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DETERMINATION OF EXTRACELLULAR FLUID VOLUME BY ^{24}Na ISOTOPE IN ANGORAS

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Introduction

Determination of the volume of the various fluid compartments of the body is important for recognizing the relationships existing between water and electrolyte household. Such studies have been performed widely, on various species of animals (Coghlan et al., 1977; Hix et al., 1959; Rico et al., 1972; Ward et al., 1972). In meat rabbits the size of the extracellular fluid volume (ECFV) has already been determined by several authors (Hahn and Hevesy, 1941; Manery and Bale, 1941; Manery and Haege, 1941; Rico et al., 1972). In Angora rabbits, however, not studies of this type have been performed so far.

This prompted us to examine the ECFV in Angora rabbits by means of ^{24}Na isotope. A further objective was to establish the optimum time of ECFV determination. A method is proposed by which it will be possible to monitor alterations in electrolyte and water household after sodium and water loading or sodium and water withdrawal. But first we have to know the size of ECFV in Angora rabbits in normal physiological circumstances.

Materials and Methods

Sixteen male Angora rabbits of 2600 to 4700 g body mass were used. The rabbits were placed in the metabolic cages. They were weighed on the day of the experiment. During the period of study food and water were withheld and the urine was collected. ^{24}Na as sodium chloride was used in the experiments for ECFV determination (activity 200 MBq/ml). On arrival

it was diluted to 1,6 MBq/ml with sterile 0,9 % NaCl. 1 ml of the appropriate solution (1,6 MBq/ml) was injected into the animals.

The experiment was carried out in two parts. In Experiment 1 we wanted to know when ^{24}Na reaches equilibrium with the extracellular fluids post injection. Four rabbits received the isotope intravenously (iv.), whereas another four intraperitoneally (ip.). In the 5th, 10th, 20th, 30th, 60th, 120th and 180th minute after isotope administration, blood samples were taken from the ear vein into a heparinized tube. At the end of the experiment (180th minute) the volume of the excrated urine was measured.

Also in Experiment 2, four rabbits received the isotope intravenously and another four intraperitoneally. Based upon the results obtained in the first experiment, blood samples were taken only on three occasions, i.e. in the 60th, 120th and 180th minute after treatment.

Blood samples taken at the above-mentioned times were examined for haematocrit value, and blood and plasma radioactivity. The volume of the collected urine, and its radioactivity, were measured. The radioactivities of samples were measured in a hollow scintillation head with a type NK-350 (Gamma, Budapest) scaler.

Most of the methods applied for determination of the fluid volume are based upon the dilution technique. The extracellular fluid volume was calculated from the injected dose, corrected for urinary losses and the plasma count rate (corrected for background). Then

$$ECFV = \frac{N_i - N_u}{N_p}$$

where N_i = activity of injected ^{24}Na in Bq/ml

N_u = " of ^{24}Na of urine in Bq/ml

N_p = " " of samples in Bq/ml

Results

The mean values of ECFV measured at different times during the experiments are shown in Table 1. In Experiment 1 the values gradually increased up to the 60th minute in intravenously treated animals and then this was followed by the onset of an equilibrium. In the case of intraperitoneal treatment the highest value was obtained in the 5th minute, whereas the lowest in the 10th minute.

Than the values increased till the 60th minute. Between the 60th

and 180th minutes a slight increase could be observed. In Experiment 2 the alterations of the mean values of ECFV measured in the 60th, 120th and 180th minute were similar to those obtained in the first experiment. With knowledge of the mean body mass and ECFV measured at the various times, the percentual proportion of ECFV can be calculated. Detailed data are shown in Table 2.
The changes of the haematocrit values during the experiments are presented in Table 3.

Discussion

In Experiment 1 in the case of intravenous administration the isotope moved directly from the intravascular space into the interstitial space at a high speed, e.g. in guineapigs 60 % of the injected Na isotope leaves the intravascular space within 1 minute (Schwarczmann, 1968). Due to the above mentioned facts, by the time of the first blood sampling (5th minute) most of the ^{24}Na entered the interstitial space. Subsequently an ever decreasing activity was measured in the plasma, wich was indicated by the elevation of the values of ECFV. This elevation lasted up to the 60th minute. At that time the isotope was already homogeneously distributed; however, parallel with the onset of equilibrium the isotope gradually moves from the extracellular space back towards the intravascular space, and ^{24}Na is being excreted. Since the isotope is gradually excreted via the kidney, the ^{24}Na activity of the plasma decreases.

When the isotope was injected intraperitoneally the isotope first has to get absorbed from the abdominal cavity into the intravascular space, the lowest ^{24}Na activity was measured in the 5th minute. The isotope must have entered blood circulation, through the capillary membrane, between the 5th and 10th minute: this accounted for the fact that the highest plasma ^{24}Na activity was measured in the 10th minute simultaneously with the lowest value of ECFV. Subsequently the values were similar to those found after intravenous administration, i.e. equilibration of ^{24}Na in the extracellular fluid was probably achieved within 60 minutes. Simultaneously, isotope excretion began in the kidneys.

On the basis of the results obtained with the ^{24}Na isotope administered intravenously and intraperitoneally, the value measured in the 60th minute was accepted as the ECFV of Angora rabbits. Results of Experiment 2 confirmed this, since with both modes of administration the alterations of the values of ECFV measured in the 60th, 120th and 180th minute were

similar to those obtained in the first experiment.

