Werk

Titel: Zoologia

Jahr: 1963

PURL: https://resolver.sub.uni-goettingen.de/purl?312899653_0008|log6

Kontakt/Contact

Digizeitschriften e.V.
SUB Göttingen
Platz der Göttinger Sieben 1
37073 Göttingen

info@digizeitschriften.de

ACTA F. R. N. UNIV. COMEN. VIII.—4., ZOOLOGIA

ACTA

FACULTATIS RERUM NATURALIUM UNIVERSITATIS COMENIANAE

TOM. VIII. FASC. IV.

ZOOLOGIA

PUBL. IX.

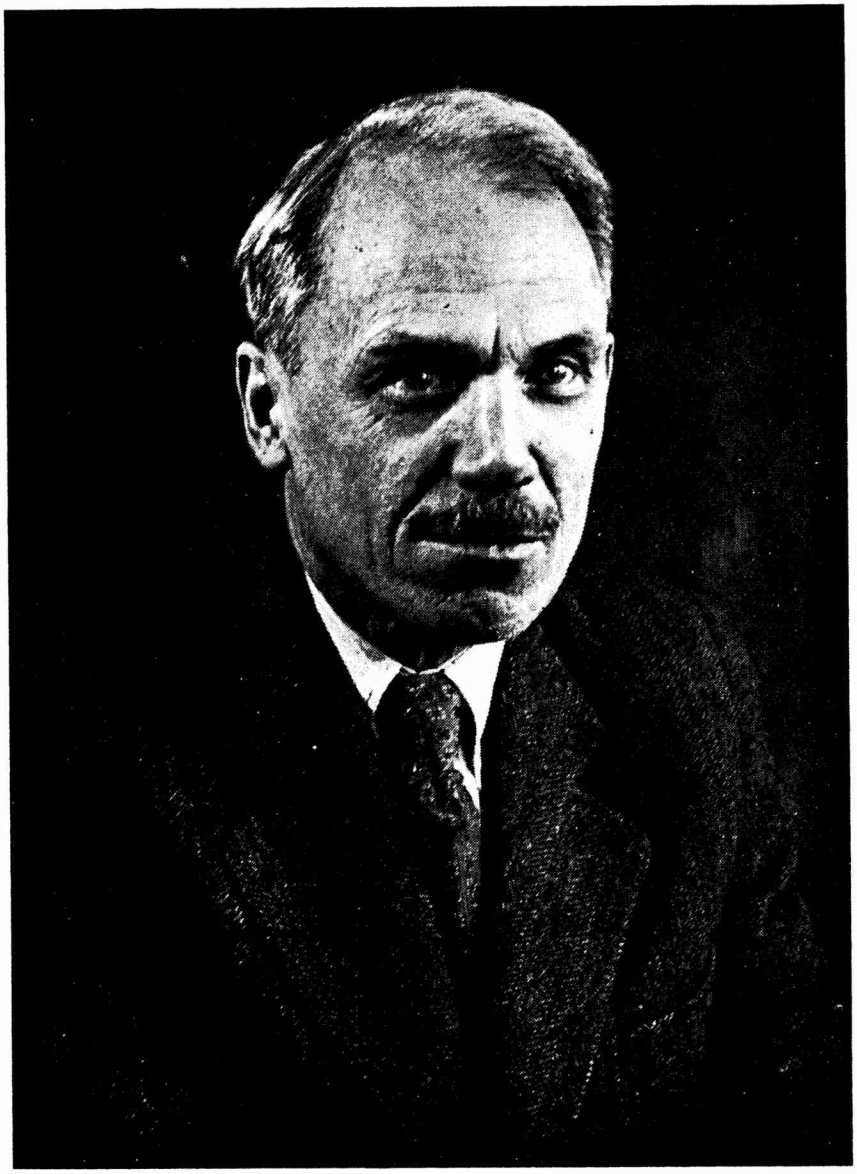
PRÁCE VENOVANÉ PRI PRÍLEŽITOSTI
10. VÝROČIA UMRTIA DOC. Dr. M. J. VÁGNERA

1963

SLOVENSKÉ PEDAGOGICKÉ NAKLADATEĽSTVO BRATISLAVA

Просим обмена публикаций
Austausch von Publikationen erbeten
Prière d'échanger des publications
We respectfully solicit the exchange of publications
Se suplica el canje de publicaciónes

Sborník Acta facultatis rerum naturalium universitatis Comenianae. Vydává Slovenské pedagogické nakladateľstvo v Bratislave, Sasinkova 5, čís. tel. 458-51. Povolilo Poverenícstvo kultúry číslom 2265/56-IV/1. — Tlač: Tisk, n. p., provoz 3, Český Těšín



Doc. RNDr. M. J. VÁGNER (11. I. 1893 – 27. VIII. 1953)

This year we paid homage to the memory of Doc. RNDr. M. J. Vágner – the founder of the branch of animal physiology at the Chair of zoology (Faculty of natural sciences Comenius University in Bratislava) on the occasion of the anniversary of the decennary of his death.

On this occasion we dedicate the submit works about the physiology of proteins to the commemoration of this scientist of renown and exemplary pedagogue.

Authors

ACTA F. R. N. UNIV. COMEN. VIII–4, ZOOLOGIA, 1963

ACTA FACULTATIS RERUM NATURALIUM UNIVERSITATIS COMENIANAE

TOM. VIII. FASC. IV. ZOOLOGIA 1963

Blood-serum proteins of rabbits under normal and radiative conditions

Š. Paulov

Introduction

Presently many research institutions are intensively occupied with the study of physiological as well as physico-chimical properties of proteins, because proteins have primordial importance for living organisms. During last years there was a notable accumulation of important results some of which concern directly the basic knowledge about life processes and life as a whole. Great progress obtained in this region of research is due principally to the perfection and worldwide use of such special analytic methods as electrophoresis, polarography and radio-isotope-labelling technique.

Under the great number of animal proteins most attention is attracted by the study of blood-serum proteins. This is based on following important facts:

1. Blood-serum proteins have a high metabolic activity.
2. In organism these proteins have important transport functions for a lot of fundamental compounds.
3. Blood-serum proteins are in dynamic equilibrium with tissue proteins.
4. Blood-serum proteins are frequently influenced by different pathological phenomenons.
5. An important circumstance is in the fact that blood-serum proteins are relatively easily accesible to experimental examinations.

This exceptional position of blood-serum proteins led a great number of scientific workers to pay special attention to this substance and this as well in sound organisms as in organisms after different interventions through mechanical, chemical or radiative influences. Under these factors primordial attention receives now the study of changes in blood proteins through irradiation.

Blood-serum research in normal and radiative conditions in our laboratories demanded to secure the experimental possibilities for applications of isotope-labelled elements and to verify the special research methods and their applicability in our present working conditions. There was a necessity to consult advanced scientific authorities. Our research was enhanced by the fact, that in this new field we had no tradition and no appropriated measuring technical means were at our disposal. We take the permission to express on this place our sincere and grateful thanks and acknowledgements to all external or internal co-workers for their effective help during the experiments as well as for their good will and moral support, which enabled us to complete our experiments and to publish this study.

Applied methods

1. Division of blood-serum proteins by means of paper electrophoresis.

The paper electrophoresis is today the most known analytic method for the study of protein physiology. The method is a relatively new one (from the fourthieth years of this century), but there is an enormous number of variations, simplifications and adaptations of this method to special aims. There is also an practically countless number, of measurements made by this method and a great number of these measurements are of basic importance. The importance of paper electrophoresis increases when we take in consideration that it can be used in parallel with other analytic methods, i. e. principally with radioisotope labelling and with polarography.

For our purpose we used mainly papers published by following authors: Dittmer (1956), Hořejší (1956), Wolstenholome et al. (1956), Lederer (1956), Bier (1959), Michalec et al. (1959), further there are our experiences gained when working in the Oncological Institute in Bratislava

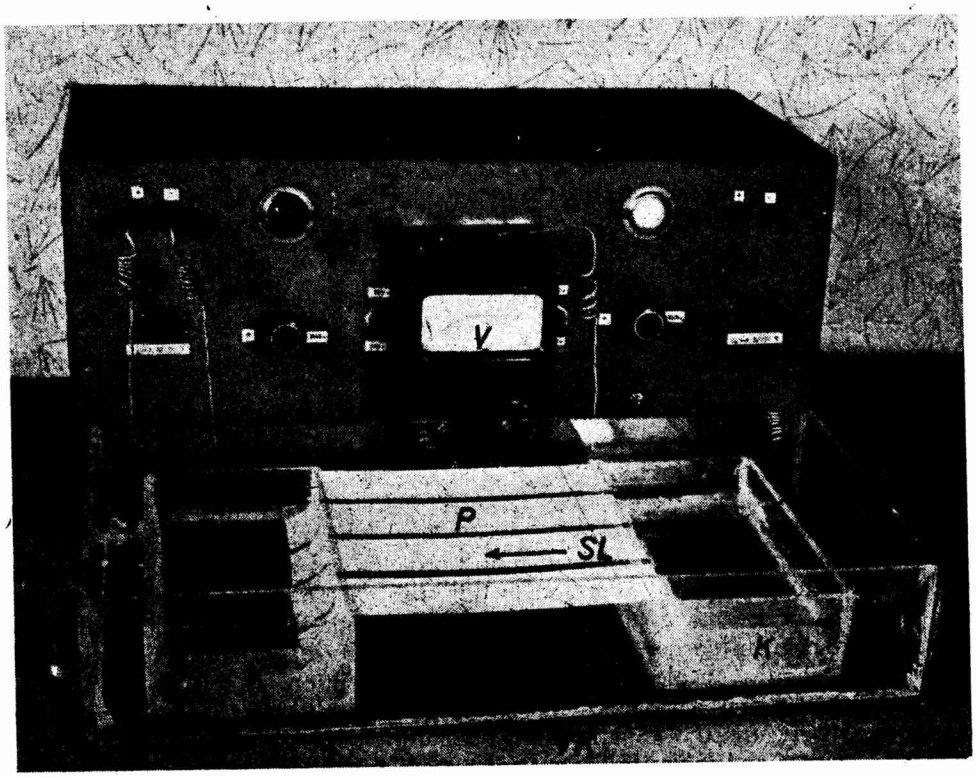


Fig. 1. Schematic diagram of the paper electrophoresis in wet chamber.

Z – *d-c* source,
V – current and tension measuring instrument,
VK – wet chamber of plexi-glass,
K – wessels of plexi-glass with veronal acetate buffer (vessel volume was 700 ml, their distance was 170 mm),
P – strips of filter paper Whatman 1 (4 strips in one wet chamber),
SL – start line for application of serum (distance from the wessel border was 20 mm),
+, – polarity indication,
← – direction of fractions migration.

as well as some experimental studies made on this institute (Hnilica—1958, Hupka—1959). Some partial informations provided from published papers of a number of authors as will be stated later.

In order to obtain good separation of protein fractions on the paper support, we have adopted constant experimental procedure which consists: 1. in the choise of a suitable experimental arrangement of the electrophoresis, 2. choise of a sutable source of electrical current, 3. provenience and quality of the paper support, 4. composition and ionic strenght of the buffer, 5. unified procedure of staining and elution of electrophoretograms, 6. unified procedure for quantitative evaluation of protein fractions.

Experimental arrangement which best suited to our possibilities is the electrophoresis with the so called „wet chamber" containing horizontally tended strips of filter paper, as in the photography on fig. 1.

As a source of current we used an electronic rectifier, giving a tension from 0—380 V with possibility of maximum current load 100 mA. (The circuit schematic is on fig. 2.) When separating protein fractions, we used a tension of 250 V (ten-

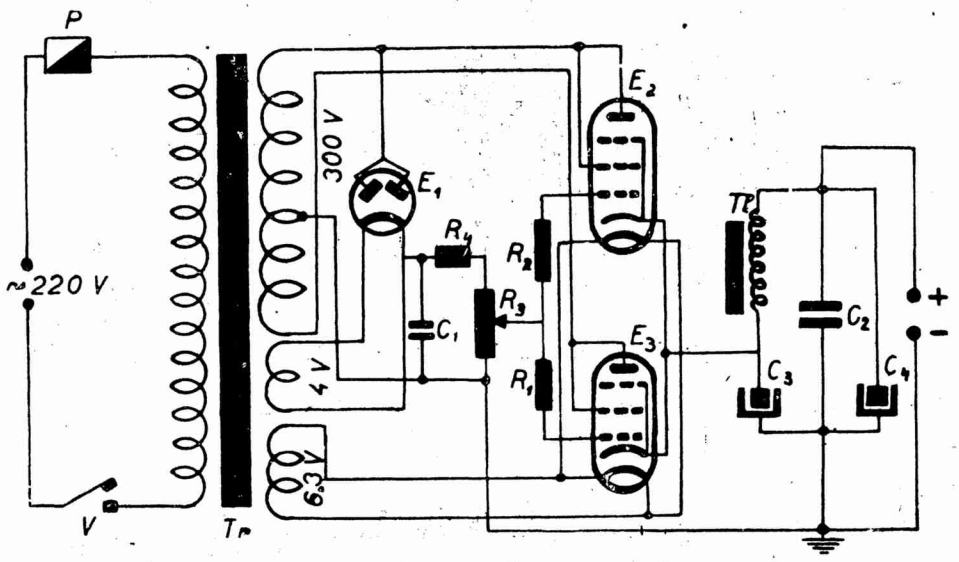


Fig. 2. Source of adjustable *d-c* (schematic diagram).

R_1 — 100 kΩ 0.25 W
R_2 — 100 kΩ 0.25 W
R_3 — 1 MΩ pot.
R_4 — 100 kΩ 0.25 W
E_1 — AZ 1 E_2 — EBL 21 E_3 — EBL 21
P — fuse 1 A 250 V, V — switch 250 V /2 A
Tr — transformer 2×300 V 80 mA, 1×4 V 1 A, 1×6.3 V 2 A,
Tl — choke 8 H 60 mA.
C_1 — 4 μF/450 V
C_2 — 1 μF/450—500 V
C_3 — 32 μF/450 V
C_4 — 32 μF/450 V

sion gradient 14,7 V/cm) with a curent of 9 mA. The total time for separation was 5 hours. As electrodes we used platinum wire 1 mm in diameter which we placed in the plexi glass containers opposite to the side on which the paper strips were immerged. We used no labyrinth in the containers. We enhanced so changes in pH in the strip region.

Among the wide range of paper supports we have choosen the filter paper Whatman 1. We cut paper strips 280 mm long and 40 mm broad. Before tending them

between the containers we immerged them in the buffer used by us and dried them out partially between leafs of clean filter paper. Strips being so prepared, we tended them between the containers and applied on them 0.03 ml of the serum by means of a micropipette. After separation of fractions, we dried the electrophoretograms at room temperature in horizontal position.

The rapidity of displacement of protein fractions and their sharp delimination is primordially influenced by the pH and the molarity of the buffer used. In our experimental conditions we obtained good results with alcalic veronal acetat buffer pH—9 ionic strenght —0.06, prepared after Dittmer (1956). In this buffer there dissociate with preponderance carboxyl groupes, the proteins having negative charge displace themselves in electric field from cathode to anode. Yeoman (1959) obtained a remarkable result when through addition of calciumlactate to the veronal-acetat buffer he obtained up to 11 fractions. Aronsson et al. (1958) obtained 9 fractions of serum protains when using the so called TRIS-buffer.

Separated proteins which we obtain on the paper strips are not visible by eye.

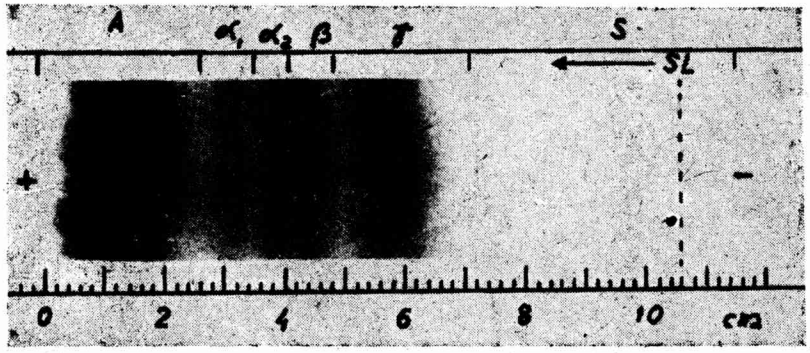


Fig. 3. Rabbit serum electrophoresis with well separated albumins and globulins alpha$_1$, alpha$_2$, beta and gama.

SL — start line, *S* — section from start to the beginning of gama globulin fraction.
In the upper part we marked the minima of staining between fractions. A 2 mm scale is in the lower part of the fig.

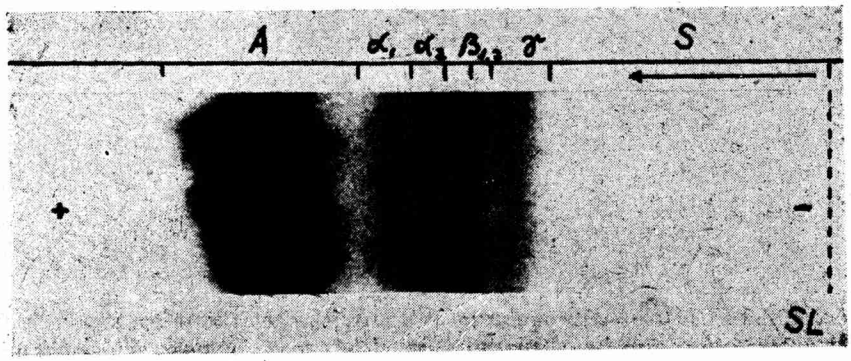


Fig. 4. Electrophoreogram of rabit serum with distinctly separated beta subfractions.

A — albumins,
S — section from start to the beginning of gama globulin,
α_1, α_2, $\beta_{1,2}$, γ — globulins,
Sl — start line where the serum is applied,
←— direction of fractions migration from cathode to anode.

We must stain them with a suitable colorant and afterwards wash out the superfluous colorant. There is a lot of staining procedures in the literature. We made a good experience with a solution of 1 % bromphenol blue in methylalkohol saturated with sublimate. In this solution we immerged the strips 10 minutes (8 strips in 1 liter of solution). The background was decouloured in three baths 0.5 % acetic acid and this always with immersions of 10 minutes in each bath. Special attention was paid to the fact that the solution of bromphenol-blue must not be „old" (but we did not use solutions older than half a year) and that after one serie of strips (8 strips) the solution was renewed. Electrophoretograms so stained were dried in horizontal position and at room temperature.

Under described conditions we separated the proteins of rabbit blood-serum in 5 fractions: albumins and globulins α_1, α_2, β and γ as it is on fig 3. Division of beta globulins in subfractions was successful only for some rabbits (fig. 4).

The determination of protein fraction concentration was made in the way that we cut first the strips in places of minimum coloration as it is to be seen in the upper part of fig. 3. The fractions so separated were eluated in a 2.5 % solution of natriumcarbonate in 25 % methylalkohol during 1 hour. We measured afterwards extinctions in a colorimeter LP with two photoelements and using an interference filter with transparence at $\lambda = 600 \pm 5$ mμ.

For graphical evaluation of fractions we used the registration apparatus of a polarograph Heyrovský. A si-

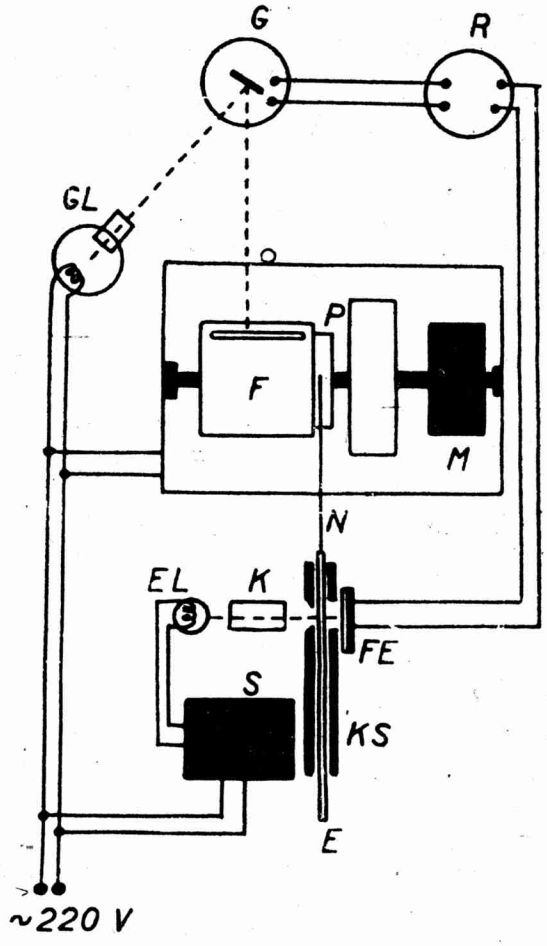


Fig. 5. Application of Heyrovský's polarograph for photodensitometric measurements and registering of fractions in stained electrophoreograms.

G – mirror galvanometer,
R – sensibility reductor,
GL – light source of the galvanometer,
F – registering photographic camera,
P – a plexi-glass disc for winding up of the string which pulls the electrophoreogram,
N – string for synchronous displacement of the electrophoreogram,
E – electrophoreogram,
M – electromotor driving the registration drum of the photocamera and the plexi-glass disk,
El – lamp for illumunation of the electrophoreogram,
S – stabilisation device for the lamp EL,
K – condensor system of the lamp EL,
FE – selenium photoelement,
KS – a metalic frame with the light slit.

miliar apparatus was described by Miettinen et al. (1953), Opplt et al. (1953), Kutaček et al. (1958). We destribed such an application in an anterior publication (Paulov — 1961). The method consists in illuminating the electrophoreogram and in measuring the fraction of light passing through the stript on a photoelement coupled with a galvanometer. A schema of such an arrangement representing a registering photodensitometer is on fig. 5. It may be said, that we used this way of measuring the fraction intensities only when we sought a demonstrative proof of some phenomenon.

The graphical registration of fractions after cutting the strips in 1—2 mm broad bands and their consecutive elutions was not found suitable for our experiments, as it was connected with errors not to be neglected. This method is no more recomended even in the litterature.

