C57BL/6J マウスの個体の放射線感受性と臓器のポーラログラフ特性*

上野陽里
京都大学医学部放射能基礎医学教室（主任：菅原努教授）
昭和41年6月30日受付

本実験で C57BL/6j のマウスの個々の放射線感受性と，中枢神経系，胸腺，脾，肝のポーラログラフ的特性との関係について追求した．ポーラログラフに於ける Brdicka 蛋白波の第一反応は蛋白質SHに対応し，第2反応は，非蛋白質SHに対応する．前者は生体にとつて重要なSH酵素を，後者は放射線防護部分を対応すると考えると，両者の比はある種の放射線防護能力を示す示標と考えられる．

一方すでに著者によつて示された方法に従つて，一系統内のマウスの個々の放射線感受性を予知し，予知されたマウスの放射線感受性に従つて，上記示標の平均を求め，それと実験による放射線感受性と比較した．このさい，著者によつて提案されたパラメーターを用いる．若干の例外を除いて各臓器の示標の大きいマウスは放射線感受性が低かつた．この両者は両対数グラフでほゞ直線的であつた．このことからマウスの個体の放射線感受性と体内SH物質量について討論された．

RADIOSENSITIVITY OF INDIVIDUAL MOUSE AND THE POLAROGRAPHIC PROPERTIES OF ITS ORGANS IN C57BL/6j MICE

Yori Ueno
Department of Experimental Radiology
(Director: Professor T. Sugahara)
Faculty of Medicine, Kyoto University, Kyoto

Introduction

Recently, many evidences have been obtained by Fukuda /14/ and many other authors that the chemical compounds having sulfhydryl and disulfide groups in their chemical structures are remarkably protective against radiation injury. The protection by this kind of compounds is found in the experiments with irradiation not only in vitro but also in vivo. The evidences that the sulfhydryl and disulfide included in the chemical protective agents combines with a target substance, account directly for the protection against radiation injury of the target which is demonstrated by the technique of ESR/2, 44/. The interpretation as to the mechanisms of protection represented with sulfhydryl and disulfide at the level of macromolecules, however, is not always appropriate for the results from the experiments at the level of mammals. Sugahara et al. /36/ have suggested the possibility of protection through unknown pharmachological action of sulfhydryl and disulfide. With reference to the pharmachological action, some authors /15, 16, 19/ have described that the substances including sulfhydryl and disulfide induce anoxia in the tissues, Pany /21/ has reported that the substances inhibit the activity of histaminase and Phol et al. /23/ has reported that the uptake of labeled thymidine to DNA and the uptake of labeled cytidine and uridine

*Contribution No. 36 from Department of Experimental Radiology, Faculty of Medicine, Kyoto University, Kyoto.

to RNA are accelerated by the substance, Pohl et al. did not refere to the relationship of this pharmacological action to the protection against radiation injury.

On the base of study as to the mechanism of protection with the substance including sulfhydryl and disulfide against radiation injury, Bacq /2, 3, 7/ and Bacq and Alexander /3/ took note of the problem whether the amount of naturally existing sulfhydryl and disulfide in tissues is one of factors affecting the radiosensitivity of the mammals or not. Though the property of the natural chemical protective agents is defined insufficiently, the substance including sulfhydryl and disulfide free from protein molecule seems to play a main role in the chemical protection (according to Ueno /37/,, Sörbo /35/, Konstantinova /18/ and Révész et al. /30, 31, 32/). Furthermore, Bott and Lundgren /8/, Ueno and Sugahara /41/ and Casperson and Révész /11/ using bacteria, mammalian tissues and mammalian cells cultured in vitro as materials of their experiments reported that the ratio of contents of nonprotein sulfhydryl and disulfide compounds to the content of protein sulfhydryl and disulfide compounds, increased with the increase of radioresistance of materials.

