

# APPLICATION OF LIQUID SCINTILLATION COUNTING TO BIOLOGY AND MEDICINE

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## INTRODUCTION

INVESTIGATORS in the many and diverse fields of the biological and medical sciences have little interest in specific details and mechanisms of the liquid scintillation process. Their knowledge of the complex electronics essential to a liquid scintillation counting device is slight and very apt to remain so. Any discussion therefore, of the applications of liquid scintillation counting to biology and medicine cannot be expected to reveal ingenious improvements in either scintillators or their associated electronics.

The biologist's and the doctor's interest in liquid scintillation counters is much the same as it is in an analytical balance or a spectrophotometer. Although it would be nice to know enough about the physics of forces and of light to be able to design a more sensitive balance or a better spectrophotometer, the biologist does well to understand enough biology to apply intelligently tools designed by others to the solution of problems in the life sciences.

This report attempts, therefore, to discuss the general features of biological and medical investigations which are responsible for the demands such investigations place upon design specifications of liquid scintillation counters. Included also are examples which illustrate the effective application of liquid scintillation counting to biological and medical problems. No attempt is made to review completely all such applications, and most of the examples given are those originating in the Biological and Medical Research Group of the Los Alamos Scientific Laboratory.

## NATURE OF BIOLOGICAL AND MEDICAL INVESTIGATIONS

Biological variability is responsible for the most arduous demands placed upon a tool applied to biological problems. Individual members of an apparently homogeneous population of living systems do not all respond alike to the same stress or treatment, and the limits of normal response are usually quite wide in comparison with physical measurements. Such variability usually necessitates statistical interpretation of results which calls for large numbers of determinations. Large numbers of determinations require that the method of measurement be simple and involve as little sample preparation as possible. Simplicity and speed are often more to be desired

than very high precision and accuracy, since biological variations are frequently the limiting factors in interpretation of results. Many important investigations of living processes involve kinetic studies. Determination of rate processes in systems having a high degree of individual variability also demands large numbers of measurements.

In applying radioactive isotopes to studies of living systems, it is necessary always to keep in mind that radiation may actually damage the living systems and thereby compromise the results. It is desirable, therefore, to keep the amount of isotope administered as low as possible, which frequently necessitates a high degree of sensitivity in the detection device. This is especially true when applied to isotopic tracer experiments on human subjects. The AEC's licensing system for the use of isotopic tracers in human experiments rigorously specifies maximum dose levels that can be administered.

Of importance also in biological studies is the wide variation in nature and composition of samples. Biological samples may be organic or inorganic. They may consist of chemically pure substances such as amino acids, steroids and sugars, or complex mixtures such as animal excreta, whole organs and tissues, or suspensions of bacterial cells. Their solubility in the usual non-polar scintillator solvents may vary from complete miscibility to essentially complete insolubility. Samples also may be completely inert to the scintillation process or they may be highly effective quenchers.

Biological investigations also require wide variations in sample size. Studies of natural radioactive levels or levels of contamination from world-wide radioactive fallout may require unusually large samples to supplement the sensitivity of detection. At the other extreme, the amount of sample available may necessitate measurements on extremely small samples. Examples of the latter case are equilibration studies between the blood stream and the cerebral spinal fluid, or kinetic studies of the anterior chamber of the eye.

Biological and medical investigations very frequently require nondestructive testing. An obvious example of this latter requirement is the measurement of build-up of radioactivity in people as a result of world-wide fallout. Possibility of recovery of the sample for further study is a very attractive feature of the internal sample scintillation counting method.

Last, but not least, is the requirement that the measuring device be rugged, stable and dependable. Many biological and medical investigations are carried on in institutions where physicists and electronics engineers are not readily available. Since the experimenter frequently has little or no knowledge of instrumentation, equipment requiring as little servicing as possible is highly desirable.

In summary, biological and medical investigations by nature call for counting systems with the greatest of versatility. Among the requirements are (a) analyses of large numbers of samples with a minimum of processing; (b) high sensitivity; (c) wide adaptability as to variations in sample size;

(d) accommodation of wide variations in nature and chemical composition of the sample; (e) potentiality for nondestructive testing; and (f) dependable operation with a minimum of servicing. This, of course, is an impossible order for a single instrument, but liquid scintillation systems show considerable promise. Instrumentation with the above criteria in mind is extremely important to investigators in all fields of the life sciences.

#### APPLICATIONS OF LIQUID SCINTILLATORS TO BIOLOGY AND MEDICINE

Liquid scintillation counting is the most important recent development in the application of radioisotopes to biology and medicine. Development of small-volume internal-sample counting techniques<sup>1-5</sup> has greatly simplified measurement of weak beta-emitting isotopes in biological materials. Construction of large  $4\pi$  gamma detectors<sup>6,7</sup> has increased sensitivity and sample size capability to the point where measurements at or below natural background levels are possible. These detectors are finding their greatest application to *in vivo* measurements. Their sensitivity is such that metabolic studies of gamma-emitting isotopes may be safely performed on normal human subjects.

