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RATES OF TURNOVER AND BIOLOGIC DECAY OF CHLORIDE AND CHLORIDE SPACE IN THE DOG DETERMINED WITH THE LONG-LIFE ISOTOPE, CL^{36}

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IN THE past, tracer studies of chloride in biologic experiments have been confined to the use of Cl^{38}, but because of its rapid rate of physical decay ($T\frac{1}{2} \simeq 37$ minutes), its use has been limited to acute experiments. Recently, Cl^{36} ($T\frac{1}{2} \simeq 2 \times 10^6$ years) became available from the Oak Ridge National Laboratories. Although behavior of this radioelement in man is of primary interest, its use in human subjects has been prohibited by the Atomic Energy Commission, pending accumulation of data concerning its rate of turnover in experimental animals. The material, therefore, has been employed to observe the rate of turnover in six dogs studied under controlled metabolic conditions. Because the observations are entirely new and are concerned with data previously unavailable and because of the implications of electrolyte metabolism, edematous states, chloride balance, rate of renal excretion of chloride, rate of exchange of chloride in the blood plasma and also because of the importance in atomic warfare, these investigations are reported.

METHOD

Six young dogs were quarantined for several days and then transferred to separate metabolic cages. Five dogs were normal; one (No. 7012) was found to have amebiasis and was observed for only three days. For three days before the experiment was begun, the dogs were allowed to become stabilized and to establish metabolic equilibrium on the same diet employed during the experiment.*

Each dog was then anesthetized with an intravenous injection of pentobarbital sodium. A cannula was inserted into a jugular vein, clotting of the cannula being prevented by periodic moistening of the stilette with heparin. Cl^{36}, as NaCl, in a dose of 1,000,000 counts per minute per 10 pounds of body weight and in approximately 2 c.c. of water, was injected in an average of 11 seconds (6 to 23) into a vein of a hind leg. The solution contained approximately 18.0 mg. of NaCl. Samples of 1 c.c. of blood were collected from the jugular vein every 5 to 15 seconds for 2 minutes, every 30 seconds for the next 3 minutes, every 1 to 5 minutes for the next 20 minutes, and then every 10 minutes for the remainder of the 60 minutes of the study. Any feces or urine excreted during the period was also collected.

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*Pard, a product of Swift and Company. It varies in chloride content from 0.24 to 0.48 per cent by weight, the average being 0.36 per cent. Therefore, 1.63 Gm. of chloride per pound (454 Gm.) of food was employed as the intake of chloride.

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The dog was then returned to his cage. Urine and feces were collected every morning. The bottom pan in the cage was rinsed twice, this having been found by experiment to be sufficient washing, and the washings were studied for radioactivity. The dog was then weighed. Food and water remaining from the previous 24 hours were measured and another known quantity of each was placed in the cage. A sample of blood was collected on alternate days. Collections of urine and blood were continued until the radioactivity of both reached background.

The volume of the urine and rinsing water and weight of the feces were recorded. Aliquots of the urine, rinsing waters, Formalin suspension of the feces, and blood serum were counted by means of thin mica window Geiger counters.*

After necessary considerations were given to background, volume of the samples, weight of the dogs, and injected dose, it was possible to express the concentration of Cl^{36} in counts per minute per cubic centimeter in terms of an injected dose of 1,000,000 counts per minute per 10 pounds of body weight. It was also possible to express the data in terms of total counts per minute of Cl^{36} excreted in the urine and feces.

RESULTS

(1) Early Concentration-Time Course of Cl^{36} in the Blood Serum.—From the blood sera collected during the short intervals of time after injection of the tracer it was possible to record the rapid decline of concentration of Cl^{36} occurring during the first 60 minutes. The results are summarized by Fig. 1. This curve was analyzed by methods previously described.[1, 2, 3] The individual curves are not shown, since they did not differ significantly from the average curve (Fig. 1).

The curve of concentration-time course was found to be composed of at least four major exponential regression rates expressed by the equation

$$CN_t = 1250e^{-2.079t} + 680e^{-0.308t} + 144e^{-0.0077t} + 674e^{-0.000128t},$$

where CN_t is the concentration of Cl^{36} in counts per minute per cubic centimeter of serum at any time,
t represents time in minutes after injection of the tracer, and
e is the base of the natural log.

Comment: The concentration-time course for the Cl^{36} in serum is similar to that reported by Gellhorn and associates[4] in dogs for Na^{24} as well as that reported by Cowie, Flexner, and Wilde[5] for Cl^{38} in guinea pigs. As stated previously, the regression curves represent complex biologic phenomena which are expressions of chemical and physical processes too complicated to interpret properly at this time. The curves have an unlimited number of exponential components, but the four major regression rates are employed for simplification. The initial one is concerned to a large extent with mechanical mixing in the blood stream immediately following the injections. The other regression rates are related to movement of Cl^{36} from the blood serum into compartments at different rates, for example, erythrocytes, interstitial fluid, chambers of the

*Neutron bombardment of KCl results in the production of K^{42} and S^{35} in addition to Cl^{36}. The material remained in this laboratory for several months to insure physical decay of K^{42} ($T\frac{1}{2}$ 12.4 hr.). Although the material had been processed at Oak Ridge National Laboratories to remove S^{35} ($T\frac{1}{2}$ 88 days), the purity was checked by decay curves and Feather's curve. All tests indicated that Cl^{36} was the only radioactive isotope injected into the dogs. As an additional precaution, all samples were counted with the use of an aluminum filter 10.335 mg. per square centimeter to eliminate any S^{35} which may have been present.

