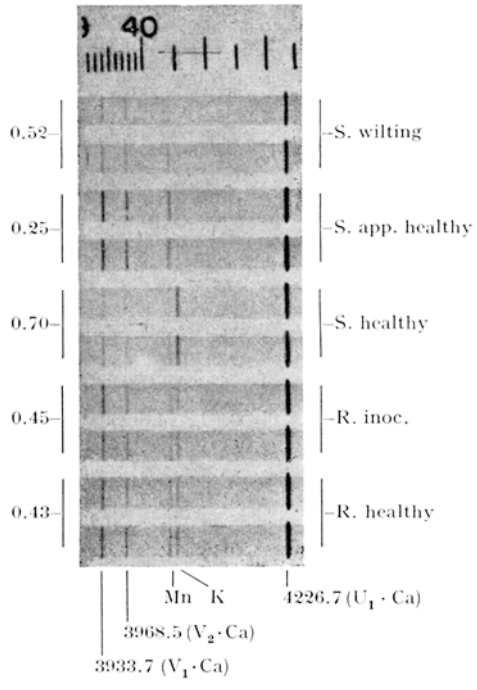
transmission values (calculated from microphotometer readings and given on the left side of the spectra in the Figure) give us an idea of the relative amounts present, the two being inversely proportional to each other.

The insignificant change in ionized calcium in the resistant plants following infection, as against the marked one in infected susceptible plants, falls in line with the trend in general ionic pattern reported earlier[2]. It was noted that a considerable loss of potassium, and an increase in the amounts of other metals generally, brought about an ionic imbalance in the infected susceptible plants, whereas the ionic ratios in the resistant plants remained little affected. As was observed in the case of magnesium and manganese, the strongest lines for ionized calcium also were in the infected susceptible plants looking healthy (non-wilting). Although at present it is not possible categorically to state the reasons for these *in vivo* ionic changes, a few possibilities are suggested.

Two groups of substances implicated in many vascular wilts are extracellular toxins and enzymes produced by the pathogens concerned. In the *Fusarium* wilt of cotton, both the phytotoxin fusaric acid and pectic enzymes are considered to play a significant role[3].



Duplicate spectra of leaf ash from 18-day old cotton plants, resistant (R) and susceptible (S) to *Fusarium* wilt.

One of the possibilities suggested here is that the extra calcium may be transported along the conduction stream by fusaric acid as a complex to the leaves where it may dissociate releasing the metal, as proposed for iron in wilting tomato plants by GÄUMANN et al.[4]. The second is that the disintegration of the middle lamella by fungal pectic enzymes may release the metallic ions. It is well known that pectic materials in the middle lamella occur mostly as calcium and magnesium pectates. Studying the pectic enzyme contents in leaves of the same varieties of cotton plants, SUBRAMANIAN[5] found that the resistant plants normally contained more of these enzymes than the susceptible (note the stronger lines for ionized calcium in these), and that the former did not show much change on infection while the latter showed increased amounts of enzymes.

A point to be noted here is that it is the infected but non-wilting susceptible plants that reveal the highest amounts of ionized calcium (Figure). What stage these plants represent during this wilt pathogenesis remains to be ascertained. It is obvious from their ionic imbalance[2] that they are only apparently healthy (on the 18th day after inoculation) but not really so. It is likely that these have outlived their contemporary wilting plants only because of a slower progress of pathogenesis, and they offer interesting material for further investigations[6].

Zusammenfassung. Blattanalysen von 18 Tage alten, gegen den Welkepilz *Fusarium vasinfectum* widerstandsfähigen oder anfälligen Baumwollpflanzen zeigten einen erhöhten Gehalt an Calcium-Ionen in den infizierten, anfälligen Pflanzen; am stärksten war der Effekt in denjenigen anfälligen Pflanzen, die infiziert worden waren, aber keine Welkesymptome zeigten. Die Steigerung kommt möglicherweise durch den Zerfall von Toxin-Metall-Komplexen oder durch den enzymatischen Abbau von Pektinstoffen zustande.