For determination of fraction concentrations we used calibration diagrams. About the necessity to use these diagrams we reported in an other publication (Paulov—1962 b). Following descriptions frequently found in some manuals (Dittmer—1956, Slavík—1960), the extinction values are simply additioned — $\Sigma\ (E_1 \ldots . E_n)$ and their sum is taken for 100 p. c. The value of each fraction (x) is then determinated from its extinction E_x through the relation:

$$x = \frac{E_x \,.\, 100}{\Sigma\ (E_1 \ldots . E_n)}$$

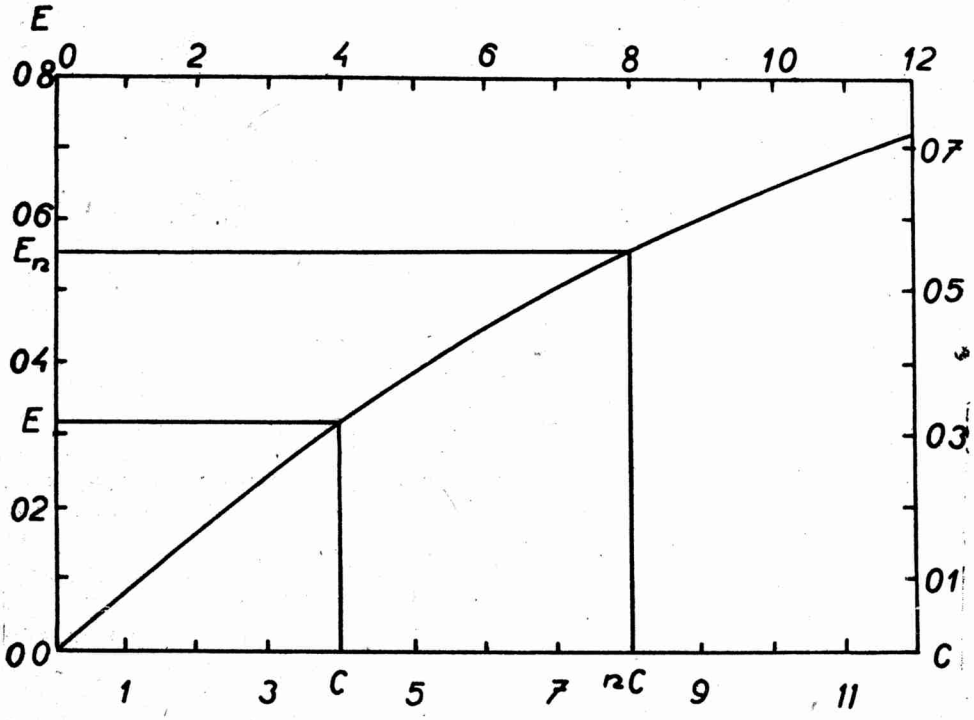


Fig. 6. Graphical relation between protein concentration (C) and extinction (E) — calibrating chart.

On the horizontal axis C are the protein quantities in a constant unity volume (or to these quantities corresponding bromphenol blue concentration). The other coordinate gives the extinction (E).

Such a calculation could be naturally justified only when there were a linear relation between extinction and concentration in a sufficiently great intervale of extinctions. But a linear relation could be found only for very narrow regions of extinction. In general, when a concentration rises n-times there is no corespondent rise of n-times in extinction. We may write this so that (fig. 6)

concentration $C \rightarrow$ extinction E

and $nC \rightarrow E_n$
but we cannot write that $nC \rightarrow nE$.
We have usually $E_n < nE$.

Therefore it is necessary for determination of fraction cocentrations and their relations together to use calibration diagrams corresponding to the quantities of proteins (to the coloration by bromphenol blue) and from these we may then calculate the concentration of a fraction from relation:

$$x = \frac{C_x \,.\, 100}{\Sigma \ (C_1 \ldots\ldots C_n)}$$

where C_x is the protein (colour) concentration determined from a calibration diagram giving the relation between concentrations and extinctions. $\Sigma \ (C_1 \ldots\ldots C_n)$ is the sum of all concentrations.

In practics we frequently eluate albumins in greater volums as globulins in order that we obtain extinctions on approximately the some section of calibration diagram. In this case we do not multiply the extinctions by the dilution degree but the amount of proteins (of stain) derived from the calibration diagram.

We did not use correction factors for determination of extinctions of isolated fractions. In literature, they are only seldon used, they are of different heights for every analysis method and give nonuniform results for different cases. The introduction of these factors is in general problematic. Our results have to be evaluated with regard to the method used. In order to obtain reproducibility of our experiments we strictly maintained equal conditions and methods for separation, staining and for quantitative evaluation.

2. *Polarography of serum proteins separated by electrophoresis*

There is at present an intense activity in the polarographic analysis of proteins. The proof of it are some of the most recent studies: Březina et al. (1952), Ivanov (1961), Heyrovský et al. (1962).

The expansion of paper electrophoresis has facilitated polarographic analysis with individual fractions. This method of analysis was studied for the first time by Homolka (1953) with application of Brdička reaction. This author determinates a characteristical protein double-wave in solutions in which he immerged paper sections with pure fractions. The fractions on these paper bands are indirectly identified with help of a parallel cut strip, which is stained as usually. This method has found very soon its applications (which minor variations) for human and veterinary medicine as well as for basic research (Bartík et al. — 1954, 1955 a, 1955 b, Ostrowski et al. — 1955, Homolka et al. — 1956, Bille Helaers — 1958, Kalous — 1960, Paulov et. al. — 1963).

In our laboratory facilities we utilized the method of Homolka (1953). We have modified the processus in that we do not make it with dry and pure fractions but with fractions coloured with bromphenol blue. This our modified method can be described as follows: Stained fractions are cut in sections places of minimum

coloration and are immersed in solution of 0.05 N KOH in 0.9 p. c. NaCl. After 45 minutes we add to the solutions equal quantity of the Brdička solution of Co $(NH_3)_6Cl_3$ (H o m o l k a – 1956). Afterwards we immediately measure the protein double-wave on a polarograph and the protein quantity (staining density) on a photocolorimeter. In this way we obtain higher values of the protein double-wave compared with pure non stained fractions (fig. 9). We reported more explicitly on this matter in another publication (P a u l o v et al. – 1963).

For measuring the protein double-wave we utilized the Heyrovský polarograf type V–301 B. We used for anode a platinum wire 1 mm in diameter wound in 12 spiralls on a capillar glass tube. We used cathodic polarization, 4 V accumulator, sensibility 1/50, duration of one Hg–drop was 4.0 sec., speed of mercury current was 2.61 mg/sec. The circuit diagram of the polarograph with mercury drop electrode is on fig. 7. Fig. 8 demonstrates the method of detemination of the protein double-wave *h*.

For evaluations of the polarographic activity of isolated serum components we use always calibration diagrams corresponding to human serum-albumins (fig. 10). The quantities of proteins increases linearly in the coordinate system, but the heights of the protein doble-wave do not increase linearly. Quantities of proteins on the *X* coordinate indicate linearly increasing quantities of polarographicaly active groups. Through extrapolation of protein double-waves we operate no more with protein quantities but with quantities of polarographically active groups. When f. i. we compare individual components among themselves we cannot compare measured protein double-wawes (as this is reproduced in the majority of publications) but we compare already determined quantities of polarographically active groups. (Here the same interpretation takes place as we used concerning the colorimetric method of determination of serum protains.) As a proof we refer to the table Nr 1, where we compare the method of determination actually in use with the proposed method.

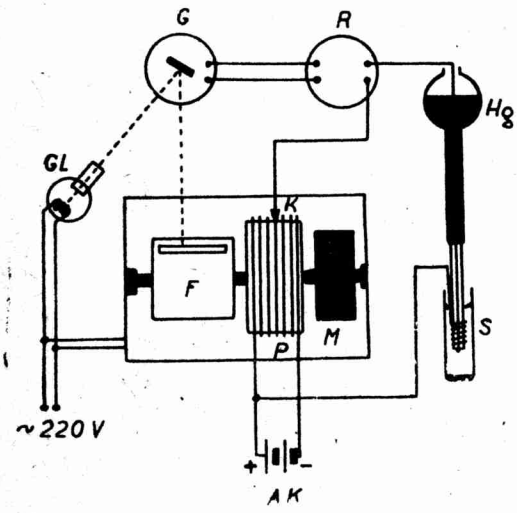


Fig. 7. A diagram of the connections of Heyrovský's polarograph with a mercury drop electrode.

G – mirror galvanometer,
R – sensibility reductor,
Gl – light source of the galvanometer,
F – registering photographic camera,
P – a potentiometer wire,
Ak – 4 volt accumulator,
M – motor driving the potenciometrical disc with the photographical camera,
K – sliding contact,
Hg – mercury vessel,
S – polarographic vessel with capillary tube and platinum anode.

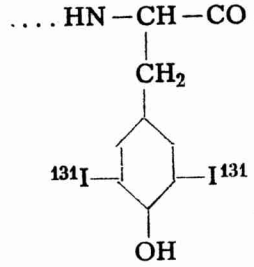
3. *Labelling of blood-serum proteins with radioactive isotopes*

Actually two principaly methods are used for preparation of labelled proteins: a) suitably arranged chemical reaction in vitro (mostly with ^{131}I), b) biosynthesis in a living organisme under utilisation of labelled aminoacids (mostly with ^{14}C, ^{35}S etc.). Both methods have their special advantages and disadvantages. We can say that for study of catabolic phenomenons the methods in vitro are preponderant for

anabolic phenomenons study the biosynthetic methods prevail.

a) *Labelling of serum proteins in vitro with radioactive iodine* ^{131}I

For chemical method of labelling we used the radioactive iodine. This labelling method is based on processus of elementary iodine liberation from NaI^{131} and on its recombination in a suitable buffer solution with amino-acid tyrosine in form. There are many methods of preparation for iodinized proteins described in litterature as



well a lot of modifications, of methods, differing through the choise of buffer solutions, through the experimental technics of iodination, and through the protein used (Pressman et al. — 1950, Shulman et al. — 1950, Francis et al. — 1951, Gilmore et al. — 1954, Cohen et al. — 1956,

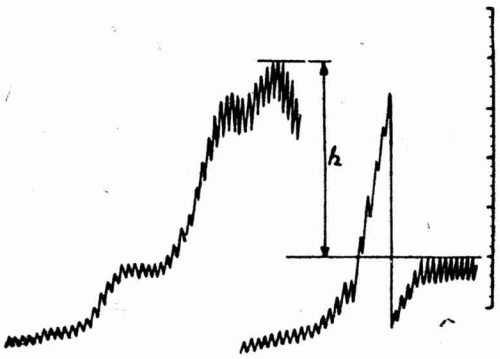


Fig. 8. Determination of the height protein double-wave (*h*).

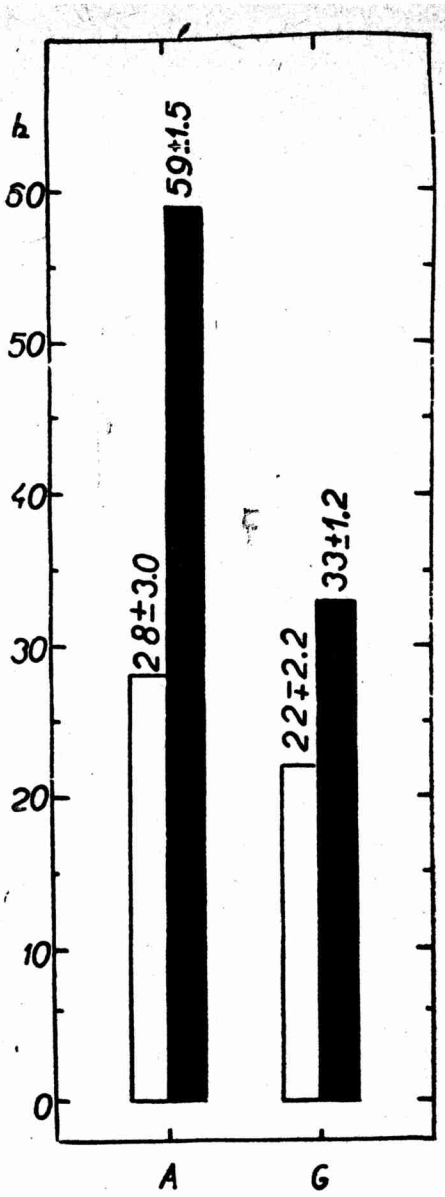


Fig. 9. Relation between the height of the protein double-wave in serum albumins and proteins and the method of fraction preparation after their separation on paper.

□ — clear non stained fractions,
■ — fractions stained with bromphenol blue,
h — measured height of double-wave in mm,
A — albumins, *G* — globulins.

Mc Farlane — 1956, Hughes — 1957, Liebster et al. — 1957, Lubran et al. — 1957, Mc Farlane — 1958 and many others).

In literature we find frequently citations of publications of Mc Farlane (1956) whose labelling method is recommended. The use of this method is advantageous, because it appears as the most reliable for studying of protein catabolism. We persuaded ourselves in this sense experimentally (Paulov et al. — 1959, Paulov — 1961).

The Mc Farlane method is relatively complicated especially as to the technical side of the processus of labelling. We started from the chemical reaction proposed by this author and adapted the technical processus to our laboratory possibilites. The modified processus is now: We fill an injection syringe with a mixture of blood-serum with glycine buffer pH 9.3. In a second syringe we fill the to called iodination mixture containing from iodide liberated radioactive iodine. Both syringes are fixed on needles which have two opposed little targets of rostless

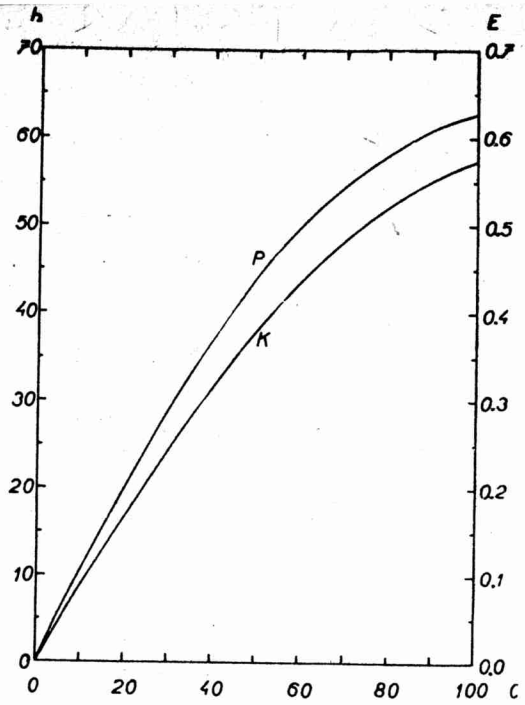


Fig. 10. Calibration charts.

C — protein concentration (or the corresponding concentration of bromphenol blue or of polarographically active groups),
h — height of the protein double-wave in mm,
E — extinction,
P — calibration chart determined polarographically,
K — calibration chart determined colorimetrically.

Table 1. Methods of determining quantities of polarographically active groups of serum albumins ane globulins.

Fractions	Fractions in per cent (colorimetric determination)	Applied volume of 0.05 N KOH in ml	Polarographicaly determined height h in mm	Usullay applied method for determining the relation A:G		New method for determining the relation A:G		
				Height h multiplied by dilution	Relation: A:G in per cent	Height h transposed in protein quantity after calibr. curve	Precedent values multiplied by dilution	Relation A:G in per cent
Albumins	69.8	15	59	177	84.3	**83**	249	87.4
Globulins	30.2	5	33	33	15.7	36	36	12.6

steel on their ends. The pistons of both syringes are slowly pushed with equal speed and so on a relatively large surface a contact is established where the two solutions mix together. The flow of protein solution falls in a vessel. Non reacted rests of the original solutions are separated through dialysis.

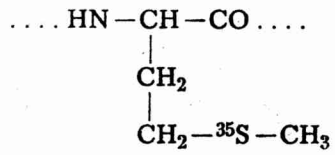
Fig. 11. Apparatus used for labelling of the blood serum proteins with radioactive iodine.

After dialysis the protein solution was intravenously injected in rabbits in quantities of 10 ml with a contents of 400 mg of proteins containing 250—300 μC ^{131}I. The specific activity was approximately 0.7 μC/mg of proteins (Bloom et al. — 1958 report that the quantity of ^{131}I has to be not greater than 100 μC/mg).

b) *Incorporation of methionine ^{35}S to serum proteins*

For the biosynthetic method of labelling we used the faculty of methionine ^{35}S to incorporate in blood serum proteins. This labelled aminoacid (with a specific

activity of 1 mC ^{35}S/1.85 mg aminoacid) was intravenously applied in rabbits in total quantities 0.27 mC/1000 g of living weight. Aminoacide was incorporated in protein molecules in the forme:



c) *Measurement of protein radioactivity*

When determining the height of radioactivity in aplied proteins it is not usual to indicate obsolute intenzities of radioactivity but to indicate only the isotope quantity through impulse number related to unity quantity of proteins determined under equal conditions.

It is true that the caracterization of radioactivity by imp/mg/min is a relative indication and depends on many conditions related to the measurements (distance of the preparation to G. M. tube, absorption through the preparation, type of the counting tube and electronic apparatus, atc.) but practically we may consider this method as sufficiently exact for execution of metabolic experiments.

For determination of radioactivity of proteins separated electrophoreticaly on paper we used the so called quadratic section method in both cases of measurements of ^{131}I as well as of ^{35}S. In this procedure we cut separated quadrangular sections smaller than the mica window of a G. M. tube from stained electrophoretograms and placed them under the window of a G. M. tube.

The continuous distribution of activity in fractions was measured through autoradiography and subsequent photodensimetric measurement of the roentgenological films used for this purpose. We used here the modified Heyrovský polarograph in the way described above when we described the evaluation method of electrophoretograms. This method was applied principally for demonstrative work. (In this connection we refer to the publication of O e f f—1954 a, who found a relatively good correspondence between the height of activity after photometrical determination from autoradiograms and the activity measured with G. M. counter.)

Results

1. *Physiological and physico-chimical caracterisation of serum proteins of rabbits in normal conditions*

a) *Quantity spectrum of blood serum proteins*

The knowledge of quantities of protein fractions in blood serum is of prime importance for the comprehension of physiology of these substancies. There was a lot of differing spectra described for many animals. Differencies are yet even

among spectra for one species of animals given by different authors. This is the case with the blood serum of rabbits. We wished to verify experimentally the validity of published results, to compare our measurements with the literature known spectra, and to determine its individual variability for rabbits.

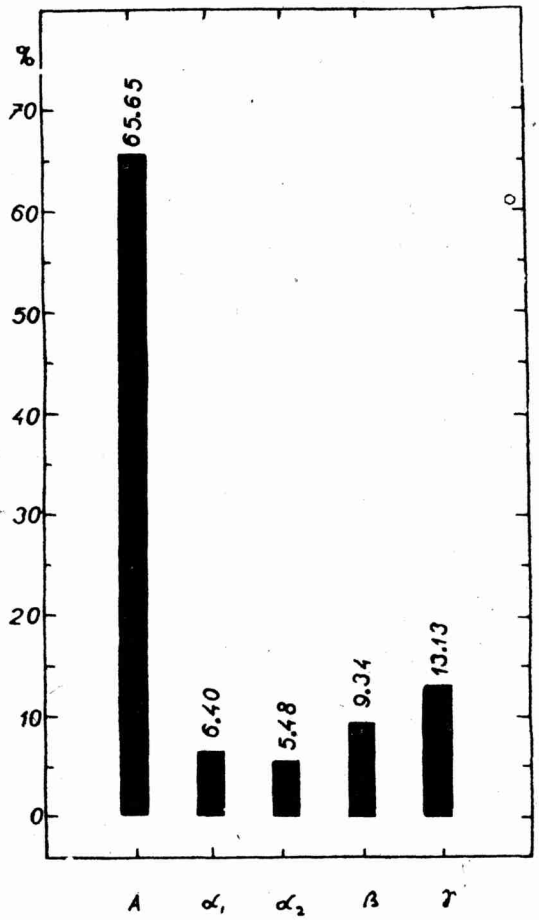


Fig. 12. Quantities of protein fractions in blood serum of sane rabbits (a mean value from 38 animals in p. c.).

In studying the protein spectra of animals we must start from exactly stipulated experimental conditions and we must use animals with suitable caracteristics. We may remember here that measuring results depend not only of the method aplied but equally from breed, age, sex, nutrition and keeping conditions, heigth over sea-level, method of taking blood samples, etc. (Mahnert — 1920, Muralt et al. — 1948, Hořejší — 1956, Myant et al. — 1959, Seniów — 1957, Lózsa — 1962). In our experiments we used rabbits of the Vienna blue-eyed breed, having approximentaly equal age and weight. We experimented with male rabbits, the control experiments on female rabbits gave similiar results in protein fraction quantities. (The total protein concentration in bloodserum was approx. of 6.2 p. c. Lustig et al. — 1937 indicate quantities of 4.362 — 6.560, Košmider et al. — 1960 give 5.20 — 8.00 p. c.)

Data on quantities of protein fractions with 38 rabbits are in table 2. A relatively great individual variability is evident from this table. This constatation forced us further to evaluate a given phenomenon for one animal and only afterwards to proceed to study the phenomenon with other animals.

Numerical data taken from table 2 are evaluated in table 3 and on fig. 12. In table 3 and in following tables we calculate the aritmetic mean value $\overline{X}$ as in formula

$$\overline{X} = \frac{\Sigma x}{n}$$

Table 2. Quantities of protein fractions in blood serum (in p. c.) of 38 rabbits.

Nr	Albumin	Globulin				Nr	Albumin	Globulin			
		α_1	α_2	β	γ			α_1	α_2	β	γ
1	66.5	5.6	5.2	7.6	15.1	20	68.0	6.7	5.4	10.9	9.0
2	69.0	5.8	5.3	6.8	13.1	21	63.5	6.3	5.9	7.6	16.7
3	63.1	6.0	3.6	7.3	20.0	22	67.0	7.1	5.2	10.1	10.6
4	67.7	5.7	5.3	7.2	14.1	23	63.0	7.0	6.0	11.0	13.0
5	70.0	6.4	3.6	7.9	12.1	24	64.8	8.2	5.0	10.0	12.0
6	68.8	6.3	6.9	9.5	8.5	25	60.8	8.0	7.0	10.0	14.2
7	68.9	7.0	6.6	9.2	8.3	26	67.0	7.0	4.0	11.6	10.4
8	71.6	6.2	5.9	5.8	10.5	27	63.5	6.2	5.4	9.5	15.4
9	62.8	7.3	6.1	9.3	14.5	28	71.0	4.4	4.6	7.5	12.5
10	67.9	6.4	5.8	7.2	12.7	29	63.0	7.0	5.0	11.5	13.5
11	58.8	8.6	6.3	9.5	16.8	30	70.6	5.2	4.6	7.2	12.4
12	69.0	6.0	5.0	9.9	10.1	31	63.1	7.1	6.0	11.0	12.8
13	70.0	5.5	4.5	9.5	10.5	32	68.9	4.3	5.3	8.7	12.8
14	61.1	8.0	6.5	13.4	11.0	33	63.6	6.3	6.0	10.0	14.1
15	66.0	4.0	3.4	9.8	16.8	34	67.1	6.7	6.0	9.6	10.6
16	65.0	5.9	5.0	8.8	15.3	35	70.0	4.8	4.3	8.2	12.7
17	59.0	7.0	5.4	12.2	16.4	36	70.6	6.4	5.2	8.0	9.8
18	67.6	5.5	5.8	9.4	11.7	37	59.6	6.0	5.4	8.3	20.7
19	53.2	8.2	10.5	14.7	13.4	38	63.6	6.9	5.3	9.4	14.8

and dispersion following to equation

$$\sigma \pm = \sqrt{\frac{\Sigma (x - x)^2}{n - 1}}$$

Our experimental results are in part similar to the known literature data, but

Table 3. Mean values of quantities of protein fractions in blood serum of 38 rabbits with indicated standart deviation (σ) and extremal values (X_{min} and X_{max}).

