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Muscle Electrolyte Composition Determined by Neutron Activation A Preliminary Study of Dehydration in Infants.[1]

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INTRODUCTION

Because muscle tissue is abundant, easily approachable and of relatively constant composition, both hydrosaline and energetic metabolism can be easily studied in the muscle area.

The activation analysis method, recently applied by Bergström (1) to the study of this material allows electrolytes (Na, K, Cl) and phosphor determinations, on small muscular fragments taken by needle biopsies. This method has been adapted to the irradiation characteristics of the BR_1 and BR_2 reactors of the C.E.N. Mol (Belgium). In order to increase the speed and precision of the determination of induced activity, a new technique of measurements was devised.

The purpose of this work, of which preliminary results are described in this paper, is a direct observation of the muscular hydrosaline equilibrium in some states of infantile dehydration.

MATERIAL AND METHOD

Needel biopsy technique. The technique of the needle muscular biopsy was described by Bergström (1), who modified the Pollen and Bickel needle, which is pointed and hollow with a window at its distal end. A cylinder with a sharp rim fits into the needle and permits the sampling of material engaged in the window. Finally, a stylet may be introduced in the cylinder in order to extract the sample (Fig. I).

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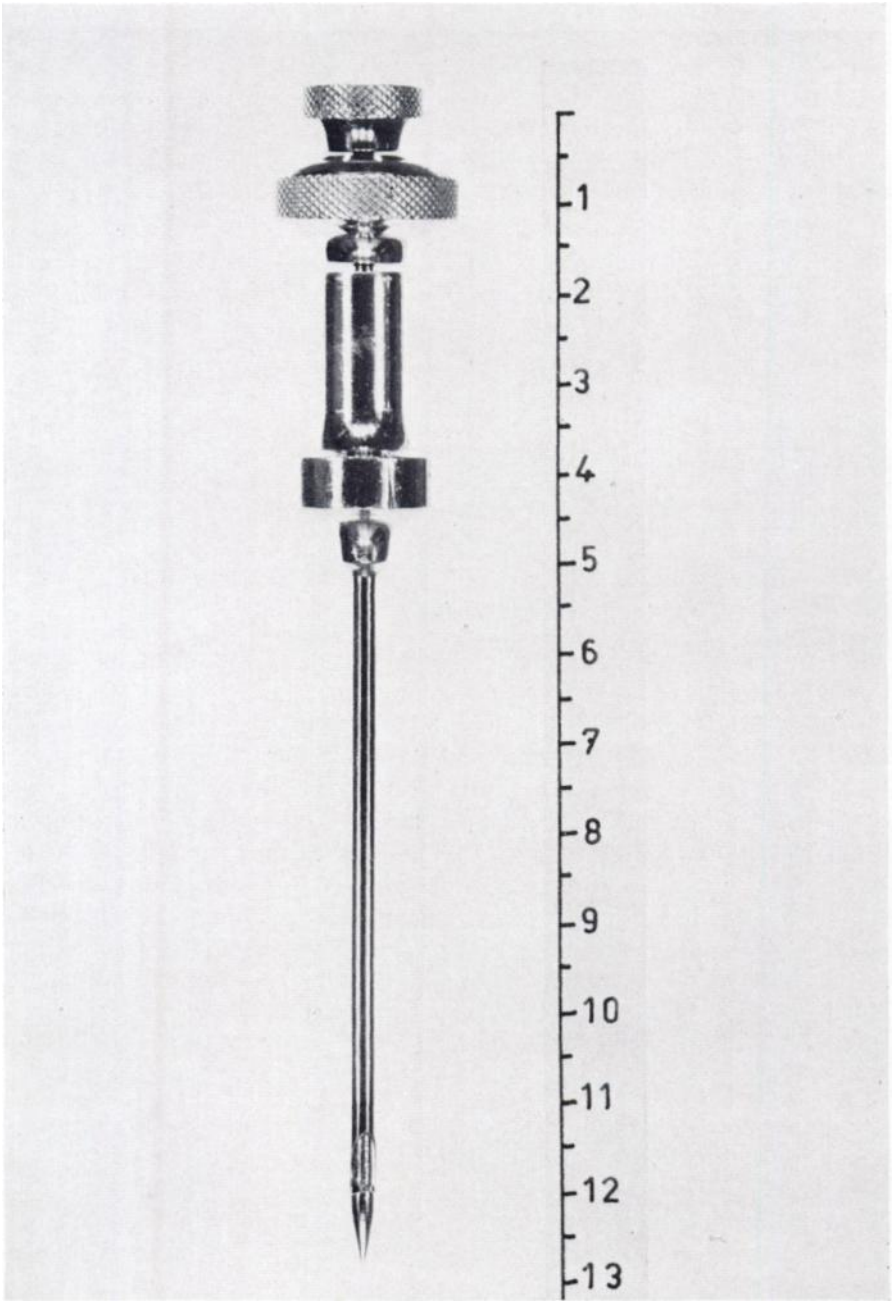

