VARIATION OF COMPOUNDS RESULTED FROM THE PRINCIPAL BIOCHEMICAL CHANGES, ON ZONES, DURING THE RIPENING OF RAW RUMANIAN SAUSAGE

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During the ripening of sausages there are produced many biochemical changes from which result different compounds that contribute at the flavour and specific taste of the product together with those of the condiments, smoke compounds and salts mixture. Many papers treated separately different aspects of this problem, concerning one or other of the compounds, trying to know the complex mechanism of the ripening.

Bianchi (1), Giolitti (2), Maillet and Henry (3), Ninivara, Pohja, Comulainen (4), Körmendy and Gantner (5), Pezacki and Duda (6), Sokolov and Djabbarova (7) identified the free aminoacids from certain dry sausages, especially by qualitative and semiquantitative paper chromatography or paper electrophoresis. Most of them agree that in dry sausages an accumulation of free aminoacids takes place, especially in the first stage of the ripening (5,6).

In the dry sausage there is a great amount of fat, that can produce by hydrolysis free fatty acids, on which Maillet and Henry (3), Szeredy (8), Dolezalek (9) presented certain data, while Duda (10) determined the quantitative changes of individual free fatty acids. Also Cantoni, Molnar, Renon and Giolitti (11), using gaschrom-

tography, made a study on the volatile and un volatile free fatty acids and on the carbonylic compunds, atributting a great role to the micrococs in the production of this compounds from the fat.

Owing to the importance of this problem, some research was started at the Food Research Institute of Bucarest, in order to know better the biochemical changes in the Rumanian dry sausage (Sibiu salami), during ripening.

We have already found, in previous experiments, that the free aminoacids amount increased very much in the first stage, at the same time with the great development of microflora and decreased a little in the final one (12). There were no important differences between the salami obtained in natural and artificial climate conditions (12), as well as between the smoked and un smoked ones (13,14),

In the present paper we studied the proteolytical and lipolytical changes on the whole, using characteristic indexes, in order to have a better view of these changes, considering that the resulted compounds contribute together at the flavour of the product.

We determined these compounds from the first stage of salami paste till the final product. Considering that the biochemical changes will be different in the middle and in the external zone of the sausage, the determinations were made on two sections, outer and inner of each stick, conventionally delimited.

Material

The sausage, known under the name "Salam de Sibiu" is a raw, smoked and dry product, optained from pork, lard and a mixture of salts and condiments. The salam used in these experiments was obtained during loo days in artificial climate conditions of ripening. The determinations were made on samples obtained from 3-4

sticks, taken at 1,7,17,40,50,67,89,101,120 days. Before use, it was kept in a refrigerator at 2-5°C. After cutting the sticks in 5 cm long pieces and after stripping off the rind two zones were separated, the inner and the outer ones, with 3/4, respectively 1/4, from the stick radius, in order to obtain two parts equal in weight. The comminution was made in conditions of cold, to prevent the fat separation.

We used chored material-for the moisture, pH, salt, determinations-and differents extracte from it, for the other ones.

Extract preparation

a. - Extraction with 80% ethyl alcohol, for free aminoacids determination: 20 g minced material was extracted with 200 ml 80% ethyl alcohol in a homogenizer type Atomix. After standing a night in a refrigerator, it was filtered at low temperature too.

b. - Extraction with 5% trichloracetic acid, for amononitrogen and ammonia-nitrogen determination: lo g comminuted material was extracted with loo ml 5% trichloracetic acid, cooled before use, in an Atomix type homogenizator and immediately filtered in the refrigerator.

c. - Fat extracts in chloroform (1:3) for free acidity and peroxide number and (1:10) for carbonyl value and benzidine test.

5g chopped material was placed with 5g anhidrous sodium sulfate and 50 ml solvent, in a stoppered bottle which, after closing, was strongly shaken. After standing in a water bath at 45-50°C for 5-10 min, the bottle being not completely closed, it was centrifuget at 3000 rpm for lo minutes and decanted in a stoppered cylinder, through a funnel with some cotton wool in it.

The fat content was determined on lo ml extract in an alumi-

nium dish, by evaporation and drying at 105°C.

Methods

The proteolytical changes were studied by determining amino-nitrogen, ammonia-nitrogen and free aminoacids.

Amino-nitrogen was determined by the spectrophotometric analysis of copper complexes of aminoacids, after Spiess and Chambers (15), with some modifications: using an extract in trichloracetic acid, a previous neutralization with IN sodium hydroxide against tymolphtalein was necessary and the standard curve was made with 0.3% -alanin in 5% trichloracetic acid.

Ammonia-nitrogen was determined by the spectrophotometric evaluation of the indophenolic dye resulted from the reaction between the NH_4^+ ion and sodium hypochlorite and phenol, according to Rachovan and Tzviling (16) with some modifications. We used the extract with trichloracetic acid, tenfold diluted, from which 5 ml were neutralized with the necessary amount of IN sodium hydroxide, separately determined, brought to 10 ml with the above mentioned reagents. The standard curve was made with a solution of $(NH_4)_2SO_4$ in 5% trichloracetic acid (4 NH_4^+/ml).

Free aminoacids were determined by monodimensional ascending paper chromatography, after extracts purification on strong acidc cation exchange resin. A previous identification was made by bidimensional paper chromatography (17). The quantitative determination was made spectrophotometrically (18).

The lipolytical changes were studied by determining the free fatty acids, the peroxide-and carbonyl-number and by benzidine test, on the fat extracted from salami.

Peroxide number was determined by the iodometric method on 10

ml chloroform extract diluted with 15 ml acetic acid.

Carbonyl number was determined with 2.4-dinitrophenyl-hydrazine according to Henick,Benca,Mitchell (19) on 2.5 ml chloroform extracted fat, in a 25 ml volumatric flask. The extintion was measured at 440 m and the carbonyl number was calculated according to Birden,Lauchard and Lowry (20).

Benzidine- test was made, according to Koudela (21), on 20 ml chloroform extracted fat.

Results and Discussions

With the values of the compounds resulted by proteolysis and lipolysis, number of diagrams was constructed. These curves show a clear difference in the variation and in the amount of these compounds from the two zones,the external and internal one,of the salami stick, during ripening, as well as the chemical characteristic indexes.

The moisture (fig.1) of the sausage decreased in a normal way, faster in the superficial layer, by evaporation, and more slowly in the internal one. The average humidity curves of the two zones devaite stepwise one from another, so in the middle of the drying-ripening period (40-50 days) the difference of the two zones consistency.

The pH showed an irregular variation,to 5,4 till 6,9 during the ripening,with some tens greater in the outer zone. Though free acidity of fat was increasing continuously,as well as one will see further,the pH did not vary systematically,showing that there is no concordance between them, thus confirming the affirmations of Coretti (23).

Salt content. The salt molecules migrated from the outer zo-

ne towards the inner one, more wet than the other, because of the particular affinity of sodium ion for water. So,in the inner zone,the salt content was higher than in the outer one, for example in the finished product 5.5 g % against 3.76%.

The proteolytical changes, with formation of aminoacids and other nitrogen-compounds, were more intensive in the internal zone of the sausage. They were studied by determining amino - nitrogen,ammonia-nitrogen and free aminoacids.

