dose of "cold" B_{12} to dilute the radiovitamin so much so that none of it would be deposited or retained in the organs described. In the group of patients who received 1 mg of "cold" B_{12} 7 days after the injection of the radiovitamin there was no alteration in the pattern or plateau over the liver, spleen or kidney during the period of observation (up to 86 days). This would suggest that vit. B_{12}, once it reaches these organs, is not readily displaced by further administration of the vitamin.

The experiment in which amethopterin was administered for one week prior to Co^{60} B_{12} was investigated because of the intimate relationship of folic acid and vit. B_{12} in nucleoprotein synthesis. However, no alteration in the pattern of radioactivity distribution was observed. It is possible that a longer period of folic acid antagonist administration is necessary to affect vit. B_{12} deposition.

Conclusions. (1) Prior administration of 30 μg of "cold" B_{12} or 10 mg of amethopterin daily for 1 week, or injection of 1 mg of "cold" B_{12} 7 days after intramuscular injection of 1 μg of Co^{60} vit. B_{12}, does not alter rate of disappearance from site of injection or pattern of distribution of radioactivity over liver, spleen and left kidney. (2) Intravenous injection of 1 μg of Co^{60} B_{12} is followed by a more rapid appearance of radioactivity over the organs named. (3) Intramuscular injection of 1 mg of "cold" B_{12} simultaneous with 1, 2 or 24 hours after the administration of 1 μg of Co^{60} B_{12} reduces height of radioactivity over organs named but does not prevent its localization there.

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Role of Various Tissues in Metabolism of C^{14} Labeled Priscoline in the Rat.* (22530)

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Priscoline (tolazoline) hydrochloride is well known today, clinically as an effective peripheral vasodilating agent and pharmacologically as an adrenergic blocking agent. In a previous study, C^{14} labeled Priscoline administered to rats was rapidly excreted in the urine almost entirely unchanged(1). That a small amount of the drug is changed in the body was suggested by recovery of traces of C^{14} in the expired air and by the separation of small amounts of a radioactive metabolite in the urine. The method of analysis described in the present study permitted the detection of minute amounts of radioactive Priscoline and this metabolite in tissues as well as in urine, and made possible further investigation of the roles of various tissues in the turnover of Priscoline.

Methods and materials. The Priscoline used in this study had a specific activity of 1 μc/mg, and was labeled at the single benzyl carbon between the phenyl and imidazoline rings.‡ Sprague-Dawley albino rats weighing from 167 to 253 g were injected intraven-

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‡ The C^{14} labeled Priscoline hydrochloride was very kindly supplied by Ciba Pharmaceutical Products, Summit, N. J.

ously with 7.95 mg/kg of radioactive Priscoline hydrochloride and were sacrificed after 1- and 2-hour intervals. Aliquots of urine and bladder washings and tissue homogenates were assayed for total C^{14} with internal gas flow Geiger counters. Tissues were analyzed for unchanged Priscoline and radioactive metabolites by the following procedure. To each 5 ml aliquot of tissue homogenate were added 25 ml of absolute methanol and 0.2 ml of concentrated ammonium sulfate. The supernatant was removed by centrifugation, and the precipitate was further extracted with 20 ml of absolute methanol. The protein precipitate contained no detectable C^{14}. The combined methanol extract was diluted to 50 ml, and a 15 ml aliquot was added to an equal amount of water in a separatory funnel containing about 2 mg of unlabeled Priscoline hydrochloride as a carrier. The 50% methanol solution was extracted once with 30 ml of carbon tetrachloride to remove fats and other substances soluble in organic solvents. No significant C^{14} was detected in this fraction. The 50% methanol fraction was made basic with ammonium hydroxide to a pH of over 11.1 and extracted 3 times with 30 ml portions of chloroform. The chloroform extract of each biological sample was evaporated to dryness, care being taken to avoid volatilization of the basic Priscoline. The radioactive residue was transferred with 4 small portions of ethyl acetate saturated with 0.1 M HCl onto a small Solka Floc or macerated cellulose column prepared with the same solvent system(2). An additional 14 ml of this solvent was used to elute a small radioactive fraction, while the unchanged Priscoline which remained on the column was eluted with 10 ml of a mixture of 55 parts of 95% ethanol, 45 parts of chloroform, and 1 part of acetic acid. Aliquots of the 50% methanol or aqueous fraction, the unchanged Priscoline, and the small chloroform extracted fraction were assayed for C^{14}. The results are expressed as millimicrocuries of C^{14}, which are equivalent to micrograms of administered Priscoline hydrochloride. Samples of urine were similarly analyzed, except that the precipitation of proteins was omitted. Control analyses were performed on samples of various tissue homogenates and urine to which known amounts of radioactive Priscoline were added.

