Mitotische und endomitoseähnliche Teilungsstadien an vier durch Reblausspeichel gereizten Stellen eines jungen Blattes von *Vitis Rupestris* St. Georges.

Anzahl Kerne	Interphasen		Teilungsstadien					
			mitotische und endomitotische		mitotische Anaphasen		Endoanaphasen	
	Anzahl	%	Anzahl	%	Anzahl	%	Anzahl	%
1711	1690	98,8	21	1,2	4	0,2	2	0,1
1761	1710	97,1	51	2,9	6	0,3	14	0,8
1941	1890	97,4	51	2,6	4	0,2	19	1,2
1727	1675	97,0	52	3,0	13	0,7	5	0,3

Naturgemäss werfen nun diese Versuche und Beobachtungen die Frage nach der biologischen Wirkung des Reblausspeichels auf. Hier hatte die Feststellung, dass die Zellkerne des Gallengewebes mehreren Grössentypen angehören, bereits zu der Vermutung geführt, dass diese polyploid sind und dass der Reblausspeichel endomitotische oder doch wenigstens endomitoseähnliche Vorgänge verursacht[2]. Dem direkten Nachweis dieser Polyploidisierungsvorgänge stellten sich jedoch zunächst erhebliche Schwierigkeiten entgegen, da diese bei der Reblausgalle – genau so wie bei vielen anderen Objekten[3] – im allgemeinen maskiert verlaufen und nur durch schwierige Analysen der Kernstrukturen sicher erfasst werden können.

Trotzdem findet man jedoch unter bestimmten Bedingungen (Gewächshaus, Rebsorte) während der ersten Entwicklungsstadien einer Galle Kernbilder, die auf die Polyploidisierungsvorgänge selbst hindeuten. Die Metaphasechromosomen erscheinen – abweichend von der für Angiospermen charakteristischen Endomitose – kontrahiert, oft sogar überkontrahiert, und rücken – gewöhnlich der Kernmembran anliegend – in der Anaphase auseinander, ohne dass sich die Tochterpaare vollkommen trennen. Im ganzen erinnert dieser Vorgang einerseits an die für manche tierische Objekte charakteristische Endomitose mit kontrahierten Metaphasechromosomen, andererseits aber an eine Stathmokinese mit darauffolgender Restitutionskernbildung.

Allerdings ist durch die Beobachtung dieser endomitoseähnlichen Vorgänge allein noch nicht erwiesen, dass diese primär durch den stofflichen Reiz der Reblaus verursacht werden. Sie könnten vielmehr auch als Folgeerscheinung der bereits in Gang befindlichen, morphologisch und histologisch feststellbaren Gallenbildung aufgefasst werden. Um diese für die Kausalanalyse der Gallenbildung entscheidende Frage zu bearbeiten, wurde junges, reaktionsfähiges Blattgewebe dem Gallenreiz ausgesetzt und einige Stunden später die unmittelbar hiervon betroffenen Zellaerale (je etwa 2000 Zellen) untersucht. Dabei stellte es sich heraus, dass die Polyploidisierung bereits zu einem Zeitpunkt einsetzt, an dem noch gar keine morphologischen und histologischen Veränderungen am Blatt wahrgenommen werden können (Tabelle)[4].

Es darf also – soweit dies die hier angewendeten biologischen Untersuchungsmethoden erlauben – als sicher angenommen werden, dass das galleninduzierende Sekret der Reblaus primär auf den Zellkern wirkt und hier polyploidisierende Vorgänge bedingt.

Ob der Reblausspeichel darüber hinaus noch andere, für die weitere Entwicklung und Gestaltung der Galle erforderliche Reize[5] ausübt, kann vorerst noch nicht entschieden werden. Es liegen jedoch bisher keine Beobachtungen vor, die für eine solche Annahme sprechen könnten.

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Résumé

La transmission expérimentale de la salive du Phylloxéra peut stimuler les jeunes feuilles de la vigne se trouvant encore dans la phase de végétation à former des galles. A cet égard, l'endopolyploidisation du tissu foliaire qui est exposé à la stimulation de la formation gallicole prouve un événement fondamental de la cécidogenèse.

