On voit donc que ces résultats permettent d'envisager l'organisation des granules comme un procès en deux étapes: la première comportant une synthèse moléculaire de ribonucléoprotéines, la seconde la formation des granules eux-mêmes à partir de ce matériel. On sait combien, par de nombreux aspects, les virus sont proches des granules cytoplasmiques: il serait tentant de supposer que le schéma de synthèse des granules auquel conduisent nos observations s'applique également à la multiplication des virus (et des bactériophages); en effet on sait que le pouvoir infectieux, lors des synthèses de virus, apparaît brusquement et augmente très rapidement après une période de latence où il reste extrêmement faible. Il est possible que cette période de latence corresponde à l'étape de synthèse moléculaire et que le pouvoir infectieux n'apparaisse que lors de la seconde étape: c'est-à-dire après organisation des particules à partir de ce matériel transformable.

Des recherches en cours nous permettront, nous l'espérons, de préciser de tels procès qui nous paraissent directement liés aux conditions d'autocatalyse des systèmes protéiques. JACQUES PANIJEL

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Summary

It has been observed that during the spermiogenesis of *Ascaris megalocephale* a rapid and considerable elimination of R.N.A. takes place. This process is due to the dissociation of cytoplasmic granules into molecular elements (supernatant). Reciprocally, during the phagocytosis of R.N.A. eliminated by the parietal cells, formation of granules from the supernatant has been observed. The conditions of synthesis of cytoplasmic granules and the relations with the elements of molecular size (supernatant) are discussed.

Preparation of Rabbit Anti-Serum for Rat-Liver Mitochondria. Its Action on Mitochondria Succinoxidase

Many techniques have been described for the isolation in a state of high purity of nuclei, mitochondria, and microsomes from cells[1].

In this paper the preparation of rabbit anti-rat liver mitochondria serum and its action on mitochondria succinoxidase are described.

Mitochondria were isolated from rat liver according to HOGEBOOM *et al.*[1]. After washing 4–5 times in 0·88 M sucrose solution to remove traces of foreign proteins, they were suspended in 3 ml of 0·15 M NaCl and injected intravenously into a normal rabbit. 8 inoculations were made at 3 day intervals, using BESREDKA's technique to avoid anaphylactic shock. Antibodies cannot be demonstrated by agglutination, since spontaneous agglutination of mitochondria occurs in the presence of electrolytes. The immune serum produced a rapid heavy agglutination, which is different from the microagglutination produced by salts, but it was found that an entirely similar phenomenon occurs with normal sera of rabbits, rats, and guinea pigs.

Lysis in presence of complement may be employed, but great caution must be used, since after a few hours spontaneous lysis occurs also without immune serum. Lysis is specific only if it occurs within 1–2 hours. Microscopic observation shows a rapid swelling of the mitochondria in presence of immune serum and complement, which is followed by complete destruction. When lysis occurs, a thick protein precipitate can be observed. But the best method for antibody demonstration was complement fixation. The titrations were performed by using serial dilutions of inactivated immune serum (1·0 ml), 0·05 ml of fresh guinea pig complement pooled from the blood of 3 animals, and 0·5 ml of a mitochondrial suspension containing the mitochondria isolated from 1 g of rat liver or kidney in 10 ml of saline. The tubes were incubated at 37·5°C for 1 hour, and sensitized sheep cells were added as usual.

In presence of rat liver mitochondria, the titers were up to 1:1280, in presence of rat kidney mitochondria up to 1:50 in some instances, whereas no fixation was observed when mitochondria from either liver or kidney of mice, guinea pigs or rabbits were used.

The action of anti-mitochondria serum on the succinoxidase system contained in mitochondria from both liver and kidney was tested in the Warburg apparatus, with 0·2 M sodium succinate added (Table I).

