58% increase in *Tm* was observed without a similar change in *S.* Thereafter, a decrease in *Tm* to nearly original levels was observed. Administration of growth hormone from another species (ovine) again produced a rise in *Tm* in at least one of the same animals. Evidence suggests that growth hormone exerts a regulatory influence on hepatic excretory function.

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Mitomalcin: A New Proteinaceous Antileukemic Antibiotic Produced by *Streptomyces malayensis* (nov. sp.)* (33750)

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We wish to report the isolation of a streptomycete from Malayan soil capable of producing a fermentation metabolite which exhibits highly significant activity against murine lymphocytic Leukemia L-1210 (1). This culture, classified as a morphologically unique streptomycete, is given the name *Streptomyces malayensis.*

A highly stable variant,[1] isolated from a glucose (1%), yeast extract (1%), K_2HPO_4 (0.05%) agar medium, and possessing light mouse gray aerial hyphae,[2] consistently produces fermentation broths exhibiting significant activity against Leukemia L-1210.

The active principle (NSC-113233) has been given the name mitomalcin (2).

The fermentation is most effectively carried out by first preparing a preform inoculum incubated for 24 hr at 28° on a reciprocating shaker.[3] Broth potency is optimized on

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[1] The parent culture, upon repeated slant transfer, loses its ability to produce the active antileukemic principle necessitating extensive microbiological examination of its susceptibility to natural variation.

[2] Electron microscopy reveals the unusual presence of hair-like projections on the spores.

[3] A high preform mycelium volume is generated when a medium consisting of 2% cornstarch and soybean meal, 0.5% yeast extract, 0.005% $MnCl_2$, $CuSO_4$, and $ZnSO_4$, 0.2% $CaCO_3$, and 0.25% NaCl is used.

24-hr fermentation in 0.5% cerelose, 0.1% NZ amine YTT, beef extract and KCl, 0.5% peptone and $CaCO_3$, and 0.4% glycerol to which is added a 2% inoculum concentration of the matured preform. If the fermentation is monitored as a function of time, one observes, on periodic aliquot removal, that maximum antibacterial[4] and antileukemic activity occurs simultaneously between 24–28 hr after inoculation and declines steadily thereafter. The active principle is removed from the culture filtrate by adsorption onto DEAE-cellu'ose followed by elution with a 4% NaCl solution, dialysis and decolorization over Dowex-1X4 (phosphate form) resin from which emerges,[4] on lyophilization, a white amorphous antibiotic active on L-1210 at 10 mg/kg (T/C 210%).[5] Turbidimetric assay employing *Staphylococcus aureus* no. 5 indicates that the sample possesses, at this point, 20% of the achievable potency.

Final purification is achieved by column chromatographic techniques utilizing DEAE Sephadex A-50 and Sephadex G-100, the separation in both instances being monitored by ultraviolet absorption (250 and 280 mμ) and antimicrobial activity.[4] The product obtained is a white, pyrogen-free, readily water soluble, nondialyzable, acidic,[6] protein,[7] readily denatured by organic solvents and on extended exposure to aqueous systems. It is indefinitely stable when maintained as a lyophil and achieves optimum therapeutic efficacy when administered freshly reconstituted in isotonic saline.

Mitomalcin exhibits an ultraviolet spectrum similar to many proteins, viz., λ_{min} 260; λ_{max} 276; λ (sh.) 290; λ (brd. sh.) 325–330 mμ. A small amount of carbohydrate (5%) was detected by the standard indole method (3) but it is noteworthy that a single symmetrical peak was observed on ultracentrifugation sedimentation analysis. A molecular weight of 17,400 was calculated from gel chromatography data (4).

Analysis[8] for the more commonly occurring amino acids demonstrates the presence of aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, leucine, isoleucine, tyrosine, phenylalanine, lysine, histidine, and arginine. We are currently pursuing degradation studies with the objective of establishing whether a structural subunit is responsible for the antileukemic effect of the drug.