##### *Small volume internal-sample counting*

Liquid scintillation counting of internal samples (where the sample is mixed with the scintillator system) may be accomplished in a variety of ways, depending on the nature and characteristics of the sample. If soluble in the usual scintillator solvent, the sample may be dissolved directly in the scintillator and counted as a homogeneous system. Such substances as sterols and lipids which are toluene-soluble are easily counted in this way<sup>4</sup>. A number of toluene-insoluble substances are water-soluble. These may be counted by employing a solvent which increases the miscibility of water in the scintillator system. Alcohol-toluene and 1,4-dioxane-naphthalene systems are useful in this way for counting sugars, salts, body fluids such as urine and blood serum and tritium water itself.<sup>8,9</sup> A special case of homogeneous counting is the determination of contemporary C<sup>14</sup>-activity by converting samples of terpenes to *p*-cymene, which serves both as the sample and the scintillator solvent.<sup>10</sup>

Other samples are more effectively counted in a heterogeneous system in which the sample is suspended in the scintillator system. This method is directly applicable to samples which are insoluble in toluene, dioxane and water. Examples of this class are certain inorganic materials, such as barium sulfate and animal and plant tissues, bacteria, amino acids, etc. These materials may be suspended directly in the scintillator system in a finely divided state<sup>11</sup> or they may be incorporated along with the scintillator into a gel.<sup>12-14</sup> In the case of direct suspension, shaking between each count or extrapolating back to zero time in a series of undisturbed counts is necessary. Use of a thixotropic gel<sup>13</sup> or aluminum stearate<sup>12,14</sup> provides stable suspensions.

Optically dense concentrations of suspended white solids do not impair counting efficiency, since they only scatter the light but do not absorb it. Colored materials lower efficiency by light absorption but in low concentrations may still be counted by using an internal standard. The use of an internal standard is desirable in any case, since it also compensates for quenching effects of the sample on the scintillator and provides for direct conversion from counts to disintegrations.

The Biomedical Research Group of the Los Alamos Scientific Laboratory has counted the following isotopes as internal samples in small-volume liquid scintillation counters:  $H^3$ ,  $C^{14}$ ,  $Na^{22}$ ,  $S^{35}$ ,  $Ca^{45}$ ,  $I^{131}$ ,  $Cs^{134}$ ,  $Cs^{137}$ ,  $U^{233}$ ,  $U^{235}$  and  $Pu^{239}$ ,  $H^3$ - $C^{14}$  and  $H^3$ - $Na^{22}$  have been simultaneously assayed in double tracer experiments. The following materials have been counted as suspensions:<sup>11</sup>  $BaC^{14}O_3$ , phenylalanine- $C^{14}$ ,  $BaS^{35}O_4$ , benzidinium- $S^{35}O_4$ ,  $Ca^{45}$ -oxalate,  $Ca^{45}CO_3$ ,  $C^{14}$ -labeled bacteria,  $Ca^{45}$  and  $Sr^{90}$ -labeled bone salts<sup>15</sup> and  $C^{14}$ -labeled rat bone, testis, spleen and muscle.

Scintillation counting of internal samples was originally devised to measure the weak beta-particles from tritium and  $C^{14}$ . The presence of hydrogen and carbon in all living matter makes isotopes of these elements extremely important in biological investigations. It is not possible to review all of the applications of liquid scintillation counting of tritium and  $C^{14}$  in such studies. Only a few illustrative examples are given.

### Tritium counting

Tritium in the form of water (HTO) has been extensively used in studies of water physiology<sup>16-18</sup>. Figure 1 shows the concentration of tritium (as HTO)

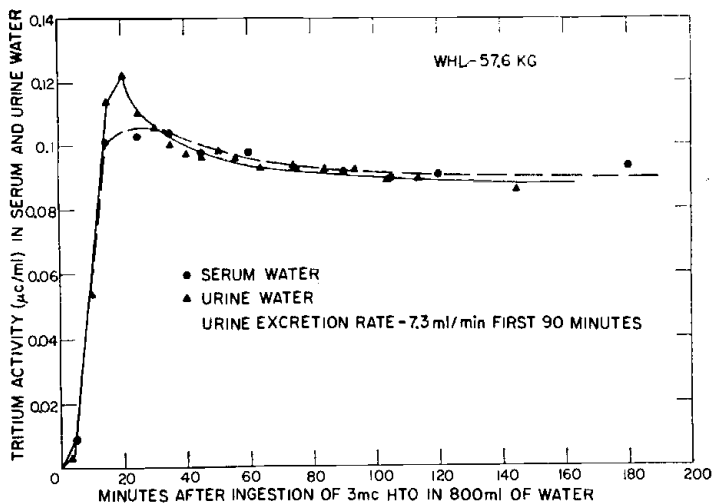


Fig. 1. Tritium activity in urine and blood serum following ingestion of HTO.

