Behaviour Of Polyhedral Bodies And Host-Cells Of Silkworms In Alcohol

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BEHAVIOUR OF POLYHEDRAL BODIES AND HOST-CELLS OF SILKWORMS IN ALCOHOL

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The proteins of polyhedral bodies, which are produced in some tissues of virus-diseased insects, were investigated in detail by Bergold.[1] The contents of virus particles in polyhedral bodies are only about five per cent, and the major part of polyhedral bodies is composed of non-infectious protein. The biological meaning of this protein or its relation to virus and host-cell is still uncertain. In order to discuss whether the protein is a kind of normal component of the host cell or an abnormal one newly produced by the virus infection, it is necessary to investigate any special characteristics of the protein.

In this paper, the solubility was investigated for polyhedral bodies in comparison with the cellular components of the host. On the solubility of polyhedral bodies, there is a report that they are soluble in acid as well as in alkali.[5] The writer, however, was surprised to find that polyhedral bodies were dissolved only with great difficulty in acid, but very easily in alcohol after treatment with acid.

EXPERIMENTAL

(1) *The effects of acid on polyhedral bodies*

Some specimens of polyhedral bodies prepared from virus diseased silkworms by different methods respectively were suspended in HCl of various concentrations from 0.05 to 2.0 *N* at room temperature or with warming, and were observed under a micro-

scope for a period extending through several days. Polyhedral bodies were generally resistant to acid solutions of these concentrations. However, polyhedral bodies which had been kept in a solution of between 0.1 and 0.25 *N* HCl for several days were deformed a little. Some of them were beginning to dissolve. Others had granulated. But the majority showed no change whatsoever. After a period of one month no more change took place. Warming to 60°C was also not effective. Moreover, the previous history of specimens examined seemed to have no effect. For instance, there was no difference in reaction to acid between the polyhedral bodies prepared from silkworms which had been corrupted for a long time and those from fresh tissue. The specimen which had been soaked in water for eighty days also did not suffer any effects from the acid even after 24 hours.

It is known that jaundice-diseased blood which is free of polyhedra is inactive after being subjected to a hydrogen ion concentration of more than pH 5, while the polyhedral bodies possessed activity even after standing at pH 2 for 24 hours.[6] This suggests that the membrane of the polyhedral bodies, the embeding material, or both may be acid-proof. If only the membrane were acid-proof, the polyhedral bodies whose membranes were destryed mechanically would suffer more or less change by acid. However, no change, even after 24 hours, was observed as a result of the acid treatment of polyhedral bodies which had previously been crushed between slide and cover glass. When two parts of 25 per cent HNO_3 were added to one part of the aqueous suspension of polyhedral bodies, about 40 per cent of them were destroyed but not dissolved.

In a droplet of dilute HCl, polyhedral bodies which were located at the outer part of the droplet were observed to be deformed. This appears to be due to the gradual increase of the hydrogen ion concentration by evaporation. Such an assumption was supported by the results obtained from this experiment: 0.1 ml. of 0.2 *N* HCl was repeatedly added to polyhedral bodies suspended in 2 ml. of water at intervals of one to three days. Two days after the second addtion of HCl, polyhedral bodies, more than 40 per cent were swollen, granulated, or divided (Fig. 1). A day after the third addition, all suffered deformation: 30 to 40 per cent of them became remarkedly swollen, and granular

masses became visible in the interior of all the other polyhedral bodies.

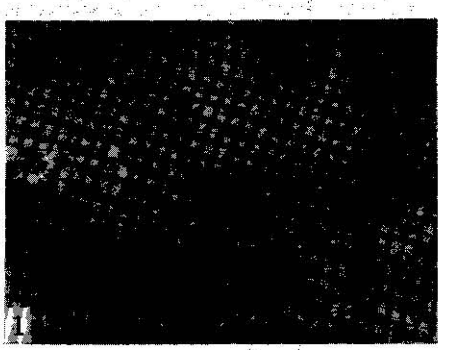

Fig. 1: Polyhedral bodies were deformed into three types by successive addition of dil. HCl at intervals of some days. Two days after the second addition. ×450

2) *Dissolution of polyhedral bodies in alcohol*

The Schmidt-Thannhauser-Schneider method[4] for the estimation of phosphorous distribution in tissues was applied to polyhedral bodies, and it was observed that the major part of them which had been previously washed with cold trichloroacetic acid solution dissolved in alcohol. The suspicion that this might be due to lipids existing in the polyhedral bodies was refuted by the fact that the ones previously treated with alcohol and a boiling mixture of alcohol and ether were dissolved in alcohol as well as the untreated ones. It is very interesting that polyhedral bodies are insoluble in alcohol without pretreatment with acid. Of the acids tried, trichloroacetic acid is the strongest in effect, hydrogen chloride is rather weak and acetic acid has no effect.

