THE INFLUENCE OF SEASONAL CHANGES IN THE DETERMINATION OF SELENIUM IN LIVER OF VARIOUS ANIMALS BY NEUTRON ACTIVATION ANALYSIS AND HIGH-RESOLUTION GAMMA SPECTROMETRY

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ABSTRACT: Harthoorn, A.M.[1]; Turkstra, J[2]. **The influence of seasonal changes in the determination of selenium in liver of various animals by neutron activation analysis and high-resolution gamma spectrometry** (1976). *Journal South African Veterinary Association* (1976) 47 No. 3, 183-186 (En). [1]Nature Conservation Division, P. Bag X209, 0001 Pretoria. [2]Atomic Energy Board Pelindaba, Republic of South Africa.

Selenium levels in the liver of animals living in the Umfolozi Game Reserve in Natal and in the Sabi Sand Nature Reserve in the Eastern Transvaal were studied by instrumental neutron activation analysis. The distribution of the selenium content was followed for about 16 months and attempts have been made to explain seasonal fluctuations of the selenium level.

INTRODUCTION

Nutritional Myopathy due to selenium deficiency has been recognised as an important factor in domestic livestock[3]. The possibility of selenium deficiency has also been investigated in zoo animals[11]. Overt myopathies are likely to occur when animals from selenium deficient areas are subjected to stress. Paradoxically the danger of this type of myopathy developing during transit has increased since improved methods of chemical capture techniques and tranquillisation have been developed. These methods have eliminated the necessity for keeping animals in holding pens on artificial feed for an interim period as was the procedure when using mechanical capture methods.

The literature on myopathies in captured wild animals has been reviewed by Harthoorn and Young[5]. Selenium deficient areas have been well documented in the United States, less well known in other countries, and virtually undocumented in Africa.

Selenium levels in normal biological tissue range from 0,005 to 0,5 ppm[2] with the result that only ultramicromethods can be used for selenium analysis. Colorimetry[4] and fluorimetry[6] have been utilized for the quantitative analysis of selenium in biological materials. A more modern analytical technique, neutron activation followed by high resolution gamma-ray spectrometry, has also been used extensively for the analysis of trace elements in biological tissues because of its inherent high sensitivity for many elements[10].

Five stable isotopes of selenium exist in nature. Table 1 gives the relevant nuclear data for radionuclides which are produced from selenium by neutron activation[7]. Only three of these radionuclides, namely ^{75}Se, ^{77M}Se and ^{81}Se, can be obtained with high specific activity. Neethling, Brown and De Wet[8] employed the shortlived radionuclide ^{77M}Se for the instrumental radioactivation analysis of selenium in biological material. Because of possible interferences associated with this fast technique, satisfactory results cannot generally be obtained at very low concentrations, unless a thin NaI(Tl) scintillation crystal or a Ge(Li) detector is applied[1]. The virtually pure beta emitter ^{81}Se has been used to a limited extent[2]. The radionuclide most commonly used for the determination of selenium in biological material is ^{75}Se[9]. A limitation of the use of this radionuclide is the length of irradiation time required to achieve a high activity, but the long half-life ($t_{\frac{1}{2}}$ = 120 days) allows sufficient time for careful radiochemical separation and activity measurements. It was therefore decided to investigate the use of high-resolution gamma spectrometry for the direct determination of selenium in liver samples of wild animals at regular intervals over a period of 16 months.

MATERIALS AND METHODS

Preparation of the liver samples:

About 30 liver samples weighing approximately 30 g were collected from animals culled for various purposes from two areas in South Africa namely the Umfolozi Game Reserve in Natal and the Sabi Sand Nature Reserve in the Eastern Transvaal. The animals investigated were impala, white rhinoceros, blue wildebeest and warthog. The samples were immediately placed in standard bottles containing formalin solution. Subsequently pieces of liver tissue weighing approximately 2 g were taken from the centre of the sample, finely chopped, heated to a constant weight at 108°C and then ground in a agate mortar to obtain a more or less homogeneous powder. Approximately 500 mg of liver powder was accurately weighed into polyethylene containers and sealed.

