pigs with severe ascorbic acid deficiency were made. 2. Plasma concentrations of these steroids were found to be approximately 10 times those found in normal guinea pigs despite the virtual absence of adrenal ascorbic acid. 3. Scorbutic animals treated with ascorbic acid for 5 days prior to sacrifice had circulating 17-hydroxycorticosteroids in amounts not significantly higher than those of control animals.

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Effects of Chloramphenicol on Incorporation of Glycine-2-C^{14} into Mammalian Tumor Cell Proteins and Purines.* (20474)

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It has been demonstrated by Wisseman *et al.*(1) that the antibiotic d(—) *threo* chloramphenicol is effective in inhibiting protein synthesis in susceptible microorganisms such as *E. coli*. They have further demonstrated that higher concentrations of l(+) *threo* chloramphenicol do not give this effect, and that concentrations of the d(—) threo isomer which effectively stop protein synthesis (50 μg/ml) permit nucleic acid purine and ribose synthesis to proceed for a significant time. Since viable suspensions of mammalian tumor cells were available and had been shown to carry on incorporation of glycine-2-C^{14} into purines and proteins(2), it was of some interest to test the effects of chloramphenicol isomers on this mammalian cell system.

Methods. The Ehrlich carcinoma cells were obtained by intraperitoneal injection of 6-10-week-old Rockland strain white mice with approximately 10^7 cells per mouse. The cells were withdrawn from mice after 5-7 days, under light ether anesthesia with a hypodermic syringe and No. 18 needle. At this time the suspension was approximately 90% tumor cells. The Gardner lymphosarcoma (6C3HED) cells were obtained in similar fashion from C3H mice 7-10 days after injection, at which time the suspensions were approximately 96% tumor cells. The cells were washed 3-4 times by suspending in isotonic saline and centrifuging. Warburg respirometer vessels of 60 ml capacity were used as reaction vessels. Incubation was for one hour at 38°C. Each vessel contained a total of 12 ml of medium, consisting of Robinson's medium with glucose and bicarbonate(3), 150 μg glycine-2-C^{14}† and cells equivalent to 30 mg dry weight. The chloramphenicols‡ were weighed dry into each such flask and dissolved in the medium. The cells were added just prior to incubation, except where otherwise indicated. Purines and proteins were isolated and counted for radioactivity as described earlier(2). Paper spots as counted contained 200-300 μg of purine. Counting was continued until statistically significant to within 5%.

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† Specific activity 20,600,000 counts/min/mg; obtained from Tracerlab, Inc., on allocation by the U. S. Atomic Energy Commission.

‡ The d(-) threo chloramphenicol and l(+) threo chloramphenicol were obtained through the courtesy of Parke-Davis and Co. and Major Charles L. Wisseman, Jr., respectively.

TABLE I. Incorporation of Glycine-2-C^{14} into Components of Ehrlich Carcinoma Cells in the Presence of Various Concentrations of d(—) Threo Chloramphenicol.

Chloramphenicol, μg/ml	Protein Radioactivity, c/min./mg	Protein % inhibition	Nucleic acid adenine Radioactivity, c/min./mg	Nucleic acid adenine % inhibition	Nucleic acid guanine Radioactivity, c/min./mg	Nucleic acid guanine % inhibition
0	3970		1280		2770	
250	3550	10	1340	-5	2970	-7
500	3570	10	1390	-9	2720	2
1000	2950	26	1120	12	1760	36
1500	2700	32	790	38	1110	60
2500	2645	33	770	40	1100	60

Radioactivities are corrected for self-absorption characteristics. Each figure is the mean of results from 3 experiments with duplicate reaction vessels.

TABLE II. Effects of Chloramphenicol Isomers on Incorporation of Glycine-2-C^{14} by Ascites Cell Tumors *In vitro*.

Addition, μg/ml	Radioactivity, c/min./mg Protein	Adenine	Guanine
Ehrlich carcinoma			
0	3990	1290	2980
1000 d(—) threo	3190	1060	1875
2000 d(—) "	2710	1005	1280
1000 l(+) "	3670	1295	1910
2000 l(+) "	3790	1120	1105
1000 d(—) " *	3150	1030	1875
1000 l(+) " *	3750	1070	1250
6C3HED			
0	3650	1050	1210
1000 d(—) threo	1270	965	361
2000 d(—) "	310	757	182
1000 l(+) "	1460	988	556
2000 l(+) "	184	965	194

* Preincubated 30 min. at 0°C.

Each figure represents avg of results in 3 to 5 experiments in the Ehrlich carcinoma, 2 experiments in the 6 C3HED.

Results and discussion. Data are presented in Table I to show the effects of varied levels of d(—) *threo* chloramphenicol upon the incorporations by Ehrlich carcinoma cells. Significant effects are obtained only at much higher levels than are required to inhibit susceptible bacteria. In Table II are data in which the 2 chloramphenicol isomers are compared in experiments with 2 types of tumor cells. In experiments with Ehrlich carcinoma cells the inhibition of incorporation of the radioactivity into protein is significant only with the isomer that has antibiotic properties. This specificity does not hold for the lymphosarcoma cells. The level of inhibition varied considerably from one experiment to another in the Ehrlich carcinoma cells, but the relative extent of inhibition of the 3 components studied held constant. This raised the question as to whether the permeability of the cells to the drug was a variable. As seen in Table II, preincubation of the cells with the drugs at 0°C did not increase the inhibition.

The results obtained by Tyner *et al.*(4) (Chart 16), indicated that synthesis of the individual nucleotide components of tumor nucleic acids was at a rate in each case parallel with the requirement for that nucleotide in the nucleic acid composition. The inhibition of guanine synthesis, without a comparable inhibition of other synthetic processes involved in growth, might lead to interference with the growth process as a whole, possibly by leading to modified nucleic acid composition. In this connection it is of interest that triethylene melamine, which is known to be an inhibitor of a number of animal tumors, inhibited the incorporation of glycine-2-C^{14} into nucleic acid guanine of Ehrlich carcinoma cells 66% at 30 μg per ml. The incorporation into protein and into nucleic acid adenine was not affected. It would be appropriate to test the *in vivo* effect of these isomers of chloramphenicol on various tumors.

It is worthy of note that when the incorporation of glycine into bacterial purines was finally inhibited, the entry into both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) purines was affected(1). The 2 nucleic acids were not separated in the experiments reported here and a 60% inhibition of incorporation of glycine-2-C^{14} into guanine may well represent complete blocking of entry into DNA.

Summary. The effects of 2 of the isomers of chloramphenicol, one the antibiotic, the other inactive toward bacteria, were tested on 2 *in vitro* systems consisting of mouse ascites

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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