of the fact that, in the material normally fixed by methanol, losses and solubility of both nucleic acids are the same (Table II). An explanation of the high losses of DNA in the course of substitution cannot be attempted on the basis of the investigation outlined in this paper. MIYAJI and PRICE[6] have stated that in a solution devoid of electrolytes the disintegration of sodium deoxypentose nucleate is speeded up by a temperature rise and slowed down by presence of electrolytes. The conditions used in our experiments favoured the disintegration of DNA molecules in this respect. It may be assumed that the disintegration is high because of the incomplete denaturation of DNA in the substituted material. It remains unclear why there is a difference in the solubility of RNA and DNA in identical experimental conditions.

Conclusions. (1) In the methanol-substituted tissue, no losses of RNA were found whereas DNA content was diminished by 17%. (2) Methanol-substituted material subsequently extracted by water loses a certain amount of tissue substance, which was expressed by a 16% loss in dry weight as compared to the control. DNA is partly eluted as well since its content in tissue is about 76%. RNA is not extracted by water. (3) Methanol substitution may be recommended as a method for RNA determination but is inadequate for quantitative determination of DNA.

Résumé. Les auteurs ont déterminé le contenu en acides nucléiques dans le foie du rat après la congélation-dessication dans du méthanol. Après cette technique, une grande partie de DNA était soluble dans l'eau.

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Department of Histology and Embryology, Medical Academy, Warsaw (Poland), November 6, 1964.

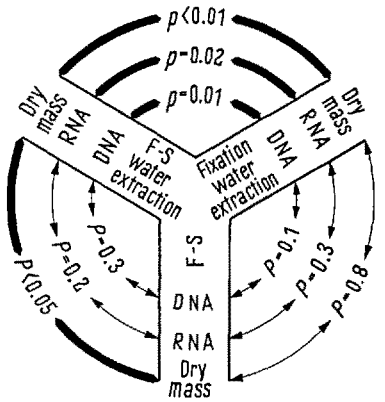

Fig. 2. Statistical significance of the differences of dry mass and nucleic acid content in the experimental groups.

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Effect of Polyribonucleotides on Amino Acid Incorporation by Chloroplast Ribosomes

That chloroplasts can synthesize protein has been demonstrated by many workers[1–4]. Ribonucleoprotein particles have recently been isolated from chloroplasts by MIKULSKA et al.[5]. While this work was in progress, two reports[6,7] were published regarding the protein synthesis by chloroplast ribosomes. We wish to report our results on the effect of polyribonucleotides on amino acid incorporation by chloroplast polyribosomes.

Chloroplasts were isolated from spinach leaves according to the method of GORHAM[8]. 10–20 g fresh chloroplasts were disrupted with 20 vol 0.10 *M* Tris buffer pH 7.0 by constant stirring in cold for 1 h and then centrifuged at 10,000 *g* for 30 min. The pellet constitutes the grana, and the supernatant stroma thus obtained was further subjected to centrifugation at 105,000 *g* for 2 h. The pellet constitutes the RNP-particles and from the supernatant pH 5 fraction was isolated. Both the fractions were dispersed in a minimal amount of 0.10 *M* Tris buffer pH 7.0. The total volume was adjusted to 2–5 ml according to the initial amount of chloroplasts taken. Polyribonucleotides were prepared with *Azetobacter vinelandii* polynucleotide phosphorylase, according to the method of GRUNBERG-MANAGO et al.[9]. After 1 h incubation the reaction was stopped with 1 ml of 1 *M* perchloric acid. The precipitate was washed 7–8 times with 10 ml 0.2 *M* perchloric acid, after dissolving once in KOH, and reprecipitated with perchloric acid. Finally the precipitate was dispersed in a few drops of NH_4OH and the volume was made up to 1.0 ml. An aliquot of this was plated and counted. Protein was estimated by Biuret test[10], after removing chlorophyll with alcohol and acetone whenever necessary.

Ribosomal particles were isolated from chloroplasts and, once sedimented at 105,000 *g*, it was found to be difficult to get into clear solution and therefore the stroma and pH 5 fractions were subjected to analytical ultracentrifugation. Ribosomal particles of various sizes (20 S – 130 S) were found to be present in the stroma fraction as has been shown in the Figure. With pH 5 only 20 S particles were obtained. When RNP particles and pH 5