[2] F. ANDERS, Verh. dtsch. Zool., Erlangen *1955*, 421; Exper. *11*, 322 (1955).

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[4] Im normalen Blatt der Rebe ist die Endomitose eine zwar natürliche, aber offenbar seltene Erscheinung. Allerdings bestehen hinsichtlich ihrer Häufigkeit sortentypische Unterschiede.

[5] Es wäre hier namentlich an zellteilungsfördernde Reize zu denken, zumal bei der Reblausgalle meist – aber nicht immer [vgl. W. NIKLOWITZ, Phytopath. Z. *24*, 299 (1955)] – eine ausgesprochene Zellproliferation beobachtet werden kann. Da es sich jedoch hierbei in den Fällen, in denen eingehendere Untersuchungen möglich waren, meist nicht um echte mitotische, sondern um amitotische Zellteilungen und «Zellunterteilungen» handelt, kann vermutet werden, dass diese mit einer vorausgegangenen Endopolyploidisierung in Beziehung stehen und somit Sekundärvorgänge sind, in deren Verlauf höhere Polyploidiegrade auf niedere reduziert werden.

Renal Ammonia Production as a Model for the Study of Enzyme Adaptation in Mammals*

Induction is defined as an increase in cellular enzyme concentration as a consequence to the application of an inducer, such as the substrate of an enzyme. Enzyme induction (adaptation) in microorganisms was first observed by WORTMANN[1] and has been studied extensively in recent years[2]. While the existence of this phenomenon has generally been accepted for microorganisms, it is still doubtful whether it occurs in mammalian cells. Since the first claim of induction of pancreatic enzymes by PAVLOV[3], reports on induction of salivary amylase, pancreatic enzymes and hepatic argin-

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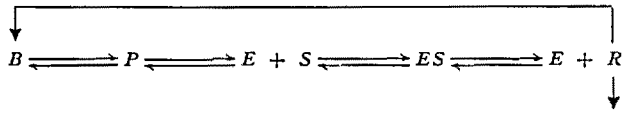
ase have been published[4]. Most of these studies are open to criticism, since one or more of the following criteria were not fulfilled: (1) Enzyme concentration must increase on a cellular basis to exclude hyperplasia, changes in hydration, etc., (2) the enzyme assays should be so designed that substrate and cofactor availability does not limit enzyme activity and (3) nonspecific alterations due to changes in the nutritional or hormonal status of the animals must be excluded.

MANDELSTAM[5] recently analyzed the kinetics of enzyme induction in microorganisms and proposed the following working hypothesis: The enzyme (E) is in a reversible equilibrium with its inactive precursor (P) which again is in an equilibrium state with the protein pool (B) of the cell. The substrate or any other inducer which will activate the enzyme system will cause an increased number of enzyme-substrate complexes (ES) and thereby shift the equilibrium in a way which will tend to increase enzyme synthesis. This is depicted in Figure 1.



B Protein pool; P precursor; E enzyme; S substrate; ES enzyme-substrate complex; R reaction product.

Fig. 1. – Mass action theory of enzyme induction[5].

The above hypothesis can be applied to the rise in renal glutaminase activity concomitant with increased ammonia excretion in acid loaded guinea pigs. Urinary ammonia is produced by enzymatic hydrolysis of glutamine[6]. Administration of acid to mammals leads to an acute as well as chronic increase in ammonia excretion. While the acute increase must be considered as a stimulus or 'kinetic change', the slow increase occurring over several days is probably due to induction or adaptation of the ammonia producing enzymes in the kidney. The most important enzyme for urinary ammonia production is glutaminase I, a phosphate activated enzyme with an optimal pH of 7·5, which splits glutamine into ammonia and glutamic acid[7]. This enzyme is primarily localized in the papillary and collecting ducts of the renal medulla[8]. Following stimulation and induction by daily administration of ammonium chloride, an increase in enzyme activity in the kidney was observed in rat slices[9] and homogenates[10] and in guinea pig homogenates[11]. The increase in enzyme concentration in the kidney and the *in vivo* ammonia excretion were roughly proportional[10].

