Whole Body Radioactivity Measurement and Significance

By G. E. SCHIFFERDECKER, W. V. KESSLER, and J. E. CHRISTIAN

The total body potassium in humans was determined by measuring the K^{40} radioactivity in a whole body liquid scintillation counter. These values were used to study the lean body mass and the fat content as a function of age and physical fitness. The effect of an anorectic-tranquilizer drug combination and food substitute therapy on the body composition of obese humans undergoing weight loss was also investigated. The techniques reported for the *in vivo*, nondestructive detection and measurement of low levels of γ-emitting radionuclides in large animals and man has opened up entirely new opportunities for research of significance to the pharmaceutical sciences, which heretofore has not been possible.

THE FIRST WHOLE body measurements of the radioactivity in humans were made with high pressure ionization chambers. These were constructed independently by Sievert (1) in Stockholm, Sweden, and by Burch and Spiers (2) in Leeds, England. These detectors were developed because of a need for instruments capable of measuring small quantities of radioactive materials which were present in the body as a result of contamination. A neutrino detector, a large volume liquid scintillation counter designed and used for the neutrino program of Cowan and Reines (3), was adapted to whole body counting after a few modifications.

The first liquid scintillation counter designed specifically for whole body counting of humans was reported by Anderson, *et al.* (4), at the Los Alamos scientific laboratory. A 2π large volume liquid scintillation counter located at Purdue University was used in the studies reported here. The design and basic operating characteristics of this counter have been previously described (5).

The recent development of whole body liquid scintillation counters for the *in vivo* nondestructive detection and measurement of low levels of γ-emitting radionuclides in large animals and man has opened up entirely new opportunities for research of significance to the pharmaceutical sciences. The *in vivo* estimation of total body potassium by counting naturally occurring K^{40} has led to techniques for the determination of body composition in terms of lean body mass and total body fat (6–8) and the study of the effect of many factors, including drugs, on body composition. In addition to the determination of the natural γ-emitting radionuclides, the same techniques may be used to determine minute amounts of radioisotopes introduced into the living system. The results reported in this paper illustrate some of the potential applications.

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EXPERIMENTAL

Operational Characteristics of the 2π Liquid Scintillation Counter. -A description of the detector

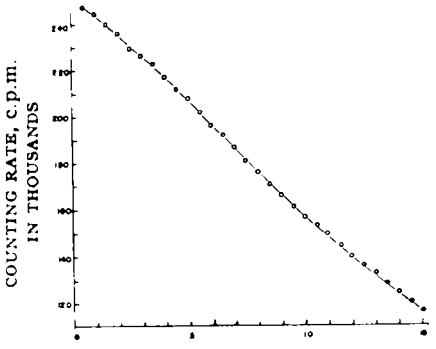

Fig. 1.—Effect of vertical placement of a Cs 137 source.

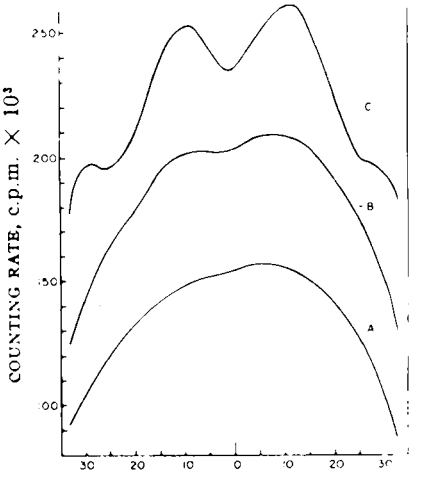

Fig. 2.—Effect of horizontal placement of a Cs-137 source. Key: A, source 10 in. above conveyor; B, source 5 in. above conveyor; C, source on conveyor.

TABLE I.—POTASSIUM, LBM, AND FAT VALUES OF HUMANS OF NEARLY CONSTANT WEIGHT OVER 12 WEEKS

Subject[a]	Age, Yr.	Wt., Kg.	Potassium,[b] Gm.	LBM,[b] Kg.	Fat,[c] Kg.
A	45	71.7	136.5 ± 3.5	51.0 ± 1.4	20.7
B	30	68.1	144.5 ± 3.5	54.0 ± 1.4	14.1
C	37	65.8	141.9 ± 3.4	53.1 ± 1.3	12.7
D	24	61.3	130.9 ± 2.9	48.9 ± 1.1	12.4
E	26	73.5	163.1 ± 3.0	61.1 ± 1.1	12.4
F	27	74.9	179.4 ± 3.0	67.1 ± 1.1	7.8
G	24	65.4	92.2 ± 2.8	34.4 ± 1.0	31.0
H	27	55.4	124.0 ± 3.2	46.2 ± 1.1	9.2

[a] All subjects were males except subject G. [b] Average of ten to 12 individual values taken at approximately weekly intervals. The observed standard deviations are indicated. [c] The fat content was calculated by difference between the total body weight and the average LBM.