Fig. 1a. Needle[1] used for muscle biopsy similar to that used by Bergström (1) but modified for pediatric investigation.

[1]Manufactured in Belgium by SIMAL (GEMBLOUX).

The biopsy is taken in the external muscular mass of the thigh. After local anaesthesia, the skin is opened two or three millimeters and the puncture biopsy is made through this orifice. The sample is immediately blotted with filter paper to remove surface blood. Visible pieces of fat and connective tissue are discarded.

The sample is then suspended on a zirconium hook of known weight (0.4-0.6 mg). Then the hook and specimen are weighed on a Cahn electro-balance. We have noted that the activity induced in platinium by the activation interferred with the muscular electrolyte activity spectrum. We therefore prefer using zirconium instead of platinium as initially described by Bergström.

Each sample is weighed five times at 30-second intervals. The weights are then plotted against the time elapsed since the biopsy was taken (Fig. 2).

The decrease of weight of the biopsy because of evaporation is linear during 10 minutes; since the first determination is done after approximately one minute 30 seconds, the straight line joining the points can be extrapolated to zero time. This point gives us the exact weight of the tissue at the moment of sampling (Fig. 2).

During all these measurements and subsequent manipulations, the biopsy remains suspended on the hook.

ANALYTICAL METHODS

The degree of hydration of the specimen is determined by weighing before and after it is dried to constant weight in an oven at 105° centigrade. Generally, the musclar samples are left in the oven overnight. Flear and Florence (2) have noted that for small fragments weighing between 10 and 14 mg a constant weight is obtained after 30 minutes drying at 105° centigrade.

Fat is extracted with petroleum ether (40°-60°). Petroleum ether was used because it was similar to the method described in the literature for fat extraction from small samples (1,2). After each extraction the sample is weighed. A constant weight is obtained after two or three extractions and dryings. Petrol ether washings were discarded because it was found on several occasions that they contained no measurable amounts of electrolytes. The fat content is calculated by the difference in weight.

The amount of sodium, chlorine, potassium and phosphorus can be determined on the sample used for analysis of fat and water content.

The available neutron flux at the C.E.N. (Mol–Belgium) is 4.10^{11} neutrons/cm^2/sec. in BR1 reactor and 10^{14} neutrons/cm^2/sec. in BR2 reactor. When a biological sample is irradiated with neutrons for a short period at these fluxes, most of induced radioactivity emanates from the sodium, chlorine, potassium and phosphorus isotopes (Table I).

Thus, conditions are favourable for the determination of these elements in biological materials.

The principal components of proteins and other organic substances (hydrogen, carbon, nitrogen and oxygen) do not produce enough radioactive isotopes to disturb the measurement of other elements.

The biopsy in individual polyethylene flasks are irradiated in groups of six in the BRI (30 min.) with a neutron flux of 4.10^{11}/cm^2/sec.