in the blood and urine of a normal man as a function of time after ingestion of HTO. Using the dioxane-naphthalene liquid scintillation counting system, these results were obtained almost as fast as the samples were collected. From these data, it is possible to estimate (a) gastric hold-up time as about 4 min; (b) time of equilibration of water between the gastrointestinal tract and the blood as about 25 min; (c) rate of equilibration of water between the blood and the total body fluids as about 90 min; (d) total body water as 66% of body weight; (e) percentage lean body mass as 87% of body weight; (f) percentage gross body fat as 13% of body weight; and (g) rate of turn-over of body water (with additional measurements) as about 12 days.

SHUMWAY *et al.*,<sup>19</sup> with the collaboration of the Los Alamos Scientific Laboratory, has introduced a novel application of liquid scintillation tritium counting with the determination of body fat in beef cattle. Such measurements are important in tests of feeding efficiency.

Figure 2 shows the tritium activity in body water of steers following intravenous injections of HTO. From these data, body fat was calculated and

TRITIUM ACTIVITY IN BODY FLUIDS OF STEER FOLLOWING INJECTION (IV) OF HTO

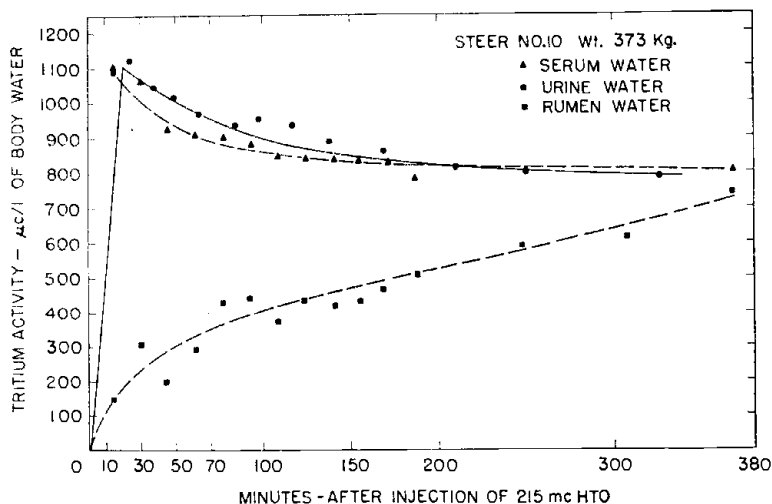


Fig. 2. Tritium activity in body fluids of a steer following intravenous injection of HTO.

when compared to conventional fat extraction methods gave good results. The method permits determination of body fat before sacrifice of the animal. The half-time of the body water turn-over in steers averaged 6.4 days. The lower curve shows the rate of equilibration of water between the blood and the contents of the rumen. These data were obtained from three animals with rumen fistulas and show an equilibration time of about 7hr.

An application of tritium liquid scintillation counting to a botanical problem is illustrated in Fig. 3. As an essential part of the determination of the

relative biological effectiveness of tritium beta-radiation (using growth inhibition of the apical cells of the root tip) it was necessary, for purposes of dosage calculation, to measure the rate of diffusion of HTO into and out of the bean root tip.<sup>20</sup> Bean roots were immersed in HTO for increasing periods of time, after which the tips were clipped off and placed in known volumes of absolute alcohol. After the root tip was dehydrated, an aliquot of the alcohol was measured for tritium activity. Other roots were immersed in HTO until equilibrium was reached. The HTO was then allowed to diffuse out into a known volume of ordinary water and aliquots withdrawn at intervals and counted. As expected, diffusion half-times of water into and out of the root tip were equal and averaged about 2 min.

The ease with which tritium can be measured by internal liquid scintillation counting affords almost limitless applications of HTO to biological studies of diffusion phenomena and water physiology and balance.

### Carbon-14

Applications of C<sup>14</sup>-liquid scintillation counting to biological and medical investigations are far too numerous to review. A few examples are given only as illustrations.