Washed mitochondria isolated from 1 g liver or 0·5 g kidney were evenly suspended in 9·5 ml phosphate buffer (p_H 7·4) in Warburg vessels (3·0 ml of mitochondrial suspension + 0·2 ml sodium succinate and 0·2 ml 20% KOH in each vessel). Each determination was made in triplicate. 0·1 ml of inactivated anti-rat

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Table I

Action of rabbit anti-serum for rat liver mitochondria on succinoxidase activity of purified mitochondria. (Average values of the percentual acceleration of activity.)

No. of exps.	Source of mitochondria	Liver		Kidney	
		serum	serum + complement	serum	serum + complement
7	rat	−10·31 ± 2·35	+20·16 ± 7·09	+17·01 ± 2·31	+30·60 ± 6·48
2	mouse	+20·13 ± 0·34	+30·64 ± 5·48	–	–
1	guinea pig	+16·16	+24·12	+ 7·12	+24·13
1	rabbit	+21·49	+22·79	+18·94	+25·75

liver mitochondria serum or 0·05 ml of fresh guinea pig complement were added respectively. Control values for immune serum plus succinate and for complement plus succinate were subtracted. The O_2-uptake was followed for 40 minutes and the results summarized in Tables I and II.

Table II

Action of normal sera on succinoxidase activity of purified mitochondria (average values of the percentual acceleration of activity)

No. of exps.	Source of serum	Source of mitochondria	Liver	Kidney
5	guinea pig	rat	+16·84 ± 4·03	+24·55 ± 1·63
2	guinea pig	mouse	+13·38 ± 2·14	+14·40 ± 3·0
2	guinea pig*	rat	+26·93 ± 3·98	+15·68 ± 1·89
1	rabbit	rat	+20·40	+12·60
1	rat	rat	+ 7·90	+12·20
1	rat	rabbit	+ 9·34	+10·32
1	rat	guinea pig	+10·05	+12·40
1	horse	rat	+13·31	+12·10
1	man	rat	+25·45	+22·40
1	dog	rat	+15·22	+13·60

* Inactivated serum.

Immune serum had only a weak inhibitory action on succinoxidase activity of rat liver mitochondria: addition of complement accelerated to some extent. The succinoxidase activity of rat kidney mitochondria was always accelerated by immune serum with and without complement. While the inhibiting action of immune serum on rat liver mitochondria is probably due to a reduction of the surface area following agglutination, the acceleration produced by complement can be explained by the fact that lysis releases the enzyme into solution and facilitates the contact with the substrate.

Normal guinea pig serum has an accelerating action on succinoxidase of mitochondria from both liver and kidney of the rat. Thus the acceleration occurring in presence of immune serum and of complement is probably unspecific.

The accelerating effect of normal guinea pig serum persists after heating at 56°C for 30 minutes: sera from rabbit, man, mouse, and dog also accelerate the reaction.

The succinoxidase activity of mitochondria decreases after incubation with normal serum for 1 hour at 37·5°. It was supposed that the accelerating action of normal sera was due to serum lipase which brings the enzyme nearer to the substrate by splitting the peripheral lipid surface of mitochondria. The decrease of activity observed after incubation at 37·5°C for 1 hour, which shows that rapid inactivation of the enzyme occurs in presence of normal serum, is in support of this hypothesis.

Experiments with the following inhibitors of serum lipase were performed: benzaldehyde, o-, m-, and p-tri-cresylphosphate (HOTTINGER, BLOCH[1]) (Tables III, IV).

The inhibiting activity of these compounds on serum lipase was tested in the Warburg apparatus with 0·03 M tributyrin as substrate in the presence of 0·1 ml fresh guinea pig serum. The hydrolysis of tributyrin was inhibited as follows: 50% in the presence of 5 mg benzaldehyde; 96% in the presence of 1 mg o-tri-cresylphosphate; 60 and 30% respectively with 10 mg of m- and p-tri-cresylphosphate. The same inhibitors were added to systems containing mitochondria, sodium succinate and serum. The results are summarized in Tables III and IV. It was found that these inhibitors antagonize almost completely the accelerating effect of normal sera on mitochondrial succinoxidase and can also delay the inactivation of the enzyme incubated with serum at 37·5°C for 1 hour.

