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USE OF RADIOACTIVE HYDROGEN FOR MEASUREMENT IN VIVO OF TOTAL BODY WATER*

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ABSTRACT

The problem of *in vivo* determination of total body water in man was approached using the radioactive isotope of hydrogen (tritium) in the form of water. The tritium was obtained by cyclotron bombardment of beryllium and was then converted to water. A method was developed for the determination of the activity of this water. This involved modification of the Geiger-Muller counter tube so as to permit introduction into the tube of measured amounts of radioactive water vapor, alcohol and argon. It was possible to evacuate and rinse the tube before each determination. Water of known activity was injected into two rabbits and one man and the plasma activity determined after a period of equilibration (close to one hour in the man). The body water of the human subject determined in this way agreed to within less than 1 per cent with the value calculated from specific gravity assuming body water to constitute 73.2 per cent of the lean body mass. The values obtained for the two rabbits agreed to within less than 5 per cent with the water content determined by desiccation and weighing.

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BACKGROUND

The total water content of the human has been estimated occasionally by the desiccation of cadavers (1); however, an in vivo method for the determination of total body water is desirable and preferable in many applications. Total body water has been demonstrated to be a relatively constant fraction, mean 73.2 per cent, of the fat-free body mass in normal adults of various species of mammals, and it has been suggested (2) that total body water of the normal, adult human may be estimated from measurement in vivo of total body fat by the specific gravity method and computation of the fat-free body mass (3,4). This procedure is rather limited in usefulness, however, as aberrations in water balance cannot be detected. The bulk of the research has been directed toward finding a readily recognizable chemical entity which, when introduced into the body, will distribute itself equally in the total body water. Sulfanilamide was proposed (5,6), but later rejected (7) because of unequal distribution in body tissues. Urea has been suggested (6,8), but rejected (9,10) because of the variability in the formation of endogenous urea, as well as unequal distribution. Thiourea has been tried (11,12), but rejected (13,14) because of its unequal distribution. Potassium also was considered (15,16), but later was demonstrated (17,18) to be distributed in the body potassium rather than in the body water. Methods involving the differential solubility of inert gases in body fat and water have been considered theoretically (19), but have not been verified experimentally.

It is of interest that the first substance to be used for the measurement in vivo of total body water should have been the most successful. Hevesy and Hofer (20), in a short note described the use of deuterium oxide for this measurement in man in 1934. Since that time the technic has been utilized for measurement of total body water in rats (21), and in guinea pigs (22). Very recently it has been repeated in man (23). With the advent of radioactive hydrogen of mass 3, i.e., tritium (24), it appeared practicable to utilize tritiated water (HTO) for the measurement of total body water.

METHODS

Preparation of HTO.- Beryllium targets, which had been subjected to deuteron bombardment in the cyclotron for neutron production, were obtained through the courtesy of the Department of Terrestrial Magnetism, Carnegie Institution of Washington. These contained tritium formed by the reaction, $Be^9 + D^2 \longrightarrow Be^8 + H^3$. (25). The tritium was collected in the form of HTO by dissolving the beryllium target in 6 N HCl in a vacuum system, passing the evolved hydrogen and tritium over copper oxide at 550° C., and collecting the water in a liquid air trap. The apparatus is shown schematically

in figure 1. A more quantitative recovery of tritium was effected by this method than by heating the beryllium target to red heat in an atmosphere of hydrogen gas. With ordinary precautions, the water collected in the trap was found to be sufficiently sterile and pyrogen-free for intravenous injection in human subjects. In a typical case, a yield of approximately 2 ml. of water was obtained in this manner from a beryllium target 1 x 1 x 1/32 inches. The tritiated water was rinsed with sterile water into a vaccine bottle to a total volume of about 6 ml., and an aliquot was removed for activity measurement of the final stock solution.