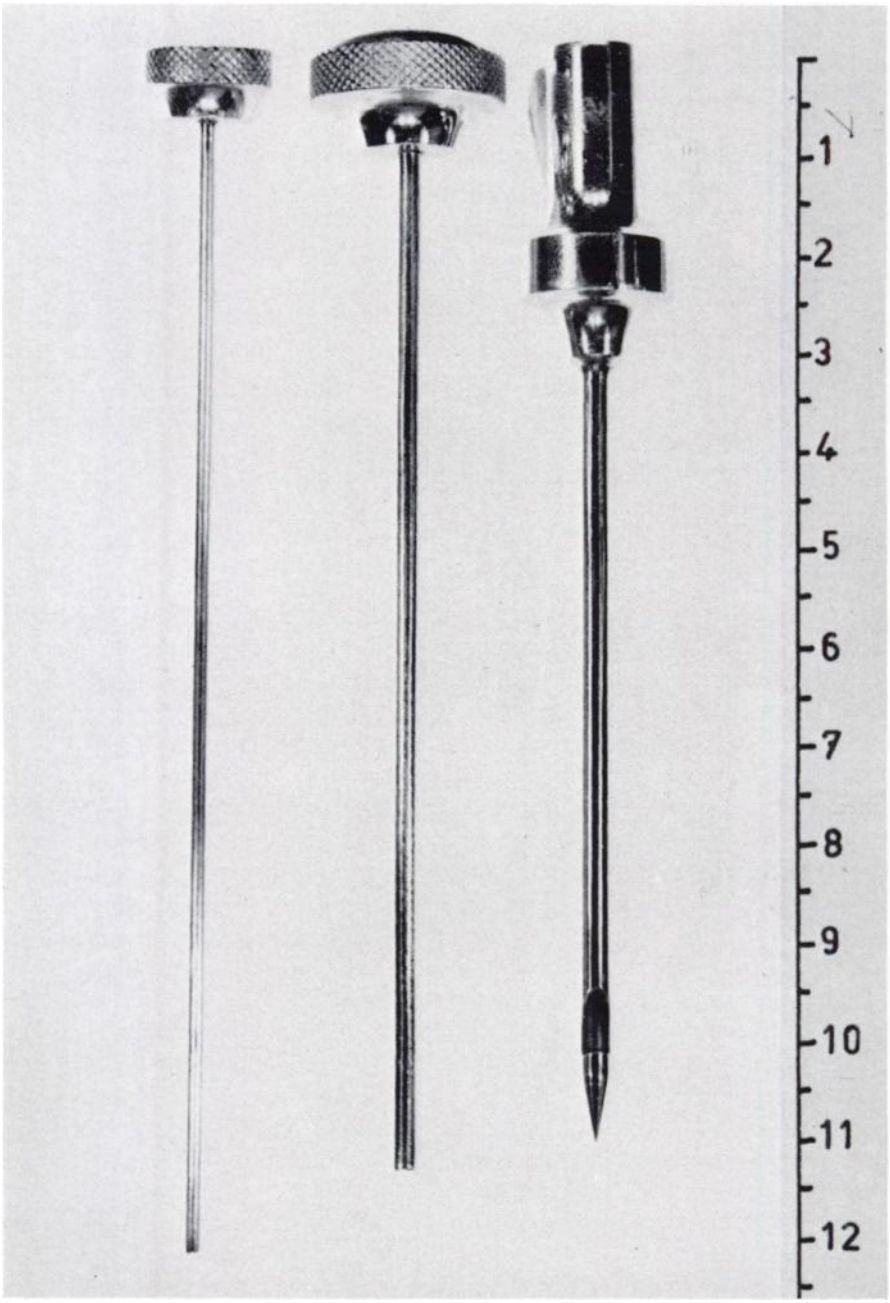

Fig. 1b. From right to left: 1) Hollow needle window diameter (0.6 cm). 2) Cylinder with a sharp rim (diameter 0.2 cm). 3) Stylet (diameter 0.15 cm).

The isotopes produced are Cl^{38} with a half-life of 37.5 min. emitting 1.60 Mev and 2.15 Mev gamma rays and Na^{24} with a half-life of 15 h. emitting 1.37 and 2.75 Mev gamma rays (Table I).

A sample of 1 ml of a solution of NaCl of known concentration, (O.OIM), is irradiated simultaneously with each group of biopsies. After irradiation, this solution is diluted to 10 ml and two samples of 0.5 ml are pipetted as standards. The amounts of Na and Cl in the biopsies are determined by comparison with these standards.

