THE DEPROTEINISATION OF NUCLEOPROTEINS

by

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The methods at present in use for the isolation of nucleic acids usually involve the intermediate isolation of nucleoproteins and subsequent removal of the protein. This deproteinisation may be accomplished by precipitation of the proteins with strong salt solutions (BANG[1]), denaturation of the proteins at a chloroform-aqueous interface (SEVAG, LACKMAN AND SMOLENS[2]) or precipitation of the proteins by means of anionic detergents (MARKO AND BUTLER[3]; KAY AND DOUNCE[4]). The most widely used method is that of SEVAG *et al.*, by which complete deproteinisation of nucleoproteins is usually achieved. It suffers from the disadvantage, however, that it is extremely tedious, particularly in the case of bacterial nucleoproteins where often up to 25–30 successive treatments with chloroform and octanol are required. The other two methods have not had such wide application, particularly with regard to the bacterial nucleoproteins (but *cf.* SNELLMAN AND WIDSTROM[5]), but they do seem to offer certain advantages over the SEVAG method with regard to time and labour.

With these considerations in view it was considered desirable to study the deproteinisation of nucleoproteins in detail in order to obtain a method which was fairly rapid, gave good yields of nucleic acid and achieved essentially complete removal of protein. The three methods mentioned above and a fourth which involved the use of chloral hydrate as a protein precipitant were therefore investigated.

EXPERIMENTAL

Analytical methods

Nitrogen estimations were carried out by the micro-Kjeldahl method of MA AND ZUAZAGA[6] using the distillation apparatus of MARKHAM[7]. *Phosphorus* was estimated by the method of JONES, LEE AND REACOCKE[8].

The isolation of the nucleoproteins

In this investigation special attention was paid to the bacterial nucleoproteins, which were isolated from *Aerobacter aerogenes* and *Mycobacterium phlei* by the method of JONES[9]. Thymus nucleohistone was prepared by the MIRSKY AND POLLISTER[10] procedure. The solutions of the nucleoproteins in 1 *M* sodium chloride were freed from cell debris by centrifuging at 20,000 *g*.

Attempted deproteinisation using strong salt solutions

Since saturated sodium chloride has been used successfully to deproteinise thymus nucleohistone (BANG) and *Myco. phlei* nucleoprotein (SNELLMAN AND WIDSTROM), it appeared probable that strong salt solutions would accomplish the deproteinisation of other bacterial nucleoproteins. The effect of saturated sodium chloride and saturated ammonium sulphate on the nucleoproteins of *Aerobacter aerogenes* and *Myco. phlei* were investigated as follows:

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Two separate aliquots of the nucleoprotein solution were saturated with sodium chloride and ammonium sulphate respectively and a third aliquot retained as control. The solutions were kept at 0° C for 48 hours, centrifuged and aliquots (0.3 ml) of the clear liquid diluted to 5.0 ml with 1 *M* sodium chloride for measurement of the optical density in the region 220–310 mμ.

The results indicated that with saturated sodium chloride a small amount of material was precipitated in the case of the nucleoprotein of *Aerobacter aerogenes* and none in that of *Myco. phlei*, and with saturated ammonium sulphate much more material was precipitated in both cases. In none of the cases however was there complete precipitation, and it was apparent that nucleic acid and protein were precipitated simultaneously.

Deproteinisation with sodium dodecyl sulphate

The use of sodium dodecyl sulphate (S.D.S.) for the deproteinisation of thymus nucleohistone has been reported by MARKO AND BUTLER. It was of interest therefore to study the action of S.D.S. on the nucleoproteins of *Aerobacter aerogenes*, *Myco. phlei* and thymus.

The optimum S.D.S. concentration for the precipitation of protein from the nucleoproteins was determined as follows:

A series of tubes was made up containing nucleoprotein solution (2.5 ml), sodium chloride, water and S.D.S. solution so that the final volume was 5.0 ml, the sodium chloride and nucleoprotein concentration remained constant and the S.D.S. concentration varied over the range. The solutions were kept at 15° C for 18 hours and then centrifuged at 15° C at 7000 *g*. Aliquots (2.5 ml) of the supernatant liquid were treated with calcium chloride (1 *M*) and ethanol (1 vol.). (This reagent completely precipitated both nucleic acid and protein), the precipitate collected and dissolved in 0.1 *M* sodium citrate (4.5 ml). Aliquots (2 × 1 ml) were taken for both phosphorus and nitrogen determinations. The results are recorded in Table I.