Amino-nitrogen, $N\text{-}NH_2$ (fig.2), increased at first,having in both zones the same value till the 17-th day,when it became twofold against the initial salami paste (0,629 g % as compared to 0,329 g %).Further,the both zones began to differentiate,the amino-nitrogen becoming gradually greater in the internal zone tiall about threefold at the middle of the ripening period(o.925 g % the 50-th day),while in the outer zone,it remained practically unchanged. Finally, the Sibiu salami had in the inner zone and in the outer zone a threefold, respectively twofold amount of amino-nitrogen compared to the initial one. The higher values of the inner section show a more active proteolysis in the middle of the stick, the microorganisms and enzymes being favoured by the humidity.

Amuonia-nitrogen $N\text{-}NH_3$ (fig.3), formed either by hydrolysis of glutamine,or by desamination of different aminoacids,varied gradually,with small differences between the two zones,a little more in the external one. These differences remained practically constant,the amount of ammonia-nitrogen rising stepwise during ripening. A more sensible increase appeared in the final period, because of the more intensive desaminations in both zones.

Free aminoacids (fig.4 a .....4m) final products of the proteolysis, showed also a stepwise variation. Most of them showed a continuous and marked increase in the first stage (40-50 days), the phase of primary phenomena of proteolysis, which corresponds to our previous experiments with other batches of salami (12,14) and agree with the observations of Körmandy and Gantner (5). Only the glutamine and arginine decreased rapidly during the maturation.

In the last stage of ripening, most of the aminoacids, after reaching the highest value, remained almost unchanged .

The variation of free aminoacids is different in the two zones, especially after 17 days; most of them have higher values in the inner zone, which agree with the values that we obtained for the amino-nitrogen and with the observations of Pezacki and Duda (6). This shows also that the proteolysis continued for a longer time in the inner zone than in the outer one, because of the humidity, which favoured the enzymes activity.

Moreover, it must be mentioned that the aminoacid differences between the two zones began to occur at the same time with the appearance of the mould on the rind.

The lipolytical changes. Under the action of some enzymes and of the atmospheric oxygen in the salami fat, phenomena of hydrolysis and oxidation occured.

The hydrolytical changes (fig.5) lead to the fat splitting into free fatty acids and glycerine. During the ripening the free fatty acids content of the fat increased till it became in the product-tenfold greater in the outer zone (9.75 g % oleic acid) and eightfold in the inner one (7.75 g % oleic acid), showing a very intensive and continuous hydrolysis.

In the first 40 days, including also the smoking stage, the free acidity of the fat increased continuously, being the same in both zones. After the 45th day, the fat acidity of the external zone became much greater than that of inner one, The curves became then almost parallel. This fact showed that in the outer zone the lipolysis was more intensive, due to the lipolytical enzymes and to the mould ifluence. As a matter of fact, the differentiation of the free acidity of the two zones took place simultaneously with the mould development on the salami stick.

The oxidative changes of the salami fat, due to the atmospheric oxygen, which diffuses through the rind and due to the lipoxydases, lead to the formation of peroxydes, carbonylic combinations and free lower acids. These changes were studied by determining the peroxide-, carbonyl-, benzidine number (table 1).

Peroxide number showed values from 0.7 - 2 mVal θ_2/kg, without sensible differences between the two zones. These low values are in agreement with the observations made - on the whole stick- by Maillet and Henry (3) and Szeredy (8).

Total carbonyl value, given by the aldehydes, ketones and ketoacids resulted from fat oxidation, presented little increase during ripening, more evident after smoking, because of the carbonylic compounds of the smoke. These low values, without marked differences between the two zones, showed that little alterations took place by oxidation.

Benzidine test with little increases, showed that there are a few aldehydes present in salami.

Generally, the low values of the indexes that characterize the fat oxidation showed that this one is reduced due especially to the anti-oxidizing action of some smoke substances. On the other

hand,the mentioned indexes record only the extant oxidation compounds remained uncombined,a part of them giving Maillard type reactions with aminoacids.

We must mention that the investigated salami was a good product,corresponding to the inquired organoleptic and chemical conditions.

Concluding remarks

This work represents a contribution to a more detailed knowledge of the biochemical changes that take place in the dry sausage,particulary the Sibiu salami,during the ripening,studying the proteolytical and lipolytical changes in two zones,interoal and external,of the salami stick.

We found a clear difference of the variation and of the amount of changes between tha two zones during the ripening.

The most intensive changes were the lipolytical ones with formation of free fatty acids,more marked in the outer zone,during the whole period of maturation.

On the contrary,the proteolytical changes,from which are resulting free aminoacids,were more intensive in the inner zone and they took place especially in the first stage(1 1/2-2 months)of the ripening.

These biochemical changes are reflected together in the flavour of the salami. Between the two zones were observed sensible differences which appeared gradually,starting especially in the second part of the ripening (after 40-50 days)and increasing towards the final stage. While the outer zone,which represents half of the stick weight,had a velvet- like taste,almost unsalted the inner zone had a marked salty taste associated with

a pleasant,specific and well-balanced,due especially to the free aminoacids,and a more pronounced flavour.

On the whole,the rumanian dry salami(Sibiu Salami) that we studied had a good taste and an agreable flavour.

TABLE 1.

Determinations of fat oxidation products

Determinations	Zone	1 day paste	7 days	17 days	4o days	5o days	67 days	89 days	1o1 days
Peroxide value mVal O_2/kg	E	o.7	o.8	1.o	1.2	1.9	1.3	1.5	1.4
	I	o.7	o.8	o.9	1.o	1.7	1.5	1.2	1.2
Carbonyl value g CO/g	E	9.6	1o.2	13.9	13.o	13.5	13.9	14.5	14.9
	I	9.6	1o.o	12.o	12.8	13.o	13.5	14.2	13.9
Benzidine test E1 cm(1 g fat in I 25ml so lution)	E	o.2o	o.25	o.37	o.44	o.57	o.5o	o.35	o.49
	I	o.2o	o.22	o.24	o.37	o.5o	o.46	o.34	o.45

E= external zone I= internal zone

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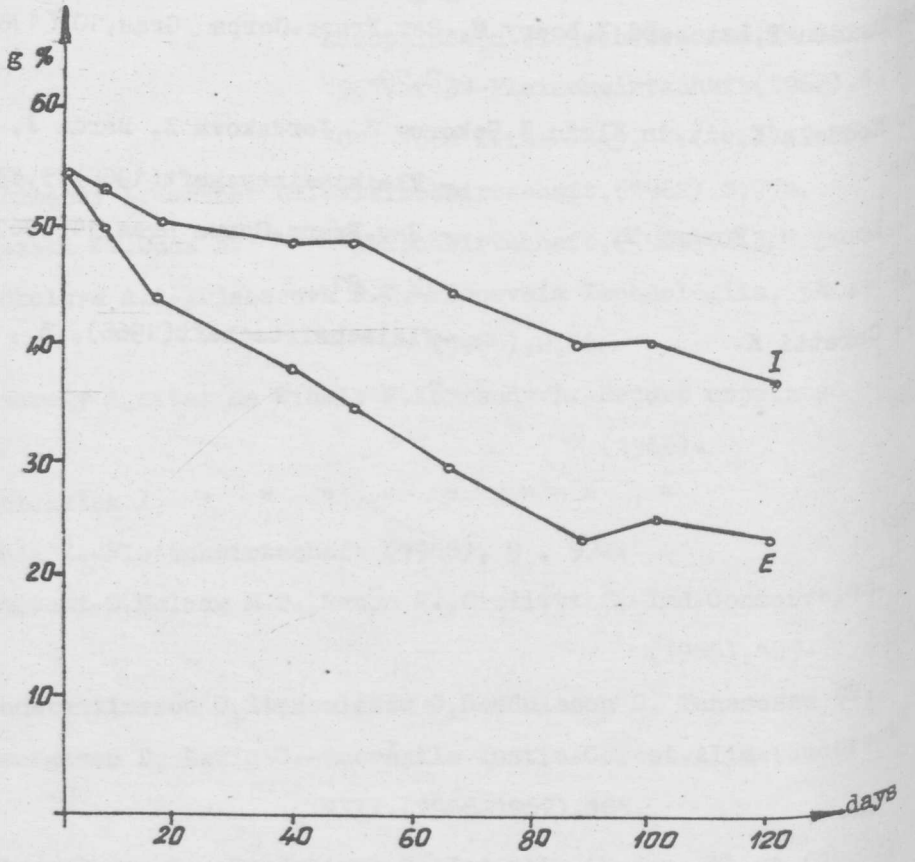


