Entstehung der Protamine

Aus den unreifen Testikeln kann man weder mit Säure ein Protamin noch mit Lauge eine Nukleinsäure extrahieren. Hier scheint die Nukleinsäure mit einem komplizierten Protein nicht dissoziabel verbunden zu sein. Dieses Protein dürfte zweifellos mehr, vielleicht sogar alle Aminosäuren enthalten. Wenn nun die Spermatozoen ausgebildet werden und heranreifen, dann wird dieses Eiweiss umgeformt, viele Aminosäuren verschwinden aus ihm, bis schliesslich eine Kombination übrigbleibt, die ausreicht, die Erbeigenschaften zu übertragen. Möglicherweise tritt auf einem Zwischenstadium der Umformung ein Histon auf.

Es verschwinden vor allem die für den Betrieb wichtigen Aminosäuren: Tryptophan, Methionin, Phenylalanin, Tyrosin, Asparaginsäure und Glutaminsäure. Übrig bleiben mehr oder weniger «träge» Aminosäuren. Da nach Ansicht der Genetiker der Kern der Eizelle wahrscheinlich ebenso einfach gebaut ist wie der der Samenzelle, drängt sich die Frage auf, wie aus diesem einfachen Substrat, das nach der hier entwickelten Ansicht den Genen zugrunde liegen soll, die komplizierten Fermentproteine aufgebaut werden. Diese enthalten sehr viele Aminosäuren, vor allem Tyrosin, das überhaupt keinem wirksamen Eiweiss fehlt. Das Material, mit dem die Gene zum Ferment ergänzt werden, befindet sich im Zytoplasma der Eizelle; wäre an sich auch im Zytoplasma der Samenzelle enthalten. Aber letzteres scheint für die Befruchtung und Entwicklung nicht nötig zu sein. Denn es gelang uns, Saiblingseier mit isolierten und gründlich gewaschenen Saiblingskernen künstlich zu befruchten[1]. Sie haben sich wie natürlich befruchtete entwickelt, und die jungen Fischlein unterschieden sich weder in der Gestalt noch in der Farbe noch in der Munterkeit von den anderen. Wenn wir den Vorgängen nachgehen, wie eine Aminosäure nach der anderen aus dem Zytoplasma ein Gen zu einem Ferment ergänzt, bietet sich uns eine neue Gelegenheit, etwas von dem Wirken jenes unbekannten Prinzips zu erfahren, das die Lebensvorgänge ordnet.

Summary

The nuclei of spermatozoa from several species of trout, herring, sturgeon and salmon consist of nucleoprotamines only.

The ratio of phosphorus to arginine almost reaches 1:1 in nucleoprotamine of trout, salmon and herring. Some of the phosphoric acid residues in nucleo-sturine are neutralized by other basic amino acids.

The nucleic acid of the nuclei is exclusively of the desoxyribose type. Ribonucleic acid occurs in cytoplasm of the sperm only.

The molecular weight of nucleoprotamine is about $1{\cdot}3 \times 10^6$. One single nucleus of a certain trout species (Saibling) consists of $4{\cdot}5 \times 10^6$ nucleoprotamine molecules as roughly calculated.

Eggs of a trout species have been artificially fertilized, and from those eggs young fish could be raised which behaved and looked normally.

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STUDIORUM PROGRESSUS

Electrophoretic Studies of Milk

Investigations on centrifuged milk of dairy cows

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Introduction. – The results of the electrophoretic investigations on colostrum, which appeared in the first publication of this series[2], showed the presence of eight fractions (*A* to *H*) in the milk. In view of the results obtained, we ascribed a casein character to the *B* and *G* components and a globulin character to the *H* component. It was not possible to determine the specific nature of the other protein fractions (*A*, *C*, *D*, *E* and *F*) and the place of the albumin peak or peaks in the electrophoresis diagrams. The object of this investigation was to obtain additional information on these problems.