Preparation of the standards:

A stock solution analytical grade selenium dioxide was prepared and diluted to contain 2 µg Se per ml of solution. Reference standards used were approximately 0,5 ml of the solution in polyethylene containers. The weights of the standard solutions were recorded and, after careful evaporation to dryness, each container was sealed.

NBS Bovine Liver Standard Reference Material (SRM 1577) containing 1,1 ± 0,1 µg selenium per gram of sample was also used as a reference standard. Accurately weighed samples (~ 250 mg) of this standard were sealed in polyethylene containers.

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TABLE 1 : NUCLEAR DATA FOR RADIONUCLIDES PRODUCED FROM SELENIUM BY IRRADIATION IN A THERMAL REACTOR[7]

Target isotope	Abundance (%)	Activation cross-section (barn)	Product isotope	Half-life	Gamma-ray energy (keV)	Activity of Se after activation for one half-life $0 = 1 \times 10^{12}$ n cm^{-2} sec^{-1} (mC/g)
^{74}Se	0,87	30	^{75}Se	120 d	97 121 136 199 265 280 305 401	25*
^{76}Se	9,02	22	^{77m}Se	17,5 s	162	97*
^{78}Se	23,52	0,38	^{79m}Se	3,9 m	96	2,9
		0,05	^{79}Se	$6,5 \times 10^4$y	nil	—
^{80}Se	49,82	0,08	^{81m}Se	57 m	103	1,5
		0,53	^{81}Se	18,6 m	1 % gamma 272 280 550 560 830	25*
^{82}Se	9,19	0,05	^{83m}Se	60 s	350 650 1 010 2 020	0,46
		0,004	^{83}Se	25 m	225 358 520 710 833 1 060 1 310 1 880 2 290	0,04

Irradiation:

For convenience of counting in the determination of selenium, the samples and standards were irradiated separately and in fixed sequence for precisely 90 seconds. The relative value of the integrated neutron fluxes were determined for some samples and standards by using gold monitor samples. Irradiations were done in the pneumatic facility of SAFARI-1, an ORR-type reactor, in a thermal neutron flux of $2,89 \times 10^{13}$ n cm^{-2} sec^{-1}. Westcott's epithermal index, r, for this irradiation, position is 0,0087.

Measurement of gamma activity:

Gamma spectrometry of the irradiated samples and standards was done exactly 20 seconds after each irradiation by placing them in a fixed position from the Ge(Li) detector. The detector used was a 50 cm^3 coaxial Ge(Li) diode (Princeton Gamma Tech.) connected to an uncooled TC 135 M Tennelec preamplifier. The output pulses were amplified by a TC 200 Tennelec amplifier. Spectrum analysis was performed on an Intertechnique 4 000-channel analyzer (Model SA 44). The resolution of this counting system is 3 keV (full width at half maximum) for the 1 333 keV photopeak of ^{60}Co. Data for peak analysis were recorded on magnetic tapes which were processed by computer. Yule's[12] smoothed first derivative method was applied to obtain the true peak counts under the photopeaks of interest.

RESULTS

A gamma spectrum of a liver sample after 90 seconds of irradiation and 20 seconds of decay time is shown in Fig. 1. The 162 keV photopeak of ^{77}Se is well separated from other gamma photopeaks. No interference due to the 198 keV photopeak of ^{190} can be observed.

It was observed by radioactivation that the Bovine Liver Standard used contains 1,14 µg of selenium per gram of sample compared to the prepared selenium reference standard. Fig. 2 shows the seasonal variation in selenium content in the liver of the animals.

