Serum Ceruloplasmin in Mice with Ehrlich Ascites Carcinoma

High values of serum ceruloplasmin were reported by several authors in human patients with malignant neoplasms. SULLIVAN and HART[1] observed this increase of ceruloplasmin in sera of patients with metastatic carcinoma. These findings were confirmed by PINEDA, RAVIN and RUTENBURG[2] describing high levels of the serum oxidase in human cases with hepatic metastasis and malignant bile duct obstruction. NEISH[3] reported in rat liver azo dye carcinogenesis, an initial inhibition of serum ceruloplasmin followed by a rise to normal levels in spite of the development of the tumors (cholangiocarcinomata). THOMAS and CONSTANTINESCU[4], THOMAS, OLINESCU and CONSTANTINESCU[5] working with rats with Jensen carcinoma and O-Ya tumor, respectively, obtained significant elevations of serum ceruloplasmin. Our studies[6] in rats with a transplantable fibrosarcoma showed that the neoplastic process is accompanied by very high values of serum ceruloplasmin activity only comparable to those obtained in acute inflammation produced by i.m. administration of turpentine oil.

The Ehrlich ascites tumor is widely used in biochemical investigations. However, serial data on serum ceruloplasmin activity in mice bearing this important kind of carcinoma are lacking. The present paper will describe the results obtained on this subject.

Material and methods. Male Swiss mice (25 g mean body weight) have been used throughout all experiments; they received ad libitum a standard balanced diet and water. A group of 16 mice was used as normal non-inoculated controls. The other mice used were injected, by i.p. route, with a clear non-hemorragic cell suspension of Ehrlich ascites carcinoma removed from a mouse treated similarly 10 days before. The ascitic fluid diluted in physiological saline at the concentration of 2.0×10^6 Ehrlich cells/0.1 ml was immediately administered, at this dose level, to the recipient animals.

Mice were killed by decapitation at 4, 7, 10 and 14 days after the inoculations, blood samples were collected and the sera were separated and stored at $-15\,^{\circ}C$. The maximum time of storage was 24 h. Ceruloplasmin was determined enzymatically by the method of RAVIN[7], and values were expressed in mg/100 ml of serum. The significance of the differences between neoplasm bearing animals and control mice were statistically evaluated by the *t*-test[8].

Results and discussion. From the results presented in the Table, it is clear that the neoplasm does not induce significant variations in serum ceruloplasmin levels up to the seventh day after inoculations. However, in more advanced stages (10 and 14 days post-inoculation) of Ehrlich carcinoma development statistically significant decreases of the enzyme were obtained.

The results of the experiments clearly show that the response of mice to the inoculation of Ehrlich carcinoma is different from that observed in human patients[1,2] with a variety of malignant tumors and in rats bearing experimental neoplasms[4-6]. It seems that the depression of the oxidase levels in mice secondary to the injection of Ehrlich carcinoma is a property of that tumor. In this connection it is interesting to remember that MAYER[9] found Ehrlich tumor cells in the heart and blood vessels of liver, kidney and meninges of mice from 7 days onwards post-inoculation. Perhaps the presence of Ehrlich metastasis in the liver or the systemically circulating carcinoma cells interferes in some way with the synthesis of the enzyme, since low levels of serum ceruloplasmin were only seen in the advanced stages of evolution.

Résumé. On montre que la présence du carcinome d'Ehrlich provoque une diminution statistiquement significative des taux de la céruloplasmine sérique des souris aux dixième et quatorzième jours après l'inoculation.

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Serum ceruloplasmin in mice bearing Ehrlich ascites carcinoma

Days after inoculation	No. of mice	Ceruloplasmin[a] Mean ± S.E.M.[b]	*t*-Test
4	9	19.7 ± 1.3	t 0.023, $P > 0.5$
7	10	19.3 ± 1.2	t 0.048, $P > 0.5$
10	8	13.5 ± 0.6	t 3.166, $P < 0.01$
14	9	10.2 ± 0.3	t 5.202, $P < 0.001$
Non-inoculated Controls	16	19.2 ± 1.7	

[a] mg/100 ml of serum. [b] S.E.M., standard error of mean = $\sqrt{\frac{\Sigma (x - \bar{x})^2}{n (n-1)}}$

[1] J. F. SULLIVAN and K. T. HART, J. Lab. clin. Med. *55*, 260 (1960).
[2] E. P. PINEDA, H. A. RAVIN and A. M. RUTENBURG, Gastroenterology *43*, 266 (1962).
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[9] K. D. MAYER, Br. J. exp. Path. *47*, 537 (1966).
[10] One of us (R.R.A.) is working with a research grant from Conselho Nacional de Pesquisas (National Research Council of Brasil).

