agreement with the electron-microscopic observations of FERNÁNDEZ-MORÁN[1]. These bodies make up some 2 to 3% of the total volume of axoplasm. Most of the bodies are more refractive than the surrounding axon structure. The axoplasm is easily extruded from the cut end of a fibre, yet its structure remains coherent. The axon can easily be pulled out from the myelin tube and stretched to several times its original length without breaking. In a stretched state the vesicles become ovoid or fusiform.

As noted by FERNÁNDEZ-MORÁN, previous light-microscopic research has failed to show any details in the normal, fresh axon, not even with the use of dark field, polarization or phase contrast.

A. WILSKA and M. KOTILAINEN

Institute of Physiology, University of Helsinki, March 15, 1953.

Zusammenfassung

Durch Anwendung eines abgeänderten Lichtmikroskops werden Strukturen in lebendfrischen Nervenfasern, die lichtoptisch bisher unsichtbar gewesen sind, deutlich gesehen.

[1] H. FERNÁNDEZ-MORÁN, Exp. Cell Res. *1*, 143, 309 (1950); *3*, 5 (1952).

Separation and Identification of the Degradation Products of Purines and Nucleic Acids on Acid Hydrolysis by Circular Paper Chromatography

In the course of our investigations on the separation and identification of purine and pyrimidine bases by circular paper chromatography[1], we investigated the nature of the products formed after acid hydrolysis of purines and nucleic acids, using the reagents, sodium 1:2 Naphthoquinone 4-sulphonate (FOLIN's reagent) in acetone[2], ninhydrin in acetone[1] and the contact printing technique using photographic paper and filtered ultraviolet light[3] for the identification of the substances separated on the chromatogram. While this work was in progress, FRICK[4] reported the formation of amino acids in acid hydrolysates of adenine by paper chromatography. He also observed the formation of two ultraviolet spots in addition to the strong ninhydrin-positive spot in the position of glycine after acid hydrolysis of adenine. In view of the above findings of FRICK and our observations on the marked differences observed in the hydrolysis products of adenine, nucleic acids, adenosine, adenylic acid, adenosine-3-phosphoric acid on the one hand and those of the other purines and pyrimidines on the other prompted us to report our observations.

Adenine (25 mg) was hydrolysed with (3 cm^3) 6 Normal hydrochloric acid by autoclaving at 15 lbs. pressure for 6 h. After removing the acid, the solution was spotted in 3 aliquots of 5 μl on the circumference of a circle drawn at the centre of the filter paper (Whatman No. 1, 24 cm diameter) and developed with n-butanol-acetic-acid-water according to the procedure described by GIRI and RAO[1]. After drying, the chromatogram was cut into three sectors, each containing the hydrolysis products of adenine separated. One of the sectors was sprayed with 0·2% ninhydrin in acetone[1] and the other with FOLIN's reagent[2]. The third sector was placed on a photographic paper and exposed to filtered ultraviolet light and developed according to the procedure described by MARKHAM and SMITH[3] (Fig. 1).

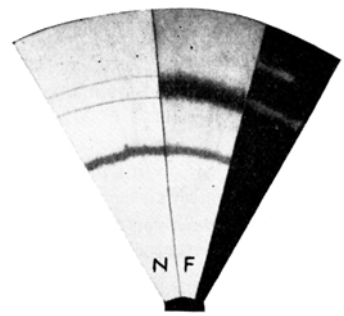

Fig. 1.—Different sectors of circular paper chromatogram of adenine hydrolysate treated as indicated below. *N* Sprayed with ninhydrin. *F* Sprayed with FOLIN's reagent. *U* Contact print of the sector taken in the ultraviolet.

It was observed that the adenine hydrolysate gives three clear bands on the chromatogram: (1) The band with the lowest R_f value (0·38) gives purple colour with ninhydrin and green colour with FOLIN's reagent and occupies the position of glycine on the chromatogram; (2) the second band with R_f value (0·69) higher than the first band gives faint yellow colour with ninhydrin (outlined with pencil in Fig. 1) and intense purple with FOLIN's reagent. It is also characterised by ultraviolet absorption as it appears on the contact print; and (3) the third band which appears on the sector obtained by contact printing technique is due to unhydrolysed adenine. Figure 2 is the reproduction of the chromatogram of adenine and adenine hydrolysate obtained by contact printing technique. It shows the formation of another band characterised by the ultraviolet light absorption, in addition to the one relating to adenine.