Nitrogen mustard (methyl-bis( $\beta$ -chloroethyl)amine hydrochloride) injected intra-arterially proximal to a tumor is reported to be more effective in cancer therapy than when injected intravenously. Intra-arterial administration was believed to give a higher concentration of drug in the tumor and lower concentration in normal tissues distal to the point of injection. Suspension counting of tissue homogenates was used by BOONE *et al.*<sup>21</sup> to study the

TABLE 1  
*Distribution of C<sup>14</sup>-Nitrogen Mustard Following IV and IA Injection*

Compound	Tissue	Nitrogen mustard ( $\mu\text{g eq/g}$ )	
		IV injection	IA injection
$  \begin{array}{c}  \text{*CH}_3 \\    \\  \text{N} \cdot \text{HCl} \\  / \quad \backslash \\  \text{CH}_2 \quad \text{CH}_2 \\    \quad   \\  \text{CH}_2 \quad \text{CH}_2 \\    \quad   \\  \text{Cl} \quad \text{Cl}  \end{array}  $	Sternum	9.4	6.6
	Thymus	14.1	11.3
	Testes	5.8	3.7
	Spleen	12.8	8.1
	Kidney	78.8	62.3
	Marrow†	4.5	13.8
	Tumor†	12.9	54.3

\*Position of C<sup>14</sup> label.

†Right leg below point of injection.

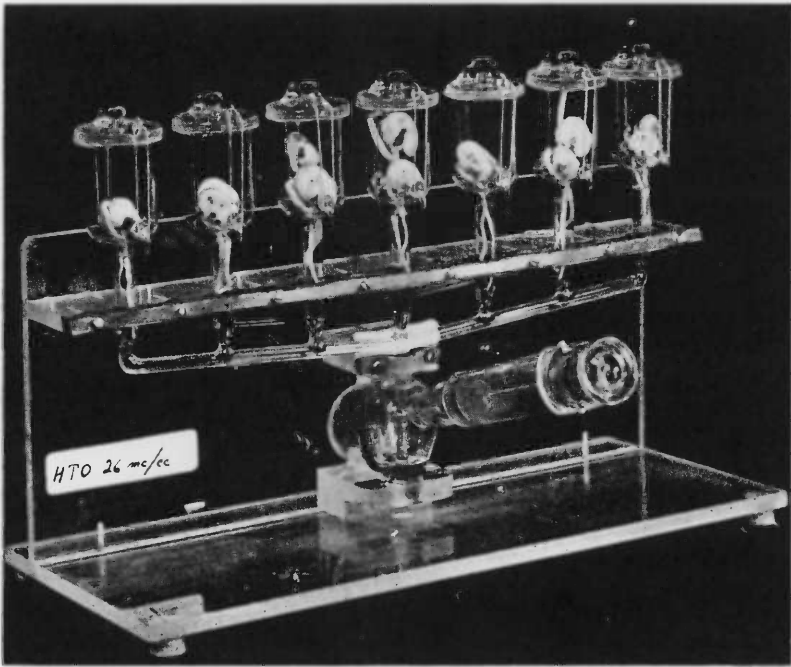


Fig. 3. Method of studying rate of uptake of HTO by bean sprouts.

localization of  $C^{14}$ -nitrogen mustard in normal and tumor tissues of rats five minutes after intra-arterial and intravenous administration. The data are summarized in Table 1 and definitely show higher concentrations of  $C^{14}$ -activity in the tumor and lower concentrations in other parts of the body following intra-arterial administration.

Data in Fig. 4 demonstrate an extremely convenient application of  $C^{14}$ -liquid scintillation counting to counter-current extraction separation of the metabolites of  $C^{14}$ -labeled compounds.<sup>22</sup>  $C^{14}$ -labeled caffeine was administered to rats for several days and the urine collected. After preliminary processing, the urine was placed in a Craig counter-current extraction device and

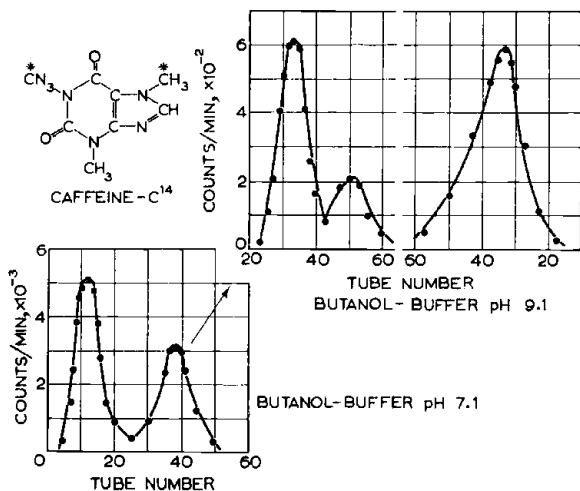


Fig. 4. Counter-current partition of the metabolites of  $C^{14}$ -caffeine.

extracted with a butanol-buffer system. The separation process of the urinary metabolites was conveniently followed by withdrawing small aliquots of the various phases from each tube with a hypodermic needle and a syringe, injecting directly into the liquid scintillator and counting. No chemical processing of the aliquots was required. Although the results were not absolute, they were relative and permitted calculation of the partition coefficients. They could have been made absolute by using an internal standard during the counting operation.

