

2, 7, 12 und 17 Tage alte Plasmocytomzellkulturen von Fall I wurden mit bis zu 2×10^{-6} ID 50 aller drei Poliotypen beimpft. Während diese Infektionsdosis Kontrollkulturen von Nierenepithelzellen von Menschen und Affen innerhalb 24 h vollständig zerstört, konnten bei den Plasmocytomzellkulturen innerhalb einer Beobachtungszeit von mehr als 6 Wochen in bezug auf die Plasmocytomzellen keine Veränderungen festgestellt werden, die auf die Wirkung der Polioviren zurückzuführen gewesen wären. Die Plasmocytomzellen schienen sich in den beimpften Röhren besser zu entwickeln als in den Kontrollröhren. Neben *Plasmocytomzellen* beobachteten wir in den beimpften Röhren auch das Überleben einer ansehnlichen Zahl von *Makrophagenzellen*. In Kulturen des zweiten Falles haben wir Überleben der Plasmocytomzellen nach Beimpfung mit 12×10^{-6} ID 50 festgestellt.

Soviel uns bekannt ist, sind die beschriebenen Plasmocytomzellen die ersten Zellen, die in der Ausgangskultur eine scheinbar absolute *Resistenz gegenüber dem zytopathogenen Effekt aller drei Poliovirustypen* aufweisen. Es ist noch nicht abgeklärt, ob diese Resistenz allen Plasmocytomzellen oder allen Zellen der Plasmazellenreihe zukommt – und ob sie durch die spezifische metabolische Aktivität dieser Zellen erklärt werden muss. In Anbetracht eines möglichen Zusammenhanges dieser Resistenz mit der Proteinsekretion der Plasmocytomzellen sei auf den starken zytopathogenen Effekt der Polioviren auf Leberzellen in Gewebekulturen hingewiesen. Die Möglichkeit, dass die beobachteten Zellen unspezifische Inhibitorensubstanzen oder gegen Poliomylitisviren gerichtete Antikörper bilden, kann gegenwärtig noch nicht ausgeschlossen werden. Die verminderte Gamma-Globulinsynthese im Falle I spricht allerdings nicht für diese Hypothese; denn Polioantikörper scheinen vorwiegend der Gamma-Globulinfraktion anzugehören.

Wir beabsichtigen das Verhalten der Plasmocytomzellen sowie der in diesen Kulturen ebenfalls resistent erscheinenden Makrophagen auch gegenüber anderen Virusarten zu prüfen. Untersuchungsergebnisse über die Poliovirusaktivität und über mögliche funktionelle Leistungen der Zellen in diesen Plasmocytomzellkulturen sollen in einer späteren Publikation erörtert werden.

R. CRAMER

Staatliches bakteriologisches Laboratorium, Stockholm, den 9. Dezember 1955.

Summary

The reaction of the plasmocyte cell cultures from a case of myeloma with decreased gamma globulin synthesis and from a case of myeloma with increased gamma globulin synthesis was tested in relation to all 3 types of polio virus.

In both cases the plasmocytoma cells proved to be resistant to the well-known cytopathogenic effect of these viruses in tissue culture. Macrophages in the culture of one case seemed also to be resistant.

Formation of Asparagine and Increase in the Free Amino Acid Content in Virus Infected Leaves of *Abelmoschus esculentus*¹

Increase in the formation of free amino acids has been reported by many workers², asparagine formation in virus infected plants is known only in few cases³. The present investigation indicates that in alcohol soluble extracts of "Yellow-Mosaic" virus-infected leaves there is an increased formation of asparagine and of a new ninhydrin-reacting substance which separates from asparagine only in one sector where 0.001 ml of sample solution was spotted.

Leaves of *Abelmoschus esculentus* (*Hibiscus esculentus*) were plucked from the third and the fourth nodes at about 11 a.m. (I.S.T.) in the month of September 1955. Equal weights of all the three sets viz., "Green and Healthy", "Yellow-Mosaic and diseased" and "Completely Yellow and Severely Diseased" were frozen at -5°C for 15 min and the extraction carried out in cold 90% alcohol with constant stirring for 0.75 h. The hydrolysate was prepared by hydrolyzing the residue with 6 N HCl and autoclaving at 15 lb pressure for 1 h. Alcoholic solutions were evaporated and then dissolved in minimum quantity of alcohol. Further, all the solutions were centrifuged at 2000 revolutions per min for 0.50 h and the clear liquid spotted on Whatman No. 1 filter paper discs at their respective places. 0.001 ml, and 0.003 ml of alcoholic extracts of all the three sets of leaves were spotted to ensure the appearance of all those amino acids present in lower quantities as well as to separate the overlapping bands which do so only in dilute solutions.