By microscopic examination, it was observed that no change took place on the polyhedral bodies treated with alcohol alone, and that the ones treated only with acid lost their gloss. The polyhedral bodies pretreated with trichloroacetic acid swelled instantly and then went on to dissolve as a result of the addition of alcohol. When *N* HCl was used as the pretreating reagent, string- or ribbon-like material flowed out from the bodies (Fig. 2).

By such a treatment, as by alkaline treatment,[8] polyhedral

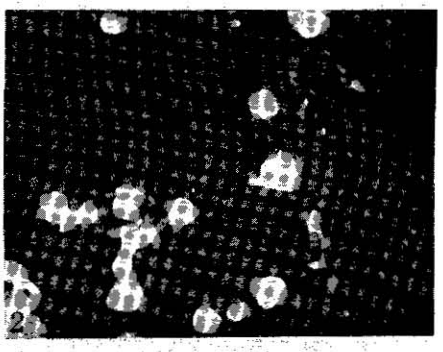

Fig. 2: String-like material flowing out from polyhedral bodies pretreated by HCl as a result of alcohol treatment. Phase contrast. ×700

bodies became so thin that electron micrographs showing their inner structure could be taken (Fig. 3).

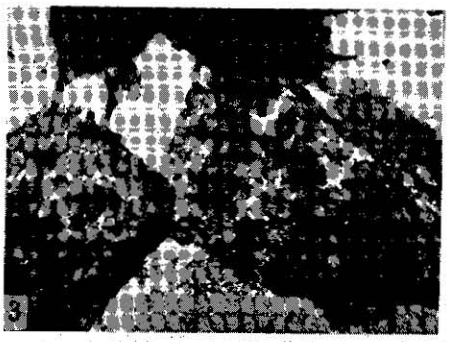

Fig. 3: Electron micrograph of polyhedral bodies treated with acid and alcohol. Many rods are observed in polyhedra. No shadow casting. ×7000

The material thus extracted by alcohol, after treatment with trichloroacetic acid, amounts to about 70 per cent in nitrogen and 20 per cent in phosphorus of polyhedral bodies. The residue remaining after the alcohol treatment is only about 30 per cent of the original weight (Table 1).

Material insoluble in alcohol was removed as follows: by being placed at 37°C for 30 minutes, polyhedral bodies were dissolved in 0.5% Na_2CO_3 solution, and the insoluble material was centrifuged off. The precipitate produced from the supernatant

Table 1. The distribution of nitrogen and phosphorus in polyhedral bodies.

Fraction	mg.	Nitrogen		Phosphorus	
		mg.	%	γ	%
Acid soluble	68.0	0.11	0.8	87.9	37.4
Alcohol soluble		9.76	71.6	55.1	23.4
Alcohol-ether soluble		0.11	0.8	3.5	1.5
DNA	32.0	3.66	26.8	59.0	25.1
RNA				22.0	9.4
Protein				7.7	3.3
Total	100	13.86	100	235.2	100

Acid soluble material and "lipid" were removed in accordance with Schmidt-Thannhauser-Schneider's procedure.[4] Nucleic acids were estimated by original method of Schmidt-Thannhauser.[3]