#### Measurement of $Sr^{90}$ and $Ca^{45}$ in bone minerals

FOREMAN<sup>15</sup> of the Los Alamos Laboratory has applied gel suspension counting to the determination of the partition of  $Sr^{90}$  and  $Ca^{45}$  between the organic matrix and the mineral portion of bone. Bone samples were extracted with

ethylenediamine, which removed all organic matter and left a completely white, insoluble residue of bone mineral. This material was pulverized and suspended in a gel system consisting of scintillator plus about 8% aluminum or potassium stearate and counted. The results definitely showed that fixation of calcium and strontium in bone was similar and confined almost entirely to the bone mineral. The method has been applied successfully also to the determination of plutonium in the mineralized portion of bone.

#### APPLICATIONS OF LARGE-VOLUME $4\pi$ DETECTORS

The Los Alamos whole body liquid scintillation counter has proven extremely useful in biological and medical investigations. Its design and operational characteristics have been reported in a number of articles.<sup>7, 23-26</sup> The Biomedical Research Group of the Los Alamos Scientific Laboratory has designed whole body detectors in a variety of sizes, and VAN DILLA and ANDERSON<sup>27</sup> have reported the characteristics of a large-volume detector used to measure radium and mesothorium content of dogs at the University of Utah. Figure 5 shows a whole body liquid scintillation gamma detector used for rats and mice. Application of these detectors to biological and medical studies has just begun. A few of the applications which have been made at the Los Alamos Laboratory are given below.

##### *Retention and excretion of radioactive isotopes*

Measurements of retention and excretion of radioactive isotopes are important in studies of animal nutrition and physiology. Turn-over rates are also of importance in present calculations of maximum permissible levels of radioactive materials in air and water. RICHMOND at Los Alamos<sup>28</sup> is using whole body liquid scintillation counting techniques to study retention and excretion rates of many gamma-emitting isotopes under a variety of conditions. The amount of the isotope retained by the animal is readily determined by periodic *in vivo* counting. Samples of excreta are collected in polyethylene bottles diluted to the volume of the animal and counted in the same counter, as an independent check on retention. Measurements of tissues and organ distribution can be made by sacrificing the animal, excising the organ or tissue and counting in a volume equivalent to that of the whole animal. Figure 6 shows the effect of dietary sodium intake on the retention of  $\text{Na}^{22}$  by the mouse. These data were collected merely by varying the dietary regimen and counting the animals periodically in the whole body counter. Determinations required no chemical processing, and each animal served as its own control. Normal retention and excretion curves (Fig. 7) may be broken down and analyzed into body compartments and rates using first order kinetics. Innumerable applications of these techniques to studies of animal nutrition should be possible.

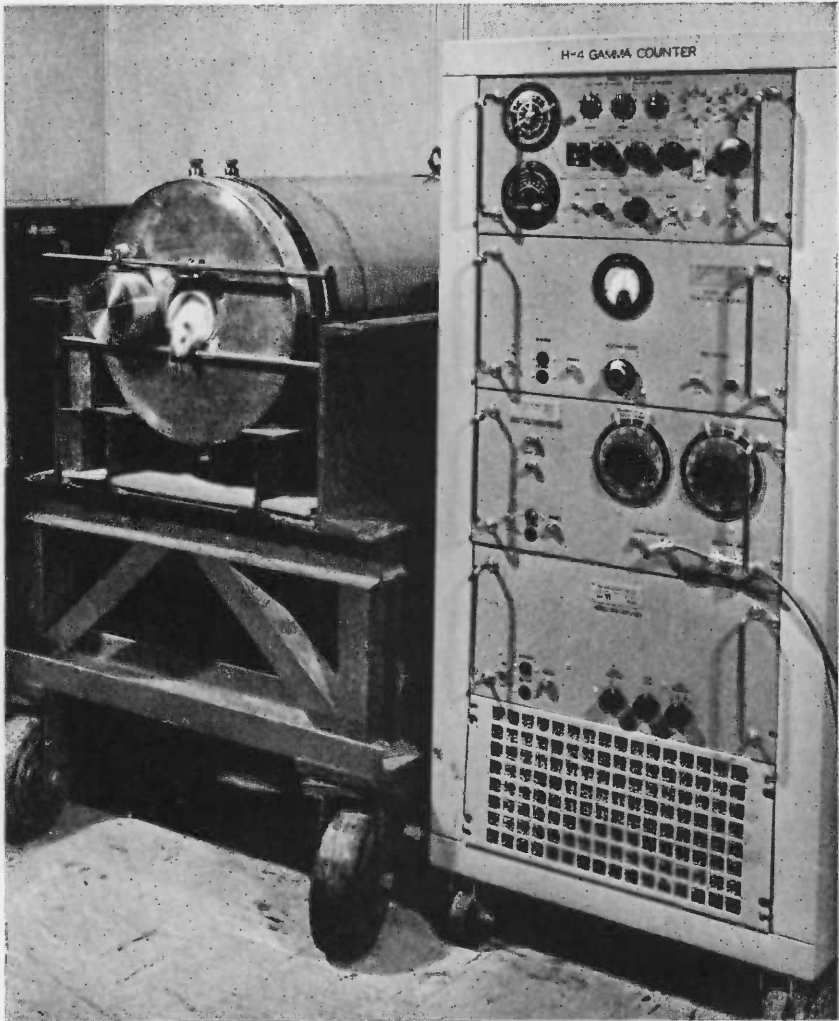


Fig. 5. Whole body liquid scintillation gamma detector for small animals.

