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APPLICATION OF NEUTRON ACTIVATION ANALYSIS TO THE STUDY OF THE VARIATIONS OF THE CONCENTRATION OF TRACE ELEMENTS IN VARIOUS ORGANS OF RAT AS A FUNCTION OF AGE

B. MAZIÈRE, C. LOC'H, O. STULZAFT, A. GAUDRY, D. COMAR

Commissariat à l'Energie Atomique – Département de Biologie, Service Hospitalier Frédéric Joliot – 91406 Orsay (France)

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In order to know the normal distribution of trace elements is rats, in terms of age, we have determined the elemental composition of different organs and body tissues by neutron activation analysis following an animal experiment during which the rats raised outside of any kind of pollution, were fed with a defined diet. The values observed for Co, Zn, Mn, Se, Fe, Cs, Rb, Hg, Cd in heart, body muscles, liver, kidney and testis are represented in table and figures. Except for renal accumulation of Hg and Cd, the more interesting fact revealed in this work, in the accumulation of Fe in body muscles and testis.

Introduction

Most research in animal biology requires the use, and consequently a knowledge, of "reference" values, referred to as "normal" values. The collection of such values is always difficult; and the lower the values, the more difficult it is to obtain them. We need only regard lists in the literature of "normal" concentrations prepared for trace elements to recognize this.[1,2] Knowledge of such concentrations in trace elements is particularly interesting for nutrition physiopathology and chronic toxicology, especially for problems associated with pollution.

In order to validly obtain these reference values from laboratory animals, several conditions must be fulfilled in advance:

- diet must be completely defined,
- the animal must be raised outside of any atmospheric or environmental pollution,
- comparative measurement must be taken at various moments in the life of the animal in order to detect eventual variations in such elemental concentrations; this latter condition implies the fact that diet must be exactly the same throughout the experiment.

In order to know the normal distribution of trace elements in rats as accurately as possible, we determined the elemental composition of different organs and body tissues by neutron activation analysis following an animal experiment conforming to the above-mentioned principles.

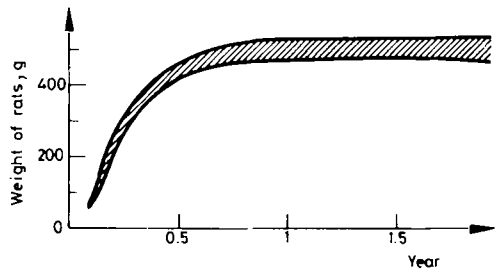

Fig. 1. The growth curve of the rats studied

Materials and methods

Animal experiment

In order to be able to determine the largest number of elements in the samples taken, the number of animals in each age group was necessarily limited.

It was consequently important to have available "standardized" animals. We used S.P.F. rats of the Sherman strain, known for their regular growth.*

As of fecundation, the mothers, raised under completely sterile conditions, were fed with the same diet (UAR DO4) as that used during the two years of experiments. This diet is sterilized and ensures animals of a normal growth up to the adult stage; as of this stage weight remains constant.

As of the end of weaning (22 days), 30 young male rats were placed in metabolic cages entirely made of plexiglass and located in an air-tight plastic chamber fed with filtered air. Temperature and humidity were closely controlled. Food and drink (spring water with a low mineral content) were given "ad libidum".

The animals were weighed regularly and the growth curve represented by Fig. 1 (average weight and standard deviation) indicated that all of the rats studied developed in a comparable manner.

The animals were broken down into groups of 3 and sacrificed at intervals of increasing time (from 15 days to 3 months). The rats were anaesthetized and then bled to death. The various organs were removed in a dust-free chamber using plas-

* The animals were supplied by the C.S.A.L. of the C.N.R.S. in Orleans.

tic dissecting instruments. The organs removed were immediately conditioned in polyethylene flasks, freeze-dried and crushed at the temperature of liquid nitrogen.

Preparation of samples and irradiation

Approximately 50 mg of dried organ powder were removed and conditioned in silica glass vials.

The first 20 microns of thickness were removed, using a solution of hydrofluoric acid in order to reduce contamination of the sample from the irradiation container.[3] After additional freeze-drying, the samples were weighed and the vials sealed.

The standards were constituted by solutions of spectrographically pure elements, deposited in quartz tubes on ultra-pure glucose and freeze-dried. All the standards needed for the many analyses of this experiment were simultaneously prepared from the same solutions.