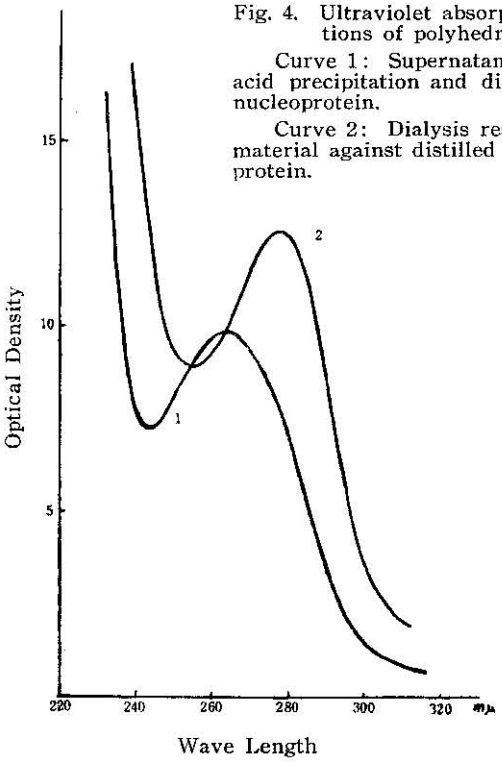

Fig. 4. Ultraviolet absorption spectra of two fractions of polyhedral bodies.

Curve 1: Supernatant fluid remaining after acid precipitation and dialysis is characteristic of nucleoprotein.

Curve 2: Dialysis residue of acid precipitable material against distilled water is characteristic of protein.

fluid by acidification with acetic acid until pH 5.5 to 6.0 was washed once with distilled water and then dialyzed against distilled water for several days in a refrigerator. The dialyzed residue was completely dissolved away in alcohol after reextraction with trichloroacetic acid. The precipitate with acetic acid was insoluble in alcohol, but on further addition of a small portion of trichloroacetic acid solution, white turbidness immediately disappeared.

The ultraviolet absorption spectrum of the acid soluble material, free of deposit which arose during dialysis, was characteristic of nucleoprotein. On the other hand, the dialysed residue, namely, alcohol soluble material, gave the absorption curve specific to polyhedral bodies or ordinal proteins (Fig. 4).

3) *Nature of alcohol soluble material*

Though the alcohol extract produced no precipitate by the addition of alkali, it did by the addition of 6*N* HCl. The addition of ether instantly caused white strong turbidness. If the remaining acid was neutralized or if the alcohol extract was prepared from residue washed once with water after the acid treatment, heavy and sticky material was precipitated suddenly by the addition of ether. This gave strong colour reactions of protein such as the biuret and the xanthoproteic reactions. The absorption spectrum of the alcohol extract was similar to ordinal proteins containing tyrosine: the alcohol extract exhibited an absorption maximum at 278 mμ and a minimum at 251 mμ (Fig. 5).

The precipitate produced by adding ether to alcohol extract redissolved with difficulty in alcohol, but easily in 0.01/N NaOH. The addition of alcohol, even in excess, to this alkali solution caused white turbidness, which, however, disappeared by the addition of acid.

From the data mentioned above, the alcohol soluble material is obviously protein or a derivative of protein. The evidence that it is not small peptide induced from protein by the rather caustic treatments, but is rather high molecular protein, was given by the experiment of ultracentrifugal sedimentation. The alcohol fraction consists of two components, as is shown in Fig. 6-A. The sedimentation constants (uncor.) of each component are 1.5 and 11 in Svedberg (s) respectively.

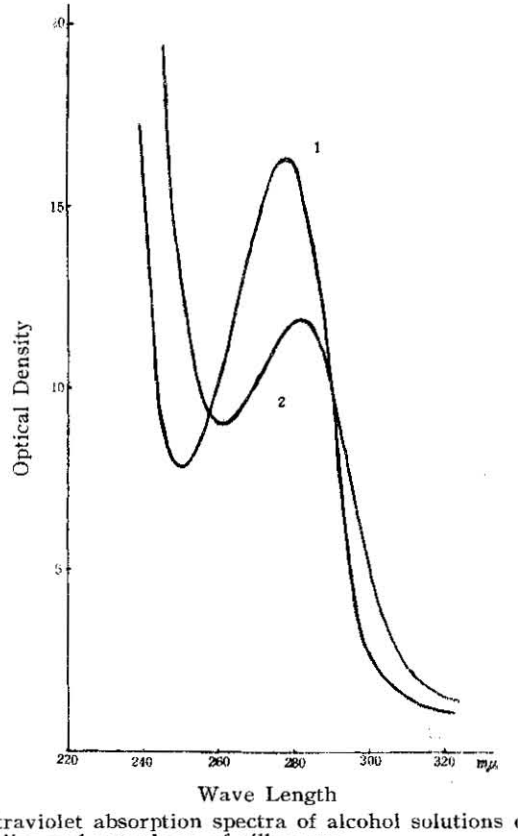

Fig. 5. Ultraviolet absorption spectra of alcohol solutions of polyhedral bodies and cytoplasm of silkworms.
Curve 1: Polyhedral bodies 1 mg.—N/ml.
Curve 2: Cytoplasm of silkworms 1 mg.—N/ml.