TABLE I

THE EFFECT OF SODIUM DODECYL SULPHATE CONCENTRATION ON THE DEPROTEINISATION OF NUCLEOPROTEINS

S.D.S. concentration %	*Aerobacter aerogenes nucleoprotein (1.0 M NaCl)*			*Myco. phlei nucleoprotein (1.0 M NaCl)*			*Thymus nucleohistone (1.0 M NaCl)*		
	N(γ/ml)	*P(γ/ml)*	*N/P*	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*N(γ/ml)*	*P(γ/ml)*	*N/P*
0	185.0	49.6	3.72	80.5	28.4	2.83	135.8	40.1	3.38
0.1	106.0	47.8	2.22	55.9	25.6	2.18	93.4	41.4	2.25
0.2	96.4	47.1	2.01	52.5	26.7	1.97	81.5	39.0	2.09
0.3	95.5	44.8	2.14	44.6	22.1	2.02	90.0	41.4	2.17
0.4	104.0	48.0	2.16	68.5	29.3	2.34	86.1	40.1	2.15
0.5	128.2	49.6	2.58	63.8	29.9	2.14	87.5	42.1	2.08

It may be seen that the the minimum N/P ratio was obtained with 0.2% S.D.S. in the case of *Aerobacter aerogenes* and *Myco. phlei* and over a wide range (> 0.2%) in the case of thymus nucleohistone.

The optimum sodium chloride concentration for protein precipitation was determined in a similar manner using the optimum S.D.S. concentrations found above.

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From the results (Table II) it was apparent that the optimum sodium chloride concentration was 1.00 *M* in the case of *Aerobacter aerogenes* and thymus nucleoproteins and 0.50–0.75 *M* in the case of the nucleoprotein of *Myco. phlei.*

TABLE II

THE EFFECT OF SODIUM CHLORIDE CONCENTRATION ON THE DEPROTEINISATION WITH SODIUM DODECYL SULPHATE

Sodium chloride concentration	*Aerogenes aerobacter nucleoprotein (0.2% S.D.S.)*			*Myco. phlei nucleoprotein (0.2% S.D.S.)*			*Thymus nucleohistone (0.2% S.D.S.)*		
M	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*N(γ/ml)*	*P(γ/ml)*	*N/P*
0.40	72.7	31.8	2.28	56.4	25.2	2.24	128.0	40.9	3.14
0.50	76.0	34.8	2.18	50.0	26.2	1.90	134.5	41.5	3.24
0.75	91.3	49.2	1.86	52.7	27.6	1.90	85.2	38.0	2.24
1.0	90.6	52.0	1.75	68.8	27.6	2.50*	79.8	38.0	2.10
1.25	94.5	50.5	1.87	70.8	28.2	2.50*	78.0	37.2	2.10
1.50	98.0	51.0	1.92*	74.8	28.8	2.59*	—	—	—

* Precipitates not easily separated by centrifugation.

It appeared probable that the optimum sodium chloride and S.D.S. concentrations would be affected by the nucleoprotein concentration. The optimum nucleoprotein concentration was determined therefore. The results are recorded in Table III.

From the results it was apparent that the bacterial nucleoproteins could be partly deproteinised by treatment with S.D.S. The detergent, sodium chloride, and nucleoprotein concentrations were important and for the best results it was necessary to work out the optimum conditions.

TABLE III

THE EFFECT OF NUCLEOPROTEIN CONCENTRATION ON THE DEPROTEINISATION WITH SODIUM DODECYL SULPHATE

Aerobacter aerogenes nucleoprotein (0.2% S.D.S.; 1.0 M NaCl)				*Myco. phlei nucleoprotein (0.2% S.D.S.; 0.75 M NaCl)*				*Thymus nucleohistone (0.2% S.D.S.; 1.0 M NaCl)*			
*Nucleoprotein concentration (mg/ml)**	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*Nucleoprotein concentration (mg/ml)**	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*Nucleoprotein concentration (mg/ml)**	*N(γ/ml)*	*P(γ/ml)*	*N/P*
0.53	60.5	22.5	2.69	0.268	33.4	13.8	2.40	0.670	49.5	22.0	1.83
0.795	121	38.0	3.20**	0.403	46.2	20.8	2.22	1.30	94.1	51.6	1.82
1.06	98.0	45.5	2.16	0.536	52.7	27.7	1.90	2.00	145	79.0	1.84
1.33	125	50.6	2.47	0.670	71.0	34.0	2.08	2.48	210	98.4	2.14
1.59	244	67.8	3.60**	0.805	91.6	40.7	2.23	3.42	344	136	2.50
1.86	228	81.6	2.79***	—	—	—	—	—	—	—	—
2.12	276	86.4	3.20***	—	—	—	—	—	—	—	—