Methods. – By high-speed centrifuging of milk, various portions of the casein are separated out, according to their content in the milk and the applied centrifugal force. Using this principle, three tests were carried out, two on normal milk and one on colostrum. Before dialysis the milk was centrifuged at 7000; 25000 and 38000 × gravity respectively. The milk as well as the sera prepared in this way were dialysed as usual against Michaelis veronal-sodium buffer[3] of pH 8 and ionic strength 0·1. Electrophoresis was carried out at 2°C with a current strength of 25 mA. For this purpose we used the Strübin apparatus[4], described in detail in the numerous publications of Wiedemann[5]. In addition to the electrophoresis tests, the casein content on normal milk samples was determined, while complete chemical fractionation was carried out on the colostrum sample, according to the method of Rowland[6], but with the difference that the albumin was calculated from the difference between the total proteins and the casein plus globulin.

Experimental results and discussion

The results of the electrophoretic examination show in the first place that the quickly-moving component *A* (only 1–2% of the total diagram), which was also found in the former investigation, is identical with the η-component found by Moore and Lynn[7], and is consequently not to be considered as a normal part of the diagram. At any rate, this does not modify the conclusions drawn from the former investigation . To avoid any confusion, the designations of the different components (*A-H*) have not been changed, though the component *A* was not included in the relative percentages.

In Table I the results of the chemical fractionation are shown, and in Table II those of the electrophoretic examination of the two normal milk samples.

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Table I

Results of the chemical fractionation of samples of normal milk before and after centrifuging

Nature of the sample	Applied centrifugal force	Total N mg/100 g	Casein-N	
			mg/100 g	as percent of total N
Milk(test 1) .		522·4	400·8	76·7
Serum I . .	7,000 × gravity	339·9	209·5	61·6
Serum II . .	25,000 × gravity	269·7	138·5	51·4
Milk(test 2) .		437·8	339·9	77·6
Serum I .	7,000 × gravity	307·3	207·8	67·6
Serum II .	25,000 × gravity	172·5	74·0	42·9
Serum III .	38,000 × gravity	143·5	40·8	28·4

In test 1, two sera were prepared by centrifuging whole milk at 7,000 and 25,000× gravity. The casein nitrogen content, as percent of the total nitrogen, was 76·7% in the milk and decreased to 51·4% in serum II. The results of the electrophoretic examination show that the *B* component decreased from 52·0% in the milk to 36·4% in serum II, while the *G* component increased slightly from 16·4% to 18·1%. Our former investigation on colostrum proved the casein character of *B* and *G* components, and this is now confirmed by the great decrease of the *B* component and also by the very slight rising of the *G* component. The fact that the *B* component presents a great decrease, and that the *G* component only varies slightly, shows that the *B* casein fraction has a much lower stability than the *G* fraction. Since the *C* component decreases from 7·9 to 6·1%, it must be concluded that this fraction has also a casein character. The components *D* and *E* increase from 8·4 and 5·4% in the milk to 16·7 and 11·0% in serum II. From this increase we may ascribe an albumin character to these fractions. The component *H* increases from 3·5 to 7·5%, as was to be expected in view of its globulin character, indicated by the former investigation.

In test 2, three sera were prepared by centrifuging whole milk at 7,000; 25,000 and 38,000× gravity respectively. The casein nitrogen content, as percent of total nitrogen, which was 77·6% in the milk, decreased to 28·4% in serum III. The results of the electrophoretic examination show, first of all, a decrease in the *B* component from 50·5% in the milk to 22·8% in serum III. The content of the second casein fraction, the *C* component, in the milk was 9·3% and in serum III 3·6%, while the third casein fraction *G* decreased from 19·6 to 17·1%. From these results, the same conclusion can be drawn as from test 1: the *B* and *C* components settled much faster than the *G* casein component. The two albumin fractions *D* and *E* increased greatly in the sera. The contents in the milk were 7·5 and 4·0% and in serum III 22·1 and 14·0%. As far as the globulin fraction *H* is concerned, it must be observed that this fraction is divided into two components, H_1 and H_2. The concentrations increased from 2·5 and 1·9% in the milk to 7·1 and 5·9% in serum III.

