route of administration on fat storage and excretion of ethynylestradiol-3-cyclopentyl ether (EECPE) were studied in rats. The EECPE was tagged with ^{3}H in the steroid nucleus and with ^{14}C in the cyclopentyl group in order to be able to trace the de-etherification process. Over a range of oral doses of 20 μg to 12.5 mg, there was a proportional increase in the concentration of compound in body fat and brain. The storage increment exceeded the dose increment suggesting that with increasing dose more of the compound escaped metabolic alteration. Nearly twice as much radioactivity was found in fat, brain, and blood of rats in which EECPE had been injected parenterally (iv, ip, sc) rather than administered orally. Evidence was sought but not found that EECPE is metabolized by the gastrointestinal tract; in fact, orally and intravenously administered EECPE were excreted in qualitatively similar patterns in bile and urine. Excretion of radioactivity following treatment of rats with cyclopentanol-^{14}C led to the conclusion that EECPE follows two basic metabolic pathways: (i) de-etherification with urinary excretion of cyclopentanol derivatives; and (ii) conjugation and biliary excretion of metabolites in which the ether linkage is still intact.

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Incorporation of Glycine-1-^{14}C into Liver Glutathione in Zinc Deficient Rats (32866)

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Du Vigneaud, *et al.* in 1931 (1) reported that glutathione (GSH) can inactivate insulin *in vitro*. Recent studies by Tomizawa and his associates (2–4) clearly indicate that the destruction of insulin in the liver is due primarily to the enzyme, glutathione insulin transhydrogenase. This enzyme degrades insulin by promoting reductive cleavage of the insulin molecule by GSH into its A and B chains. Thus, alterations of liver GSH content may bear a significance in the regulation of insulin-degrading activity. Since crystallization of insulin requires traces of zinc (5), and since the plasma insulin level is decreased in zinc deficient rats (6), it is of interest to determine the role of zinc on GSH synthesis. The present communication describes some experiments concerning incorporation of radioactivity from glycine-1-^{14}C into liver GSH and tissue proteins from normal and zinc deficient rats.

Materials and Methods. Male rats 21–24 days of age weighing 40–60 gm of McCollum strain from our own colony were randomly divided into two groups. The first group received a diet low in zinc. Its percentage composition was as follows: sucrose, 65.97; dried egg white, 15.0; casein hydrolyzate (salt free) 3.0; corn oil, 10.0; zinc free salt mixture, 5.74;[1] and vitamin supplements, 0.49.[2] The zinc content of the diet was 2 to 3 ppm as determined by the Jarrell Ash direct reading spectrometer. The second control group re-

TABLE I. Growth Performance and Feed Efficiency in Zinc Deficient and Control Rats.

Dietary condition	No. of rats	Body wt. Initial (gm)	Body wt. Final in 16 days (gm)	Average daily feed intake/rat (gm)	Feed efficiency (gm of feed/gm of wt. gain)
Zn-fed	7	40 ± 2[a]	89 ± 6.1	7.0 ± 0.7	2.3 ± 0.2
Zn-deficient	7	40 ± 4	53 ± 7.5[b]	3.5 ± 0.7[b]	5.2 ± 2.1[b]

[a] Mean ± SD.
[b] $p < 0.01$.