Fig. 2.—A contact print of a sector of a circular paper chromatogram of adenine and adenine hydrolysates taken in the ultraviolet using a chlorine filter. *A* Adenine, *A* (*h*) Adenine hydrolysate.

Other compounds of adenine and nucleic acids were similarly subjected to acid hydrolysis and chromatogramed. Figure 3 is the reproduction of the chromatogram showing the bands of the substances separated on the filter paper, present in the hydrolysates of adenine, ribonucleic acid (R.N.A.), Desoxyribonucleic acid (D.N.A.), Adenosine, Adenylic acid, and Adenosine-3-Phosphoric acid. It was observed that all the substances containing adenine give, in addition to the band occupying the position of glycine band, another band

[1] K. V. GIRI and N. A. N. RAO, Nature *169*, 923 (1952); J. Ind. Inst. Sci. *34*, 95 (1952).
[2] K. V. GIRI and A. NAGABHUSHANAM, Naturwissenschaften *23*, 548 (1952).
[3] R. MARKHAM and J. D. SMITH, Biochemical J. *45*, 294 (1949).
[4] G. FRICK, Nature *169*, 759 (1952).

[1] K. V. GIRI and N. A. N. RAO, Nature *169*, 923 (1952); J. Ind. Inst. Sci. *34*, 95 (1952).
[2] K. V. GIRI and A. NAGABHUSHANAM, Naturwissenschaften *23*, 548 (1952).
[3] R. MARKHAM and J. D. SMITH, loc. cit.

with higher R_f value than glycine band, giving an intense purple colour with FOLIN's reagent and faint yellow colour with ninhydrin reagent and characterised by ultraviolet absorption. This band was not present on the chromatograms of other purine and pyrimidine hydrolysates investigated, viz., Guanine, Xanthine, Cytosine, Uracil, Thymine, Hypoxanthine, Uric acid, etc., which gave a band only corresponding to glycine.

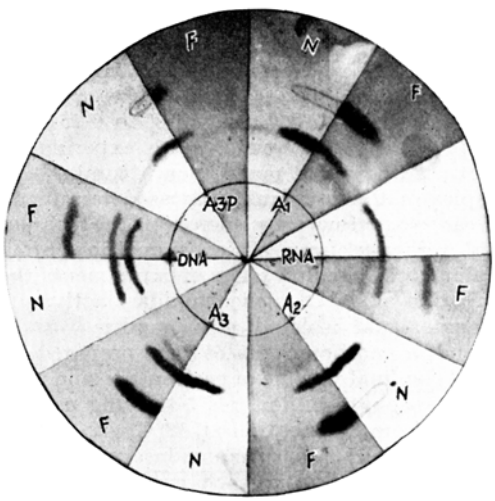

Fig. 3.—Circular paper chromatogram of acid hydrolysates of adenine (A_1), R.N.A., D.N.A., Adenosine (A_2), adenylic acid (A_3), and Adenosine-3-Phosphoric acid (A.3.P). *N* Sectors sprayed with ninhydrin. *F* Sectors sprayed with FOLIN's reagent.

The substances formed in the acid hydrolysates of nucleic acids, adenine and adenine compounds, giving intense purple colour with FOLIN's reagent, was identified tentatively as 4(5) amino-5(4) imidazole carboxamidine as its absorption maximum (λ_{max} – 287 mμ) is in agreement with that given by CAVALLIERI *et al.*[1], who prepared it in 10% yield by hydrolysing adenine sulphate with 6 N · HCl for 2 h at 150°C and established its constitution unequivocally by its conversion to isoguanine. The formation of this compound from adenine and nucleic acids even under mild conditions of hydrolysis (with N · HCl for 1 h at 100°C) was an another interesting observation made during the course of our investigations.

The appearance of this band on the chromatogram is specific for adenine compounds and nucleic acids. From the foregoing, therefore, it will be seen that the observed reaction of adenine and its compounds and nucleic acids on acid hydrolysis, is of importance in the analysis of nucleic acids, particularly in the determination of the component purines in a nucleic acid. These observations seem to call for greater care in the interpretation of the results obtained by spectro-photometric determination of purines after acid hydrolysis of nucleic acids.