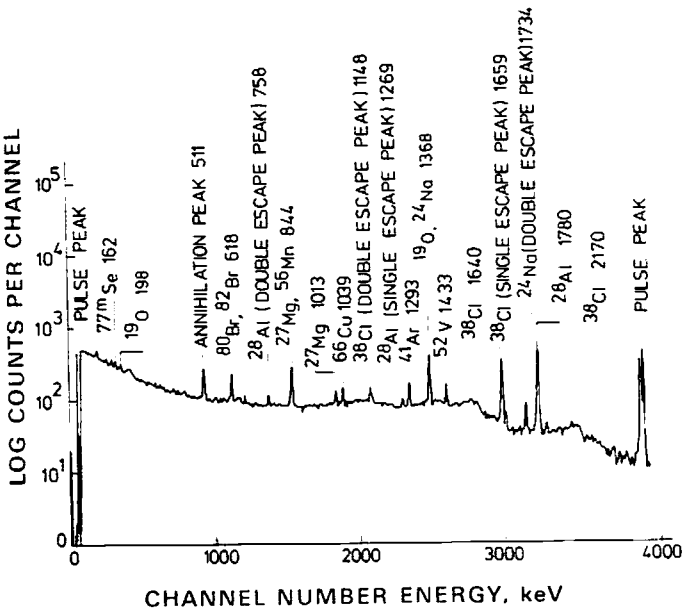

FIG. 1

The results obtained for the selenium concentration in the liver samples are given in Table 2.

Table 2: SELENIUM CONTENT IN ANIMAL LIVER DETERMINED BY NEUTRON ACTIVATION ANALYSIS.

Date animal killed	Neutron Activation Analysis Se (ppm)
73-09-02	0,78
73-12-21	0,76
73-12-21	0,64
74-01-07	0,71
74-01-07	0,74
74-02-05	0,54
74-02-05	0,88
74-03-04	1,01
74-03-08	0,99
74-03-26	1,14
74-04-20	1,14
74-04-23	0,68
74-05-10	0,94
74-05-23	1,35
74-06-12	1,28
74-06-18	0,72
74-07-02	0,82
74-07-28	1,1
74-08-08	0,59
74-08-21	0,68
74-09-07	0,5
74-09-23	1,11
74-10-07	1,22
74-10-25	0,99
74-11-02	1,04
74-11-23	0,86
74-12-07	0,69
74-12-19	0,45
75-01-09	0,83
75-01-21	0,97

DISCUSSION

The results of the analyses show remarkable conformity considering that the samples were derived from a number of different species and several geographical locations. The animals include grazers such as warthog (25), white rhinoceros (1) and blue wildebeest (1), and the impala (3) which is a facultative browser. All the animals follow a definite curve; even the impala which are shown separately, show the same trend. The work on wild animals is inevitably done on smaller numbers than on cattle where large numbers of samples are obtained from slaughter houses.

A few of the results do not ift into a general curve. Exceptionally low values were found in lactating and, in one case, pregnant warthog. The unnumbered dots represent impala which, possibly as facultative browsers, appeared to deviate somewhat from the others and were therefore excluded from the averages. These impala samples were derived from animals in predominantly overstocked land at Sabi Sand Game Reserve.

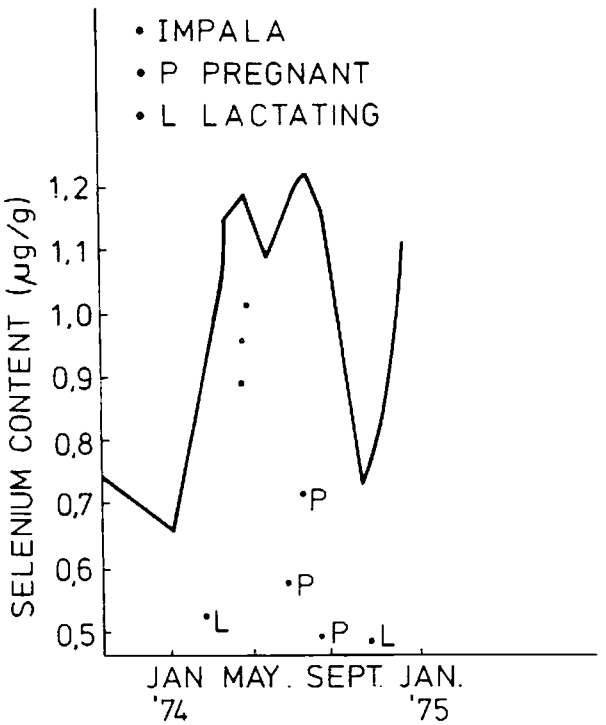

FIG. 2

The first low levels occur in September, dropping gradually until December (Fig. 2), after which the values commence to rise steeply, reaching peak values in April and May. The levels do not follow the same pattern as the rains. The first substantial rains fell in September, but the rise in liver selenium content occurred in December and January. This suggests a low selenium content of the first flush of herbage.