Measurement of HTO activity.- The maximum energy of the tritium beta ray has been reported to range from 0.0095 Mev to 0.015 Mev (26, 27). This low energy necessitates the employment of special means of counting. Water vapor at 1 to 2 mm. Hg pressure may be introduced into a Geiger-Muller counter tube without interference with its performance; consequently, the activity of tritium may be measured in the form of HTO vapor (28,29). Figure 2 is a schematic representation of the vacuum system used for the measurement of water radioactivity in the vapor state. This system was a modification of that employed by Black and Taylor (30), and the operating procedure also was similar in some respects. The counting circuit was a high speed circuit constructed in this Institute, and was equipped with six scaling circuits ranging from 2 to 64. The counter tube (B) consisted of a copper window-screen cathode 5 cm. in diameter and 30 cm. in length with a 10 mil tungsten wire anode, all sealed in a glass envelope with a total volume of approximately 1 liter. A lead shield about 2 inches thick was placed around the counter tube, and served to reduce the background count to approximately one half. The exchangeable test tubes (F), (G), and (H) contained inactive water, the unknown active water sample, and anhydrous ethyl alcohol, respectively. After affixing the tubes to the manifold, these solutions were degassed by twice freezing with liquid air and evacuating while frozen. The storage bulb (E) was filled with dry argon through stopcock 14 to slightly less than atmospheric pressure as indicated on the attached open end manometer (M). A low range McLeod gage (J) was used to measure pressures of 0.1 micron to 0.1 mm. Hg. The water vapor pressures of 0.4 mm. to 2.2 mm. Hg introduced into the counter tube were measured on a high range McLeod gage (L), which operated on a compression factor of about 7.5. Closed end manometers (C) and (D) indicated pressures in the counter tube and manifold, respectively.

In preparing for a count, the manifold and counter tube were evacuated to a pressure less than 0.3 microns. The system was tested for leaks by closing stopcocks 8 and 10, and measuring the pressure periodically by means of McLeod gage (L). Water vapor from the radioactive sample (pure water or plasma) was then carefully introduced into the system through stopcock 6, taking care that the vapor pressure did

not greatly exceed 2 mm. Hg by observing the manifold manometer (D). After diffusion equilibrium was reached, as indicated by constant readings on McLeod gage (L), the temperature was recorded, stopcock 2 was turned to close the counter tube, and the manifold was evacuated. Alcohol vapor was introduced by opening wide stopcock 7 and then slowly opening stopcock 2 to admit 2.5 cm. Hg of alcohol vapor into the counter tube, as indicated by a total pressure of 2.7 cm. Hg on manometer (C). Stopcocks 2 and 7 were closed, and the manifold again evacuated. Next, 2.0 cm. Hg of argon were introduced into the counter tube in similar fashion, making a total pressure of 4.7 cm. Hg in the counter tube. Finally, the tube was permitted to stand for ten minutes before counting was begun.

The tube, filled as described above, had a plateau of 300 volts and was operated at 1400 volts which corresponded to the mid-point. Three measurements of at least 20,000 counts each usually were made with each filling of the tube. Since it was found that the rate of counting increased somewhat during the first 20,000 counts and then leveled off, the mean of the second and third measurements was used. In 90 per cent of the determinations, these two counts were within 1.1 per cent of their mean.

Background determinations were carried out in an identical manner ith ordinary water substituted for the active water component. This method resulted in a reproducible mean background value of 3.58 counts per second, standard error of the mean ($\sigma/\sqrt{N}$) $\pm$0.03 counts per second. Twenty-five individual background determinations were made during the course of the experiments to obtain the mean value.

Simple evacuation of the counter tube was insufficient to obtain a return to normal background values following the use of active water samples, thus the tube was alternately evacuated and flushed eight times with inactive water vapor at about 17 mm. Hg pressure, followed by a room air rinse and re-evacuation to 0.3 micron. This procedure was employed after each activity measurement.