So on the basis of the two experiments done with 16 animals, accepted the value measured in the 60th minute, the percentual size of ECFV (^{24}Na space) was 25,66 ± 0,84 % (mean ± sd) of the body mass in Angoras.

Using ^{24}Na isotope, for meat rabbits Manery and Bale (1941) reported an ECFV of 25 %. At the same time, also with ^{24}Na isotope, Hahn and Hevesy (1941) reported a higher value (ECFV = 28 %). Contrarily, Rico et al., (1972) determined, using ^{35}S isotope, a smaller (22,5 ± 1,5 %) ECFV in meat rabbits.

Multiple blood sampling resulted in a significant blood loss for which the animal could not compensate rapidly by mobilizing its blood stores. This accounts for the observation that in Experiment 1, where blood samples were taken altogether on seven occasions, a larger decrease of the haematocrit value was found (iv. 9,33 %; ip. 13,64 %) than in Experiment 2 in which only three blood samples were taken (iv. 2,6 %; ip. 2,4 %). It can be seen that the number of blood samplings should be as low as possible to reduce blood less.

If we accept the value determined in the 60th minute as the ECFV of Angora rabbits, a single blood sampling, performed one hour after the application of the ^{24}Na isotope will be sufficient to determine extracellular fluid volume of Angoras.

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Table 1.

The values of the ECFV as a function of time (ml)

Mode of isotope administration	Time of blood sampling (min)						
	5	10	20	30	60	120	180
	mean ± sd						
Intravenous (n = 4)	844 ± 48	954 ± 58	1057 ± 53	1106 ± 54	1173 ± 76	1186 ± 76	1192 ± 75
Experiment 1							
intraperitoneal (n = 4)	1055 ±143	795 ± 59	841 ± 27	873 ± 30	942 ± 60	976 ± 26	1000 ± 50
Intravenous (n = 4)	-	-	-	-	748 ± 94	766 ±103	778 ±104
Experiment 2							
intraperitoneal (n = 4)	-	-	-	-	757 ± 68	764 ± 62	770 ± 59

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Table 2.

Extracellular fluid estimated by ^{24}Na dilution in angora rabbits

Mode of isotope administration	^{24}Na space (% body weight)		
	60 th minute	120 th minute	180 th minute
	mean ± sd		
Intravenous (n = 4)	26,70 ± 0,47	26,96 ± 0,49	27,13 ± 0,45
Experiment 1			
intraperitoneal (n = 4)	25,43 ± 0,75	26,38 ± 1,23	26,80 ± 1,28
Intravenous (n = 4)	25,33 ± 0,85	25,90 ± 0,94	26,33 ± 1,02
Experiment 2			
intraperitoneal (n = 4)	25,23 ± 0,47	25,48 ± 0,29	25,70 ± 0,25

Table 3.

Change of the haematocrit (Ht %) in the experiments

Time of blood sampling (min)	Experiment 1		Experiment 2	
	iv.	ip.	iv.	ip.
5.	37,5	44,0		
60.			42,0	39,5
180.	34,0	38,0	41,0	38,5

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The isotope was applied by two different manner, given intravenously and intraperitoneally to adult Angora bucks. Distribution of ^{24}Na was investigated in two experiments. During the **first experiment**, the time of homogenous distribution of isotope was determined on the basis of activities measured 5, 10, 20, 30, 60, 120 and 180 min after the application. This developed in the 60th min. According to this, the extracellular (EC) fluid volume was investigated in the **second experiment** only 60, 120 and 180 min after the application and the values obtained were compared to the corresponding values of the first experiment. Percental size of extracellular fluid volume was calculated, which was 25,66 $\pm$ 0,84 % of the body mass in Angoras. According to the comparable results of the two experiments, it has been pointed out that extracellular fluid volume of Angoras can be determined by ^{24}Na isotope in a single blood sample taken 60 min after the application of the isotope.

MESSUNG DES EXTRAZELLULAREN WASSERAUMES IN ANGORAKANINCHEN MITTELS ISOTOPS ^{24}NA

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Das Radionuklid ^{24}Na wurde erwachsenen, männlichen Angorakaninchen iv. und ip. eingespritzt, und dann die Verteilung der Radioaktivität in 2 Versuchen gemessen. **Im 1. Versuch** hatte man an Hand der 5, 10, 20, 30, 60, 120, und 180 Minuten nach der Injektion gemessenen Radioaktivität festgestellt, dass die Verteilung 60 Minuten nach der Injektion homogen war. **Im 2. Versuch** prüfte man den extrazellularen Wasserraum (EC-Raum) nur mehr 60, 120 und 180 Minuten nach der Injektion, und verglich die Messwerte mit den im 1. Versuch erhaltenen betreffenden Werten. Aus den Messwerten errechnete sich das Verhältnis an EC-Raum (% der Körpermasse) im Organismus des Angorakaninchens, es wurde ein Wert von 25,66 $\pm$ 0,84 % gefunden. Die in den beiden Versuchen erhaltenen Werte waren miteinander gut übereinstimmend, man glaubt deshalb, dass eine einmalige Blutentnahme 60 Minuten nach der Injektion von ^{24}Na für die Messung der Radioaktivität genügt, um den Prozentsatz an EC-Raum bestimmen zu können.