	Albumin	Globulin			
		α_1	α_2	β	γ
$\bar{x}$	65.65	6.40	5.48	9.34	13.13
$\sigma \pm$	4.11	1.07	1.15	1.85	2.86
x_{min}	53.2	4.0	3.4	5.8	8.3
x_{max}	71.6	8.6	10.5	13.4	20.7

Table 4. A review of published data about quantities of blood serum proteins of rabbits determined through paper electrophoresis (in per cent).

Author	Number of animals	Albumin	Globulin				
			α_1	α_2	β_1	β_2	γ
Scheifarth, Berg 1952	16	62.20 72.60	5.78 10.13	2.22 6.02	4.34 10.55	1.89 3.33	6.11 12.70
Andreoni, Dompé, Russo 1955	8	61.1	4.2	7.0	12.5		14.5
Corsini, Grazia 1957	8	53.31 ± 4.06	12.67 ± 1.89		12.92 ± 1.54		21.05 ± 4.44
Runák 1961	22	59.08 ± 0.25	6.12 ± 0.08	5.70 ± 0.14	9.10 ± 0.17		20.42 ± 0.22
Paulov	38	65.65 ± 4.11	6.40 ± 1.07	5.48 ± 1.15	9.34 ± 1.85		13.13 ± 2.86

there are some differencies (table 4). It would be no wise to look for a complete concordance, as no case there were identical experimental conditions. Our results are in a great extend to be regarded from the standpoint of our experimental conditions.

Conclusion: The quantity spectrum of blood-serum proteins in rabbits is typical for species but a relatively great individual variability is observed.

b) *The quantity spectrum of polarographically active groups of serum proteins*

Up to now, the research works in the field of polarographical activity of proteins using the Brdička reaction are exclusively executed on human proteins. For animal proteins we can consult only some studies of veterinary character (Bartik et al. — 1954, Bartik et al. — 1955 a, b), occasionally we find some isolated reports concerning usual laboratory animals and connected to a research concerning different life functions. There are no analyses from this viewpoint for rabbits and so we applied our modified method in our experimental conditions on such research. (When analysing quantities of polarographically active groups we proceed as described in the methodic part of this publication.)

Data about measured quantities of polarographically active groups in serum proteins of individual experimental animals as well as the summary evaluation are in table 5. We can see, that there is a relatively great individual variability so in the quantity spectra of proteins as well as in the quantities of polarographically active groups.

When we represent graphically side by side mean quantities of polarographically active groups and mean quantities of proteins (fig. 13) we observe a discordance

Table 5. Quantities of polarographically active groups of serum proteins of rabbits in per cent.

Nr	Quantity of proteins in per cent (colometric determination)					Quantity of polarographically active groups in per cent (polarographical determination)				
	Albumin	Globulin				Albumin	Globulin			
		α_1	α_2	β	γ		α_1	α_2	β	γ
1	66.5	5.6	5.2	7.6	15.1	82.4	3.8	3.2	4.0	6.6
2	69.0	5.8	5.3	6.8	13.1	79.7	4.7	3.5	5.1	7.0
3	63.1	6.0	3.6	7.3	20.0	79.6	3.3	2.6	4.9	9.6
4	67.7	5.7	5.3	7.2	14.1	81.0	4.3	3.3	4.6	6.8
5	70.0	6.4	3.6	7.9	12.1	83.9	3.5	3.1	4.5	5.0
6	68.8	6.3	6.9	9.5	8.5	91.3	1.8	1.6	2.5	2.8
7	68.9	7.0	6.6	9.2	8.3	90.5	2.1	1.9	2.8	2.7
8	71.6	6.2	5.9	5.8	10.5	91.0	2.1	1.5	2.6	2.8
9	62.8	7.3	6.1	9.3	14.5	84.1	3.5	3.0	4.2	5.2
10	67.9	6.4	5.8	7.2	12.7	89.6	2.4	2.0	2.6	3.4
$\bar{x}$	67.63	6.27	5.43	7.78	12.89	85.31	3.15	2.57	3.78	5.19
$\delta \pm$	2.83	0.54	1.12	1.20	3.43	79.6	1.02	0.60	0.98	2.31
x_{min}	62.8	5.6	3.6	5.8	8.3	4.77	1.8	1.5	2.6	2.7
x_{max}	71.6	7.3	6.9	9.5	20.0	91.3	4.7	3.5	5.1	9.6

of these two spectra and can see that they are typical for the animal species. It is visible that albumins bind more polarographically active groups than globulins. This discrepancy between albumins and globulins is seen more discintly from a more generalised diagram in fig. 14.

For individual globulin fractions we have a relatively linear relation between globulin quantities and quantities of polarographically active groups. This relation is more evident when we set the sum of each of these parametres equal to 100 and determine each globulin fraction relatively to this sum (fig. 15).

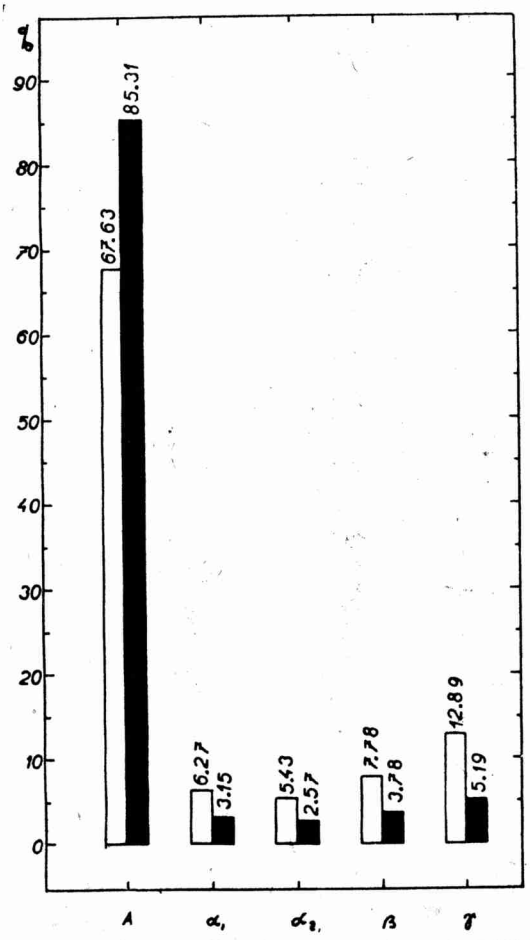


Fig. 13. Quantity spectrum of proteins ☐ and quantity spectrum of polarographically active groups in fractions ■ (in p. c.).

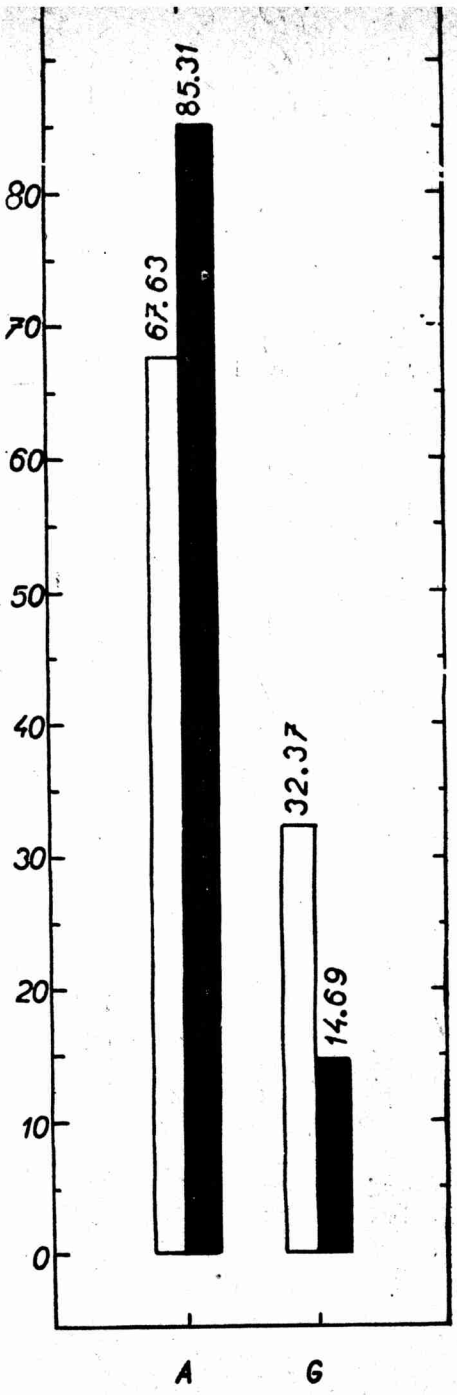


Fig. 14. Quantities of polarographically active groups ■ in serum albumin and globulins and quantities of proteins ☐ (in p. c.).

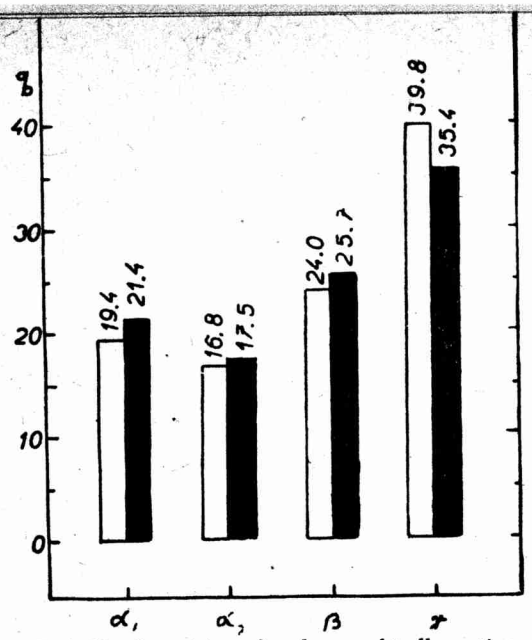


Fig. 15. Quantities of polarographically active groups ■ and quantities of individual serum globulins □ in p c.

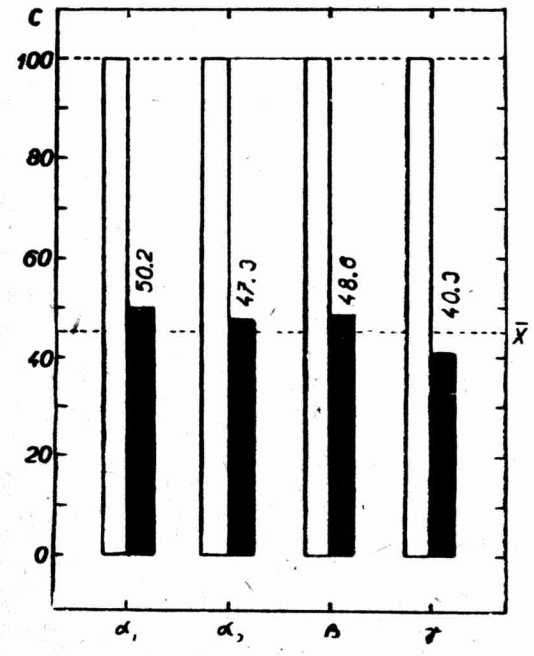


Fig. 17. Quantities of polarographically active groups ■ relative to unity quantities of globulins □

$\bar{X}$ – arithmetic mean value of quantities of polarographically active groups in total globulins.

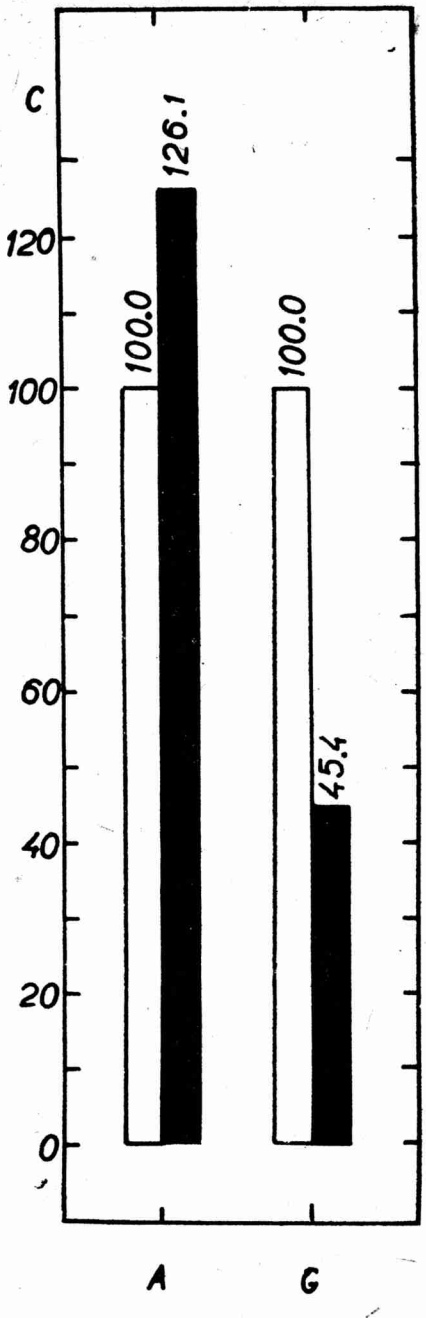


Fig. 16. Quantities of polarographically active group ■ relative to unity quantities of albumins and globulins □

Dependency between the quantity of proteins and the quantity of polarographically active groups gets more evident when we calculate the quantities of polarographically active groups on unitary quantities of proteins (fig. 16). Such a transformations gives that for albumin there are three times more polarographic active groups (exactly 2.78 times) against globulins. Similiarly we can utilise such a relation for globulin fractions alone (fig. 17). We see there that the quantities of polarographically active groups for different fractions are equal.

Conclusion: The quantity spectrum of polarographically active groups of serum proteins is typical for the species and does not corespond to protein quantities.

In albumins there are more polarographically active groups as in globulins, and globulin fractions have approximately equal quantity of polarographically active groups.

c) *Spectrum of quantities of radioiodine ^{131}I incorporated in vitro to serum proteins*

In usual tracing processus with radioactive isotope is effected on formerly from a mixture isolated protein and afterwards follow their catabolism in a living organism. In our experiments we labelled a mixture of proteins (the whole serum) and after labelling we separated the mixture through paper-electrophoresis in individual fractions. In these fractions we determined the quantities of bound radioiodine. As all fractions of the mixture are simultaneously in equal reaction-conditions, the quantity of bound radioiodine can substantially help us in better comprehension of the specifity of individual fractions.

Numerical data on bound iodine in ten samples of serum as well as summary evaluation are in table 6. Mean values of fraction quantities and quantities of bound

Table 6. Quantities of bound radioactive iodine ^{131}I in blood serum proteins of rabbits in per cent.

Nr	Quantity of proteins in per cent (colorimetric determination)					Quantity of radioactive iodine in per cent (measurement with GM-counter)				
	Albumin	Globulin				Albumin	Globulin			
		α_1	α_2	β	γ		α_1	α_2	β	γ
1	70.0	5.1	4.9	9.5	10.5	84.0	1.3	4.0	3.7	7.0
2	65.0	5.9	5.6	8.5	15.0	82.4	1.5	3.9	3.8	8.4
3	66.0	4.0	3.4	9.8	16.8	84.3	2.0	4.3	3.5	5.9
4	70.0	4.8	4.3	8.2	12.7	82.1	2.1	4.9	4.0	6.9
5	68.0	6.7	5.4	10.9	9.0	81.0	2.6	5.3	5.1	6.0
6	67.0	7.1	5.2	10.1	10.6	83.7	1.6	5.2	3.8	5.7
7	64.8	8.2	5.0	10.0	12.0	75.0	3.0	6.5	6.2	9.3
8	71.0	4.4	4.6	7.5	12.5	82.9	2.9	4.0	3.5	6.7
9	70.6	5.2	4.6	7.2	12.4	79.9	1.9	4.4	4.0	9.8
10	69.6	6.4	4.0	9.5	10.5	84.3	1.2	4.7	2.8	7.0
$\bar{x}$	68.20	5.78	4.70	9.12	12.20	81.96	2.01	4.72	4.04	7.27
$\sigma \pm$	2.35	1.32	0.67	1.21	2.30	2.84	0.64	0.79	0.95	1.42
x_{min}	64.8	4.0	3.4	7.2	9.0	75.0	1.2	3.9	2.8	5.7
X_{max}	71.0	8.2	5.6	10.9	16.8	84.3	3.0	6.5	6.2	9.8

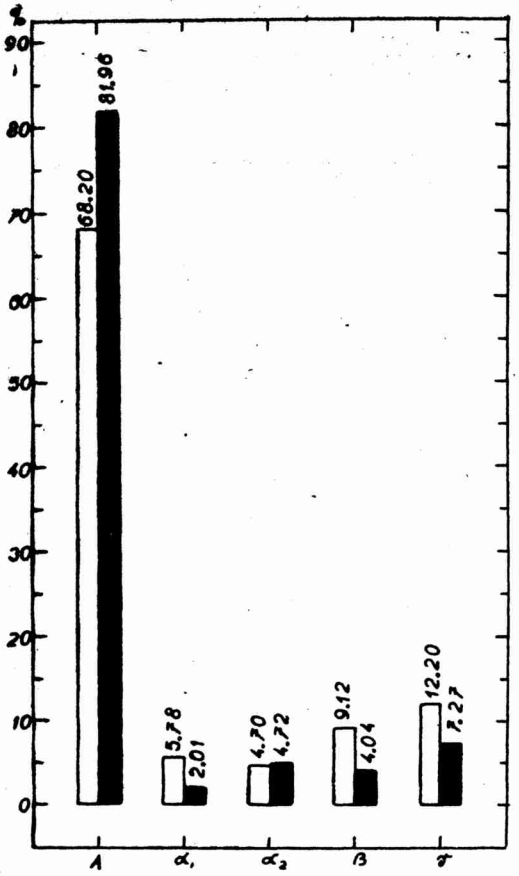


Fig. 18. Quantity spectrum of protein fraction and quantity spectrum of bound radioactive iodine (in p. c)

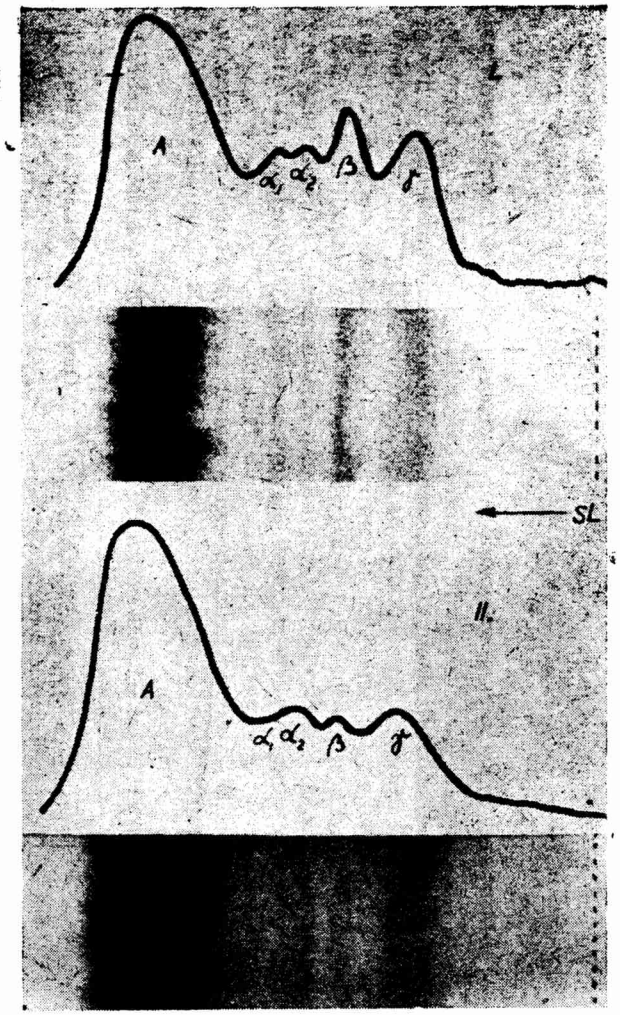


Fig. 19. A documentary photography about ^{131}I bound in blood serum proteins.

I. — electrophoreogram and the corresponding diagram,

II. — autoradiogram from the upper electrophoreogram with a graphical evaluation. SL-start line and migration direction in electrical field.

radioiodine over all fractions are graphically demonstrated on fig. 18, documentary photo is on fig. 19. The relation between albumins and globulins is shown graphically on fig. 20.

These data and diagrams indicate that albumins accept much smaller quantities of iodine compared to globulins and that individual globulin fractions show inequal tendency of "incorporating" iodine. This is best seen from fig. 21, where the percentual relations among globuline fractions are indicated.

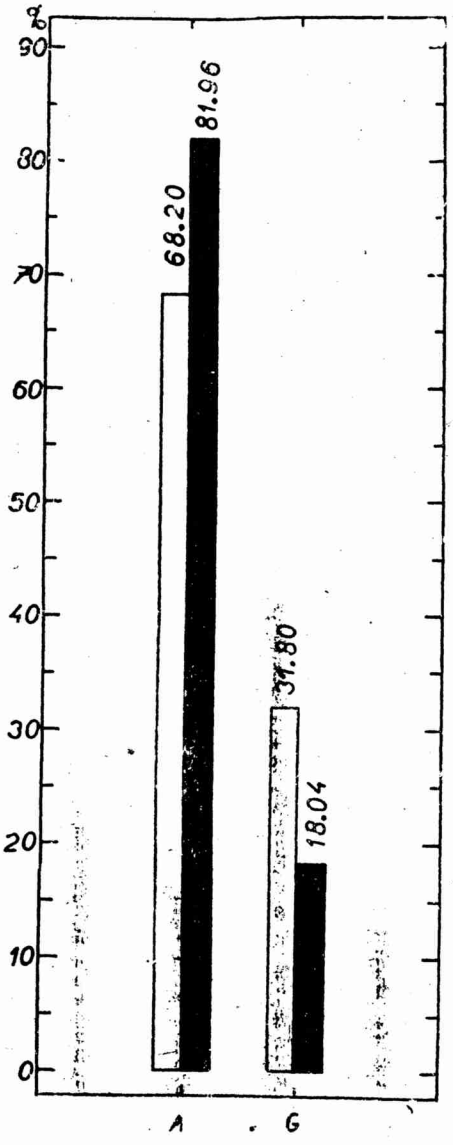


Fig. 20. Quantities of bound radioiodine in serum albumins and globulins expressed in a p. c. relation.

The relation between quantities of bound iodine and quantities of albumins and globulins will be more clear when we refer the radioiodine quantities of proteins (fig. 22). Thus we can verify that f. i. albumins bound two times more radioiodine as the total of globulins (exactly 2.12 times). A calculation for equal quantities of individual globulin fractions is on fig. 23. This diagram indicates that most iodine is bound by globuline alpha 2, then comes gama, beta and the smallest quantity is by alpha 1 globulin.

Conclusion: The quantity spectrum of bound radioactive iodine in serum proteins is typical and is in no correspondence with the protein quantities.