The experiments suggest that accelerating effects of normal sera are due to the activity of serum lipase.

I am indebted to Miss G. MISLAP, of the Hygiene Institute of the University of Basle, for supplying o-, m-, and p-tri-cresylphosphate.

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Table III

Inhibition with lipase inhibitors of acceleration of succinoxidase activity of rat mitochondria determined by guinea pig normal serum (average values of the percentual acceleration of activity)

No. of exps.	Lipase inhibitors	Liver		Kidney	
		Serum	serum + lipase inhibitor	serum	serum + lipase inhibitor
4	Benzaldehyde	+23·39 ± 2·67	+4·33 ± 2·25	+29·48 ± 2·92	+ 3·70 ± 3·66
3	O-tri-cresylphosphate	+21·95 ± 1·84	0	+28·54 ± 1·85	+ 0·42 ± 0·39
2	M-tri-cresylphosphate	+23·44 ± 3·95	+5·82 ± 0·29	+27·64 ± 0·7	+ 9·98 ± 0·21
2	P-tri-cresylphosphate	+21·65 ± 0·72	+8·14 ± 1·43	+26·95 ± 2·66	+10·71 ± 0·63

Table IV

Acceleration of succinoxidase activity of rat mitochondria by guinea pig normal serum before and after incubation for 1 hour at 37·5°C. Action of lipase inhibitors added at the beginning of incubation (Average values of the percentual acceleration of activity)

No. of exps.	Inhibitor added	Liver		Kidney	
		before incubation	after incubation	before incubation	after incubation
3	none	+30·08 ± 2·88	−24·98 ± 1·10	+35·53 ± 3·09	− 15·53 ± 1·82
2	O-tri-cresylphosphate	+23·84 ± 1·75	− 0·74 ± 0·05	+30·27 ± 0·29	− 1·46 ± 0·45
1	M-tri-cresylphosphate	+26·40	− 3·12	+22·16	− 3·16
1	P-tri-cresylphosphate	+24·17	− 6·12	+36·18	− 5·16

Résumé

L'auteur a obtenu dans le lapin un sérum antimitochondre du foie de rat. Le sérum antimitochondre a une faible action inhibante sur la succinoxydase des mitochondres du foie du rat, tandis qu'il accélère l'activité des mitochondres du rein. Les sérums normaux de cobayes, de lapins, de rats, de souris, de chiens et d'hommes ont une action accélérante qui est supprimée après incubation à 37,5°C pour 30 minutes.

L'auteur démontre, par les inhibiteurs de la lipase du sérum, que l'action des sérums normaux doit être rapportée à cet enzyme.

Enzymatic Activity and Protein Amounts in the Liver

VIRTANEN and coll.[1] relate interesting observations on the behaviour of certain enzymatic activities of *Escherichia Coli, Torulopsis utilis, Pseudomonas fluorescens*, when the protein amount of the cell of the microorganisms is reduced by the development of such microorganisms on satisfactory culture media.