Fig. 1.

Amino - nitrogen

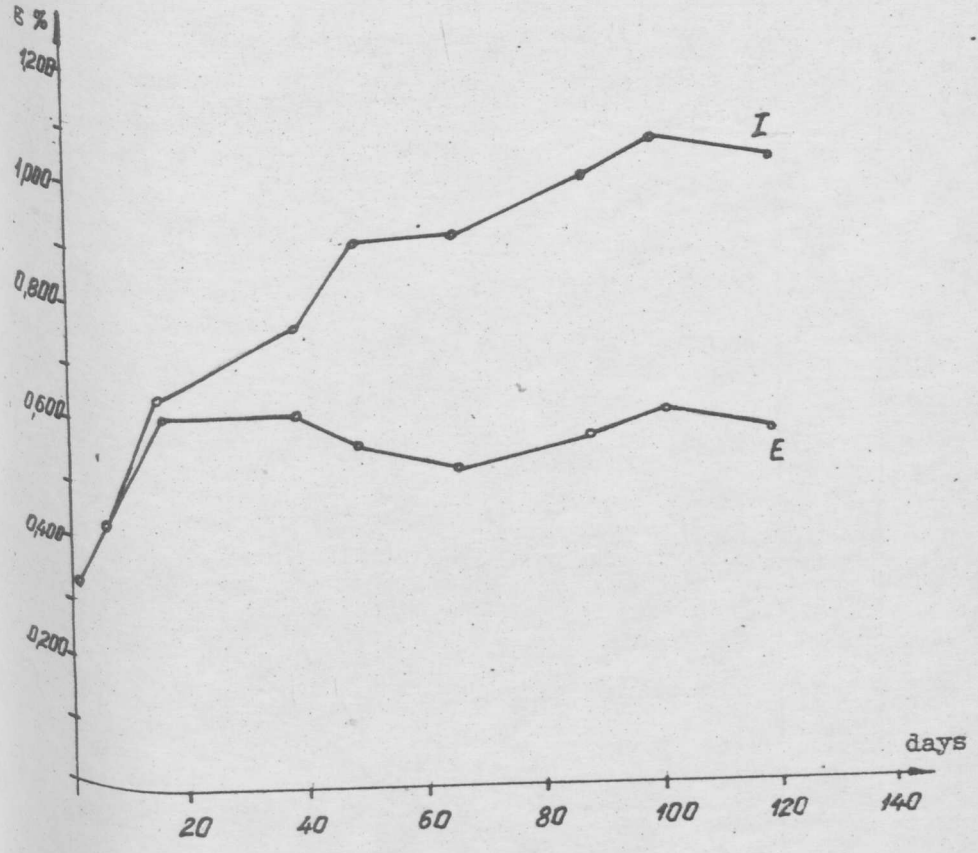


Fig. 2.

Ammonia - nitrogen

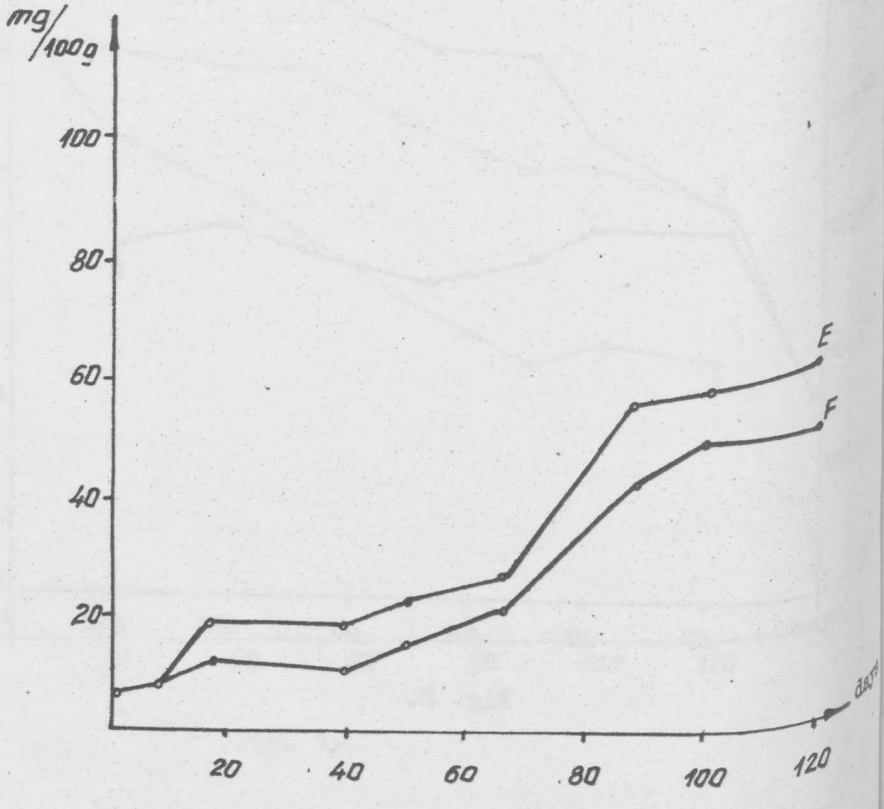


Fig. 3.

