

EXCRETION OF ACID HYDROLASES (β -GLUCURONIDASE AND ACID PROTEASE) DURING MOULTING OF *PHILOSAMIA RICINI* (SATURNIIDAE: LEPIDOPTERA)

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ABSTRACT

Enzymic activity variations of β -glucuronidase and acid protease have been studied in the developing eri silkworm *Philosamia ricini* during larval-larval moult. The activity of both the enzymes increases at moulting time and then decreases. It has been suggested that in *P. ricini*, acid hydrolases are got rid of via cast off cuticles once their functions are over. The excreta are devoid of any acid hydrolase activity.

INTRODUCTION

OCCURRENCE of lysosomes in insect tissues and their possible role in insect metamorphosis have been well recognized¹⁻⁵. Increasing activity of lysosomal hydrolases has also been observed during metamorphosis of insects and anurans. However, there still remains a wide gap concerning the endogenic behaviour and control of lysosomal enzymes during insect metamorphosis.

Barker and Alexander⁶ detected the presence of acid phosphatase in the empty puparia of *Musca domestica* while Varute⁷ demonstrated β -glucuronidase activity in the excreta of the metamorphosing anuran tadpole of *Rana tigrina*. Varute and Sawant³ also demonstrated that the difference in β -glucuronidase activity observed between the pharate-adult and the adult blowfly, was detectable in the empty puparia left behind by the adult insect after emergence.

Available literature revealed that studies on the fate of increased acid hydrolases during histolysis in insects are scant. Therefore, in the present communication β -glucuronidase and acid protease activity variations, in the developing larva of the eri silkworm *Philosamia ricini*, during larval-larval moult have been studied and also an attempt has been made to explore the fate of these enzymes after adult emergence.

MATERIALS AND METHODS

P. ricini was reared as described earlier⁸. Three lots of 6 larvae each, starved for 6 hr were picked at random from insect colonies of known age and stage of development. They were separately weighed, chilled and homogenized with glass-distilled water in a Potter-Elvehjem type all-glass homogenizer to 10%

(W/V) tissue concentration. The homogenates were strained through muslin cloth.

The cast off cuticles (50-60) of the ecdysed larvae were washed and homogenized in ice-cold distilled water, the homogenate strained through muslin cloth and diluted with distilled water to a known volume.

Excreta pellets of the larvae (2-3 g) were thoroughly homogenized to a 10% (W/V) concentration in ice-cold distilled water and the homogenate allowed to stand at 4°C for one hr. It was then centrifuged (5000 g, 4°C) and the supernatant employed for enzymic assays.

Acid protease was assayed by the method of Matsushita and Iwami⁹ and β -glucuronidase by that of Fishman¹⁰. Proteins were estimated by Lowry's method¹¹. Assays were made in duplicates in all the four homogenates—the additional homogenate being the mixture of the three homogenates. The experiments were repeated three times. The results varied between 11-19%.

RESULTS AND DISCUSSION

The acid hydrolases—acid protease and β -glucuronidase (figures 1 and 2) register significant enhanced activity at the time of each moult and an equally significant decreased activity after the cuticle is cast off.

Excreta reveal total absence of any acid hydrolase activity whereas the fresh cast off cuticle shows a very marked activity of both the acid hydrolases. The loss of activity of the enzymes in the newly ecdysed larva is suggestive of its recovery in the cast off cuticle (table 1).

