In monkeys given a large amount of the drug, the number of cells affected is approximately the same as the total number of red cells. With smaller doses, the effect is proportionally less. Webster(1) comments on the fact that supravital preparations have "the advantage that the smaller Heinz bodies can be seen and thus the beginning stages of the phenomenon can be detected and followed." It should be remembered that a red cell affected by phenylhydrazine to such an extent that a Heinz body forms is also most likely to be affected to such a degree that either the cell or a portion of it will persist in distilled water and thus be counted in the total number of Heinz bodies. This technic could be used in routine clinical hematologic examinations and, if proven satisfactory, would be of value when drug or industrial poisonings are suspected.

Summary. Heinz bodies have been demonstrated in the blood of monkeys following the oral administration of phenylhydrazine hydrochloride. These bodies disappear from the circulating blood in normal monkeys more rapidly than they do from the blood of splenectomized animals. A quantitative method for determining the number of Heinz bodies has been described. This method is based upon the observation that Heinz bodies are not hemolysed in distilled water. A comparative study is made of the number of Heinz bodies free in the plasma in blood smears stained with a combination of Wright's and Giemsa's stains with those remaining after treatment with distilled water. The latter technic is both simple and rapid.

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Adenosine Triphosphate from Barium-Acid Insoluble Residues. (18450)

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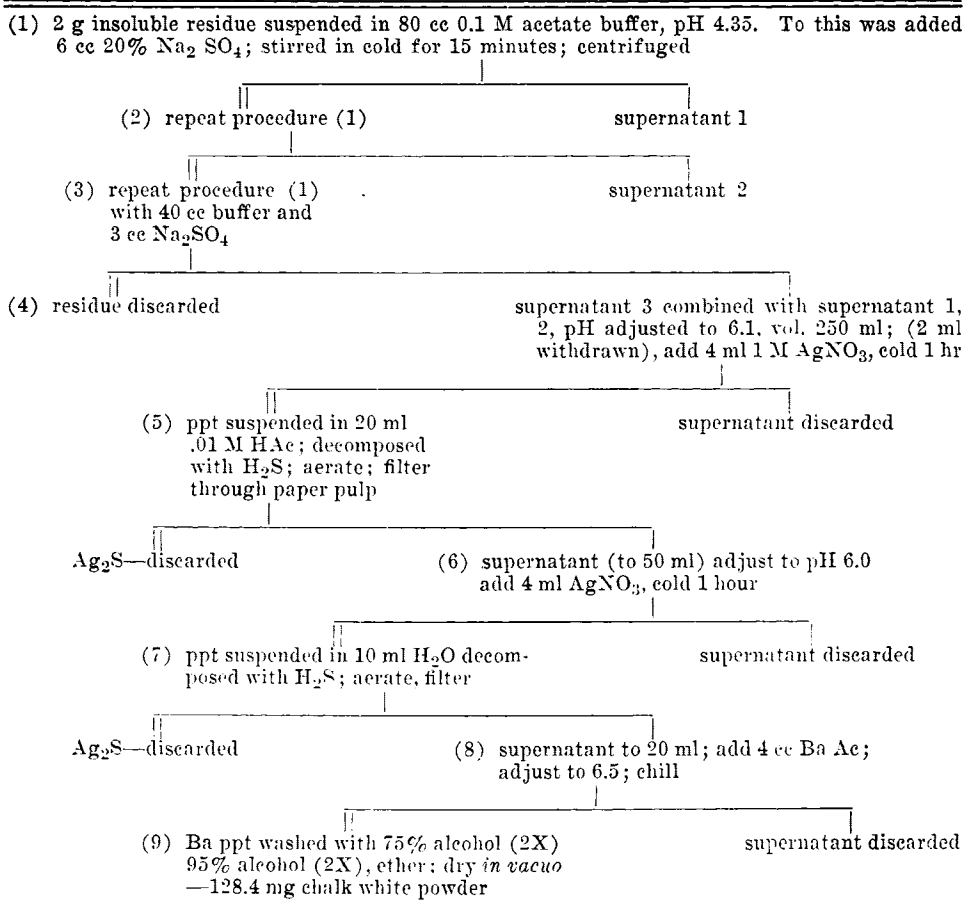
The purpose of this paper is to demonstrate that the customarily discarded barium-acid insoluble residues obtained in the classical methods for the extraction of adenosine triphosphate may contain considerable amounts of a Ba ATP which differs markedly in solubility from ordinary ATP and to outline a procedure for its isolation. We first became interested in these residues following a relatively unsuccessful attempt to prepare ATP from whelk muscle by the method of Needham(1). The yield from the barium-acid soluble fraction of this material was disappointingly low while the acid insoluble residue was many times larger than the corresponding fraction obtainable from mammalian muscle. The procedure reported here, originally worked out for dealing with the whelk residue, has also been applied to the much smaller residues from rabbit skeletal muscle and beef heart with qualitatively similar results.

1. Needham, D. M., *Biochem. J.*, 1942, v36, 113.

Methods and Results. From approximately a bushel of whelks (Busycon sp?), obtained from a local fish market, 4.082 kg of foot muscle were removed. The muscle was homogenized in Waring Blendors in cold 10% trichloracetic acid, filtered through cheese cloth, and reextracted with an equal volume of cold 5% T.C.A. The filtrates were combined and centrifuged in a Sharples centrifuge. The residue was discarded and the supernatant fluid adjusted to pH 8.2 with concentrated NaOH. A precipitate formed which was centrifuged out and discarded. The cloudy supernatant was treated with 1 volume of cold ethyl alcohol; the precipitate which came down was removed by centrifugation and to the supernatant solution (12.6 liters) was added another 6.3 liters of alcohol; no additional precipitate was formed. The clear supernatant fluid (approximately 18.0 liters) was treated with 300 cc of 25% barium acetate, and placed in the cold room over night. The next morning the barium pre-

TABLE I.



cipitate was collected, and suspended in H_2O; after centrifugation, the precipitate remaining was once more suspended in H_2O. The final precipitate obtained corresponds to the barium insoluble, alcohol insoluble fraction from which adenosine triphosphate is generally isolated.