The relationship of LD 50/30's of various mouse strains to a polarographic property related with sulfhydryl and disulfide compound of the organs in mice of corresponding strains being described in the previous report /41/, the present study deals with the relationship of the radiosensitivity of individual mouse belonging in one strain to the polarographic property in corresponding individual mouse. The polarographic property will be discussed later.

Before the present study being started, it is requested to classify the mice with the same age, sex and same housing conditions into several classes, according to their radiosensitivities. The prediction of radiosensitivity of individual mouse in one strain with the practically same biological, genetic and housing conditions has been reported by Pospisil /24, 25, 26, 27, 28, 29/ and Ueno /38, 39, 42/. The principle proposed in the reports is that the difference in radiosensitivities of mice belonging in one strain with same conditions is caused mainly from the difference in physiological states of the mouse. It is well known that in spite of the same biological, genetic and housing conditions of mice, they have many variations in the peripheral blood cells counts, haemoglobin content, amount of urine, Na/K ratio in the urine, body weight etc, which seem to be depended on their various physiological states. For determining the physiological state of individual mouse before irradiation, it is naturally requested to find a suitable biological index to represent the physiological state of the animal. As one of the most suitable forms, Leiderman and Shapiro /20/ have proposed the mean square successive difference, expressed as δ^2 by them. The δ^2 value is able to be induced from many biological scales, that is, the amount of urine, body weight etc. The author has succeeded in proving some relationship of the radiosensitivity of individual mouse to δ^2 calculated from body weights before irradiation in a given strain of mice.

Method

The mice of C57BL/6j of both sexes were used throughout the experiment, which were supplied from the animal room of our department through brother sister mating. The mice were housed in an individual cage at fifty days of age and fed a laboratory chow (Funabashi Farms) and water ad libitum. The body weights were measured every 10:00 a.m. for ten days. The mean square successive difference (δ^2) was calculated from the daily measured values of body weights, according to the following expression;

$$\delta^2 = \frac{\Sigma(X_i - X_{i+12})}{n-1}$$

Xi is a time series of continous variables sampled at times i=1, 2,...n. The mice were classified into four groups A, B, C and D in order of δ^2 from small to large.

Polarographic technique was as follows; the organs were homogenized in Potter's homogenizer. The homogenates were divided into two groups for the measurements of the first and second reactions of Brdicka's catalytic wave. For the first reaction, 1.0 ml of homogenate was denatured by adding 0.1 ml of 1.0 N KOH and further 0.5 ml of distilled water. For the second reaction, 1.0 ml of homogenate was filtrered after deproteination by the addition of 1.0 ml of 20% solution of sulphosalicylic acid and 0.5 ml of distilled water and the filtrate was used as material for measurement. The filter paper used was Toyo Filter Paper No. 5A with 5.5 cm in diameter (Toyo Roshi Kaisha Ltd.).

At the measurement, 0.9 ml of materials were added into 5 ml of the cobaltic solution with 0.1 M NH_4Cl, 0.001 M Co $(NH_4)\cdot Cl_3$ and 0.08 M NH_4OH at both reactions. All polarographic waves were recorded with 50 μA of sensitivity at the temperature of 25°C by the Shimadzu Pen Recording Polarography Model PR-2 (Shimadzu Seisakusho Co. Ltd.) with Shimadzu automatic corrector. The heights were assumed to correspond to the amount of sulfhydryl and disulfide group polarographically active in the materials. In the first reaction, the detection is made in the group included in the homogenate which are mainly bound on the protein molecule and in the second reaction in the group included in nonprotein compounds such as a polypeptide /9, 10, 33. As the index representing the polarographic property of homogenate was used the ratio of the wave heights of the first reaction to that of the second reaction.

The mice in the second series to determine the radiosensitivity were irradiated by Toshiba Deep Therapy Unit Model KXC-18-2A (Tokyo Shibaura Electric Co. Ltd.) with the following factors; 24 mA, 190 kVp, the filter of 1.0 mm Cu and 0.5 mm Al and the output of about 58 R/min. The target distance to the center of the material was 50 cm. A Radocon Model 575 dosimeter was placed in the center of the irradiation field for monitering.

