Whether these nucleotides are associated with some inert material from which they are removed with difficulty or whether they represent some polymerized form of these compounds, or both, we are not prepared to say at this time.

Summary. A procedure has been described for the isolation of adenosine triphosphate from barium-nitric acid insoluble residues prepared from whelk foot muscle and rabbit striated muscle. The ATP so isolated is indistinguishable physiologically from ATP prepared according to the standard procedure of Needham but is only slightly soluble in strong acid. It is present in the smooth muscle of the whelk in much larger amounts than in striated rabbit muscle. Treatment of a similar residue from beef heart yielded a compound which chemically and physiologically resembled ADP.

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Effect of Intravenous Digitoxin on Fluid Distribution in Hospitalized Males without Cardiovascular Disease.* (18451)

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(Introduced by G. T. Harrell)

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Although the actions of digitalis on the heart are well known, its effect on the distribution of body fluids in normal individuals have not been reported. Because the cardiac glycosides are similar in chemical structure to other cyclopentenophenanthrene derivatives which are known to affect salt and water metabolism—for example, the sex hormones and the adrenal cortical hormones—they might be expected to produce some alteration in the distribution of fluids in the body.

The present study was conducted in order to determine the effect of intravenous digitoxin on the plasma and extracellular fluid spaces in normal individuals.

Material and Methods. Subjects. Serial studies were performed on 5 male patients who had been hospitalized for non-cardiovascular disorders and who had no obvious disturbances of salt and water metabolism. These individuals were ambulatory and were on a house diet. No attempt was made to control their intake of salt and water. *Material.* Commercial crystalline digitoxin, prepared from Digitaline nativelle and dissolved in a 40% solution of alcohol, was used in all cases. The solution contained 0.2 mg of digitoxin per ml of fluid. *Methods.* The methods which were used to determine the *hematocrit,* the *plasma volume* and the *thiocyanate space* have been described previously (1). In preliminary experiments it was demonstrated that the thiocyanate ion apparently reached a state of equilibrium in the extracellular fluid space 3 hours after injection(2); the 3-hour values, therefore, have been used for calculations and comparisons. Since the total volume of blood withdrawn for each complete study did not exceed 40 ml, the blood loss would not have affected the circulating blood volume appreciably. The *serum protein concentration* was determined by the Kingsley biuret method(3). This value and the plasma volume were used in calculating the *total circulating protein* content.

Plan of the experiment. A baseline deter-

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mination of all the quantitative measurements described above was made on each individual at least a day prior to the administration of digitoxin, and the subject was weighed at this time. After completion of the baseline studies, a total of 1.2 mg of digitoxin was administered intravenously to each patient. This amount was divided into 2 equal doses of 0.6 mg each, given one hour apart. Five hours after the first injection of digitoxin, venous blood was withdrawn and the solution containing T-1824 (Evans Blue) and sodium thiocyanate was injected intravenously. The plasma and total blood volumes were measured on samples drawn 10 minutes later. The thiocyanate space was calculated from a sample drawn 3 hours later (8 hours after the initial injection of digitoxin). The total serum protein concentration was determined on samples drawn 5, 7, and 8 hours after the first dose of glycoside; and the hematocrit, on the samples drawn at 5 and 8 hours. In order to determine whether the alterations in fluid distribution were reversible, the entire battery of tests was repeated 72 hours after the injection of digitoxin.

Method of statistical analysis. Each patient served as his own control. In order to test the significance of the results, the differences between the baseline value and the values observed at the specified time intervals after the administration of digitoxin were determined for each individual. The mean difference and the standard error were then calculated; from these values, "t" was obtained.

Results. (Table I). A progressive decrease in the mean serum protein concentration during the first 8 hours was found. The decrease at 8 hours (0.75 g per 100 cc) was statistically significant ($t = 8.4$). The mean values for the total circulating protein content before the administration of digitoxin, and 5 and 72 hours after its administration were 215, 235, and 200 g, respectively. The increase of 20 g at 5 hours was significant ($t = 4.95$, $P = < 0.01$).