Extrapolation of animal experimental results to man is one of the greatest uncertainties associated with the interpretation of biological and medical investigations. Using whole body counting, retention and excretion studies of gamma emitting isotopes in mice, rats, dogs, monkeys and man are being

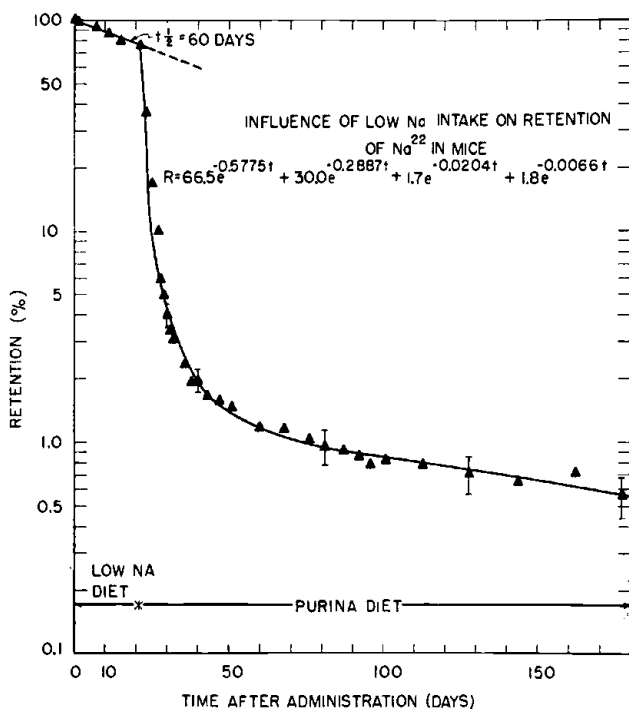


Fig. 6. Retention of Na<sup>22</sup> in mice maintained on sodium-deficient and normal diets.

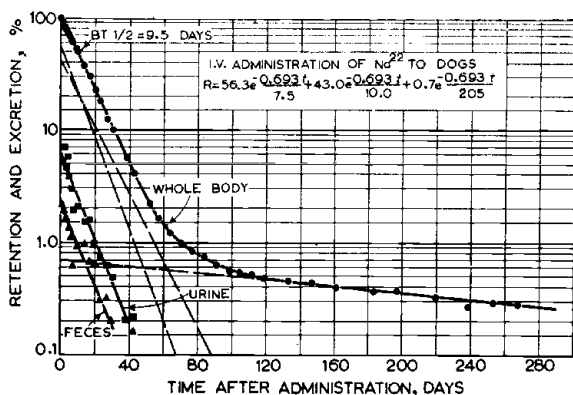


Fig. 7. Normal retention of intravenously administered Na<sup>22</sup> in dogs.

conducted in a search for inter-species metabolic correlations.<sup>29</sup> Figure 8 shows the results of an inter-species retention study with  $Rb^{86}$ . Figure 9 summarizes inter-species correlation studies of retention of the alkali metals.

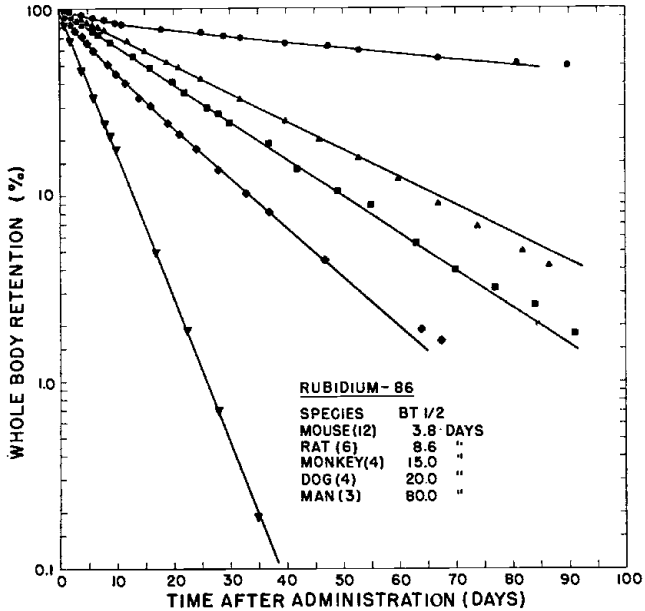


Fig. 8. Rate of retention of  $Rb^{86}$  in various species.

