treatment with x-rays alone, but in mice pretreated with cysteine this depression of leukocytes was only temporary and followed by increase in cell number. This increase was lacking in mice pretreated with high doses of cysteine. These results suggest that small doses of cysteine stimulate and high doses depress some cellular metabolic processes, perhaps, the activity of hematopoietic tissue as suggested by Cronkite(5,6). Since intraperitoneal route was most efficient for protection of mice by small doses of cysteine and for inducing their death by high doses, it may be presumed that some abdominal organs are the site of action of cysteine.

It follows from the experiments of the Series D that pretreatment with small doses of cysteine acted in internally (intraperitoneally) irradiated mice in a way similar to the effect of high doses in externally irradiated mice *i.e.*, it reduced their life span. It is reasonable to presume that this phenomenon is effected by a similar mechanism, *i.e.*, the increased vascular permeability of irradiated tissues enables cysteine to reach some vitally important tissues faster and in higher concentration.

Conclusions and summary. 1. CFW and dba mice were pretreated with small (125 and 250 mg/kg) doses of cysteine (pH 1.0 or 6.5) before irradiation with lethal doses of x-rays (600 r). It was found that 10 to 14 days after irradiation the percentage of surviving animals was consistently higher in cysteine pretreated group than in irradiated not pretreated controls. These results illustrate "protective" effect of cysteine against radiation. 2. In new mice prepared with high doses (more than 800 mg/kg) of cysteine the x-ray treatment was followed immediately by death or agony. In mice treated by one of these factors alone, the condition was affected only after several days. Thus, the rapid lethal effect of combined treatment with cysteine (high doses) and x-rays is interpreted as potentiation of one factor by the other. 3. This effect of high doses of cysteine was absent in x-ray treated mice possessing copious peritoneal exudate due to the growth of free S-37 cells in the peritoneal fluid. This failure of cysteine is attributed to inactivation (oxidation) of cysteine by some factor of the peritoneal fluid. In these mice with exudate pretreated with small doses of cysteine, the decrease in the cell number (in the exudate) induced by irradiation was followed by a steady increase, while in irradiated controls the decrease continued until their death. 4. Pretreatment with small doses of cysteine (intravenously) had unfavorable effect on survival of mice injected (*i.p.*) with radioactive colloidal gold. 5. An explanation of the above phenomenon as manifestation of cysteine penetration from blood stream into tissues after increase of blood vessels permeability by high doses of irradiation is offered.

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Use of N-Acetyl 4-Aminoantipyrine (NAAP)* in Measurement of Total Body Water. (18928)

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Previous studies have demonstrated that antipyrine is a satisfactory substance for use in the estimation of total body water(1). Antipyrine, however, does not entirely fulfill

* N-acetyl 4-aminoantipyrine may be purchased from the special chemicals division, Winthrop-Stearns, Inc., 1450 Broadway, New York City.

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the requisites of an ideal agent to use for the measurement of body water. It is bound to plasma proteins to the extent of about 10%. Compensatory binding in tissues, presumably, accounts for the fact that the substance diffuses throughout the body in close proportion to the water content. Again, a correction must be applied for the antipyrine metabolized during the period of measurement. This involves the analysis of three consecutive plasma levels.

In the study of the intermediary metabolism of aminopyrine (4-dimethylaminoantipyrine) a metabolite, N-acetyl 4-aminoantipyrine (NAAP), was found which also distributes in tissues in proportion to their water contents (2). In contrast to antipyrine, NAAP is negligibly bound to plasma proteins. Since its rate of metabolism is essentially nil, its volume of distribution may be calculated from a single plasma sample and a urine sample. Furthermore, the determination of NAAP does not require an ultraviolet spectrophotometer. For these reasons, it is believed that NAAP has certain advantages over antipyrine in the estimation of total body water.

Chemical methods. NAAP in organ tissues is estimated by a procedure previously described(2). NAAP in plasma is assayed in the filtrate after zinc hydroxide deproteinization. The urine (generally diluted 1 to 50) is handled similarly. NAAP is hydrolyzed in acid and the resulting 4-aminoantipyrine is estimated by diazotization and coupling with alpha naphthol.