The Thorium-Series in Cigarettes and in Lungs of Smokers

From 1958 through 1965 W. V. MAYNEORD et al.[1-3], using an α-spectrometric method, carried out an extensive investigation about the level of the 2 natural radioactive series of thorium and radium occurring in man and his environment: soils, vegetation, animal tissues, human foods, drinking-water, human tissues. On this trend, TURNER and RADLEY[4] found ^{226}Ra and ^{228}Ra in all ashes of cigarette brands issued from around the whole world. Comparing the radon inhaled during smoking and that inhaled continuously with ambient air these authors concluded the first inhaled radioactivity must be negligible compared with the second one. Measuring ^{210}Po in

whole cigarettes, ash, butt, total smoke and main stream (inhaled) smoke, RADFORD and HUNT[5] showed that about half of the polonium of smoke, or 20–25% of ^{210}Po in cigarettes must be inhaled with the main stream and deposited with the smoke particles in alveolar epithelium. Supposing the radioactive particles are carried out with a mucus flow of 3 cm^3, having an average transit time through the bronchial epithelium of 36 h, the total calculated doses amount to 36–100 rems in 25 years for a smoker of 40 cigarettes daily. In further researches on ^{210}Po in man, HILL[6] measured too in different raw tobaccos ^{210}Po activity of 240 to 670 pCi kg^{-1}. Analyzing the smoking process, he finds that about 10% of polonium is drawn from cigarettes in the main stream to the lungs. Assuming a thickness of 100 μm for the irradiated surface and a transit time of 10 days, he calculates a dose rate of 1 rem per year in smoker's lungs.

In the previous papers the investigation of smoking has been limited to ^{210}Po and the remainder of the ^{226}Ra- and ^{228}Ra-series seems not have been considered. Yet TURNER and RADLEY[4] measured ^{226}Ra and ^{228}Ra in cigarettes both in the same magnitude, with activities of 7 up to 22 pCi per 100 g raw tobacco. And HILL[7] in a former paper has shown in normal lung ash, the accumulation of measurable amounts of ^{232}Th, ^{238}U and nearly all their decay products. Lastly, HOLTZMANN et al.[8], in measuring ^{210}Pb and ^{210}Po in the wood rings of trees (oaks and hickory), found equally ^{226}Ra with a 2–10 times lower activity than that of the previous isotopes. It seems that the roots of plants are able to draw radium directly from the soil but that a higher activity issued from the ambient radon in air is adsorbed by the leaves (MAYNEORD et al.[3]). In tobacco plants, ^{226}Ra and ^{228}Ra are taken up in soil and the decay products of ^{222}Rn as ^{210}Po are deposited on the leaves through rain.

At the opportunity of monitoring the lungs of workers exposed during several decades to the smoke of arc-lamps releasing thorium vapors, I observed that the thorium-series is accumulated by the lungs and that very small amounts induced acute injurious effects. An accumulation of 5 up to 10 mg thorium leads, after 30–40 years exposure, to severe fibrosis with dyspnoea and permanent incapacity for working. For this reason I considered here, firstly, a possible contamination of the lungs through the decay products of thorium in tobacco, a hazard which seems not have been studied so far.

The Thorium-Series in Cigarettes. The advantage of the γ-spectroscopy over the α's is that ashing and chemical treatment are no longer necessary and the material

