mine and a synergistic effect on toxicity of serotonin in the mouse. (2) Heparin interferes with this compound 48/80 effect on lethal intoxication with histamine or serotonin by direct interaction with compound 48/80 but does not appear to alter individual toxicities of respective amines. (3) It is suggested that degranulation of mast cells in response to compound 48/80 is a protective function of connective tissue in which the releaser drug is sequestered by heparin of the mast cell. The associated release of amines is thought to be a manifestation of this interaction between mast cells and compound 48/80 and only indirectly related (if at all) to lethal effects of this drug.

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Rapid Sensitive Method for Determining H^3-Water in Body Fluids by Liquid Scintillation Spectrometry.* (25125)

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The assay of tritium water by liquid scintillation spectrometry is difficult for 2 main reasons: a) water is not efficient in transferring the excitation energy of tritium radiation to the phosphor; and b) water is insoluble in the known aromatic solvents (toluene and xylene) which are most effective in making this transfer. In the methods of Kinard (1), Hayes and Gould(2), and Okita *et al.* (3), alcohol is used to solubilize water with xylene or toluene. This results in considerable dilution of the H^3 in the water sample, thus limiting these methods to samples of tritium water with high radioactivity. Furst *et al.* (4) were the first to report that naphthalene can restore, to a large extent, the counting efficiency lost by addition of water to dioxane solutions containing tritium. This observation formed the basis of the more efficient assay developed by Langham *et al.*(5). A method for increasing still further the efficiency of counting tritium water in biological fluids is described here. Some of the conditions that influence the efficiency of the assay of tritiated water by the dioxane-naphthalene

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system have been studied. It is shown that the sensitivity of H^3-water measurements in biological fluids can be greatly augmented by a preliminary distillation of the water in these fluids with benzene. The method evolved is simple in operation, and does away with the need for internal standards(6). It is capable of detecting about 5.7 x 10^{-5} μc of H^3-water in almost all tissues.

Experimental. A Packard Tri-Carb Model 314 spectrometer was used for H^3 determinations; counting was carried out with a discriminator window setting of 10-90, a voltage of 1340 volts, and a deep-freeze setting of + 3°C. Water was removed from reagent grade benzene by distilling it until the distillate became clear. The benzene remaining in the distilling flask was dry enough for the present experiments. The dioxane and naphthalene were Eastman Kodak products (catalogue Nos. 2114 and 168, respectively), and they were used without further purification. 2,5-diphenyloxazole (PPO), 1,4,-bis-2(5-phenyloxazolyl)-benzene (POPOP), and α-naphthylphenyloxazole (NPO) were purchased from Arapahoe Chemical Inc., and were of scintillation grade. The H^3-water was obtained from the National Bureau of Standards.

Results. Conditions affecting H^3-counting efficiency of dioxane solution containing various amounts of H^3-water. Optimum conditions for liquid scintillation counting of 1-, 2-, and 3-ml samples of water, each containing the same amount of H^3 and each made up to a total volume of 15 ml with dioxane (6.6, 13.6, and 20% of H^3-water in dioxane, respectively), were determined by studying the effects of varying concentrations of naphthalene, PPO, POPOP, and NPO on the counting rates. The results are given in Table I. Exp. 1-3 show that, for 20% solutions, the maximal permissible concentration of naphthalene is about 116 g/liter. At higher concentrations the solutions separate into 2 layers. Since little difference was observed in counting rates of Exp. 1 and 2, the conditions of the former were chosen for further study. Increasing the concentration of PPO decreased the counting rate (Exp. 4). Exp. 5-8 indicate that NPO is inferior to POPOP as a secondary phosphor or spectrum shifter(6). The use of higher concentration of POPOP (0.25 g/liter) seems warranted because of higher counting rate in Exp. 8 than in Exp. 6. The most efficient system for counting 20% H^3-water in dioxane is provided by conditions shown in Exp. 8. Exp. 9 and 10 indicate that smaller volumes (10 ml and 5 ml, respectively) of H^3-water can be assayed without loss of counting efficiency, a finding in agreement with results of Davidson and Feigelson (7). However, the conditions of these 2 experiments are not recommended for counting volumes smaller than 3 ml because, as shown in Exp. 11-19, more efficient conditions were found. For example, in Exp. 13 and 19, counting efficiencies of 11 and 15% were found for solutions containing 13.3 and 6.6% of H^3-water, respectively. The conditions used in these 2 experiments are therefore recommended when less than 3 ml of water is available for counting. By reducing the amount of each constituent shown in Exp. 19 by one-third, as little as 333 μl of H^3-water can be counted with an efficiency of 15%. The data of Table I were obtained by using pure H^3-water-dioxane solutions. Similar results were to be expected with water of body fluids provided the water could be isolated in sufficiently pure form. In their studies on the H^3-water turnover in rats, Thompson and Ballou(8) used a Dean-Stark trap for collecting water during distillation of a tissue with benzene. We also found that almost pure H^3-water can be obtained from plasma, blood, and urine by distilling them with dry benzene.