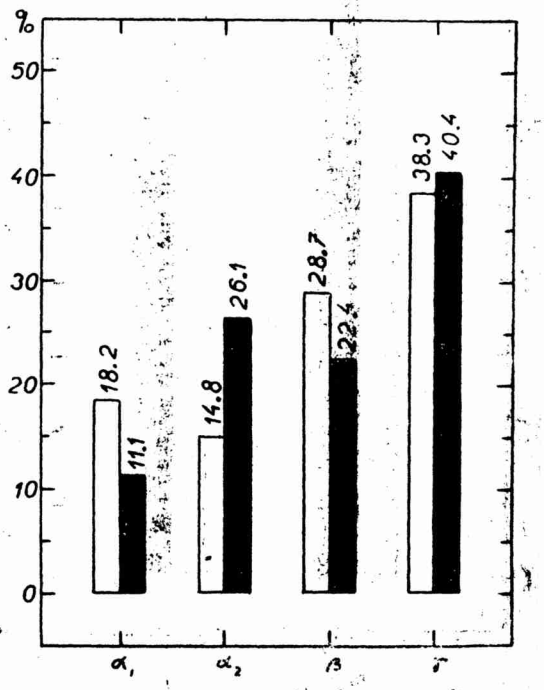


Fig. 21. Quantities of radioactive iodine bound on individual globulin fractions in p. c.

Serum albumins get more radioactive iodine then globulins. Individual globulins have different abilities to bind radioiodine.

d) *Catabolism of blood serum proteins determined witd* ^{131}I *labelled proteins*

All compounds in living organisms being in constant and regular renowation, the blood serum proteins are subject to incessant variations in composition. These variations are denoted in a summary way as metabolic processes with two antagonistic and mutually corresponding anabolic and catabolic processus. It were just the tracing methods with radioactive isotopes, which brought new insights in the metabolic changes of blod serum proteins.

In the methodic part of this study we mentioned that the rapidity of catabolism of blood serum proteins depends on the labelling processus. We have naturally to respect the inequal catabolic speed of protein fractions of the blood serum of an animal species and the fact there is an unequal catabolism of the same protein fraction with different animal species. Generally is indicated that biosynthetically labelled proteins in vivo have a lesser spreed of catabolism as proteins

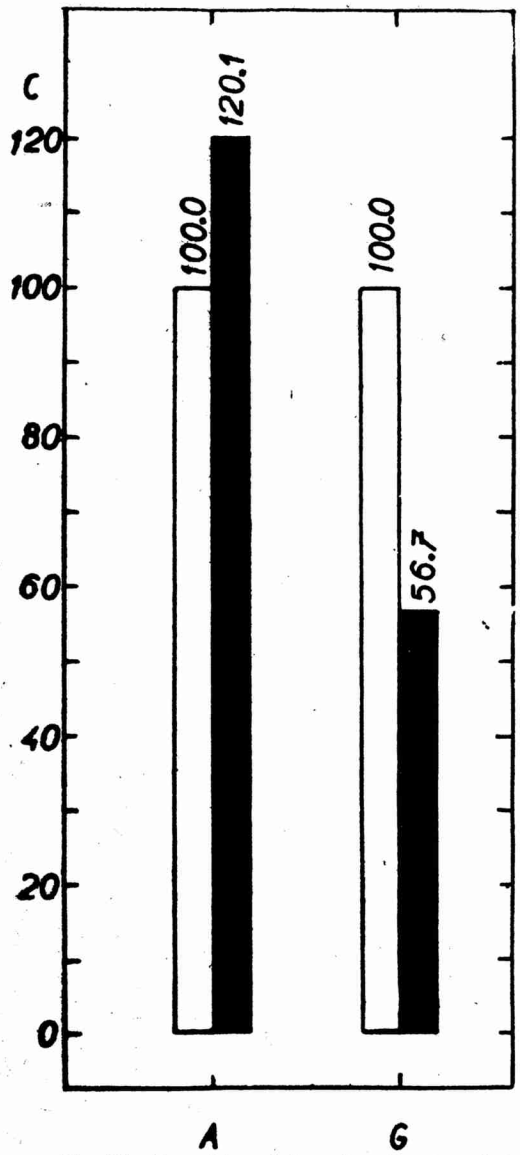


Fig. 22. Quantities of bound radioiodine ■ related to unity quantities of albumins and globulins □

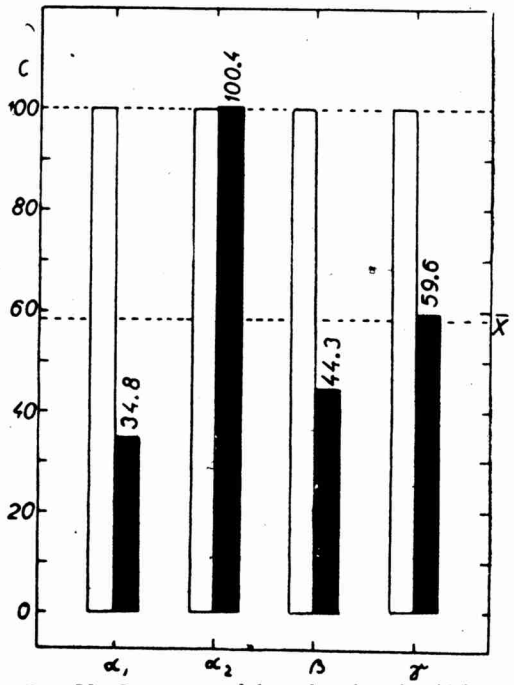


Fig. 23. Quantities of bound radioiodine ■ related to unity quantities of globuline fractions □

labelled chemically in vitro (Goldsworthy et al — 1957). This may be not always true (Walter et al. — 1957). The most used method after Mc Farlane (1956) with proteins labelled with ^{131}I in vitro gives similiar results as biosynthetically labelled proteins.

Up to now published data on blood serum protein half-life indicating the catabolism are resumed in table 7. From this table we can see, that half-life values differ with authors. In the same vay have our results to be judged from the standpoint respecting the labelling method used.

In literature we find many proofs concerning variations of catabolism speed of the same protein fraction in different animal species. For dogs the experimental data gave Wasserman et al. (1951), Dixon et al. (1952), Goldsworthy et al. (1958), for rats Niklas et al. (1952), Campbel et al. (1956), Wiggans et al. (1957), Jeffay et al. (1958), Walter et al. (1958), for sheep Campbell et al. (1961). Especially the work of Dixon et al. (1952) is remercable. It contains data for many animal species. An extraordinary great attantion was given especially to the study of the catabolism of human blood serum proteins: Sterling (1951), Berson et al. (1953), Margen et al. 1956, 1957, Gitlin (1957), Bennhold et al. (1959), Takeda et al. (1959), Cohen et al. (1961). Problems of catabolism of foreign proteins were studied by Latta (1951), Terres et al. (1960).

In our experimental conditions we used for an investigation about the speed of catabolism of blood serum proteins the labelling with ^{131}I as described previously.

The animals used for the investigation received 3—4 days before beginning of the experiments as well as during the experiments drinking water with NaJ (100 mg/1 l water) in order to enhance the capturation of iodine from the labelled proteins through the thyroid gland (Mc Farlane — 1956) and in order that the thyroid gland may not secondary influence the distribution of active iodine over serum proteins (Hradec et al. — 1954, Lamarque et al. — 1958). Such drinking water was also sprayed over victuals.

Labelled blood-serum proteins were applied to rabbits intravenously in the border ear vein. Five minutes afterwards a blood sample was taken from the border vein on the opposite ear. Further samples were taken in 1—3 days intervals during the whole time of approximately 2 weeks.

From blood samples we prepared serum and this was separated through paper electrophoresis into albumins and globulins. After staining we measured the specific radioactivity and after this we determined colorimetrically the fraction concentration.

Values of measured ^{131}I activities in protein fractions corrected as to the physical half-life of the radioisotope were inscribed in a semilogarithmic coordinate diagram in the way that the beginning value of activity (e. i. 5 minutes after application) in albumins as well as in globulins was taken as 100 p. c. Activities on following days were then determined in p. c. A curve was interpolated through the measured points and from this curve we concluded on the character of catabolism in blood serum proteins.

The sinking of acivity in serum proteins shows a characteristic form, which is in good accord with literature data (fig. 24). In 2—3 days the activity sinks rapidly. This rapid sinking is designated as distribution phase. Further the sinking is slow and in semilogarithmic coordinates apperars to be linear. This phase is designated as metabolic (degradation) phase. Through extrapolation on this section

Table 7. Values of biological half-lives in days of serum proteins of rabbits after different authors.

Author	Fraction	Biological half-life in days	Remarque
Dixon et al. 1952	^{131}I—glob. ^{131}I—glob.	4.6± 0.8 5.7± 1.2	Fractionation with alkohol Fractionation with amonium sulphate
Stevens et al. 1953 b	^{35}S—alb. ^{35}S—glob.	7.1 5.0	methionine methionine
Oeff 1954 c	^{131}I—alb. $^{131}I\alpha_1$—glob. $^{131}I\alpha_2$—glob. $^{131}I\beta$—glob. $^{131}I\gamma$—glob.	4.7—4.9 2.9—3.4 2.3—2.7 3.1—3.3 3.2—3.5	
Mc Farlane 1956	^{14}C—alb. ^{14}C—glob. ^{131}I—alb. ^{131}I—glob.	7.9 6.1 7.0 4.0	^{14}C aminoacids ^{14}C aminoacids „Jet-iodination" of oxalate plasma in glycine buffer at pH 9.3
Cohen et al. 1956	^{131}I—alb. ^{131}I—glob.	8.4—7.5—7.0 6.2—6.0—4.0	Different ways of iodination
Smoličev 1957	^{35}S—alb. $^{35}S\alpha_1$—glob. $^{35}S\alpha_2$—glob. $^{35}S\beta$—glob. $^{35}S\gamma$—glob.	16.5 18.0 10.5 8.8 8.3	methionine
Schultze 1957	$^{35}S\beta$—globulin $^{35}S\gamma$—globulin	4.2 6.8	methionine
Schultze et al. 1957	$^{35}S\beta$—glob. $^{35}S\gamma$—glob.	4.1 6.8	methionine
Penn et al. 1957	^{14}C—alb. ^{14}C—alb. ^{14}C—alb. ^{14}C—alb. ^{14}C—alb. ^{14}C—alb.	10.5 10.8 11.4 11.7 12.4 15.7	^{14}C—glycine ^{14}C—alanine ^{14}C—glut. acid ^{14}C—tyrosine ^{14}C—arginine ^{14}C—lysine
Paulov	^{131}I—alb. ^{131}I—glob.	6.90±0.18 4.04±0.13	Iodination with targets in glycine buffer at ph 9.3

we determine the values of biological half-life. (A mathematical foundation for this expression is given by Matthews — 1951 and Schwieg — 1953).

Biological half-life as determined on ten rabbits are in table 8. On fig. 25 there is a summary diagram which gives character of catabolic processus of serum albumins and glebulins with indicated values of mean biological half-life. We see

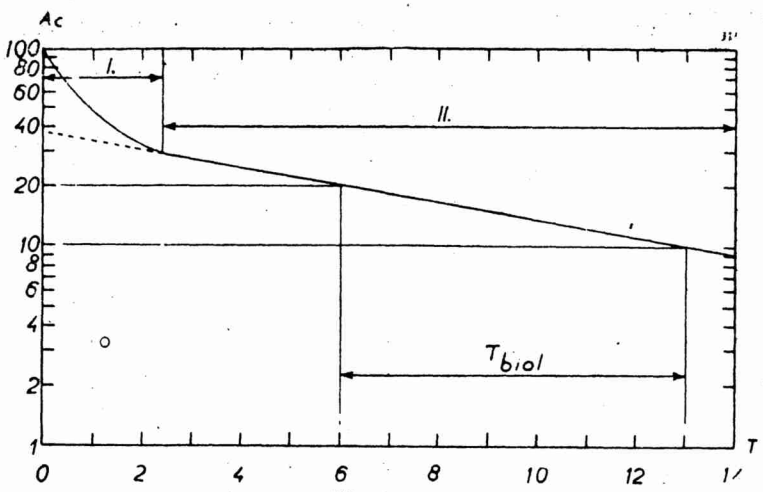


Fig. 24. Radioactivity ^{131}I decay in serum proteins.

Ac — height corresponding to the logarithmic value of the specifical activity in p. c.
T — time in days,
I. — distributive (mixing) phase,
II. — metabolic (degradation) phase,
T_{biol} — determination of the biological half-life through extrapolation.

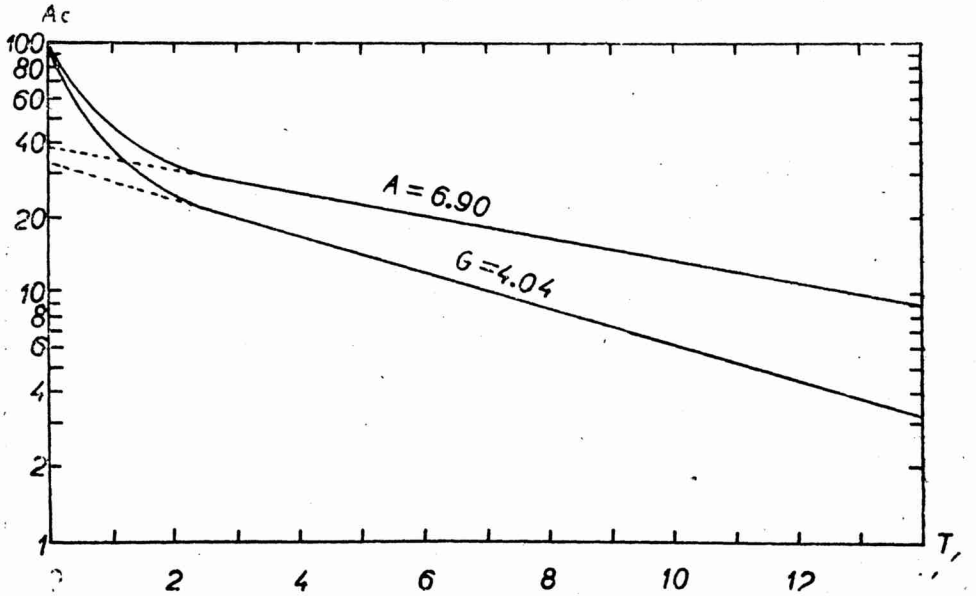


Fig. 25. Catabolism character of serum albumins and globulins measured through the ^{131}I labelled proteins.

Numbers given for albumins (*A*) and globulins (*G*) are the mean values of biological half-times found for 10 rabbits.

that globulins are more rapidly decomposed than albumins. Determined values T_{biol} are in some cases near the values found in literature, they are to be viewed from the standpoint of the method here used.

Conclusion: Serum globulins show a more rapid catabolism than serum albumins.

Table 8. Values of biological half-lives of serum albumins and globulins in sane rabbits in days.

Nr	Biological half-life in days		Quantity of fractions in per cent	
	Albumin	Globulin	Albumin	Globulin
1	7.0	4.2	70.0	30.0
2	7.0	4.0	65.0	35.0
3	7.0	4.2	66.0	34.0
4	6.9	4.1	70.0	30.0
5	6.5	3.8	68.0	32.0
6	7.0	4.1	67.0	33.0
7	7.0	4.0	64.8	35.2
8	6.7	4.0	71.0	29.0
9	6.8	4.1	70.6	29.4
10	7.1	3.9	69.6	30.4
$\bar{x}$	6.90	4.04	68.20	31.80
$\sigma \pm$	0.18	0.13	2.36	2.36
x_{min}	6.5	3.8	64.8	29.0
x_{max}	7.1	4.2	71.0	35.2

e) *Quantity spectrum of incorporated methionine ^{35}S in serum proteins*

A great number of authors occupied themselves with the problems of blood serum proteins in many animal species. Remerquable results were obtained, but experimental data are ofter discordants. In this chapter we try to verify published experimental results and complete them with data about the catabolism of serum proteins. We are principally interested in a determination of the quantity spectra of incorporated methionine, as especially this side of the problem was until now not studied sufficiently in detail in literature.

Methionine ^{35}S was applied to the border ear vein of a rabbit and blood samples were taken from the same vein on the opposite ear in intervals of 5 minutes, 1, 2, 4, 6, 8, 10 and 12 hours after application. From blood samples we prepared the serum which was separated in fractions through paper electrophoresis. The protein fractions were then stained with bromphenol blue and cut into sections in places of minimum coloration. The sections were measured as to their specific activity ^{35}S with a G. M. counter. After this measurement we immerged the sections in eluation solutions and determined the quantities of protein fractions.

The values of activities in individual serum proteins were noted in a diagram and a curve was afterwards interpolated through the measuring points. We

obtained so a picture of the incorporation of methionine in individual protein fractions as a function of time (fig. 26).

In our experiments are have found that a maximum of incorporation of methionine in all serum proteins is attained in 6—8 hours after its applications. In literature we find different data concerning the obtention of this maximum as well as of the summary heigth of this incorporation (Niklas et al. — 1952, Kurochtina — 1954, Green et al. — 1955, Okulov — 1956, Smoličev et al. — 1957, Rodionov et al. — 1959). Our data are partially in concordance with indications of these authors.

When determining quantity spectra of incorporated aminoacid we used the blood sample taken 12 hours after application. The values of determined specific activities (in p. c.) for 5 experimental animals are noted in table 9. As a prof about the distribution of the incorporated aminocaid we include here the fig. 27, where an autoradiogram is completed with a diagram of the fraction distribution.

The quantities of incorporated aminoacid in individual fractions

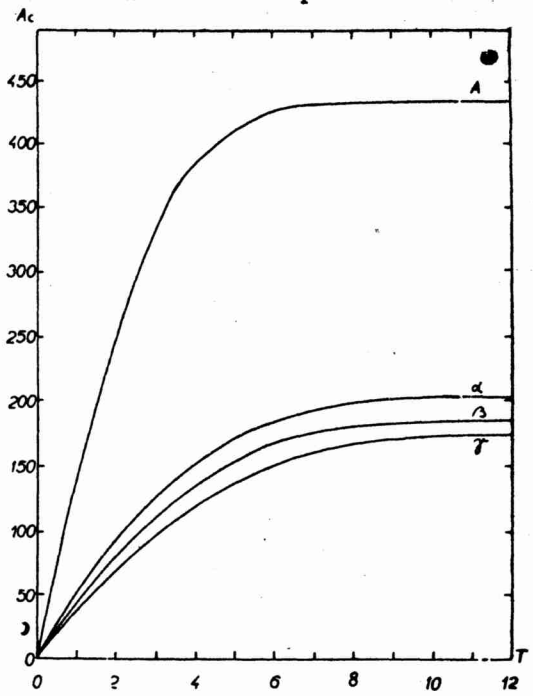


Fig. 26. Character of incorporation of methionine ^{35}S in serum proteins.
T — time in hours,
Ac — specific activity ^{35}S (imp/min/mg).

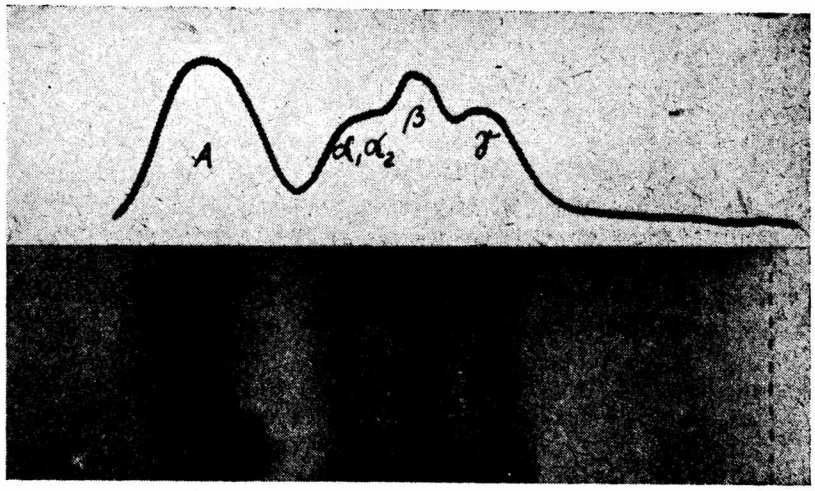


Fig. 27. A documentary photography concerning methionine ^{35}S quantities incorporated in serum proteins at the time of maximal incorporation (12 hours after aminoacid application).
Autoradiogram and the corresponding graphical evaluation.

(their mean values) can be demonstrated graphicaly. We obtain so again a spectrum as it was the case with bound ^{131}I and with polarographically activy groups (fig. 28). The spectrum here obtained is specific in his character and shows that quantities of incorporated aminoacid do not correspond to the quantities of protein fractions. So f. i. albumins incorporate less of aminoacid than globulins (fig. 29). Specific relations are equally as to the distribution of incorporated aminoacid in individual globulin fractions (fig. 30).

Table 9. Quantities of incorporated methionine S^{35} in proteins of the blood serum proteins of sane rabbits 12 hours after intravenous application of aminoacid.

Nr	Quantity of proteins in per cent (colorimetric determination)				Quantity of methionine S^{35} in fractions in percent (measurement with GM-counter)			
	Albumin	Globulin			Albumin	Globulin		
		α	β	γ		α	β	γ
1	63.1	13.1	11.0	12.8	46.1	21.0	17.1	15.8
2	68.9	9.6	8.7	12.8	44.4	20.4	18.3	16.9
3	63.6	12.3	10.0	14.1	42.4	19.8	19.1	18.7
4	67.1	12.7	9.6	10.6	41.8	20.9	19.2	18.1
5	70.0	9.1	8.2	12.7	44.0	20.6	17.7	17.7
$\bar{x}$	66.54	11.36	9.50	12.6	43.74	20.54	18.28	17.44
$\sigma \pm$	3.09	1.80	1.09	1.25	1.68	0.45	0.90	1.12
x_{min}	63.1	9.1	8.2	10.6	41.8	19.8	17.1	15.8
x_{max}	70.0	13.1	11.0	14.1	46.1	21.0	19.2	18.7

The relation between quantities of incorporated aminoacid and of proteins will be more explicit, when we express the quantities of incorporated aminoacid on individual quantities of protein fractions (fig. 31 and 32). This shows us that equal quantities of albumins incorporate two and half less aminoacid than globulins (exactly 2.56 times). But even globulin fractions do not equaly incorporate methionine.

A different degree of methionine incorporation in individual protein fractions points to their unequal anabolism. This property of unequal anabolism speed must be on the other side compensated by catabolic processus.

Conclusion: Methionine ^{35}S incorporates in blood serum proteins with a determined speed and a maximum of incorporation is obtained in 6—8 hours after intravenous application of aminoacid.

The distribution of methionine quantities in protein fractions gives a spectrum

which shows that the incorporated aminoacid quantities are not directly proportional to protein quantities. A higher proteosynthesis is observed for globulins than for albumins. This unequal anabolism is compensated by unequal speed of their catabolism. In fractions with reduced anabolism is reduced their catabolism, in fractions rapidly created there is equally a higher speed of their decomposition.