The amino acids and amides were separated by the horizontal migration-multiple sector chromatographic technique of RANJAN *et al.*⁴ and the common bands of glutamic acid + threonine and serine + glycine + aspartic acid were separated using MCFARREN'S technique⁵ (Solvent: Phenol saturated with buffer of pH 12).

The acid hydrolysate of all the three sets of leaves revealed the same range of amino acids; leucine + isoleucine + phenylalanine (average Rf value 0.83 band No. 1 and 2), valine + methionine (0.72; 3), tyrosine (0.67; 4) and alanine (0.56; 5), glutamic acid + threonine (0.51; 6), glycine + aspartic acid (0.45; 7), arginine (0.39; 8) and histidine + lysine (0.35; 9).

The alcoholic extracts of "Yellow-Mosaic" and "Completely Yellow" leaves showed a general increase in the concentration of the amino acids over that of healthy leaves (*cf.* BAWDEN⁶). Leucines + phenylalanine (av. Rf 0.83; I) and valine + methionine (av. Rf 0.70; II) were new developments in diseased leaves only. However, they were traceable in a sector where 0.003 ml of healthy leaf extract was kept but were in such small

¹ B. N. UPPAL, P. M. VARMA, and S. P. CAPOOR, *Curr. Sci.* 9, 227 (1940).

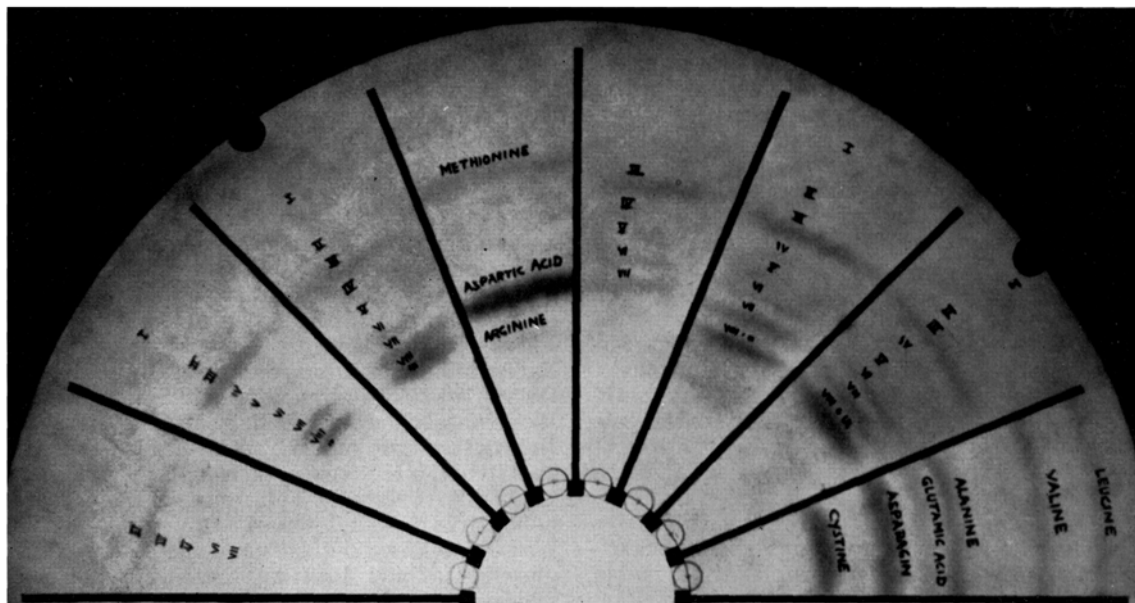
² L. F. MARTIN, A. K. BALLS, and H. H. MCKINNEY, *Science* 87, 329 (1938). – M. MENIGHINI and C. C. DELWICHE, *J. Biol. Chem.* 189, 177 (1951). – B. COMMONER and V. NEHARI, *J. Gen. Physiol.* 36 (6), 791 (1953). – M. M. LALORAYA and GOVINDJEE, *Nature* 175, 907 (1955). – S. RANJAN and T. RAJARAO, *Flora* (Jena) 143, 87 (1956).