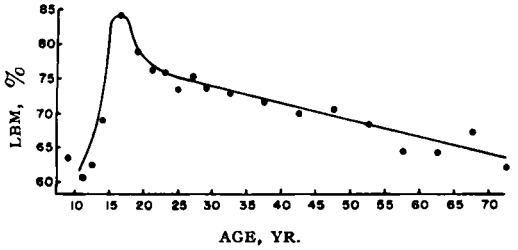

Fig. 3.—Average per cent lean body mass values of male humans as a function of chronologic age.

tank, electronics, steel shield, sample conveyor system, stability, and reproducibility of the counter has been previously reported (5). The high voltage supply for the photomultiplier tubes was operated at 1580 v. The high voltage supplied to each tube was adjusted with a variable series resistance so that all tubes had the same gain. The base discriminator and window settings for the K^{40} channel were 16.0 and 10.0, respectively; those for the Cs^{137} channel were 4.0 and 5.0, respectively. The base discriminator range of 100 pulse height units corresponded to about 85 v. for both the K^{40} and Cs^{137} analyzers. The maximum window width of 10 pulse height units was 20% of the base discriminator range. This corresponded to a maximum window width of about 17 v. The counting efficiency for a Cs^{137} point source[1] was 30.5% and for a K^{40} source (3.32 Kg. potassium chloride) was 18.6% when these sources were positioned in the center of the sample well.

The effect of horizontal and vertical positioning on the counting rate of a Cs^{137} source in the sample well was studied. The source was positioned in a plastic tube supported on wooden holders. The vertical displacement of the source was made at the center of the sample well with 1-minute measurements of the counting rate being made at $^1/_2$-in. intervals up to a height of 15 in. Figure 1 is a plot of these data. The effect of horizontal displacement of the point source was determined at the bottom of the sample well and 5 and 10 in. above the bottom. At each height, determinations were made at 1-in. intervals over the full length of the detector tank. Figure 2 is a plot of these data.

Body Composition Studies of Humans.—Total body potassium, lean body mass (LBM), and total body fat values of five different groups of humans have been calculated from the K^{40} content determined by liquid scintillation counting procedures. The average K^{40} activity of each subject was calculated from four 1-minute determinations. This value was corrected for background, background depression, and variation in efficiency of the detector. The total body potassium was determined by dividing the corrected activity in counts per minute by the counter efficiency and 177, the number of γ rays emitted per minute per gram of potassium. Using the factor 68.1 meq. of potassium per kilogram of LBM,[2] the LBM was calculated from the body potassium.

Based on the LBM concept described by Behnke (12), body fat was estimated by substracting the LBM from total body weight.

Reproducibility of Potassium, LBM, and Fat Measurements.—A group of eight subjects whose weight remained nearly constant was measured periodically (weekly when possible) for potassium, LBM, and fat content over a period of 12 weeks to establish reproducibility. The results are presented in Table I. The reproducibility of the measurements over the 12-week period was between 2 and 3%. The reproducibility measured on three of the subjects over an 8 to 10-month period was 3.1, 3.3, and 3.5%.

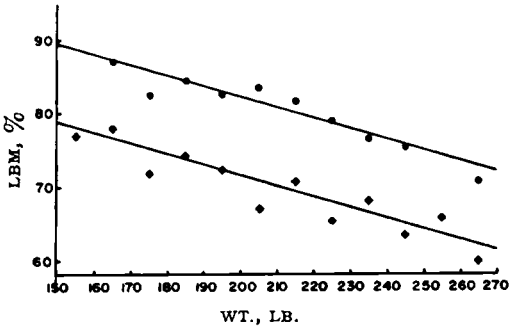

Fig. 4.—Relationships between per cent lean body mass and body weight of male humans of average and above average physical fitness. Key: ●, = above average physical fitness; ◆, average physical fitness.

Average Per Cent LBM of the Human Male As A Function of Age.—A group of 930 males ranging in age from 8 to 75 years was measured for potassium and LBM. Figure 3 shows the average per cent lean body mass as a function of their chronological age.[3]

The per cent LBM increases with age from 8 years up to a maximum at 16, then decreases sharply to about age 20 and assumes a gradual decline with increasing age. The structure of this curve, although not identical in detail, is similar to previously reported data by Anderson and Langham (13) and Onstead (14) in which potassium concentration (grams per kilograms) is reported *versus* age. Both groups report no significant differences between the male and female until approximately the age of puberty, after which the female curve drops rapidly to age 16. Beyond age 16, there is a gradual decline in potassium concentration with age paralleling the

[1] Model RT-2, Nuclear Chicago Corp.