Table 1. Number and distribution of mice according to δ^2 calculated from daily measured body weight

group δ^2	A 0.00–0.29	B 0.30–0.59	C 0.60–0.89	D 0.90–	total
number of mice in the experiment to get average of ratio ····· 1st series					
under the unanaesthetization	16	18	2	10	46
under the anaesthetization	28	18	2	8	56
number of mice in the experiment to get average maximum tolerant dose···· 2 nd series	26	9	2	2	39
number of mice in the experiment to get mean survival time ···· 2 nd series					
under the unanaesthetization	31	18	6	4	59
under the anaesthetization	20	24	7	3	54
total	121 47.6 %	87 34.3 %	19 7.5 %	27 10.6 %	254 100.0 %

A cylindrical box, 15 cm in diameter and 3 cm in height, containing eight radical compartments was used to house the mice individually during irradiation.

For the determination of radiosensitivity, three methods of irradiations were prepared. The first was to determine the maximum tolerant dose of individual mouse, that is, thirty-nine mice irradiated repe-

atedly with 200 R whole body irradiation every Tuesday and Friday, twice a week till the death of mice. The second was done to determine the mean survival time (day) after the acute whole body irradiation of 800 R to the anaesthetized mice and unanaesthetized mice. The mice used in the second and third methods were 113 mice. The number of mice in each group classified by δ^2 in the second series were shown in Table 1.

In each group, the average of the ratio and average radiosensitivity were calculated and were compared with each other. The mice in the first series were used for the determination of the ratio and that in the second series were used for the determination of radiosensitivity. The mice used to determine the ratio were sacrified with the cervical dislocation at the tenth day after putting into the individual cage and the brain, liver, spleen and thymus were removed and weighed. The treatment was carriedout at 11:00 a.m. to avoid the influence of the circadian changes in the organ weights /22/. After weighing, the organs were chilled at once in 5 ml of the distilled water. In this series, 102 mice were sacrified under the condition of unanaesthetization and anesthetization with 30 mg/kg of nembutal through the intraperitoneal injection. The number of mice in each group classified by δ^2 was listed in Table 1.

Results

1. The relationship of the maximum tolerant dose to the average of the ratio.

Figure 1. Relationship of the average of the ratio to the average maximum tolerant dose of irradiated mice

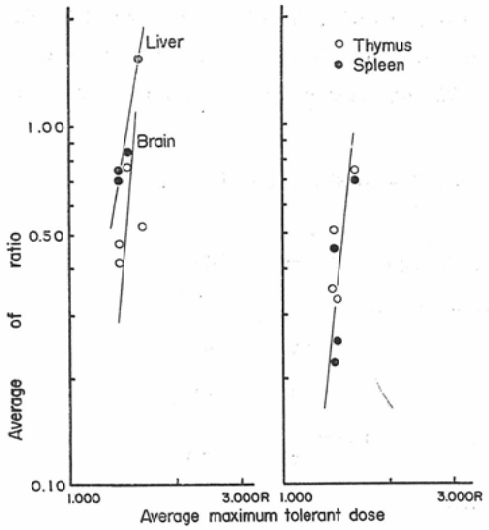
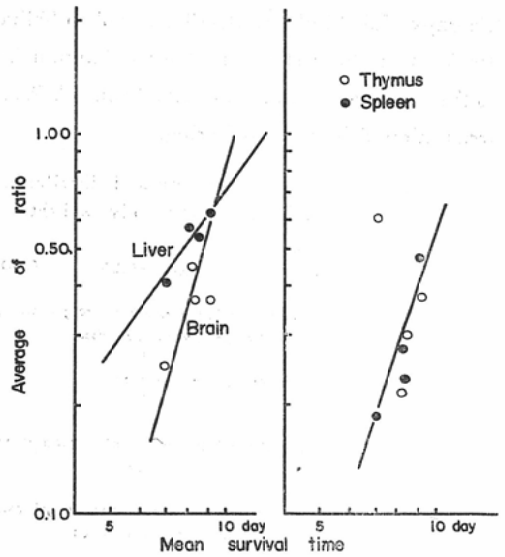