Enzyme concentration is rarely the limiting factor for the activity of a biocatalytic system. More important are substrate availability, intracellular pH and the presence of effectors. We observed nevertheless that ammonia production by renal glutaminase I becomes limiting for ammonia excretion *in vivo*, if production is stimulated acutely by the administration of acid[12]. Using this system for a study of enzyme induction, it is therefore not only possible to measure enzyme concentration (E) *in vitro*, but also the reaction product (R) as excreted *in vivo*. Since in the guinea pig physico-chemical factors have little influence on ammonia excretion as compared to dog and man[13], this animal is particularly well suited for induction studies.

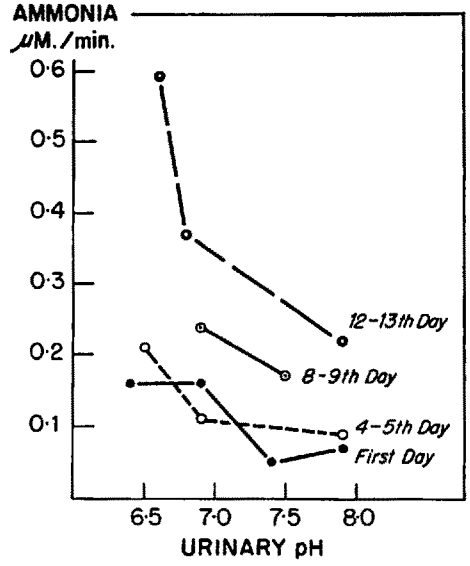


Fig. 2. – Increase in ammonia response with relation to urinary pH during induction.

In the present investigation ammonia excretion was followed prior to and following administration by stomach tube of an acid load of 0·5 *mM* ammonium chloride per 100 g body weight. Six animals weighing 540–580 g were used. The animals were kept in metabolic cages, and the urine collected for 1 h prior to (basal excretion) and 1 h after the stimulus had been applied. The administration of ammonium chloride served two purposes, first, providing a maximal stimulus for ammonia excretion and, second, inducing glutaminase I. The concentration of this enzyme in the whole kidney at the end of the induction period, i.e. 14 days approximately doubles[11].

Since the acid-base balance of the animal is a good measure of the extent of acute stimulation of the ammonia producing mechanism, the individual results are correlated with the urinary pH. As shown in Figure 2, ammonia excretion progressively rose to reach a peak at the 12–13th day after the beginning of the daily administration of ammonium chloride. For any given pH level ammonia excretion approximately doubled during induction. But even if the urinary pH is not taken into consideration, a similar pattern is observed. Figure 3 shows the response to the stimulus (ammonium chloride) on a particular day as compared to the basal excretion on that same day. The ammonia response following an acid load prior to induction was taken as 1, and the response during induction expressed as per cent of the preinduction response. Peak induction was observed between the 6th and 10th day, and a linear relationship

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between the increased response and the time involved was observed.

The induction rate, i.e. the induction occurring per unit time, can either be logarithmic or linear, depending on whether the reaction product (R) is used as building material for the enzyme (E) or is taken out of the equilibrium, as shown in Figure 1. Since the end product, ammonia, is excreted in the urine and thereby taken out of the equilibrium, the observed linear response is consistent with MANDELSTAM's hypothesis. It may be concluded that the hypothesis of MANDELSTAM cannot only be applied to enzyme induction in microorganisms, but also to enzyme adaptation in mammals.

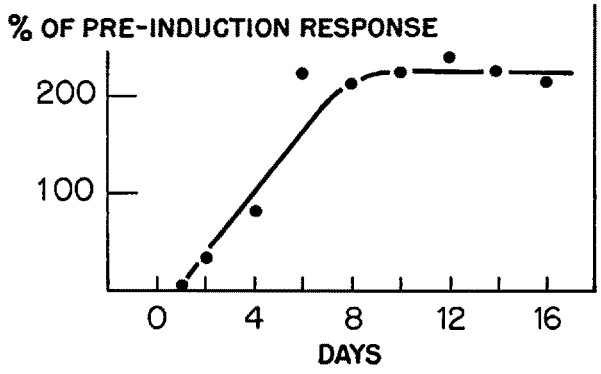

Fig. 3.—Increase in ammonia response during induction.