The actual activity of a given HTO solution was determined by making duplicate counts on at least four separate fillings of the counter tube containing different pressures of water vapor. The water vapor pressures were corrected to 0° C., and a plot was made of the observed counts per second against the corrected pressure. The slope of the best line through these data, including the mean background value of 3.6 counts per second, was determined by the method of least squares, and represent the counts per second per mm. Hg of water vapor, corrected for background. A sample determination is illustrated by table 1. The standard error of estimate for all activity values obtained by this method in the present experiments ranged from $\pm$1.1 per cent to $\pm$5.8 per cent of the slope value, and the mean standard

error was ±3.7 per cent. Because the volume of the counter tube was constant, values of activity expressed in this fashion are directly proportional to the concentration of HTO in the water sample being measured, and may be used as such without further conversion. The long half life of tritium, 31 years (27), eliminated the need for a decay correction during the course of the experiments.

EXPERIMENTAL

Approximately 1 ml. of tritiated water was prepared from a beryllium-target, 1.9 x 1.9 x 0.08 cm., which had received 20 hours of bombardment in a 25 Mev deuteron beam. A 1:1000 dilution of the sample was found to have an activity of 15.3 counts/sec/mm. Assuming a counting efficiency of at least 70 per cent from the geometry of the system, this activity corresponds to an absolute value of approximately 2×10^7 counts/sec/ml. of the original sample.

A standard solution of HTO was prepared with an activity of 54.2 counts/sec/mm. (table 1). Dilutions of 1:2 and 1:10 of the standard with inactive water also were prepared, and the activity of each was measured. In figure 3 the activity is plotted against the relative HTO concentration expressed as a fraction of the concentration of the standard solution. The straight line relationship is taken as evidence of the validity of the method over the count range shown. It was found that the linearity fell off when samples possessing an activity higher than 60 counts/sec. were employed. For this reason highly active samples were measured by aliquot dilution to a suitable range. The deviation from a straight line also occurred when water vapor pressure in excess of 2 to 3 mm. Hg was used in the counter tube.

A stock solution prepared from another target was found, by aliquot dilution, to contain 2128 counts/sec/mm. Two adult male rabbits were fasted, with drinking water ad libitum, for 24 hours preceding the experiment. Rabbit A received 5.09 ml. of the stock solution from a calibrated syringe directly into the blood stream by an ear vein at the rate of 1 ml. per 15 seconds. Blood samples were withdrawn by heart puncture into centrifuge tubes containing heparin at intervals of 21, 58 and 132 minutes following the injection. The plasma was separated and stored in the frozen state for subsequent activity measurement. Rabbit B received 1.96 ml. of the stock solution, and blood samples were withdrawn at intervals of 33 and 61 minutes in like manner.

As shown in table 2, calculations were made of the total body water content following measurement of the plasma tritium activity. The simple formula, $A_1 \times V_1 = A_2 \times V_2$, was used for this purpose. A_1 is the activity of tritium, expressed as counts/sec/mm., in the stock solution, V_1 is the volume in ml. of the stock solution injected,

A_2 is the activity of tritium in a body fluid, e. g., plasma or urine, after equilibrium has been established, and V_2 is the volume in ml. of body water. V_2 may be converted to units of weight by multiplying by the density of water at 38° C., 0.993, and may then be expressed as a fraction of the total body weight. Total body weight of the rabbits was taken as the depilated weight.

Total body water also was estimated on the basis of a constant fraction, 73.2 per cent, of the lean body mass, after measurement of the total body fat by the body specific gravity method (2). Specific gravities of 1.040 and 1.047 of the eviscerated carcasses were obtained for rabbits A and B, respectively. These correspond to fat contents of 29.1 and 25.3 per cent of the total body weight. The total body water estimates by this method are shown in table 3.