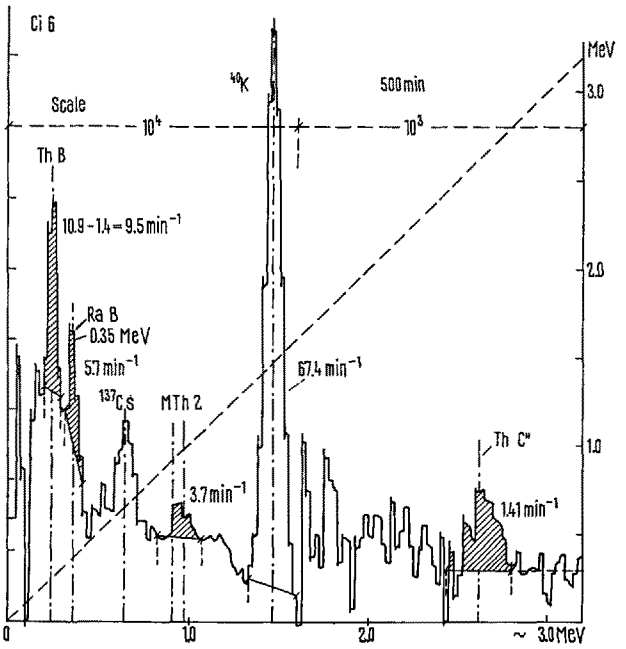

Fig. 1. Spectrum of 120 cigarettes of an usual brand. One sees, beside ^{40}K and ^{137}Cs, the ThB, MTh$_2$ and ThC'' main γ-peaks. One fourth of the 0.35 γ's of RaB is subtracted from the 0.24 γ's.

[1] R. C. TURNER, J. M. RADLEY and W. V. MAYNEORD, Br. J. Radiol. *31*, 397 (1958).
[2] W. V. MAYNEORD and C. R. HILL, Nature, Lond. *184*, 667 (1959).
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[4] R. C. TURNER and J. M. RADLEY, Lancet *1960*, 1197.
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[6] C. R. HILL, Nature, Lond. *208*, 423 (1965).
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[8] R. B. HOLTZMAN and F. H. ILCEWICZ, Argonne National Laboratory Report ANL-7615, 38 (1968–69).
[9] W. V. MAYNEORD and C. R. HILL, in *Radiation Dosimetry* (Academic Press, New York 1969), vol. 3, p. 401.

Table I. γ-composition of the 3 windows and calibration

Main lines (MeV)	Window-width (MeV)	Nuclides	γ's MeV	Intensity in the window (%)	min^{-1} per mg Th-equiv.
0.239	0.198–0.280	ThB (^{212}Pb)	0.239	47	
		MTh$_2$ (^{228}Ac)	0.209	3	
			0.270	2	18.8
		ThX (^{224}Ra)	0.241	4	
		ThC'' (^{208}Tl)	0.277	1	
0.91–0.97	0.82–1.06$_5$	MTh$_2$ (^{228}Ac)	0.912	23	
			0.966	20	
			0.836	1.5	4.6$_8$
			0.796	1.5	
		ThC'' (^{208}Tl)	0.860	3	
2.615	2.43–2.80	ThC'' (^{208}Tl)	2.615	36	2.7$_8$

Branching ratio for ThC: 0.36.

of samples can be measured in raw conditions. In a whole-body spectrometer with a steel room, according to MARINELLI[10] and MILLER[11], described elsewhere[12], the γ-spectrum of cigarette brands having a ready sale in Switzerland, was recorded. 120 cigarettes of each brand, each bought in 3 or 4 different shops, were put in a cylindrical Plexiglas box, gas-tight closed, in order to obtain equilibrium of the decay-products of thoron (^{220}Rn). Each box has been placed against and coaxial in front to the 8 inches diameter NaI(Tl)-Crystal. Background and full box were recorded one after another for 500 min each. Every sample has been measured twice, respectively with 2 energy scales of 1.6 and 3.2 MeV. Figure 1 shows such a spectrum.

For the calibration of the spectra, a source of old $Th(NO_3)_4$. 4 H_2O 'analar', purity 98.4%, has been used in conditions reproducing the average solid angle and the autoabsorption of the cigarette samples. This spectrum includes 5 clearly defined peaks at 0.06, 0.24, 0.58, 0.91–0.97 and 2.62 MeV. As tobacco always contains radium, we did not use the 2 peaks which may be superposed or mingled with one of the radium-series. The 0.06 MeV's is too near the 0.078's and the 0.58 is nearly superposed upon the 0.61-line of RaC. Thus 3 peaks only may be used for calibration, namely these of ThB (0.24), MTh_2 (0.91–0.97) and ThC'' (2.62). Window-width, composition of the peaks and calibration in min^{-1} per mg Th-equivalent are given in Table I. The radiations frequency, expressed as percentage of the total decays, is, as one knows, somewhat uncertain. The intensity of the 0.242-γ's of RaB, which appears in the ThB-window, is determined after its ratio to the 0.352-γ of the same nuclide and deduced from that peak.