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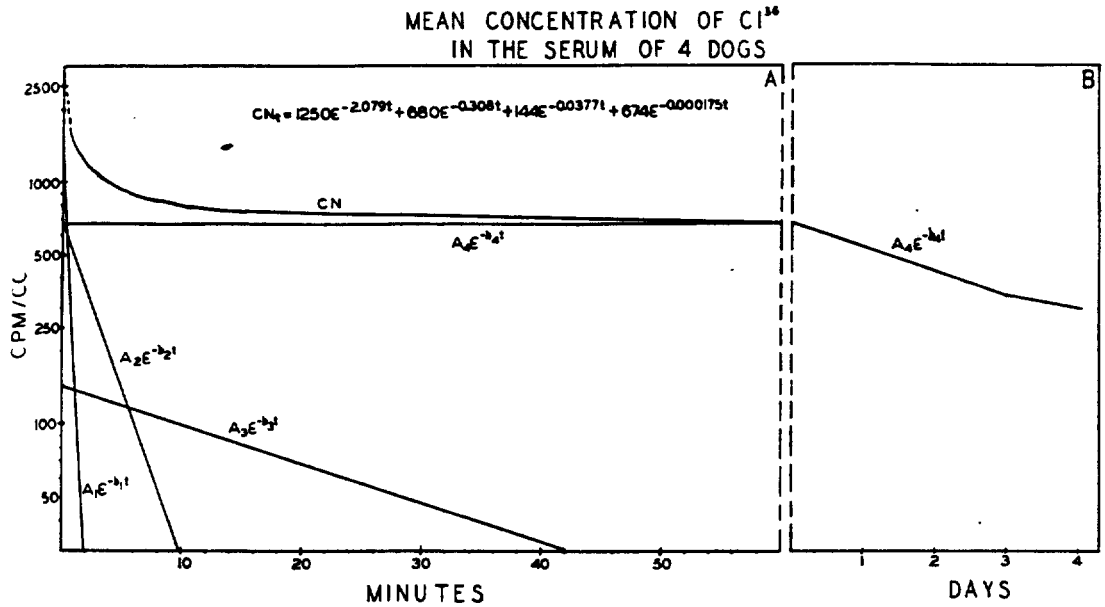

Fig. 1.—*A*, The mean curve of concentration-time course of Cl^{36} for the serum of four dogs for 60 minutes analyzed into 4 regression rates.
B, The mean curve of concentration-time course of Cl^{36} for the serum of the same four dogs for the first 4 days of the experiments. The rate of regression in concentration is identical with the slowest rate of part *A* of this figure.

eye, spinal fluid, and bone, as well as urine and feces. These problems, previously discussed in detail,[2, 3] are much the same for almost all such tracer studies.

Studies on rats and rabbits by Manery and Haege[6] indicated that Cl^{36} penetrated a space equivalent to that of sodium in one hour but that it continued at a slower rate to penetrate certain tissues, especially the brain. Winkler and associates[7] studied the decay of Cl^{36} in blood serum of dogs and considered equilibrium to be established in about one hour. This observation was corroborated in our experiments, the mean curve of the concentration-time course approaching a straight line by the end of 60 minutes (Fig. 1). From the individual curves of the five dogs (Fig. 2), it is evident that the early regression curves decline precipitously. Furthermore, the fourth exponential shown in the equation for the regression curve of Fig. 1 represents excretion primarily. This is evident from the fact that the curves of regression in concentration of Cl^{36} in the serum and of the Cl^{36} mass in the body as a whole are practically parallel, particularly during the first few days before cumulative errors in collection are of consequence and before the counts diminish to a range in which errors in counting are significant (Fig. 2). Another observation supporting the existence of equilibrium at the end of one hour is the fact that the serum concentration for one hour calculated from the slow regression rate was within a few counts of that actually observed from the study of rapid regression in serum concentration at the end of 60 minutes. Therefore, for practical purposes, in the normal dog the equilibrium of distribution is reached relatively quickly.

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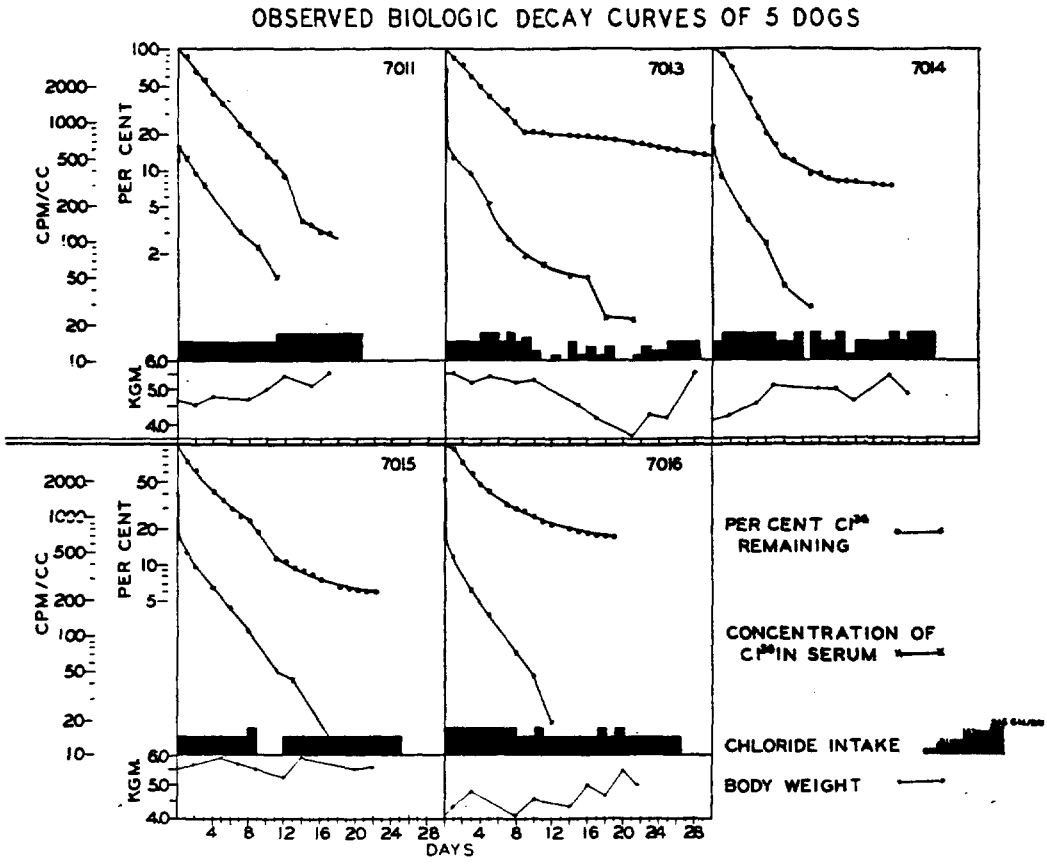

Fig. 2.—The biologic decay rates of Cl^{36} for five dogs. The percentage remaining in the body, dosage minus that accounted for in feces and urine, is calculated as

$$\%N_t = \left(1 - \frac{\sum_{s=1}^{t} k_s}{N_o}\right) \times 100, \text{ where}$$

N_o = injected Cl^{36} in counts per minute; k_s = Cl^{36} excreted in urine and feces during the sth day after injection, expressed in counts per minute; $\%N_t$ = the percentage of injected Cl^{36} not excreted by the end of the tth day after injection. The daily variations in body weight and intake of chloride are shown.