Finally, total body water of the rabbits was measured by the weight change during desiccation. The two animals were dissected into small pieces, care being taken to avoid tissue loss in the process, and placed in flat pans. The tissue was dried in a hot air blast oven at 90° C. for 13 days, and weighed at intervals during this period. After seven days weight loss occurred at a very slow constant rate, increasing from the seventh to the thirteenth day by less than 1 per cent of the total weight loss; consequently, the weight loss after seven days of drying was arbitrarily assumed to represent the total body water. The weight loss data are given in table 4, and the total body water estimates by this procedure are included in table 3.

A stock solution of HTO of high specific activity was prepared from a beryllium target which had received more than 100 hours of bombardment. After removal of an aliquot for activity measurement, 5.09 ml. of the solution were introduced in the antecubital vein of a 19 year old normal male human subject, 184 cm. tall and weighing 70.78 kg. At intervals of 30 minutes, one, two, and three hours, blood samples were withdrawn from the antecubital vein, and the plasma was stored for tritium activity measurement. Total body water content was calculated from these measurements and the results are given in table 3. The body specific gravity of the subject was found to be 1.077, corresponding to 11.0 per cent total body fat. Total body water content calculated from these data is also given in table 3.

DISCUSSION

From the data in table 3 it appears that tritiated water may be used for the estimation of total body water within 10 per cent of the true value, an error comparable with that in the deuterium oxide method. Much of the error is probably attributable to the uncertainty in the measurement of activities of one half to two times background value, which was unavoidable in the present instance. The anticipated

availability of tritiated water of higher specific activity, and improvements in the technic of measurement of tritium activity may be expected to lessen the present error considerably. A possible source of error, which is physiological in nature and inherent in isotope dilution procedures, particularly when the isotope is introduced directly into the blood stream, is the high plasma level of the isotope during the period of equilibration. This results in loss of the isotope through the kidney, and unless taken into account, leads to high diluent volume values. The error may be minimized by slow injection of the isotope to avoid very high diffusion gradients, as was done in these experiments, or by administration through the gastro-intestinal tract.

The time for the bulk of the HTO to reach equilibrium with the body water in man after intravenous injection is apparently close to one hour. This agrees well with the time for equilibration of D_2O found by Moore (23), and may be compared with the value of 2.5 hours found by Hevesy and Hofer (20) when D_2O was taken by mouth. Equilibration time was apparently shorter in the case of the rabbits, and is estimated to be not over 30 minutes. Flexner, et al. (22) found that equilibrium in the guinea pig, following injection of D_2O, was practically completely established in 10 to 12 minutes. It would appear then, that the rate of equilibration in the various mammals is related to the cardiac output per unit of fat-free body mass.

The value for total body water of the human subject, by HTO dilution and taking the mean of the one, two and three hour determinations (table 3), was 64.7 per cent of the body weight. The total body water value calculated as 73.2 per cent of the lean body mass of this subject was found to be 65.2 per cent of the total body weight, thus providing further substantiation for the concept of the relative constancy of the water content of the lean body mass of normal adult mammals (2). For their subject, Hevesy and Hofer (20) found a value of 63 per cent for total body water, which is likewise in accord with generally accepted values. On the other hand, Moore (23) obtained a relatively high value of 72.5 per cent for the total body water of his subject who, although apparently quite lean, did not appear abnormally lean from the height and weight data given. A somewhat high value of total body water of 67.85 per cent was also reported by Mitchell, et al. (1) on a cadaver whose water content was measured by desiccation. Total fat content was found to be 12.51 per cent, and the water content of the lean body mass of the specimen may be calculated to be 77.6 per cent. Acute heart attack was given as the cause of death, and if edema occurred owing to cardiac decompensation prior to death, that might account for the high water value in this case.

The two rabbits used in the present experiment were obese, containing 25 to 30 per cent total body fat, and the total body water was correspondingly lower. There was fair agreement between the desiccation

method and the body specific gravity method for estimation of the total body water. The HTO dilution method also gave good agreement in the case of rabbit A, whereas in the case of rabbit B the method appeared to give a slightly high value for water content. This may have been due to the low activities of plasma tritium measured in the latter case. In any event the water content, expressed as per cent of the total body weight, was decidedly low for these animals.