* Based on the assumption that the solution contained only nucleoprotein and that the nitrogen content of the nucleoprotein was 15%.

** A cloudy suspension was produced which could not be clarified by centrifugation.

*** A light precipitate was formed which collected at the top of the solution upon centrifuging.

Comparison of the S.D.S. deproteinisation method with the chloroform method

A solution of thymus nucleohistone in 1 *M* sodium chloride was deproteinised completely by shaking with chloroform and octanol (12–13 times) according to the method of SEVAG, LACKMAN AND SMOLENS. The nucleic acid was precipitated with

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ethanol, dissolved in distilled water, dialysed and freeze-dried, to give a white fibrous product 342 mg (P; 7.85%; N, 13.50%; N/P, 1.72, $[\varepsilon]_P = 7540$).

An equal volume of the 1 *M* sodium chloride solution of thymus nucleohistone was treated with 0.4% S.D.S. in 1 *M* sodium chloride (1 vol.) and allowed to stand for 15 hours at 0° C. The precipitated protein was removed by centrifugation (15 min) at 20,000 *g*. The nucleic acid was precipitated with ethanol (2 vols.), the precipitate redissolved in distilled water and sufficient sodium chloride added to make the concentration 1 Molar. The last traces of protein were removed by three treatments with chloroform and octanol. The nucleic acid was then isolated in the usual way when 694 mg of product (P, 7.85%; N, 13.3%; N/P, 1.69 $[\varepsilon]_P$ 7250; S < 0.05%; analysis carried out by Mr. D. S. LETHAM) were obtained.

Deproteinisation with chloral hydrate

Chloral hydrate has been used to extract starch from various sources (MEYER AND BERNFELD[11]; BOURNE, STACEY AND WILKINSON[12]) and gave a product almost free from protein. It appeared therefore that chloral hydrate may act as a protein precipitating reagent. This was shown to be the case in preliminary experiments in which chloral hydrate at pH 7 and 0° C was shown to completely precipitate γ-globulin. Moreover it was found that in strong salt solutions and at pH 6.5 and 0° C, chloral hydrate was able to almost completely deproteinise various nucleoproteins. The optimum conditions for this deproteinisation were determined therefore.

Determination of the optimum sodium chloride concentration

At sodium chloride concentrations above and below the optimum, protein and nucleic acid tended to precipitate together. The optimum conditions was taken therefore as the point of minimum nucleic acid precipitation as indicated by the phosphorus content of the supernatant liquid.

A series of tubes each containing nucleoprotein solution (5 ml), chloral hydrate solution (a freshly prepared 30% solution in acetate buffer pH 6.5, ionic strength 0.10) (5 ml), and sodium chloride of the appropriate concentration, were allowed to stand at 0° C for 15 hours and then the solution centrifuged at 7,000 *g* for 30 minutes. An aliquot (5 ml) of the supernatant liquids was removed from each tube, treated with uranyl acetate-trichloroacetic acid (MACFADYEN'S[13] reagent) (5 ml), the precipitate collected, washed and redissolved in 0.2 *M* sodium hydroxide (5.0 ml). Aliquots (2 × 1 ml) were taken for phosphorus determinations.

TABLE IV

THE EFFECT OF SODIUM CHLORIDE CONCENTRATION ON DEPROTEINISATION WITH CHLORAL HYDRATE

Sodium chloride concentration (M)	% *Phosphorus not precipitated*		
	Aerobacter aerogenes nucleoprotein	*Myco. phlei nucleoprotein*	*Thymus nucleohistone*
0.5	4.8	6.0	30.3
1.0	18.6	9.1	53.7
2.0	93.1	90.6	82.0
3.0	87.1	85.5	72.5
4.0	36.4	79.8	69.2
5.0	14.8	68.0	—

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The results shown in Table IV indicate that the optimum sodium chloride concentration for minimum nucleic acid precipitation was 2.0–3.0 *M*.