In order to differentiate the gamma ray characteristics of Na^{24} from Cl^{38}, the measurements are made by gamma spectrometry.

The equipment used requires a well-type NaI crystal detector of 3″ × 3″. The gamma spectrum is accumulated in the memory of a 400 channel analyser (Intertechnique) for five minutes and automatically recorded on a magnetic tape. The following sample is measured and recorded immediately afterwards. A perfect stability of the spectrum is obtained by means of a drift stabilizer, "Stabimat", centered on the Na^{24} 2.75 MeV photopeak.

The quantitative measure of Na^{24} is calculated from the 2.75 MeV photopeak rather than the 1.37 MeV photopeak, which is distorted by the K^{42} 1.53 MeV photopeak.

A reference spectrum of Na^{24} is recorded on a magnetic tape in the same conditions as those of the samples. To obtain the relative activities, this spectrum or one of its multiples is subtracted, by means of an intermediate computer (RG

TABLE I

ISOTOPE OF SODIUM, POTASSIUM, CHLORINE AND PHOSPHORUS OBTAINED BY NEUTRON ACTIVATION.

	Half life	*Capture cross section* (*σ. act.*)	γ	β
Na^{24}	15.0 h	0.52 barn	1.37 Mev 100% 2.75 Mev 100%	1.39 Mev 100%
K^{42}	12.5 h	1.17 barn	1.53 Mev 18%	3.54 Mev 82% 1.98 Mev 18%
Cl^{38}	37.5 min.	0.56 barn	1.60 Mev 31% 2.15 Mev 47%	1.11 Mev 31% 2.77 Mev 16% 4.81 Mev 53%
P^{32}	14.3 days	0.21 barn	—	1.71 Mev 100%

23 Intertechnique), from that of the samples and of the standards. Correction for decay gives the values of Na in the samples compared to the standards.

In order to measure chlorine and after substraction of the sodium spectrum, the same procedure is used with a reference spectrum of chlorine-38.

By activation of potassium and phosphorus, K^{42} and P^{32} are formed. These isotopes are measured by β spectrometry because P^{32} is a pure β emitter and the 1.53 MeV photopeak of K^{42} is partially hidden by the 1.37 MeV photopeak of Na^{24}.