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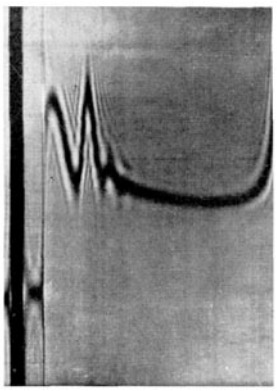
fraction were incubated with phenylalanine-1-C^{14}, there was an increase in phenylalanine incorporation with time up to 1 h. From Table I it is evident that for optimal incorporation RNP particles, pH 5 fraction, and GTP were required, but the absolute requirement for ATP and generating system could not be demonstrated. It was later observed that adenylate kinase activity was associated with RNP fraction. If any one of them was omitted, there was an inhibition in phenylalanine incorporation. Chloramphenicol was found to be inhibitory. The optimal pH for the incorporation was recorded to be around 7.0 and linear with increased concentration of RNP and pH 5 fractions. When grana was tested with phenylalanine, a very low activity was detected; but when the system was reconstituted, i.e. RNP + pH 5 (stroma) combined with grana, 55% inhibition was recorded. The effect of poly U in this case was noted after treating the RNP particles with pancreatic RNase for 15 min. The data are given in Table II. Half of the incorporation was diminished after the short treatment with RNase, but with the addition of poly U the incorporation could be fully restored. Without this treatment there was no effect of poly U. 40% inhibition in phenylalanine incorporation was observed when poly A was added. RNA isolated from RNP particles could restore 85% of phenylalanine incorporation. Since it has been observed that with the addition of grana phenylalanine incorporation was inhibited, it was thought possible that grana might have some inhibitor. When tested for the presence of RNase, a high activity was found to be associated with the grana, but very little activity was detected with intact chloroplasts or with stroma fraction. The results are given in Table III. When sedimentation patterns of pH 5 and stroma fractions are compared, it is apparent that ribosomal aggregates exist in the chloroplast. For amino acid incorporation all ingredients are required, except ATP generating system. As a matter of fact, adenylate kinase is present in RNP fraction. Since addition of poly U does not stimulate the incorporation of phenylalanine, it has been thought possible that the messenger RNA in the chloroplasts might be more stable and associated with the ribosomes. It has been demonstrated in bacterial and mammalian systems that poly U encodes phenylalanine[11–13]. It is expected that this coding is operative in this case as well. In fact, after brief RNase treatment, phenylalanine incorporation has been stimulated by poly U[14]. With higher concentration of poly A, not more than 40% reduction has been obtained, indicating possibly a limiting association with poly U. RNA isolated from RNP particles could restore 85% phenylalanine incorporation, possibly suggesting the



Sedimentation pattern of stroma fraction. 0.8% in buffer pH 7.5, 42,040 rpm, bar angle 30°. Peaks correspond to 20, 80, 100 and 130 S respectively.

Table 1. Phenylalanine incorporation by ribonucleoprotein particles. Complete system contains the following: RNP, 1.97 mg; pH 5, 1.98 mg; ATP, 2 μmoles; GTP, 1 μmole; KCl, 10 μmoles; $MgCl_2$, 5 μmoles; PEP, 3 μmoles; pyruvate kinase, 0.20 mg; phenylalanine-1-C^{14}, 1 μmole of specific activity 1.4×10^5 cpm/μmole; grana, 4–5 mg and 12.5 μmole chloramphenicol/ml whenever used. Incubated at 37° for 60 min

Incubation mixture	mμm/mg P
Complete	0.80
– RNP	0.50
– ph 5	0.48
– GTP	0.44
– ATP generating system	0.80
+ Chloramphenicol	0.30
Grana	0.20
RNP + pH 5 + Grana	0.35

Abbreviations: RNA, ribonucleic acid; RNase, ribonuclease; Poly A, polyadenylic acid; Poly U, polyuridylic acid. ATP and GTP, corresponding nucleoside triphosphates; PEP, phosphoenol pyruvate. Tris, tris(hydroxymethyl)aminoethane; RNP, ribonucleoprotein particle.

Table II. The effect of poly U and poly A in the incorporation of phenylalanine. Pretreatment with pancreatic RNase (100 μg) for 15 min and others are same as mentioned in Table I. RNase is removed by centrifugation at 105,000 *g* for 2 h. RNA isolated from RNP particles, 2 O.D.

Incubation mixture	mμm incorporated
1. Complete	1.40
2. 1 + poly U	1.40
3. 1 + RNase	0.65
4. 3 + RNA (RNP)	1.20
5. 3 + poly U (1 O.D.)	1.35
6. 5 + poly A (1 O.D.)	0.90
7. 5 + poly A (2 O.D.)	0.85

Table III. RNase activity in chloroplast. In the assay system 3 O.D. RNA was used. The reactions were stopped by the addition of uranylacetate in perchloric acid. Increase in O.D. in acid soluble fraction was determined at 260 mμ against reagent blank; 0.001 O.D. increase per min per mg of protein has been taken as 1 unit

	unit/min/mg of P
Grana	32
Stroma	8
Chloroplast	3

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stable nature of that RNA. Further evidence is also adduced from the fact that Actinomycin D cannot reduce appreciably the ribosomal RNA synthesis in chloroplast (unpublished). As regards to the inhibition of phenylalanine incorporation by grana, it seems that after disruption of the chloroplasts RNase activity increases by at least tenfold. It is apparent from this study that chloroplast has ribosomal aggregates and follows the same coding principle in protein synthesis[15].

Zusammenfassung. Partikel mit Ribonucleoprotein aus Spinatchloroplasten wurden isoliert und als wahrscheinliche Loci der Proteinsynthese bestimmt. Nach Chloroplastenaufschluss hemmte die Granafraktion mit hoher Ribonucleaseaktivität den Aminosäureneinbau. Der Einbau von Phenylalanin wird durch Polyuridylsäure gefördert und durch Polyadenylsäure nur nach Vorbehandlung der Ribosomen-Aggregate mit Ribonuclease gehemmt.