Chemical analysis

In order to determine the largest number of elements possible, we used the three possibilities offered by neutron activation:

- individual analysis after chemical separation,
- multielement analysis by direct γ-spectrometry using a Ge(Li) diode without chemical separation,
- multielement analysis after chemical separation.

In order to confirm the results obtained with one of these techniques each sample was analyzed by at least two of these techniques.

For multielement analysis after chemical separation, we used an original technique recently described.[4]

In order to measure simultaneously radioelements of short and long periods with a maximum of accuracy, separation (Fig. 2) was carried out in a shielded chamber following intensive irradiation (integrated neutron flux of approximately 10^{19}). After rapid wet-ashing and distillation of volatile elements, the samples were taken up in phosphoric acid and fixed on series of 3 anion and cation exchange resin columns. Three different elutions were then carried out on the last group of cationic resins. Finally, all the elements constituting the sample were separated into 7 groups (3 resins, 3 eluates, 1 distillate) the radioactivity of which was then measured with a Ge(Li) diode.

The separation device used was semi-automatic and measured 24 elements in each of the 4 samples simultaneously processed. The time needed for this was sufficiently rapid so that the detection of radioisotopes with periods close to an hour was possible (^{165}Dy, ^{87m}Sr, ^{56}Mn . . .).

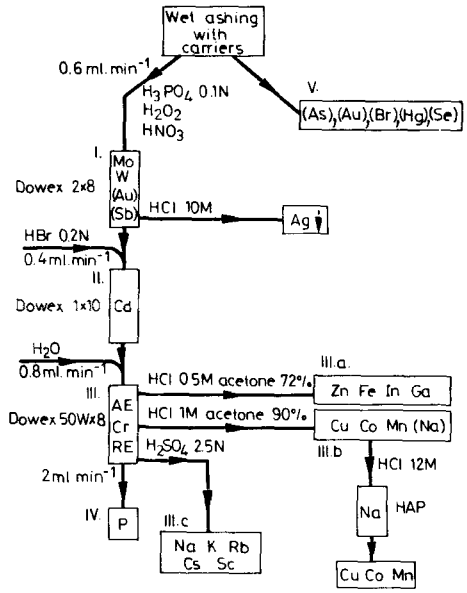

Fig. 2. Separation scheme

In addition to elements ordinarily measured by activation analysis (Fe, Co, Ni, Zn), this method allowed us to measure with a statistic accuracy close to 1% rare earths such as La, Ce, Sm, Eu and Dy existing in our samples at concentrations often less than a tenth of a part per billion (ppb).

Results

Among the ten organs removed, only five have been completely analyzed at this time: heart, body muscle, liver, kidney and testicles. The results cover three categories of elements:

- "essential" trace elements (Co, Zn, Mn, Se, Fe)
- trace elements without any biological role defined at present (Cs, Rb),
- trace elements recognized as "contaminants" (Hg, Cd).

The values observed in terms of age for each of these elements (ppm dry weight) are represented in Figs 3–11; each of the points is the mean of the results obtained on 6 samples removed from 3 animals of the same age. The shaded zones define the values included between ± 1 σ around the mean value calculated on the last 8 points or provided by a regression line in the case of elements the concentration of which varies with age.

Table 1
Mean contents of elements in various organs of rats

Element	Content, ppm dry weight				
	Heart	Kidney	Liver	Muscle	Testis
Co	0.30 ± 0.04	0.96 ± 0.12	0.37 ± 0.05	0.04 ± 0.01	0.08 ± 0.02
Fe	525 ± 80	460 ± 100	630 ± 100	A D* (39–114)	A D (98–586)
Mn	1.53 ± 0.17	3.10 ± 0.40	7.50 ± 0.80	0.34 ± 0.04	2.30 ± 0.30
Se	1.66 ± 0.15	5.40 ± 1.00	3.80 ± 0.40	0.71 ± 0.07	6.80 ± 0.40
Zn	74 ± 9	100 ± 8	103 ± 10	46 ± 10	180 ± 18
Cs	0.053 ± 0.008	A D (0.096–0.060)	0.059 ± 0.006	0.122 ± 0.012	0.10 ± 0.014
Rb	26.8 ± 2.5	30.4 ± 3.0	40.2 ± 3.5	29.6 ± 2.5	53.0 ± 3.5
Cd	0.127 ± 0.086	A D (0.049–0.4)	0.06 ± 0.02	0.05 ± 0.019	–
Hg	0.22 ± 0.08	1.11 ± 0.13**	0.14 ± 0.03	0.18 ± 0.06	0.19 ± 0.06

*A D: age dependent (20 days–22 months);
**: after the 5th month.