In test 3, four sera were prepared by centrifuging milk, sampled at the end of the colostrum period. From the results of the chemical fractionation, given in Table III, it appears that the original colostrum contains 76·1% casein nitrogen, as percent of the total protein nitrogen; in the sera this figure drops to the values 65·5, 55·4, 32·3, and 28·6%. The albumin and globulin nitrogen percent increases from 12·1 and 11·8% in the colostrum to 36·1 and 35·3% in serum IV.

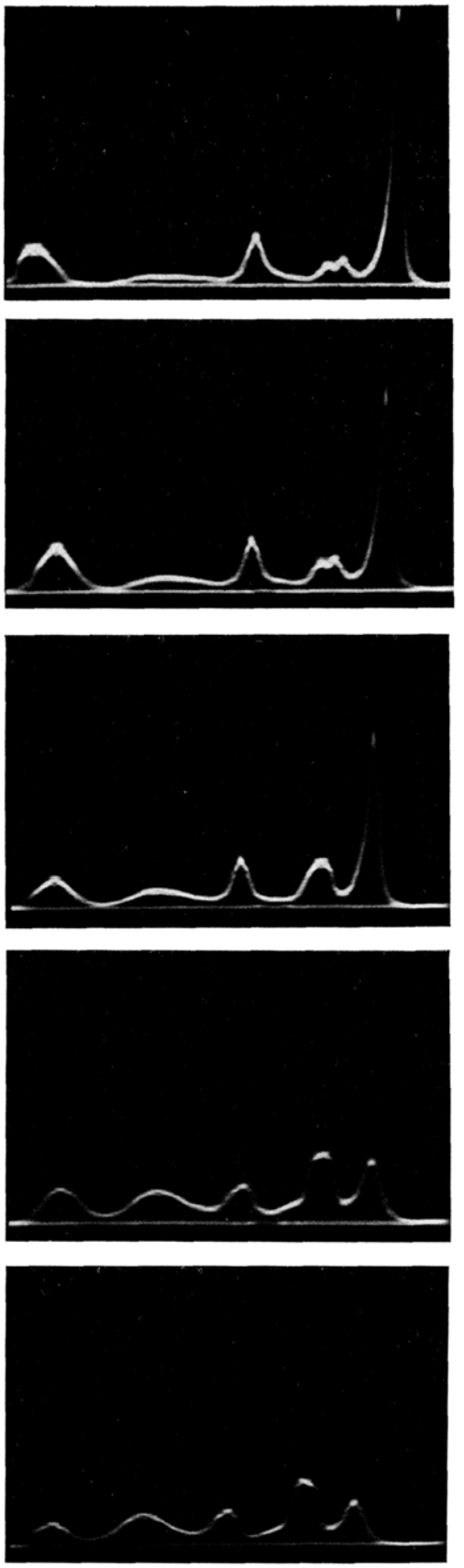

Fig. 1. –Electrophoresis diagrams of milk and the sera, prepared by centrifuging with a centrifugal force varying from 7,000 to 38,000 × gravity. Test 3 (Colostrum)-Rising Boundaries.

Table II

Results of the electrophoretic examination of samples of normal milk before and after centrifuging

