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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Received Sept. 11, 1968. P.S.E.B.M., 1969, Vol. 130.

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.

* Contribution from the Department of Biochemistry, School of Agriculture and Life Sciences, and School of Physical Sciences and Applied Mathematics. Paper No. 2700 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina. Supported in part by a grant from the Herman Frasch Foundation and Public Health Service Research Grant No. AM-13055-01 from the National Institute of Arthritis and Metabolic Diseases.

TABLE I. Composition of Diets.

Dietary ingredients	Control (%)	0.75% zinc (%)
Casein	19	19
Corn starch	63	61.6
Vegetable fat[a]	10	10
Mineral mix[b]	4	4
Cellulose	2	2
Vitamin mix[c]	2	2
Choline chloride	0.1	0.1
Zinc carbonate	—	1.4

[a] "Wesson Oil," Hunt-Wesson Foods, Fullerton, Calif.

[b] Salt Mixture W, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[c] Matrone *et al.*, J. Nutr. **86**, 154 (1965).

In the second experiment 36 weanling rats were divided into 2 groups of 18 each. One was fed the control diet and the other the 0.75% zinc diet. In this experiment the pre-period extended over 2 weeks and the treatment period over 96 hr. During the pre-period the blood of all animals was monitored for serum CPA and level of hemoglobin. At the end of the pre-period the 18 rats in the control group were divided into 6 trios, and the zinc-fed rats were divided up in a similar manner. Trios from the 6 control groups were assigned at random to the following treatments administered i.p.: saline control, 100 μg of Cu^{2+}, 400 μg of Fe^{2+} or Fe^{3+}, 100 μg of Cu^{2+} + 400 μg of Fe^{2+} or Fe^{3+}. The ferrous iron was administered as ferrous ammonium sulfate in 0.014 *M* citric acid and the ferric iron as ferric ammonium citrate and copper as $CuSO_4 \cdot 5H_2O$. The same procedure was followed for the 6 trios in the zinc-fed group.

In both experiments during the pre-period as well as the treatment period the animals were fed and watered *ad libitum*. The animals, which were individually caged, were weighed weekly.

Hemoglobin values were determined by the method of Shenk *et al.* (5); ceruloplasmin activity was determined by the procedure of Houchin (6) as modified by Rice (7); and copper and iron concentration of wet-ashed tissues were determined by direct reading in a model 303 atomic absorption spectrophotometer (Perkin-Elmer Corporation, Norwalk, Conn.).

Results. Expt. I. As shown in Table II animals fed the high zinc diet have lower liver copper, liver iron, and serum copper levels. The most marked difference was found in serum copper. The average serum copper for the control animals was 97 μg/100 ml whereas for the zinc-fed animals it was reduced to 2 μg/100 ml. The ceruloplasmin and hemoglobin values are presented in Table III. At the end of 5 weeks the CPA of the zinc-fed animals had dropped to 0, whereas those of the controls show CPA of 22 IU. In the treatment period of 48 hr the control rats administered either saline or copper showed a significant and similar rise from around 23–24 IU to 38–39 IU. For the zinc-fed animals the saline-treated group showed no change in CPA, remaining at the 0 level. In contrast the zinc-fed rats administered copper showed a highly significant rise from 0 to 15 IU. As shown in Table III during the pre-period the controls showed a rise in hemoglobin while zinc-fed rats showed a decrease. Although the hemoglobin values of the zinc-fed animals were much lower than the control animals in the treatment period, no changes were observed in either group attributable to saline or copper.

Expt. II. Two findings appeared clear from Expt. I: First, the rapid decline of CPA, and second, the ability of i.p. administered copper to partially restore the CPA in serum of these animals. The objective of the second experiment was to delineate these two effects more precisely. A second objective was to determine the effect of iron on the regeneration of CPA. A corollary interest, particular-

TABLE II. Effect of 0.75% Zinc Intake on Tissue Iron and Copper Levels in Pre-period of Expt. I.[a]

	(μg/g of dry tissue)		Serum copper[b]
	Liver copper[b]	Liver iron[b]	(μg/100 ml)
Control	14	186	97
Zinc, 0.75%	3	87	2

[a] Each value is a mean of 8 animals.

[b] Difference between control and zinc-fed rats is significant at $p \leq 0.005$.

TABLE III. Ceruloplasmin and Hemoglobin Values of Rats in Expt. I.