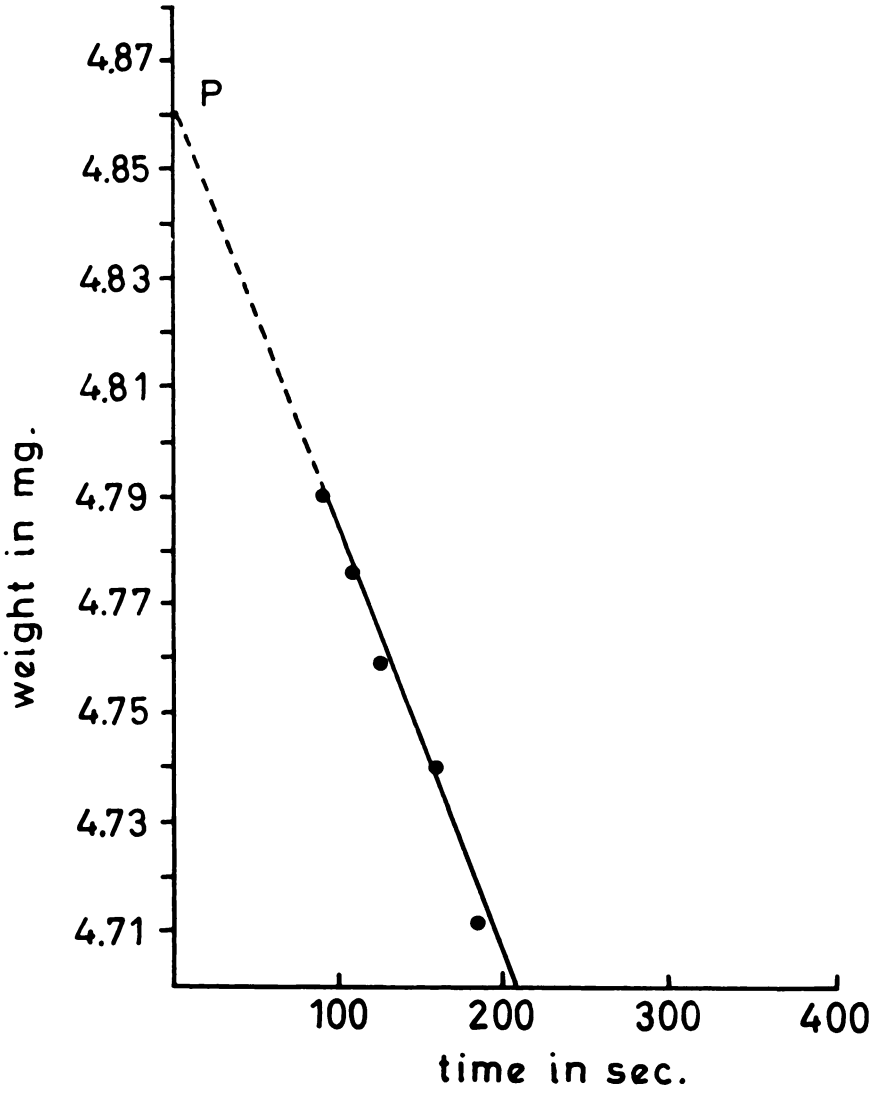

Fig. 2. Weight curve of a muscle sample extrapolated to zero time. P. extrapolated weight of the biopsy at the moment of sampling.

After determination of Na and Cl, the samples and two standards of KH_2PO_4 are irradiated for ten minutes with a BR2 flux of 10^{14} neutrons/cm^2/sec.

Afterwards, they are kept for 24 hours to obtain a sufficient decrease of Cl^{38} and Mn^{56} activity. The biopsy is then digested in 3 ml of 7N warm nitric acid containing a carrier of KH_2PO_4 10^{-2}M.

Three samplings of the solution are pipetted off on counting trays and dried under an infrared lamp.

The counting trays are then placed under an antracene crystal mounted on a photomultiplier connected to a monochannel analysor.

To measure K^{42} (max β emission : 3.5 MeV), and in order to avoid the detection of P^{32} (max. : 1.7 MeV) the monochannel analysor window width is 2 MeV and the lower level is 2 MeV. However, the result must be corrected because of the presence of sodium-24. In reality, the gamma rays of 2.75 MeV can be detected by the Compton effect in anthracene. Moreover, the simultaneous detection of Na^{24} β ray (1.39 MeV) and of the Compton event of one of the γ radiations of Na^{24} can give an impulse exceeding the 2 MeV threshold fixed at the monochannel. In the material studied, this error can reach 25% of the total activity.

The measurement of P^{32} is done on the same counting trays after 10 days, allowing time for the decay of K^{42} and Na^{24}.

In this case, the monochannel window set from 0,4 to 1,8 MeV does not detect the S^{35} and Ca^{45}.

The K and P contents of the muscular sample are calculated in reference to the standards.

PRELIMINARY RESULTS AND DISCUSSION

Preliminary results are shown in Table II and III. Table II points out the values obtained by chemical and neutron activation methods in normal children, and shows that there is no significant difference between the results obtained by the two methods.

We emphasize the fact that all our biopsies were done on children, because the values are different from those obtained in adults and cannot be compared. Similarly, Bergström's values for normal adults by the neutron activation method are identical with those found by other authors (3, 4, 5, 6, 7, 8) by chemical methods and we may therefore assume that the values found by the two methods are comparable.

Table III shows quantities of total water and electrolytes found in children with two different acute dehydration states (Isotonic and hypertonic dehydrations). We have classified patients as hypertonic if serum sodium concentrations are 150 mEq/liter or higher.

The preliminary results seem to indicate a rapid participation by muscular tissue in water and electrolyte movements which are characteristics of the above states. Moreover, these variations are generally similar to those obtained in the plasma. This seems to show that the muscular water osmolarity is well depicted by the plasmatic ionogram. In certain conditions, however, the interpretation of the data is ambiguous.

Our results are expressed, as usual, in relation to dry fat free solid. Thus we assume that the dry tissue remains constant during all pathological processes. This assumption is probably true in acute states of dehydration occuring in previously healthy children, but we have noted that this material was modified in some cases of chronic malnutrition (7).