L. SARASWATHI-DEVI and T. S. SADASIVAN

University Botany Laboratory, Madras (India), July 18, 1961.

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Effect of Metabolic Inhibitors on the Release of Histamine by Anaphylatoxin and by Antigen *in vitro*

Previous results obtained in this laboratory[1], demonstrated that mast cell alterations and histamine release caused by compound *48/80*[2] in rat tissue *in vitro*, are readily inhibited by agents (dinitrophenol, salicylate, thiopental, anoxia, cyanide, etc.), which interfere with oxidative phosphorylation or Krebs cycle oxidations. The action of these inhibitors was shown to be markedly reduced by the presence of glucose in the incubation medium. This indicated that this substrate was able to supply the metabolites needed by the histamine-releasing process, even under conditions in which aerobic energy metabolism is fully blocked. A similar effect of glucose has been described for the action of compound 48/80 on cat tissue kept under anoxia[3], as well as for the action of several naturally occurring histamine liberators acting on rat tissue[4,5]. The present report extends these observations to conditions of histamine release by endogenous

[1] A. M. ROTHSCHILD, I. VUGMAN, and M. ROCHA E SILVA, Biochem. Pharmacol. *7*, 248 (1961).
[2] Condensation product of *p*-methoxiphenethylmethyl amine with formaldehyde.
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Tab. I. Release of histamine from sensitized rat and guinea-pig tissue by antigen *in vitro*

Incubation conditions	Histamine release (%) Rat diaphragm Mean ± S.E.	P[a]	Guinea-pig lung Mean ± S.E.	P[a]
Controls	9.9 ± 3.6 (6)[b]		11.9 ± 2.5 (5)[b]	
NaCN, 1 m*M*	3.9 ± 0.9 (6)		2.6 ± 0.6 (5)	
NaCN + glucose[c],	7.3 ± 1.6 (6)	< 0.1	11.8 ± 2,4 (5)	0.02
NaCN (no antigen)	3.8 ± 1.3 (6)		2.6 ± 0.4 (5)	
Controls	8.2 ± 1,2 (7)		11.9 ± 2.5 (5)	
DNP, 0.3 m*M*	4.7 ± 0.8 (7)		2.1 ± 0.7 (5)	
DNP + glucose,	13.1 ± 1.6 (7)	< 0.01	4.6 ± 0.5 (5)	< 0.01
DNP (no antigen)	4.2 ± 0.9		2.6 ± 0.4 (5)	
Controls (aerobic)			20.2 ± 3.8 (8)	
Anoxia			4.5 ± 0.6 (8)	
Anoxia + glucose,			12.4 ± 4.0 (8)	0.05
Anoxia (no antigen)			2.8 ± 0.6 (2)	

[a] Significance ('t' test) of the effect of glucose on the action of the inhibitor. [b] Number of experiments. [c] 4.5 m*M*.

Tab. II. Release of histamine from guinea-pig lung slices by anaphylatoxin

Incubation conditions	Histamine release (%) Mean ± S.E.	P[a]
Controls	10.6 ± 1.3 (5)[b]	
NaCN, 1 m*M*	2.0 ± 0.3 (4)	
NaCN + glucose, 4.5 m*M*	11.8 ± 3.7 (4)	0.025
NaCN (no anaphylatoxin)	1.8 ± 0.3 (3)	
Controls	10.6 ± 1.3 (5)	
DNP, 0.3 m*M*	1.7 ± 0.3 (5)	
DNP + glucose, 4.5 m*M*	8.7 ± 0.7 (5)	0.01
DNP (no anaphylatoxin)	1.6 ± 0.4 (3)	
Aerobic controls	10.6 ± 1.3 (5)	
Anoxia	3.8 ± 1.0 (4)	
Anoxia + glucose, 4.5 m*M*	13.2 ± 4.0 (4)	0.05
Anoxia (no anaphylatoxin)	2.5 ± 0.9 (2)	

[a] Significance ('t' test) of the effect of glucose on the action of the inhibitor. [b] Number of experiments.

factors, e.g., *in vitro* anaphylaxis in the rat and guinea-pig and anaphylatoxin action on guinea-pig lung tissue.

