tumor cells. In Ehrlich carcinoma cells the antibiotic isomer inhibited the incorporation of glycine-2-C^{14} into protein and nucleic acid purines. The other isomer inhibited incorporation into the purines to the same extent, but did not appreciably affect entry into proteins. In lymphosarcoma cells (6C3HED), both isomers were equally effective in inhibiting the incorporation into proteins and purines. Incorporation into guanine was inhibited much more than into adenine. It is suggested that this might indicate tumor inhibiting properties. The levels of the antibiotic necessary to give significant inhibition are much higher than are required to affect bacteria.

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Some Interrelations of Dietary Protein, Molybdenum, Riboflavin and Calories on Liver and Intestinal Xanthine Oxidase.* (20475)

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Previous studies(1-3) have described the dependence of liver and intestinal xanthine oxidase upon the dietary intake of protein, the xanthine oxidase factor, riboflavin(4) and calories(2). The additional studies herein reported on the interrelationship of the various factors were made possible by the identification of the xanthine oxidase factor as the trace element molybdenum(5,6). The following questions were studied. 1) How much does a "pure" deficiency of riboflavin and/or molybdenum affect the concentration of xanthine oxidase in the liver and intestine, and does a specific deficiency of either factor alter the oxidase:dehydrogenase ratio of the enzyme? 2) Is the loss of xanthine oxidase from these tissues during starvation due to an inadequate protein intake, the caloric restriction, or a combination of such factors? 3) Does the molybdenum content of the diet influence the protein effect on tissue xanthine oxidase? The latter question has a bearing on the use of liver xanthine oxidase concentrations as a criterion of the dietary value of a protein(7).

Experimental. Riboflavin, Mo and calories. Weanling male albino rats were divided into 4 groups and fed purified diets which were identical in composition with the one previously described(1), except that the diets contained 24% Labco casein and 65% glucose. The diets were varied by adding or omitting molybdenum and riboflavin, as shown with the results. When present, riboflavin and Mo (as Na_2MoO_4) were added to the diet at 4 and 1 mg per kg, respectively. These diets were fed *ad libitum*, and the daily food consumption of the rats consuming the control diet No. 4, containing riboflavin and Mo, was measured. A fifth group of rats was fed the same diet but was given daily only 23% of the total food intake eaten by the control group. A sixth group of rats was fed a diet containing 96% casein, 4% salts, the vitamin supplement and 1 mg Mo per kg diet, but these rats received daily only 25% of the amount of food eaten by the control group. These intakes were established so that Group 6 would receive the same daily caloric intake as Group 5 and the same daily protein intake as the control Group 4. After feeding the diets for 2 to 3 weeks the livers and intestines were analyzed for xanthine oxidase in the presence and absence of methylene blue by the methods previously described(2). The results are shown in Table I.

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TABLE I. Effect of Variations in Dietary Riboflavin and Molybdenum on Liver and Intestinal Xanthine Oxidase in Weanling Rats.

No.	Diet B_2	Diet Mo	Days on diet	Body wt Start	Body wt End	No. of rats	Liver XO† —	Liver XO† +MB	Intestinal XO† —	Intestinal XO† +MB
1	+	—	17	50	134	8	24 ± 2.4	38 ± 4.1	3 ± 1.0	12 ± 2.7
2	—	+	14	51	67	7	26 ± 3.0	42 ± 3.8	21 ± 2.7	40 ± 3.1
3	—	—	14	47	57	6	28 ± 2.6	42 ± 2.9	3 ± 1.7	15 ± 2.8
4	+	+	17	51	104	8	27 ± 2.9	47 ± 3.8	39 ± 3.2	58 ± 4.6
6*	+	+	13	52	39	3	25	39	39	64

* A 96% casein diet fed at 25% of level of diet 4 eaten *ad libitum*. Diets 1-4 contained 24% casein.

† Liver and intestinal xanthine oxidase values, determined in presence and absence of methylene blue (MB), have been recorded as net cmm O_2 consumed per 20 min. per Warburg flask containing 283 mg fresh tissue; values are the mean ± stand. error of the mean.

TABLE II. Lack of Effect of Partial Caloric Restriction, with and without a Simultaneous Reduction in Protein Intake, on Rat Liver and Intestinal Xanthine Oxidase.

