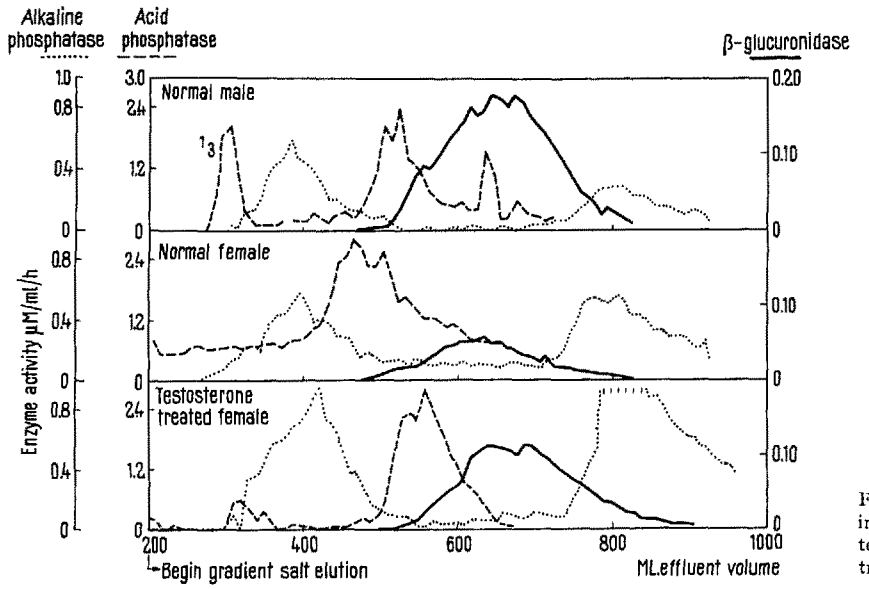


Fig. 2. Distribution of the enzyme activities in the chromatograms of male, female, and testosterone-treated female rat kidney extracts.

in a broad notched peak near the centre of the chromatogram, the first notch corresponding to the peak 6–7 space and the second to the peak 8–10 space. In the treated female a small amount of enzyme was eluted with protein peak 1; the activity was only 10% that of the male acid phosphatase in the same location.

The results presented above indicate that the sex-dimorphism of rat kidneys is not reflected in major, qualitative differences in their protein pattern.

Of the enzymes tested in our experiments, some were separated in multiple peaks; for each enzyme, however, the distribution of the multiple chromatographic forms was essentially the same in male and female pattern, with the exception of acid-phosphatase. The first peak of acid phosphatase activity was in fact missing in the female chromatograms and appeared upon testosterone treatment. It is conceivable that this particular form of acid-phosphatase in the kidney is under hormonal control. According to previous findings[8,9], it was found that the androgens influence the total activity of β-glucuronidase and of alkaline-phosphatase.

*Riassunto.* Sono state frazionate le proteine solubili estratte dal rene di ratto, mediante cromatografia su colonna di DEAE-cellulosa. Quattro enzimi sono stati localizzati nelle varie frazioni. Sono stati paragonati i profili cromatografici del rene del maschio, della femmina e della femmina sottoposta a trattamento con androgeni.

R. L. CHESANOW, M. LETIZIA SALVI, and P. U. ANGELETTI

*Istituto di Chimica, Facoltà di Medicina, Università Cattolica Roma (Italy), October 3, 1963.*

[8] C. D. KOCHAKIAN and E. ROBERTSON, Arch. Biochem. *20*, 114 (1950).
[9] G. RIOTTON and W. H. FISHMAN, Endocrinology *52*, 692 (1953).

## Alteration of Enzyme Activities in Detached Leaves and their Counteraction by Kinetin

Detached leaves are characterized by an intense protein breakdown and a respiratory drift[1]. The nature of respiratory drift, especially the mechanism of the increase in respiratory rate associated with the yellowing of tissues, is not fully understood. Kinetin, a compound capable of maintaining the normal protein level in detached leaves[2], appears to be a useful tool for the elucidation of the problem whether or not the respiratory changes taking place in detached leaves are causally connected with protein breakdown.