It should be remembered that the term "equilibrium" is employed to indicate equilibrium of distribution. The physiologic problem of steady state is a different one, the distinction becoming evident when comparisons of the regression curves of Figs. 1 and 2 are made.

(2) Rates of Turnover of Chloride Between the Blood Serum and Extraseral Compartments.—From the mathematical approach described by Merrell and associates[1] and employed previously for Na^{24} studies in man,[2] the proportion of serum chloride leaving the blood serum per minute and the proportion of extravascular chloride returning to the blood serum per minute were calculated. The proportion of the total chloride not in the blood serum, as well as that in the blood serum, was also calculated.

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Briefly, the approach to the calculations was as follows: If an equilibrium for the curve of the concentration-time course shown in Fig. 1 is assumed to have been reached at the end of about 60 minutes and if mechanical mixing in the serum is considered to be complete at t_o, and since from the general equation of concentration-time course

$$CN_t = A_1e^{-b_1t} + A_2e^{-b_2t} + A_3e^{-b_3t} + A_4e^{-b_4t}, \quad (1)$$

therefore

$$R_s = \frac{A_1b_1 + A_2b_2 + A_3b_3 + A_4b_4}{A_1 + A_2 + A_3 + A_4}, \quad (2)$$

where R_s represents the proportion of serum Cl^{36} leaving the serum per unit of time (one minute). This equation holds since in Equation (1) the coefficients A_1, A_2, A_3, and A_4 represent the relative sizes of the compartments of the Cl^{36} in the serum from which Cl^{36} is leaving, the exponents b_1, b_2, b_3, and b_4 represent the fractions of the respective compartments which leave the serum per unit of time (one minute), and the sum of the coefficients ($A_1 + A_2 + A_3 + A_4$) represents the serum concentration at t_o if equilibrium of distribution of the isotope had been achieved at that time.

$$\text{Therefore, } q = 1 - \frac{A_4}{A_1 + A_2 + A_3 + A_4}, \quad (3)$$

where $\frac{A_4}{A_1 + A_2 + A_3 + A_4}$ is the proportion of total Cl^{36} which is in the serum, and

q is the proportion of total Cl^{36} in the chloride space other than the blood serum.

Then the proportion of Cl^{36} outside the serum which is returning to the serum per unit of time (one minute), R_e, may be represented by the equation

$$R_e = R_s\left(\frac{1 - q}{q}\right). \quad (4)$$

The mean values for the four dogs are shown in Table I. Since Cl^{36} labels the Cl^{35}, then it is evident from Table I that about 75 per cent of the total body chloride was outside the blood serum. The entire chloride of the serum was turned over or exchanged in about one minute. The data also show that about one-third of the chloride outside the serum returned to the serum each minute and about one-fourth moved out of the serum each minute, or about one-half of the entire chloride of the body passed into and out of the serum per minute. Therefore, if a dog weighing 10 pounds contains 5.5 Gm. of chloride, then 2.75 Gm. chloride passed into or out of his serum per minute, which is equivalent to an exchange of 165 Gm. of chloride per hour or 3,960 Gm. Cl or 6,600 Gm. NaCl per day. Thus, the mass of NaCl passing in and out of the serum daily is about 1.2 times the weight of the dog. These quantities would be larger if the chloride mass were larger, as the data in these experiments suggest.

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TABLE I. MEAN VALUES OF DISTRIBUTION AND TURNOVER OF CHLORIDE IN BLOOD SERUM AND EXTRACELLULAR COMPARTMENTS FOR FOUR DOGS (DOGS 7012, 7013, 7015, AND 7016)

q = Proportion of total chloride not in the serum	0.7547
1 - q = Proportion of total chloride in the serum	0.2453
R_s = Proportion of seral chloride leaving the serum per minute	1.02
R_e = Proportion of nonseral chloride returning to the serum per minute	0.3315
$R_s \times (1 - q) = R_e (q)$ = Proportion of total body chloride moving in *or* out of the serum per minute	0.2502
$R_e + R_s$ = Proportion of total body chloride moving in *and* out of the serum per minute	0.5004

Comment: The rate of exchange of Cl^{36} and of Cl^{35} as calculated is based upon the *assumption* that the influence of mechanical mixing in the blood stream is completed early, within about one minute, and therefore contributes relatively little to the initial rapid rate of regression. This assumption, obviously, is not entirely valid. Nevertheless, the calculations were deemed interesting and justifiable since the studies of others[8] would suggest that "transfer" of the freely diffusible ions, such as Na, Cl, or K, from the blood stream through the walls of the capillary endothelium is so rapid that mechanical mixing is the limiting factor. In other words, the rate with which the electrolyte is brought to the capillaries where "transfer" can occur determines the rate of "transfer." This concept is probably true, for the surface area of the capillaries of man is about 6,000 square meters over which flow only about 3 liters of plasma several micra thick. It is, therefore, most probable, if similar anatomic and physiologic comparisons exist in the dog, that the rates of turnover for the dog are of the order described, although the absolute values certainly are subject to error.

The rapid rate with which the tracer reaches equilibrium of distribution throughout the body is consistent with the rapid rates of diffusion across the walls of the capillaries. The significance of these rapid rates of turnover of chloride in electrolyte metabolism and abnormal states of electrolyte and water metabolism, such as congestive heart failure and generalized edematous states, remains unknown and subject almost entirely to conjecture at this time.

(3) Biologic Decay Rates.—The biologic decay rates observed in five dogs are summarized by Table II and Fig. 2. The terminology employed has been described previously[9] and is briefly as follows:

(a) $C_{1/2}$ represents the length of time required for the concentration of the isotope to reach one-half its value after equilibrium of distribution of the isotope has been reached. The mean $C_{1/2}$ value for the serum was found to be 2.51 days, with extremes of 2.16 and 3.00 days.