Experiment N°	Nature of the sample	*B*	*C*	*D*	*E*	*F*	*G*	H_1	H_2
1. Relative percentage concentrations of the different fractions									
243	Milk (test 1)	52·0	7·9	8·4	5·4	6·4	16·4	3·5	—
241	Serum I	44·0	6·8	12·4	9·1	4·3	17·9	5·5	—
242	Serum II	36·4	6·1	16·7	11·0	4·2	18·1	7·5	—
249	Milk (test 2)	50·5	9·3	7·5	4·0	4·7	19·6	2·5	1·9
247	Serum I	42·5	9·9	10·6	5·9	6·1	18·6	3·9	2·5
246	Serum II	34·1	6·5	16·9	10·3	6·3	17·2	4·9	3·8
245	Serum III	22·8	3·6	22·1	14·0	7·4	17·1	7·1	5·9
2. Mobilities (x 10^{-5}) of the different fractions									
243	Milk (test 1)	7·7	7·0	6·4	5·9	4·7	3·6	1·9	—
241	Serum I	7·8	7·1	6·4	5·8	4·8	3·8	2·0	—
242	Serum II	7·7	7·1	6·4	5·7	4·6	3·7	1·9	—
249	Milk (test 2)	7·4	6·8	6·3	5·9	4·7	3·5	2·2	1·4
247	Serum I	7·6	7·0	6·4	5·9	4·5	3·5	2·0	1·2
246	Serum II	7·7	6·9	6·3	5·8	4·7	3·6	2·5	1·5
245	Serum III	7·6	7·1	6·4	5·9	4·7	3·7	2·4	1·1

Table III

Results of the chemical fractionation of the sample of colostrum before and after centrifuging

Nature of the sample	Applied centrifugal force	Total N mg/100 g	Casein-N	Albumin-N	Globulin-N
			as percent of total protein-N		
Milk (test 3)		720·0	76·1	12·1	11·8
Serum I	7,000 × gravity	547·9	65·5	17·4	17·1
Serum II.	25,000 × gravity	442·4	55·4	22·7	21·9
Serum III	38,000 × gravity/10′	304·4	32·3	34·1	33·6
Serum IV	38,000 × gravity/30′	286·9	28·6	36·1	35·3

Table IV

Results of the electrophoretic examination of the sample of colostrum before and after centrifuging

Experiment No.	Nature of the sample	*B*	*C*	*D*	*E*	*F*	*G*	H_1	H_2
1. Relative percentage concentrations of the different fractions									
254	Milk (test 3)	48·8	10·0	6·3	4·0	5·2	13·2	2·5	10·0
257	Serum I	37·9	12·0	8·5	5·5	5·0	14·6	2·7	13·8
252	Serum II	31·3	10·6	11·6	7·8	4·3	14·3	3·3	16·8
255	Serum III	19·9	3·6	15·8	10·9	6·9	11·2	5·7	26·0
250	Serum IV	15·7	3·0	17·2	11·7	7·0	9·9	7·3	28·2
2. Mobilities ($\times 10^{-5}$) of the different fractions									
254	Milk (test 3)	7·6	6·9	6·3	5·9	4·9	3·7	2·8	1·8
257	Serum I	7·5	6·8	6·2	5·8	4·9	3·6	2·5	1·6
252	Serum II	7·7	7·1	6·4	5·9	4·7	3·7	2·5	1·6
255	Serum III	7·6	7·0	6·4	5·9	4·6	3·6	2·5	1·6
250	Serum IV	7·6	7·1	6·4	5·9	4·6	3·6	2·6	1·6

Examination of the photographs of the electrophoresis diagrams, obtained before and after centrifuged colostrum (Fig. 1), shows clearly the changes in composition of the milk proteins. Since the visual course of the protein composition is similar in all three tests, both on the rising and descending boundaries, only the rising boundaries of test 3 are reproduced.

The results obtained from the electrophoresis diagrams, given in Table IV, show that the first and largest casein component *B* drops from 48·8% in the milk to 15·7% in serum IV. The second and smallest casein component *C*, first increases slightly from 10·0 to 12·0% and afterwards drops to 10·6, 3·6 and 3·0%. The third casein component *G* shows first a small increase from 13·2 to 14·6% and afterwards a decrease to 14·3, 11·2 and 9·9%. The concentrations of the two albumin components *D* and *E* rose from 6·3 and 4·0% in the colostrum to 17·2 and 11·7% in serum IV. With regard to the globulin, we also found two components, H_1 and H_2, which increased from 2·5 and 10·0% in the milk to 7·3 and 28·2% in serum IV.