The average values of the Th-content for 120 cigarettes is given in Table II for 8 different trade marks. The S.D. amounts to $\pm$ 0.04 mg Th per sample. Knowing the activity of one mg ^{232}Th equals 109 pCi, we see that our samples have activities higher by a factor of about 2 than the activities found by TURNER and RADLEY[4] for ^{228}Ra in raw tobacco. In the last column of Table II, the thorium weight is given for 9000 cigarettes corresponding fairly well to the annual supply of a smoker of 25 cigarettes daily. These weights, it is worth while to note, are not at all negligible, even if 10% only is inhaled into the lungs.

The 0.35 γ's of RaB is present in all samples (Figure 1), but, the build-up time of one month for radon having not been observed, the activity of the radium-series has not been calculated. The ^{137}Cs activity varies strongly from one sample to another, this activity depending on the latitude of the plantation from which the tobacco comes. ^{137}Cs is very high in Turkish tobacco and fully missing in that of Rhodesia. Potassium, a physiological element, appears to be nearly constant in the 8 samples of cigarettes.

The Thorium-Series in Lungs of Smokers. So far, it seems that the lung activity of smokers has not been observed in vivo. In the whole body spectrometer previously described[12], the γ-spectrum of the chest has been recorded on 10 smokers and 2 non-smokers in the steel room. After a short check on superficial activity of the head, hands and forearms, a shower was taken by the subject before dressing in paper pyjamas. In the steel room, the standard NaI(Tl)-crystal has been set against the chest of the subject, anterior close to the sternum, at mid-height of both lungs. Before or after the record of 50 min exposure, the background of a Plexiglas phantom of the chest is registered negatively.

Figures 2 and 3 show the spectra recorded in this way on 2 smokers. One sees again the 3 peaks of ThB, MTh_2 and ThC''. They are not so clearly defined as for cigarette samples because, with intensities in about the same figure, the recording duration is 10 times shorter. Further, owing to some fallout nuclides such as ^{144}Ce (0.134 MeV), ^{141}Ce (0.145 MeV), ^{125}Sb (0.176 MeV) and the backscattering-limits of ^{137}Cs and ^{40}K, the lower limit of the ThB peak is often so badly defined as in Figure 3. We took into account the cases only where the 3 peaks (ThB, MTh_2 and ThC'') are simultaneously present. On account of predominance of the back ground values in the calculation, the standard deviations shown in Figures 2 and 3 for the MTh_2 and ThC'' windows are constant in all our measurements.

The distribution of thorium in lungs being unknown, the calibration was performed in a conventional geometry with a central source of 252 mg thorium, set on the axis 14.3 cm from the crystal front, with a filter of 3.25 g cm^{-2}

[10] L. D. MARINELLI, Br. J. Radiol., Suppl. 7, 38 (1957).
[11] C. E. MILLER, Argonne National Laboratory Report ANL-5829, 144 (1957).
[12] G. JOYET and A. BAUDRAZ, Experientia *24*, 865 (1968).

Table II. Equivalent-thorium 232 weight in cigarettes

Spectrum	Trade mark	Tobacco	Weight for 120 cigarettes (g)	Th-Equiv. weight (mg) for cigarettes No. 120	9000
Ci 1	Parisiennes	Maryland FJB	107.0	0.24_7	$18._5$
Ci 2	Lucky Strike	Turkish-American BAT	124.7	0.27_4	$20._6$
Ci 3	Craven A	Virginia, English CL	128.2	0.34_4	$25._8$
Ci 4	Gauloises bleues	Caporal RF	112.8	0.17_9	$13._4$
Ci 5	Mary Long	Maryland BAT	100.2 (F)	0.12_2	$9._2$
Ci 6	Kent, King Size	? PLCo	109.5 (F)	0.47_8	$35._9$
Ci 7	Chesterfield	Turkish-American LM	125.3	0.21_2	$15._9$
Ci 8	Camel	Turkish-American RJR	121.2	0.33_6	$25._2$

(F), filter cut up. 1 mg Th = 109 pCi. S.D. $\pm$ 0.04.