To throw some light on the possible mechanism of respiratory drifts in detached leaves, the level of a number of oxidative enzymes was measured according to conventional methods[3] in cell-free homogenates from detached and intact barley leaves. It has been shown that upon detachment the activity of a number of enzymes including glucose-6-P dehydrogenase, 6-P-gluconic dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, dehydroascorbic acid reductase, peroxidase, inorganic pyrophosphatase markedly increases over the control. The most characteristic changes were experienced with the pentose phosphate shunt dehydrogenase. The activation of G-6-P dehydrogenase as a function of time after detachment is shown in Figure 1. It is also indicated that the increase in enzyme activity parallels the respiratory increase suggesting a possibility of causal relationship. Simultaneously

[1] W. O. JAMES, *Plant Respiration* (University Press, Oxford 1953).
[2] A. E. RICHMOND and A. LANG, Science *125*, 650 (1957).
[3] K. KISBÁN, L. DÉZSI, M. HORVÁTH, J. UDVARDY, and G. L. FARKAS, Acta bot. Acad. Sci. Hung., in press.

with the increase in G-6-P dehydrogenase activity and the respiratory rise, the malonate sensitivity of $O_2$-uptake decreases (Figure 2) and phenolics accumulate. This latter phenomenon was also observed by other workers[4].

The above findings are compatible with the view that the respiratory increase in detached leaves is associated with an increased participation of the hexose monophosphate pathway in the overall respiratory process. The higher level of pentose phosphate pathway dehydrogenases, among other factors, might be involved in the regulation of alternative respiratory pathways. The level of G-6-P dehydrogenase in barley tissues is low compared to the *in vivo* values of $O_2$-uptake. Therefore, a regulatory function of G-6-P dehydrogenase activity as a pacemaker seems possible. It should also be stressed that the metabolic alterations described above are known to occur in diseased tissues as well[5-7] and evidence is accumulating to show an increased participation of the hexose monophosphate shunt in the respiration of diseased plants, at least in some stages of disease development[8-11]. Therefore, the metabolism of diseased tissues and that of the detached leaves reveals a number of similar characteristics[7].

As to the nature of increase in enzyme levels in detached leaves, we came to the conclusion that it cannot be due to *de novo* enzyme protein synthesis. Experiments with G-6-P dehydrogenase have shown that the augmentation in enzyme activity cannot be inhibited by treating detached leaves with 0.25 mg/ml crystalline ribonuclease or $10^{-5}M$ 2,4-dinitrophenol, compounds known to block protein synthesis. By contrast, the increase in enzyme activity was markedly inhibited when the leaves were placed in $10^{-5}M$ kinetin (Table). Concomitant determination of soluble proteins in kinetin-treated barley leaves has shown a constant or even increasing protein level as compared to intact plants. Therefore, it seems likely that the higher level of G-6-P dehydrogenase (and other enzymes) in detached leaves is due to protein breakdown. Probably a proteolytic liberation of latent enzymes from inactive form (ribosomes?) is involved. Proteolytic activation of enzymes in microsomal preparations has recently been described[12,13].

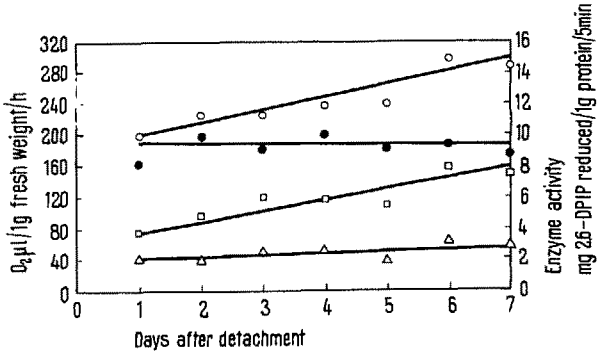


Fig. 1. Respiratory drift and the activation of G-6-P dehydrogenase in detached barley leaves. ● $O_2$-uptake of control leaves. ○ $O_2$-uptake of detached leaves. △ G-6-P dehydrogenase activity in control leaves. □ G-6-P dehydrogenase activity in detached leaves. Average values from four experiments.