4a

Lysine

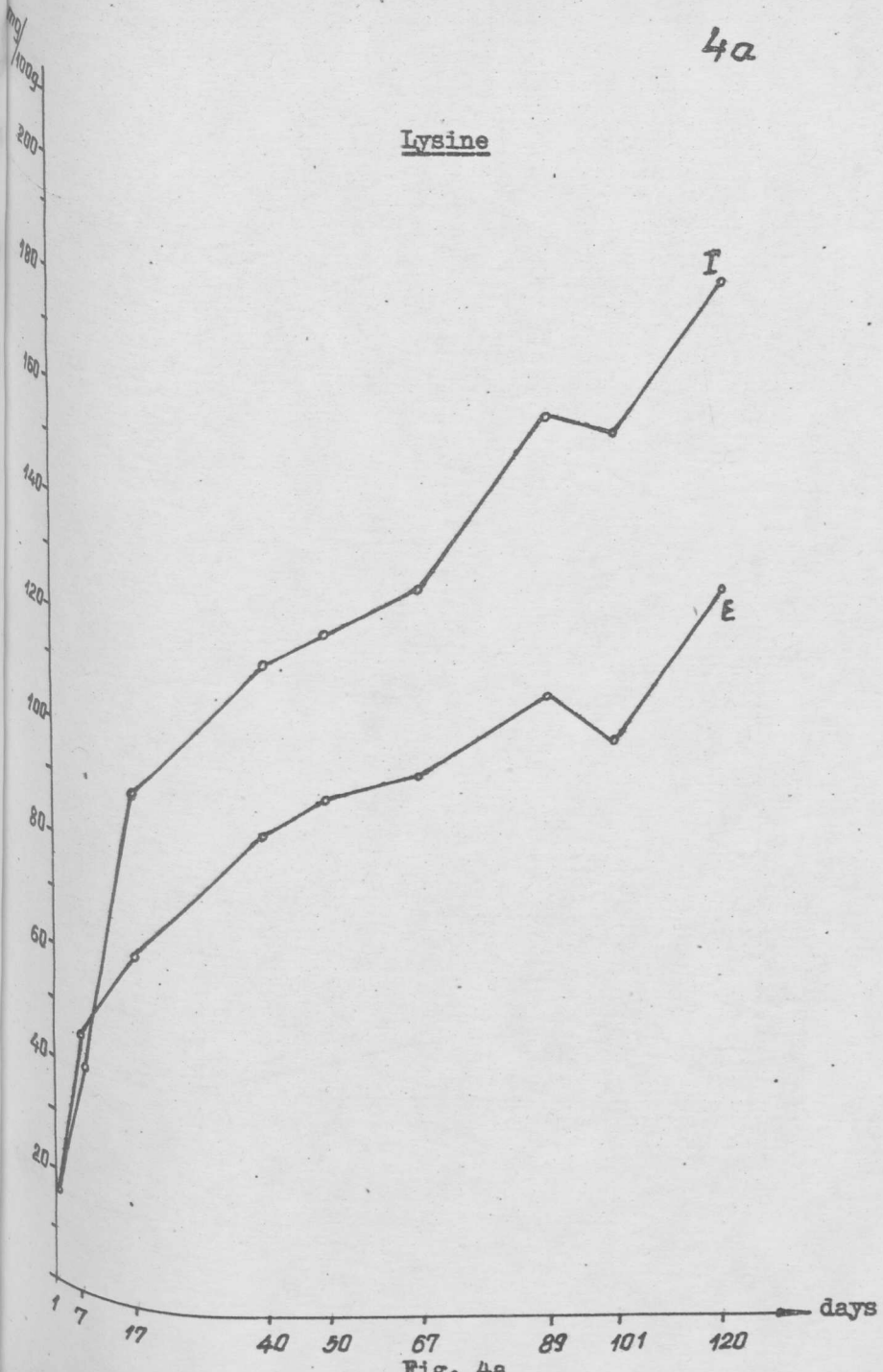


Fig. 4a

Arginine

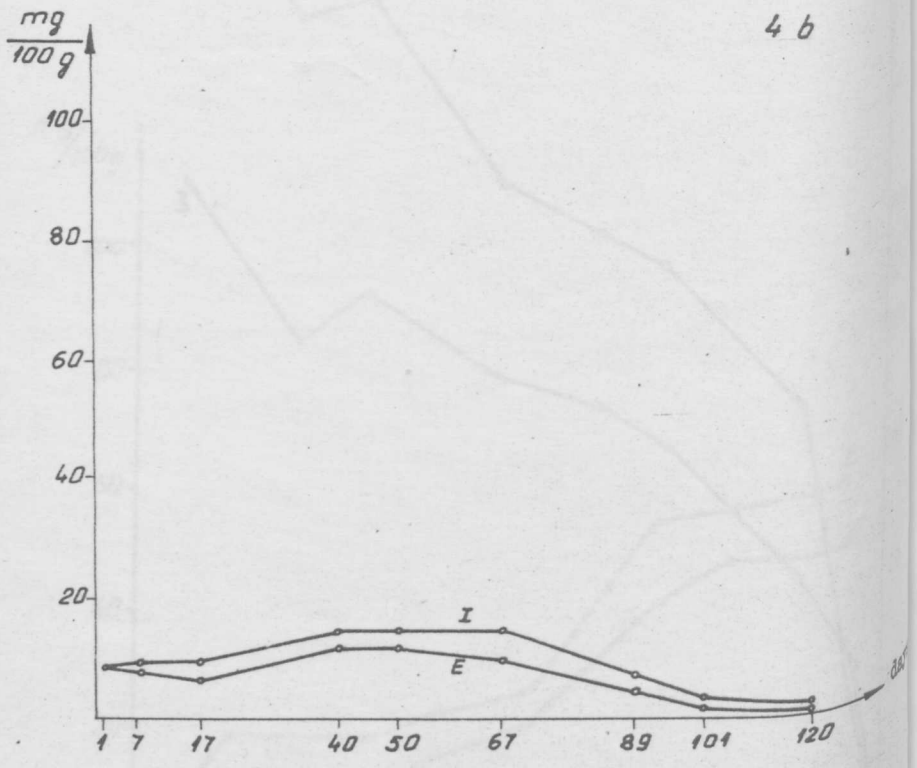


Fig. 4b

4 c

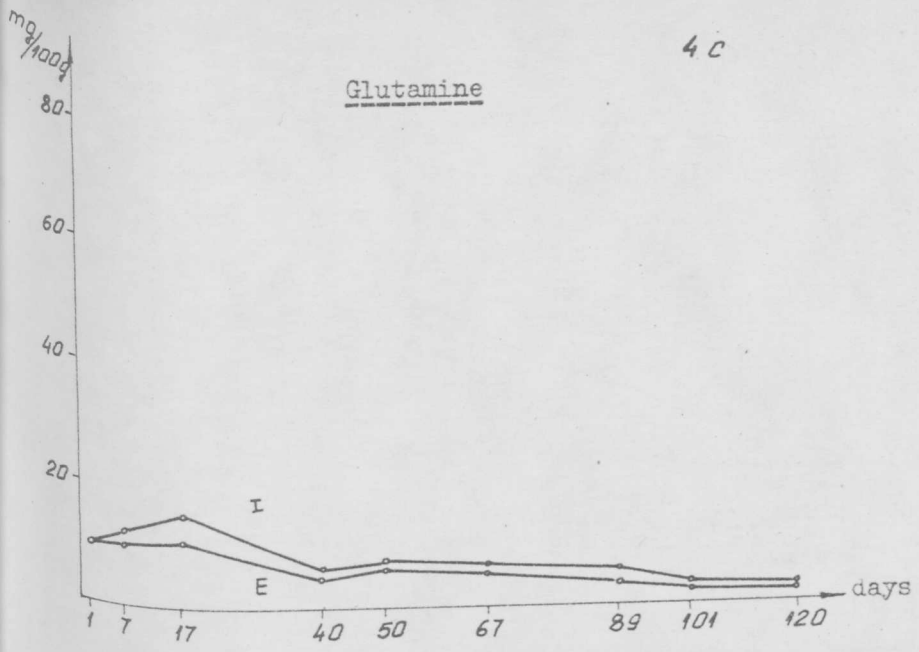
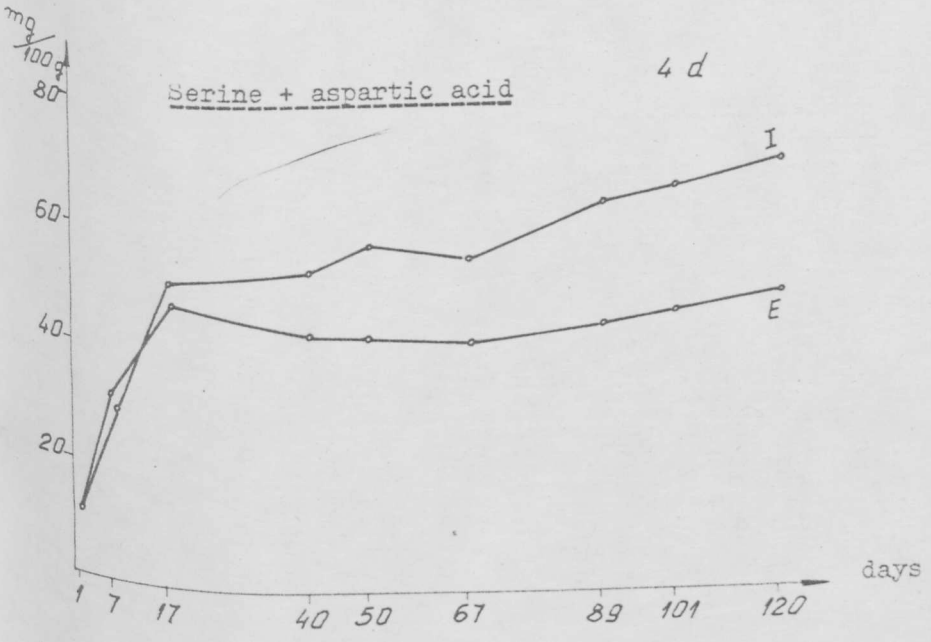


Fig. 4c

4 d



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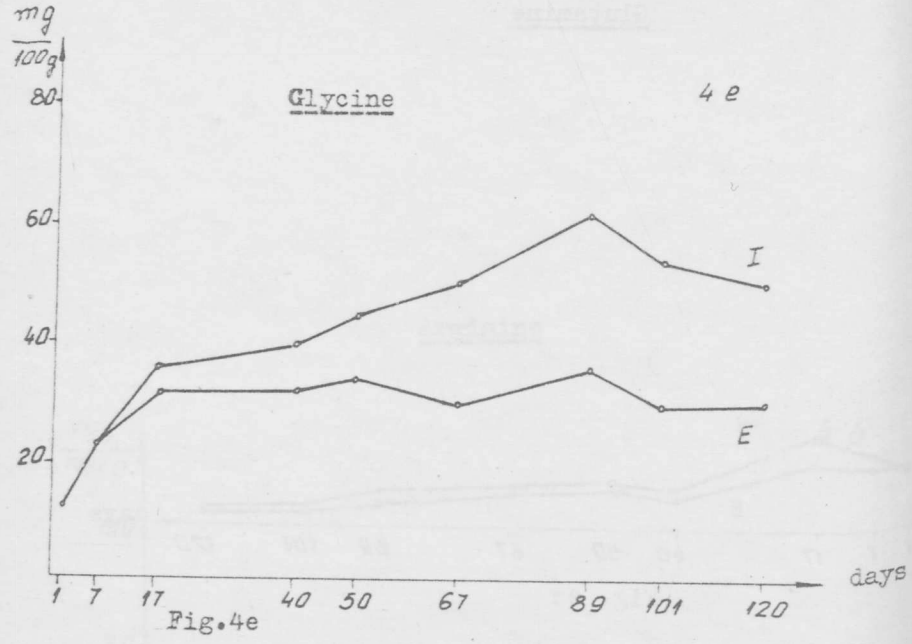