Biological activity. Whole broth ultrafiltrates are found to be highly active against L-1210, exhibit weak *in vitro* antibacterial activity, and cytotoxicity. The final product, obtained pyrogen-free from G-100 Sephadex, produced significantly increased extensions of survival time on both Leukemias L-1210 and P-388.

Evaluation of this product in mice implanted with L-1210 demonstrated that daily intraperitoneal dosage from days 1–9 following implantation produces a maximum tolerated dose of 1.5 mg/kg, a maximum effectiveness of 276% of control survival time at 0.5 mg/kg and a minimum effective dose of 0.03 mg/kg.

Dosage regimen studies disclose equal efficacy (T/C 280–325%) when the compound is administered intraperitoneally on day 1 only; days 1–5; days 1 and 9 only; days 1, 5, 9; and when injected in divided doses given every 3 hr on day 1 only: days 1 and 9 only; and on days 1, 5, and 9.

The results of standard serial dilution tests (5) show minimum inhibitory concentrations against common gram-positive microorganisms in the range of 0.39–6.25 μg/ml. No activity is evident against gram-negative species.

The results of cell culture tests carried out according to the method of Toplin (6) reveal cytotoxicity for HeLa cells at a concentration of 0.17 μg/ml and a lethal effect at a concentration of 1.7 μg/ml. In contrast, the results obtained against a normal mouse embryo cell line reveal cytotoxicity at 5 μg/ml and a lethal effect at 13 μg/ml.

[4] Determined by agar plate assay with *Bacillus subtilis* (ATCC 6633).

[5] T/C = (mean survival time treated animals/mean survival time control animals) × 100.

[6] Electrophoresis isoelectric point, 3.4.

[7] Approximately 95% as determined by the biuret assay method.

[8] Measured on a Technicon AutoAnalyzer.

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The value of Leukemia L-1210 in the detection of chemotherapeutic agents which may be effective against clinical neoplasia was recently established (7). Mitomalcin is currently undergoing preclinical pharmacologic evaluation in our laboratories.

Summary. A culture variant of *S. malayensis* elaborates a metabolite (mitomalcin) which displays striking activity against Leukemias L-1210 and P-388 when administered intraperitoneally in single, daily, and interrupted dosage schedules. The purified protein is active *in vitro* against common gram-positive microorganisms and is approximately 30-fold more cytotoxic to the HeLa cell than to a normal mouse embryo cell line.

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Iron and Copper Effects on Serum Ceruloplasmin Activity of Rats with Zinc-Induced Copper Deficiency* (33751)

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In the present study two experiments were conducted to further determine the effects of high zinc diets on iron and copper metabolism in the rat. Previous studies (1) showed no interference by 0.75% zinc in the diet on ferritin synthesis but an impairment of iron incorporation into or release from ferritin. Further studies into this toxicity revealed a rapid depletion of ceruloplasmin activity (2). The copper-containing protein ceruloplasmin of serum may have a biological role in promoting the rate of iron saturation of transferrin as well as in stimulating iron utilization by acting as a ferroxidase (3, 4). The present report concerns the interrelationship between serum ceruloplasmin activity (CPA) and iron metabolism as affected by a high level of zinc in the diet.

Materials and Methods. Weanling male Dublin-Sprague derived rats (Dublin Laboratories, Dublin, Va.) averaging 50–60 g were used. In Expt. I prior to the pre-period, 30 weanling rats were divided into 2 groups of 15 each. One group was fed the control diet, while the other group was fed the diet containing 0.75% zinc (Table I). Starting at the second week of the pre-period, blood was taken weekly from the tail by nicking the end with a razor and analyzed for CPA, serum copper, and hemoglobin. At the end of 5 weeks 8 of the control animals and 8 of the zinc-fed animals were killed. The livers were excised and analyzed for iron and copper. Six of the 7 remaining animals from each group were divided into 2 trios. One of the trios was administered saline and the other 100 μg of Cu^{2+} as $CuSO_4 \cdot 5H_2O$ intraperitoneally (i.p.) in 1-ml volume. The 2 trios in the zinc-fed group were treated in a similar manner.

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