Figure 2. Relationship of the average of the ratio to the mean survival time of irradiated mice under the anaesthetization

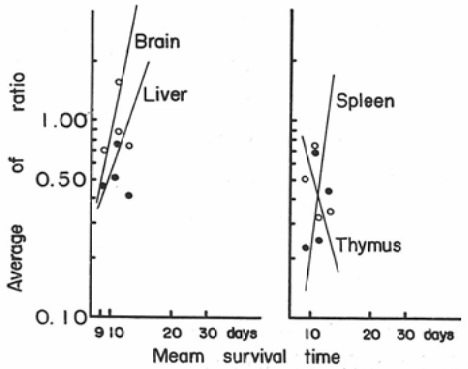


The relationship of the average maximum tolerant dose to the average of the ratio of organs in four groups were illustrated in Figure 1. The relationships showed the linear forms in log-log scale.

2. The relationship of the mean survival times to the average of the ratio.

The relationships of the mean survival times to average of the ratio of organs were illustrated in Figure 2 and 3. A linear relationship in the experiment used the anaesthetized mice was shown on a log-log paper, though one point of thymus was deviated from the line drawn along the other three points, as illustrated in Figure 2.

Figure 3. Relationship of the average of the ratio to the mean survival time of mice irradiated under the unanaesthetization



The relationship in the experiment used the unanaesthetized mice was roughly linear, as illustrated in Figure 3. In comparison with the results in Figure 2, the lines were near the vertical, the lines of the brain was almost vertical and as for the line responsible for the thymus, the contrary relationship was shown.

Discussion

At the results from the present study, the increase of the ratio of organs appears likely tobeaccompanied with the increase of radioresistance of mice. The lines illustrated in Figure 1 were more vertical in comparison with the lines in Figure 2. Since the physiological and morphological states of mice irradiated repeatedly were not similar at every time of irradiation in the time series of experiment to determine the maximum tolerant dose and further more the ratio were determined in the irradiated mice, the values do not represent one at each time of irradiation repeatedly give. This seems to be one of causes to make the lines more vertically. In addition, the method to determine the maximum tolerant dose is not free completely from the risk to include the waste dose given at the time near death. They are considered as causes disturbing the clearly linear relatinship, through making the line more vertical as illustrated in Figure 1.

The posibility that the chemical protective agents involving SH compounds reduce the oxygen content in the tissues, has been reported /15, 16, 19/, but contrarily there are few reports as to the possibility that the treatment to reduce the oxygen content in the tissues increase the amount of protective SH compounds in the tissues. In the present experiment, the ratio were compared among the conditions of anesthetization and unanaesthetization, since it is believed by some authors /34/ that the anaesthetization might reduce the oxygen content in the tissues and this mechanism seems to be one of the causes to reduce the radiosensitivity. The average values of all organs tested were, however, smaller in the anaesthetized mice. The results did not support the assumed possibility. But, comparing the mean survival time of anaesthe-

Table 2. Relationship of radiosensitivity to the average of the ratio

treatment	unanaesthetization	anaesthetization
mean survival time (day)	11.3	8.3
average of ratio for four group		
liver	0.98	0.55
brain	0.55	0.36
thymus	0.49	0.38
spleen	0.41	0.30

tized mice with that of unanaesthetized mice, the latter is larger than the former as shown in Table 2. The larger mean survival time in the latter, therefore, was accompanied with the larger average of the ratio in the latter. According to the reports of Doherty /12/, the anaesthetization has little or no effect as protective agents and Evants et al. /13/ have reported the sensitization by anaesthetization with pentobarbital in some condition of their experiment. Furthermore, Bacq and Alexander /5/ have suggested that anoxia is not the main mechanism responsible for radio-protection by SH compounds for mammals. Therefore, the results that the mean survival time of anaesthetized mice was shorter than that unanaesthetized mice in the present study, seem to be reasonable on the base of the results as to the ratio as described above.