mg
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Glycine
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89
101
120
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Fig.4e

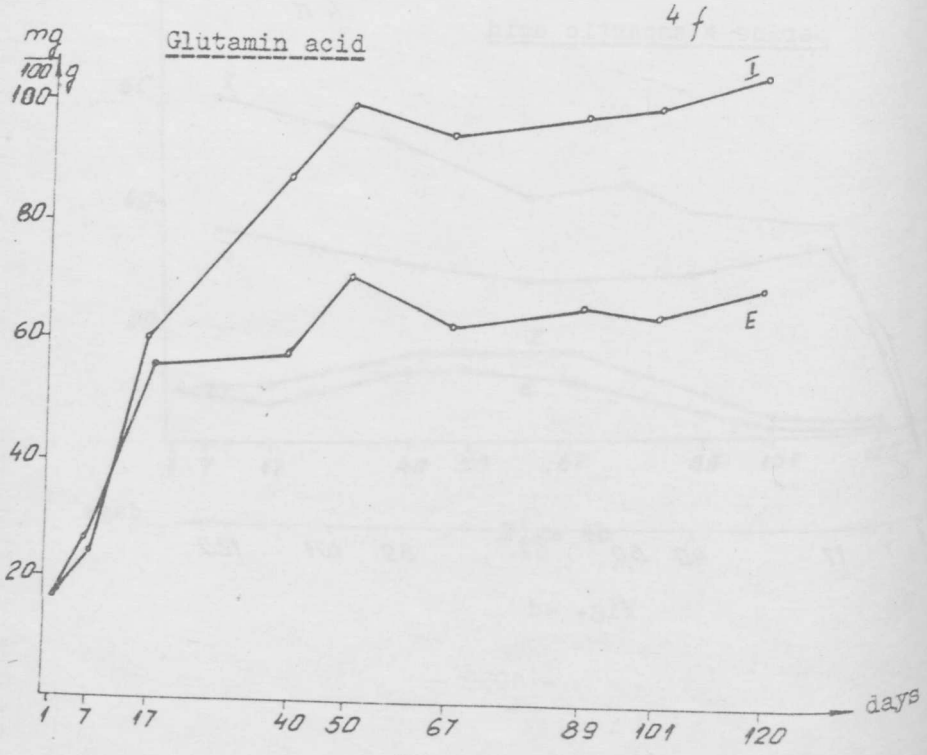
4 f
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Fig. 4f