Increased activity of lysosomal acid hydrolases

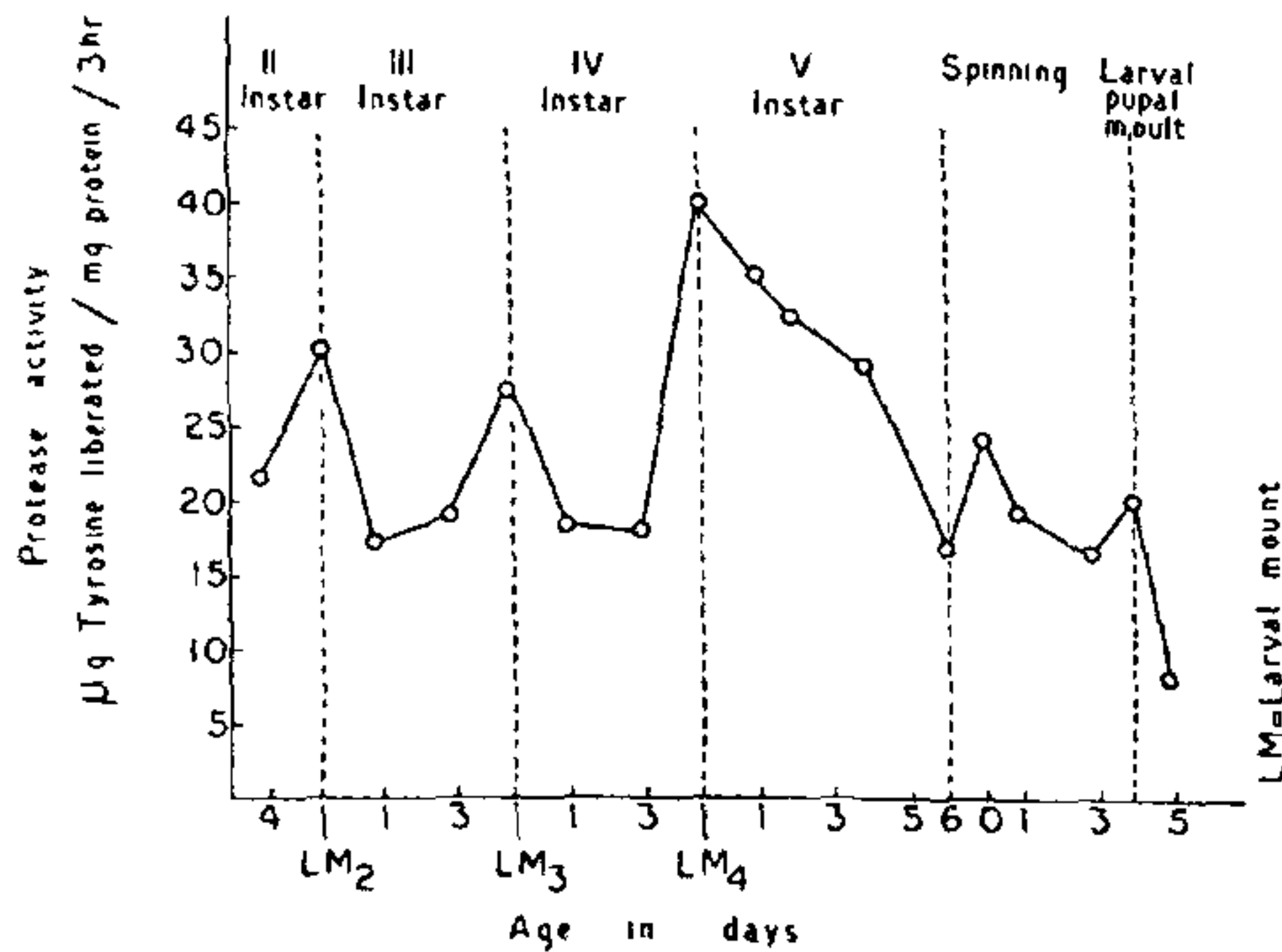


Figure 1. Variation in protease activity during successive moulting in *Philosamia ricini*.