Plexiglas against the crystal. The window-widths being the same as in Table I, the calibration constants are, in min^{-1} per mg Th-equivalent, 3.39 for ThB, 2.14 for MTh_2 and 0.84_5 for ThC″. On the whole, in these tests, the standard deviation of a thorium determination in man amounts to 0.6 mg Th-equivalent.

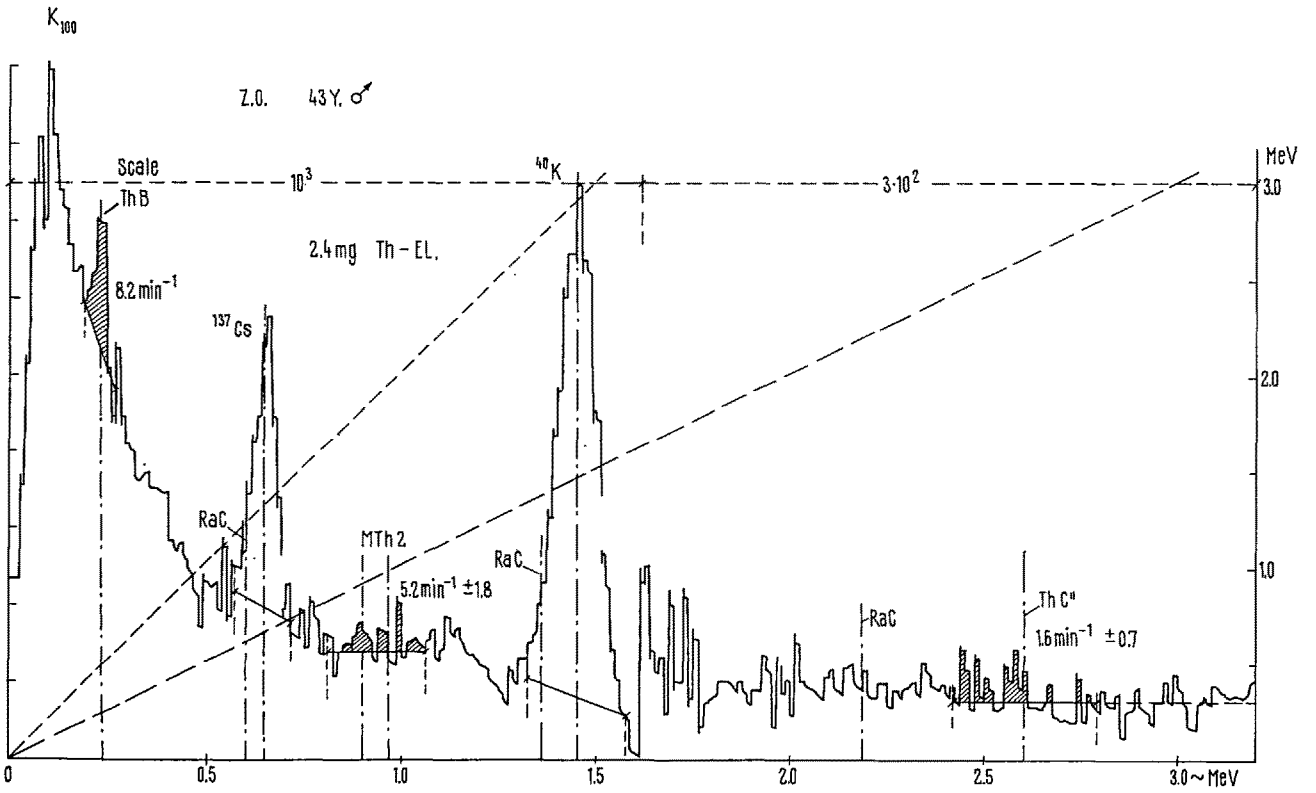

Fig. 2. Spectrum of the chest of a 43 years old smoker, smoking 35 cigarettes daily since his twentieth. One sees the ThB, MTh_2 and ThC″ peaks.