Methods. Rats were sensitized to bovine serum albumin (BSA, fraction V, Armour Laboratories) by three subcutaneous injections (of 10 mg each) of alum-precipitated BSA given on alternate days; the animals were used 20 to 30 days following the last injection. Guinea-pigs were sensitized by a single intraperitoneal injection of 100 mg of egg albumin, and used two weeks afterwards. Rat serum anaphylatoxin was prepared by incubation with agar (Difco), in the manner described by ROTHSCHILD and ROCHA E SILVA[6]. *In vitro* anaphylaxis in rat tissue was evoked by adding BSA, (0.1%), to pieces of sensitized rat diaphragm suspended in Krebs-Ringer phosphate buffer, pH 7.3 at 37°C. Guinea-pig lung slices were prepared according to UMBREIT et al.[7]; approximately 100 mg samples (5–8 slices), were added to each incubation flask; antigen concentration was 0.1%. In the anaphylatoxin experiments, the activated rat serum containing this principle was present at 1:4 dilution in the final incubation mixture. Pre-incubation periods (without antigen or anaphylatoxin but with inhibitor) were 20 min; following the addition of antigen, incubations were continued for 10 min. Details of the incubation procedure and histamine bio-assay have been described[1].

Results. The first part of Table I shows that histamine release from sensitized rat diaphragm by antigen, is fully blocked by 0.3 m*M* dinitrophenol (DNP) or 1 m*M* sodium cyanide. However, when glucose is present in the medium, DNP action is no longer apparent, and cyanide block is also extensively reversed. These results are identical with those described for the inhibition of histamine release by compound 48/80[1], and suggest that similar metabolic pathways are followed by the cell in its response to antigen and the synthetic histamine liberator, respectively.

The second part of Table I indicates that these results are also obtained in experiments on guinea-pig lung anaphylaxis. Cyanide, DNP and anoxia (induced by placing the incubation mixture in evacuated Thunberg tubes and adding antigen from the side-arm) caused full inhibition of histamine release. These effects were significantly reversed by glucose in all cases, although full return to control values was only obtained in the case of cyanide.

It has been shown that besides its histamine releasing capacity *in vitro*[8], anaphylatoxin causes changes in guinea-pig mast cells which closely resemble those induced by antigen[9]. Table II shows that the histamine releasing action of anaphylatoxin is highly susceptible to inhibition by DNP, NaCN or anoxia, but that, like in the experiments with antigen, the inhibitions are readily reversed by glucose.

In view of these results it seems warranted to postulate that the histamine-releasing actions of compound 48/80, of antigen and of anaphylatoxin depend on metabolically active mast cells, and furthermore, that in the presence of glucose this activity can take place under conditions in which aerobic metabolism is blocked. It seems probable that this action of glucose is connected with the functioning of the Embden-Meyerhof cycle; further work will however be required to define the biochemical tie between glucose metabolism and cellular response to histamine releasing stimuli. Studies on this relationship are at present underway in this laboratory.

Zusammenfassung. Es wird durch *in vitro*-Anaphylaxie an Rattenzwerchfell und Meerschweinchenlunge gezeigt, dass die Freisetzung von Histamin durch Dinitrophenol, Cyanid und Sauerstoffmangel gehemmt wird. Diese Hemmung kann aber durch Zufügen von Glukose durchbrochen werden. Die anaphylaktische Freisetzung von Histamin ist also an stoffwechselaktive Mastzellen gebunden.

A. M. ROTHSCHILD
with the technical assistance of
J. U. BARRETO

Department of Pharmacology, Faculty of Medicine of Ribeirão Prêto, University of São Paulo (Brazil), July 27, 1961.

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