2. *Changes in blood serum proteins of rabbit under influence of ionizating radiation*

A certain nomber of changes produced in blood serum proteins of irrariated animals is known actually. Through knowledge of these changes we can often conclude on the degree of lesion of irradiated organism. Experimental data often

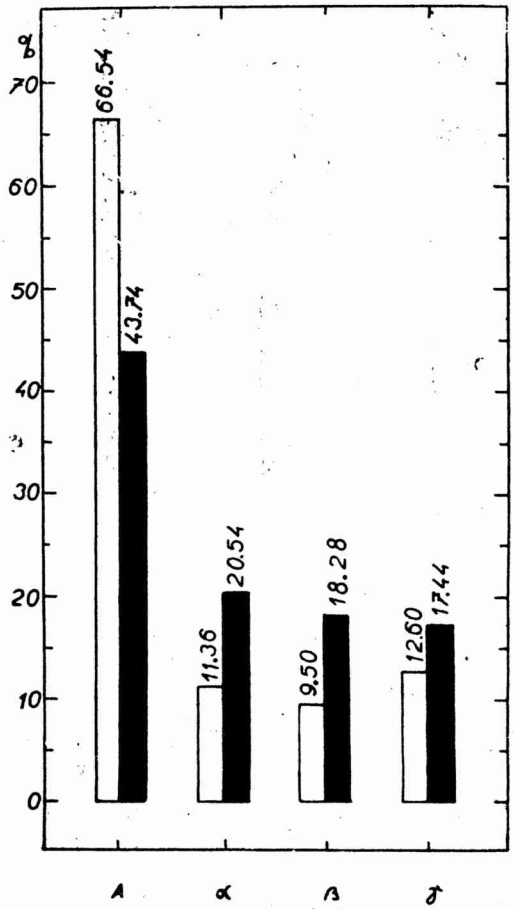


Fig. 28. Quantity spectrum of incorporated ^{35}S methionine ■ and quantity spectrum of protein fractions □ (in p. c.).

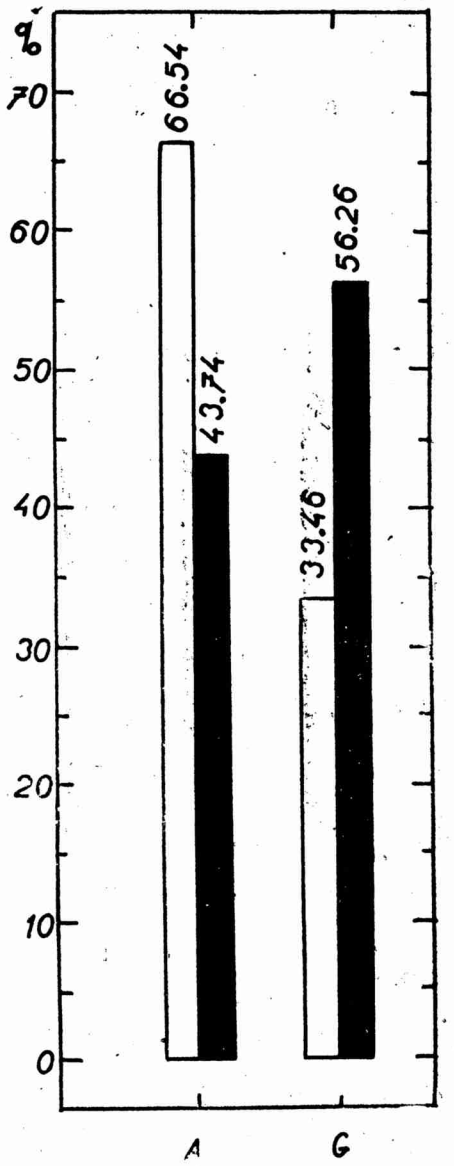


Fig. 29. Quantities of incorporated methionine ■ in serum albumins and globulins and quantities of these fractions □ in p. c.

show discrepancies and differencies are especially in their summary evaluations. We refered to these problems more in our work P a u l o v (1961).

In relation with the research about some properties of blood serum proteins of sane rabbits we have undertaken a study of the blood serum proteins of irradiated rabbits. In following chapters we give the main data of our study.

Ve used radioactive cobalt Co^{60} (giving 400 gekv Ra) as a source of ionizing gama radiation. The rabbits were submitted to a single irradiation over the whole body in a distance of 1365 mm (centrum of the rabbit) from the source of radiation.

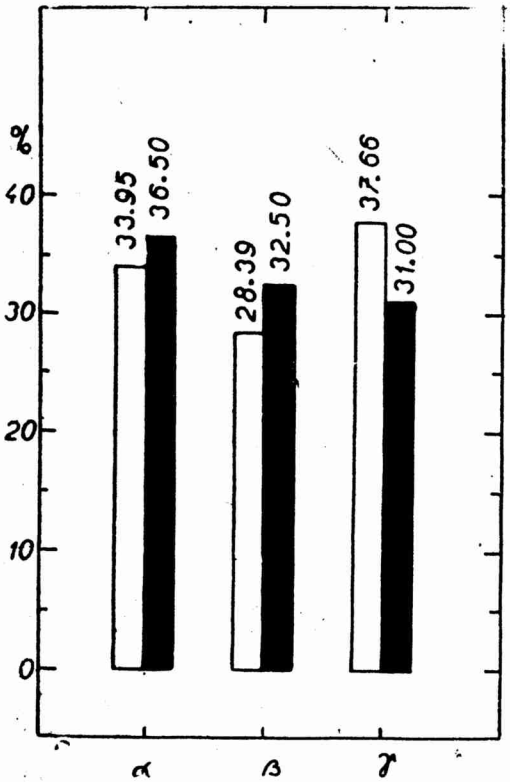


Fig. 30. Quantities of incorporated methionine ■ in individual globulin fraction □ in p. c.

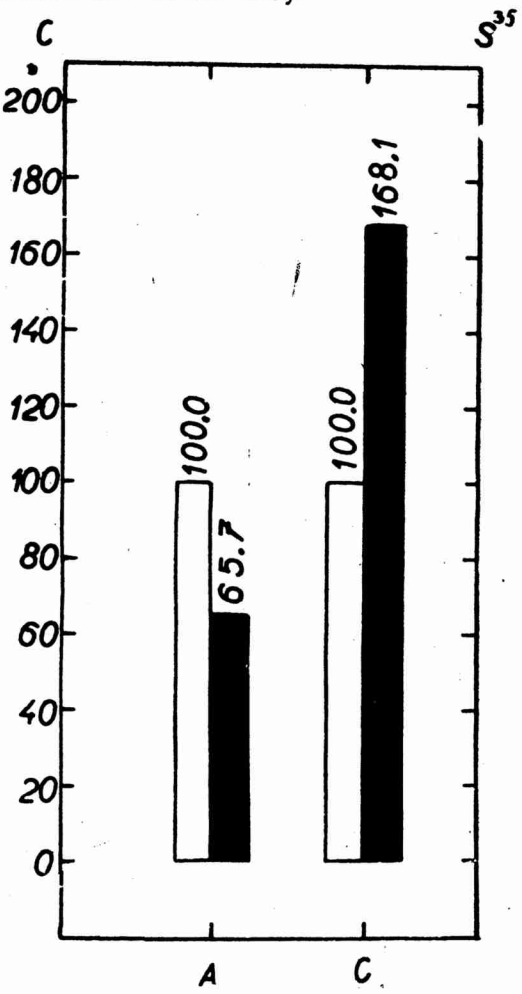


Fig. 31. Quantities of incorporated methionine ■ related to unity quantities of albumins and globulins □

The quantity of irradiation was determined in air with a Siemens-universal dosimeter (2 r/min). In experiments we used dosis of 800 r, 1000 r and 1100 r. (LD 50/30 for gama radiation ^{60}Co is after R u s t et al. — 1955 equal to 1094 r. For rtg radiation on rabbits is the lethal dosis 750—825 r as indicated by B a c q et al. — 1955).

a) *Changes in quantities of serum proteins in irradiated rabbits*

In the majority of publications is described that in irradiated organisms a reduction of albumins and an increase of globulins is produced. The increase

is mainly due to alpha and beta globulins whereas gama globulins show a reduction. This character of changes is not always confirmed as there are other changes of blood proteins especially in individual serum globulins. The character of these changes in postradiative period can vary. Frequently we observe that changes in the spectrum of protein quantities are typical for each experimental animal (each individuum).

With rabbits the changes in blood serum proteins were described by a certain number of authors: Stender et al. (1953), Andreoni et al. (1955), Meyniel et al. (1955), Ivanov et al. (1956), Fastjušenko et al. (1957), Smoličev (1957), Blochina et al. (1959), Košmider et al. (1960 b), Paulov (1961), Waldschmidt et al. (1961). Changes in serum proteins of irradiated guinea-pig described Kohn (1950), Meyniel et al. (1955), of irradiated pigs describes Grigorjan et al. (1959), of fishes Epštejn et al. (1959), of rats Gjessing et al. (1950), Suplee et al. (1952), Höhne et al. (1953), Westphal et al. (1953), Fisher et al. (1955), Höhne et al. (1955), Bauer et al. (1956), Winkler et al. (1956), Gross et al. (1958), Lózsa (1962), of mice Shekarchi et al. (1960), of dogs Muntz et al. (1949), Gornatzer et al. (1953), Li-

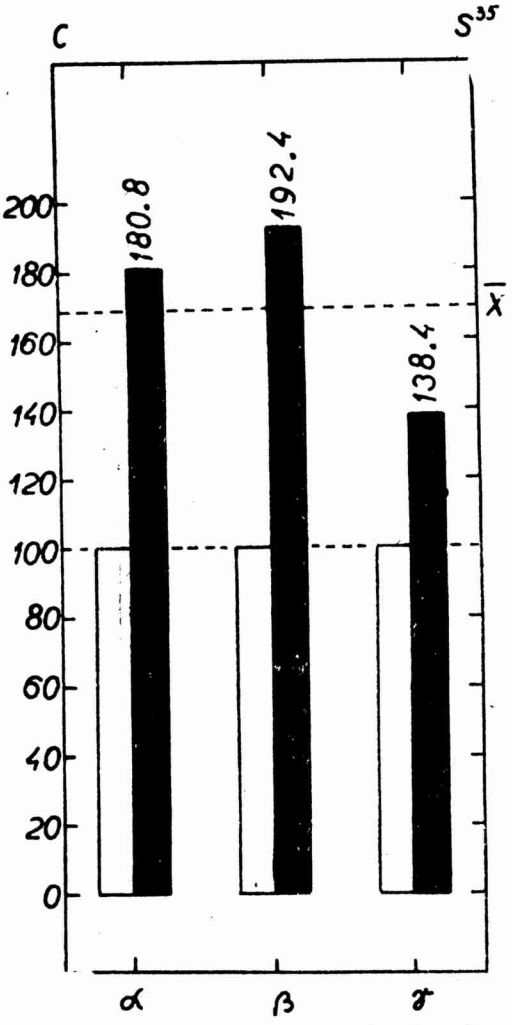


Fig. 32. Quantities of incorporated methionine ■ related to unity quantities of globulin fractions □.
$\overline{X}$ – arithmetical mean value of in total globulins incorporated aminoacid.

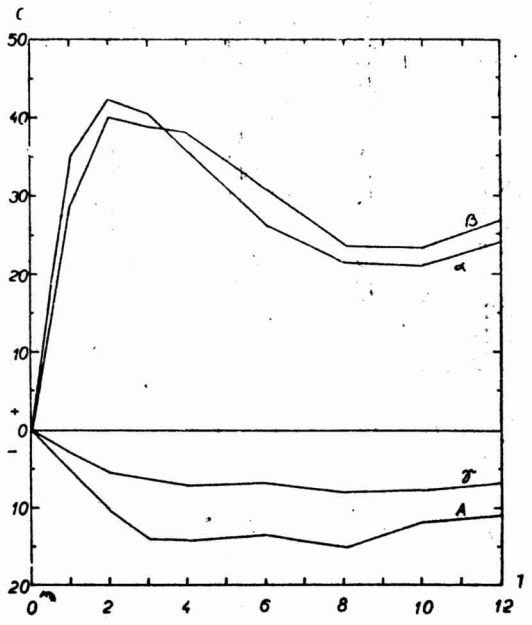


Fig. 33. Changes in serum protein quantities of a rabbit irradiated with a single whole-body dosis of 800 r.
T – time in days after irradiation,
C – change in serum protein quantities in p. c.

binzon (1959), Zícha et al. (1959), and of men Mahnert (1920), Jammet et al. (1959), Nišivaki et al. (1959), Veit (1953).

Our experimental results are in general in good accord with the data published. We observed in all rabbits a reduction of albumins and gama globulins and an increase of alpha and beta globulins (fig. 33).

Conclusion: In irradiated rabbits there is a reduction of albumin and gama globulins and an increase of alpha and beta globulins.

b) *Apparition of new proteins of type X in serum of irradiated rabbits*

In the metodic part of this publication we refered to the introduction of an S section. It is the section from the starting line to the beginning of the gama globulin fraction. We observed often that this section stains in a relatively intense way with bromphenol blue on serum of irradiated rabbits. This may indicate the presence of some proteins.

We studied more profoundly this section for rabbits irradiated with a dosis of 1100 r (6 animals). We observed that appear even 1—2 distinctly differenciated fractions in the S section. We designed these fractions as $X_{1,\,2}$. More in detail we described this in an other publication (Paulov — 1962 a). (These proteins are not identical with the so called reactive proteins C — Riley et al. 1962, Tukačinskij et al. — 1961 but this are new up now in literature non described fractions.)

The new fractions show besides a good affinity to bromphenol blue a small mobility in electric field and possess minimal quatities of polarographically active groups (determined after Homolka — 1953). They do not colour with Sudan black B — a stain characterising the lipoproteids (after Swahn — 1952). These fractions do not appear in rabbits irradiated with smaller dosis and were not observed in serum of with 1100 r irradiated rabbits during their postradiative period but only before the death.

We suppose that the apparition of these proteins in serum of irradiated rabbits hangs together with a last natural protection of the organism against radiative influences. We can nevertheless equally suppose that this are corporal proteins which get in blood as a consequence of general destructive changes caused by ionization, this in order that they may be transported to another place of action or made ready for expulsion from the organism.

Conclusion: In serum of irradiated rabbits with a dosis of 1100 r new proteins $X_{1,\,2}$ were identified appearing in blood before the exit of the animal on irradiation dissease. These proteins were until now not described in literature by other authors.

c) *Changes in catabolism of serum proteins under influence of ionizing radiation*

Among the plurality of methods which begin to be used nowadays for the study of changes in proteins of irradiated organisms, there are in foreground the labelling methods with radioactive isotopes. We hawe found only a few publications in literature about application of these methods to irradiated organisms. Smoličev (1957) administred to rabbits methionine ^{35}S together with ^{32}P radiating and states that all serum proteins are more rapidly decomposed. Steinfeld et al. (1957), Yalow et al. (1957), Bloom et al. (1958) observed that irradiated proteins are more rapidly expulsed from organisms.

In order to determinate the speed of catabolism in serum proteins of irradiated rabbits we used labelling of serum proteins with ^{131}I. Labelled ^{131}I serum proteins

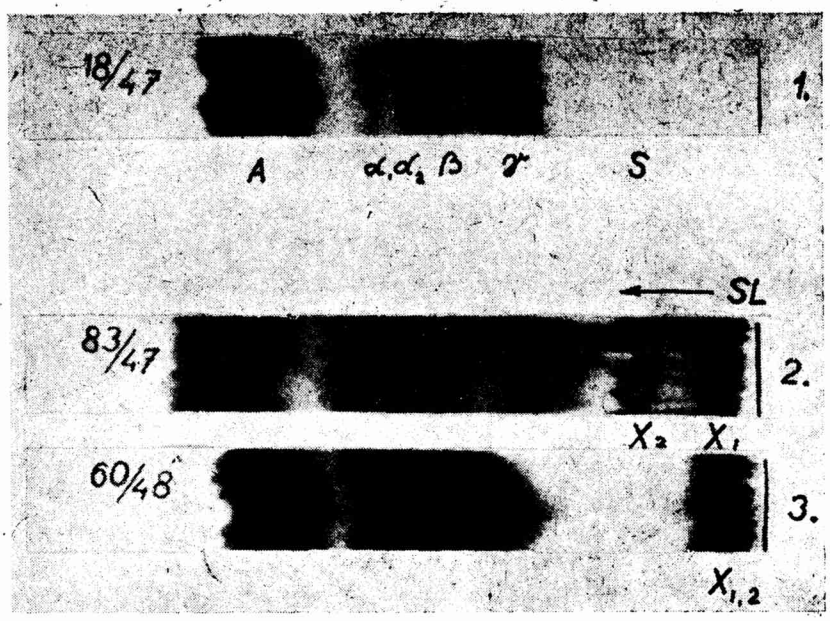


Fig. 34. A documentary photograph of the apparition of *X* type proteins in serum of irradiated rabbits.

1. spectrum of a sane non-irradiated rabbit,
2. spectrum of a rabbit 12 days after irradiation (2 days before exitus) containing distinctly the $X_{1,2}$ fractions,
3. spectrum of a rabbit 6 days after irradiation (1 day before exitus) with a distinct *X* fraction,

S — section from start to the beginning of the gama globulin fraction,
SL — start and direction of fractions migration.

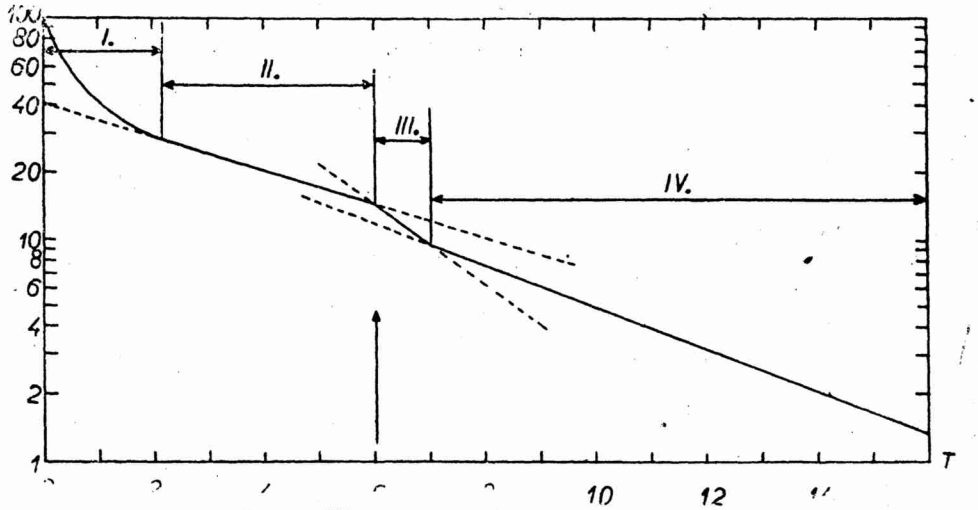


Fig. 35. Character of sinking of ^{131}I activity in blood serum proteins of rabbits after irradiation.

Ac — logarithmic value of the specific activity ^{131}I in p. c.,
T — time in days,
I. — mixing (distribution) phase,
II. — metabolic (degradation) phase,
III. — first postradiative degradation phase,
IV. — second postradiative degradation phase,
↑ — indicates the data of irradiation of the rabbit under experiment.

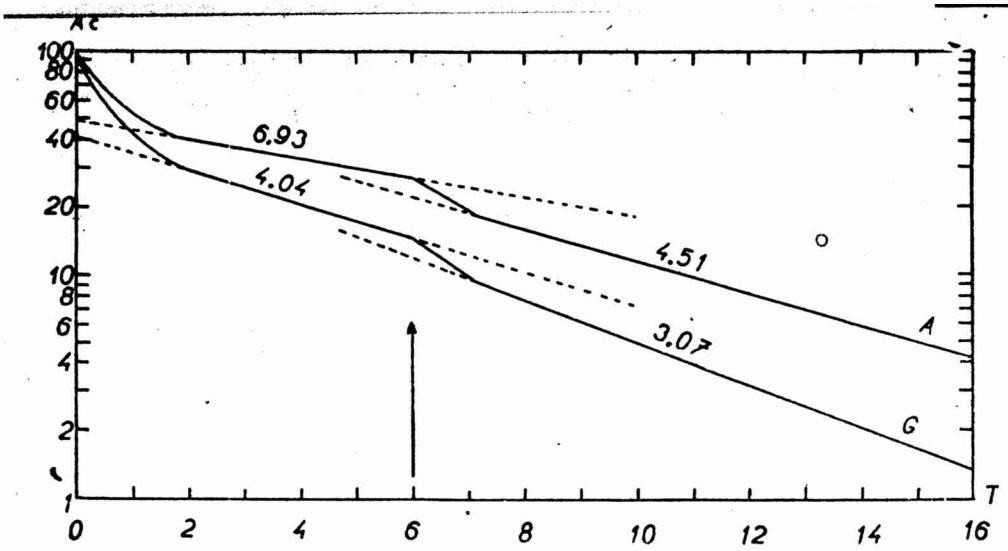


Fig. 36. Sinking of the ^{131}I activity of serum albumins and globulins of rabbits after an irradiation of 800 r.

Ac — logarithmic value of the specific activity ^{131}I in p. c.,
T — time in days,
↑ — data of rabbit irradiation,
A — albumins, *G* — globulins with mean values of biological half-lifes indicated.

Table 10. A change in biological half-lifes of serum albumins and globulins of rabbits after one irradiation of the whole body with a dosis of 800 r.

Nr	Biological half-life in days before irradiation		Biological half-life in days after irradiation	
	Albumin	Globulin	Albumin	Globulin
1	6.7	4.0	4.1	3.0
2	7.0	4.1	4.8	3.2
3	7.1	3.9	4.0	2.8
4	7.0	4.1	4.2	3.1
5	6.9	4.0	4.2	3.1
6	7.0	4.0	5.2	3.0
7	6.8	4.2	5.1	3.3
$\bar{x}$	6.93	4.04	4.51	3.07
$\sigma \pm$	0.14	0.08	0.50	0.16
x_{min}	6.7	3.9	4.0	2.8
x_{max}	7.1	4.2	5.2	3.3

were applied intravenously into the border ear vein and after one week the rabbits were irradiated with unique dosis of 800 r on the whole body. Through measurement of the ^{131}I activity in irradiated rabbits we distinctly observed two well differenciated phases of accelerated sinking of activity (we denominated them as the first and second postradiative degradation phases). The first phase lasts approximately 1 day after irradiation, the second comes aftervards and has in the whole rest of the postradiative periode a linear character when followed in a semilogarithmic diagram (fig. 35). Such phases were not until now described for irradiated animals in literature. The accelerated reduction of ^{131}I activity it not caused by primary ionization (Paulov — 1961) but has a metabolic character. The sinking of activity described can evidently besides an accelerated catabolism include even other factors (f. i. a dilutive effect etc.). We get an accelerated sinking of specific ^{131}I activity even when we make a correction on equal quantities of proteins (Paulov — 1960 b).

Measured values of biological half-life of irradiated rabbits (7 animals) are noted in table 10, mean values are graphically represented in diagram on fig. 36.