ceived the same low zinc diet with a supplementation of 80–90 ppm of zinc as zinc carbonate. All rats were housed in individual stainless-steel cages. Feed and deionized water were freely available. After feeding for 16–21 days, and overnight fasting, each rat was injected intramuscularly with 5 μC glycine-1-^{14}C (specific activity, 22.3 μC/μM) for each 100 gm of body weight. Four and 6 hours later animals were sacrificed by decapitation. Whole liver, kidney, pancreas, and a portion of small intestine were removed as rapidly as possible. The concentrations of liver GSH and nonprotein thiol compounds were determined according to the methods of Patterson and Lazarow (7), and Grunert and Phillips (8), respectively. For the isolation of ^{14}C-labeled GSH, the outline procedure of Goldzieher *et al.* (9) was followed. The cadmium–glutathionate precipitate was dissolved in 2% sulfosalicylic acid to a final volume of 2 ml. The preparation of the protein fraction from various tissues was a modification of a procedure previously described by Wool and Krahl (10). The frozen sample was allowed to soften and a 5% homogenate was made with ice-cold water in a Potter-Elvehjem homogenizer. An equal volume of cold 10% trichloroacetic acid (TCA) containing unlabeled glycine (0.01 *M*) was added to the homogenate. Following a 30-min stand at room temperature, the suspension was centrifuged, and the sediment was washed twice with 5 ml of 5% TCA. The residue was then dissolved in 3 ml of 88% formic acid and 0.6 ml of 30% peroxide and the mixture was incubated for 30 min at 25–30°C. The resulting precipitate was collected by centrifugation, washed three times with acetone, once with ethyl ether, and allowed to dry overnight. The fat free product was dissolved in a small volume of 1 *N* NaOH. The concentrations of ^{14}C-labeled protein and GSH were determined in a Tri-carb-liquid scintillation spectrometer using the diotol solvent of Herberg as a liquid scintillator (11). Protein contents in tissues were estimated according to the method of Lowry *et al.* (12).

Results and Discussion. The results in Table I reveal growth retardation and low feed efficiency in the rats on a zinc low diet. A majority of the zinc deficient rats also showed such external symptoms as alopecia and scabby skin lesions.

Table II shows that the concentrations of nonprotein SH compounds and GSH in the livers of zinc deficient rats were significantly higher than those of zinc supplemented controls. These changes were observed as early as 1 week of experimental feeding. No further increase of liver GSH was noted when the rats were deprived of zinc for 2 or 3 weeks.

Table III illustrates the incorporation of glycine-1-^{14}C into liver GSH in zinc deficient and zinc supplemented rats. The mean value of total GSH in liver of zinc deficient rats was 60% more than that of zinc adequate animals.

[1] The following minerals were supplied (mg/100 gm of diet): $CaHPO_4$, 2.716; $CaCO_3$, 0.957; Na_2HPO_4, 0.67; NaCl, 0.383; KCl, 0.670; $MgSO_4$, 0.287 $FeC_6H_5O_7 \cdot 5H_2O$, 0.19; $MnSO_4$, 0.012; KIO_3, 0.010; $ZnCO_3$, 0.012; $CuSO_4 \cdot 5H_2O$, 0.001. To prepare zinc low diet, $ZnCO_3$ was eliminated from the mixture.

[2] Vitamins were supplied at the following levels per 100 gm of diet: thiamine HCl, 0.24 mg; riboflavin, 0.6 mg; pyridoxine HCl, 0.30 mg; Ca-pantothenate, 2.0 mg; niacin, 4.0 mg; inositol, 10.0 mg; biotin, 0.2 mg; folic acid, 0.2 mg; cyanocobalamin, 0.005 mg; choline chloride, 100.0 mg; 2-methyl-1 4-napthoquinone, 1.0 mg; α-tocopherol, 6.0 mg; vitamin A, 2300 IU; vitamin D_3, 330 IU.

TABLE II. Effect of Zinc Deficiency on Liver Nonprotein SH Compounds and GSH Levels.[a]

Dietary condition	Weeks	No. of rats	Nonprotein SH compounds	GSH
Zn-fed	1	5	300 ± 56[b]	206 ± 45
Zn-deficient		5	514 ± 59[c]	344 ± 37[c]
Zn-fed	2	5	318 ± 63	193 ± 35
Zn-deficient		6	575 ± 49[c]	341 ± 87[c]
Zn-fed	3	7	—	209 ± 16
Zn-deficient		7	—	319 ± 67[c]

[a] μmoles/100 gm of liver.
[b] Mean ± SD.
[c] $p < 0.01$.

Its specific activity, expressed as cpm/mg of liver GSH was about 71% higher at 4 hours and 51% at 6 hours in zinc deficient rats as compared to their respective controls. When data were calculated as cpm/gm liver and percentage of injected dose, zinc deficient rats again had twice as much GSH radioactivity as their controls following the injection of ^{14}C-labeled glycine.