[2] This is the average value of the published chemical analyses of the potassium content of human lean body mass (neutral fat extracted) (9–11).

[3] The age groups plotted contained from 14 to 113 persons (average group size, 46), except for the last four groups which had ten or less persons.

phenyl)-3-butanone (*B*, Fig. 1). In addition, very small amounts of 2-(*p*-chlorophenyl)-3-methylbutadiene-1,3 and *p*-chlorophenyl *tert*-butyl ketone were present. Any delay in fractionation allows the substituted butadiene to polymerize readily (probably catalyzed by peroxides) by a 1:4 addition to form an amorphous insoluble polymer.

A literature search produced no reference to *p*-chlorophenyl *tert*-butyl ketone. It was, therefore, necessary to synthesize this compound for comparison with the conjugated ketone detected in the reaction mixture. The first attempted synthesis of this ketone was from chlorobenzene and trimethyl-acetyl chloride *via* a Friedel-Crafts reaction. A ketone was separated by careful fractionation of the reaction product, but infrared absorption studies indicated that it was not a conjugated ketone. An alternate synthesis from *p*-chloroacetophenone and methyl iodide furnished the desired ketone.

Investigators have established rules for predicting the "migration aptitude" and percentage migration which can be expected for symmetrical pinacols (3), but no satisfactory rules have been formulated to predict the extent of migration of the groups of an unsymmetrical pinacol (4). Collins, in a recent review, states: "Every unsymmetrically substituted glycol can rearrange in two conceivable ways, depending upon which of the two hydroxyl groups is lost during reaction." (See Fig. 1.)

To confirm that 2-methyl-2-(*p*-chlorophenyl)-3-butanone was formed in almost quantitative yield and that it had not resulted from rearrangement during fractionation, a typical reaction mixture was subjected to a selective oxidation process for methyl ketones (5). A substantially quantitative yield of a mixture of *p*-chlorophenyl-dimethylacetic acid along with a small amount of *p*-chlorobenzoic acid was obtained. The mother liquor and washings from the oxidation process were worked up, and a small amount of nonoxidized ketone was isolated as the crystalline 2,4-dinitrophenylhydrazone. The powder X-ray diffraction pattern of this crystalline hydrazone was identical with that of the hydrazone prepared from the fractionated 2-methyl-2-(*p*-chlorophenyl)-3-butanone. The possibility that *p*-chlorophenyl *tert*-butyl ketone might form a 2,4-dinitrophenylhydrazone with difficulty or not at all was considered, but studies with the synthetic ketone prepared for comparison revealed that it formed the 2,4-dinitrophenylhydrazone even more readily than the unconjugated isomer.

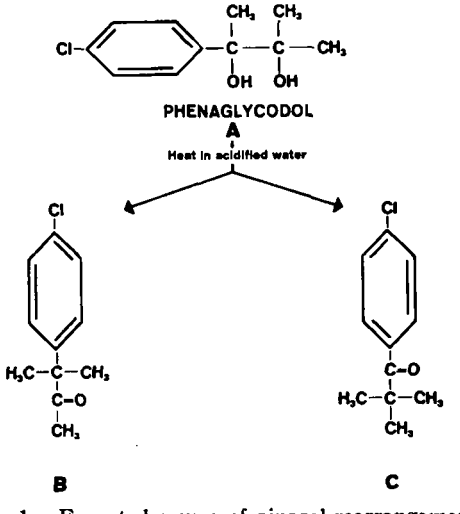

Fig. 1.—Expected course of pinacol rearrangement.

PHARMACOLOGICAL STUDIES

2-Methyl-2-(*p*-chlorophenyl)-3-butanone which had been purified by fractionation was studied in mice for neurosedative activity and in rats for anticonvulsant activity. Quite unexpectedly, this ketone displayed one-third to one-half the pharmacological activity of phenaglycodol. The acute toxicity (LD_{50} 1650 ± 50 mg. per Kg.) by mouth in white mice was about one-half that of phenaglycodol. Since *p*-chlorophenyl-dimethylacetic acid is a possible metabolite of phenaglycodol, the toxicities of the sodium salt by mouth and after intravenous injection in white mice were determined.