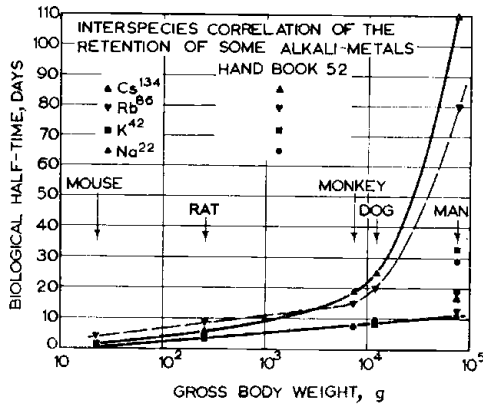


Fig. 9. Inter-species comparison of retention of alkali metal isotopes.

In this figure, the biological half-time of the principal component of the retention curve is plotted against the gross body weight of the species. The data indicate that the biological turn-over times for man might be seriously underestimated if extrapolated directly on a weight basis from the results obtained

on lower animals. One of the most important applications of *in vivo* whole body counting is the collection of human data. The great sensitivity of the detectors makes possible radioactive tracer studies on human volunteers without administering more than 1/100th to 1/1000th of a maximum permissible daily dose of the isotope.

*Gross body composition studies*

One of the first applications of the Los Alamos Human Counter was the study of gross body composition through measurements of total body potassium by counting the naturally occurring  $K^{40}$ . Figure 10 shows  $K^{40}$  measurements on 185 human subjects plotted against gross body weight in pounds.<sup>30</sup> Body potassium is largely confined to muscle tissue, therefore, the  $K^{40}$  measurements should reflect the lean body weight or muscle mass. With appropriate corrections,  $K^{40}$  determinations should provide a method of estimating the lean/fat ratio. The diagonal line in Fig. 10 was drawn through the  $K^{40}$  points determined on children. Those adult points falling below the

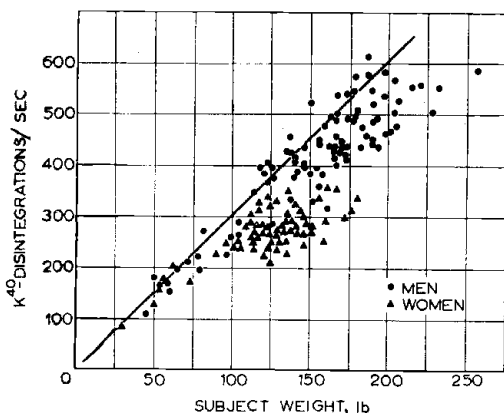


Fig. 10. Correlation between  $K^{40}$  measurements and gross body weight in normal human subjects.

line indicate varying amounts of fat on the subjects. That varying amounts of fat are responsible for deviations below the line is readily shown by the data in Fig. 11. Representative subjects, some of which varied greatly from the basic lean/fat ratio, were given tritium water and their fat computed by tritium dilution, counting the tritium by internal liquid scintillation counting. This figure shows the subjects'  $K^{40}$  measurements plotted against body weight in pounds before and after correcting for body weight determined by tritium measurements. Their lean body weights showed an excellent correlation with the  $K^{40}$  measurements.<sup>31</sup> These measurements were sufficiently impressive that the Los Alamos Scientific Laboratory, in collaboration with the Fitzsimons General Hospital, undertook experiments to measure gross body

composition by tritium dilution,  $K^{40}$  determination and body density measurements determined by under-water weighing.<sup>32</sup> These data are shown in Table 2.

TABLE 2  
*Fat Content of Humans in Percentage of Body Weight*

Subjects	Body density method	HTO dilution method	$K^{40}$ counting method
26 males	23.9	24.8	19.7
7 females	31.1	37.5	37.1

Gross body composition measurements are extremely important to the nutritional laboratories of the Armed Forces for studies of effect of dietary and other stress on soldiers in the field.

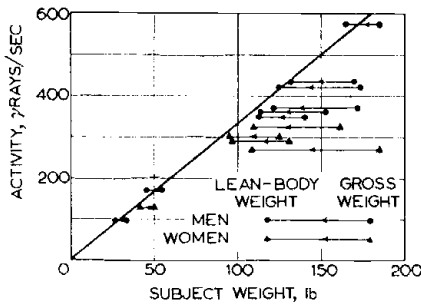


Fig. 11. Correlation between  $K^{40}$  content and gross and lean body weights of normal human subjects.

