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.

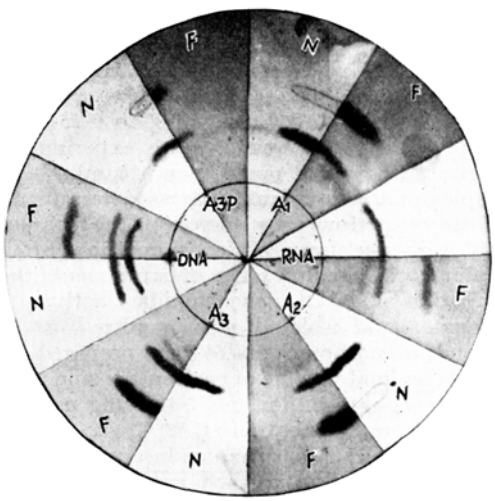

Fig. 3.—Circular paper chromatogram of acid hydrolysates of adenine (A_1), R.N.A., D.N.A., Adenosine (A_2), adenylic acid (A_3), and Adenosine-3-Phosphoric acid (A.3.P). *N* Sectors sprayed with ninhydrin. *F* Sectors sprayed with FOLIN's reagent.

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.

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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.

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[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).

Als Versuchsobjekt diente in allen Versuchen die «Toronto»-Variante CN 2000 von *C. diphtheriae*, die von Dr. F. V. LINGGOOD, Wellcome Research Laboratories, Beckenham, England, freundlichst zu unserer Verfügung gestellt wurde.

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Arzneimittelfabrik Orion Oy., Mikrobiologische Abteilung, Helsinki, Finnland, den 6. Mai 1953.

Summary

The possible role of tricarboxylic acid cycle in the metabolism of *C. diphtheriae* was studied. The occurence of succinic, malic, citric, pyruvic, oxaloacetic, and α-ketoglutaric acid in the culture filtrate and, on the other hand, the dehydrogenation of succinic, malic, citric, and acetic acid by freeze-dried bacteria could be demonstrated.

On the Inactivating Properties of Liver Homogenates on Succinoxidase Activity of Isolated Mitochondria

Many techniques have been described in recent years permitting the fractionation of the cell. It was found that many enzymatic activities, and particularly those concerned with the respiratory processes, are localized in the "mitochondrial fraction" (HOGEBOOM *et al.*[1]; SCHNEIDER[2]). It was established in a previous paper of the author[3] that fresh serum from many kinds of animals produces slight acceleration of the mitochondrial succinoxidase activity, which is changed to a strong inhibition after incubation at 37°C for 30 min. This effect was attributed to the lipase content of the tested sera.

It seemed interesting to study if a similar inactivating property is present also in liver homogenates. This paper shows that some inactivating principles, probably of enzymatic nature, are in fact contained in the supernatant fluid after the sedimentation of mitochondria and microsomes from liver homogenates.

Mitochondria were isolated with 0·25 *M* sucrose from 10% liver homogenates. White rats from a selected strain and weighing 90–100 g, were used in these experiments. After a first centrifugation at 1500 g for 15 min, which produced the sedimentation of tissue débris, undamaged cells, nuclei, red cells and some mitochondria, the main portion of mitochondria was sedimented by a second centrifugation at 12,000 g in the SS-I SERVALL Centrifuge, in the cold room at 5°C. The supernatant fluid after this centrifugation, which contained microsomes and the soluble part of the cytoplasm, was submitted to a new centrifugation at 22,000 g for 2 h. The sediment was mainly made up of microsomes. Mitochondria were washed twice with 0·25 *M* sucrose, and then suspended in 0·067 *M* phosphate buffer solution (pH 7·4). Tests for succinoxidase activity were made manometrically in a WARBURG conventional apparatus at 38°C, by measuring the O_2-uptake of a suspension of mitochondria corresponding to 0·25 g of the tissue in the presence of 0·0125 *M* sodium succinate, 0·2 ml 30% KOH being placed in the central well of the vessels. Neither $CaCl_2$ nor cytochrome *c* were added in the main experiments, because it was observed in previous work that no influence is exerted by these substances when the experiments are made with an excess of enzyme material. Succinoxidase activity of homogenates was similarly studied, a suspension corresponding to 0·25 g of tissue beeing placed in each vessel. Each experiment lasted 1 h, but readings were made every 10 min. N content of samples of the preparations was determined at the end of each experiment by microkjeldhal technique and QO_2 (N) values (i.e. mm^3 O_2 consumed in 1 h/mg of N) were calculated. In a first series of experiments the effect of incubation at 38°C on succinoxidase activity of liver homogenates and isolated mitochondria was studied. Mitochondria and homogenates were prepared from 1 g liver, and the final volume of each suspension was 12 ml, 0·067 *M* phosphate buffer (pH 7·4) being used as a suspension medium. Incubation at 38°C was performed in a thermostat, and the succinoxidase activity of 3 ml samples withdrawn from each suspension was determined at periods of 0,1, 2, 3 h. The whole procedure was made in sterile vessels, in order to avoid any bacterial interference. Sterility controls were made at the end of each experiment. Table I shows that, while the enzymatic activity of the homogenates decreases strongly as a consequence of incubation at 38°C, that of the mitochondrial suspension decreases at a very lower rate. After 3 h of incubation, succinoxidase activity of homogenates is decreased 47·5%, that of mitochondria only 16·9%.

Table II

Difference of activity between mitochondria isolated from fresh homogenates and mitochondria isolated from homogenates incubated at 38°C

Time of incubation	Mitochondria from fresh homogenates	Mitochondria from incubated homogenates
Non-incubated	218·0	
1 h	215·0	170·1
2 h	200·6	140·6

In a second series of experiments, succinoxidase activity of mitochondria isolated from homogenates incubated at 38°C for 1 h and for 2 h was compared with that of mitochondria isolated from a fresh homogenate

Table I

Influence of incubation at 38°C on succinoxidase activity of liver homogenates and mitochondria (The data are expressed at $QO_2(N) \pm \sigma$ = standard deviation)

	Number of experiments	Non-incubated controls	Samples incubated for		
			1 h	2 h	3 h
Homogenates	5	51·6 ± 0·9	40·8 ± 1·9	33·1 ± 0·9	27·1 ± 1·4
Inactivation %			20·9	35·8	47·5
Mitochondria	5	216·5 ± 2·3	210·7 ± 0·4	199·3 ± 2·0	179·5 ± 2·1
Inactivation %			2·5	10·5	16·9

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