Criteria	Pre-period No. of rats	2 weeks	5 weeks	Treatment period i.p. inj.	No. of rats	0 hr	48 hr
Ceruloplasmin activity (IU)							
Controls	15	17.6	22.1	Saline	3	23.2	38.4
				100 μg Cu^{2+}	3	24.6	38.2
Zinc-fed	15	2.5	0	Saline	3	0	0
				100 μg Cu^{2+}	3	0	15.1
Hemoglobin (g/100 ml)							
Controls	15	9.7	13.0	Saline	3	10.7	9.9
				100 μg Cu^{2+}	3	11.5	10.9
Zinc-fed	15	7.3	3.1	Saline	3	2.4	2.2
				100 μg Cu^{2+}	3	2.2	2.3

ly in view of the work reported by Osaki and co-workers (4) of the ferroxidase activity of ceruloplasmin, was to determine whether or not the valency of the iron might influence the results.

The effects of i.p. injection of ferrous and ferric iron were not significantly different. Therefore, the results were combined and are shown in Fig. 1. As presented in Fig. 1A, control rats administered saline maintained a relatively constant level of CPA of approximately 27 units both in the pre- and treatment periods. The zinc-fed rats showed a precipitious drop in CPA within 1 week which remained at this low level throughout the experiment. As shown in Fig. 1B, the control rats injected with the combination of iron and copper showed during the treatment period a highly significant ($p \leq 0.01$) rise in CPA which was greater ($p \leq 0.01$) than copper administered alone or iron administered alone. In fact copper administered alone effected no change in the CPA of these rats. For the zinc-fed rats either copper alone or the combination of copper and iron resulted in a highly significant ($p \leq 0.01$) rise in CPA over that of the group administered iron alone. Further analysis of the data showed a highly significant difference between the group administered copper alone and those administered copper and iron as a combination during the last 2 sampling periods (48–96 hr). During this period the CPA was higher for the group receiving the combination of iron and copper.

The hemoglobin values are shown in Table IV. In general there was a similar decline in hemoglobin values during the 96-hr treatment period for all experimental groups. This was attributed to the loss of blood due to frequent sampling.

Discussion. The results with the zinc-fed rats in Expt. II, showing the decrease in CPA and the subsequent increase in CPA after

TABLE IV. Hemoglobin Values (g/100 ml) of Rats in Expt. II.

	Pre-period No. of rats	0 week	1 week	2 weeks	Treatment period i.p. inj.	No. of rats	0 hr	24 hr	48 hr	96 hr
Control rats	18	10.4	12.2	11.6	Cu	3	11.8	9.9	9.5	9.4
					Fe	6	11.3	9.9	8.2	9.2
					Cu + Fe	6	11.6	10.0	8.5	9.2
Zn-fed rats	18	10.8	10.3	7.4	Cu	3	8.1	6.6	5.6	4.5
					Fe	6	7.4	6.4	5.4	4.6
					Cu + Fe	6	6.7	6.2	5.1	4.9

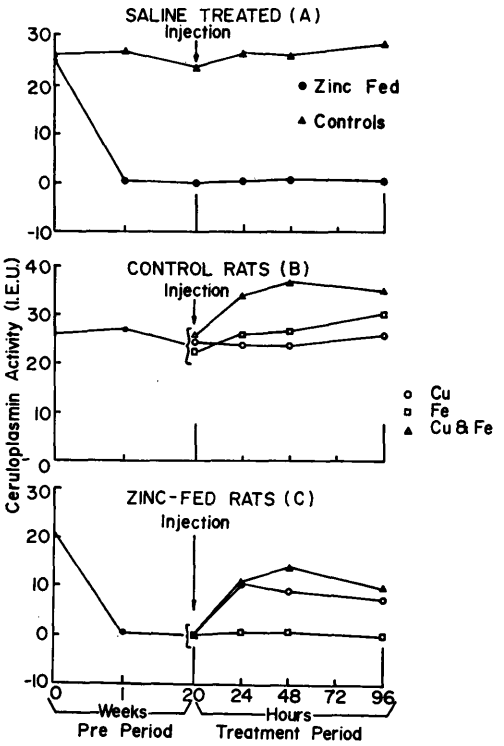

FIG. 1. Ceruloplasmin activity values of rats in Expt. II.

the administration of copper, substantiate the results found in Expt. I and confirm those reported by Minato and Ogiso (8). In addition, results of Expt. II indicate a copper—iron interaction on the level of CPA of both control animals and zinc-fed animals. Since only enzyme activity was measured, it cannot be determined from these experiments whether or not the increase in activity brought about by combining copper and iron was due to a stimulatory effect or due to *de novo* synthesis of ceruloplasmin.