Orientation experiments with irradiated rabbits after dosis of 400 r and 600 r showed a similiar accelerated catabolism and determinated values of biological half-life did not differ substantially from those for rabbits irradiated at 800 r.

Conclusion: In irradiated rabbits it comes to an accelerated catabolism of serum albumins and globulins in two postradiative degradation phases.

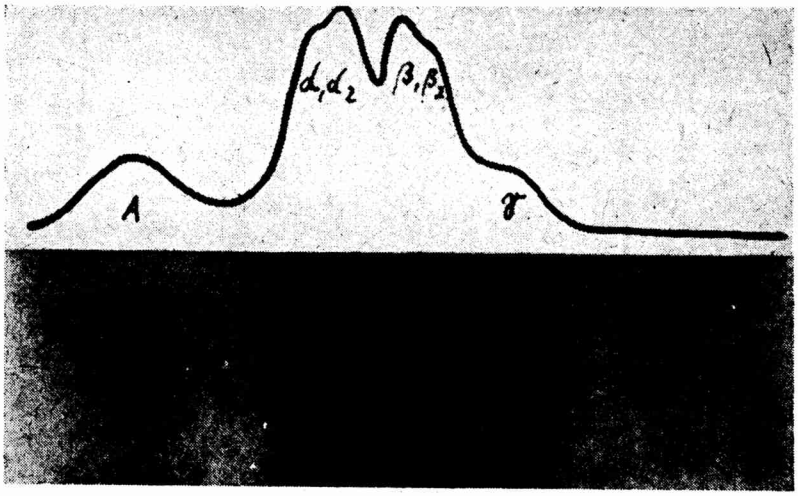


Fig. 37. Documentary photograph of the incorporation quantity of methionine ^{35}S into blood serum proteins of a rabbit irradiated with a dosis of 800 r.
The blood sample from the irradiated rabbit was withdrawn 17 hours after irradiation (12 hours after application of the aminoacid).

d) *Changes in anabolisme of blood serum proteins in irradiated rabbits*

In literature we find a lot of freqently discordant data about changes in proteosynthesis of irradiated organisms. Stevens et al. (1953 a) observed an increased incorporation of methionine into albumins as well as into globulins (more in globulins) for irradiated rabbits. Similiar results notes Okulov (1956). Rodionov et al. (1959) states an increase of methionine incorporation for

irradiated dogs and this only in globulins. Smoličev (1957) notes a reduced protheosynthesis of irradiated rabbits. Gabrieli et al. (1955) measure an increase of methionine incorporation in alpha and beta globulins and a reduction of albumins in irradiated rats. Fedorov (1961) observes a similiar phenomenon for irradiated dogs. Nikolaev et al. (1959) note an increase of proteosynthesis of plasmatic and body proteins in irradiated mice. In a number of works there are nonconcordant indications on proteosynthesis of other bodily proteins (Hempelmann et al. — 1950, Alexandrov — 1956, Kay et al. — 1956, Kumta et al. — 1957, Richmond et al. — 1957, Janoušek et al. — 1960 a, b, c).

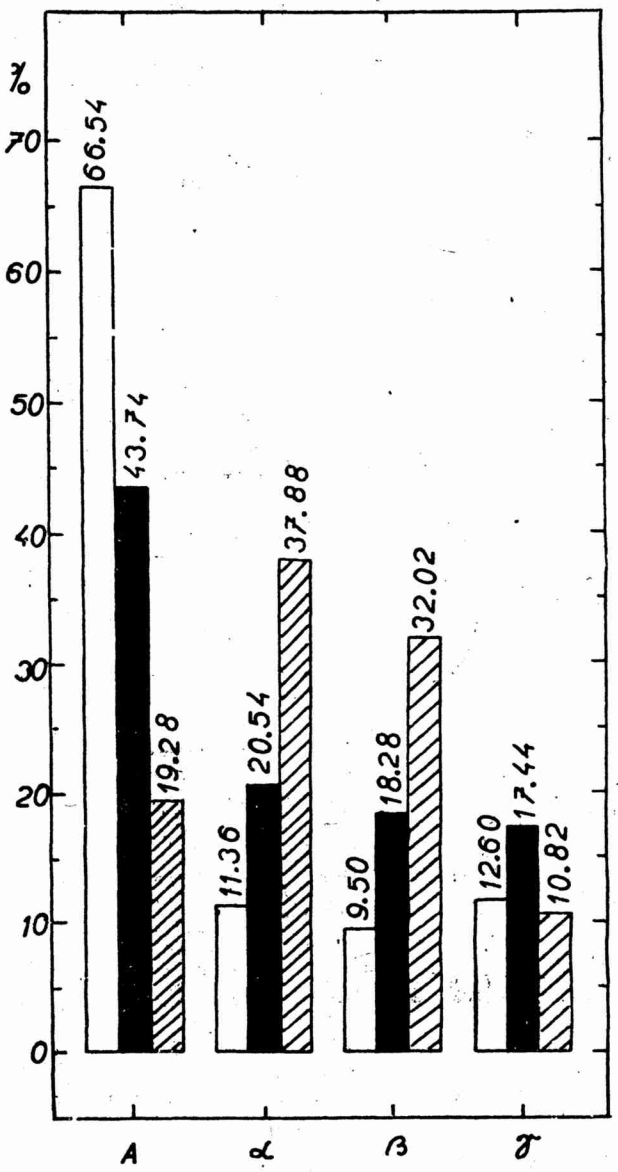


Fig. 38. Quantity spectrum of the blood serum proteins ▭, quantity spectrum of incorporated methionine ^{35}S in serum proteins of controll animals ■ and spectrum of incorporated methionine for irradiated rabbits after a dosis of 800 r ▨ (in p. c.).

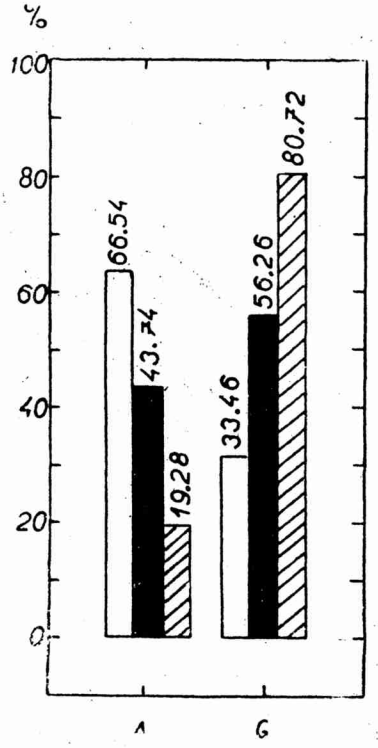


Fig. 39. Quantities of methionine ^{35}S incorporated in serum albumins and globulins controll rabbits ■ of irradiated rabbits ▨ and quantities of fractions ▭ in p. c.

From the publications mentionned in results that we cannot uniformly take for valable the statement as to the proteosynthesis in irradiated organisms being enhanced or being accelerated through irradiation. Yet some specificity is to be observed as to the proteosynthesis in individual proteins. We have naturally to take with a certain reserve the opposite results published concerning the same problem studied by different authors.

We occupied us also with the question of proteosynthesis in irradiated rabbits. Five hours after irradiation with a dosis of 800 r we applied to rabbits intravenously methionine ^{35}S and 12 hours later we withdraw blood sample in order to determinate the degree of incorporation in individual fractions. In our experimental conditions we observed that in

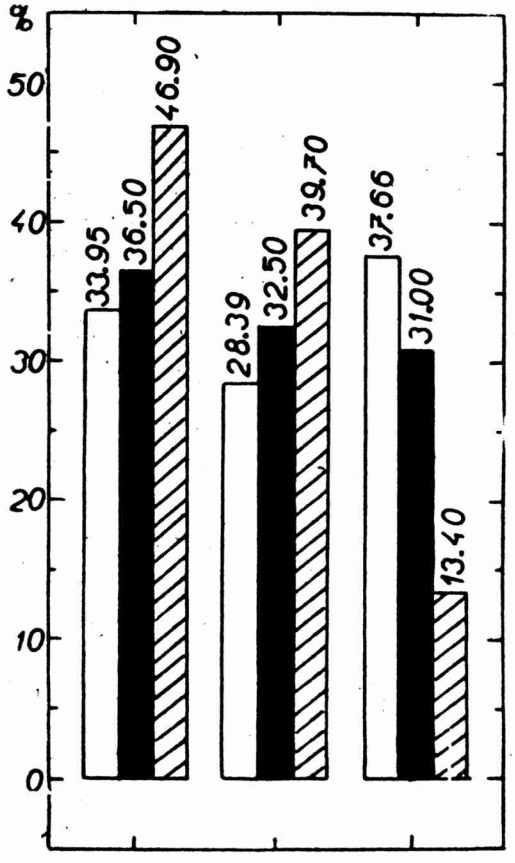


Fig. 40. Quantities of methionine ^{35}S incorporated in serum globulins of controll rabbits ■, of irradiated rabbits □ and quantities of individual globulin fractions ▨ in p. c.

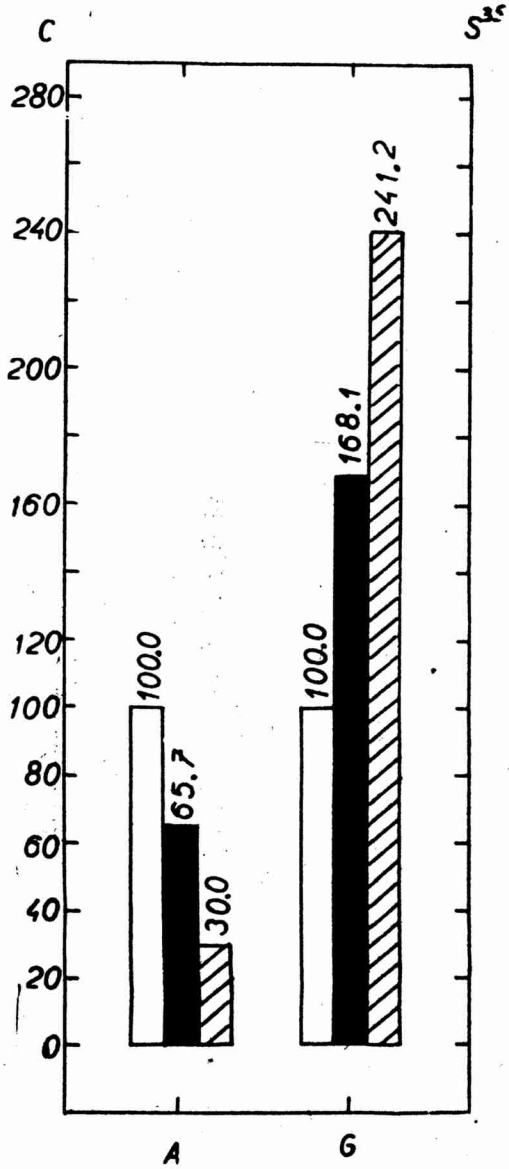


Fig. 41. Quantities of methionine ^{35}S incorporated in serum albumins and globulins of controll rabbits ■ and of irradiated rabbits ▨ related to unity quantities of fractions □

irradiated rabbits an increased proteosynthesis of globulins sets in and that albumins and gama globulins show a sinking creation. Increased proteosynthesis of globulins is caused only through increased proteosynthesis of alpha and beta fractions (fig. 37, 38, 39, 40 and table 11).

When we calculate the quantities of incorporated aminoacid on unitary quantities of proteins, we can see for irradiated rabbits in equal quantities of albumins there is less aminoacid than in equal quantities of globulins (fig. 41) and the relation between these quantities attains 8 times (exactle 8.04 times). With the help of a similar calculation for individual globulin fractions we can ascertain that alpha and beta globulins have by far a higher incorporation of aminoacid than globulins (fig. 42).

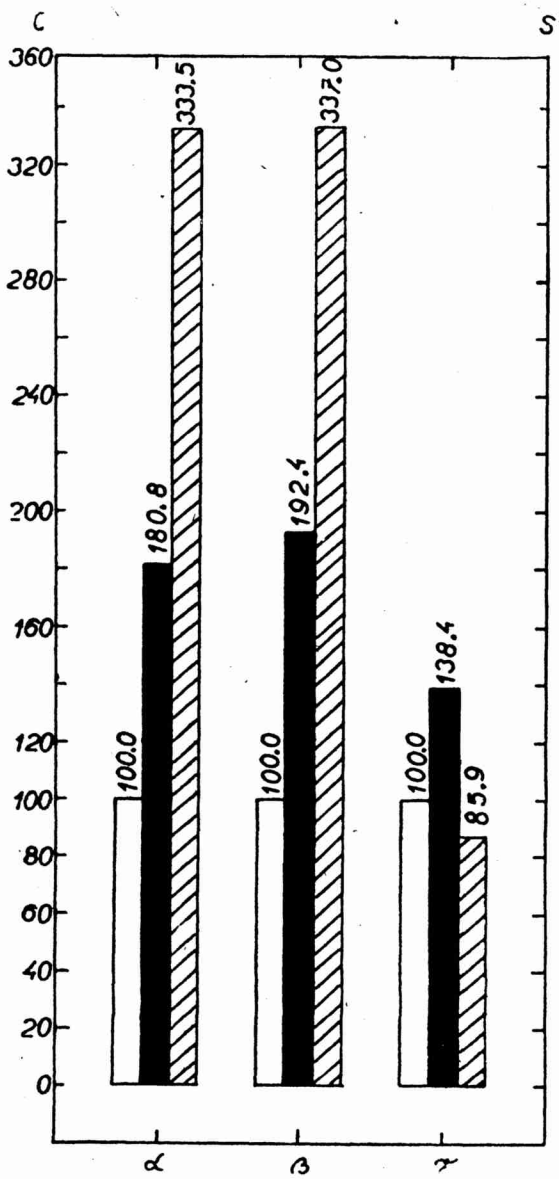


Fig. 42. Quantities of methionine ^{35}S incorporated in serum globulins of controll rabbits ■ and of irradiated rabbits ▨ related to unity quantities of globulin fractions □

We give these changes in relation with the determined changes in protein quantities and in the speed of catabolism in the following way: An increase in albumin quantities with irradiated rabbits is caused by their accelerated catabolism and by a reduced proteosynthesis. An increase of globuline quantities is a result of their increased proteosynthesis (principally for alpha and beta globulins) which overbalances even their accelerated catabolism.

Conclusion: In irradiated rabbits it comes to a reduced proteosynthesis of serum albumins and gama globulins and to an increase of the proteosynthesis of total globulins principally by a higher creation of alpha and beta globulins.

Summary

In this publication some problems are studied concerning experimental methods applied for research in the physiology of blood serum proteins. On the basis of experimental constatations following conclusions are submitted:

1. Quantity spectrum of blood serum proteins is typical for the species.

2. Quantity spectrum of polarographicaly active groups of blood serum proteins is typical for the species and is in no accord with the quantities of protein fraction.

3. Quantity spectrum of labelled ^{131}I in vitro for serum proteins is typical and in no accord with the quantities of protein fractions.

4. Blood serum proteins fractiones have an uneqal catabolic and anabolic speed.

5. The spectrum of incorporated methionine ^{35}S in serum proteins (after obtaing maximum incorporation) is typical and is in discordance with the quantities of protein franction.

6. In irradiated rabbits there appear typical changes in their quantity spectra of blood serum proteins and sometimes appear proteins of the type *X*.

7. Changes in quantities of serum proteins in irradiated rabbits are caused by changes in catabolism and anabolism.

Table 11. Quantities of incorporated methionine S^{35} in blood serum proteins of sane and irradiated rabbits with radiation dosis of 800 r.

Nr	Quantity of methionine S^{35} (in per cent) in sound rabbits determined 12 hours after application of amino-acid				Quantity of methionine S^{35} (in per cent) in irradiated rabbits (17 hours after irradiation and 12 hours after application of aminoacid)			
	Albumin/	Globulin			Albumin	Globulin		
		α	β	γ		α	β	γ
1	46.1	21.0	17.1	15.8	20.3	35.8	31.1	12.8
2	44.4	20.4	18.3	16.9	16.9	37.3	32.2	13.6
3	42.4	19.8	19.1	18.7	19.4	35.7	31.6	13.3
4	41.8	20.9	19.2	18.1	18.5	40.7	33.3	7.5
5	44.0	20.6	17.7	17.7	21.3	39.9	31.9	6.9
$\bar{x}$	43.74	20.54	18.28	17.44	19.28	37.88	32.02	10.82
$\sigma \pm$	1.68	0.45	0.90	1.12	1.84	2.12	0.86	3.30
x_{min}	41.8	19.8	17.1	15.8	16.9	35.7	31.1	6.9
x_{max}	46.1	21.0	19.2	18.7	21.3	40.7	33.3	13.6

References

Alexandrov S. N.: Ob izmenenii skorosti vključenija indikatora v belki organov oblučennych životnych. Dokl. Ak. nauk SSSR, 106, 1:153, 1956.

Allison J. B., Wannemacher R. W., Russel T., Mc Coy J. R.: The uptake of S^{35} from methionine by plasma proteins in protein-depleted and tumor-bearing dogs. Cancer Res., 18, 4:394, 1958.

Andreoni O., Dompè M., Russo A. M.: Proteine e lipoproteine seriche in conigli sottoposti a panirradiazione. Ricerca Sci. 25, 6:1393, 1955.

Armstrong S. H., Mc Leod K., Wolter J., Kukral J.: The persistence in the blood of the radioactive label of albumin, gama globulins, globulins of intermediate mobility studied with S^{35} and paper electrophoresis. J. Lab. clin. Med., 43:918, 1954.

Aronsson T., Grönwall A.: Improved separation of serum proteins in paper electrophoresis — a new electrophoresis buffer. Science Tools, LKB Instr. J., 5, 2:21, 1958.

Bacq Z. M., Alexander P.: Fundamentals of radiobiology. London, 1955.

Balachovskij S. D., Balachovskij I. S.: Metody chimičeskogo analiza krovi. Moskva, 1953.

Balle-Helaers E.: Dosage polarographique des fractions d'électrophorèse. Clin. Chim. Acta., 3:51, 1958.

Bartík M., Havassy L.: Polarografia krvných sér domácích zvierat. Veter. čas., 3, 4:270, 1954.

Bartík M., Havassy J.: Biochémia a fyziológia bielkovín krvných sér domácich zvierat. Veter. čas., 4, 3—4:131, 1955 a.

Bartík M., Havassy J.: Biochémia a fyziológia krvných sér domácich zvierat. Veter. čas., 4, 1:22, 1955 b.

Bauer R., Piller S., Schneider G.: Tierexperimentelle Serumeiweissuntersuchungen bei Körperganzbestrahlungen mit und ohne Sieb. Strahlentherapie, 100, 1:16, 1956.

Benjamin E., Reuss A.: Röntgenstrahlen und Stoffwechsel. Münch. med. Wchschr., 53:1860, 1906.

Bennhold H., Kallee E.: Comparative studies on the half-life of I^{131}-labeled albumins and nonradioactive human serum albumin in a case of analbuminemia. J. clin. Invest., 38, 5:863, 1959.

Berson S. A., Yalow R. S., Schreiber S. S., Post J.: Tracer experiments with I^{131} labeled human serum albumin. J. clin. Invest., 32:746, 1953.

Betz E. H.: Irradiation et métabolisme protéique. Rev. franc. études clin. et biol., 3:387, 1958.

Bier M.: Electrophoresis, theory, methods and applications. New York, 1959.

Blochina V. D., Šalnov M. J.: Stravnitelnoe izučenije belkovych frakcij plazmy krovy posle odnokratnogo vozdejstva protonami vysokych energij i rtg lučami. Bjul. exp. biol. i med., 8:49, 1959.

Bloom H. J. G., Crockett D. J., Stewart F. S.: The effect of radiation on the stability of radioiodinated human serum albumin. Brit. J. Radiol., 31, 367:377, 1958.

Boyd G. A.: Autoradiography in biology and medicine. New York, 1955.

Brdička R.: Polarographic cystine and protein tests. Research, 1, 1:35, 1947.

Březina M., Zuman P.: Polarografie v lékařství, biochemii a farmacii. Praha, 1952.

Bronský D., Freeark R. J., Hyman S., Armstrong S. H.: The persistence in the blood of the radioactive label of albumins, gama globulins and globulins of intermediate mobility. J. Lab. clin. Med., 50:577, 1957.

Burnazjan A. J., Lebedinskij A. V.: Radiacionnaja medicina. Moskva, 1960.

Campbell R. M., Cuthbertson D. P., Matthews C. M., Mc Farlane A. S.: Behaviour of ^{14}C— and ^{131}I—labelled plasma proteins in the rat. Int. J. Appl. Rad. Isotopes, 1:66, 1956.

Campbell R. M., Cutbertson D. P., Mackiel W., Mc Farlane A. S. Philipson A. T., Sudsaneh S.: Passage of Plasma albumin into the intestine of the sheep. J. Physiol., 158, 1:113, 1961.

Clouet D. M., Ball C. O. T., Meneely G. R., Hahn P. F.: Electrophoresis of plasma proteins and ascitic fluid of dogs with radiation cirhosis. Proc. Soc. Ex. Biol. Med., 87:362, 1954.

Cohen S., Holloway R. C., Matthews C., Mc Farlane A. S.: Distribution and elimination of I^{131} and C^{14} labelled plasma proteins in the rabbit. Biochem. J., 62, 1:143, 1956.

Cohen S., Freeman T., Mc Farlane A. S.: Metabolism of ^{131}I labelled human albumin. Clin. Sci., 20, 2:161, 1961.

Cornatzer W. E., Engelstad O., Davison J. P.: Effect of whole body *x*-irradiation on blood constituents. Am. J. Physiol., 175:153, 1953.

Corsini F., Grazia G.: Le proteine del siero di sangue di coniglio. Arch. Sci. biol. (Bologna), 41:595, 1957.

Curtis H. J.: The biological effects of radiations. Annu. rev. Physiol., 41, 1951.

Dienstbier Z., Arient M., Kofránek V.: Nemoc z ozáření se zvláštním zřetelem ke krevním změnám. Praha, 1957.

Dittmer A.: Papierelektrophorese. Jena, 1956.

Dixon F. J., Maurer P. H., Deichmiller M. P.: Half-lives of homologous serum albumins in several species. Proc. Soc. exp. Biol., 83:287, 1953.

Dixon F. J., Talmage D. W., Maurer P. H., Deichmiller M.: The half-life of homologous gamma globulin (antibody) in several species. J. Exp. Med., 96:313, 1952.

Ellinger F.: Medical radiation biology. Illinois, 1957.

Epštejn J. A., Lavrovskaja N. F.: Vlijanie ionizirujuščego oblučenija na belkovyj obmen ryby. Biochimija, 24, 4:592, 1959.

Fastjušenko O. V., Varšavskij B. M.: Izmenenija nekotorych pokazatelej belkovogo obmena pri chroničeskom i ostrom vozdejstvii ionizirujuščego izlučenija. Trudy vsesoj. konf. med. radil.: 121, 1957.

Fedorov J. V.: Ob izmenii belkovogo obmena u životnych pri ostroj lučevoj bolezni. Med. radiobiol., 9:82, 1961.