Threonine

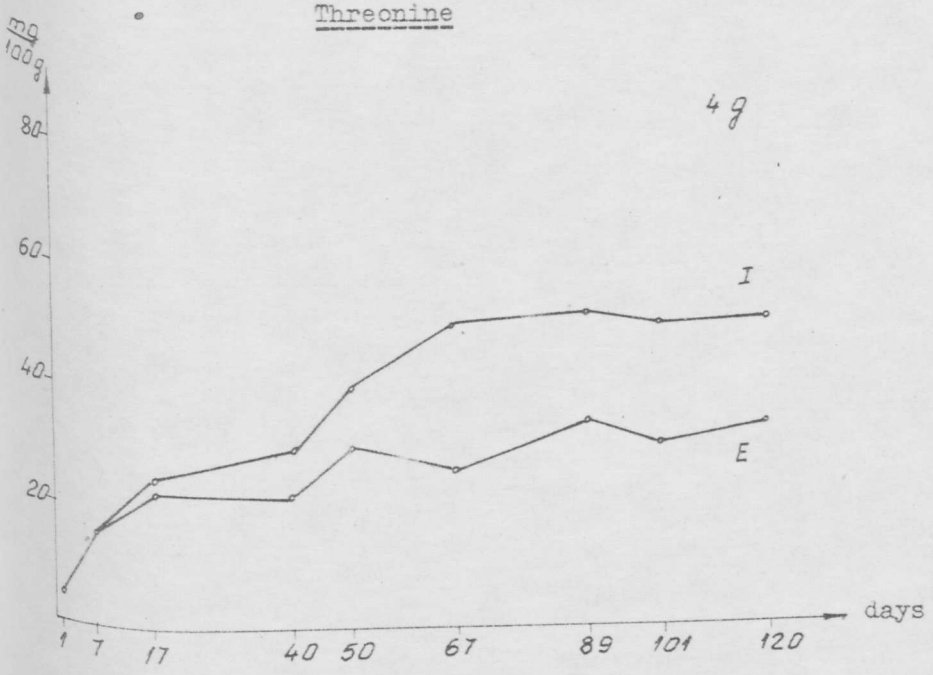


Fig. 4g

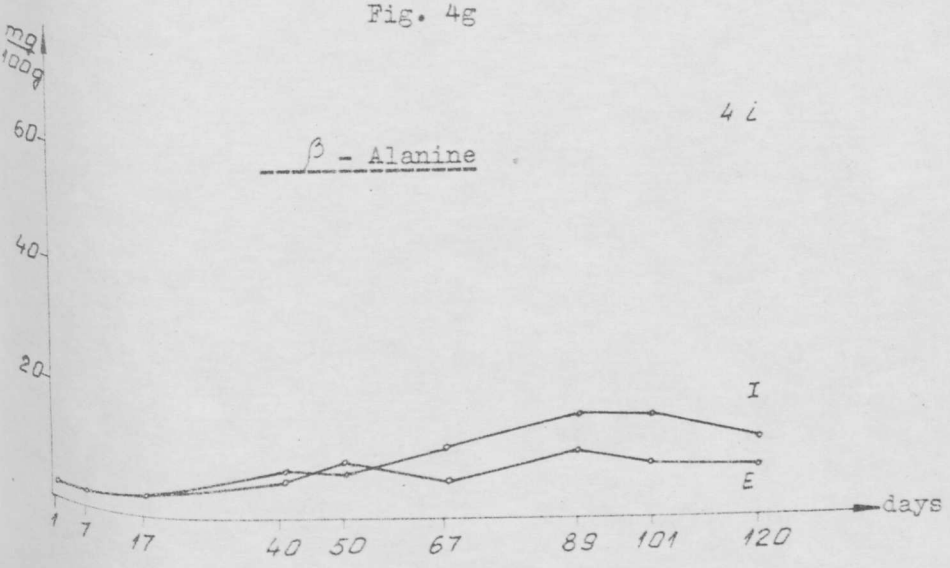
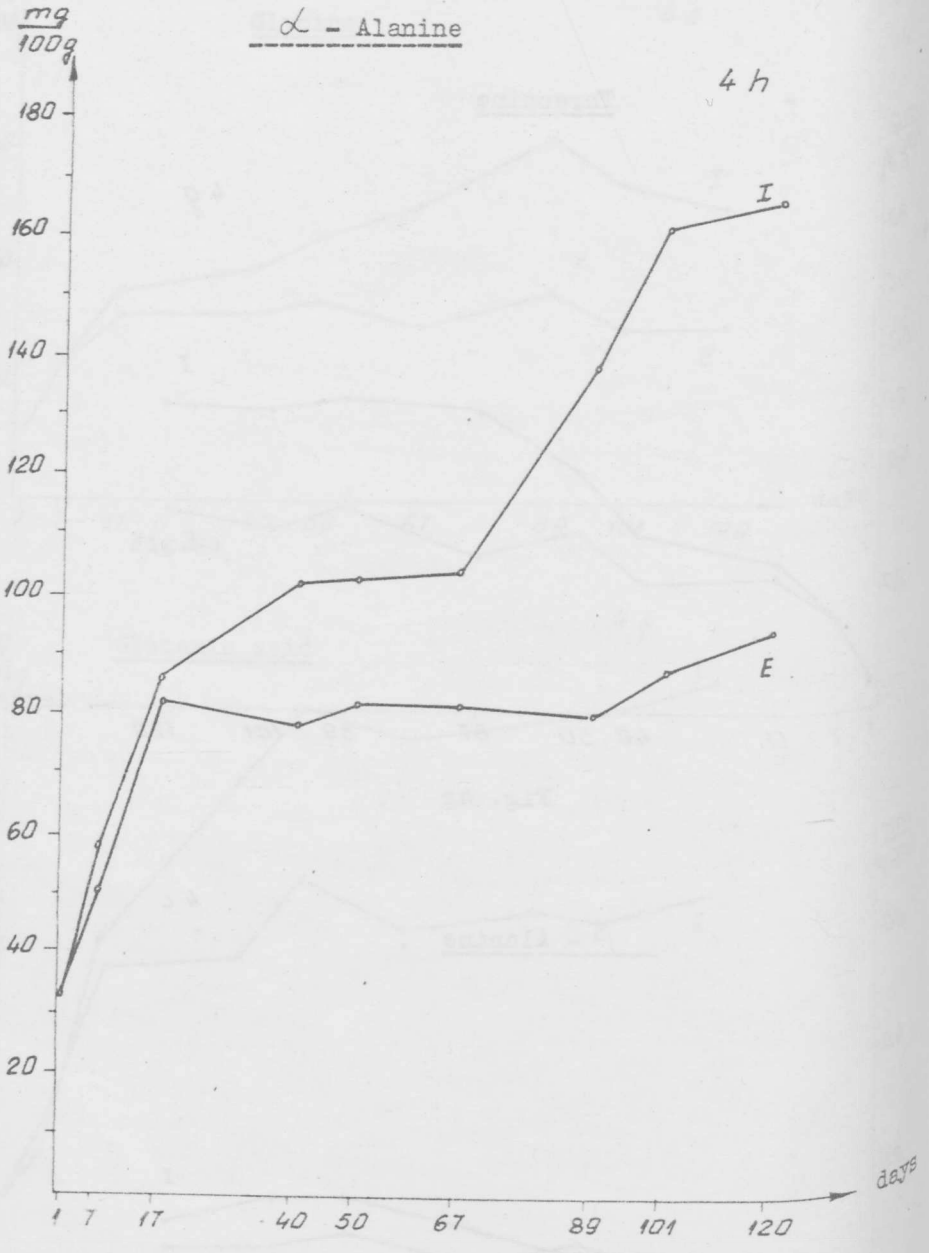


Fig. 4i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160
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Fig. 4h