In view of the varying course of component *F* in the three tests, its protein character is still unknown.

Conclusions. From the investigations on non-centrifuged and centrifuged milk (normal milk and colostrum) we may conclude that three casein fractions (*B*, *C* and *G*), two albumin fractions (*D* and *E*), and one or two globulin fractions (H_1 and H_2) occur in milk. Of the three casein fractions, the *B* component probably corresponds with the α-casein, which is well known in the literature while the *G* component would be identical with the β casein. This hypothesis is justified by the relationship, in concentration as well as in mobility, between the values found for α and β casein by CHERBULIEZ[1], HOSTETTLER[2], NITSCHMANN[3], and WARNER[4], and our values. From our results it appears moreover that the component *G* is more stable than component *B*. This observation is not in accordance with the results obtained by HOSTETTLER[2], who found an almost constant proportion between α and β casein in the sediments obtained by centrifugation the milk at 1,000 to 17,000× gravity.

An albumin character is attributed to the components *D* and *E* because of their increase in concentration in the sera proportional to the separated casein quantity. Recent research confirms that the albumin, found by application of the chemical fractionation contains at least two fractions, among them the well known β lactoglobulin of PALMER[5]. The mobility of this lactoglobulin is, according to DEUTSCH[6], 6·1 and according to BRIGGS and HULL[7] $6{\cdot}3 \times 10^{-5}$. We also found that the lactalbumin contains two fractions, *D* and *E*, of which the *D* component (mobility 6·2 to $6{\cdot}4 \times 10^{-5}$) should correspond with the β lactoglobulin. The results confirm the globulin character of the *H* component, indicated by the former investigation; however we notice that the fraction is sometimes divided into two components H_1 and H_2.

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Résumé

L'électrophorèse et le fractionnement chimique furent effectués sur des laits et leurs sérums obtenus en soumettant le lait à des forces centrifuges 7000 à 38000 fois supérieures à la pesanteur. De nos résultats, il ressort que dans les sérums obtenus avec des forces centrifuges croissantes, la caséine et les fractions électrophorétiques *B*, *C* et *G* diminuent, alors que l'albumine et la globuline de même que les fractions *D*, *E* et *H* augmentent. Il en résulte également que les fractions *B*, *C* et *G* correspondent à la caséine (*G* est plus stable vis-à-vis de la force centrifuge que *B*), *D* et *E* à la lactalbumine totale et *H* à la globuline. Il est possible que la fraction *A* soit identique au composant *n* de MOORE et LYNN, *B* à la caséine alpha, *D* à la bêta lactoglobuline de PALMER et *G* à la caséine bêta.

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EXPLICATIONES

Fundamentals for Prophylaxis and Therapy of Premature Ageing

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Real understanding of natural processes can only be acquired by studying them as a *whole* and not by dividing them into independent parts. This is especially the case for research, prophylaxis and therapy of premature ageing in man. Modern antisenetics require a whole system of prophylactic measures, well founded experimentally and clinically and taking the organism and its surroundings as a *dynamic whole;* besides this, special symptomatic methods are needed against the heterochronical, heterometrical, and heterotopical signs of ageing. Such facts and observations as are known at present are very dispersed but can form the fundamentals of prophylaxis and therapy of premature ageing.

I. *Energetic Fundamentals*

Our studies of the age-problem, extended over many years, have led us to the conclusion that ageing of an organism is a consequence of the *irreversibility* of its individual evolutional energy. Therefore the whole problem of age therapy involves energetics, and the possible reversibility of the regressive symptoms of ageing. Of course such a reversibility in man should not be understood in the sense of a direct homodrome re-metamorphosis of an old man into a child, but means a lasting conservation of life energy at the level of "useful productive" age. This can be established by the following three facts:

(1) Any organism is an *open* system and hence the process of ageing is not subject to the law of entropy. This means in practice that ageing can be retarded or accelerated from *outside*.

(2) As is well known, the human organism during its life uses only one third of its life energy. This fact is in

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