Fischer M. A., Magee M. Z., Coutler E. P.: Studies on the serum proteins of the *X*-irradiated rat. Arch. Bioch. Biophys., 56:66, 1955.

Foss O. P.: Paper electrophoresis of I^{131}—labelled serum proteins. Sc. J. clin. Lab. Invest., 10:418, 1958.

Francis G. E., Mulligan W., Wormall A.: Labeling of proteins with I^{131}, S^{35} and P^{32}. Nature, 167:748, 1951.

Freeman T.: The biological behaviour of normal and denaturated human plasma albumin. Clin. chim. acta, 4:788, 1959.

Gabrieli E. R., Chang C. H.: Change of electrophoretic pattern and incorporation of radioactivity into plasma proteins following whole body *X*-irradiation. Fed. proc., 14:53, 1955.

Gilmore R. C., Robbins M. C., Reid A. F.: Labeling bovine and human albumin with I^{131}. Nucleonics, 12:65, 1954.

Gitlin D.: Distribution dynamics of circulating and extravascular I^{131} plasma proteins. Ann. New York Acad Sci., 70:123, 1957.

Gjessing E. C., Chanutin A.: Studies on the proteins and lipids of plasma fractions of *x*-ray irradiated rats. Arch. bioch., 27:191, 1950.

Goldsworthy P. D., Volwiler W.: Comparative metabolic fate of chemically (I^{131}) and biosynthetically (C^{14}—or S^{35}—) labeled proteins. Ann. New York Acad Sci., 70:26, 1957.

Goldsworthy P. D., Volwiler W.: Mechanism of protein turnover studied with cystine S^{35}, lysine C^{14} doubly labeled plasma proteins of the dog. J. Biol. Chem., 230:817. 1958.

Goldwater W. H., Entenman C.: Nature of serum protein changes in the *x*-irradiated dog. Am. J. Physiol., 188:409, 1957.

Green H., Anker H. S.: Kinetics of amino acid incorporation into serum proteins. J. gen. Physiol., 38:283, 1955.

Grigorjan M. S., Brutjan A. S.: Dinamika obščego belka i belkovych frakcij u svinej pri lučevom poraženii. Izv. Akad. nauk Armenskej SSR, 12, 4:39, 1959.

Gross S., Wronska T., Sysa J.: Zmiany skladu bialek surowicy krowi u szczurow nasvietlanych promienami roentgena. Med. Pracy, 9:107, 1958.

Hempelmann L. H., Carr S., Frantz J. D., Masters R., Lamdin E.: Effect of body exposure to *x*-rays on rate of incorporation of C^{14} carboxyl-labeled alanine into mouse protein. Federation Proc., 9:183, 1950.

Heyrovský J., Kůta J.: Základy polarografie. Praha, 1962.

Hnilica L.: Niektoré vlastnosti histonu z telacieho brzlíka. (Kand. diz. práca), Bratislava, 1958.

Höhne G., Jaster R., Künkel H. A.: Der Einfluss von Cystein auf die Strahleninduzierten Veränderungen im Serumeiweiss der Ratte. Klin. Wschr., 31:910, 1953.

Höhne G., Künkel H. A., Anger R.: Die Serumeiweisskörper der Ratte nach Roentgenganzbestrahlung mit 3000 r. Klin. Wschr., 33: 284, 1955.

Homolka J.: Elektroforeticko-polarografický výskum povahy krevních bílkovín. ČLČ, 23:633, 1953.

Homolka J.: Chemická diagnostika v dětském věku. Praha, 1956.

Homolka J., Mydlil V.: Bílkoviny krevní u kojenců s hlediska kvantity a kvality. ČLČ, 95, 5:113, 1956.
Hořejší J.: Bílkoviny krevní plasmy. Praha, 1956.
Hradec J., Sablík J., Klumpar J., Jiroušek P.: Studium metabolismu bílkovin pomoci radioaktivních isotopů. Čsl. fysiol., 3, 2:186, 1954.
Hughes W. L.: The chemistry of iodination. Ann. New York Asad. Sci., 70:3, 1957.
Hupka Š.: Sledovanie interakcie medzi histonom izolovaným z telacieho brzlíku a bielkovinami plazmy. (Kand. diz. práca), Bratislava, 1959.
Ivanov I. D.: Poljarografija belkov, enzymov i aminokyslot. Moskva, 1961.
Ivanov I. I., Balabucha V. S., Romancev E, F., Fedorova T. A.: Obmen veščestv pri lučevoj bolezni. Moskva, 1956.
Jammet H., et al.: Étude de six cas d'irradiation totale aiguë accidentell. Rev. fran. ét clin. biol., 4, 3:210, 1959.
Janoušek V., Kornalík F., Neuwiert J., Pokorný Z.: Metabolismus methioninu a cysteinu S^{35} v játrech po ozáření. Acta Univ. Carol. Med., 10:74, 1960 a.
Janoušek V., Kornalík F., Neuwirt J., Pokorný Z.: Vliv ozáření na inkorporaci methioninu S^{35} a cysteinu S^{35} do tkáňových bílkovín. Acta Univ. Carol. Med., 10:79, 1960 b.
Janoušek V., Kornalík F., Neuwirt J., Pokorný Z.: Vylučování anorganické a organické S^{35} po ozáření. Acta Univ. Carol. Med., 10:84, 1960 c.
Jeffay H., Winzler R., Donnelly J. S.: The metabolism of serum proteins. J. biol. chem., 231:101, 1958.
Kalous V.: Polarographische Bestimmung des Mucoproteins MP-1 nach der Trennung der Sera durch Papierelektrophorese. Coll. Czechoslov. Chem. Commun., 25:878, 1960.
Kay R. E., Entenman C.: The conversion of glycine C^{14} to protein C^{14} and $C^{14}O_2$ in liver slices from *x*-irradiated rats. Arch. Biochem. Biophys., 62:419, 1956.
Kohn H. J.: Changes in blood plasma of guinea-pig during acute radiation syndrome. Am. J. Physiol., 162:703, 1950.
Kośmider S., Myślak Z.: Polarograficzne badania białek surowicy krwi królików po napromienianiu. Arch. Immunol. Ter dośw., 8:531, 1960 a.
Kośmider S., Piekarski B., Petelenz T., Wierny L.: Badania elektroforetyczne białek surowicy krwi królików w chorobie popromiennej. Arch. Immunol. Ter. dośw., 8:747, 1960 b.
Kumta U. S., Gurnani S. U., Sahasrabudhe M. B.: Investigations on the fate of methionine S^{35} in irradiated rats. J. Sci. Industr. Res., 16, 5:111, 1957.
Kunkel H. G., Tiselius A.: Electrophoresis of proteins on filter paper. J. gen. Physiol., 35:89, 1951.
Kurochtina T. P.: Izučenije intenzivnosti obnovlenija belkov plazmy v organizme pri pomošči metionina S^{35} Biochimija, 19:16, 19. 4.
Kutaček M., Kratochvil L.: Primenenie elektroforeza na bumage dlja issledovanija i razdelenija belkov syvorotki moloka zdorovych i bruceloznych korov. Biochimija, 23:471, 1958.
Lamarque P., Thibaud E., Mercier J.: Étude radioactive de l'électrophoréogramme du sérum de malades traités par l'iode radioactif I^{131}. Radioisotopes in Sci. Res., 110, 1958.
Latta H.: Experimental hypersensitivity in the rabbit. J. Immunol., 66: 635, 1951.
Lederer M.: An introduction to paper electrophoresis and related methods. 1955 (ruský preklad, Moskva, 1956).
Libinzon R. E.: Biochimičeskie izmenenia pri dejstvii bolšich doz ionizirujuščej radiacii. Biofizika, 4:89, 1959.
Liebster J., Babický A., Kozel J., Liss E., Sydov G.: Příprava bílkovín značkovaných ^{131}J. Čsl. biológie, 6:227, 1957.
Lózsa A.: Factors influencing the normal values of the serum protein fractions in the albino rat. Acta Physiol. Hung., 21, 2:127, 1962.
Lubran M., Moos D. W.: The determination of serum albumin concentration using ^{131}I—labelled albumin. Clin. chim. acta, 2:246, 1957.
Lustig B., Ernst T.: Über den Eiweisszucker, Eiweissgehalt und Kohlenhydratindex der Sera und Körperflüssigkeiten verschiedener Tierarten. Biochem. Ztschr., 289:365, 1937.
Mahnert A.: Beiträge zum Studium der Veränderungen des Blutserum — Eiweissgehaltes unter verschiedenen Einwirkungen. Hoppe-Seyler's Z. physiol. Chem., 110:1, 1920.
Margen S., Tarver H.: Comparative studies on the turnover of serum albumin in normal human subjects. J. clin. Invest., 35, 10:1161, 1956.
Margen S., Tarver H.: The deiodination of proteins labeled with I^{131}. Ann. New York Acad. Sci., 70:49, 1957.

Matthews Ch.: The theory of tracer experiments ^{131}I-labelled plasma protein. Physics in Med. a Biol., 2, 1:36, 1951.
Mc Farlane A. S.: Labelling of plasma proteins with radioactive iodine. Biochem. J., 62:135, 1956.
Mc Farlane A. S.: Efficient trace-labelling of proteins with iodine. Nature, 182, 4627:53, 1958.
Meyniel G., Mende S.: Étude électrophoretique des protéines du sérum chez les animaux irradiés. Bull. Soc. chim. biol., 37, 1:127, 1955.
Miettinen J. K., Moisio T.: A self-recording strip photometer for paper electrophoresis and paper chromatography. Acta chem. Scand., 7:1225, 1953.
Michalec Č., Kořínek J., Musil J., Ružička J.: Elektroforesa na papíře a v jiných nosičích. Praha, 1959.
Muntz J. A., Barron E. S. G., Prosser C. E.: Studies on the mechanisms of action of ionizing radiations. Arch. Bioch., 23, 3:434, 1949.
Muralt G., Notter B.: Die Bluteiweisse im Hochgebirge. Helv. Physiol. Pharmacol. Acta., 6:649, 1948.
Myant N. B., Osorio C.: Serum proteins including thyroxine-binding proteins, in maternal and foetal rabbits. J. Physiol., 146:344, 1959.
Niklas A., Maurer W.: Über die Neubildung einzelner, getrennter Serum-Eiweissfraktionen nach oraler Gabe von S^{35}-l-Methionin an Ratten. Bioch. Z., 323:89, 1952.
Nikolaev A. J., Oster N. R.: Ob izmenenii skorosti vyvedenija radioizotopov C^{14} i S^{35} iz belkov organizma pri lučevoj bolezni. Med. Ž. Uzb., 12:12, 1959.
Nišivaki J., Kojama J., Mijoši K., Kumatori T., Takajama Š.: Případ Bikini. Praha, 1959.
Oeff K.: Umsatz von radioaktiven Serumeiweissfraktionen I. Z. exp. Med., 123:294, 1954 a.
Oeff K.: Umsatz von radioaktiven Serumeiweissfraktionen II. Z. exp. Med., 123:309, 1954 b.
Oeff K.: Umsatz von radioaktiven Serumeiweissfraktionen. III. Z. exp. Med., 123:434, 1954 c.
Okulov N. M.: Vlijanie ostroj lučevoj bolezni na izmenenie vsasyvaemosti metionina iz želudočno-kišečnogo trakta. Med. radiol., 5:41, 1956.
Opplt J. J., Kutáček M., Loštinský C., Čižimský J.· Nová modifikace klinické mikroanalysy bílkovín tělných. ČLČ, 92, 23:624, 1953.
Ostrowski W., Oszast Z.: Badania nad elektroforetycznymi i polarograficznymi zmianami w surowicy krwi chorych z gruźlicą skóry leczonych tiosemikarbazonem. Polski tygod. lekar., 10:982, 1955.
Paulov Š.: Účinky ionizačného žiarenia Co^{60} na metabolizmus sérových proteínov u králikov sledované stopovacou technikou rádioaktívnymi izotopami. (Kand. diz. práca), Bratislava, 1960 a.
Paulov Š.: Účinky ionizačného žiarenia Co^{60} na metabolizmus značkovaných sérových proteínov u králikov. Babákova sb., 16:159, 1960 b.
Paulov Š.: Použitie methioninu S^{35} a NaJ^{131} pri štúdiu metabolizmu sérových proteínov u ožiarených králikov. Sb. thesí II. cel. Sjezdu spol. biochem., 23, Praha, 1960 c.
Paulov Š.: Príspevok k poznaniu pohyblivosti sérumalbumínu pri elektroforéze na papieri. Biológia SAV, 15:916, 1960 d.
Paulov Š.: Einwirkungen von Ionisationsstrahlung auf den Metabolismus von Serumeiweissstoffen bei Kaninchen. Biol. práce SAV, 7/2, 1961.
Paulov Š.: The apperance of new proteins in the serum of irradiated rabbits. Folia biol., 8:186, 1962 a.
Paulov Š.: Príspevok k fotokolorimetrickému určovaniu koncentrácie krvných bielkovín delených elektroforézou na papieri. Biológia SAV, 17:690, 1962.
Paulov Š., Gírethová G., Kostolanská A.: Použitie roztokov KOH pri určovaní koncentrácie bielkovín rozdelených elektroforézou na papieri. Biológia SAV, 17:841, 1962.
Paulov Š., Gírethová G., Veselovský J.: Modifikácia elektroforeo-polarografickej analýzy sérových proteínov. Čs. fysiol., 12:284, 1963.
Paulov Š., Hupka Š.: Príspevok k poznaniu biologických poločasov bielkovín krvného séra značkovaných ^{131}I u králikov. Biológia SAV, 14:142, 1959.
Penn N. W., Mandels S., Anker H. S.: On the kinetics of turnover of serum albumin. Bioch. Biophys. Acta, 26:349, 1957.
Pressman D., Eisen H. N.: The zone of localization of antibodies. J. Immunol., 64:273, 1950.
Richmond J. E., Ord M. G., Stocken L. A.: The effect of *x*-radiation in vivo on protein and nucleoprotein metabolism in the rat. Biochem. J., 66:123, 1957.
Riley R. T., Coleman M. K., Hokama Y.: *Cx*-reactive protein responses in the rabbit after whole-body irradiation. Rad. Res., 13:148, 1960.

Rodionov V. M., Čudinovskich A. V., Antokolskaj Ž. A., Loboda L. A.: Vključenija metionina S^{35} v syvorotočnye belki oblučennych sobak perenesčich krovopoterju. Bjul. exp. biol. i med., 6:43, 1959.
Rusnák J.: Príspevok k poznaniu vazomotorických zmien pri klinickej a experimentalnej atheroskleroze. (Kand. diz. práca), Bratislava, 1961.
Rust J. H., Folmar G. D., Lane J. J., Trum B. F.: The lethal dose of total body cobalt Co^{60} gama radiation for the rabbit. Am. J. roent., 74:135, 1955.
Scheiffarth F., Berg G.: Serumeiweissbestimungen bei Ratten und Kaninchen mit der Papierelektrophorese. Z. ges. exp. Med., 119:550, 1952.
Schultze B.: Biologische Halbwertszeit einzelner Globulin Fraktionen beim Kaninchen. Biochem. Z., 329:144, 1957.
Schultze B., Maurer W.: Über im Organismus des Kaninchens nach Injektion von S^{35} Beta und Gama Globulinen entstehende S^{35} Albuminen. Biochem. Z., 329:127, 1957.
Schwiegk H.: Künstliche radioaktive Isotope in Physiologie, Diagnostik und Therapie. Berlin, 1953.
Seniów A.: Quantitative pattern of electrophoretic serum fractions in healthy rabbits. Zool. Pol., 8:283, 1957.
Shekarchi J. C., Makinodan T.: Electrophoretic analyses of sera from mice protected from lethal *x*-radiation. Int. J. Rad. Biol., 2:353, 1960.
Shulman N. R., Tagnon H. J.: Proteolytic activity determined with a substrate tagged with radioactive iodine. J. biol. chem., 186:69, 1950.
Slavík K.: Biochemické vyšetřovací metody. Praha, 1960.
Smoličev E. P.: Elektroforetičeskoe issledovanie dinamiki vključenija metionina v belkovye frakcii syvorotki krovi krolikov pri vozdejstvii masivnych doz beta radiacii. Trudy Vsesoj. konf. med. radiol., 100, Moskva, 1957.
Steinfeld J. L., Paton R. R., Flick A. L., Milch R. A., Beach F. E., Tabern D. L.: Distribution and degradation of human serum albumin labeled with I^{131} by different techniques. Ann. New York Acad. Sci., 70:109, 1957.
Stender H. St. Elbert O.: Tierexperimentelle Untersuchungen über die Beeinflussung des Serumeiweissbildes durch eine Ganzbestrahlung mit 500 und 1000 r. Strahlentherapie, 89:275, 1953.
Sterling K.: The turnover rate of serum albumin in man as measured by I^{131} tagged albumin. J. clin. Invest., 30:1228, 1951.
Stevens K. M., Gray J., Schwartz M. S.: Effects of irradiation on anabolism of antibody and of serum albumin and globulin. Am. J. Physiol., 175:141, 1953 a.
Stevens K. M., Gray J., Schwartz M. S.: Catabolism of antibody and of serum albumin and globulin. Am. J. Physiol., 175:147, 1953 b.
Suplee H., Hauschild J. D., Entenmann C.: Plasma proteins and plasma volume in rats following total-body *x*-irradiation. Am. J. Physiol., 169:483, 1952.
Swahn B.: A method for localisation and determination of serum lipids after electrophoretical separation on filter paper. Scand. J. Clin. Lab Invest., 4:98, 1952.
Takeda Y., Franks J. J., Reeve E. B.: Preparation and behaviour in man of appareatly satisfactory ^{131}I-labelled human serum albumin. Fed. Proc., 18:155, 1959.
Terres G., Hughes W. L., Wolins W.: Whole-body measurement of radioactivity as a means of following in vivo the degradation of I^{131} labelled proteins in mice. Am. J. Physiol., 198:1355, 1960.
Tukačinskij S. E., Moisieva V. P.: *Cx* reaktivnyj belok pri lučevoj bolezni. Biochim. i biofiz., 8:48, 1961.
Veit H.: Das Verhalten der Serumproteine bei der Bestrahlung gynäkologischer Erkrankungen. Strahlentherapie, 90:148, 1953.
Waldschmidt-Lietz E., Keller L.: Über den Einfluss der Bestrahlung auf die Zusammensetzung der Serumproteine. Hope-Seyler's Z. physiol. Chem., 323:88, 1961.
Walter H., Haurowitz F.: Turnover of young and old serum proteins. Science, 128:140, 1958.
Walter H. et al.: The metabolic fate of injected homologous serum proteins in rabbits. J. biol. chem., 224:107, 1957.
Wasserman K., Mayerson H. S.: Exchange of albumin between plasma and lymphy. Am. J. Physiol., 165:15, 1951.
Westphall U., Priest S. G., Stets J. F., Selden G. L.: Influence of whole body *x*-irradiation. Am. J. Physiol., 175:424, 1953.
Wiedermann D.: Klinický význam elektroforesy na filtračním papíře. Lék. listy, 7, 22:543, 1952.

Wiggans D. S., Burr W. W., Rumsfeld H. W.: Metabolism of serum proteins. Arch. Bioch. Biophys., 72:169, 1957.
Winkler C., Paschke G.: Protein content and composition of rat serum as related to amount of whole-body x-irradiation. Rad. Res., 5:156, 1956.
Wolstenholme G. E. W., Millir E. C. P.: Ciba foundation symposium on paper electrophoresis. London, 1956.
Yalow R. S., Berson S. A.: Chemical and biological alterations induced by irradiation of I^{131} labeled human serum albumin. J. clin. Invest., 36:44, 1957.
Yeoman W. B.: A study of paper protein electrophoresis with special reference to a new method of subfractionation. Clin. chim. Acta, 4:523, 1959.
Zícha B., Kalousová V., Kučera K.: Changes in the serum proteins of dogs subjected to x-ray irradiation. Physiol. Bohemoslov., 8:137, 1959.

Adresa autora: *Katedra zoológie PFUK, Bratislava, Moskovská ul. 2.*
Do redakcie dodané 15. XII. 1962.

BIELKOVINY KRVNÉHO SÉRA KRÁLIKOV V NORMÁLNYCH A RADIAČNÝCH PODMIENKACH

Š. Paulov

Súhrn

V predkladanej práci sa rešia niektoré problémy pracovných metód používaných pri štúdiu fyziológie bielkovín krvného séra a na podklade experimentálnych výsledkov sa podávajú tieto závery:

1. Spektrum množstiev bielkovín krvného séra je druhove typické.

2. Spektrum množstiev polarograficky aktívnych skupín sérových proteínov je druhove typické a nezhoduje sa s množstvami bielkovinových frakcií.

3. Spektrum množstiev naviazaného J^{131} in vitro u sérových proteínov je typické a nezhoduje sa s množstvami bielkovinových frakcií.

4. Bielkoviny krvného séra sa vyznačujú nerovnakou rýchlosťou katabolizmu a anabolizmu.

5. Spektrum množstiev inkorporovaného mentionínu S^{35} v sérových proteínoch (po dosiahnutí maxima inkorporácie) je typické a nezhoduje sa s množstvami bielkovinových frakcií.

6. U ožiarených králikov vyskytujú sa typické zmeny v spektre množstiev bielkovín krvného séra a niekedy bielkoviny typu *X*.

7. Zmeny v množstvách sérových proteínov ožiarených králikov sú podmieňované zmenami ich katabolizmu a anabolizmu.

БЕЛКОВЫЕ ВЕЩЕСТВА КРОВЯНОГО СЕРУМА У КРОЛИКОВ В ОБЫКНОВЕННЫХ И РАДИАЦИОННЫХ УСЛОВИЯХ

Ш. Паулов

Резюме

В предлежащей работе решаются некоторые проблемы рабочих методов применяемых при исследовании физиологии кровяного серума и на основании экспериментальных результатов подаются следующие выводы:

1. Спектр количеств белковых веществ кровяного серума является по виду типическим.

2. Спектр количеств полярографически активных групп серумовых протеинов является по виду типическим и не согласуется с количествами белковинных фракций.

3. Спектр количеств навязанного J^{131} in vitro у серумовых протеинов является типическим и не согласуется с количествами белковинных фракций.

4. Белковые вещества кровяного серума отличаются неодинаковой скоростью катаболизма и анаболизма.

5. Спектр количеств инкорпорованного метионина S^{35} в серумовых протеинах (после достижения максимума инкорпорации) типический и не согласующийся с количествами белковинных фракций.

6. У облученных кроликов настают типические перемены в спектре количеств белковинных веществ кровяного серума и иногда встречаются белковые вещества типа *X*.

7. Перемены в количествах серумовых протеинов у облученных кроликов обусловляемы переменами катаболизма и анаболизма.

ACTA F. R. N.: UNIV. COMEN. VIII–4., ZOOLOGIA, 1963

ACTA FACULTATIS RERUM NATURALIUM UNIVERSITATIS COMENIANAE

TOM. VIII. FASC. IV. ZOOLOGIA 1963

Quantity spectra of polarographically active groups of blood serum proteins in fishes (t i n c a t i n c a L.) and in frogs (R a n a t e m p o r a r i a L.)