(b) $U_{1/2}$ represents the length of time required for one-half the injected tracer to be excreted in the urine. The mean $U_{1/2}$ was found to be 3.50 days, the extremes being 2.75 and 4.00 days.

(c) $E_{1/2}$ represents the length of time required for excretion of one-half the injected tracer, by all routes, i.e., urine, feces, saliva, tears, and so forth. The mean $E_{1/2}$ was found to be 3.40 days, the limits being 2.75 and 3.80 days.

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TABLE II. CERTAIN OBSERVED BIOLOGIC DECAY PERIODS AND PERCENTAGE RECOVERY OF Cl^{36} IN FIVE DOGS

DOG	$C_{1/2}$ (DAYS)	$U_{1/2}$ (DAYS)	$E_{1/2}$ (DAYS)	BACKGROUND OBTAINED		Cl^{36} RECOVERED		
				SERUM (DAYS)	URINE (DAYS)	URINE (%)	FECES (%)	TOTAL (%)
7011	3.00	3.33	3.33	16.0	18.0	95.51	1.38	96.89
7013	2.50	4.00	3.80	30.0	31.0	81.16	5.89	87.05
7014	2.50	2.75	2.75	15.0	21.0	89.32	3.51	92.83
7015	2.40	3.80	3.50	22.0	21.0	92.48	1.72	94.20
7016	2.16	3.66	3.66	20.0	20.0	82.87	.60	83.47
Mean	2.51	3.50	3.40	20.6	22.2	88.27	2.62	90.89
Max.	3.00	4.00	3.80	30.0	31.0	95.51	5.89	96.89
Min.	2.16	2.75	2.75	15.0	18.0	81.16	.60	83.47

Thus in the normal dog on the diets indicated (Fig. 2) one-half the chloride of the entire body is renewed about every three and one-half days.

Obviously, it is not possible to determine with absolute accuracy the time when all of the tracer is excreted or when all has been collected, because the excretion is exponential in nature and when low concentration of the isotope is reached and the background levels are approached, large errors are introduced. Nevertheless, it was interesting to note the length of time required for the counts in the serum to reach background (Table II), the mean being 20.6 days, with extremes of 15 and 30 days. The mean for the urine was found to be 22.2 days and the extremes were 18 and 31 days. These time values are essentially seven to eight times the $C_{1/2}$ and $E_{1/2}$ values, a quantitative relationship to be expected in an exponential phenomenon with absolute values of such order.

For reasons similar to those just mentioned, the percentage of the injected isotope which was recovered in the excreta is subject to error. Nevertheless, the mean total recovery was 90.8 per cent of the amount injected, the extremes being 82.9 and 96.9 per cent.

Comment: The rapid rate of renewal of chloride in the body of the dog indicates the dynamic nature of the Cl ion in its body. This dynamic state of the chloride ion is supported by the rapid exchange between the chloride compartments of the body, the rapid rate with which equilibrium of distribution is established, and the rapid turnover of chloride in the body as a whole.

Reasons for differences in the $C_{1/2}$ and $E_{1/2}$ values are unknown. They may be due in part to a small daily loss in Cl^{36} during collection, measuring, cleaning the cages, and other such procedures, as well as possible gradual fixation of the Cl^{36} in compartments with relatively slow rates of exchange. Although such compartments may exist, definite ones are not known. It is possible that the rate of turnover of chloride in bone may be as slow as it is for sodium.

The significance of the rapid rate of turnover of chloride in the body and, of course, of the radioactive form can be appreciated in other biologic problems, such as planning of tracer studies with chloride or bromide, metabolic balance studies, bromide poisoning and its therapy, bromide therapy, atomic warfare, and exposure to radiochloride and bromide. These and other implications become evident with consideration of the biologic applications of the observations.

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The influence of intake of chloride, change in size of compartment, and other factors upon $U_{1/2}$, $E_{1/2}$, and $C_{1/2}$ are discussed later. For more detailed information reference may be made to similar discussions applied to Na^{22} studies in man.[9, 10]

These tracer studies corroborate data already available which show that only a small amount of chloride escapes from the body in the normal stool. The excretion of the Cl^{36} represented 2.6 per cent (range 0.6 to 5.9 per cent) of the total tracer administered. The difference between the values for $U_{1/2}$ and $E_{1/2}$ is due to that excreted in the feces. These studies also demonstrate the protective role played by the intestinal tract over the electrolyte stores of the body. On the basis of such findings many physiologists believe it permissible to ignore a study of the feces in certain types of metabolic studies of electrolytes.

(4) Chloride Space.—The chloride space was estimated from the regression curves shown in Fig. 2. The data indicate that equilibrium of distribution of the tracer is reached by the end of the first day and almost completely by the end of the first hour. The concentration of the isotope, CN_o, in the serum at t_o was, therefore, determined by extrapolating the curves of serum concentration (Fig. 2) to t_o. This value was then divided into the injected dose N_o for the dog. The resultant is the "serum equivalents" of chloride space in cubic centimeters. The mean chloride space in "serum equivalents" was found to be 34.6 per cent of the body weight of the dog, the extremes being 32.4 and 38.0 per cent (Table III).

TABLE III. CALCULATION OF Cl^{36} "SPACE" IN "SERUM EQUIVALENTS" IN FIVE DOGS*

DOG	AMOUNT INJECTED N_o (CPM)	INITIAL CONCENTRATION CN_o (CPM/C.C.)	Cl^{36} SPACE N_o/CN_o (C.C.)	BODY WEIGHT (GM.)	Cl^{36} SPACE AS % BODY WEIGHT
7011	1,025,000	620	1653	4654	35.52
7013	1,225,000	670	1828	5562	32.87
7014	900,000	580	1552	4086	37.98
7015	1,200,000	680	1765	5448	32.40
7016	900,000	640	1406	4086	34.41
Mean	1,050,000	638	1641	4767	34.64
Max.	1,225,000	680	1828	5562	37.98
Min.	900,000	580	1406	4086	32.40

*By use of the extrapolated value for the initial concentration (CN_o) of Cl^{36} in the blood and the injected dose (N_o) in counts per minute.