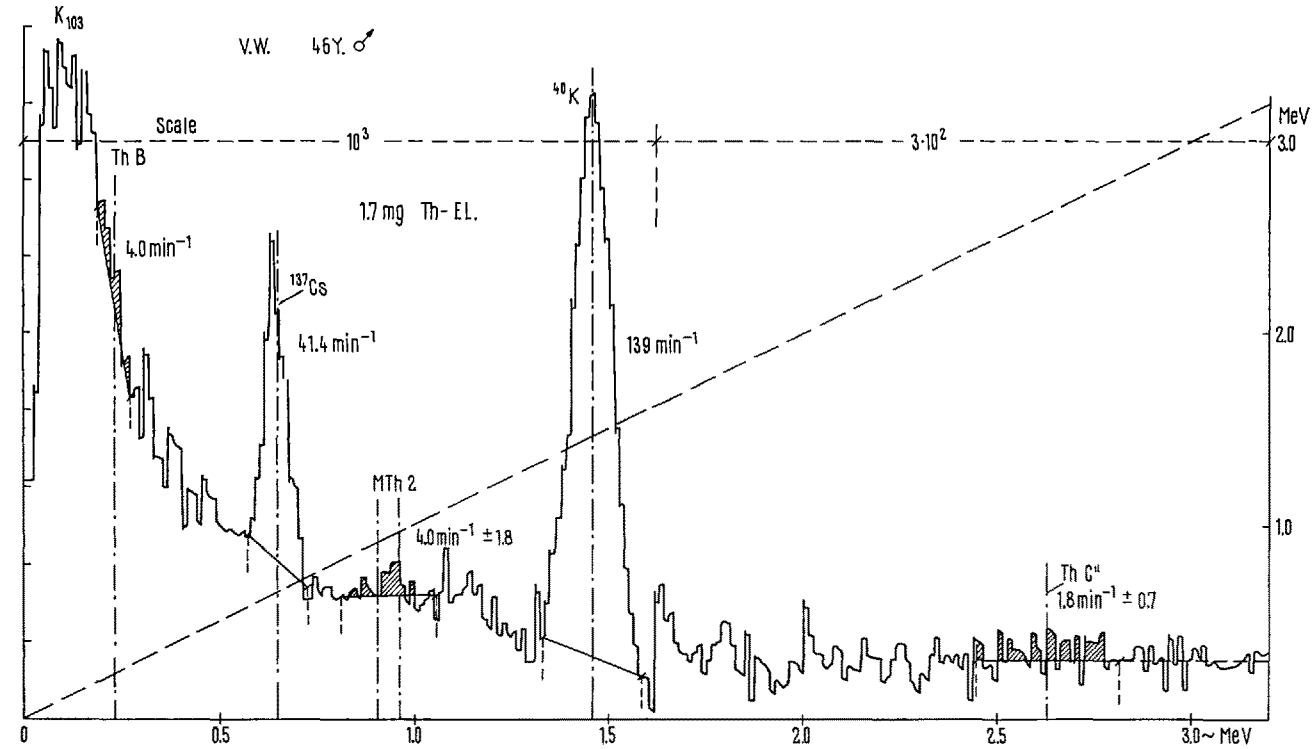

Fig. 3. Spectrum of the chest of a 46 years old smoker, smoking 25 cigarettes daily since 15 years. One sees the MTh_2, ThC″ and – less clearly – the ThB peaks.

Table III. Smokers and non-smokers: ^{232}Th-equivalent in lungs

Spectrum	Subject	Sex	Age	Cigarettes daily	Trade mark	^{232}Th-Equiv. (mg)
Smokers						
K_{104}	K.A.	♂	44	25	Marlboro (F)	0.8
K_{103}	V.W.	♂	46	25	Stella (F)	1.7
K_{100}	Z.O.	♂	43	35	Dumouriez (F)	2.4
K_{99}	C.R.	♂	30	20	Parisiennes (F)	0
K_{98}	H.F.	♂	58	25	Marocaines (F)	0
K_{97}	W.V.	♀	26	25	Gauloises (F)	0
K_{93}	L.S.	♂	54	35	Mary Long (F)	1.7
K_{92}	S.M.	♂	64	40–30	Frégate (F)	0
K_{91}	D.K.	♂	40	30	Parisiennes	2.0
K_{53}	H.D.	♀	51	25	Muratti Amb. (F)	1.8
Non-smokers						
K_{101}	B.C.	♀	22	0	–	0
K_{102}	B.M.	♀	21	0	–	0

(F), filter. 1 mg Th = 109 pCi. S.D. $\pm$0.6.