Š. P a u l o v, S. K m e ť o v á

In the course of studying the quantities of polarographically active groups of serum proteins in different animals we have found, that there is no accordance between the spectra of polarographically active groups and the spectra of quantities of protein fractions and that both spectra are typical in species.

In the proposed work we have taken for our aim to analyse the above-mentioned types of spectra in *Tinca tinca* L. and in *Rana temporaria* L., for which the analyses we determined the spectra by method described in the work P a u l o v (1963).

The quantity spectrum of serum proteins as well as the spectrum of polarographically active groups in *Tinca tinca* L. are represented on fig. 1, in *Rana*

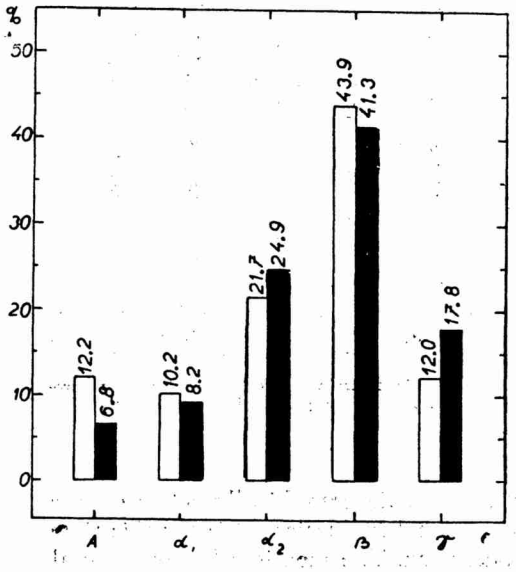


Fig. 1. Quantity spectrum of proteins and quantity spectrum of polarographically active groups in fractions (in p. c.) in *Tinca tinca* L.

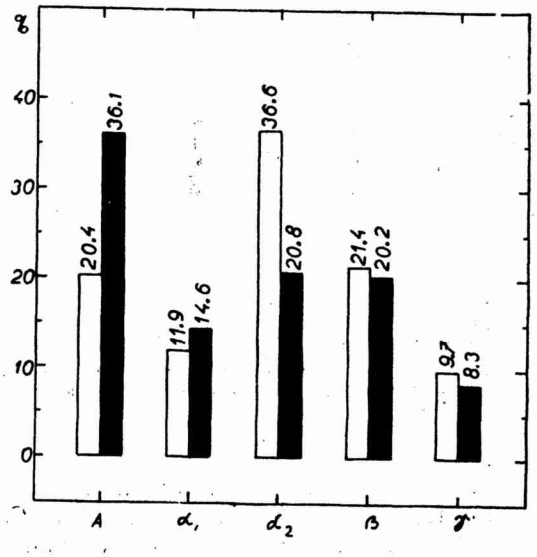


Fig. 2. Quantity spectrum of proteins and quantity spectrum of polarographically active groups in fractions (in p. c.) in *Rana temporaria* L.

temporaria on fig. 2. From figures mentioned it is evident that both spectra are quite different and that they are typical for species of animals.

On fig. 3 there are shown the quantities of polarographically active groups in relation to unit quantities of serum proteins in *Tinca tinca*, on fig. 4 there are theese data for *Rana temporaria*.

From above-mentioned experimental results may be concluded that spectrum

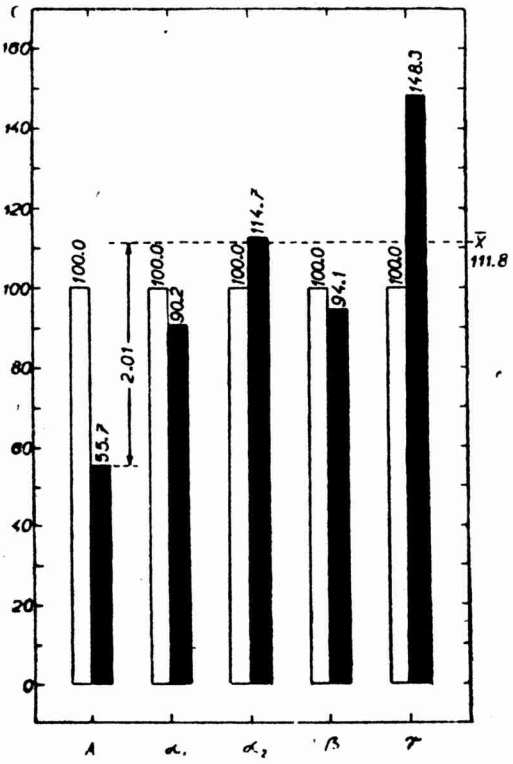


Fig. 3. Quantities of polarographically active groups ■ relative to unity quantities of serum proteins □ in *Tinca tinca* L.
$\overline{x}$ – arithmetic mean value of quantities of polarographically active groups in total globulins.

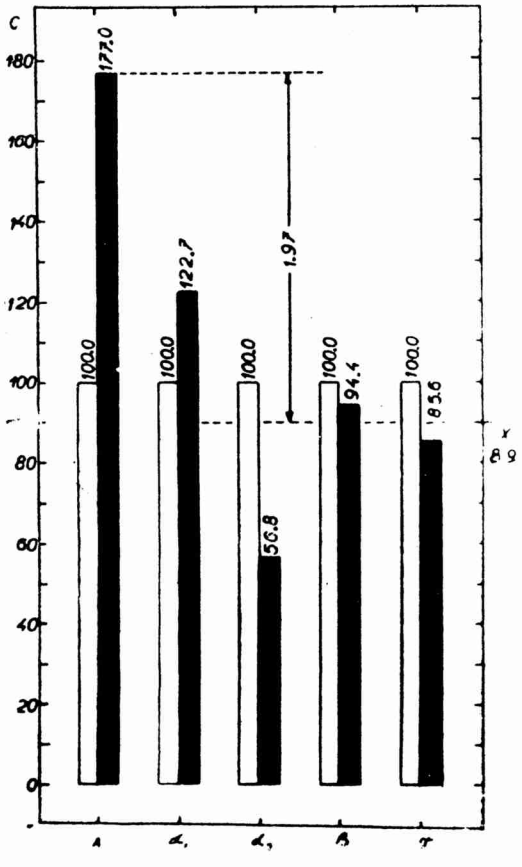


Fig. 4. Quantities of polarographically active groups relative to unity quantities of serum proteins □ in *Rana temporaria* L.
$\overline{X}$ – arithmetic mean value of quantities of polarographically active groups in total globulins.

of serum-protein quantities as well as spectrum of polarographically active groups are for *Tinca tinca* and for *Rana temporaria* typical in species. It is for this time not possible to give a generally valuable conclusion. The mentioned experimental results are only a part of a larger complex of problems in the comparative physiology of blood serum proteins.

Summary

The authors analyse the quantity spectra of blood serum proteins and the quantity spectra of polarographically active groups in these fractions in fishes *Tinca tinca* L. and in frogs *Rana temporaria*. 2. The authors conclude that both spectra are typical in species.

References

Paulov Š.: Blood-serum proteins of rabbits under normal and radiative conditions. Acta F. R. N. Univ. Comen., 8:175, 1963.

Adresa autorov: *Katedra zoológie PrFUK, Bratislava, Moskovská ul. 2.*

Do redakcie dodané 15. XII. 1962.

SPEKTRÁ MNOŽSTIEV POLAROGRAFICKY AKTÍVNYCH SKUPÍN BIELKOVÍN KRVNÉHO SÉRA RÝB (*Tinca tinca* L.) A ŽIAB (*Rana temporaria* L.).

Š. Paulov, S. Kmeťová

Súhrn

Autori v predkladanej práci analyzujú spektrá množstiev bielkovín krvného séra, ako aj spektrá množstiev polarograficky aktívnych skupín v týchto frakciách u rýb *Tinca tinca* L. a žiab *Rana temporaria* L. Zisťujú, že obe spektrá sú druhove typické.

СПЕКТРЫ КОЛИЧЕСТВ ПОЛЯРОГРАФИЧЕСКИ АКТИВНЫХ ГРУПП БЕЛКОВЫХ ВЕЩЕСТВ КРОВЯНОГО СЕРУМА У РЫБ (*Tinca tinca* L.) И ЛЯГУШЕК (*Rana tamporaria* L.)

Ш. Паулов, С. Кметьова

Резюме

В предлагаемой работе авторы анализируют спектры количеств белковых веществ кровяного серума как также спектры количеств полярографически актирных групп в тех фракциях у рыб *Tinca tinca* L. и у лягушек *Rana tamporaria* L. Было установлено, что оба спектры являются по виду типическими.

ACTA F. R. N. UNIV. COMEN. VIII.—4, ZOOLOGIA 1963

ACTA FACULTATIS RERUM NATURALIUM UNIVERSITATIS COMENIANAE
TOM. VIII. FASC. IV. ZOOLOGIA 1963

About some changes in hen eggs during the incubation

J. Veselovský

In our laboratories we are studying some physiological problems in incubated eggs, because there is not enough attention devoted to this research.

1. The total weight during the incubation-rime. We have

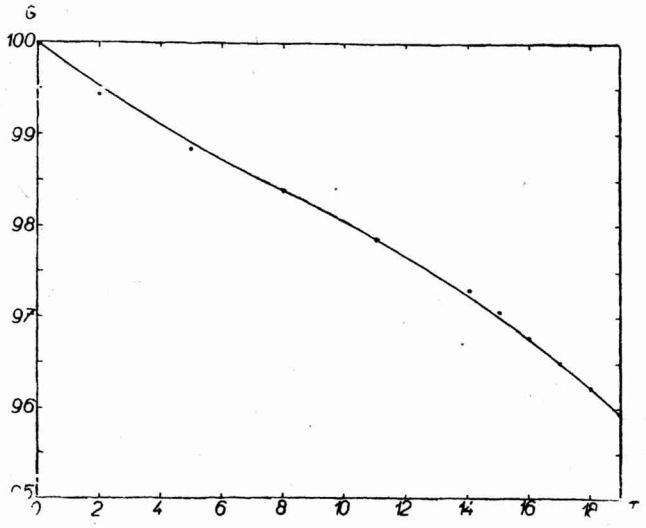


Fig. 1. Changes in weight of incubated eggs.

ascertained, that the weight of eggs (12 pieces) during incubation constantly diminished. This decrease was regular and attained approximatelly 2 grams in 30 days. The whole dynamics of the weight changes is drawn on fig. 1 an on table 1.

The asserted experimental results of weight changes are typical. It is possille to set them partially in correspondence with the literary data of authors as Teodorjan et al. (1961), in which we found but orientative and unre gularly obtained data.

2. The consistence of the egg-white. We have investigated the white of eggs in a series of eggs, in which we have followed the weight changes in

Table 1. The table of changes of weight in incubated eggs.

Nr		Days of incubation										
		0	2	5	8	11	14	15	16	17	18	19
1		60.40	60.05	59.75	59.50	59.25	59.00	58.90	58.80	58.70	58.55	58.45
2		59.30	59.00	58.75	58.43	58.10	57.85	57.75	57.65	57.55	57.45	57.35
3		54.50	54.10	53.85	53.65	53.35	53.05	52.85	52.70	52.55	52.40	52.25
4		58.10	57.80	57.55	57.35	57.12	56.87	56.75	56.60	56.45	56.35	56.20
5		60.30	60.04	59.72	59.42	59.17	58.87	58.75	58.60	58.45	58.30	58.15
6		50.35	50.10	49.75	49.50	49.25	48.95	48.85	48.70	48.55	48.40	48.25
7		51.85	51.55	51.25	51.05	50.75	50.50	50.40	50.25	50.15	50.00	49.85
8		56.90	56.68	56.35	56.05	55.80	55.45	55.30	55.15	54.95	54.80	54.60
9		51.40	51.15	50.75	50.55	50.30	50.00	49.85	49.70	49.55	49.40	49.25
10		55.40	55.05	54.65	54.40	54.05	53.70	53.55	53.35	53.20	52.95	52.75
11		51.10	50.90	50.65	50.45	50.25	50.00	49.90	49.80	49.65	49.55	49.45
12		48.20	47.75	47.30	46.85	46.45	45.90	45.70	45.45	45.15	44.95	44.75
$\bar{x}$	g	54.82	54.51	54.19	53.93	53.65	53.34	53.21	53.06	52.91	52.76	52.61
	%	100.00	99.43	98.85	98.38	97.87	97.30	97.06	96.79	96.52	96.24	95.97
$\delta \pm$		4.15	4.21	4.25	4.25	4.27	4.31	4.33	4.35	4.38	4.39	4.41
x_{min}		48.20	47.75	47.30	46.85	46.45	45.90	45.70	45.45	45.15	44.95	44.75
x_{max}		60.40	60.05	59.75	59.50	59,25	59.00	58.90	58.80	58.70	58.55	58.45

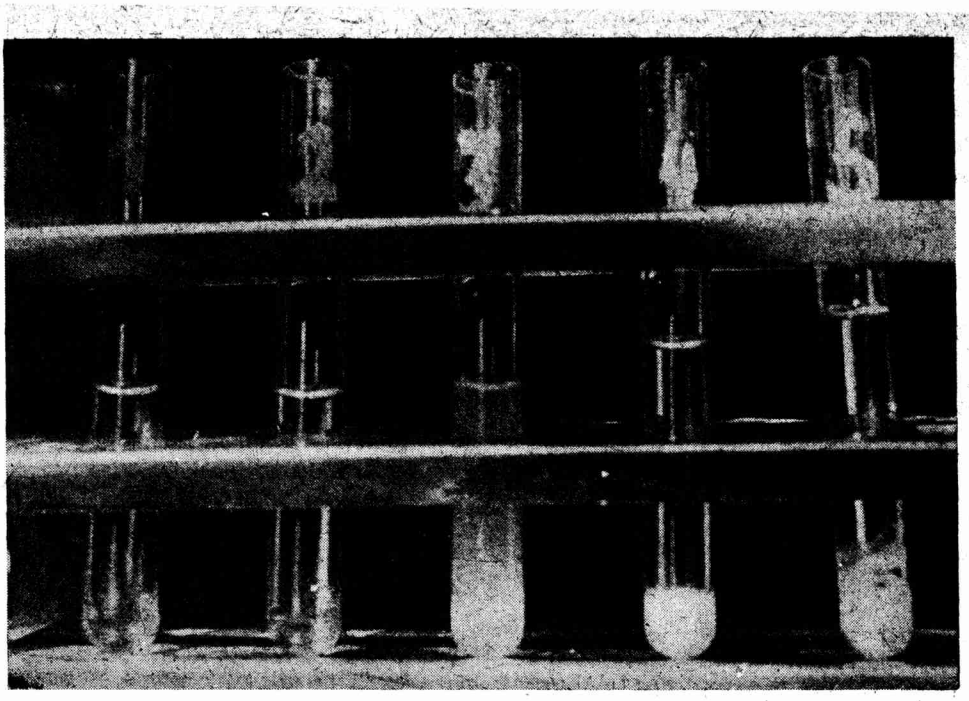


Fig. 2. The densification of egg-white and the falling out of gel from albumin-solutions.

1-th	– control test	(dilution 1:2)
2-th	– 5-th dav of incubation	(dilution 1:2)
3-d	– 8-th day of incubation (clouding)	(dilution 1:2)
4-th	– 11-th day of incubation (gel)	(dilution 1:4)
5-th	– 14-th day of incubation (gel)	(dilution 1:4)

intervals of two or three days. We have diluted this eggs-white for the necessity of further researches in a proportion 1:2 till 1:4 with veronal-acetate buffer pH 9 (according to Dittmer 1956). We have ascertained that with the proceeding incubation it there is a densification of egg-white and that it begins at las to create a kind of gel. This gelatinous substance (see fig. 2) is indissoluble in concentrated mineral acids. (For this time it is not possible to give a more precise identification of this gel.)

3. Proteins concentration in egg-white. A diluted egg-white was divided by electrophoresis on paper in ovoalbumins and ovoglobulins (by Paulov —1961). After staining the fractions with bromphenol blue, we cut the strip on places of minimal stain intensity and eluted the sections partly in 0,05 N KOH, partly in 2,05 % carbonate and 25 % methylalcohol. The values of extinctions were checked through calibrated charts of concentrations. The values befre incubation were set at 100 %, while the successive values were determined by comparisom. We have found out that in course of the incubation it came to an increase of protein concentration in egg white. There was a growth of concentration in the shape of an S curve with a rapid ascent in the 5-th — 8-th day. The change of ovoglobulin fraction is shown for documentation on fig. 3. Ovoalbumins show a similar change too. We had no succes to find until now in accessible special literature the mentioned character of changes.

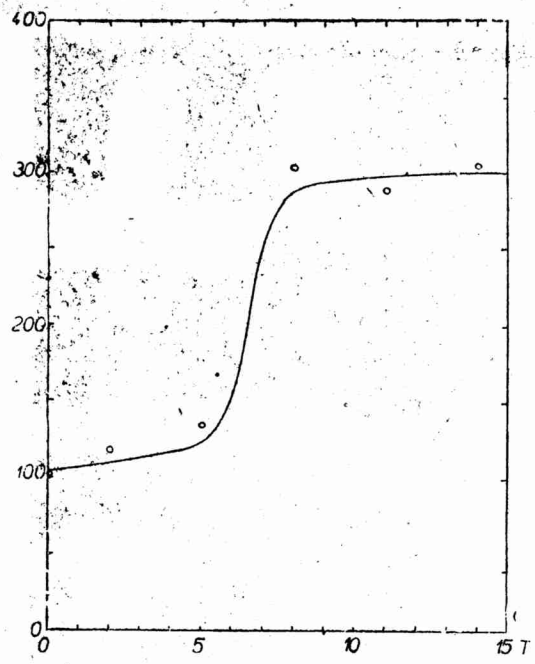


Fig. 3. The change of concentration of ovoglobulins during the incubation.

T represents the incubation-time in days, *G* — the change of concentration in percent in comparison with the control test.

In egg and egg-white the obtained results show that take place intensive changes in determined dependencies of time. Their detailed study will be the object of our further investigations.

Sumary

Author analyses some weight changes in incubated eggs as well as some alteretions in egg-white. The weight reduction is approximatively linear in time and the concentration of proteins rises corresponding to an S formed curve with incubation progress and showing a critical change on the 5th day. During incubation a formetion of gel of egg-white was observed. This was not diluted in conc. hydroxides or concentrated anorganic acids.

References

Dittmer A.: Papierelektrophorese, Jena, 1956.

Mátl J.: Liahnutie hydiny, Bratislava, 1959.

Paulov Š.: Einwirkungen von Ionisationsstrahlung auf den Metabolismus von Serumeiweißstoffen bei Kaninchen, Biologické práce SAV 7/2, Bratislava, 1961.

Teodorjanu N., Burlaku G., i Opresku St.: Izučenie zarodyščevogo i poslezarodyščevogo obmena veščestv u cypljat porod Leggorn i Rod-Ajland i u ich metisov v svjazi s javleniem geterozisa, Rev. Biol. 6, 4, 449, 1961.

Adresa autora: *J. Veselovský, Katedra zoologie UK Bratislava, Moskevská 2.*

Do redakcie dodané 15. 12. 1962.

O NIEKTORÝCH ZMENÁCH SLEPAČÍCH VAJEC POČAS INKUBÁCIE

Súhrn

J. Veselovský

Autor v práci analyzuje niektoré váhové zmeny u inkubovaných vajec, ako aj niektoré zmeny vo vaječnom bielku. Zisťuje, že pokles váh inkubovaných vajec je približne lineárny a že v bielku s postupujúcou inkubáciou stúpa koncentrácia proteínov podľa S-ovitej závislosti s kritickou zmenou na 5. deň. V priebehu inkubácie pozorovalo sa vypadávanie gélu z bielka, ktorý sa nerozpúšťal v koncentrovaných hydroxydoch ani v koncentrovaných minerálnych kyselinách.

О НЕКОТОРЫХ ПЕРЕМЕНАХ КУРИНЫХ ЯИЦ В ТЕЧЕНИЕ ИНКУБАЦИИ

Я. Веселовский

Резюме

Автор анализирует в настоящей работе некоторые перемены веса у инкубированных яиц, как также некоторые перемены в яичном белке. Он установляет, что понижение весов инкубированных яиц является приблизительно линеарным и что в белке с продвигающейся инкубацией повышается концентрация протеинов по зависимости S-видной с критической переменой в 5 день. В течение инкубации наблюдалось выпадивание желе из белка, которое не растворялось ни в концентрированных гидроксидах ни в концентрированных минеральных кислотах.

ACTA FACULTATIS RERUM NATURALIUM UNIVERSITATIS COMENIANAE

sú fakultný sborník určený k publikáciám vedeckých prác interných a externých učiteľov našej fakulty, interných a externých ašpirantov a našich študentov. Absolventi našej fakulty môžu publikovať práce, v ktorých spracovávajú materiál získaný za dobu pobytu na našej fakulte. Redakčná rada vyhradzuje si právo z tohto pravidla urobiť výnimku.

Práce musia byť doporučené katedrou. Práce študentov musia byť doporučené študentskou vedeckou spoločnosťou a príslušnou katedrou.

Publikovať možno v jazyku slovenskom alebo českom, prípadne v ruskom alebo anglickom, francúzskom alebo nemeckom. Práce podané na publikovanie majú byť písané strojom na jednej strane papiera, ob riadok, tak aby jeden riadok tvorilo 60 úderov a na stránku prípadlo 30 riadkov. Rukopis treba podať dvojmo a upraviť tak, aby bolo čo najmenej chýb a preklepov. Nadmerný počet chýb zdražuje tlač a ide na účet autora.

Rukopis upravte tak, že najprv napíšete názov práce, pod to meno autora. Pracovisko, pokiaľ je na našej fakulte, sa neuvádza. Iba tam, kde je viac spolupracovníkov a niektorý z nich je z mimofakultného pracoviska, sa uvádzajú všetky pracoviská. Tiež tam, kde práca bola vypracovaná na dvoch pracoviskách, treba ich obidve uviesť.

Fotografie načim podať na čiernom lesklom papieri a uviesť meno autora, zmenšenie a text pod obrázok. Kresby treba previesť tušem na priehladnom papieri (pauzák) alebo na rysovacom papieri a taktiež uviesť meno autora, zmenšenie a text pod obrázok.

Každá práca musí mať resumé v ruskom a niektorom západnom jazyku. K prácam, publikovaným v cudzom jazyku, načim pripojiť resumé v slovenskom (českom) jazyku a v jazyku západnom v prípade publikácie v ruskom jazyku, alebo v ruskom jazyku v prípade publikácie v jazyku západnom. *Nezabudnite pri resumé uviesť vždy názov práce a meno autora v rovnakom poradí ako v základnom texte.* Za správnosť prekladu zodpovedá autor.

Autori dostávajú stĺpcové a zlámané korektúry, ktoré treba do 3 dní vrátiť. Rozsiahlejšie zmeny behom korektúry idú na ťarchu autorského honoráru. Každý autor dostane okrem príslušného honoráru i 50 separátov.

Redakčná rada.