Using a sodium chloride concentration of 2.5 *M*, the optimum chloral hydrate and nucleoprotein concentrations were determined in a similar manner to that described for the S.D.S. experiments. The results are recorded in Tables V and VI.

TABLE V

THE EFFECT OF CHLORAL HYDRATE CONCENTRATION ON THE DEPROTEINISATION OF NUCLEOPROTEINS

Chloral hydrate	*Aerobacter aerogenes nucleoprotein*			*Myco. phlei nucleoprotein*			*Thymus nucleohistone*		
Concentration	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*N(γ/ml)*	*P(γ/ml)*	*N/P*
0.00	—	—	—	398	89.6	4.44	144	42.5	3.38
0.05	117	40.1	2.90	—	—	—	—	—	—
0.10	113	42.0	2.69	—	—	—	—	—	—
0.15	101	38.7	2.60	—	—	—	—	—	—
0.30	90.8	40.6	2.23	—	—	—	—	—	—
0.40	86.0	39.5	2.18	336	88.0	3.81	—	—	—
1.0	—	—	—	332	89.0	3.72	—	—	—
1.25	76.4	39.6	1.93	—	—	—	—	—	—
2.0	—	—	—	307	85.8	3.59	—	—	—
2.5	78.2	38.0	2.06	—	—	—	—	—	—
3.0	—	—	—	254	84.0	3.03	—	—	—
4.0	—	—	—	128	70.0	1.82	83.8	37.6	2.20
6.0	—	—	—	136	74.0	1.83	—	—	—
8.0	—	—	—	—	—	—	55.3	27.2	2.02
10.0	—	—	—	148	74.0	1.97	52.1	27.0	1.93
12.0	—	—	—	—	—	—	52.7	27.0	1.95
16.0	—	—	—	169	74.1	2.27	80.8	28.8	2.80
20.0	—	—	—	164	68.5	2.38	—	—	—

TABLE VI

THE EFFECT OF NUCLEOPROTEIN CONCENTRATION ON DEPROTEINISATION WITH CHLORAL HYDRATE

Aerobacter aerogenes nucleoprotein (2% chloral hydrate)				*Myco. phlei nucleoprotein (6% chloral hydrate)*				*Thymus nucleohistone (10% chloral hydrate)*			
Nucleoprotein concentration (mg/ml)	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*Nucleoprotein concentration (mg/ml)*	*N(γ/ml)*	*P(γ/ml)*	*N/P*	*Nucleoprotein concentration (mg/ml)*	*N(γ/ml)*	*P(γ/ml)*	*N/P*
1.05	48.4	25.6	1.89	0.85	42.9	26.7	1.61	0.41	81.8	23.0	3.52
2.10	107	59.1	1.81	1.70	93.5	58.4	1.61	0.82	40.2	22.5	1.78
3.15	161	85.0	1.89	2.55	137	83.0	1.65	1.23	(did not clear		
—	—	—	—	3.40	200	107	1.86	—	on centrifugation)		

Chloral hydrate achieved considerable deproteinisation of the nucleoproteins. The bacterial nucleoproteins formed compact precipitates on centrifugation, but in the case of thymus nucleohistone, however, the protein formed a bulky gelatinous precipitate and a large proportion of the thymus nucleic acid was co-precipitated with the protein.

The effect of chloral hydrate on nucleic acids

In order to investigate the effect of chloral hydrate on nucleic acids the following experiment was carried out:

Two tubes were prepared containing highly polymerised thymus DNA (15 mg)

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in 3 *M* sodium chloride solution (10 ml). To one tube there was added a freshly prepared solution of chloral hydrate (30%) in 0.10 *M* acetate buffer pH 6.5 (10 ml) and to the other tube, 0.10 *M* acetate buffer, pH 6.5 (10 ml). After mixing, the tubes were left at room temperature (24 h) and treated with ethanol (2 vols.). The fibrous nucleic acid was collected, redissolved in distilled water and dialysed (120 h) against repeated changes of 0.10 *M* sodium chloride. The extinction coefficients, phosphorus contents, and viscosities of the resulting nucleic acid preparations were determined. The viscosities of the chloral hydrate-treated and control sample were almost identical and the $[\varepsilon]_P$ values were 6706 and 6977, respectively. Thus there appeared to be no indication of degradation of the nucleic acid by chloral hydrate. Nucleic acids treated with chloral hydrate did not contain detectable amounts of chlorine and therefore there was no combination of chloral hydrate with the nucleic acid.