When their nitrogen content diminishes, the activity of certain enzymes [enzymatic systems of respiration (*Escherichia coli, Torulopsis utilis*), catalase (*E. coli, Torulopsis utilis, Pseudomonas fluorescens*), proteolytic enzymes (*E. coli, Torulopsis utilis, Pseudomonas fluorescens*), saccharase (*Torulopsis utilis*)], remains almost the same as in that of the normal cell; other enzymes, on the contrary [saccharase (*E. coli*), lactase (*E. coli*), formic dehydrogenase (*E. coli*), enzymatic system of the anaerobe glycolysis (*E. coli*)], show a remarkable decrease in their activity. Therefore in the cells of microorganisms and perhaps of animal organisms, certain enzymes must be present (VIRTANEN and WINKLER[2]) which are indispensable for life and which maintain their activity even when the nitrogen content is reduced, and other enzymes, not indispensable for life, which lose most or all their activity when the nitrogen content of the cell is reduced. Yet, MILLER[3] observed in the liver of fasting rats after 7 days a decrease in the activity of some enzymes (catalase, alkaline phosphatase, xanthine dehydrogenase, cathepsin) of the same degree or higher than the protein loss of the tissue. HARKNESS, SEIFTER, NOVIC, and MUNTWYLER[4] also in the liver of rats receiving a protein-deficient diet, found a decrease in the concentration of A co-enzyme, which is essential for the sulfamide acetylation, proportional to the reduction of the protein concentration in the liver. Therefore the behaviour of the enzymes in the tissues of animals deficient in nitrogen analogous to that observed in micro-organisms is not yet ascertained.

To examine the question, I compared the activity of some enzymes (esterase, phosphomonoesterase, pyrophosphatase, adenosintriphosphatase, dipeptidase, arginase, catalase, succinic dehydrogenase, choline dehydrogenase) of the liver of rats receiving a qualitatively and quantitatively suitable diet, with that of rats receiving a protein deficient diet, followed by a remarkable decrease of the nitrogen content of the tissue.

Therefore 10–12 weeks old rats (♂) were fed during 25 days on a diet composed of saccharose (87%), olive oil (8%), a salt mixture (4%), dry yeast (1%), with the addition of a suitable amount of thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, inositol, choline, axerophthol, calciferol, tocoferol, 2-methyl-1,4-naphtoquinone. Ten rats fed with the same diet, but with casein substituted for part of the saccharose (24%), were used as animals of control. After 25 days, the rats were killed by decapitation, after fasting 18 hours. Immediately their liver was removed and used to test the enzymatic activity as a homogenate, prepared in Potter and Elvehjem's apparatus.

The esterase was determined according to LENTI[1]. Phosphomonoesterase and adenosintriphosphatase were measured in accordance with methods suggested by CAFIERO[2]. The determination of dipeptidase was obtained by measuring the carboxyl groups liberated in the glycil-glycine hydrolysis after formaldehyde blocking of the aminic groups. The arginase was determined according to LENTI[3]. For the catalase we used the method suggested by VON EULER and JOSEPHSON[4] Succinic dehydrogenase and choline dehydrogenase were tested, measuring the O_2 loss in Warburg's apparatus.

The quantity of water, lipids and nitrogen was ascertained in parts of the liver. Water was calculated from the difference of weight after drying to 103°, lipids after ether extraction of the dry material. Nitrogen was determined on dried and lipide-free material using Kjeldahl micromethod.

Under the above mentioned conditions, the liver of the rats receiving a protein-deficient diet shows, as has been long known, a decrease of nitrogen content and an increase of lipide content in relation to the liver of animals receiving a quantitatively and qualitatively normal diet, while the water content is not appreciably modified (Table I).

Table I

Water, lipid and nitrogen content of the liver of rats receiving a normal and a protein-deficient diet

Diet	Water g/kg	Lipide g/kg	Nitrogen g/kg
Of control	677 (643–699)	54 (31–79)	30 (20–35)
Protein-deficient . . .	685 (639–717)	96 (62–130)	19 (13–23)
Difference in %	−1·2	+77·8	−36·7

The behaviour of the examined enzymatic activities differs highly. Phosphomonoesterase shows a very remarkable increase. Esterase, catalase, choline dehydrogenase show a remarkable decrease, a less remarkable decrease is to be observed for pyrophosphatase, adenosintriphosphatase, arginase, and succinic dehydrogenase. Dipeptidase remains unchanged (Table II).

From such various changes of the activity of the examined enzymes it is impossible to assume, at least under the adopted experimental conditions, a definite relation between the concentration of the above-mentioned enzymes and the protein concentration of the liver. Even a division of the liver enzymes into indispensable and

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