Results of the first measurements in 10 smokers and 2 non-smokers are given in Table III. In 5 smokers, thorium appears for a certainty, ranging from 1.7 to 2.4 ± 0.6 mg. It is uncertain or negligible in the 5 other cases. In the 2 non-smokers, none of the 3 peaks is present.

The potential hazards of the uptake of the thorium-series in lungs is dependent on the radiation dose delivered to the tissues. The calculation of this dose is subordinated to the distribution of radioactive particles in the lungs. For an improbable homogeneous distribution of 2 mg thorium with all decay products, in a conventional lung mass of 480 g (according to SNYDER[13]) and ΣE(RBE) = 360 MeV for all α-rays emitted in the series, the accumulated dose for 30 years amounts to 92 rems, or a dose rate of 3 rems per year. These values should amount to 276 rems and 9 rems respectively in a more likely inhomogeneous distribution limited for instance to a third of lung mass only[14]. Such accumulated or yearly doses would be just acceptable for occupational workers exposed to ionizing radiation, but they are not permissible for the whole population at large. For this type of population, according to the last regulation, the maximum permissible dose rate suggested for other organs than gonads is 1.5 rem per year (K. Z. MORGAN[15]).

According to my own experience, the serious of the hazard issued from thorium inhalation has been shown in 2 workers exposed to the thorium vapors of an arc-lamp[16]. The first one, after 42 years exposure, having accumulated 6.5 mg Th-equiv., had a fibrosis with dyspnoe and a reduction of his working capacity. The second one, after 46 years exposure in the same workshop, showed a lung burden of 10.6 mg Th-equiv. and had to undergone a surgical lobectomy. With a severe dyspnoe, he had a permanent working incapacity[17].

In conclusion, this first study, which shows the accumulation of the thorium-series in lungs of some cigarette smokers, has to be carried on systematically on a large number of smokers with improved precision (larger crystal, 14 inches diameter × 8 inches height, background reduction with inactive photomultipliers and a lengthenings of measurement time). Further, in the same apparatus, the spectrum of lungs and parts of lungs (lymph glands and tumors especially) has to be recorded post mortem[18].

Résumé. Le spectre de cigarettes courantes révèle systématiquement la série radioactive du thorium, à raison de 9 à 36 mg de ^{232}Th-equiv. pour 9000 cigarettes, la consommation annuelle d'un grand fumeur. Dans l'anthropospectromètre, le thorax de la moitié des fumeurs mesurés montre les raies de la même série avec 1,7 à $2,4 \pm 0,6$ mg de ^{232}Th-équiv. par sujet.

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[13] R. S. SNYDER in K. Z. MORGAN and J. E. TURNER, *Principles of Radiation Protection* (J. Wiley, New York 1967), p. 330.

[14] In this calculation, I admit the lungs burden of the thorium-series is constant, that is an equilibrium between intake and elimination has been reached. Further, I suppose that ^{232}Th is present in the chain, what is not at all sure. The peak of MTh_2 (^{228}Ac) with a half-life of 6.1 h being always present, one may only affirm, therefore, that ^{228}Ra is present too. The energy of ^{232}Th α' rays being weak (4.0 MeV), the absence of the head of the series would not modify substantially this dosimetry. Lastly it is for simplicity that the lung burden is given in mg thorium-equivalent instead as activity units. One must remember that the activity of 1 mg thorium-element equals 4.03 des. sec^{-1} = 109 pCi.

[15] K. Z. MORGAN, in *Principles of Radiation Protection* (Eds. K. Z. MORGAN and J. E. TURNER; J. Wiley, New York 1967), p. 508

[16] The carbon electrodes of the arc-lamp showed clearly all the characteristic γ-lines of the Th-series.

[17] The investigation of these cases has been performed on behalf of 'Schweizerische Unfall-Versicherungsanstalt'.

[18] Acknowledgments are due to the Swiss National Fund for Scientific Research which financed all the equipment for the whole-body spectrometer and specially to Prof. Dr. HANS STAUB for support and helpful discussions. Further I should like to thank Mrs. M.-L. JOYET for her constant technical collaboration and all the voluntary smokers and non-smokers for their kind understanding help.

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[20] Theses researches were performed before leaving this laboratory mid-January 1970. Paper delivered at '12ème Table Ronde sur l'exploration fonctionnelle par les isotopes radioactifs' on May 23, 1970, Strasbourg.