Estimation of NAAP in plasma and urine. To 2 ml of plasma or diluted urine (containing 20 to 60 μg of NAAP) in a 50 ml flask, add 3 ml of water and 2 ml of zinc reagent (100 g of $ZnSO_4 \cdot 7H_2O$ and 40 ml of 6N sulfuric acid, diluted with water to 1 liter). Add 2 ml of 0.75N NaOH dropwise with continuous swirling of the flask and shake for a half minute. After 10 minutes, transfer to a test tube, and centrifuge. Transfer 4 ml of the clear supernatant fluid to a colorimeter tube and add 1 ml of 2.5 N HCl. Cover the tube with a glass marble to prevent evaporation and heat in a boiling water bath for 30 minutes. Cool and add 0.5 ml of 0.2% sodium nitrite solution and place in refrigerator for 10 minutes. Then add 0.5 ml of 1% ammonium sulfamate solution and mix. Wait 3 minutes and add 0.1 ml of a 5% solution of resublimed alpha naphthol in absolute alcohol, followed by 1 ml of 4N NaOH. Let stand for about 5 minutes and read at 490 mμ in a photoelectric colorimeter. A reagent blank run through the procedure is used for the zero setting.

Standards are prepared by taking 4 ml of a known solution of NAAP (5 to 10 μg per ml), adding acid, and hydrolyzing and colorizing as with the unknowns. Standards should be run concurrently with the unknowns since there are slight daily variations in color intensity.

NAAP added to plasma in amounts from 15 to 30 μg per ml has been estimated with an average recovery of 101.5% with a standard deviation of 2.4% (112 recoveries).

Results. Distribution of NAAP in body tissues. Two dogs were given NAAP intravenously. Four hours later, they were killed by an intravenous injection of air. The tissue concentration of NAAP was measured and calculated in terms of tissue water. Tissue water was determined by drying at 95-100°. The concentration of NAAP in the water of the tissues was almost identical with that of plasma water (Table I). These results indicate that NAAP achieves a uniform concentration in all the fluid compartments of the body.

The extent to which NAAP is bound to nondiffusible constituents of plasma was determined by dialysis against isotonic phosphate of pH 7.4 at 37° for 20 hours. The results indicated binding of less than 3%.

Urinary excretion of NAAP. One gram of NAAP was administered intravenously to each of 6 normal human subjects. Forty-eight hour urine collections showed a recovery of NAAP which averaged 92% (range 82 to 100%). Thus the disappearance of NAAP from the body can be almost entirely accounted for by its renal excretion and only a minor fraction of the compound is metabolized over a period of 3 hours (0-15 mg of a 1 g dose).

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TABLE I. Distribution of N-Acetyl 4-Aminoantipyrine (NAAP) in Water of Dog Tissues. Dog 1, weighing 7.3 kg, received 2.1 g and dog 2, weighing 13.7 kg, received 2.5 g of NAAP intravenously. The tissues were examined 4 hr later.

Tissue	NAAP in tissue water, mg per l		Ratio, tissue water NAAP/ plasma water NAAP	
	Dog 1	Dog 2	Dog 1	Dog 2
Plasma	317	142		
Red cells	310	141	.98	1.00
Liver	340	139	1.07	.98
Spleen	308	133	.97	.94
Muscle (leg)	334	144	1.05	1.01
Muscle (back)	315	144	.99	1.01
Lung	316	152	1.00	1.07
Heart	308	158	.97	1.10
Brain	268	137	.85	.97
Cerebrospinal fluid	278	123	.89	.87
Avg			.97	.99

TABLE II. Distribution of N-Acetyl 4-Aminoantipyrine (NAAP) Between Plasma and Transudates. Subjects with peripheral edema, ascites, and pleural effusion were given 1 g of NAAP intravenously and the plasma and transudate were obtained 5½ to 8 hr subsequently.