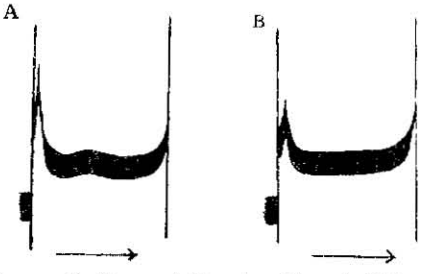

Fig. 6. Patterns of ultracentrifugal sedimentation.
A: Alcohol solution of polyhedral bodies:
56,100 r.p.m. 26 mts. 20°C.
s: 12 and 1.5 in Svedberg (uncorrected)
B: Alcohol solution of cytoplasm inoculated with viruses:
56,100 r.p.m. 43 mts. 16.5°C.
s: 1.5 in Svedberg (uncorrected)

4) *The behaviour of host cell nuclei and cytoplasm to alcohol*

It has been demonstrated that the major part of the polyhedra consists of the protein characteristic by the fact that it dissolves very easily in alcohol, though this phenomenon might be caused as the result of denaturation caused by the acid treatment. It is, however, necessary to investigate whether such a material exists in the cells of the host insect. To this purpose, fractions of cell nuclei and cytoplasm were prepared and analysed.

The cell nuclei were isolated from tissue of dissected silkworms (*P*21, 4th instar) by mincing in very dilute ice-cold citric acid using a Waring blendor and controlling always at pH 4.0, by filtration through cloths, and by washing and centrifuging repeatedly some ten times with citric acid solution at pH 4.0. The preparations of nuclei obtained by this procedure were almost pure judging from microscopic appearance.

To the supernatant fluid obtained after removal of the nuclei in dilute citric acid, 50 per cent trichloroacetic acid was added in amounts to make a final concentration of 5 per cent. The precipitate produced by this treatment was regarded as the fraction of the cytoplasm.

The isolated nuclei were dried in a vacuum after dehydration with acetone and were then analyzed through the procedure used for polyhedral bodies. In the case of the cytoplasm, the precipitate by trichloroacetic acid was treated from the second step (alcohol extraction) after being washed with a small volume of water. The amounts of the components in the cytoplasm were expressed on the basis of the residue defatted with alcohol and ether treatments.

In the nuclei, no phenomenon similar to that in the polyhedral bodies was observed. Upon completing the step of the removal of lipids (extraction by trichloroacetic acid, alcohol, and a mixture of alcohol and ether) it was observed that the weight of the nuclei had been reduced by about 20 per cent. Only 7 per cent nitrogen and 1 per cent phosphorus were detected in the alcohol soluble fraction of nuclei (Table 2). The alcohol extract of cattle spleen nuclei contained a little more nitrogen and phosphorus and was positive for biuret reaction but gave no deposit with ether (Table 3).

Table 2. The distribution of nitrogen and phosphorus in nuclei of silkworms.

Fraction	mg.	Nitrogen		Phosphorus	
		mg.	%	γ	%
Acid soluble		—	—	7.7	0.7
Alcohol soluble	19.4	1.25	7.4	18.0	1.6
Alcohol-ether soluble		—	—	6.7	0.6
Residue	80.6	13.92	82.2	1111.3	96.1
Total	100	16.89	100	1143.7	100

The "residue" means the material which remained after extraction with trichloroacetic acid, alcohol, and alcohol-ether mixture, and it consists of nucleic acids and protein.

Table 3. The distribution of nitrogen and phosphorus in nuclei of spleen.