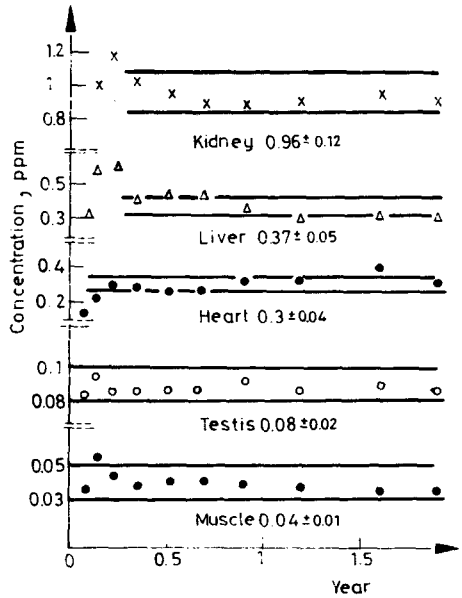

Fig. 3. Variations in cobalt concentration in terms of age

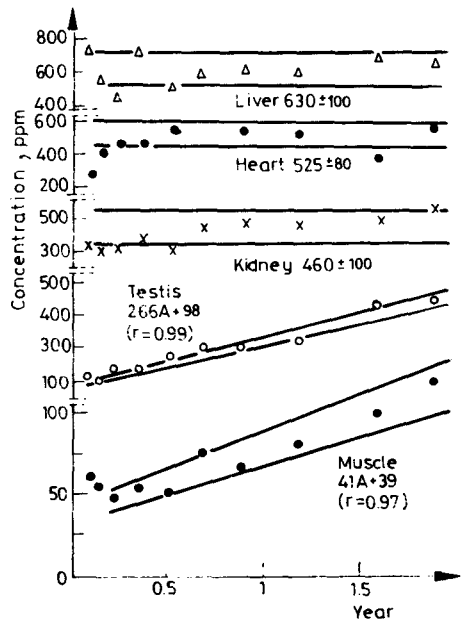

Fig. 4. Variations in iron concentration in terms of age

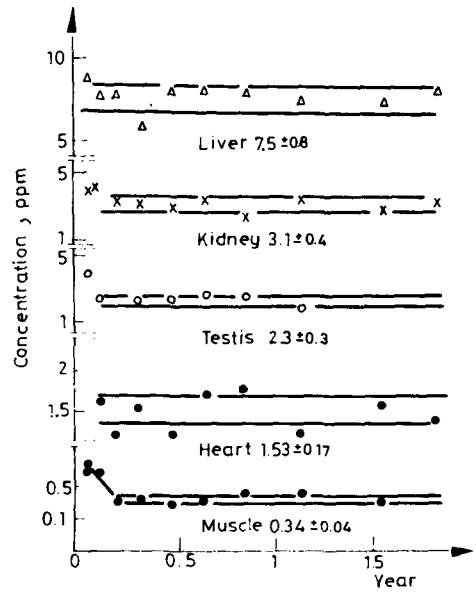

Fig. 5. Variations in manganese concentration in terms of age

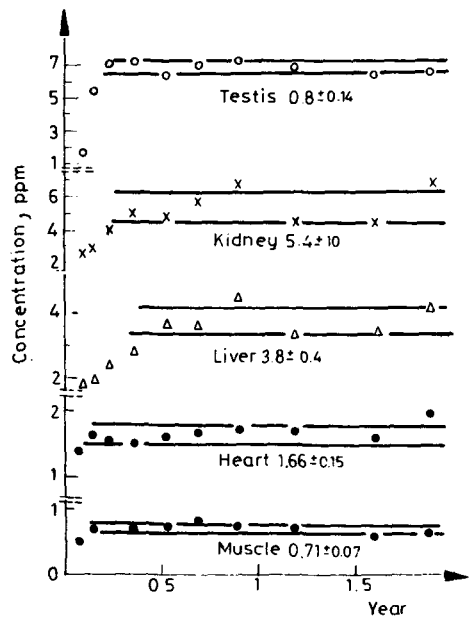

Fig. 6. Variations in selenium concentration in terms of age

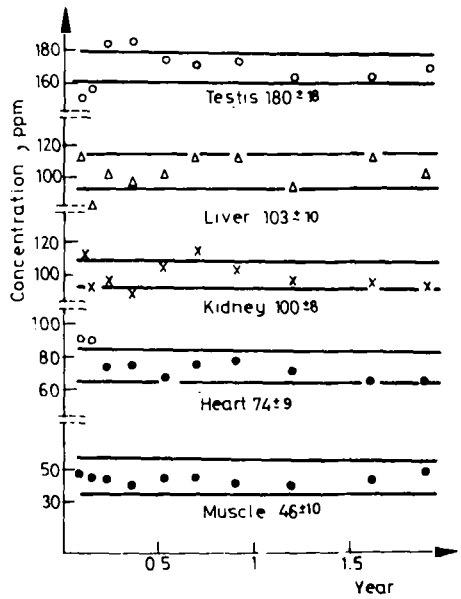

Fig. 7. Variations in zinc concentration in terms of age

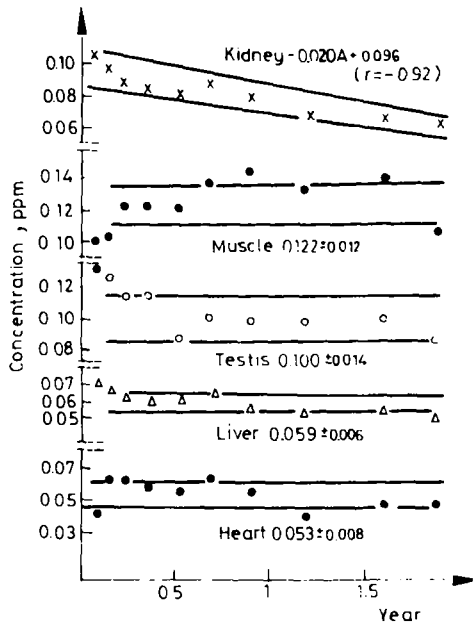

Fig. 8. Variations in cesium concentration in terms of age

