Discussion. Specific features characterize each of the 5 known methods for analysis of Tris-(THAM) and make each one more fit for some purposes than for others. The method(3) based on the use of C-14 tagged Tris-(THAM) is the most accurate, but is very costly and is not applicable to humans. Linn and Roberts' titrimetric method(5) is desirable when only minute quantities of sample can be obtained, but it requires standing over night. Clark's methods(6) seem reproducible but they require special equipment.

Though Rosen's method(4) generally is described as unsuitable for blood analysis(2, 6), its modification as described here is applicable to either plasma or urine. It yields average recoveries of over 95% of Tris-(THAM) added to biological fluids. Reproducible calibration curves are obtained, and there is no interference by amines, amino acids or Penicillin at usual levels. It seems that the protein precipitation in the modified method introduces fewer errors than the one described by Rosen(4). In addition, the modified concentration of some reagents and the use of special glassware, might be contributory factors in making this modified method suitable for both blood and urine analysis. The relative simplicity of this method, both in procedure and equipment, makes it desirable for general use.

Summary. 1. A modification of Rosen's method for Tris-(THAM) has been presented. Both Rosen's method and this modification are based on the oxidation of Tris-(THAM) by potassium dichromate after chromatographic separation to remove interfering substances. 2. Tris-(THAM) recoveries of over 95% were obtained without interference from amino acids or Penicillin. There was PAH interference by up to 35% of added PAH. 3. This method is applicable for use with small quantities of plasma and urine following injection of Tris-(THAM) in recommended pharmacological doses. 4. Pending development of a specific chemical test, this method is recommended for general use because of its simplicity and reproducibility.

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Blood Changes in Turkeys Associated with a Copper Deficiency.* (28276)

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It is known(1) that copper is an indispensable trace mineral constituent of the diet for many species of animals. Gallagher(2), however, was unable to produce an uncomplicated copper deficiency anemia in chicks. The turkey's requirement for copper has not been determined(3).

Until recently is was assumed that erythropoiesis was the only metabolic process in which copper was concerned. It is believed that copper does have a role in connective tissue integrity because copper deficient pigs(4) and chicks(5) died from internal hemorrhage as a consequence of elastic fiber degeneration and aortic rupture.

This study of copper deficiency in turkeys was initiated because many identical histopathological lesions have been described in chicks fed a copper deficient diet(5) and turkeys fed *Beta-aminopropionitrile* (BAPN)

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† Florida Agri. Exp. Station Journal Series No. 1635.

TABLE I. Composition of Basal Diet.

Ingredients	%
Cerelose	18.8
Dried skim milk	70.0
Vegetable oil	5.0
L-arginine·HCl	.7
Glycine	.5
Limestone	1.2
Defluorinated phosphate	1.3
Vitamin mixture*	1.0
Mineral mixture*	1.5

* Creech *et al.*(14), except that $CuSO_4 \cdot 5H_2O$ was eliminated from the mineral mixture.

(6). It was deemed advisable, therefore, to determine if aortic ruptures in turkeys could be produced by copper deficiencies.

Materials and methods. Day-old, Broad-Breasted Bronze turkey poults were purchased from a commercial hatchery. Male poults were used because they are known to be highly susceptible to BAPN-induced dissecting aneurysms(6). They were housed in electrically heated battery brooders with raised wire floors. Feed and demineralized water (containing 0.015 ppm or less of copper) were supplied *ad libitum* in galvanized iron troughs. The basal diet utilized in this study is shown in Table I. By analysis it contained 3.3 ppm of copper.

Five dietary levels of copper were compounded in a stainless steel paddle mixer by adding various amounts of $CuSO_4 \cdot 5H_2O$ to the basal diet. Commencing at one day of age, 5 groups of birds, consisting of 2 replicas of 10 birds each, were fed diets containing 0.0, 0.66, 1.32, 1.98, and 3.96 ppm of supplemental copper.

The poults were slaughtered at 6 weeks of age. At this time individual weights were recorded. Livers and hearts were frozen within 30 minutes at −8°C, and cytochrome oxidase activity of the left ventricle and liver of 4 birds from each experimental treatment was determined within one week, using the method of Cooperstein and Lazarow(7).