Slices of liver, kidney, lung, spleen, and heart were freshly obtained from untreated normal rats and incubated 2 hours at 38°C in Warburg vessels containing 3 ml of Krebs-Ringer phosphate solution (pH 7.4 with C^{14} labeled Priscoline at a concentration of 9.44 μg/ml. Rat diaphragm and 0.2 ml samples of plasma were similarly incubated. The center wells of the flasks contained 0.2 ml of 10% KOH. The gas phase was oxygen, and O_2 consumption was measured during the first hour. Following incubation, the slices were removed, quickly blotted, homogenized in water, and aliquots were dried on copper planchettes for the determination of dry weights and C^{14} contents of the tissues. Wet weights were estimated by conversion factors separately determined for each tissue. A 2 ml sample of buffer from each flask was analyzed for total C^{14}, unchanged Priscoline, and radioactive metabolites. Similar analyses were performed on control buffers with radioactive Priscoline which were incubated without tissue samples and on unincubated solutions containing 0.2 ml of plasma. Liver slices were also incubated for 1 hour with radioactive Priscoline in Krebs-Ringer bicarbonate buffer under anaerobic conditions. The gas phase was 5% CO_2 in nitrogen. Liberation of CO_2 was measured manometrically. Slices and buffers were analyzed as above. Liver, kidney, heart, and spleen homogenates were also incubated in media containing radioactive Priscoline. Final concentrations of constituents in each flask were 0.1 M KCl, 0.02 M sodium phosphate (pH 7.4), 1.33×10^{-4} M cytochrome c, 0.001 M Na-ATP (neutralized), 0.0033 M $MgCl_2$, 0.01 M Na-pyruvate, and 0.004 M Na-fumarate. Eighty mg of wet tissue were added as 10% homogenates in 0.154 M KCl, and enough water was added to bring the volume to 3 ml. The gas phase was oxygen and the incubation time was 1 hour. The center well contained 0.2 ml of 10% KOH. Incubated homogenates were analyzed for total C^{14}, unchanged Priscoline,

TABLE I. Recovery of Unchanged Priscoline and Radioactive Metabolite from Tissues and Urine from 5 Rats Sacrificed 1 Hour after Injection and from 3 Rats Sacrificed after 2 Hours.

	Hr	C^{14}, mμc/g tissue	50% methanol soluble fraction		Unchanged Priscoline	
			mμc/g	% of total conc.	mμc/g	% of total conc.
Liver	1	16.6	2.34	14.1	12.4	75
	2	10.6	2.82	26.6	6.7	62
Kidney	1	22.9	.95	4.2	18.0	79
	2	13.1	.80	6.1	11.0	84
Lung	1	8.3	tr*	2.0	7.4	89
	2	4.29	n*	—	3.73	87
Spleen	1	6.04	tr	2.0	5.23	87
Heart	1	4.25	n	—	3.62	85
Skeletal muscle	1	4.66	n	—	4.36	94
	2	2.80	.14	5.0	2.62	94
Skin	1	2.78	n	—	2.51	90
Small intestine	1	8.05	.36	4.5	6.53	81
	2	5.10	.42	8.2	4.02	79
Plasma	1	2.28	.26	11.5	1.74	76
	2	1.25	.18	14.4	.92	74
		% of total dose	% of total dose	% of total recovery	% of total dose	% of total recovery
Small intestine content	1	3.31	.25	7.5	2.70	82
	2	1.95	.40	20.5	1.38	71
Urine	1	36.4	.55		31.8	
	2	59.7	1.55		55.4	

* tr = trace; n = negative.

and radioactive metabolites.