K. V. GIRI, P. R. KRISHNASWAMY,
G. D. KALYANKAR, and
P. L. NARASIMHA RAO.

Departement of Biochemistry, Indian Institute of Science, Bangalore 3, India, April 15, 1953.

[1] L. F. CAVALLIERI, J. F. TINKER, and G. B. BROWN, J. Amer. Chem. Soc. *71*, 3973 (1949).

Zusammenfassung

Die Degradationsprodukte, die sich bei der Hydrolyse der Purine, der Pyrimidine und der Nukleinsäuren bilden, werden mittels runder Filterpapierscheiben chromatographisch untersucht. Adenine, Nukleinsäuren und Adeninverbindungen zeigen bei einer Säurehydrolyse ausser einem Band, das den Platz des Glycin einnimmt, noch ein weiteres Band. Dieses Band ist versuchsweise als 4(5)-amino-5(4)-imidazol Carboxamidin charakterisiert worden. Die Bedeutung dieser Ergebnisse für die quantitative Analyse der Nukleinsäuren und Adeninverbindungen wird in der vorliegenden Arbeit erörtert.

Trikarbonsäurezyklus im Stoffwechsel von *Corynebacterium diphtheriae*

Bei routinemässiger Züchtung von *C. diphtheriae* für technische Produktion von Diphtherietoxin konnte nachgewiesen werden, dass sich Bernsteinsäure, Apfelsäure, Zitronensäure, Brenztraubensäure, Oxalessigsäure und α-Ketoglutarsäure in der Kulturflüssigkeit anhäufen. Als Substrat diente ein modifiziertes Kaseinhydrolysat nach HOLT[1]. Die Züchtungsdauer betrug 5 Tage bei 34°C und die Toxinausbeute 100–120 L_f-Dosen/ml. Bernsteinsäure wurde als Silbersalz, Apfelsäure und Zitronensäure nach PUCHER *et al.*[2] sowie papierchromatographisch[3] und die Ketokarbonsäuren durch Papierchromatographie ihrer 2,4-Dinitrophenylhydrazone[4] nachgewiesen.

Das wachsende Bakterium vermochte zugesetzte Bernsteinsäure, Apfelsäure oder Zitronensäure nicht zu verwerten. Dagegen wurde Essigsäure mit einer Intensität von etwa der gleichen Grössenordnung verbrannt wie Glukose und Maltose. Auch Äthanol und Milchsäure konnten verbraucht werden.

Da die Nichtverwertbarkeit der Polykarbonsäuren vermutlich durch Permeabilitätsbarrieren bedingt ist, wurde ihre Verwertbarkeit mit lyophilisierten Bakterienzellen nachgeprüft. Dabei konnte mit der Thunberg-Technik die Dehydrierung von Bernsteinsäure, Apfelsäure, Zitronensäure und andererseits auch von Essigsäure dargelegt werden. Die Succinodehydrase erwies sich inhibierbar durch Malonsäure. Weiterhin konnte die Bildung von α-Ketoglutarsäure aus Zitronensäure und die Umbildung von Fumarsäure in Apfelsäure durch die Einwirkung von lyophilisierten Bakterienzellen unter aeroben Bedingungen festgestellt werden.

Das Auftreten der obenerwähnten Intermediate und Reaktionen im Stoffwechsel des Diphtheriebakteriums lässt vermuten, dass auch die Verwertung des Azetats möglicherweise über den Trikarbonsäurezyklus erfolgt. Untersuchungen über das Auftreten des Koenzyms A und über die Inhibierung der Azetatverbrennung durch Malonsäure sowie über das verschiedene Dehydrierungsvermögen der einerseits mit Maltose, andererseits mit Azetat gezüchteten lyophilisierten Bakterien hinsichtlich Zitronensäure werden noch fortgeführt.

[1] L. B. HOLT, Brit. J. Exp. Path. *29*, 335 (1948).

[2] W. PUCHER, A. J. WAKEMAN und H. B. VICKERY, Ind. Eng. Chem. Anal. Ed. *13*, 244 (1941). – W. PUCHER, C. C. SHERMAN und H. B. VICKERY, J. Biol. Chem. *113*, 235 (1936).

[3] M. L. BUCH, R. MONTGOMERY und W. L. PORTER, Anal. Chem. *24*, 489 (1952).

[4] D. CAVALLINI, N. FRONTALI und G. TOSCHI, Nature *163*, 568 (1949).