Subject No.	Time after NAAP administration, hr	NAAP, mg per l water	Ratio, transudate/ plasma water
1. Edema fluid	5½	18.9	.80
Plasma		23.5	
Edema fluid	6½	20.1	.89
Plasma		22.6	
Edema fluid	8	22	.97
Plasma		22.6	
2. Pleural fluid	6½	15	.73
Plasma		20.6	
3. Pleural fluid	6½	13.6	.68
Plasma		20	
4. Pleural fluid	8	7.1	.49
Plasma		14.7	
5. Ascitic fluid	7	16.6	.79
Plasma		21.1	

Time for even distribution of NAAP in body. One gram of NAAP was administered intravenously to each of 20 individuals in a total of 30 experiments. The logarithm of the plasma concentrations plotted against time (3, 4½ and 6 hours) yielded without exception a linear relationship. The rate of disappearance from plasma averaged 8% per hour with only minor variation. It may be inferred that equilibration of NAAP with body water is complete within 3 hours for normal subjects.

One gram of NAAP was administered to 5 subjects with abnormal accumulations of fluid. The concentration of NAAP in plasma water and the fluid accumulation was compared 5½ to 8 hours later (Table II). The concentration of NAAP in the transudate was usually considerably less than in plasma water, indicating incomplete distribution of the compound. To 2 of the subjects (1 and 2), 1 g of antipyrine was administered concurrently with the NAAP. In contrast to NAAP, the concentrations of antipyrine in the 2 fluids were almost identical in 5½ hours. These results suggest that in subjects with abnormal fluid depots, NAAP equilibrates too slowly between plasma and fluid accumulations to use it for estimation of total fluid.

Toxicity. No toxic effects have been observed in 30 subjects given 1 g of NAAP.

Measurement of body water with NAAP. Extrapolation procedure. A control sample of blood is drawn for the estimation of a small blank value which is used for correcting subsequent samples. One gram of NAAP in 50 ml of 5% glucose solution is injected intravenously from a burette during a period of

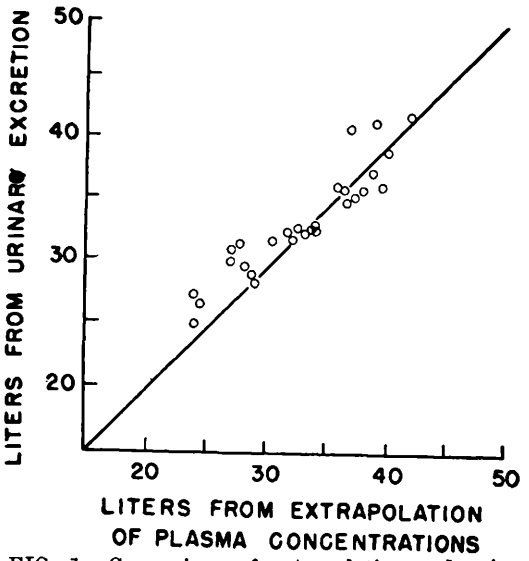

FIG. 1. Comparison of extrapolation and urine collection procedures for measuring total body water with N-acetyl 4-aminoantipyrine.

about 5 minutes. The middle of the injection period is taken as zero time. Blood samples are subsequently withdrawn at about 3, 4½ and 6 hours and the exact time noted. All blood samples are heparinized. Plasma and cells are separated by centrifugation and the plasma stored in stoppered tubes for subsequent analysis. NAAP is stable in plasma for at least several days at refrigerator temperature.

Body water is calculated from the plasma levels in the manner previously described for the antipyrine method(1).

Urine collection procedure. Since NAAP is almost quantitatively eliminated in the urine of humans, it should be possible to calculate its volume of distribution on the basis of its urinary excretion and a single plasma level. This involves the measurement of a plasma level and the urinary excretion 3 hours following the administration of NAAP. Body water is calculated as follows:

$$\text{Body water (liters)} = \frac{\text{amt of drug injected (mg)} - \text{amt excreted (mg)}}{\text{conc. in plasma water (mg per liter)}}$$

The procedure possesses two inherent sources of error: the small amount of NAAP metabolized in the body (0 to 15 mg of a 1 g dose in 3 hours) and the NAAP that is concentrated in the urine left in the ureter and pelvis after the bladder is completely emptied. These errors are in the direction of making the estimate of body water too large. The magnitude of the combined errors was assessed by comparing the volume of dilution of NAAP as measured from a single plasma level and urinary excretion, with that calculated by extrapolation of a line through 3 plasma concentrations. Comparisons for 30 subjects were in fairly good agreement; those obtained by the urine collection procedure averaging 1 liter higher than those obtained by the plasma extrapolation technic (Fig. 1). These results suggest that the errors of the urine collection procedure are not large.