Fraction	mg.	Nitrogen		Phosphorus	
		mg.	%	γ	%
Acid soluble		—	—	17.6	1.0
Alcohol soluble	20.0	2.29	15.5	79.6	4.4
Alcohol-ether soluble		—	—	18.3	1.0
Residue	80.0	11.13	75.6	1678.9	93.6
Total	100	14.73	100	1794	100

The data derived through the examination of the cytoplasm were extraordinally variable with each preparation. Specifically, the contents of nitrogen and phosphorus in the acid extract, which composed a major part of the whole, were so changeable as to make it impossible to compare samples. However, the ratios of each component to the value obtained by deducting the acid soluble phosphorus or nitrogen from their total could be compared with each other (Table 4). The alcohol soluble material from the cytoplasm was more than from the nuclei and gave the colour reaction and the ultraviolet absorption spectrum (Fig. 5) characteristic to protein. However, it gave no precipitation with the addition of ether. In the latter point, it differs from the alcohol extract of polyhedral bodies. Some differences are shown in Table

4 between the preparation obtained from the silkworms (*P*21) inoculated with viruses several hours before (Preparation 1), and other preparations obtained from healthy ones of the same strain (*P*21) (Preparation 2) or from a mixture of various strains (Preparation 3). The alcohol extract of preparation 1 produced a deposit reversible by ether. This alcohol soluble fraction gave the pattern of ultracentrifugal sedimentation showing one peak, even if it could hardly be said to be homogeneous (Fig. 6-B). The rate of sedimentation was in agreement with the main component of alcohol extract from polyhedral bodies: the sedimentation constant uncorrected for density was 1.5 in Svedberg (s). However, the quantity of alcohol soluble material of the cytoplasm was very small compared to that of the polyhedral bodies (Tables 1, 4).

Table 4. The distribution of nitrogen and phosphorus in cytoplasm of silkworms.

Fraction \ Preparation	Nitrogen %			Phosphorus %		
	1	2	3	1	2	3
Alcohol soluble	7.0	26.8	25	40.7	23.6	16.7
Residue	93.0	—	—	58.4	75.5	81.0
Total—Acid soluble	100	100	100	100	100	100

In this table the ratios of each component to the value obtained by deduction of acid soluble fraction from total nitrogen or phosphorus are shown.

Discussion

The polyhedral bodies resist acids but some components are removed and their proteins may be denatured. At any rate, the major part of them becomes soluble in alcohol by the action of acid. According to the direct observation under the microscope, the effect of acid is not so remarkable, but the polyhedral bodies are extremely deformed by slowly increasing the acid concentration. There are two or three types of deformation (Fig. 1). This suggests that all bodies are not invariably uniform. As a result of research with electron microscopy, Tokuyasu also[6,7] made such a suggestion.

It is known that the solubility of protein in alcohol is generally increased by denaturation with acid or alkali.[2] In fact, in the writer's experiences, outside the above mentioned examples, a considerable quantity of protein in the cytoplasm of cattle spleen was dissolved away in alcohol by the same procedure, but the alcohol solution produced no deposit by the addition of ether. Anyway, so far as silkworms are concerned, it may be said to be a very characteristic phenomenon that such an alcohol soluble protein occupies the major part of polyhedral bodies. Moreover, there is no such protein in the nuclei in which polyhedral bodies are produced. From these facts, it is evident that the abnormal protein must be newly produced by infection with viruses.

Interestingly, from the cytoplasm of virus-injected silkworms, a special protein was discovered which was similar to the abnormal one of polyhedral bodies at least on the points of solubility and size of protein. However, the relation of the protein to viruses or polyhedral bodies can not be discussed without further investigations.

The writer found in the nuclei of silkworms a rather small quantity of desoxyribonucleic acid and a larger quantity of ribonucleic acid in comparison with the nuclei of spleen. There may be some important relation between these findings and the facts that, on the one hand, much protein is newly synthesized as polyhedral protein in nuclei by virus infection and, on the other hand, as is well known, only a small number of virus particles including desoxyribonucleic acid is produced.

According to Bergold,[1] the polyhedral protein consists of a main component of which s is 13, and minor components of which s's are 3 and 1.5 respectively. In the alcohol extract, the s of the main component is 1.5 in contradiction to the findings of Bergold. It seems to be due to dissociation of the larger molecule during the treatments.

Summary

With regard to their behaviour in acid, there are three types of polyhedral bodies. The polyhedral bodies are dissolved in dilute acids only with difficulty, but the major part (ca. 70%) of the ones pretreated with trichloroacetic acid are easily dissolved in alcohol.

It was demonstrated by colour reactions, the absorption spectrum in ultraviolet, and ultracentrifugal sedimentation, that the alcohol soluble material is protein. In the nuclei of silkworms, such a material was not found. This indicates that the new abnormal protein is synthesized by the infection with viruses. A similar protein, though only a small quantity, was found in the cytoplasm in the early stage of virus infection. Regarding this problem, further investigation is desirable.

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