The time-course of the chloride space in "serum equivalents" was studied for one dog, Dog 7011. Corrections were made for the Cl^{36} excreted. Results are shown in Table IV. The average space was 39.6 per cent of the body weight as serum equivalents. The time-course difference between the concentration curve and the excretion curve in Fig. 2 is an index of the time-course of chloride space. When the concentration values approach background, errors in counting increase so that only the portions of the curves representing the first five days of the study should be considered to be accurate enough to warrant serious consideration. Since the curves of concentration and excretion are essentially parallel in their course, chloride space therefore remains essentially constant. The time to time variations are evident

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from the curves. Because of errors of an undetermined origin (probably in collection of excreta), the calculated space for the other dogs expanded with time.

TABLE IV. TIME-COURSE OF CHLORIDE SPACE IN "SERUM EQUIVALENT" FOR DOG 7011*

DAY t	AMOUNT Cl^{36} REMAINING N_t (CPM)	SERUM Cl^{36} CONCENTRATION CN_t (CPM/C.C.)	Cl^{36} SPACE N_t/CN_t (C.C.)	BODY WEIGHT (GM.)	Cl^{36} SPACE AS % BODY WEIGHT
0	1,025,000	620	1653	4654	35.52
1	901,000	500	1804	4597	39.24
2	678,476	386	1758	4540	38.72
3	578,047	307	1883	4654	40.46
7	242,451	123	1971	4881	40.38
9	172,641	89	1940	4994	38.85
11	124,576	54	2307	5221	44.19
Mean			1902	4792	39.62

*By use of the concentration in the serum at time t (CN_t) and the amount remaining in the body at time t (N_t).

Comment: The size of the chloride space is expressed in "serum equivalents," i.e., the volume of serum that would be necessary to provide a concentration of CN_0 if all of the radiochloride were in serum. The term "serum equivalents" was used because serum was studied and because the concentration of chloride in serum is approximately the same as that of interstitial fluid in general.

The data obtained indicate that the chloride space is larger than commonly considered, being about 35 per cent of the body weight in "serum equivalents" instead of 20 per cent. However, Winkler and co-workers[7] found it to be 25 per cent and suggested that it might be larger. From these data one of three conclusions may be drawn:

(a) The extracellular fluid space is greater than 20 per cent of body weight.

(b) Chloride is found in other compartments than the extracellular fluid in greater quantities than usually considered.

(c) Both the foregoing statements are true.

It is unlikely that the extracellular fluid space in a normal dog is as great as 35 per cent of the body weight of the animal; there are too many physiologic studies that would not support this. It is possible that the extracellular fluid space is somewhat greater than 20 per cent, since the accuracy of the methods available today for these studies has not been fully established and, therefore, may be subject to greater error than usually recognized.

On the other hand, it is probable that chloride is found in cells and bones to a greater extent than most studies indicate. If the extracellular fluid compartment is 20 per cent of the body weight, then the remaining 80 per cent of the body weight would contain 17.5 mEq. per liter of chloride in order that the total chloride space would be 35 per cent of body weight. This is true if there is homogeneity of distribution of chloride in the "tissues" of the body.

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Since there is obviously not homogeneity of distribution of chloride in the cells of the body, some tissues, such as muscle, would undoubtedly contain a much higher concentration and others a somewhat lower concentration. Heilbrunn[11] indicated that by his methods of study muscle contains 41 to 51 mEq. Cl per kilogram instead of 24 to 30 mEq. Cl per kilogram as determined by earlier procedures. Therefore, if the value for extracellular fluid space is assumed to be correct, the actual chloride space would be larger than that reported previously. It is known that chloride is found in fairly high concentration inside certain cells of the body, such as gastric mucosa, erythrocytes, renal tubular epithelium, and glomerulus of the sweat glands. It is conceivable that it may also be found in low concentration in other cells of the body. Although the concentration is low, the total amount would be large because of the large cellular mass.

It is also evident that the measurement of extracellular fluid mass based upon chloride studies is subject to considerable question. Observations must be re-evaluated or, at least, considered cautiously until the size and nature of the chloride space are more definitely established.

Variations in chloride space with change in body weight and aspects of the influence of several factors upon the rates of turnover within that space are discussed later.

GENERAL AND THEORETIC DISCUSSION

From the data presented it is evident that chloride must be intracellular in significant quantities. Therefore, it is not possible to employ measurements of chloride space as commensurate with measurement of extracellular water. If it is assumed that the extracellular water of the body is equivalent to 20 per cent of the body weight, then approximately four-sevenths of the chloride of the body is within and the remainder is outside the extracellular body fluid. The reasons for the failure to account for this remainder are not known, but the rapid rate of diffusion of chloride out of cells may be partially responsible. In addition, it has been found that if filter paper disks to which have been added sodium chloride in water at a pH of 7.3 and labeled with Cl^{36} are placed in an oven at 210° C. for twenty minutes, about 60 per cent of the chloride is lost by volatilization. Details of these studies will be reported elsewhere. Therefore, it is possible that dry ashing procedures employed to measure intracellular as well as total body chloride may result in the loss of chloride by volatilization. Such problems require further investigation before definite conclusions may be drawn.

In addition to the foregoing turnover data, there are certain calculations and theoretic considerations for chloride which can be applied to the dog as were applied previously for sodium in man.[11] For example, in the studies with sodium in man it was shown that the rate of intake of nontracer sodium and consequently the rate of output of sodium was one of the most important factors concerned in determining the rate of excretion of the tracer sodium, Na^{22}. The same was found to be true for the dog. A change in body weight and sodium space in man influenced slightly the rate of excretion and