Hematological examinations were performed on at least 10 birds in each treatment level. Microhematocrits were run at 4 and 6 weeks of age. Wright's stained blood smears were examined at 2, 3, 4, 5, and 6 weeks. At the same times, 6 to 8 drops of heparinized blood were fixed in either 2% potassium permanganate(8) or 1% osmic acid with added sucrose(9) for sectional studies with the electron microscope. This fixed blood was subsequently dehydrated in methanol and embedded in Epon 812(10) or methacrylates. Sections 1 μ in thickness were cut from Epon 812 embedded material, stained by Masson's trichome stain and examined by conventional microscopy. Surface studies of unfixed erythrocytes, lysed by water and shadowed with chromium, were also accomplished by electron microscopy. A Philips 100A electron microscope was used in all studies involving ultrastructure.

All data are discussed in terms of supplemental copper.

Results. As shown in Table II, average body weights of poults at 6 weeks indicated that 1.98 and 3.96 ppm of supplemental copper in the diet were adequate for normal weight gains. The diet which contained 1.32 ppm of supplemental copper gave increased gains above the controls; however, these gains were numerically less than those obtained with the 2 highest levels of copper. There were statistically reduced weight gains in birds fed the basal (0.0 ppm of supplemental copper) diet.

Leg weakness was seen in poults fed the 2 lowest levels of copper. However, no mortality due to internal hemorrhages occurred in any groups. Deaths beyond normal expected losses were recorded in birds fed 0.66 ppm of supplemental copper, but this was attributed to malfunctioning of the electric brooder in one replicate.

Average microhematocrits of poults at 4 and 6 weeks of age were within normal ranges reported in the literature for chicks(11).

The erythrocytes were abnormal in 7 of 12 poults fed 1.32 ppm of supplemental copper. In stained blood smears nuclear changes in the form of double nuclei, partially separated nuclei connected by bands, lobulated nuclei, nuclei with nipple and arrow-shaped extremities, tailed extremities, forked nuclei, nuclear droplets, and other bizarre manifestations were seen (Fig. 1). Spindle-shaped erythrocytes also were observed. Altered nuclei were common in smears, and several were evident in every microscopic field examined by oil im-