Method for determination of H^3 content of 1-3 ml samples of water distilled from body fluids. Three-ml sample. Fifty ml of dry benzene and the tritiated sample to be analyzed (blood, plasma or urine) were added to a 100-ml, round-bottom flask. A Dean-Stark trap, provided with a calibrated collecting tube, a stopcock, and a condenser fitted with a drying tube, was attached to the flask. The given mixture was refluxed until about 3.5 ml of water had collected in the trap; the water was drained through the stopcock into a stoppered test tube, and kept at room temperature for at least 1 hour (preferably 2) to permit minute amounts of benzene in the distillate to

TABLE I. Electrolyte and Water Content of Mesenteric Arteries.

	Na	K	Na + K	Cl	H_2O
Hypertensive rats					
Mesenteric arteries	54.8 12.5	25.1 5.2	80.1 18.0	23.5 5.2	250.5 54.1
Serum	142.0 2.7	3.9 .7		97.2 2.1	
Control rats					
Mesenteric arteries	44.7 8.4	21.8 4.3	66.0 16.2	18.8 6.1	200.8 48.3
Serum	142.5 1.1	5.0 .6		100.7 2.5	

Values are in meq/100 g of dry fat-free solids and per liter of serum. H_2O in g. Figures below each value are stand. dev.

then across superior mesenteric artery. The vessels showed no gross abnormality. The arteries were analyzed for sodium, potassium, chloride, and total water. The tissues were dried at 105°C, ground, and defatted with mixture of ethyl ether and petroleum ether. The fat-free dry solids were then extracted with 10 cc of normal nitric acid. All procedures were carried out in the original tubes. Sodium and potassium were determined by standard flame photometry. Chloride was determined by Volhard's titration method(5). Data on mesenteric arteries were obtained in 20 hypertensive rats and in 20 control rats.

Results. The animals with renal ligation were in good condition when sacrificed. They were all hypertensive with systolic blood pressures generally in 170-190 mm Hg range. Control rats were normotensive.

Values for sodium, potassium, sodium plus potassium, chloride, and water in the mesenteric arteries are shown in Table I along with levels of serum electrolytes. Combined cation values were derived from the mean of sums of sodium and potassium concentration for each rat.

In comparison with normal rats, the mesenteric arteries of hypertensive animals showed elevation of sodium, potassium, chloride, and total water. The increase was statistically valid in each instance. Sodium plus potassium content, expressed as meq/100 g of dry fat-free solids was also increased to statistically significant degree. However, the combined cations showed no significant change over the normal in terms of concentration in tissue water.

Extracellular sodium was calculated by using chloride space as a measure of extracellular fluid according to the method of Hastings and Eichelberger(6). Values obtained in this way indicated that extracellular sodium accounted for only part of the total rise of this cation, and hence that the intracellular phase contained more sodium. However, it is doubtful that chloride space is an adequate measure of extracellular volume in the mesenteric vessels. Further comment on this point is given below.

Serum sodium, potassium, and chloride in hypertensive rats showed reduction from normal levels. This was statistically valid in each instance.

Discussion. Our technic for obtaining mesenteric arteries eliminated perivascular fat almost completely, but did not remove adventitia of blood vessels. Hence, while the bulk of material taken for electrolyte analysis was probably smooth muscle, there was also a substantial amount of connective tissue. In reference to man, the vessels in the rat correspond to large arteries but they also include smaller arteries, *i.e.* terminal branches of superior mesenteric tree extending to bowel wall, and these would be comparable to vessels about 1 mm diameter in the human. Also there are numerous arterioles in the adventitia of larger mesenteric vessels.

Previous information on electrolyte composition of vessels in hypertensive rats has apparently been limited to the aorta(7). However, this structure is not concerned in the origin of hypertension nor does it participate in characteristic lesions observed so frequently in the more distal vascular bed of hypertensive rats. In contrast, mesenteric arteries of the rat are a relatively early and prime site of such disease. Hence electrolyte alterations here are apt to reflect similar changes of small arteries and arterioles throughout the body.

Our data show that there was accumulation of sodium in the mesenteric arteries of rats with acute renal hypertension. This was accompanied by rise in potassium and chloride and also by expansion of water. The last

was sufficient in magnitude so that total cation concentration in tissue water of the hypertensive rats did not differ significantly from that in control animals.

It was not feasible to partition sodium and potassium into extracellular and intracellular fractions through use of chloride space. Sometimes the extracellular fluid volume calculated on this basis in both experimental and control animals left only a very small fraction of tissue water to be assigned to cell water or even resulted in negative cell water concentration. There is some doubt in general as to whether chloride space can properly be applied to smooth muscle and to structures rich in connective tissue(8). Since the extracellular fluid space of blood vessels analyzed in this study was in question, increase in intracellular sodium and potassium in arteries could not be established even though this is quite probable.

An interesting item is the relatively high standard deviation for mean content of sodium and tissue water in the mesenteric arteries. This holds for both normal and hypertensive animals, especially the latter, where the deviation increases along with rise of mean values for sodium and water. It does not apply to potassium or chloride levels. The variability of sodium and tissue water in mesenteric arteries suggests that they constitute a labile system in this location.