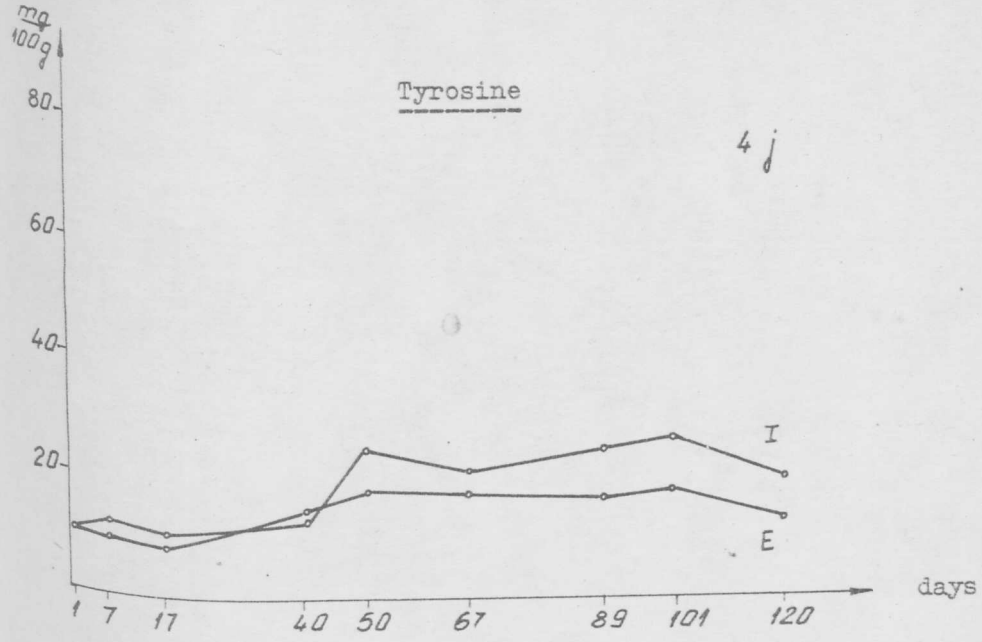
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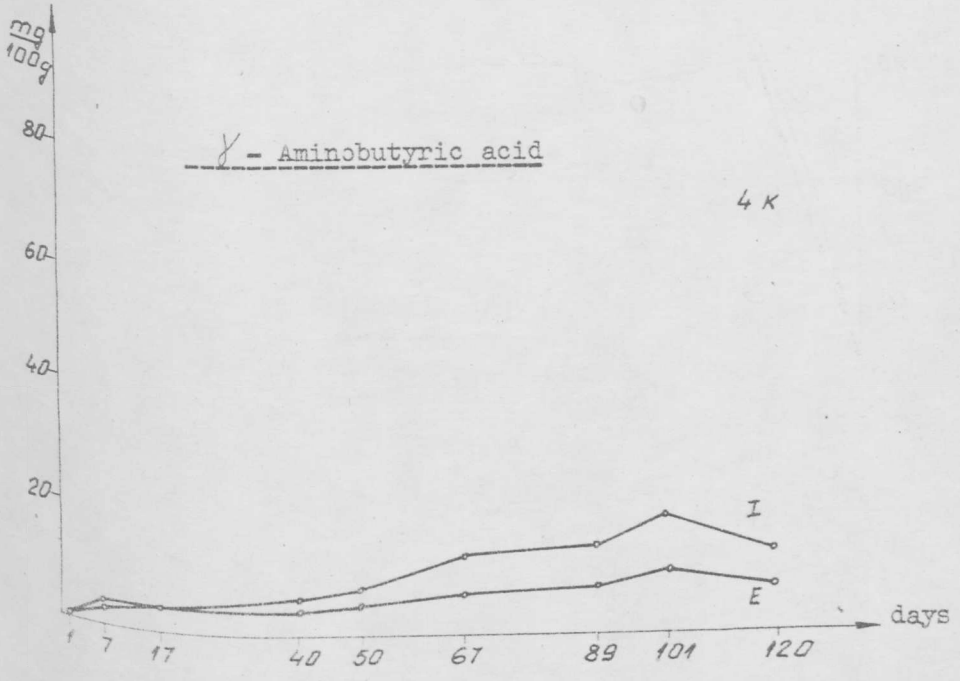
Fig. 4j


mg
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γ - Aminobutyric acid
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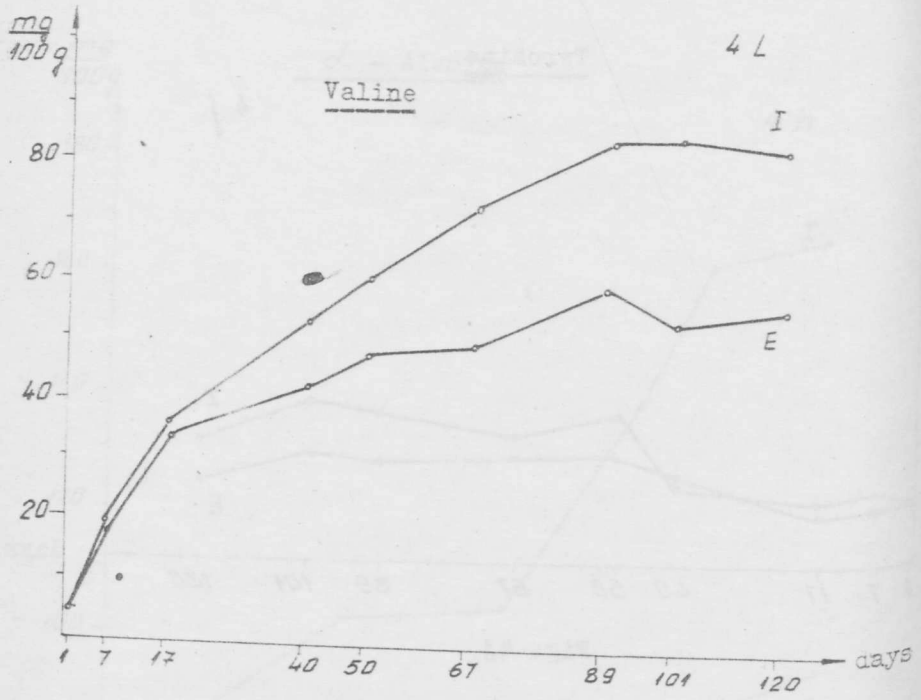
Fig. 4k


mg
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Valine
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Fig. 4L

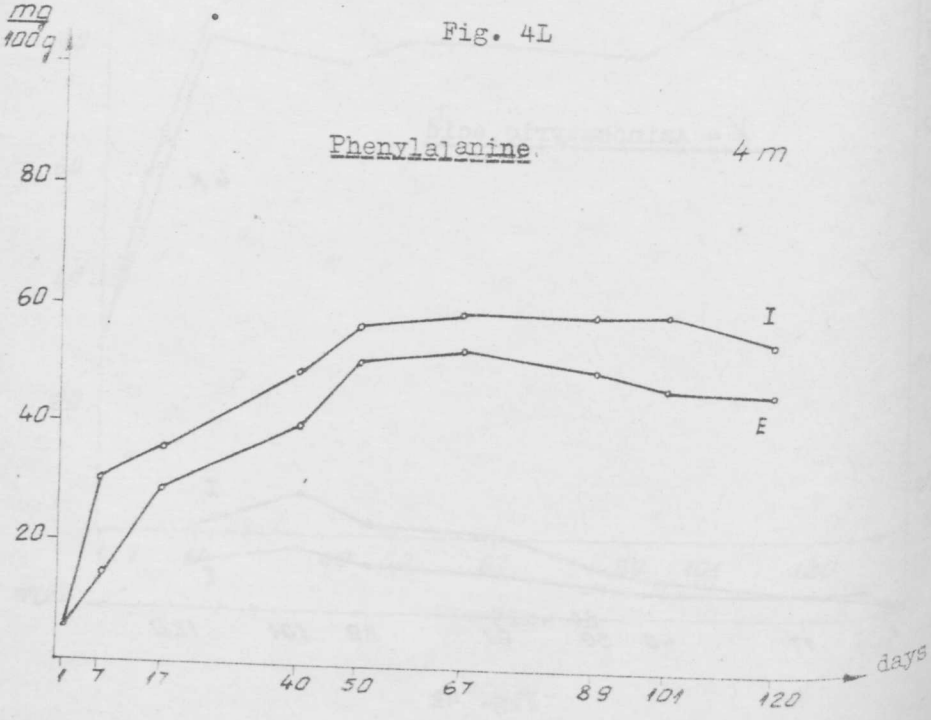
mg
100 g
Phenylalanine
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Fig. 4m