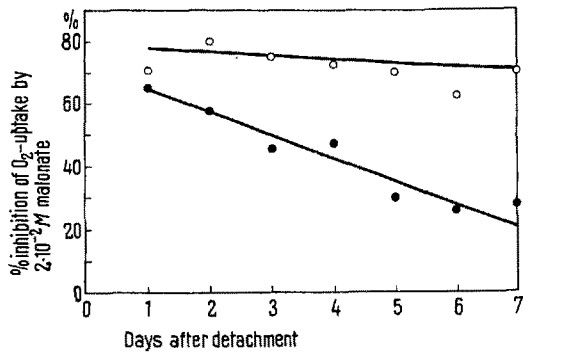


Fig. 2. Effect of detachment on the malonate sensitivity of barley leaves. ○ Control. ● Detached. Average values from four experiments.

Effect of kinetin on the activity of G-6-P dehydrogenase in detached barley leaves (representative experiment)

| Days after detachment | Enzyme activity expressed as mg 2,6-dichlorophenol indophenol reduced/1 g protein/5 min | | |
|---|---|---|---|
| | Intact (control) | Detached | Detached and treated with $10^{-5}M$ kinetin |
| 1 | 3.4 | 5.6 | 5.1 |
| 2 | 3.5 | 9.5 | 5.5 |
| 3 | 5.0 | 9.0 | 6.6 |
| 4 | 5.0 | 11.4 | 7.1 |
| 7 | 6.6 | 17.2 | 4.0 |

Kinetin affects not only the enzyme level (as measured in cell-free extracts) but the *in vivo* $O_2$-uptake as well. The respiratory drift can be prevented by kinetin to a high extent and the malonate sensitivity of respiration in detached kinetin treated leaves tends to be similar to that of intact ones. These observations are in line with a recent note on the carbon sparing effect of kinetin[14].

A detailed description of these experiments will be given in Acta bot. Acad. Sci. Hung.[3].

*Zusammenfassung.* In abgetrennten Blättern werden verschiedene Enzyme, besonders aber die Pentosephosphatcyclus-Dehydrogenasen aktiviert. Gleichzeitig steigt die Atmungsintensität an. Die Malonatsensitivität der $O_2$-Aufnahme nimmt ab. Die Ausbildung der obigen Stoffwechsellage kann durch Kinetin gehemmt werden.

J. Udvardy, M. Horváth, K. Kisbán, L. Dézsi, and G. L. Farkas

*Institute of Plant Physiology, University of Budapest (Hungary), November 25, 1963.*

[4] Z. Király, unpublished results.
[5] G. L. Farkas and Z. Király, Physiol. Plant. *8*, 877 (1955).
[6] G. L. Farkas, Ber. dtsch. bot. Ges. *74*, 382 (1962).
[7] G. L. Farkas, L. Dézsi, M. Horváth, and K. Kisbán, Phytopath. Z., in press.
[8] M. Shaw and D. J. Samborski, Can. J. Bot. *35*, 389 (1957).
[9] J. M. Daly, R. M. Sayre, and J. H. Pazur, Plant Physiol. *32*, 44 (1957).
[10] G. Loebenstein and N. Linsey, Phytopathology *53*, 350 (1963).
[11] P. Tien and P. S. Tang, Scientia sinica *12*, 565 (1963).
[12] K. McQuillen, in *Protein Biosynthesis* (Ed. R. J. C. Harris, Academic Press, London and New York 1960).
[13] M. Görlich and E. Heise, Nature *197*, 698 (1963).
[14] B. E. S. Gunning and W. K. Barkley, Nature *199*, 262 (1963).