A mean increase of 381 ml in the plasma volume and of 348 ml in the total blood volume (each roughly 10% of the baseline value) was found at 5 hours; the values at 72 hours were lower than the baseline values in 4 of the 5 subjects. The mean increase in plasma volume at 5 hours was at least suggestive ($t = 2.98$).

TABLE I. Comparison of the Baseline and 5′ Plasma Volumes.

	Baseline	5′	d
Re	3272	3841	+569
Le	3492	3966	+474
Ca	3217	3841	+624
Br	2683	3004	+321
Be	2683	2596	— 87
		Sum of d	+1901
		Mean diff.	—380.2

$$\frac{Sd^2 \quad 1{,}048{,}420}{\frac{(Sd^2)}{n} \quad -722{,}760}$$

$$325{,}660$$

$$\frac{Sd^2-\frac{(Sd^2)}{n}}{n(n-1)} \quad \frac{325{,}660}{20} = 16{,}280.$$

$$\text{Square root} = S_d = 128.0.$$

$$t = \frac{380.2}{128.0} = 2.98.$$

The decrease in the mean hematocrit value was not significant at either time interval. The decrease in thiocyanate space at 72 hours, although of considerable magnitude, was not significant. No change was observed at 5 hours. No significant alterations in body weight were noted during the period of observation, although there was a slight decrease at 72 hours.

Comment. The results of the present study suggest that changes occur in fluid distribution when 1.2 mg of digitoxin is given intravenously to individuals without cardiovascular disorders. A significant decrease in the concentration of serum proteins and an increase in the mean total circulating protein content, associated with a suggestive increase in the mean plasma volume and a decrease in the mean hematocrit, indicate that there is either (1) a primary shift of water and a secondary mobilization of labile protein into the vascular compartment or (2) a primary shift of protein containing fluid into the vascular compartment. The maximum dilution

of the blood proteins appears to have occurred at 8 hours. Unfortunately, the experiment was so designed that the plasma volume was not measured at that time.

It is known that in congestive heart failure the onset of diuresis is preceded by a fall in the specific gravity of the plasma, presumably due to an increased hydration of the blood plasma(4). The resultant increase in blood volume is thought to be responsible for the diuresis. A similar mechanism may have been in operation in the present study; this would explain the decrease in the thiocyanate space and in the body weight observed at 72 hours.

The changes which were observed in the fluid content of the vascular compartment may have been due to a primary decrease in cardiac output, with a consequent decrease in capillary pressure. However, a decrease in the venomotor tone, an increase in the interstitial fluid pressure, or both, may also cause the changes noted. Whether the results were due entirely to the glycoside's effect on the heart or whether there was also an extracardiac effect is not known. A more detailed study of the problem is now in progress.

Summary. The intravenous administration of 1.2 mg of digitoxin to 5 male subjects with no cardiovascular disorder was found to cause a significant decrease in the serum protein concentration and an increase in the total circulating protein content within 8 hours. The results were interpreted as indicating an increase in the water and protein content of the vascular compartment.

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Xanthine Oxidases in Different Species.* (18452)

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It has been assumed tacitly that the xanthine oxidase activities demonstrated in different organs from various species(1) were due to the same enzyme, and that this enzyme was similar or identical with the better-known milk xanthine oxidase. A previous study(2) showed that the enzyme occurring in rat liver was different from milk xanthine oxidase inasmuch as only the former could be inhibited by antabuse.† This antabuse inhibition was overcome by the addition of methylene blue to the aerobic system, or by heating the liver homogenate at 56° for 30 minutes. The latter treatment destroyed some of the original aerobic activity of the enzyme, but its dehydrogenating ability was not affected. This evidence was interpreted(2) as showing that xanthine oxidase occurred in rat liver as a complex of two distinct enzyme activities (dehydrogenase and oxidase) closely associated with some other unidentified constituent that conferred antabuse sensitivity upon the complex.

This report describes the application of the same techniques to other selected tissues in order to determine whether "xanthine oxidase activity" is uniformly due to the same kind of enzyme. Ignoring minor differences for purposes of generalization, the xanthine oxidase of mammalian tissues was similar to the rat liver enzyme. The xanthine oxidase of bird tissues, however, was fundamentally a dehydrogenase, since it was nearly devoid of autooxidizable characteristics. Evidence is

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