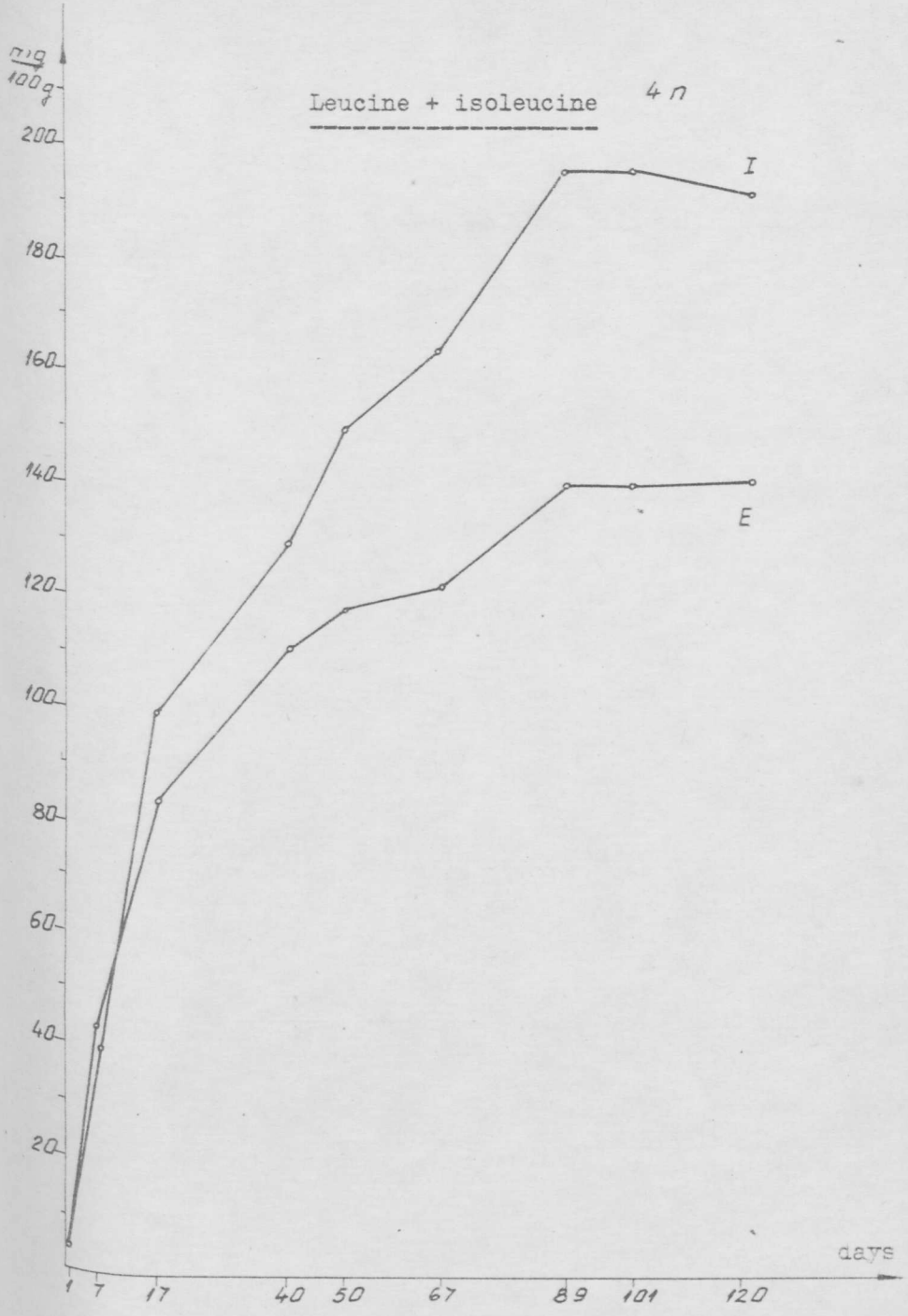
mg/100g
Leucine + isoleucine
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Fig. 4n

Free fatty acids

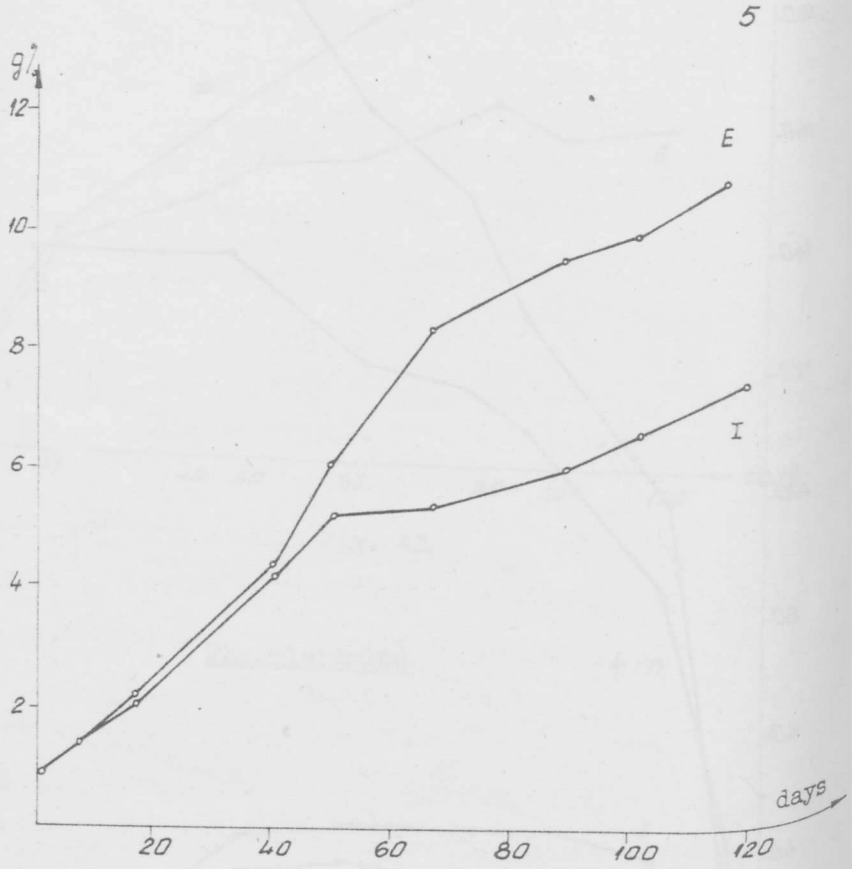

Fig. 5