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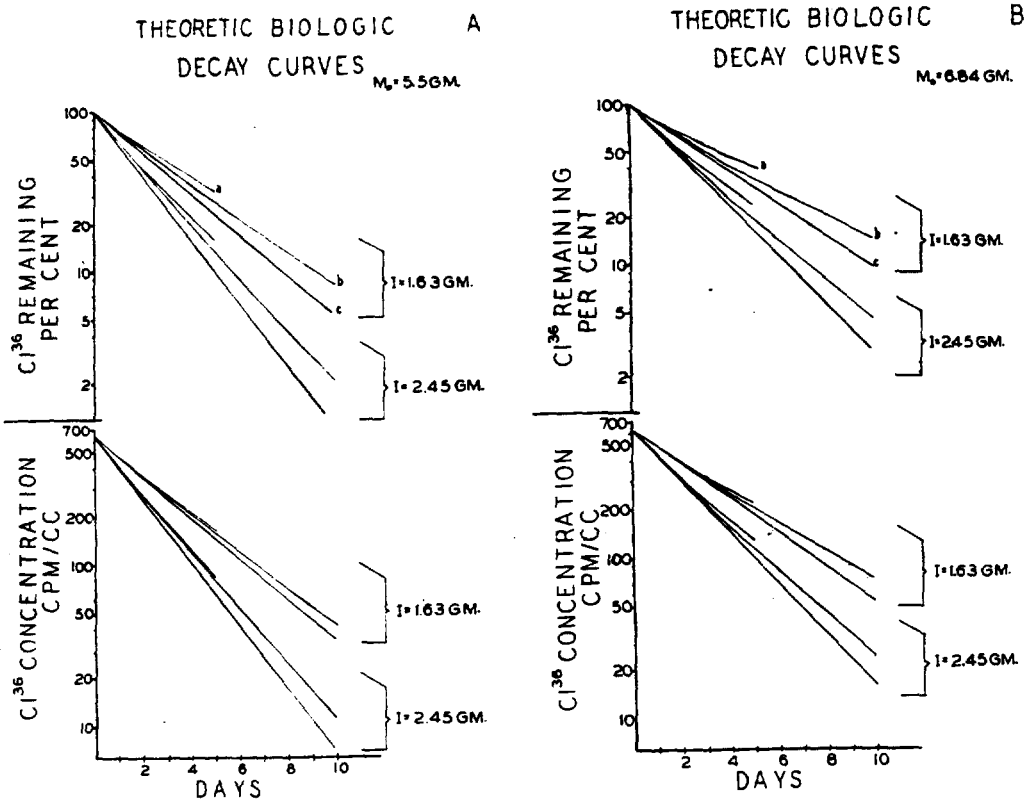

Fig. 3.—*A*, Theoretic curves showing the regressions of Cl^{36} from a dog weighing 4.54 kilograms assumed to have a chloride mass of 5.5 Gm., a value based upon data in the literature. In each group *a* would be the curve if the dog were gaining weight at a rate of 2 pounds in 5 days, *b* the curve if the dog were gaining 2 pounds in 10 days, and *c* if there were no change in weight. *I* is daily intake in grams. M_o is the total body chloride mass.

B, Same scheme as *A* except that the total body chloride mass (M_o) is considered to be 6.84 grams.

concentration of Na^{22} in the blood serum. In the dog, on the other hand, a change in body weight, such as a gain of 2 pounds in five days in a 10 pound dog or a gain of 20 per cent in body weight in five days had little influence on the theoretic rate of decrease in the concentrations of Cl^{36} in the blood serum of the dog (Fig. 3, *A*). This is to be expected since the intake of chloride in the dog is relatively high. A 10 pound dog has, according to the literature (Table V), about 5.5 Gm. of chloride in his body and his intake was 1.63 and 2.45 Gm. a day, which represents 29.6 to 44.5 per cent of the total body chloride. The same type of theoretic analyses is shown in Fig. 3, *B* for a dog with a total chloride mass of 6.84 Gm. per 10 pounds of body weight, a value suggested by the experiments. With this chloride mass a daily dietary intake of 1.63 or 2.45 Gm. represents 23.8 or 35.8 per cent of the chloride mass respectively. It is evident from Fig. 3 that on a similar rate of chloride intake the turnover of Cl^{36} is theoretically more rapid in the presence of the smaller body chloride mass. In the sodium studies the daily intake for man was about 5.0 per cent of the total body sodium. The rate of intake and output of the

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TABLE V. TOTAL BODY CHLORIDE FOR A DOG WEIGHING 10 POUNDS (4.54 KG.)*

OBSERVER	ANIMAL	WEIGHT (KG.)	Cl CONCENTRATION (MG./100 C.C. Cl SPACE)	Cl SPACE (C.C.)	CALCULATED TOTAL Cl CONTENT (GM.)	CALCULATED Cl CONTENT OF 4.54 KG. (10 LB.) DOG (GM.)
Winkler and associates[7]	Dog 1	11.0	378	2760	10.43	4.3
	Dog 2	11.4	397	2580	10.24	4.077
Harrison, H. E., Darrow, D. C., and Yarnet, H.: J. Biol. Chem. 113: 515, 1936	Dog 1	5.81	430.5	1510	6.5	5.079
	Dog 2	6.27	441.0	1756	7.74	5.6
Shohl, A. T.: Am. Chem. Soc. Mon. 82, N. Y., 1939, Reinhold Pub. Corp.	Man	70.0			85.0	5.51
Burch and associates	Mean of 5 dogs	4.54	435	1573	6.84	6.84

*Calculated from data reported in the literature and obtained in these experiments.

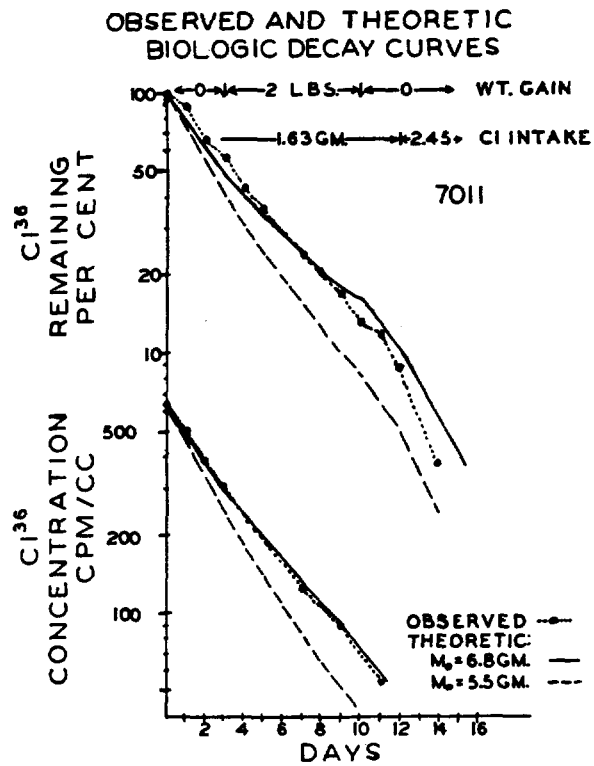

Fig. 4.—Comparison of theoretic with observed biologic decay curves. The dots represent the observed values, the solid line the theoretic curve derived from the observed intake and changes in weight if the chloride mass were 6.84 Gm., and the broken line the same curve if the chloride mass were 5.5 grams.