The problem why the line illustrated in Figure 3 are more vertical than that illustrated in Figure 2, is difficult to be solved. As one of causes seems that the experiment to determine the ratio being carried out about nine months earlier than that to determine the mean survival time, the unknown factors might effect in the results of experiment for the interval. The contrary results of thymus in Figure 3 might be explained with the reason that the thymus, especially, thymic weight being remarkably modified with many factors called stresses /24, 43/, it is not easy to maintain these factors in the same conditions, in all experiments.

The experiment to determine the intrastrain difference in one strain of mice such as the present study will always accompany many difficulties in comparison with the experiment to determine the interstrain difference among many strains. In spite of these difficulties, the present study showed the comparatibely definite relationship between the ratio and the radiosensitivity. And the results from the present study were analogous to that from author's previous studies /37, 41/, though the formers were carried out on the level of cultured cell and the individual mouse, on the other hand, the latter was carried out on the level of the strain of mice.

The reason why the high value of the ratio is accompanied with the radioresistance remains to be solved. As described in the previous reports/9, 40, 41/, the second reaction seems to represent by sulfhydryl and disulfide included in nonprotein molecule, such as, mucopolypeptide and the first reaction seems to represent by sulfhydryl and disulfide included in protein molecule, such as, SH-enzymes which are usually essential enzymes for the living of the cells. By Hatano /17/ so-called SH-enzymes are very radiosensitive in comparison with so-called non SH-enzymes. Seeming to correspond with the amount of protein molecule including SH-enzymes, the value expressed by the first reaction is allowed to describe to correspond with the amount of so-called the target site. On the contrary, the value in the second reaction, as described by Bacq /6/ and others, seems to represent the protective site. Therefore, the ratio used appears to be the reasonable index to represent the degree of protection or radiosensitivity.

The results listed in Table 2 is noted in the other point. In the condition unaffected by anaesthetization, the average values of the ratio of the brain and liver are larger than that of the spleen and thymus. The former being believed to be more radioresistant organs than the latter, the difference in the average values of the ratio of four organs is able to be noted, on the base of relationship of radiosensitivity to the ratio.

Conclusion

The present study was carried out to clarify the relationship of a polarographyic property of organs in mice to the radiosensitivity of corresponding mice in one strain. The value of the ratio of the wave heights of the first reaction to that of the second reaction of Brdicka's catalytic reaction seems to be one of reasonable indices to represent the radiosensitivity, as representing the ratio of protective site to the target site. The relationship is nearly illustrated as a line in log-log paper, except some cases. It seems that the high

value of the ratio is accompanied with high radioresistance of mouse in one strain. The data shifting from this relationship seem to be resulted from the affecting on the relationship by the unknown factors. It should be brought into consideration that the experiment as to the intrastrain difference in radiosensitivity is always accompanied by such difficulties as that are not met by us in the experiment about the interstrain difference in the radiosensitivity. In spite of these difficulties, the problem of the difference in radiosensitivity at the level of individual should be solved and the further experiments are in consideraition by the author.

Acknowledgement

The author wish to his gratitude to Professor T. Sugahara for his valuable advices and comment concerning this manuscript. The author wish to thank Mr. T. Tanaka, Miss. K. Fujimoto and Miss. H. Takagi for their technical assistance. The author is grateful to Professor Z.M. Bacq for valuable advices and comment concerning this manuscript.