In these last cases, the extracellular protein material composition is quantitatively different from that of normal muscle; the collagenous fractions become greatly augmented in comparison with the other extracellular protein fractions. Moreover the relation of total extracellular proteins to intracellular proteins changes, that is, referring the amounts of electrolytes to dry fat free weight will lead to a false interpretation. For example, the potassium values will be overestimated.

We have to admit, therefore, that reference to fat-free solids will be inadequate in some circumstances.

As do Lilienthal and his colleagues (9), some authors suggest expressing the results in terms of non-collagen nitrogen, that is, in terms of intracellular proteins and we have indeed demonstrated that non-collagen nitrogen remains constant even in severe pathological cases where collagen nitrogen is modified (10). It is impossible, however, to determine non-collagen nitrogen accurately on small fragments taken by needle biopsy. A surgical biopsy is necessary, but cannot be carried out in most cases.

TABLE II

MUSCULAR COMPOSITION IN TOTAL WATER, ELECTROLYTES AND PHOSPHORUS IN NORMAL CHILDREN, MEAN AND S.D.[1]

Column A: results obtained by neutron activation.

Column B and C: results obtained in two groups of children by chemical methods (7).

	A	*B*	*C*
Total water	354.7 ± 6.2 −9 cases	342.4 ± 5.0	346.2 ± 5.9
Na	18.4 ± 1.7 −10 cases	25.8 ± 1.0	16.8 ± 1.1
K	44.4 ± 1.2 −9 cases	42.2 ± 1.0	44.7 ± 1.3
Cl	13.5 ± 1.5 −10 cases	15.4 ± 1.1	17.4 ± 0.4
P	26.6 ± 1.4 −8 cases	29.9 ± 1.7 −6 cases	 −10 cases

[1]The results are expressed in g. for water and in mM for electrolytes and phosphorus, per 100 g. dry fat free solid.

TABLE III

PRELIMINARY RESULTS BY MUSCULAR BIOPSY ACTIVATION ANALYSIS IN SOME INFANTS WITH HYPERTONIC AND ISOTONIC DEHYDRATION.[1,2,3]

NORMAL MEANS		*HYPERTONIC DEHYDRATION*				*ISOTONIC DEHYDRATION*			
Cases		*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Total water	354 ± 6.2	337.2	315.45	277.2	313.1	352.07	334.0	331.8	284.8
Na	18.4 ± 1.7	26.6	22.6	21.8	17.2	14.8	18.5	19.4	19.7
K	44.4 ± 1.2	47.1	37.9	44.2	42.9	43.2	37.9	38.7	41.6
Cl	13.5 ± 1.5	22.3	18.3	12.1		15.2	14.5	20.3	14.3
P	26.6 ± 1.4	34.0	33.2	37.6	35.5	30.8	34.3	29.7	34.7
Na+ K {in mEq/liter plasma}	139 ± 4	176.0	162.8	171.2	156.1	138.8	141.0	152.5	148.6
Percentage of variation of electrolytes (Na+ K)									
a) in plasma		+26.2	+16.7	+22.8	+11.9	−0.1	−1	+9.1	+ 6.6
b) in muscular water		+20.1	+ 9.5	+34.4	+ 9.2	−0.6	−3.5	0	+22.4

[1] Results are expressed in grams for water and in mM for electrolytes per 100 g. dry-fat free solid.
[2] Percentage of variations of the plasmatic and muscular electrolytes in relation to the normal means has been noted.
[3] The concentration of Na + K are calculated in muscular water in mEq/l.

SUMMARY AND CONCLUSIONS

Following the work of Bergström in the adult, we have described a method of analysis of muscular electrolytes and phosphorus in children. By comparing our results with those obtained by the chemical technique we came to the conclusion that this method is reliable.

An analysis of needle biopsy specimens of muscle from children with different degrees of dehydration suggests that the muscular tissue is rapidly involved in acute hydro-electrolytic disorders. The preliminary data will be checked by the study of a larger series of cases.

Although the use of such a method is limited, it remains the only one which allows a direct study of tissue in pediatric pathology.

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