No.	Diet: Casein content, %	Diet: Amt fed, %*	Days fed	Body wt, g: Start	Body wt, g: End	No. of rats	Liver XO† —	Liver XO† +MB	Intestine XO† —	Intestine XO† +MB
4	24	100	28	120	239	13	26±1.8	47±2.5	32±1.8	48±2.5
5	24	23	22	119	85	13	31±4.2	46±5.2	35±3.5	56±3.8
6	96	25	20	125	90	10	26±2.2	44±3.7	37±2.4	57±3.9

* *Ad libitum* consumption of 24% casein diet was taken as 100% (Group 4), and Groups 5 and 6 were fed 23% and 25% respectively of this amount daily.

† Liver and intestinal xanthine oxidase values, determined in presence and absence of methylene blue (MB), have been recorded as net cmm O_2 consumed per 20 min. per Warburg flask containing 283 mg fresh tissue; values are the mean ± stand. error of the mean.

Xanthine oxidase was removed extensively from the intestine on molybdenum deficient diets whether riboflavin was present or not. When the diet contained both of these constituents, saturation levels of the intestinal enzyme were obtained, but only half as much enzyme was present when flavin was omitted. Calculation(8,9) of the oxidase:dehydrogenase ratio of the liver or intestinal enzyme showed that by this criterion the composition of the enzyme was unchanged by dietary variations in riboflavin and molybdenum. In this experiment a deficiency of riboflavin and/or molybdenum had no effect on the concentration or oxidase:dehydrogenase ratio of liver xanthine oxidase. Additional rats that had been on these diets for 4 to 5 weeks had intestinal xanthine oxidase values which were essentially identical with those shown in Table I.

Since all of the rats in Group 5 and most of the rats in Group 6 died as a result of the caloric restriction within the first 2 weeks, this portion of the experiment was repeated with older rats. The daily food consumption of Group 4, which was fed the purified 24% casein diet containing riboflavin and Mo, was measured, and 23% of this amount was fed to Group 5; 25% of this amount of the 96% casein diet was fed to Group 6. The results are shown in Table II.

Both of the groups receiving the restricted food intake did not lose xanthine oxidase from either the liver or the intestine. Complete starvation caused a loss of this enzyme from both tissues(2) but a reduction in calories to ¼ the usual food intake had no effect. This restriction of the 24% casein diet reduced the absolute daily protein intake to less than that provided by the *ad libitum* feeding of an 8% casein diet; the latter caused a marked and almost complete loss of this enzyme from both the liver and intestine(1), but when the calories were restricted simultaneously, the tissue concentration of xanthine oxidase was unaffected. An obvious difference exists between the effect of protein restriction in the presence of adequate calories and the simultaneous restriction of both protein and calories.

TABLE III. Effect of Variations in Dietary Molybdenum and Protein on Liver and Intestinal Xanthine Oxidase.

Dietary casein, %		35		24		18		8		0	
Dietary Mo, γ/kg		40	1000	40	1000	40	1000	40	1000	40	1000
Avg days on diet		21	23	23	23	19	22	23	23	13	13
Final body wt, g*		107	110	107	104	97	109	53	56	32	31
No. rats analyzed		11	14	14	12	9	11	12	12	7	7
Xanthine oxidase†	Liver	27±2.6	28±1.8	21±2.0	19±2.0	15±2.7	15±3.6	3±1.2	4±1.8	9±2.7	5±3.9
	Intestine	24±2.2	40±2.2	25±2.1	30±2.5	18±4.9	27±4.5	12±1.9	14±2.1	13±1.5	19±3.4

* Starting body wt averaged 37-39 g for all groups.

† Liver and intestine xanthine oxidase activities have been recorded as net cmm O_2 consumed per 20 min. per Warburg flask containing 283 mg fresh tissue; values are mean ± stand. error of the mean.

Protein and Mo. Weanling male rats were fed diets varying in protein content from 0 to 35% casein; each such diet contained 4 mg riboflavin and either 40 γ or 1000 γ Mo (as Na_2MoO_4) per kg. The diets were otherwise identical with the one previously described; variations in the casein content were made with corresponding adjustments of the glucose concentration. After being on the diets for 2 to 4 weeks, the rats were sacrificed and analyzed for liver and intestinal xanthine oxidase. The results are shown in Table III.