³ M. M. LALORAYA and GOVINDJEE, *Nature* 175, 907 (1955). – M. M. LALORAYA, GOVINDJEE, RAJNI VERMA, and T. RAJARAO, *Exper.* (in press).

⁴ S. RANJAN, GOVINDJEE, and M. M. LALORAYA, *Proc. Natl. Inst. Sci. (India)* 21, B 10, 42 (1955).

⁵ EARL F. MCFARREN, *Anal. Chem.* 23, 168 (1951).

⁶ F. C. BAWDEN, *J. Sci. and Industr. Res.* 13 (3), 196 (1954). – F. C. BAWDEN and N. W. PIRIE, *Ann. Rev. Pl. Physiol.* 3, 171 (1952).



Chromatogram showing amino acids and amides of healthy and "Yellow mosaic" infected leaves of *Hibiscus esculentus*. A and D Reference solutions HA(1) and HA(3)—Alcoholic extract of healthy leaves 0.001 and 0.003 ml spotted. MA(1) and MA(3)—Alcoholic extract of Mosaic leaves. YA(1) and YA(3) alcoholic extract of yellow mosaic leaves—severe infection.

From left to right: Section No. I: Ha(1); Section No. II: HA(1); Section No. III: YA(1); Section No. IV: A; Section No. V: HA(3); Section No. VI: MA(3); Section No. VII: YA(3); Section No. VIII: D.

quantities that the faint bands disappeared in no time and could not be photographed. 0.01 ml of healthy leaf extract spotted on a separate chromatogram confirmed the presence of the above-mentioned amino acids in healthy leaves also though in a lesser quantity. Tyrosine (av. Rf 0.67; III) band was extremely deep in the "Yellow-Mosaic" leaves and its intensity decreased considerably in the "Completely Yellow" leaves although its concentration still remained more in comparison to that of the healthy leaves. Aspartic acid (av. Rf 0.45; VII; absence of glycine and serine established by running the chromatogram in phenol saturated with buffer of pH 12) also increases considerably in the "Yellow Mosaic" leaves but it does not show any variation as noted in tyrosine.

The amide asparagine (av. Rf 0.395; VIII) overlapped with the new band (IX) is a new formation in the "Yellow Mosaic" and the completely yellow leaves. The intensity of this band is more in the latter case. It appears that concomitant with the severity of infection, there is an increased formation of asparagine.

Other common amino acids present in alcoholic extracts were glutamic acid (av. Rf 0.51-threonine absent; VI), alanine (Rf 0.56; V), and one more having average Rf value 0.60 (IV) which is amino-butyric acid.

Metabolic processes, leading to the formation of asparagine, increase in leucine, isoleucine, phenylalanine, tyrosine, and aspartic acid in the "Yellow Mosaic" leaves and decrease in tyrosine from "Yellow-Mosaic" to "completely Yellow" leaves, are yet to be studied and explained. Only biochemical studies on "Yellow-Mosaic" virus of "Bhindi" (*Abelmoschus esculentus*) itself can reveal which of these amino acids are the constituents of virus protein and which of these remain simply as the changed metabolites of the host.

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GOVINDJEE, M. M. LALORAYA,
and T. RAJA RAO

Department of Botany, University of Allahabad, India,
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Zusammenfassung

Papierchromatographische Analysen der freien Aminosäuren und Amide in gesunden und mit «yellow mosaic»-Virus infizierten Blättern von *Abelmoschus esculentus* zeigen neben einer Zunahme von Leucin, Phenylalanin, Valin + Methionin und Asparaginsäure die Bildung einer neuen, ninhydrinpositiven Substanz und Asparagin in den infizierten Blättern. Es werden Anregungen für weitere Untersuchungen gemacht.

Die Bildung von Fusarinsäure durch *Fusarium lycopersici* in vivo

Fusarium lycopersici Sacc., der Erreger einer Welkekrankheit an Tomatenpflanzen, bildet in Reinkultur neben andern biologisch wirksamen Stoffwechselprodukten auch das Welketoxin *Fusarinsäure* (5-n-Butyl-picolinsäure)¹. Aus Kulturen einiger verwandter pflanzen-

¹ E. GÄUMANN, ST. NAEF-ROTH und H. KOBEL, *Phytopath. Z.* 20, 1 (1952). — PL. A. PLATTNER, W. KELLER und A. BOLLER, *Helv. chim. Acta* 37, 1379 (1954). — E. HARDEGGER und E. NIKLES, *Helv. chim. Acta* 39, 223 (1956).