The information on electrolyte composition of tissues in hypertensive animals is relatively scant. Moreover there are differences of opinion in regard to both the nature and significance of the data. In Grollman's study(9) no significant change from the normal was observed in electrolyte composition of various organs, including heart of rats rendered hypertensive through choline deficient diet. Using inulin to measure extracellular volume, Ledingham(10) found no significant change in intracellular concentration of either sodium or potassium in hearts of rats with acute renal hypertension and believed that the increase in total sodium content was explained by expansion of extracellular fluid. Tobian and Binion(7) reported an increase in sodium and potassium content of the aorta in hypertensive rats and, through use of chloride space, calculated that not more than one-third of the increase in sodium concentration resulted from elevation in extracellular sodium. Greene and Sapirstein(11) found a substantial rise in total body sodium of rats with renal hypertension and believed that this could not be explained by expansion of extracellular volume. They postulated either deposition of sodium in bone or an increased amount of sodium in intracellular position.

It appears that changes in electrolytes and water which we observed in mesenteric vessels were a direct consequence of the kidney damage employed to induce hypertension. However, the precise mode of origin is not certain. Although the renal injury was severe, it did not result in significant azotemia. Renal dysfunction, resulting in retention of salt and water, is one possible mode of origin. Others include injurious agents of toxic or pressor nature derived from the injured kidneys and acting on the vessel walls. Our study did not encompass these items. Perhaps the electrolyte changes within the arteries were merely incidental to altered renal function and not causally related to the high blood pressure. An alternative is that they played a role in the origin of hypertension by creating an ionic disequilibrium which enhanced vasomotor tone.

Summary. 1) Mesenteric arteries of rats with acute renal hypertension were analyzed for electrolyte and water content. A technic is described for obtaining these vessels. 2) Arteries showed a significant rise in sodium, potassium, and chloride, as well as expansion of total water. However, total cation concentration in tissue water was not significantly altered. 3) Electrolyte changes may have resulted from renal dysfunction associated with retention of salt and water. Whether they were incidental to the hypertensive state or played a role in its genesis is not established.

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Intracellular Distribution of Vit. B_{12}-Co^{60} in Liver and Kidney of B_{12} Deficient and Normal Rats.*† (25127)

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Results of microbiological assays of fractions of mouse liver(1) indicated that Vit. B_{12} is concentrated in mitochondria. Kidneys of rats(2) contained most radioactivity 96 hours after parenteral administration of B_{12}-Co^{60}. At 48 hours(3) most radioactivity was present in supernatant fraction of kidneys in bound form, and measurements at various time intervals after administration of B_{12}-Co^{60} (4) showed that kidneys progressively lose radioactivity. Intracellular distribution of radioactivity of administered B_{12}-Co^{60} in livers of rats(3,5,6) and of $cobalt^{60}$ in organs and intracellular organelles of mice(7) was studied. The relationships of amounts of Vit. B_{12} in fractions of kidneys and livers of normal and deficient animals at different time intervals after administration of vitamin has not been studied. We studied the intracellular distribution of B_{12}-Co^{60} and $cobalt^{60}$ in kidneys and livers of normal and B_{12} deficient rats at intervals after the last injection of radioactive compounds.

Methods. Rats of the St. Louis University colony were fed soybean meal diet of Hogan *et al.*(8) for production of deficiency of B_{12} and soybean meal diet with 90 μg Vit. B_{12}/kg of diet or Purina lab chow for control animals. Rats fed these diets for 8 to 10 weeks received 1 or 5 injections (1 μc each injection at 24 hour intervals) of $Co^{60}Cl_2$ or high specific activity B_{12}-Co^{60}.‡ At intervals after last injection, animals were sacrificed and samples of liver and kidney excised, weighed, homogenized in cold 0.88 M sucrose, and cellular components separated by differential centrifugation(9). Microsomal fractions were prepared by centrifuging 1 hour at 100,000 x g in a Spinco Model L centrifuge. Each sedimented fraction was washed twice with sucrose and the sediment suspended in 5 ml of sucrose for each gram-equivalent of original tissue used. There was good agreement between cts/min§ of minced tissue, homogenates and the sum of cts/min of the fractions. Radioactivity recovered in fractions was 88% to 102% of that in homogenates. Nitrogen was determined by micro-Kjeldahl procedure.

Results. Table I shows distribution of nitrogen in fractions of liver and kidney of normal and deficient animals. Normal group contains data on animals fed laboratory chow (Table II) and soybean meal diet supplemented with 90 μg of B_{12} (Table III). Separate treatment of data on nitrogen distribution showed no difference between groups so the data were combined. Nuclear and mitochondrial fractions of liver and kidney of deficient animals contain more nitrogen than these fractions of normal animals. Conversely, microsomal fraction of tissue of normal animals

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§ Radioactivity was counted with a Tracerlab SC-57 low background well scintillation counter and an SC-18A scaler. cts/min = counts/min.