The dominant role of dietary protein in controlling the level of liver xanthine oxidase was again evident. Diets containing either 1 mg or 0.04 mg Mo per kg gave the same level of liver enzyme at each protein concentration. Hence large increases in the molybdenum content of the diet did not alter the response of liver xanthine oxidase to dietary protein. Low protein diets prevented the intestinal xanthine oxidase from responding normally to either 40 or 1000 γ of Mo/kg, but much higher levels of intestinal enzyme were obtained on a protein-free-Mo-containing diet than on an adequate protein intake without molybdenum. Hence the molybdenum content of the diet tended to dominate the control of the level of intestinal xanthine oxidase, just as protein dominated the liver enzyme. Since a dietary concentration of 40 γ Mo/kg was not enough to produce saturation levels of intestinal xanthine oxidase (*i.e.*, above 30), the diets containing adequate protein and 1000 γ Mo/kg all gave higher levels of this enzyme in the intestine. Large amounts of dietary molybdenum did not give saturation levels of intestinal xanthine oxidase in the absence of an adequate protein intake.

Summary. The omission of riboflavin and/or molybdenum from a 24% casein diet fed to weanling rats had no effect on liver xanthine oxidase within 2 weeks; a molybdenum deficiency in the presence or absence of riboflavin removed xanthine oxidase from the intestine almost completely, while a riboflavin deficiency in the presence of molybdenum gave levels of intestinal enzyme which were about one-half the saturation levels achieved by a diet containing both molybdenum and riboflavin. The oxidase:dehydrogenase activity of the enzyme present in the liver or intestine was not altered by a riboflavin or molybdenum deficiency. Restriction of the daily food intake to 23% of the *ad libitum* intake of a 24% casein diet or 25% of a 96% casein diet had no effect on liver or intestinal xanthine oxidase. The level of liver xanthine oxidase depended primarily on the protein content of the diet, and such values were the same whether the diet contained 0.04 or 1 mg of molybdenum per kg. Intestinal xanthine oxidase levels were determined primarily by the molybdenum content of the diet, but low protein diets allowed only intermediate levels of this enzyme to be achieved even in the presence of a large excess of molybdenum.

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Biological Half-Life of Endogenous ACTH.* (20476)

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The relatively high concentration of adrenocorticotrophic hormone (ACTH) in the blood of the adrenalectomized rat(1,2) makes it possible to determine the biological half-life of endogenous hormone in this animal.

Methods. Male rats from the Holtzman-Rolfsmeyer farm weighing 200 to 300 g were acclimatized for 2 weeks in the laboratory (constant lighting and constant temperature, 76 ± 2°F) before use in the experiments.

Two separate experiments were performed to determine the rate of disappearance of ACTH from the blood of adrenalectomized rats following hypophysectomy. One week (Exp. 1) or 2 weeks (Exp. 2) after bilateral adrenalectomy,† the animals were anesthetized with ether and either sham-hypophysectomized (pituitary exposed, but not removed) or hypophysectomized. The sham-hypophysectomized animals served as zero-time controls. All rats were decapitated‡ and blood was drained from the trunk into 4 volumes of glacial acetic acid. Blood samples were collected exactly one minute after sham-hypophysectomy, and 1, 2, or 3 minutes after hypophysectomy. The blood-glacial acetic acid mixtures from individual rats of each group were pooled and processed by the oxycellulose§ method(3).

Exp. 3 was designed to determine if the rapid disappearance of ACTH from the blood of adrenalectomized rats one minute after hypophysectomy could be attributed to inactivation in blood itself. Two weeks after bilateral adrenalectomy, 76 rats were exposed to ether for 2 minutes, then bled from the abdominal aorta for a period of one minute. One-third of each blood sample was added immediately to 4 volumes of glacial acetic acid; the remaining two-thirds were quickly transferred to a centrifuge tube, incubated for one minute at 37°C, and then immediately added to 4 volumes of glacial acetic acid. The non-incubated (control) and the incubated blood-glacial acetic acid mixtures were pooled separately and processed by the oxycellulose method.

The pH of the HCl-eluate of each pool of processed blood was adjusted to 4 immediately before assay for ACTH in male hypophysectomized rats by the adrenal ascorbic acid-depletion method(4). The estimate of the concentration of ACTH in blood is based on the assumption that the oxycellulose method recovers 100% of the hormone. Studies on ACTH added to hypophysectomized rat blood indicate that 96 ± 6% of the hormone is recovered(5).

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† Adrenalectomized animals were given 0.9% sodium chloride solution to drink.

‡ Decapitated in order to prevent the adenohypophysis of the sham-hypophysectomized rat from contributing to blood ACTH during bleeding.

§ Samples of oxidized cellulose (10-12% COOH) were generously supplied by the Research Laboratories, Tennessee Eastman Co.