References

1) P. Alexander and M.G. Ormerod: Symposium on biological effects of ionizing radiation at the molecular level, IAEA, Vienna, 399–410 (1962).
2) Z. M. Bacq: Reflexions sur la radiosensibilite comparee des etres vivants, Estratto dalle a Pubbl.Staz. zool. Napoli, 27, 256–260 (1955).
3) Z.M. Bacq and P. Alexander: Fundermentals of Radiobiology, 2nd Ed. Pergamon Press, Oxford, 457–483 (1961).
4) Z.M. Bacq: L'influence de certains facteurs metaboliques la radiosensibilite, J. Clin. et biol. medicale, 34, 80–84 (1963).
5) Z.M. Bacq and P. Alexander: The role of oxygen in the phenomena of chemical protection against ionizing radiation, Proceedings of a symposium held in London, 1963, "Oxygen in the animal organism", Pergamon Press, Oxford, 509–536 (1964).
6) Z.M. Bacq and P. Alexander: Importance for radio-protection of the reaction of cells to sulfhydryl and disulfide compounds, Nature, 203, 162–164 (1964).
7) Z.M. Bacq: Chemical protection against ionizing radition, Charles C. Thomas, Springfield, 240–252 (1965).
8) K.F. Bott and D.F. Lundgren: The relationship of sulfhydryl and disulfide constitutions of Bacillus cereus to radioresistance, Radiation Res., 21, 195–211 (1964).
9) M. Brezina und P. Zuman: Die Polarographie in der Medizin, Biochemie und Pharmazie, Akad. Verlag., Leipzia, 544–546 (1956).
10) M. Brezina: Polarographyic analysis of urine, Rev. of Polaragraphy (Japan), 11, 26–28 (1963).
11) O. Casperson and L. Revesz: Cytochemical measurement of protein sulfhydryl in cell lines of different radiosensitivity, Nature, 199, 155–155 (1963).
12) D.G. Doherty: Chemical protection to mammals, Radiation protection and recovery, Ed. by A. Hollaender, Pergamon Press, Oxford, 45–86 (1960).
13) J.C. Evans and L.R. Orkin: Protective effect of nitrous oxide against total-body radiation in the mouse, Nature, 195, 822–823 (1962).
14) M. Fukuda: Studies on chemical protection against radiation in Japan, Strahlentherapie, 117, 142–146 (1962).
15) E. Ya. Graevskij i M. M. Konstantinova: Issledovaniya mekhanizma protivluchevogo zashchitnogo dejstviya nekotorykh serusoderzhashchikh veshchestv, Doklady AN SSSR, 133, 969–972, (1960).
16) E. Ya. Graevskij i M.M. Konstantinova: Zavisimosti protivoluchevoj effektivnostj zashchitnykh veshchestv ot sozerzhaniya kisloroda vo vdykhaemom vozdukhe i tkaniyakh, Doklady AN SSSR, 145, 195–198 (1962).
17) H. Hatano: Actions of radiation on enzymes, Symposia of the society for cellular chemistry, No. 9, Ed. by S. Katsunuma, Maruzen Co. Ltd., Tokyo, 21–38 (1959).
18) M.M. Konstantinova: Raspredelenie Karboksil'nykh i sulfgidril'nykh grupp bekov v tkani selezenk myshej v norme i pole oblucheniya, Tsitologiya, 3, 293–299 (1963).