It will bear reiteration that variation in body fat content is sufficiently large to cause appreciable differences in the values of other body components when these are referred to total body weight. For example, the body water content may vary from 50 per cent in very obese individuals to 70 per cent of the total body weight in very lean individuals, and yet the water content may be a constant fraction of the fat-free body mass. For this reason, measurements of total body water should be accompanied by an estimate of total body fat whenever the comparison of individual values is made.

SUMMARY

1. Methods are described for the preparation of water containing the radioactive isotope of hydrogen, tritium, and for the determination of tritium activity in the form of water vapor from tritiated water and body fluids.

2. Total body water was estimated in two rabbits and a human by the dilution of tritiated water administered intravenously. The results agreed well in the case of the rabbits with the water content determined by desiccation of the carcasses, and with the water content calculated as 73.2 per cent of the lean body mass after measurement of total body fat by the specific gravity technic. In the case of the human, very good agreement was also obtained between the results from the tritium dilution method and the body specific gravity method.

3. Less than 30 minutes was required for essentially complete distribution of the tritiated water in the body water of the rabbits, and approximately one hour for the human. Consideration of these data and those of other investigators using deuterium oxide indicates a relationship between body size and rate of equilibration.

4. The tritium method at present appears to yield results with an accuracy as good as that obtaining by the use of deuterium, and greater accuracy is expected with improvements in technic and the forthcoming availability of tritium of higher specific activity.

ACKNOWLEDGMENTS

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Table 1.- Samplo method for determination of the activity of an HTO solution measured at various water vapor pressures

Vapor pressure mm. Hg (X)	Second 20,000 counts counts/sec.	Third 20,000 counts counts/sec.	Mean activity counts/sec. (Y)
0.0	-	-	3.6
0.40	25.2	26.5	25.8
0.55	33.8	34.1	34.0
0.97	56.6	57.5	57.0
1.07	60.9	60.7	60.8

$Y = 3.85 + 54.2X$ (By method of least squares)

$\sigma_Y = \pm 0.59$ counts/sec.

Table 2.- Activity of stock solutions of HTO injected into rabbits and human subjects

Stock solution employed	Dilution factor	HTO activity measured counts/sec/mm.	HTO activity of stock counts/sec/mm.	Volume injected ml.	Total HTO injected counts/sec.
RS (rabbit A)	312.5	6.81	2,128	5.09	10,832
RS (rabbit B)	312.5	6.81	2,128	1.96	4,171
ES (human)	5,000	6.08	30,400	5.09	154,736

Table 3.- Estimation of total body water by various methods in the rabbit and the human

Subject	Method	Time after HTO injection hr.	min.	Plasma HTO activity counts/sec/mm.	Total body weight kg.	Total body fat per cent	Total body water per cent
Rabbit A	Desiccation	-		-	3.502	-	55.5
	Sp. gravity	-		-	3.502	29.1	51.9
	HTO dil'n.	0	21	5.80	3.502	-	52.9
	HTO dil'n	0	58	5.37	3.502	-	57.2
	HTO dil'n	2	12	5.64	3.502	-	54.5
Rabbit B	Desiccation	-		-	2.881	-	55.9
	Sp. gravity	-		-	2.881	25.3	54.7
	HTO dil'n	0	33	2.42	2.881	-	59.4
	HTO dil'n	1	01	2.51	2.881	-	57.3
Human	Sp. gravity	-		-	70.78	11.0	65.2
	HTO dil'n	0	30	3.02	70.78	-	71.9
	HTO dil'n	1	00	3.49	70.78	-	62.2
	HTO dil'n	2	00	3.21	70.78	-	67.6
	HTO dil'n	3	00	3.40	70.78	-	64.3

Table 4.- Weight loss of two rabbit carcasses during desiccation in a hot air blast oven at 90° C.