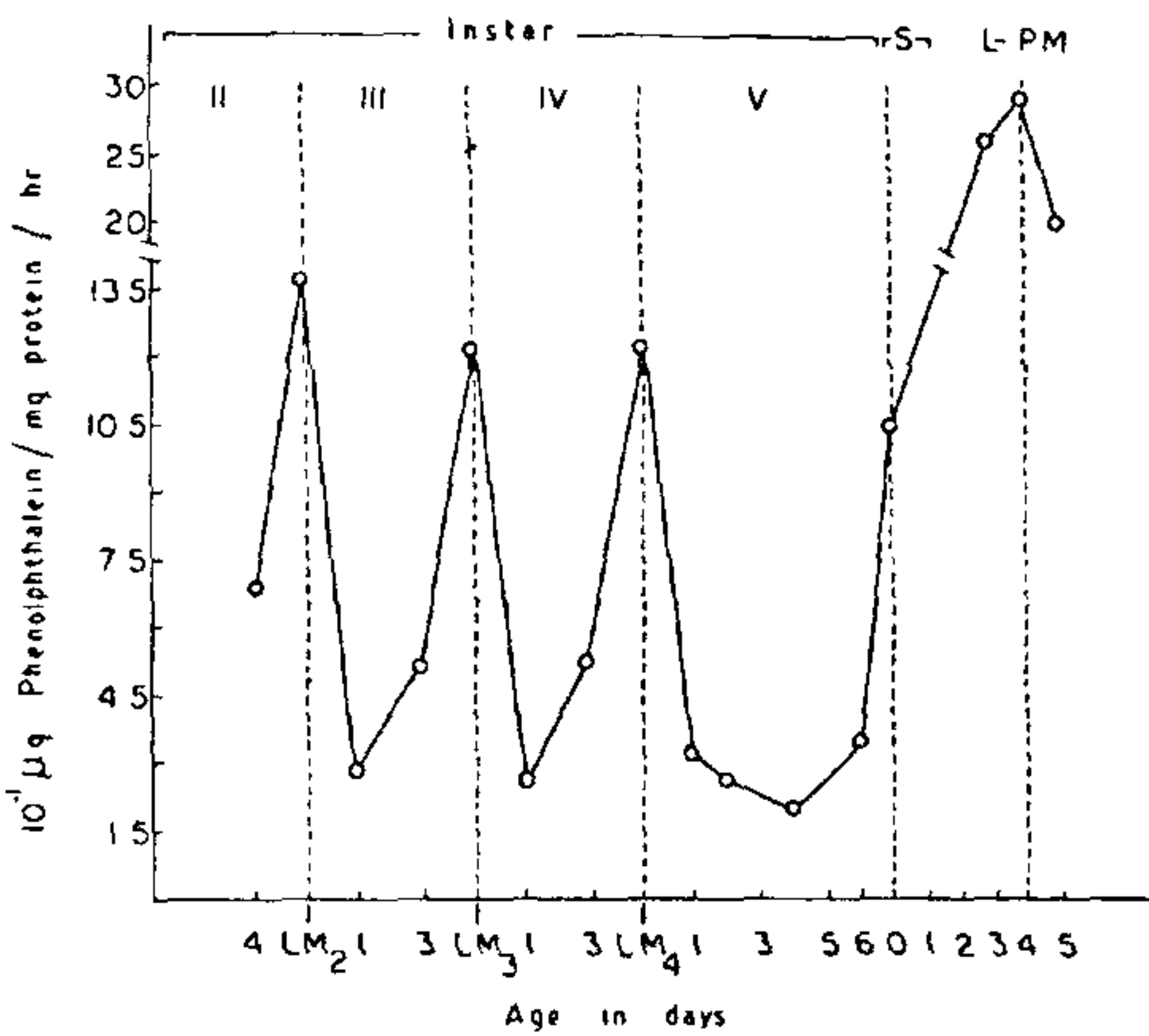


Figure 2. Variation in β -glucuronidase activity during successive moulting in *Philosamia ricini*.

Table 1 Acid hydrolases activity in the cast off cuticles of *P. ricini*

| Larval moult | Acid protease | β -glucuronidase |
|--------------|---------------|------------------------|
| LM II | 275 | 6 |
| LM III | 305 | 15 |
| LM IV | 363 | 22 |

(expressed as μg tyrosine liberated/cuticle/3h for acid protease and μg phenolphthalein liberated/cuticle/hr-for β -glucuronidase activity)

during pupation is an adaptive response to the autolytic or histolytic events occurring during the process of metamorphosis. Although De Duve and Wattiaux¹² and Strauss¹³ ascribed a dynamic role to lysosomal enzymes in the process of histolysis, their fate after the completion of the process has not been clarified. In the metamorphosing frog, Varute⁷ and Varute and More¹⁴ observed that after the completion of metamorphosis, the increased lysosomal enzymes from the degenerated tissues of the tadpole were transferred to the liver by the macrophages and into the lumen of the alimentary canal wherefrom they got excreted. Thus in anurans there seems to exist a definite mechanism to get rid of the increased lysosomal enzymes after their role is accomplished.

An anuran metamorphosing tadpole endowed with an open system is capable of acquiring its nutritive and respirative requirements from the atmosphere and is also able to excrete the undesirable waste metabolic products. On the other hand, a moulting larva or pupa having a cleidoic system has to fulfil its demands from within the puparium and also is compelled to retain the harmful waste products inside the body due to its inability to excrete them immediately.

The present study reveals that in *P. ricini* the excretion of acid hydrolases is achieved through the cuticle after their function is over. This conclusion explains the observation of Barker and Alexander⁶ who detected high phosphatase activity in the empty puparia of *Musca domestica* after adult emergence and also of Varute and Sawant³ who demonstrated the recovery of the entire hydrolases activity in the closely adhering inner membrane of the empty puparium cast off by the blowfly *Crysomya rufifercis*.

ACKNOWLEDGEMENT

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NEWS

ASSESSING BASIC RESEARCH

... "After reviewing the literature on scientific assessment, we argue that, while there are no simple measures of the contributions to scientific knowledge made by scientists, there are a number of 'partial indicators' - that is, variables determined partly by the magnitude of the particular contributions, and partly by 'other factors'. If the partial indicators are to yield reliable results, then the influence of these 'other factors' must be minimised. This is the aim of the method of 'converging partial indicators' proposed in this paper. . . . in an empirical study of four radio astronomy observatories, the method of converging partial indicators is tested, and several of the indicators (publications per researcher, citations per paper, numbers of highly cited papers, and peer

evaluation) are found to give fairly consistent results. The results are of relevance to two questions: (a) can basic research be assessed? (b) more specifically, can significant differences in the research performance of radio astronomy centres be identified? We would maintain that the evidence presented in this paper is sufficient to justify a positive answer to both these questions, and hence to show that the method of converging partial indicators can yield information useful to science policy-makers." (Reproduced with permission from: *Press Digest, Current Contents*®, No. 39, September 24, 1984, p. 14; Copy right by the Institute for Scientific Information® Philadelphia, PA, USA).

COMPUTERS AS MIND READERS

... "Computers may soon be able to accept input directly from a person's brain. At first, the systems will use electrodes placed on a person's neck and temples, but in the next decade these will be replaced by 'biochips', computer chips made of organic materials, according to 'Non-Keyboard Data Entry', a 353 page report from International Resource Development Inc.

The report cited positive uses of the technology but warned that it may permit the stealing 'through' from a person's brain. (Reproduced with permission from: *Press Digest, Current Contents*® No. 40, October 1, 1984; Copyright by the Institute of Scientific Information®, Philadelphia, PA USA)".