#### *Environmental contamination measurements*

Large-volume liquid scintillation detectors are especially adaptable to studies of fission product environmental contamination as has occurred from bomb test operations, or as might occur in the event of a reactor accident.  $Cs^{137}$  contamination of people and foodstuffs from bomb test operations is being studied with the Los Alamos counter.<sup>30</sup> Several hundred people and several thousand pounds of foods have been measured. Samples of dried blood from several packing houses are being routinely measured to collect information on the build-up of cesium in United States beef sources. Materials that have been measured for  $Cs^{137}$  fallout contamination range all the way from sacks of potatoes and carrots to a bale of alfalfa hay. The extreme sensitivity of the counter to gross fission product gamma activity and the very short counting times required makes the instrument potentially valuable for the determination of fission product contamination of food and water supplies in the event of a run-away reactor accident.

### *Determination of neutron exposure*

A large volume detector with proper calibration is also capable of measuring thermal and fast neutron exposures at or below the daily maximum permissible range. Measurement of induced  $\text{Na}^{24}$  activity in normal body sodium has already been used to estimate the thermal neutron exposure of a person who received only 3/10 of a maximum permissible daily dose of thermal neutrons from the Los Alamos water boiler reactor. Measurement of sodium-induced activity is presently being studied as a means of diagnosing exposure to fission neutrons as occurred in the Los Alamos critical assembly fatalities and as might occur at an atomic weapons detonation or reactor accident. The induced-sodium activity in monkeys that received a near lethal dose of fission neutrons from the Los Alamos fast neutron fission assembly (*Godiva*) was too high to count in the whole body counter until permitted to decay through two or three half-lives. Induced  $\text{Na}^{24}$  activity from neutron exposure is unfortunately dependent on chemical composition of tissue, mass of the body, and neutron energy. Calibration of the counting equipment for various neutron energies and conditions of exposure will be necessary before the sodium counts can be converted to absolute dose.

### *Clinical applications*

One of the most promising applications of *in vivo* whole body counting is to clinical medicine and diagnosis. Potassium measurements are often of considerable clinical and diagnostic importance. Lack of correlation between extra-cellular potassium, as measured from blood plasma, and total body potassium, which is largely intra-cellular, is one of the difficulties encountered in interpretation of the usual clinical determinations of potassium. Significant depressions of intra-cellular (hence, total) potassium have been reported for a number of diseases, among which are muscular dystrophy, steatorrhea, infant diarrhea, diabetic coma and familial periodic paralysis. Such diseases could perhaps be diagnosed and studied with facility using whole body counting techniques.

Radioiodinated-serum albumin is finding considerable application to clinical medicine and diagnosis. Iodinated serum protein studies could be conducted readily in a multiplicity of diseased states using whole body counting methods.  $\text{Fe}^{59}$ ,  $\text{Na}^{22}$  and other isotopes may possibly have their clinical and diagnostic applications extended greatly by use of whole body counting. The potentialities of whole body counting in connection with the artificial kidney to investigate *in vivo* dialysis of sodium, potassium and other isotopes suggests some interesting possibilities.

A specific example of the clinical application of whole body counting at Los Alamos is its use to follow the rate of turn-over of  $\text{Cr}^{51}$  administered as a tag on red blood cells in leukemic vs. normal patients.<sup>33</sup> Figure 12 shows the rate of turn-over of the chromium (determined by whole body counting) by a

normal man and by a patient suffering from an acute leukemia. Both curves show three rate components with half-times of 0.3, 12 and 95 days, respectively. The first component with 0.3 day half-time represents excretion of unattached  $\text{Cr}^{51}$ . Exactly what the other components represent is not clear, but the leukemic patient showed twice as much  $\text{Cr}^{51}$  excreted with the 12 day half-

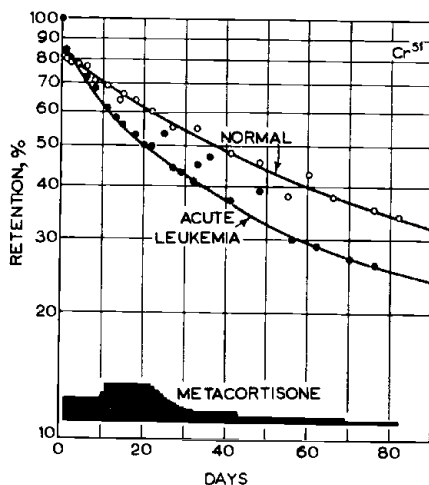


Fig. 12. Rate of turn-over of  $\text{Cr}^{51}$  in a normal and a leukemic patient given  $\text{Cr}^{51}$ -tagged red blood cells.

time as did the normal subject, even though medication brought about remission of his disease. Had therapy been ineffective, the difference might have been even greater.

Application of whole body counting to diagnosis and therapy is only just beginning. Undoubtedly, many other applications will be forthcoming as whole body counters become available to the larger hospitals.

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