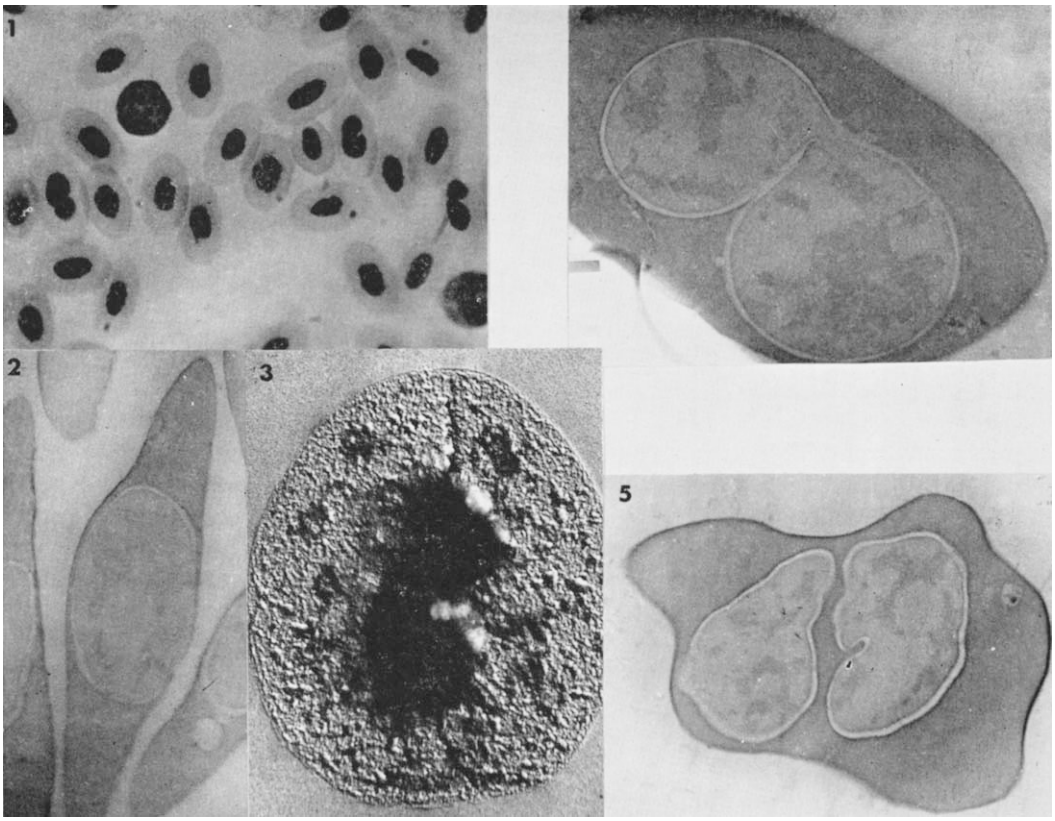

FIG. 1. Photomicrograph of blood smear showing many manifestations of abnormal nuclear morphology. Wright Stain, × 500.

FIG. 2. Micrograph of normal erythrocyte showing dense and compactly clumped chromatin material. × 5000.

FIG. 3. Micrograph of an abnormal lysed erythrocyte. The nucleus is lobulated. Chromium shadowed. × 5000.

FIG. 4. Section of an erythrocyte with a lobulated nucleus. Note diffuse distribution of the chromatin material. × 5000.

FIG. 5. There are 2 nuclei in this erythrocyte, each of which is surrounded by its own nuclear envelope. × 5000.

mersion. Such cytopathology was also evident in 1 μ stained sections of Epon 812 embedded blood. They were not observed in birds fed diets containing 1.98 and 3.96 ppm copper. The erythrocytes of poults fed 0.0 and 0.66 ppm copper were normal except for rare tailed and forked nucleated forms. Polychromasia above normal was not evident in birds in any group.

Surface and sectional electron microscope studies of erythrocytes substantiated all of the nuclear abnormalities observed under the light microscope. Double nuclei, each bound by a separate nuclear envelope, were seen as well as intermediate forms. The nuclear envelope was not well preserved in methacrylate sections. Chromatin material was more dense and more compactly clumped in normal than irregular shaped nuclei (Fig. 2). Lobulated and bean-shaped nuclei were also common in erythrocytes examined under the electron microscope (Fig. 3-5).

Cytochrome oxidase activity of the left ventricles and livers of birds fed the various levels of copper was determined. There was a significant inhibition of myocardial and hepatic cytochrome oxidase activity in birds fed 0.0 and 0.66 ppm of supplemental copper (Table II).

Discussion. In contrast to copper deficiency in chicks, mortality due to internal hemorrhage did not occur in turkey poults fed

TABLE II. Effect of Feeding Various Levels of Copper for 6 Weeks on Body Weight and Cytochrome Oxidase Activity.

Supplemental* Cu (ppm)	Avg wt (g)	Cytochrome oxidase† (O.D. change/min/mg wet wt) Liver	Heart
0	484 ± 13[a]	.114 ± .009[a]	.054 ± .005[a]
.66	533‡[b]	.114 ± .007[a]	.061 ± .008[a]
1.32	572 ± 27[bc]	.121 ± .009[b]	.085 ± .002[b]
1.98	605 ± 18[c]	.119 ± .008[b]	.088 ± .003[b]
3.96	606 ± 26[c]	.121 ± .007[b]	.084 ± .006[b]

* By analysis the basal diet contained 3.3 ppm of copper.

† Averages and standard errors of mean of triplicate analysis of 4 birds. Values with different alphabetical superscripts are significantly different by Duncan's Multiple Range Test.

‡ Due to excessive mortality caused by a faulty brooder in one replicate, the missing value was calculated according to the procedure outlined by Snedecor(15).

a low copper diet. This unusual difference among related species is interesting because poults are more susceptible to BAPN toxicosis than chicks(12), and apparently copper deficiency(5) and BAPN toxicity(6) cause similar aortic histopathological changes.

This study showed that 1.98 ppm of supplemental copper is adequate for maintenance of normal weight gains in turkeys. Thus, turkeys apparently have a slightly higher copper requirement than chickens(1).