Comparison of body water measurements made with antipyrine and NAAP. The volume of distribution of NAAP (extrapolation tech-

TABLE III. Comparison of Total Body Water in Normal Subjects Measured by N-Acetyl 4-Aminoantipyrine (NAAP) and Antipyrine.

Subject No.	Wt, kg	Total water		Difference (NAAP–antipyrine), liters
		NAAP, liters	Antipyrine, liters	
1	43.7	24	22.3	+1.7
2	47.8	27.3	28.8	—1.5
3	53.7	27	30.5	—3.5
4	56.5	27	29.6	—2.6
5	58.7	33	32.5	+ .5
6	60.4	33.8	37	—3.2
7	62	29.5	30.1	— .6
8	62.5	36.5	36.4	+ .1
9	68.6	28.2	30.4	—2.2
10	72.3	40	43.3	—3.3
11	77.3	33.4	35.8	—2.4
12	85.6	36.5	40.4	—3.9
			Mean diff.	—1.8

nic) and of antipyrine was compared following the intravenous administration of the 2 substances to 12 normal humans. The antipyrine space averaged 1.8 liters higher than the NAAP space (Table III).

Use of NAAP for the measurement of body water in the dog. NAAP disappears from the dog at a rate of about 20% per hour. Only about 60% of the NAAP administered to a dog can be accounted for in the urine; the remainder is metabolized. Only the extrapolation procedure, therefore, can be applied to the dog. A dose of about 50 mg per kg will result in plasma levels which can be measured accurately.

Summary. The use of N-acetyl 4-aminoantipyrine (NAAP) in the measurement of body water has been explored. The substance is uniformly distributed in the various tissues in close proportion to the water content, its metabolism is negligible and it is excreted slowly. The volumes of distribution of antipyrine and NAAP were compared in normal human subjects and the values found to agree well. NAAP has certain advantages over antipyrine: it is negligibly bound to plasma proteins; the value for body water may be calculated from a single plasma sample and a urine sample; its colorimetric estimation obviates the need for an ultraviolet spectrophotometer.

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Characteristic Pressure Pulses Recorded with an Esophageal Balloon in Experimental Mitral Insufficiency in Dogs.* (18929)

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This is a study of the pulsation attributable to cardiac activity which can be recorded in the esophagus at the level of the left atrium. It was undertaken to investigate the potentialities of the technic with regard to the diagnosis and possible quantitation of mitral insufficiency. The work of earlier investigators(1-3) along these same lines had not succeeded in conclusively establishing the origin and form of the various pulse waves observed in the esophagus nor was their relationship to events occurring within the left atrium ever clearly demonstrated. Even the work of Taquini(4) and of Puddu and Sibilia(5) was not completely convincing, though it tended to show that a characteristic pattern could be observed in mitral insufficiency in humans. The curves which they obtained, however, showed comparatively minor alterations from those found in normal individuals. Moreover, the patterns did not resemble either the left atrial pressure or volume curves obtained experimentally by Wiggers in dogs with acute mitral insufficiency(6).

All earlier recording had been done with air filled latex balloons attached to elastic rubber tubing. The pressure sensitive element was a rubber diaphragm. Such elastic, air filled systems allow for substantial volume displacement, and therefore record volume changes but do not accurately portray pressure variation. To render the apparatus more sensitive to pressure change the volume displacement permitted in the system was reduced by employing (1) a water filled balloon and completely water filled system; (2) polythene tubing; (3) a relatively rigid pressure

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