There appears to be a dip at the apex of the curve. This may be an artifact caused by scatter of results at this season. Other samples collected at this time which have still to be processed, will doubtlessly give substance to this section of the curve so that the trend can be more exactly determined.

The low values of 1975 are higher than those in 1974, and the curve rises earlier and more steeply. This phenomenon may be due to unseasonable rain which occurred during the winter in May. As the rainfall in 1975 was unusual both in quantity and distribution, it is likely that the 1974 section of the curve represents the more usual pattern. The difference between the two lower legs of the curve suggests that the selenium values in years of low rainfall may be lower.

As increased susceptibility to capture myopathy in wild animals during the latter part of the dry season is

generally accepted, although factors such as low forage protein value are undoubtedly a major cause, the low levels of selenium during this time of year are likely to be a contributory factor.

ACKNOWLEDGEMENTS

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BOOK REVIEW

BOEKRESENSIE

VETERINARY TOXICOLOGY

E.G.G. CLARKE and MYRA L. CLARKE

Baillière Tindall, London pp. 438, Figs. 0, Tables 5, Publ. Price R19,55

The first edition of Veterinary Toxicology by R.F. Garner became available in 1957 and soon became a standard text-book. In 1967 the third edition was published, after revision by Clarke and Clarke, with the title "Garner's Veterinary Toxicology". Eight years later, in 1975, there is once again a first edition titled "Veterinary Toxicology" by Clarke and Clarke. New sections have been added but the basic outlay of the previous edition is maintained.

In the Introduction the authors deal with the important factors of absorption, distribution, accumulation, detoxification and the elimination of toxic substances. Other basic facts concerning toxicology are also discussed in this chapter.

Part Two deals with mineral and inorganic substances, and is discussed in the format previously used; viz. : Forms and occurrence of substance, the absorption and excretion: the symptoms and lesions; the confirmation of the diagnosis and then the treatment and control.

Part Three, a chapter dealing with toxic gases and vapours, is a new addition. This is a very short chapter, only eight pages, but covers the essentials.

The following part is one of three that covers organic compounds; this chapter more specifically the drugs. This is a very wide field and most often discussed in pharmacological text-books, but the most important veterinary drugs are included in this chapter.

Part Five deals with the pesticides. The importance of the toxicology of the pesticides in veterinary medicine can never be overemphasised These compounds are becoming more and more important in agriculture and animal husbandry. Useful information could be found on all the important pesticides in use.

Organic compounds — Miscellaneous, is the heading of the following part. Substances not classified in the two previous chapters are described here, ranging from acetic acid to toxic fat disease in chickens.

The section in toxic plants forms the major portion of the book. 120 pages. Many names and description of South African plants appeared here, not always a complete description as one would prefer, but it stands to reason that this is not possible in a publication of this kind. The authors must be complimented on the manner in which they have presented Part Seven. Though the information is sometimes brief, the references given enable the reader to continue his study.

Part Eight covers the mycotoxins. This is a fairly new field and we hope that the authors will elaborate on this chapter in future editions, especially for the benefit of practitioners who often do not have specialized text-books available.

The penultimate section is a short one on venomous bites, stings and doping. This is followed by the section dealing with radio-active materials. This is a field of which every veterinarian should have some basic knowledge.

This book is once again an outstanding text-book for students, research workers and the practitioner. The way the information is presented and arranged as well as the long lists of references makes this a must on every book-shelf.

A.I.