU-S) into the cell cycle. In the human, metiamide blocks the 4-methylhistamine-induced entry of resting precursors of CFU-GM into the cycle[7]. H_2 receptor blocking is also suggested as an explanation for their in vitro inhibition of human granulo-monocyte precursors[8].

To determine whether this inhibition is really dependent on interaction with H_2 receptors we studied the effect of ranitidine and cimetidine on the growth of CFU-GM in vitro. Since the anti H_2 receptor activity of ranitidine is 4–6 times greater than that of cimetidine[9-11], it was expected to prove a proportionally more powerful inhibitor of growth if the inhibition depended solely on receptor block.

Material and methods. Normal CFU-GM cultures were prepared as described elsewhere[12]. Briefly, marrow blood samples from patients subjected to check surgery for non-neoplastic diseases were collected in preservative-free heparin, diluted with isotonic saline, and stratified on lymphoprep (density 1,077, Nyegaard, Oslo, Norway). The light density fraction was collected and washed 3 times. One $\times 10^5$ cells, resuspended in McCoy's 5A medium (supplemented with essential and non-essential amino acids, sodium pyruvate and glutamine) +15% fetal calf serum were seeded on 0.3% agar gel in 35 mm Petri dishes. Increasing concentrations of ranitidine (Zantac, Glaxo, Verona, Italy) or cimetidine (Tagamet, Smith, French & Kline, Milan, Italy) were added to these cultures. Colony growth was stimulated with 10% GCT condi-



Effect of increasing concentrations of cimetidine ■——■ and ranitidine ●- - -● on in vitro growth of normal CFU-GM (9 samples). Values are expressed as percentages of control colony number. Results are means ± SD of these percentages. Colony number in control dishes/10^5 light density cells: 82–198.

tioned medium (Gibco, Grand Island, New York, USA). After 8 days of incubation at 37 °C in a humidified atmosphere at 5% CO, 3 dishes per point were scored and all aggregates of more than 40 cells were counted as colonies.

Results and discussion. The results are shown in the figure. At concentrations of up to 250 µg/ml neither drug altered CFU-GM proliferation. Significant growth inhibition appeared at 500 µg/ml, and virtually no colonies were counted at 1000 µg/ml. 50% inhibition of growth was present at a concentration of about 600 µg/ml of cimetidine and of about 800 µg/ml of ranitidine.

The mechanism of this inhibition pattern is not clear. The blocking of H_2 receptors on stem cells seems very unlikely since ranitidine would have inhibited colony formation at relatively low concentrations if this were the case. In fact both drugs displayed the same inhibition curve. It must therefore be supposed that other mechanisms are responsible for the toxicity of both compounds for CFU-GM. On the other hand it is by no means clear whether our data mean that the risk of myelo-suppression is reduced by using ranitidine, which is clinically effective at lower blood levels and presumably bone marrow microenvironment levels than cimetidine. In fact 50% inhibition of gastric secretion in man is obtained at a plasma concentration of 0.1 µg/ml of ranitidine and at a concentration of 0.5 µg/ml of cimetidine[13-15]. Quite apart from the obvious difficulty of translating in vitro into in vivo data, inhibition was, in effect, only observed at drug concentrations higher than those seen therapeutically. Therefore, although, as far as we know, no cases of myelosuppression have as yet been reported with ranitidine, it would seem to be too early to declare it non-toxic.

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Influence of phenobarbital and TCDD on the hepatic metabolism of TCDD in the dog

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Summary. The influence of phenobarbital and TCDD pretreatment on the formation and biliary excretion of TCDD-metabolites following single doses of ^{3}H-TCDD was investigated. Without pretreatment, 24.5% of the absorbed ^{3}H-TCDD dose was excreted in the bile within 110 h. Phenobarbital did not influence this rate, whereas a single dose of 10 µg of unlabeled TCDD/kg b.wt nine days earlier resulted in a doubling of the amount of radioactive material eliminated in the bile (47.4%).
Key words. TCDD; metabolism; biliary excretion; phenobarbital; stimulation.

The pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats, guinea pigs and hamsters have been reported[1-4]. Depending on the species, the eliminiation half life of this compound was reported to vary between two and about four weeks. Feces contained most of the material excreted, some of which probably arose from hepato-biliary excretion. Bile of rats and hamsters has been shown to contain metabolites of TCDD[3,5,6]. Recently we reported that biliary excretion of TCDD-metabolites in the dog was considerable and identified the structure of some of the biotransformation products[7]. Preliminary observations suggested that TCDD increases its own hepatic metabolism and excretion, but the experimental design of the earlier study, in which large doses of TCDD were given repeatedly, was not suitable for studying these effects. In addition, data on the intestinal absorption and excretion in urine in the dog were lacking. Therefore this work was carried out under well-defined conditions of pretreatment and using low doses of the dioxin, which allowed a determination of the radioactive material in the excreta without excessive risk in handling and measuring samples.

Materials and methods. Chemicals. ^{3}H-labeled TCDD (sp. act. 40 Ci/mmole; source A. Kende, Rochester, NY) was purified by preparative gas chromatography (2 m × 2 mm i.d. all glass column, DC 560, 230 °C), resulting in a radiochemical purity higher than 98% (checked by GC). For dosage it was dissolved in corn oil, yielding solutions containing 0.75 or 0.82 µg of ^{3}H-TCDD/ml, as calculated from radioactivity measurements. Unlabelled TCDD (Dow Chemical, Midland, MI) was used without further purification. It was dissolved in benzene (0.25 mg/ml). Prior to administration 1 ml of this solution was mixed with 5 ml of corn oil and the benzene evaporated by bubbling a stream of nitrogen through the solution.

Animal experiments. A male Boxer dog underwent cholecystectomy with implantation of a Thomas cannula about 18 months before these experiments (age at the time of the surgery was approximately 1 year). In the meantime the dog