The preparation of nucleic acids using chloral hydrate

a. *Aerobacter aerogenes*

Nucleoprotein solution (25 ml) in 2.5 *M* sodium chloride solution, was treated with an equal volume of 2.5% chloral hydrate in 2.5 *M* sodium chloride, and the protein removed by centrifugation at 20,000 *g*. The nucleic acid was precipitated with ethanol, collected, redissolved in 1 *M* sodium chloride and treated with chloroform-octanol to achieve clarification. The nucleic acid was precipitated with ethanol (2 vols.), washed with 85% ethanol (twice), redissolved in distilled water, dialysed (36 h) against distilled water and freeze-dried to give a white fibrous product (28.2 mg), N, 13.5%; P, 7.95%; (N/P 1.70); $[\varepsilon]_P^{258}$ 6240.

b. *Myco. phlei*

Nucleoprotein solution (140 ml) was treated with an equal volume of 12% chloral hydrate solution (both dissolved in 2.5 *M* sodium chloride solution). After 30 minutes the solution was centrifuged at 20,000 *g* and the supernatant liquid treated with ethanol (2 vols.). The precipitated nucleic acid was dissolved in distilled water, sodium chloride added until its concentration was approximately 1 *M* and the solution shaken with chloroform and octanol twice. The nucleic acid was precipitated with ethanol (2 vols.), washed with 85% ethanol (twice), redissolved in distilled water, dialysed against distilled water and freeze-dried to give a white fibrous product (161 mg) N, 13.0%; P, 7.85; N/P, 1.66; $[\varepsilon]_P^{258}$, 6040.

DISCUSSION

It is apparent from the foregoing experimental that, of the deproteinisation methods studied, only the chloroform method of SEVAG *et al.* gave essentially complete deproteinisation and could be applied to all three of the nucleoproteins in this investigation. This method, however, besides being extremely tedious, had the disadvantage of producing low yields of nucleic acid due, most probably, to adsorption on the very large surface area of the chloroform-protein gel. This was shown to be the case during the deproteinisation of thymus nucleohistone and *Aerobacter aerogenes* nucleoprotein, also with the nucleoprotein of *Sarcina lutea* with which it was shown (in collaboration with Mr. S. K. DUTTA) that the pentosenucleic acid was adsorbed by the gel to a slightly greater extent than the deoxypentosenucleic acid (DNA). Much of the nucleic acid

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could be recovered from the gels by prolonged washing but this was an extremely tedious procedure. Since the work of CHARGAFF, CRAMPTON AND LIPSHITZ[14], and of BROWN AND WATSON[15], has shown the heterogeneous nature of DNA from a given source, it was apparent that adsorption of the nucleic acid on the protein gel was particularly undesirable when a nucleic acid preparation representative of the source was required.

Deproteinisation with strong salt solutions which is quite effective for thymus nucleohistone, was not applicable to the nucleoproteins of *Aerobacter aerogenes* and *Myco. phlei* as it appeared that they were not completely dissociated even by saturated ammonium sulphate. A similar result was reported by CHARGAFF AND SAIDEL[16] for the nucleoprotein of *Mycobacterium tuberculosis*.

Sodium dodecyl sulphate was able to remove most of the protein from the bacterial nucleoproteins as well as from thymus nucleohistone. The optimum conditions were rather critical, however, particularly in the case of *Aerobacter aerogenes*. In no instance was deproteinisation complete, and it was necessary to remove the last traces of protein by SEVAG's method. Thymus DNA prepared in this manner contained no detectable sulphur (less than 0.05%).