EXPERIMENTAL[2]

2-Methyl-2-(p-chlorophenyl)-3-butanone (B, Fig. 1).—One-hundred grams of commercial phenaglycodol was added to a solution of 20 ml. of concentrated sulfuric acid in 180 ml. of water in a round-bottomed flask. This mixture was refluxed gently with constant stirring for 16 hours. The heavier molten glycol separated to the bottom of the flask at first, but as rearrangement progressed the lighter ketonic layer rose to the surface.

The acidic mixture was cooled, transferred to a 500-ml. separator and the lower aqueous phase separated and discarded. The yellow, oily liquid was washed six times with about 80 ml. of 10% sodium chloride solution, then dried with 20 Gm. of anhydrous sodium sulfate. The dried product was filtered into a still pot which was attached to a spinning band reflux column. The column had a calculated efficiency of 28 theoretical plates. The liquid was fractionated at 3 mm. Hg and its composition was as indicated in Table I.

The fraction boiling at 105° was 2-methyl-2-(*p*-chlorophenyl)-3-butanone displaying characteristic infrared absorption bands at 5.83 μ for the unconjugated ketone, at 9.09 μ and 9.82 μ, characteristic of *p*-chlorophenyl, and at 11.96 μ, characteristic of *p*-substituted phenyl. Nuclear magnetic resonance studies supported the assigned structure.

Anal.—Calcd. for $C_{11}H_{13}ClO$: C, 67.17; H, 6.66; Cl, 18.03. Found: C, 67.18; H, 6.50; Cl, 17.62.

[2] All boiling points and melting points reported have not been corrected.

measurements emphasize the attractiveness of this method for research in the pharmaceutical sciences.

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Convenient Synthesis of 3-(γ-Aminopropyl)-5-ethoxyindole

By HUBERT W. MURPHY

3-(γ-Aminopropyl)-5-ethoxyindole has been synthesized from *p*-phenetidine and α-methyl-acetoacetate. This method involves fewer intermediates, with new and better methods for their purification.

THE PREPARATION of this indole in low yield by the use of the Japp-Klingmann reaction has been reported (1). A simpler and more satisfactory synthesis has been developed to prepare larger quantities. By this reaction the desired 4-ethoxyphenylhydrazone was prepared from 4-ethoxybenzenediazonium chloride and ethyl α-methyl-acetoacetate. The crude hydrazone was cyclized by heating in absolute alcohol containing 10% concentrated sulfuric acid to produce 5-ethoxy-2-carbethoxyindole in 40% yield. Saponification with methanolic potassium hydroxide gave an 82% yield of 5-ethoxy-2-carboxyindole. The latter was decarboxylated to furnish 5-ethoxyindole in 80% yield. 5-Ethoxyindole was reacted with acrylonitrile under pressure to yield about 80% of crude 5-ethoxy-3-indolepropionitrile. This can be crystallized from isopropanol before being reduced; however, it was found more expedient to reduce the crude nitrile. Reduction of the crude product furnished a mixture containing both primary and secondary amines from which the pure γ-(5-ethoxy-3-indolyl)-propylamine was separated by crystallization as a Schiff base from acetone. The yield, based on 5-ethoxyindole, was about 20%.

The structure of the isopropylidene was firmly established by infrared absorption: N—H 3.20; N=C, 6.01; aryl ring, 6.14, 6.27, and 6.69; and ethoxy, 8.03 and 9.56 μ. The assigned structure was supported by the nuclear magnetic resonance pattern. The isopropylidene derivative was converted practically quantitatively to an amine salt by hydrolyzing the Schiff base with a dilute aqueous solution of a strong acid.

This Schiff base can also be used as a starting material for the preparation of secondary amines (2). The ease with which the Schiff base is formed under alkaline conditions with acetone is remarkable. Since tryptamine and acetone readily furnish a similar Schiff base, this may be characteristic of 3-indolylamines.

A dicarboxylic acid was isolated from the acetone filtrate remaining from the crystallization of 5-ethoxy-2-carbethoxyindole. This acid is believed to be α-(5-ethoxy-2-carboxy-3-indolyl)-acrylic acid. Structure assignment has been made on the basis of titration data, ultraviolet and infrared absorption, nuclear magnetic resonance studies, melting point, and elemental analysis. The difference in pKa′ values (ΔpKa′, 1-2) in 66% dimethylformamide was 3.6 pH units for this acid. This is about halfway between that for succinic acid, representing an acid with free rotation of the carboxyl groups, and *o*-phthalic acid, an acid with somewhat limited rotation. It is distinctly different from maleic acid, an acid with restricted rotation. This is strong evidence against the alternative quinoline dicarboxylic acid structure. Infrared

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