This precipitate was homogenized and washed in 4 successive 300 cc aliquots of 0.2 N HNO_3. When rabbit muscle is used, most of the barium precipitate goes into solution, and a small residue remains which is discarded. In this case, however, the precipitate remaining was considerable; this was washed successively with barium acetate, alcohol, and ether and dried *in vacuo;* 6.3 g of a dry white powder were obtained. The material which had gone into solution in the nitric acid was treated with Lohmann's reagent and eventually precipitated as the barium insoluble salt; it corresponded to 359 mg of dibarium adenosine triphosphate, the molar ratio of total to labile phosphorus to adenine was 3.00:2.00:1.05.

Because of the low yield of adenosine triphosphate in the procedure described above, the nitric acid insoluble residue was reexamined. When small quantities of this material were suspended in H_2O, and the pH lowered to 1.0 little or none appeared to go into solution; after centrifugation the supernatant solution contained only traces of adenine as measured spectrophotometrically. The procedure outlined in Table I was therefore devised in an attempt to isolate additional material. In principle the method depends on bringing the nucleotides into solution by exchanging sodium for barium in suspensions

containing an excess of sodium sulfate, and then precipitating them as the silver salts. This method was first used as one of the purification steps in the isolation of adenosine triphosphate from plant tissues(2) where similar difficulties in purification were encountered.

By this procedure from 6.3 g of the insoluble residue 404 mg of a chalk white barium salt could be isolated. Analysis of this barium salt yielded the following molar ratio: total phosphorus : labile phosphorus : adenine:pentose, 3.02:1.86:1.07:1.00; purity based on pentose, 90% (calculated as Ba_2 ATP. $4H_2O$); inorganic P—none. Adenine was identified spectrophotometrically from the sharp absorption maximum at 2600 Å. In the orcinol pentose reaction(3) 74.0% of the color was developed in 7 minutes, as compared with 74% for vertebrate ATP. No free adenylic acid could be detected spectrophotometrically by the method of Kalckar using a muscle deaminase(4). The compound was indistinguishable in its behavior from ordinary vertebrate ATP in its ability to donate phosphorus to glucose in the yeast hexokinase system(5). (Table II).

This compound, however, was not readily soluble in water, even at pH 1 and had to be brought into solution by sodium exchange. When chromatographed on paper with a developer composed of 0.1 M malonate buffer at pH 6 and isoamyl alcohol, it appeared to be identical with ordinary ATP although it was evident that a very small amount of adenylic acid was present as a contaminant. But when the malonate buffer was replaced with a 0.1 M solution of barium acetate buffered at pH 4.7 with acetic acid, a marked difference in behavior was noted. With the latter developer, adenylic acid and ordinary ATP migrate at essentially the same rate and form sharply defined spots, while the compound prepared from whelk muscle leaves a long streak on the paper extending all the way from the starting point to the same level as that attained by the other compounds. This is taken to indicate that even in this relatively purified form, the solubility of the barium salt of the whelk compound differs from that of the usual mammalian preparation.

TABLE II.
Ability of Whelk and Vertebrate ATP to Donate Phosphorus to Glucose in the Yeast Hexokinase System, as Measured Spectrophotometrically. Decrease in density at 265 λ at the end of time in minutes.

		3	7	11	15	18
Sample						
A	Whelk ATP	.024	.058	.087	.105	.125
B	Vertebrate ATP	.024	.058	.084	.108	.122
C	Whelk ATP					+.005

Samples A and B each contained ATP, 0.5 ml 0.1 M malonate buffer pH 5.9, 0.1 ml $MgCl_2$ (10 mg/ml), 0.02 ml myokinase (containing 4 μg protein N), 0.1 ml 0.5 M glucose, 0.02 ml yeast hexokinase (containing 7 μg protein N), 0.03 ml adenylic acid deaminase (containing 15 μg protein N), and water to 5.0 ml. Sample C contained whelk ATP, malonate buffer, adenylic acid deaminase and water.

The same procedure was used on a nitric acid insoluble residue obtained from rabbit muscle and beef heart. From 159 mg of the rabbit residue was isolated 31.4 mg of a white barium salt which was indistinguishable from ordinary rabbit ATP. The molar ratio of total phosphorus : labile phosphorus : adenine : pentose was 3.00 : 2.00 : 1.06 : 1.00; purity based on phosphorus 83%. As in the case of the whelk, the compound contained no free adenylic acid and transferred its phosphorus to glucose in the yeast hexokinase system. By the same procedure, from 2.14 g of nitric acid insoluble residue from beef heart, was isolated 25.3 mg of a yellowish barium salt. The molar ratio of total phosphorus : labile phosphorus : adenine was 2.04 : 1.02 : 1.00, suggesting an ADP, rather than an ATP. That this compound was indeed an ADP is indicated by the observation that it is not deaminated directly by muscle deaminase, but is deaminated after first being treated with myokinase according to the method of Kalckar.

2. Albaum, H. G., Ogur, M., and Hirshfeld, A., *Arch. Biochem.*, 1950, v27, 130.

3. Albaum, H. G., and Umbreit, W. W., *J. Biol. Chem.*, 1947, v167, 369.

4. Kalckar, H. M., *J. Biol. Chem.*, 1947, v167, 461.

5. Albaum, H. G., and Lipshitz, R., *Arch. Biochem.*, 1950, v27, 102.

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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