Chloral hydrate was a quite efficient deproteinising agent for the bacterial nucleoproteins. It had the advantage that the optimum conditions were not so critical as with sodium dodecyl sulphate, although it was apparent that the protein was partly redissolved by excess chloral hydrate. In some cases almost complete removal of the protein was obtained by one treatment, but it was desirable to make sure that last traces of protein were removed by shaking once or twice with chloroform and octanol. Chloral hydrate could not be used for the deproteinisation of thymus nucleohistone, however, due to the fact that much of the nucleic acid was precipitated with the protein. The proteins precipitated from the nucleoproteins by the action of chloral hydrate could not be dissolved at pH values between 2 and 12 even after removal of the chloral hydrate by dialysis. No reaction between chloral hydrate and the nucleic acids could be detected; it appeared, therefore, that this reagent could be safely used for the preparation of relatively undegraded nucleic acids.

It is suggested that the best method for the deproteinisation of nucleoproteins is to remove most of the protein as compact precipitates with sodium dodecyl sulphate or chloral hydrate and then remove the last traces by SEVAG's method. In this way complete deproteinisation is assured, while at the same time the number of treatments with chloroform is cut to a minimum, thus saving time and avoiding the production of voluminous gels upon which nucleic acid is lost by adsorption. A method, which is the converse of this, namely removal of the majority of the protein by SEVAG's method and the last traces by sodium dodecyl sulphate, has been suggested by BERNSTEIN[17]. No comparison between these two methods can be made, as in the above paper no yields or analytical results were recorded. From the present work, however, it appears doubtful whether the final treatment with sodium dodecyl sulphate would result in complete deproteinisation.

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SUMMARY

Various methods for the deproteinisation of the nucleoproteins of *Myco. phlei*, *Aerobacter aerogenes* and thymus have been studied in detail. The deproteinising agents investigated were strong salt solutions, chloroform and octanol (SEVAG *et al.*), sodium dodecyl sulphate (S.D.S.) and chloral hydrate. It was found that strong salt solutions were ineffective for the bacterial nucleoproteins; the SEVAG method was tedious and nucleic acid was to some extent adsorbed on the gel layer. S.D.S. readily removed most of the protein but deproteinisation was not quite complete. Chloral hydrate behaved similarly in the case of the bacterial nucleoproteins, but was ineffective with thymus nucleoprotein. The best method found was to remove the bulk of the protein by the use of S.D.S. or chloral hydrate and the remainder by one or two treatments with chloroform and octanol.

RÉSUMÉ

Diverses méthodes de déprotéinisation des nucléoprotéines de *Myco. phlei*, *Aerobacter aerogenes* et du thymus ont été étudiées en détail. Les agents déprotéinisants étudiés sont des solutions salines concentrées, le chloroforme et l'octanol (SEVAG *et al.*), le dodécylsulfate de sodium (S.D.S.) et le chloral.

Les solutions salines concentrées sont inefficaces dans le cas des nucléoprotéines bactériennes; la méthode de SEVAG est laborieuse et une partie de l'acide nucléique est adsorbée sur le gel. S.D.S. élimine facilement la plus grande partie de la protéine mais la déprotéinisation n'est pas totale. Le chloral donne des résultats analogues dans le cas des nucléoprotéines bactériennes, mais est sans action sur les nucléoprotéines du thymus.

La méthode la meilleure consisterait à éliminer la plus grande partie de la protéine à l'aide de S.D.S. ou de chloral, puis à achever la purification par un ou deux traitements au chloroforme et à l'octanol.

ZUSAMMENFASSUNG

Es wurden verschiedene Methoden der Enteiweissung von Nucleoproteinen von *Myco. phlei*, *Aerobacter aerogenes* und Thymus untersucht. Als enteiweissende Reagentien wurden starke Salzlösungen, Chloroform und Octanol (SEVAG *et al.*), Natriumdodecylsulfat (S.D.S.) und Chloralhydrat untersucht.

Es wurde gefunden, dass starke Salzlösungen an Bakteriennucleoproteinen unwirksam sind; die SEVAG-Methode war langwierig und die Nucleinsäuren wurden teilweise an die Gelschicht absorbiert. S.D.S. entfernt schnell den grössten Teil der Proteine, aber die Enteiweissung war nicht ganz vollständig. Chloralhydrat verhält sich im Falle der Bakteriennucleoproteine ähnlich, an Thymusnucleoproteinen aber ist es unwirksam.

Als beste Methode wurde die Entfernung des Hauptanteiles der Proteine durch Anwendung von S.D.S. oder Chloralhydrat und des Rückstandes durch ein- oder zweimalige Behandlung mit Chloroform oder Octanol gefunden.

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