That *de novo* synthesis is not necessarily involved is supported by reports in the literature (3, 5). Osaki (3) showed in an *in vitro* system that without iron *p*-phenylenediamine dihydrochloride (PPD) is directly oxidized by the enzyme-bound copper. However, with the addition of Fe^{2+} the rate of the reaction was increased showing a stimulatory effect of the iron. Curzon and O'Reilly (9) also using an *in vitro* system reported studies on the coupled iron–ceruloplasmin oxidation system. They concluded that in addition to the direct oxidation of the PPD by ceruloplasmin there was a coupled iron–ceruloplasmin *n,n*-dimethyl-*p*-phenylenediamine (DPD) oxidation system which gives rise to an apparent activation of ceruloplasmin by iron. The results reported in this study suggest that the stimulatory effect of iron may be manifested under *in vivo* conditions as well. As shown in Fig. 1B when i.p. iron is administered to control animals, the rise of CPA obtained was greater than when copper alone was administered—although the difference is not statistically significant. The greatest rise in CPA was obtained when iron and copper were given simultaneously. The data in Fig. 1B suggest that the rise in CPA of the group administered iron alone over those administered copper alone may reflect a stimulatory effect of iron on CPA and that the rise in ceruloplasmin by the group administered iron and copper may be made up of one component attributable to the stimulatory effect of iron and anther component involving a factor other than the simple iron effect.

Summary. The results of the two experiments conducted to determine the effects of high zinc diets on CPA were as follows: The CPA dropped precipitously in approximately 1 week. The i.p. administration of 100 μg of copper induced a significant rise in CPA. The administration of iron and copper brought about a significant rise in CPA over and above that when copper alone was administered in both control and zinc-fed groups. Copper alone had no effect on CPA of the control rats while iron alone had no effect on CPA level of the zinc-fed rats. However, iron alone increased the CPA of the control rats.

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Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

Effects of Pargyline and Amphetamine upon Acute Stress Responses in Rats*† (33752)

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A number of independent studies (1–3) have shown that in rats a single dose of reserpine or chlorpromazine can cause a persistent hypersecretion of ACTH. This pituitary–adrenal hyperactivity is reflected by elevated plasma and adrenal corticosterone levels, depletion of pituitary ATCH and depletion of corticotropin-releasing factor (CRF) in the median eminence of the ventral hypothalamus. In order to understand the neuropharmacological basis of such endocrine responses to reserpine and chlorpromazine one may ask the question if there is any relationship between changes in the functional activity of biogenic amines in hypothalamus after these drugs and the hypersecretion of ACTH. Shore and Brodie (4) first showed that pretreatment of animals with a monoamine oxidase (MAO) inhibitor counteracts the central effects of reserpine and protects the released amines from metabolic biotransformation. The present paper describes the effect of pretreatment of rats with the MAO inhibitor pargyline or with amphetamine on the ACTH releasing property of reserpine or acute stress.

Materials and Methods. Adult female Wistar rats (150–175 g) were used in all experiments. The animals were fed commercial rat chow and water *ad libitum*. The rats were housed in a constant temperature and humidity room (Labline Inc., Chicago, Illinois) for at least 3 days before use and during this period they were adapted to the stress of handling and injection by giving saline (0.1 ml/100 g of body wt.) intraperitoneally for 3 consecutive days.

The doses of drugs are expressed as their salts unless otherwise specified. Pargyline hydrochloride (Eutonyl, Abbott Laboratories) was administered intraperitoneally in a dose of 50 mg/kg in saline. Amphetamine sulfate (Smith, Kline and French) was administered intraperitoneally in a dose of 5 mg/kg in saline. Control rats were given saline (0.2 ml/100 g of body wt.) by the same route. Drug-treated and control rats were sacrificed 24 hr after pargyline or 4 hr after amphetamine administration. The experiments were designed so as to sacrifice the animals between noon and 1:00 p.m. in all experiments.

Stress. The animals were subjected to an acute ether-laparotomy stress. The rats were exposed to ether vapor for 1 min by the end of which time they were completely anesthetized. The animals were then quickly subjected to laparotomy by making an incision of about 5 cm in the lumbrosacral region on one side of the animal at the adrenal region. This procedure required approximately 12–15 sec. The animals were sacrificed by decapitation 15 min from the onset of the stress. Mixed trunk blood was collected in heparinized tubes. The plasma and adrenal corticos-

* Supported by a grant from the National Institutes of Health AM-05110.

† Presented in part at the Am. Soc. Pharmacol. Exptl. Therap. meeting (Fall) 1967 in Washington, D. C.

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