regular form of the electrolyte is the most influential factor governing turnover of the tracer material.[10] Change in body weight and chloride space are relatively less influential factors than intake in the dog. This is borne out

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by Figs. 3 and 4, which show that the theoretic $C_{1/2}$ and the curves of concentration-time course and rate of excretion tend to remain little changed with large percentage changes in body weight of the dogs. The normal average chloride intake for the dog is relatively high, so that a considerable change is necessary to reflect its influence upon the curves.

Comparison of actually observed curves with theoretic ones is shown in Fig. 4. They are remarkably similar where the mass of chloride in the body is assumed to be 6.84 Gm. for the theoretic circumstances. Furthermore, the closer similarity, or almost superimposition, of the observed biologic decay curves to the theoretic one based upon a chloride mass of 6.84 Gm. than to the theoretic curve based upon a chloride mass of 5.5 Gm. is in support of the larger chloride mass as the more correct value for this dog.

The importance of these factors can be appreciated when experiments concerned with electrolyte metabolism in dogs are compared with similar experiments in man. For example, a man weighing 70 kilograms, on a low sodium diet, 200 mg. daily, and with a total body sodium of 60 Gm. is receiving 0.33 per cent of his body sodium in his daily diet. A dog weighing 4.54 kilograms (10 pounds) with a comparable total body sodium would have to receive 12.8 mg. of sodium in his daily diet to be on a comparable diet. This is difficult to achieve and makes it difficult to compare the influence of sodium upon edematous or hypertensive states in the dog with those in man. This may be true for other animals. For even better appreciation of this a comparison may be made of the intake of a normal dog on an average daily intake of sodium chloride of 2.68 or 1.05 Gm. of sodium or 1.63 Gm. of chloride, which represents 29.6 per cent of his body chloride. A man would have to take in about 43 Gm. of sodium chloride a day to be on a diet comparable to that of the dogs. However, a diet low in sodium for a dog usually may be compared only insofar as the dog's daily intake is concerned and not with respect to his total body sodium.

The theoretic considerations previously reported for sodium in man[10] have many other important applications in turnover studies of tracer substances. They are too numerous to permit mention of more than a few in this report but become evident with careful consideration of the equations. For example, three additional applications are:

(1) If there is no gain in size of compartment after equilibrium of distribution has been established and if the exchange is free and dynamic, it is possible to determine the total body chloride also from the equation[10]:

$$CN_t = CN_o e^{-\frac{I}{M_o} t} \quad \text{or} \quad M_o = \frac{I}{-\log_e \frac{CN_t}{CN_o}} \times t.$$

This general equation becomes:

$$M_o = \frac{I}{-\log_e 0.5} \times C_{1/2},$$

where M_o = mass of chloride in the animal's body in grams,

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I = daily intake of chloride in grams,

$C_{\frac{1}{2}}$ = time at which CN_t is 50 per cent of CN_o or the concentration is reduced to 50 per cent of its initial value.

Table VI shows values for the total body chloride obtained from this equation.

TABLE VI. CALCULATION OF TOTAL BODY CHLORIDE*

DOG	$C_{\frac{1}{2}}$ (DAYS)	INTAKE I (GM./DAY)	TOTAL BODY CHLORIDE M_o (GM.)	TOTAL BODY CHLORIDE M_o PER 10 LB. BODY WEIGHT (GM.)	BODY WEIGHT (GM.)	TOTAL BODY CHLORIDE AS % BODY WEIGHT $(\frac{M_o}{BW} \times 100)$	CHLORIDE CONCENTRATION $\left(\frac{M_o \times 100{,}000}{\text{SPACE}}\right)$ (MG./100 C.C.)
7011	3.00	1.63	7.05	6.8	4654	.151	426
7013	2.5	1.63	5.88	4.8	5562	.105	322
7014	2.5	2.45	8.84	9.8	4086	.216	570
7015	2.4	1.63	5.6	4.64	5448	.103	317
7016	2.16	2.45	7.6	8.44	4086	.186	541
Mean	2.51	1.96	6.99	6.896	4767	.152	435

*The mass of chloride in grams calculated to be present from the equation $M_o = \frac{I}{(-\log_e 0.5)} \times C_{\frac{1}{2}}$. The estimated concentration in milligrams per 100 c.c. is calculated from this mass of chloride and the Cl^{36} space is recorded for each dog in Table III.

In Table V is shown the total body chloride calculated from the data obtained in these experiments and from the observations of others. A chloride content of 5.5 Gm. in a dog weighing 10 pounds (4.54 kilograms) was chosen arbitrarily from the calculations shown in Tables V and VI.

(2) If the intake and body mass of the regular form of the substance are known, it is possible to predict the $C_{\frac{1}{2}}$ for a tracer substance from the equation:

$$C_{\frac{1}{2}} = \frac{M_o\ (-\log_e 0.5)}{I}\ .$$

This equation has many applications in biology and atomic warfare.

Comparisons of $C_{\frac{1}{2}}$ for dogs and man are presented in Table VII. Values obtained by calculation and experiment show remarkable agreement. This holds if there is no change in the size of the compartment. It is, therefore, possible to predict closely the biologic half-life of such electrolytes as Na and Cl from such equations.

(3) It is also possible from the same general equation to calculate the intake (I) necessary for a given rate of change and total mass of the tracer substance. For example,

$$I = \frac{M_o\ (-\log_e \frac{CN_t}{CN_o})}{t}\ .$$

SUMMARY

1. The concentration-time course in the blood serum and turnover rates within the body in acute experiments and the concentration-time course, time-course of excretion in the urine and feces, and turnover rates for the entire body were studied in six normal dogs observed continuously for eighteen to thirty-one days under controlled metabolic conditions with long-life radiochlorine, Cl^{36} ($t_{\frac{1}{2}}$ about 2×10^6 years), as a tracer substance.