Data summarized in Table IV indicate that the rate of incorporation of glycine-1-^{14}C into protein of pancreas, liver, kidney, and small intestine of zinc deficient rats was not different from that of zinc supplemented rats. It is worthwhile to point out that we have previously reported that the incorporation rate of leucine-^{14}C into pancreatic protein and its degradation were enhanced in zinc deficient rats (13). If these findings are confirmed, it would suggest that the effect of zinc on protein metabolism appears to be specific for certain amino acids rather than a general phenomena.

The present study shows that dietary deficiency of zinc results in an increase of liver GSH concentration. Furthermore, the synthesis of liver GSH from glycine-1-^{14}C was increased in zinc deficient rats. Deficiency of this trace element causes a decrease in total plasma protein of rats (14), chicks (15), and Japanese quail (16). On the other hand, the presence of relatively large quantities of GSH in various tissues suggested that this tripeptide might be an intermediate between free amino acids and proteins or functioning as a regulatory mechanism (17). However, our observations, indicating the rate of incorporation of glycine-1-^{14}C into liver protein to be unaffected by zinc deficiency, cast doubt on the metabolic relationship between GSH and protein synthesis. Although it has been

TABLE III. Effect of Zinc Deficiency on Incorporation of Glycine-1-^{14}C into Liver GSH.

Dietary condition	No. of rats	Hours after glycine injection	Liver (μmoles /100 gm)	Liver GSH (cpm/mg)	Liver (cpm/gm)	% of injected ^{14}C found in whole liver as GSH
Zn-fed	6	4	214 ± 29[a]	15240 ± 3288	10060 ± 3250	0.318 ± 0.158
Zn-deficient	5	4	323 ± 42[b]	26092 ± 2490[b]	25890 ± 3950[c]	0.784 ± 0.128[c]
Zn-fed	6	6	268 ± 28	9690 ± 1615	8575 ± 1850	0.271 ± 0.079
Zn-deficient	6	6	418 ± 84[b]	14635 ± 3005[b]	19025 ± 4861[c]	0.512 ± 0.117[c]

[a] Mean ± SD.
[b] $p < 0.05$.
[c] $p < 0.01$.

TABLE IV. Effect of Zinc Deficiency on Incorporation of Glycine-1-^{14}C into Tissue Proteins.[a]

Dietary condition	Pancreas	Liver	Kidney	Small intestine
Zn-fed	236 ± 120[b]	34 ± 16	58 ± 9	111 ± 37
Zn-deficient	274 ± 36	37 ± 13	49 ± 11	180 ± 47

[a] Five rats were used in each group and sacrificed 4 hours after glycine-1-^{14}C injection. Results were expressed as cpm/mg of tissue protein.
[b] Mean ± SD.

demonstrated that GSH is involved in the inactivation of insulin *in vivo* (2–4) and *in vitro* (1), it is not clear whether the elevation of liver GSH in zinc deficient rats is responsible for the decreased insulin-like activity as reported by others (6). Additional work concerning the effect of zinc deficiency on glutathione insulin transhydrogenase activity is in progress.

Summary. Weanling male rats receiving a diet low in zinc for 2–3 weeks had a significant increase of liver nonprotein SH compounds and glutathione over those fed an adequate zinc diet. Deficiency of this trace element caused an increase in the incorporation rate of glycine-1-^{14}C into liver glutathione moiety, but it had no effect on protein synthesis.

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Acatalasemia and Hypocatalasemia in the Dog and the Duck* (32867)

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For a variety of studies, it is of interest to have available sources of blood very low in, or completely free of, catalase activity. There are now known acatalasemic or hypocatalasemic humans (1,2), guinea pigs (3), and laboratory mice (4). The low catalase members of these species are presumed to be mutants; certainly a far higher blood catalase level is the much commoner occurrence in these species.

Before these low blood catalase sources were available, biologists wanting catalase-free or catalase-low blood would generally select the duck, which had been shown in 1952 (5) to be normally low in this enzyme.

Allison *et al.* (6) suggested that dogs fall naturally into three levels of blood catalase activity: low, intermediate, and high. Unfortunately their mode of expression of catalase activity made it difficult to compare the dog blood catalase activity with that of other species. In the course of studies on the comparative heat stability of blood catalase (7), we observed that *all* dogs we tested were either acatalasemic or hypocatalasemic. We have therefore performed a series of blood catalase assays on dogs and a few on ducks, for comparative purposes. We have also done

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