19) M.M. Konstantinova, A.G. Tarasenko i V. M. Fedoseev: Issledovanie protivoluchevoj aktivnosti N-alkil-proisvodnykh 2, 3-dimerkaptopropilamina i mekhanizma ikh dejstviya, Radiobiologiya, 4, 216–220(1964).
20) P.H. Leiderman and D. Shapiro: Application of a time series to physiology and psychology, Science, 138, 141–142 (1962).
21) J.F. Pany: Der Einfluss schwefelhaltiger Strahlenschutzstoff auf den Stoffwechsel des Histamins I. Die Wirkung von SH und Disulfibverdindungen auf die Fermente des Histaminabbaus,Gesam. Expertl Med., 137, 609–618 (1963).
22) D.J. Pizzarello, D. Issaak and K.E. Chu: Circadian rhythmicity in the sensitivity of two strains of mice to whole-body radiation, Science, 148, 286–291 (1964).
23) V. Pohl et J. Quertier: Groupes sulhydriles et morphologenese IV. Effects du β-mercaptoethanol et de l'acid-α-lipoique sur lemetabolisme des acids nucleiques chez les embryons de bacteacien et de puolet, J. Embryol. exp. Morph., 11, 293–305 (1963).
24) M. Pospisil: The urine sodium/potasium ratio and its relationship to the degree of radiation thymolysis in rats, Folia biol. (Praha), 8, 182–185 (1962).
25) M. Pospisil, L. Novak and B. Hasek: Energeticheskij balans i predokazanie radiochuvstvichel'nosti krys, Radiobiologiya, 4, 396–401 (1964).
26) M. Pospisil: The significance of electrolyte balance in the differentiation of interindividual radiosensitivity in mice, Rad. Therap., 5, 517–524 (1964).
27) M. Pospisil and Y. Ueno: Prediction of individual differences in radiosensitivity in mice, testicalar atrophy, mortality and weight changes, Folia biol. (Praa), 10, 232–238 (1964).
28) M. Pospisil, Y. Ueno and J. Sikulová: The dynamics of physiological processes in relation to radiosensitivity, Naturwissenschaften, 20, 485–486 (1964).
29) M. Pospisil, J. Sikulová and F. Sevcik: The fluctuation of urine electrolyte excretion and its relation to mortality of multiple irradiated mice, Gesam. Exptl. Med., 139, 112–121 (1965).
30) L. Revesz, H. Bergstrand and H. Modig: Intrinsi nonprotein sulphydryl levels and cellular radiosensitivity, Nature, 198, 1275–1277 (1963).
31) L. Révész and H. Bergstrand: Radiation protection by cysteamine and cellular sulphydryl levels, Nature, 200, 594–595 (1963).
32) L. Révész, and H. Modig: Cysteamine-induced increase of cellular glutathione-level, A new hypothesis of the radioprotective mechanism, Nature, 207, 430–431 (1965).
33) T. Sasai: Contribution to the development of clinical application of polarograp hyic protein wave, Rev. of Polarography (Japan), 5, 26–38 (1957).
34) E.S. Shchepot'eva, S.N. Ardashnikov, G.E. Lure' i T.B. Rakhmanova: Kislorodnyj effekt pri dejstvii ioniziruyushchikh izluchenij, Medgiz, Moskva, 131–156 (1962).
35) B. Sörbo: The effect of radioprotective agents on tissue nonprotein sulfydryl and disulfide levels, Arch. Biochem. Biophys., 28, 342–344 (1962).
36) T. Sugahara, Y. Okazawa and T. Tanaka: A biological aspects of the protective action of AET againt X-ray irradiation in mice, Acta Schol. Med. Univ. Kioto, 39, 57–63 (1964).
37) Y. Ueno: Vzrah mezi relativnim obsahem sulfhydrylovych, Cesk. fysiol., 12, 393–393 (1963) (abstract).
38) Y. Ueno and M. Pospisil: The individual difference of radiosensitivity, the mammalian level, Med. and Biol., 69, 244–246 (1964).
39) Y. Ueno: The difference in radiosensitivity of individual mouse for whole body irradiations, Constitutionall Med., 29, 1–6 (1964).
40) Y. Ueno, M. Horikawa, T. Aoyama and T. Sugahara: Radiosensitivity and SH compounds in mammalian cells, Folia biol. (Praha), 10, 39–44 (1964).
41) Y. Ueno and T. Sugahara: The radiosensitivity and nonprotein SH compounds in mammalian tissues, Nipp. Acta Radiol. 25, (5), 46–49 (1965).
42) Y. Ueno, T. Tanaka and T. Sugahara: On the body weight and its change in the inbred strains of mice and the relationship between them and radiosensitivity, Bull. Expetl. Animals, 15, 6 — 9 (1966)
43) Y. Ueno: Unpublished data,
44) K.G. Zimmer and A. Miller: New light on radiation biology from electron spin resonance studies, Current topics in radiation research, Vol. 1. Ed. by M. Ebert and A. Howard, North-Holland Publ. Com., Amsterdam, 1–42 (1965).