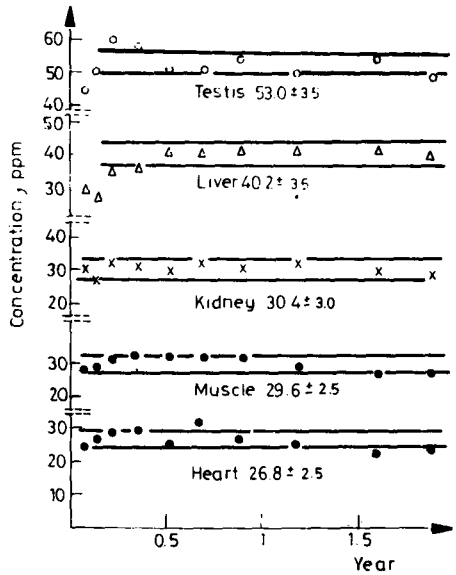

Fig. 9. Variations in rubidium concentration in terms of age

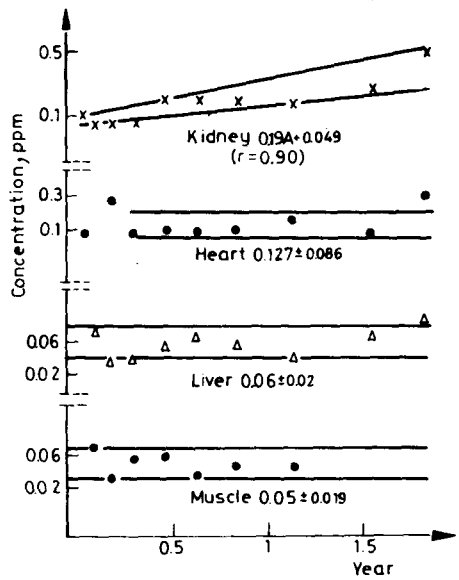

Fig. 10. Variations in cadmium concentration in terms of age

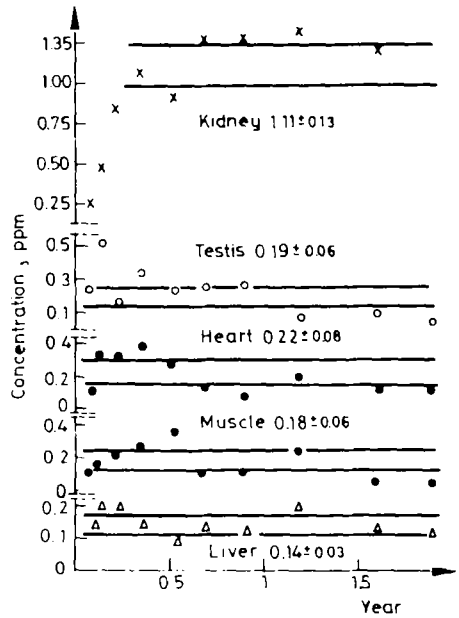

Fig. 11. Variations in mercury concentration in terms of age

It should be pointed put that for all of these elements, the accuracy of the analytical method is only slightly less than the standard deviation observed on measurements made on animals of the same strain which consequently seem to behave in a very homogeneous manner.