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Radiochemical Laboratory, Bose Institute, Calcutta (India), December 18, 1964.

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Über den Einfluss einiger oberflächenaktiver Substanzen auf den Einbau freier Fettsäuren in die Lipoide des Rattenpankreas in vitro

Im Rahmen von Untersuchungen über funktionelle Zusammenhänge zwischen Lipoidstoffwechsel und Zymogengranulaausschüttung nach Sekretingaben wurde in vitro am Rattenpankreas folgende interessante Beobachtung gemacht: In mehreren reproduzierbaren Versuchsreihen, von denen eine in der Tabelle wiedergegeben ist, konnte die Aufnahme freier Fettsäuren (FFS) in die Lipoide des Pankreas durch Zugabe von oberflächenaktiven Substanzen zum Inkubationsmedium beeinflusst werden. Die Ladung der oberflächenaktiven Substanzen war dabei von Bedeutung. Die ungeladenen Tergitol, Saccharosemonopalmitat, Rindergehirncerebrosid und Vitamin A und das schwach saure Colamin-Kephalin zeigten keinen oder nur geringen Einfluss auf die Aufnahme von FFS in das Pankreas. Jedoch erhöhten das positiv geladene Adogen 401, eine quartäre Ammoniumbase mit langer hydrophober Kohlenwasserstoffkette und das negativ geladene Laurylsulfat die Fraktion der zellulär gebundenen, d.h. mit Kochsalzlösung nicht entfernbaren FFS um das Vielfache. Es wurde nicht untersucht, ob die FFS nur an der äusseren Zelloberfläche adsorbiert waren oder bereits dem intrazellulären FFS-Pool angehörten. Im Gegensatz zum Adogen 401 erhöhte das Laurylsulfat zusätzlich den Einbau von FFS in die Neutrallipoide auf etwa das Fünffache. Keine der getesteten oberflächenaktiven Substanzen hatte eine stimulierende Wirkung auf den Einbau von FFS in die Glycerinphosphatide. Wegen seiner sauren Ladung wurde Heparin, das nicht zu den oberflächenaktiven Substanzen gezählt werden kann, unter den gleichen Versuchsbedingungen getestet. Heparin zeigte dabei keinen Einfluss auf den FFS-Einbau.

Der Einfluss von oberflächenaktiven Substanzen auf die Aufnahme von H^3-Palmitinsäure in die Pankreaslipoide

Zusätze	dpm Freie Fettsäuren	dpm/mg Neutrallipoide	dpm/mg Phosphatide
Leerwert	375	591	444
Tergitol	612	462	291
Vitamin A	405	297	300
Saccharosemonopalmitat	384	555	531
Cerebrosid	222	351	378
Colamin-Kephalin	417	449	446
Adogen 401	2823	471	428
Laurylsulfat	3310	2678	312
Heparin	417	561	387

Die Tabelle zeigt die Verteilung der Radioaktivitäten auf die verschiedenen Lipoidfraktionen. Jeweils 2 Rattenpankreas wurden in H^3-Palmitinsäure enthaltenden Albuminphosphatpuffern ± oberflächenaktiver Substanz 30 min bei 37°C unter leichtem Schütteln aerobisch inkubiert.

Die Erklärung dieser Befunde kann nur hypothetischer Art sein und geht von der Voraussetzung aus, dass oberflächenaktive Substanzen zellulär gebunden werden müssen, um zur Wirkung zu kommen. Für eine solche Bindung sind die zellulären Mucopolysaccharide, Proteine und Lipoproteide in Betracht zu ziehen. Die Bindung kann dabei direkt oder über polyvalente Ionen erfolgen. Wahrscheinlich können die ungeladenen oberflächenaktiven Substanzen im Gegensatz zu den geladenen nur schwer mit der Zelle in Wechselbeziehungen treten, was ihren geringen Einfluss auf die Aufnahme von FFS erklären würde. Dagegen wird das positiv geladene Adogen 401 vermutlich leicht an die Zelloberfläche adsorbiert und scheint dabei FFS zu binden, ohne deren Einbau in die Neutralfette oder Glycerinphosphatide zu beeinflussen. Die stimulierende Wirkung des negativen Laurylsulfats auf den Einbau von FFS in die Neutrallipoide weist auf eine Förderung der FFS-Penetration hin. Diese Verbesserung der Penetration kann durch eine teilweise Auflösung von Lipoidmembranen, wie sie von NERMUT[1] elektronenmikroskopisch an den Oberflächenmembranen von Penicillinspheroplasten in Gegenwart von Laurylsulfat beobachtet wurde, verursacht oder zumindest gefördert worden sein. Derartige Auflösungen von zellulären Membranstrukturen durch oberflächenaktive Substanzen sind ein allgemein bekanntes Phänomen und wurden vor allem bei Mikroorganismen[2,3], Erythrocyten[4,5] und sub-

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