Results. Recovery of unchanged Priscoline and metabolites from tissues of treated animals. The total C^{14} content of tissues decreased rapidly and was excreted in the urine, as was previously reported(1), and most of the C^{14} in tissues and urine was recovered as unchanged Priscoline (Table I). The highest concentrations of C^{14} and unchanged Priscoline were found in liver and kidney tissues. Of significance is the increasing proportion of the 50% methanol or aqueous radioactive fraction found in liver and small intestinal contents. About 27% of the liver C^{14} was found in this fraction after administration of labeled Priscoline. A small but significantly increasing amount of the aqueous fraction was again found in the urine(1). In control studies, no detectable C^{14} was found in the 50% methanol fraction when labeled Priscoline was added to tissue homogenates and analyzed, so that all recoveries of C^{14} in this fraction in experimental tissues were considered significant. Small amounts of C^{14} in all samples analyzed were recovered as the chloroform extracted fraction which was separated chromatographically from Priscoline. In control analyses, about 1% of the added C^{14} was recovered as this fraction, and represented either contamination, impurities, or a small error inherent in the method. *Effects of rat tissues in vitro on radioactive Priscoline.* The ratios of concentrations of C^{14} in the tissue slices to those of the respective media were taken as a measure of the ability of the tissues to concentrate the radioactive materials from solutions containing labeled Priscoline. Liver and kidney slices took up averages of 20.2 and 26.8 mμc/g, respectively, which were 2.6 and 3.4 times the corresponding C^{14} concentrations in the baths. These were significantly higher ($p<0.05$) than similar tissue to bath C^{14} ratios for lung, spleen, muscle, and heart, with averages from

1.4 to 1.7. The media obtained following incubation with various tissues were analyzed for unchanged Priscoline and the two other radioactive fractions. From 90 to 95% of the C^{14} was recovered in the fractions as compared with the total C^{14} determinations. This was almost entirely unchanged Priscoline. The only finding significantly different from control analyses was the recovery of 1.4% of the total C^{14} in the bath as the 50% methanol fraction when liver slices were incubated aerobically with radioactive Priscoline. There was only a slight indication that kidney slices might be able to form this fraction. Small percentages of C^{14} were recovered as the chloroform extracted product, but no differences were seen from control analyses. There appeared to be no correlation between the Qo_2 of any tissue and its effect on Priscoline.

Under anaerobic conditions, liver slices incubated with radioactive Priscoline did not form the 50% methanol soluble fraction. However, the average tissue slice to buffer C^{14} ratio was about 3.5, indicating that the tissue retained its ability to concentrate the radioactive material. The average Qco_2 was about 2.5.

Liver, kidney, spleen, and heart homogenates were incubated with radioactive Priscoline for 1 hour. Four or more samples were run for each tissue. Analyses of the media indicated that no radioactive metabolic products were formed during this time. The average Qo_2 values for the homogenates were 19.4 for liver, 27.4 for heart, 53.3 for kidney, and 4.5 for spleen, based on a standard of 10 mg of wet weight tissue for 20 minutes.