Birds fed 1.32 ppm of supplemental copper grew almost as well as those fed higher levels, but their erythrocytes had abnormal nuclear morphology. Similar erythrocytic changes have been observed in chicken blood(11), but it was not determined whether or not they were manifestations of cytopathology. The finding in this study that bizarre-shaped nuclei of turkey erythrocytes result from submarginal levels of copper in the diet is difficult to explain. It may be due to the ability of medium copper levels to stimulate significant increase in growth rate (Table II), but its inability to stimulate normal maturation of erythrocytes. The only other report on this blood dysfunction is one describing the presence of irregularly shaped nuclei in chicken erythrocytes following treatment with folic acid antagonists(13). It is not believed that copper acts as a folic acid antagonist at the levels fed in this experiment and reported herein. The homogeneous texture of the cytoplasm of erythrocytes and their affinity for acid dyes identified the abnormal cells described in the paper as mature erythrocytes.

Summary. Day-old turkey poults were fed 5 levels of supplemental copper for 6 weeks. There was a significant stimulation of growth in those fed 1.32 to 3.96 ppm of added copper. Microhematocrits of all turkeys were within normal ranges. The erythrocytes of birds fed 1.32 ppm of supplemental copper had abnormally shaped nuclei, while those of poults fed 1.98 and 3.96 ppm of copper were normal. Nuclear changes consisted of lobulated nuclei, duplication of nuclei, forked nuclei and other bizarre manifestations. Heart and liver cytochrome oxidase activity was greater in those birds fed 1.32 to 3.96 ppm of added copper.

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Fatty Acid Ethyl Ester Formation During Ethanol Metabolism *in vivo.* (28277)

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During recent experiments on the enzymatic hydrolysis of cholesterol esters, we observed that rat liver homogenate fractions capable of hydrolyzing cholesterol esters were also active in effecting hydrolysis of ethyl palmitate(1). Since Margolis and Vaughan have reported the esterification of ethanol and other short-chain alcohols with palmitic acid by adipose tissue microsomes(2), this suggested that formation and breakdown of fatty acid ethyl esters might occur during mammalian metabolism of exogenous ethanol. The present experiments were conducted to test this hypothesis.

Four male Sprague-Dawley rats weighing 190-210 g were each given an intravenous injection of 1 ml isotonic saline containing 60 μc of 1-C^{14}-ethanol. The total amount of ethanol injected into rats No. 1 and 2 was 0.15 ml and into rats No. 3 and 4 was 0.05 ml. In addition, rats 1 and 2 received 1 ml 40% ethanol by stomach tube 30 minutes prior to injection. This was done in order to provide a large load of ethanol for metabolism by rats 1 and 2. Since the relative rates of the pathways of ethanol metabolism are not known, the experiment was designed to examine C^{14}-ethanol metabolism at 2 time intervals, after both a small and large load of ethanol. Rats 1 and 3 were killed by cervical fracture 20 minutes following the injection and rats 2 and 4 after 2 hours. Each rat was then passed through a meat grinder and homogenized in a gallon-size blender with 3 liters of ethanol:acetone, 1:1 (v/v). Significant amounts of blood were not lost during the processing. The suspensions were allowed to stand for 2 days with intermittent shaking, to extract all tissue lipids. Portions of each extract were then evaporated just to dryness with a stream of nitrogen. The residue was dissolved in $CHCl_3$:MeOH, 2:1 (v/v) followed by addition of 1/5th vol. water, collection of the lower $CHCl_3$ phase and evaporation of the $CHCl_3$. This solvent partition removed all non-lipid materials from the residue obtained after ethanol-acetone extraction. The lipid residues were then transferred to small vials with light petroleum ether, the petroleum ether evaporated, and the residues finally dissolved in measured small volumes of benzene.

Control experiments have shown that this series of procedures, including 3 complete solvent evaporations and one $CHCl_3$-MeOH solvent partition, completely removes all unreacted labeled ethanol which might be present in the original extract.

Portions of the resultant total body lipid extract from each rat were then directly assayed for radioactivity in a Packard liquid scintillation counter. Other identical portions were hydrolyzed by heating at 60° for 1 hr under nitrogen with 4% KOH in 90% ethanol. After addition of an equal volume of water and acidification with concentrated HCl, each solution was extracted repeatedly with light petroleum ether, until no further radio-

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