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TABLE VII. COMPARISON OF INTAKES OF SODIUM AND CHLORIDE FOR MAN AND DOGS*

	ANIMAL	WEIGHT (KG.)	$C_{\frac{1}{2}}$ (DAYS)	SODIUM M_o (GM.)	SODIUM I (GM.)	SODIUM $\frac{I}{M_o} \times 100$ (%)	SODIUM NaCl† (GM.)	CHLORIDE M_o‡ (GM.)	CHLORIDE I (GM.)	CHLORIDE $\frac{I}{M_o} \times 100$ (%)	CHLORIDE NaCl† (GM.)
Condition a:	Man	70	2.51	60	16.57	27.6	42.14	85	23.47	27.61	38.68
Based on rate of biologic decay in normal dog	Dog	4.54	(2.51)§	3.89	1.07	27.5	2.72	5.5	(1.63) 1.51	27.45	2.48
	Ratio: $\frac{\text{Man}}{\text{Dog}}$	15.4		15.4	15.5		15.5	15.5	15.5		15.6
Condition b:	Man	70	(13.3)	60	(3.1) 3.13	(5.2) 5.22	(8.0) 7.96	85	4.43	5.211	7.3
Based on rate of biologic decay in normal man	Dog	4.54	13.3	3.89	.203	5.22	.516	5.5	.286	5.200	.471
	Ratio: $\frac{\text{Man}}{\text{Dog}}$	15.4		15.4	15.4		15.4	15.5	15.5		15.5
Ratios of Conditions a and b for Man and for Dog											
			b:a		a:b	a:b	a:b		a:b	a:b	a:b
	Man		5.3		5.3	5.3	5.3		5.3	5.3	5.3
	Dog		5.3		5.3	5.3	5.3		5.3	5.3	5.3

*The intake (I) of sodium and chloride calculated as necessary to produce the $C_{\frac{1}{2}}$ values shown, provided there is no change in size of compartment. The $C_{\frac{1}{2}}$ of 2.51 days is that observed for Cl^{36} in dogs. The $C_{\frac{1}{2}}$ of 13.3 is that observed for Na^{22} in normal man.[7] M_o = The total amount in grams of the element in the animal. $\frac{I}{M_o} \times 100$ = The percentage of the total Cl or Na mass represented by the intake per day.

Where M_o is constant, to shorten $C_{\frac{1}{2}}$ by a factor of 5.3, the intake (I) must be increased by a factor of 5.3. Likewise if M_o is 15.4 times as large for one animal (man) as for another (dog), then the intake (I) must be 15.4 times as large for the $C_{\frac{1}{2}}$ to be equal.

It is evident that the relationship of $\frac{I_a}{I_b} = \frac{C_{\frac{1}{2}b}}{C_{\frac{1}{2}a}}$ holds where I_a is initial intake, I_b is the necessary or new intake, $C_{\frac{1}{2}a}$ is the initial time required to reach one-half concentration, $C_{\frac{1}{2}b}$ is the desired time. Or, in an animal whose compartment size is constant, a desired change in the slope of the biologic decay curves may be obtained by changing the intake by the reciprocal of the ratio of the initial and "desired" slopes. This holds true for both positive and negative changes.

There is remarkably close agreement between calculated and experimentally obtained values.

†The daily NaCl intake required to produce $C_{\frac{1}{2}}$ value indicated.

‡Taken from Shohl, A. T.: Am. Chem. Soc. Mon. 82, New York, 1939, Reinhold Publishing Corporation.

§Values in parentheses were observed.

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2. The mean concentration-time course of Cl^{36} in the blood serum for the first 60 minutes after intravenous administration can be expressed by the multiple exponential equation

$$CN_t = 1250e^{-2.079t} + 680e^{-0.[illegible]t} + 144e^{-0.0377t} + 674e^{-0.000173t},$$

where CN_t is the concentration of Cl^{36} in the serum at any time t.

3. All the seral chloride leaves the serum each minute and about one-third of the nonseral chloride returns to the serum each minute. Thus, about 2.75 Gm. of chloride pass out of and into the serum each minute or about 3960 Gm. daily, which is equal to 6600 Gm. of NaCl or about 1.2 times the body weight of the dog.

4. The mean $C_{½}$ value for the blood serum was 2.51 days (range 2.16 to 3.00 days), the mean $U_{½}$ was 3.50 days (range 2.75 to 4.00 days), and the mean $E_{½}$ was 3.40 days (range 2.75 to 3.80 days).

5. The mean total recovery was 91 per cent of that administered (range 83 to 97 per cent), 88 per cent (range 81 to 96 per cent) being in the urine and 2.6 per cent (range 0.6 to 5.9 per cent) in the feces.

6. The mean chloride space in "serum equivalents" was 35 per cent of the body weight (range 32 to 38 per cent) and the mean total body chloride was 6.84 Gm. for a dog weighing 10 pounds (4.54 kilograms).

7. These experiments indicate that:

a. The chloride space is larger than previously reported.

b. Chloride space is not a measure of the extracellular fluid space.

c. Chloride is present in considerable quantities outside the extracellular fluid space.

d. A man weighing 70 kilograms would have to consume 43 Gm. of NaCl daily to have a dietary intake of this salt comparable to that of an average dog.

e. A diet of 200 mg. of sodium for a man weighing 70 kilograms is equivalent to a diet of 13 mg. for a dog weighing 4.54 kilograms (10 pounds). Thus the usual studies of low sodium metabolism in small animals with hypertension must be evaluated cautiously. A man of 70 kilograms must consume 15 to 22.5 pounds of food daily to take in equivalent quantities of the same food substances as a normal dog of 4.54 kilograms (10 pounds).

8. The importance of these experiments in the planning of tracer studies with chloride and bromide and understanding metabolism and excretion of chloride and bromide, bromide therapy and intoxication, edematous states, intoxication due to radiochloride and radiobromide in atomic warfare, and other physiologic processes is evident from the data.

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