Discussion. The results described here further indicate that the kidneys are the most important organs for the removal of Priscoline from the body(1,3). Analyses of various tissues and urine have shown that almost all of the administered drug is widely distributed in the body and excreted unchanged in the urine. A very small amount of the drug, however, appears to be metabolized by the liver to a product which is not extracted from alkaline aqueous solutions with chloroform, suggesting that the basic or alkaloid-like characteristics of Priscoline have been chemically lost. The appearance of increasing percentages of this 50% methanol soluble radioactive fraction in the liver ($p<0.05$) and small intestinal contents ($p<0.001$) and increasing amounts in the urine ($p<0.001$) suggest that it is slowly formed in the liver, excreted in the bile, reabsorbed by the intestine, and finally excreted in urine with the rest of the Priscoline. That the liver is capable of metabolizing Priscoline at a slow rate is further suggested by the recovery of a small amount of the same radioactive metabolic fraction following incubation of Priscoline with liver slices under aerobic conditions. Both intact liver tissue and oxygen appear to be necessary for the formation of this fraction. An oxidative degradation of the imidazoline ring is suggested by the loss of the chemically basic character of the molecule, and by the appearance of very small amounts of C^{14} in expired air(1). The positive inotropic action of Priscoline on the guinea pig heart and the inhibition of respiration of rat kidney slices have been reported as bioassay methods for determining concentrations of Priscoline in incubation solutions(4). Liver slices were reported to detoxify solutions of Priscoline at initial concentrations of 10 mg/ml. On the basis of experiments presented here, it appears that the decrease in biological potency observed by these investigators was almost entirely due to uptake of Priscoline by the liver slices, rather than to any metabolic action of the tissues.

Summary. Further studies on *in vivo* and *in vitro* metabolism of Priscoline are reported. Analyses of tissues, small intestinal contents, plasma, and urine of rats given radioactive Priscoline hydrochloride intravenously showed that almost all of the C^{14} was recovered as unchanged Priscoline both 1 and 2 hours after injection. Liver, small intestinal contents, and urine contained small but significantly increasing percentages of a 50% methanol soluble radioactive metabolite. About 27% of the C^{14} in liver was found as this fraction after 2 hours. When rat tissue slices were incubated with radioactive Priscoline under oxygen, liver and kidney took up the highest concentrations of C^{14}. Liver slices metabo-

lized small amounts of the drug to the 50% methanol soluble fraction under aerobic conditions, but not under anaerobic conditions. There appeared to be no correlation between Qo_2 values of tissues and their particular role in the disposal of Priscoline.

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Chemical and Hematological Studies on Blood of Bovine Dwarfs.*† (22531)

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Dwarfism in cattle has been reported since 1860 among many breeds of cattle in both *Bos taurus* and *Bos indicus*(1). Craft and Orr described an undersized Hereford steer with dwarf-like appearance and suggested this condition might be due to an underdeveloped thyroid and pituitary gland(2). A dwarf form morphologically similar to the preceding one has been studied more recently by various workers including Johnson, Harshfield and McCone(3), and Gregory and coworkers(4,5). This type of dwarf has a short broad head, an excessively bulging forehead, and marked prognathia. In addition to its small stature, it has a distended abdomen, a strong predisposition to bloat and breathes heavily. A premature fusion of the spheno-occipital synchondrosis in this dwarf type has been observed by Julian(6).

Due to the abnormal osteology in the dwarfs studied and their similarity phenotypically to mammalian cretins, this study of 38 "short-headed" dwarfs was initiated to understand more fully the pathological physiology present. Serum electrolytes and protein levels were run to aid in interpretation of osteological abnormalities. Cholesterol and protein-bound iodine determinations were used to assay thyroid function. Due to the lack of information on the hematology of the dwarf, blood studies were also included. The dwarfs were of both sexes and of both Hereford and Angus breeding.

Materials and methods. Samples were collected by jugular puncture prior to sacrificing for anatomical studies. All whole blood was collected in Heller and Paul's anticoagulant mixture for hematological studies. Standard methods were used for all blood counting procedures. Packed cell volumes were performed using Wintrobe's hematocrit tube. A Leitz Rouy Photometer was employed for hemoglobin determinations, using a wave length of 550 mμ, standardized by Wong's method for iron. Two hundred white blood cells were counted in all differential counts. Serum protein fractionations were carried out by the method of Wolfson, Cohn, Calvary and Ichiba(7) taking care to shake gently the span ether in the albumin determination. Saifer and Zymaris(8) have shown this prevents denaturation and approximates electrophoretic separation closely. Serum calcium was determined by the method of Clark and

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