All of these results are shown in Table 1 and the trace element contents of the diet used is detailed in Table 2.

Table 2
Trace element contents in the diet (UAR D 04) of rats

Element	Content, ppm dry weight	Element	Content, ppm dry weight
Ce	0.20	Gd	0.002
Cd	0.081	La	0.08
Co	1.3	Mn	96
Cs	0.032	Rb	8.5
Dy	0.011	Sm	0.01
Eu	0.002	Zn	90
Fe	242	Hg	0.003

Discussion

Although a serious comparison with results existing in the literature is impossible because data on diet is lacking in most instances, the values presented in this paper are within the range of generally accepted means.[1,2]

We have revealed differences in the evolution of the element concentration in each organ in terms of age. Five different behaviours were consequently found:

- A permanent steady-state: there was no significant variation in the concentration of the element in the organ during the experiment; zinc in the five organs analyzed and mercury in the liver are examples of this;
- Steady-state stabilization around puberty (50th to 60th day): this state representing the most frequent phenomenon, can be induced from a higher initial concentration (Mn in the muscle or Cs in the liver), or on the contrary, from a weaker concentration (Se in the kidneys or Mn in the heart);
- Accumulation limited in time: the evolution of the mercury concentration in the kidney well illustrates such activity. For the first 4 months, the kidney will fix approximately 0.25 ppm of mercury a month. The concentration will then be stabilized and remain constant throughout the life of the animal;
- Permanent accumulation: this phenomenon is particularly important for iron in the muscle (3.4 ppm/month) and especially in the testicles (23.2 ppm/month) as well as for cadmium in the kidneys (0.016 ppm/month).
- Permanent depletion, a rare phenomenon, was observed only for cesium in the kidneys (0.17 ppm/month).

These tables also provide a clear picture of the differences in concentration of the same element is different organs without any reference to age; the cobalt concentration in the muscle and the kidneys, for example, varies by a factor of 20.

It is remarkable to observe that the concentration of most of the essential trace elements is much higher in the myocardium than in the body muscle. This phenomenon does not appear for such elements as Cs, Rb and Hg.

Table 3
Extreme values of rare earth content in various organs of the rats

Element	Content, ppb dry weight				
	Heart	Kidney	Liver	Muscle	Testis
Ce	4 –600	5 –60	2 –30	6 –60	6 – 10
Dy	0.5 – 5	0.3 – 3	0.3 – 1	0.2 – 5	0.1 – 1
Eu	0.05– 0.4	0.01– 0.07	0.01– 0.07	0.01– 0.2	0.02– 0.09
La	3 –100	1 –55	0.3 –20	2 – 8	20 –200
Sm	0.1 – 2	0.1 – 1	0.05– 0.7	0.1 – 3	0.05– 0.4

In addition, these tables also show the high content of zinc in the testicles.

Among the elements analyzed but not accounted for on the curves below, it is interesting to note the "anarchic" behavior of rare earths. Despite accuracy in measuring these elements (approximately 5%), the dispersion of results, whatever the organ studied, is such that it is impossible to define a mean and a standard deviation and the best we can do is to quote extreme values (Table 3).

Conclusions

The first results which we have just presented allow us better to understand the normal state of a laboratory animal.

However, we should stress the fact that such results are completely meaningful only for rats raised on a diet similar to that used. In fact, previous research showed that concentrations observed for some elements such as iodine,[5] selenium,[6] manganese[7] and bromine[8] varied according to diet.

Except for renal accumulation of mercury, a phenomenon more or less accepted now and renal accumulation of cadmium already mentioned by TIPTON in man, the newest fact in research on variations of elemental concentrations in terms of age is the revelation of the accumulation of iron is the body muscle and gonads.

In addition, we can note that mercury seems to behave differently in the kidneys from one animal species to another. In man mercury is accumulated indefinitely whereas in rats it rapidly attains a saturation threshold or a level beyond which the concentration is stabilized.

In conclusion, the experiment has shown that variations in elemental concentrations in terms of age were a general phenomenon and that for some elements a steady state is apparently never attained.

This may be the cause of error in using the indicator method to study the renewal of these elements as this method assumes a steady state throughout the experiment.

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