For the time being it is convenient to interpret adaptation from a teleological viewpoint, i.e. in the case of increased urinary ammonia excretion in chronic acidosis to consider this as a mechanism protecting the animal from an excessive loss of base. This phenomenon explains the loss of diuretic effect of ammonium chloride when this drug is administered for several days. Instead of sodium and potassium, ammonium is excreted, while the other cations are retained. This same phenomenon is probably important in renal diseases with acidosis. Further investigation of induction, experimental and spontaneously occurring in diseases, is warranted, since the prognosis of many metabolic diseases is possibly dependent on the degree of adaptation.

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Zusammenfassung

Chronische Verabreichung von Säure führt zu einer erhöhten Glutaminase-I-Konzentration in der Niere und zu einer gesteigerten Ammoniakausscheidung im Urin. Das ammoniakproduzierende biokatalytische System (Glutaminase I) ist besonders gut zum Studium der Enzymadaptation (Induktion) bei Säugetieren geeignet. Der Stand der Adaptation kann nicht nur durch die Bestimmung der Enzymveränderungen in der Niere, sondern auch durch den Nachweis des Reaktionsproduktes (Ammoniak) im Urin am lebenden Tier beurteilt werden.

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The Kinetics of Sulphatase A

The anomalous kinetics of the hydrolysis of dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) by ox liver aryl sulphatase A (sulphatase A) were first noted in this laboratory[1] and similar anomalies have since been detected in the corresponding enzymes of rat[2] and human[3] liver. The reaction velocity is not directly proportional to the enzyme concentration but with incubation times of 1 h it is linearly related to the enzyme concentration raised to the power of 3/2[4]. It was suggested[4] that this effect might be due to a polymerisation of the enzyme. DODGSON and SPENCER[5] showed the fundamental anomaly to be that the reaction was not of zero order at low concentrations of enzyme and suggested an explanation for this effect in a series of competing reactions involving the enzyme, substrate and reaction products. Simultaneously, ROY[6] showed that nitrocatechol sulphate used in these studies was impure, containing up to 10% of nitropyrogallol disulphate[7] which could be removed by paper electrophoresis. It was also shown[6] that preparations of nitrocatechol sulphate obtained through its methylene blue salt did not exhibit these anomalous kinetics, the reaction being of zero order and the velocity directly proportional to the enzyme concentration. This seemed excellent evidence for the suggestion[6] that the anomalies might be due to the use of impure substrate preparations but more recent work has cast some doubt on this interpretation as specimens of nitrocatechol sulphate free from nitropyrogallol sulphate still give anomalous kinetics[8]. In view of the increasing use of nitrocatechol sulphate as a substrate in sulphatase assays it seems that a more detailed report of the observations might be of value.

In the present studies the nitrocatechol sulphate was free from nitropyrogallol disulphate and the experimental procedures were those already described in detail[9]. The enzyme was ox liver sulphatase A–3[4]. Assays were carried out at 37° in 0·15 M acetate buffer, pH 5·0, and at a substrate concentration of 0·003 M nitrocatechol sulphate.

Typical progress curves for the hydrolysis of nitrocatechol sulphate by sulphatase A are shown in Figure 1 and in Figure 2 is shown the relationship between enzyme concentration and reaction velocity. The progress curves consist typically of three parts: stage 1, in which the initial velocity decreases rapidly; stage 2, during which the velocity may fall to almost zero; and stage 3 when the velocity rises again to an almost constant value which is considerably less than the initial velocity. The relative proportions of these three stages vary considerably with changes in enzyme concentration and with those used in the original work[1] stage 2 disappears so that the reaction approximates to one of zero order. Preincubation of the enzyme in the absence of substrate did not significantly alter the shapes of these progress curves. Increasing the substrate concentration to 0·01 M caused an increased velocity during stage 1 and a pro-

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