	Days of drying							
	0	2	4	5	6	7	9	13
Rabbit A								
Carcass weight, kilos	3.502	1.705	1.600	1.581	1.565	1.557	1.552	1.544
Weight loss, kilos	0.0	1.797	1.902	1.921	1.937	1.945	1.950	1.958
Rabbit B								
Carcass weight, kilos	2.881	1.352	1.288	1.279	1.272	1.270	1.269	1.266
Weight loss, kilos	0.0	1.529	1.593	1.602	1.609	1.611	1.612	1.615

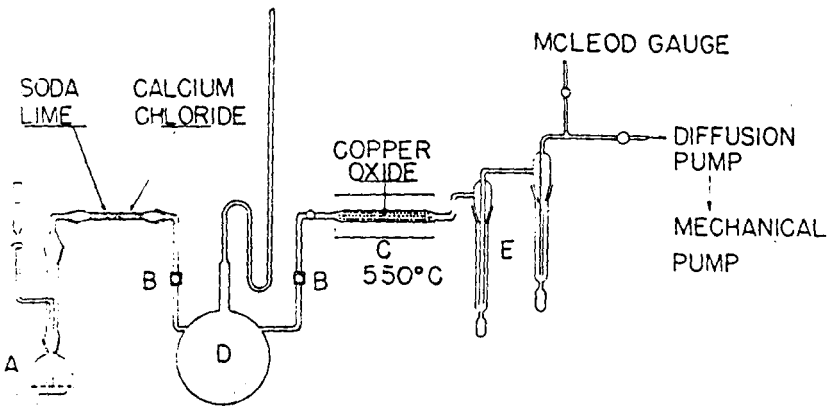

Figure 1.—Apparatus for the collection of tritiated water from a beryllium cyclotron target.

A Hydrogen generator
B Needle valves
C Electric furnace
D Storage bulb for hydrogen
E Liquid air traps

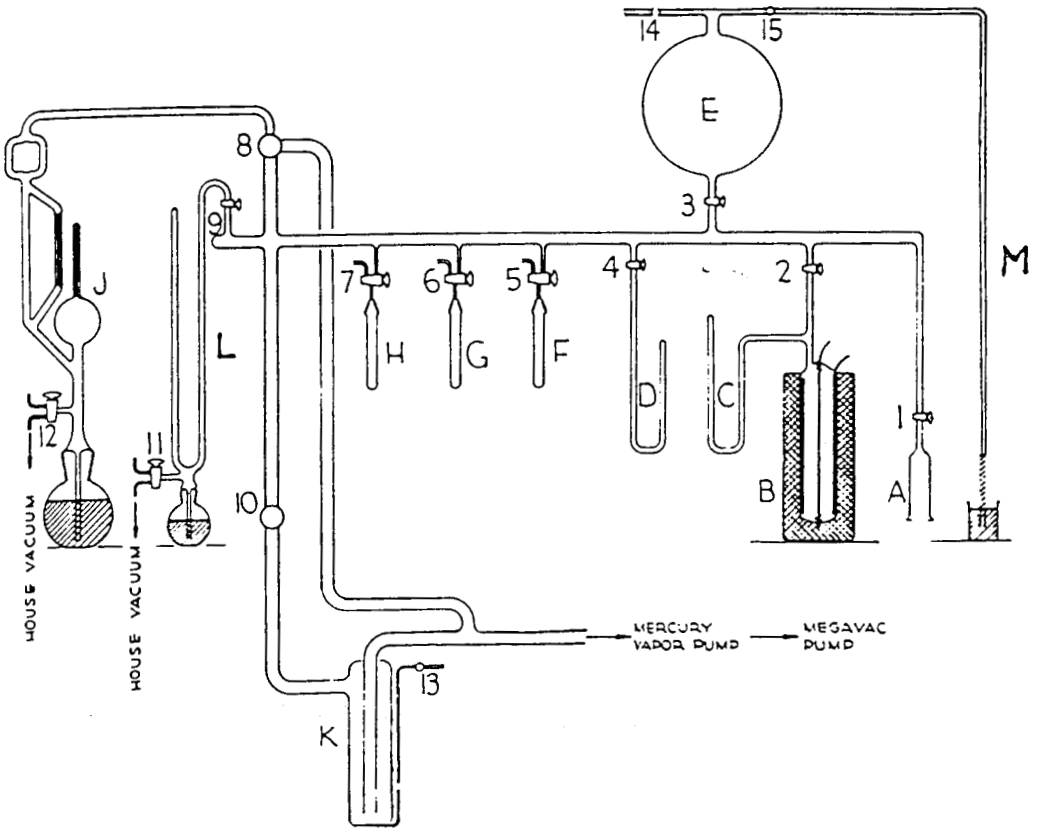

Figure 2.—Vacuum system for measuring water radioactivity

A Calcium chloride tube
B Geiger–Müller counter tube
C Manometer
D Manometer
E Storage bulb for argon
F Test tube for inactive water
G Test tube for radioactive water
H Test tube for ethyl alcohol
J Low range McLeod gauge
K Liquid air trap
L High range McLeod gauge
M Manometer for approximate argon pressure

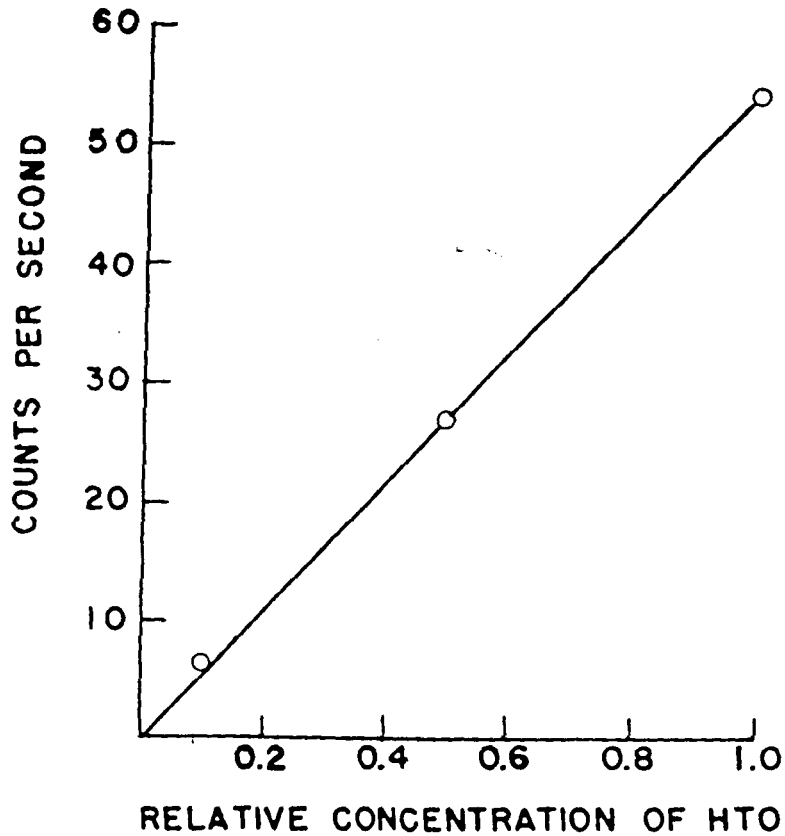

Figure 3.—Relationship of activity to concentration of radioactive water (HTO). The activity of each of the above solutions was actually determined by making counts on at least five separate fillings of the counter tube containing different pressures of water vapor. The slope of the line obtained by plotting the observed counts per second against the vapor pressure corrected to 0°C. gave the activity of the solution in counts per second per mm. Hg water vapor pressure corrected for the background.