

when the amounts of coenzyme A and choline acetylase (therefore CoA acetylating system) are increased, suggests that these compounds do not affect the enzymatic transfer of acetic group from acetyl-coenzyme A to choline, but they inhibit the acetylation of coenzyme A. This, moreover, was likely, because our substances inhibit sulfanilamide acetylation also in pigeon liver extracts<sup>2, 10</sup>.

*Acknowledgement.* We wish to thank Dr. E. MUSSINI for his cooperation in carrying out chromatographic controls on acetyl-coenzyme A.

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November 14, 1957.*

#### Riassunto

Numerosi derivati di acidi acil-aromatici si dimostrano attivi nell'inibire la acetilazione della colina catalizzata dal Coenzima A.

Si ritiene che questa inibizione si realizzi attraverso una inibizione della acetilazione del Coenzima A.

<sup>10</sup> S. GARATTINI, C. MORPURGO, and N. PASSERINI (in press).

### Serological Properties of Poly-L-Tyrosine Derivatives<sup>1</sup>

SELA *et al.*<sup>2</sup> have reported that guinea pigs are sensitized by the injection of polytyrosyl-gelatin, but not by gelatin alone, nor by a copolymer of tyrosine and aspartic acid. Since gelatin has been found to be antigenic in some species<sup>3</sup>, it is not clear whether the antigenicity of polytyrosyl-gelatin is due to its gelatin moiety or to its polytyrosyl residue. We have investigated, therefore, the serological behaviour of two polytyrosine derivatives. One of them, poly-L-tyrosine-azophenylarsonate (PTA) was prepared by coupling polytyrosine ( $n = 45$ )<sup>4</sup> with an excess of diazotized arsanilic acid. The other, poly-L-tyrosyl-gelatin-azophenylarsonate (PTGA) was obtained in the same manner from poly-L-tyrosyl-gelatin<sup>2</sup>. Rabbits were injected subcutaneously with four 30 mg doses of PTA or PTGA directly or after addition of alum and neutralization with alkali. The first three injections were given in intervals of 3 days, the last injection after a further week. One week later the animals were bled and their sera tested with PTA, PTGA and also with arsanil-azo-bovine- $\gamma$ -globulin (AsBGG) prepared from one gram of bovine  $\gamma$ -globulin (Armour) with 0.1 g of diazotized arsanilic acid. Neither PTA nor PTGA gave any precipitates. However, the serum from a rabbit injected with alum-PTGA gave a distinct precipitin test with AsBGG. When 4.5 ml of the serum were incubated with 0.5 mg AsBGG, a precipitate was obtained which was not noticeably soluble on ad-

dition of 0.5 ml of a 2% solution of AsBGG. The insoluble residue, after washing, weighed 2.2 mg; colorimetric comparison of its solution in 1% NaOH with a standard solution of AsBGG showed that it contained 0.2–0.3 mg AsBGG. When three 2 ml samples of the same immune serum were incubated with 0.2 mg PTA, PTGA or AsBGG, only the last substance gave a precipitate; it contained approximately 0.1 mg AsBGG.

We conclude from these results that poly-L-tyrosine-azophenylarsonate is not an antigen, but that poly-L-tyrosyl-gelatin-azophenylarsonate, if injected with alum as adjuvant, induces formation of precipitins which combine with the tyrosine-bound azophenylarsonate groups. Evidently, the nonantigenic poly-tyrosyl-azophenylarsonate acquires antigenic properties by its combination with gelatin.

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#### Zusammenfassung

Polytyrosin und Polytyrosylgelatine wurden mit diazotierter Arsanilsäure gekoppelt und in Lösung oder nach Fällung mit Alaun und Alkali Kaninchen injiziert. Die injizierten Substanzen wurden von keinem der Sera präzipitiert. Hingegen präzipitierte das Serum der mit gefällter Arsanilazo-polytyrosyl-gelatine injizierten Tiere Arsanilazo-Rinderserumglobulin. Wir schliessen daraus, dass Arsanilazopolytyrosin nicht als Antigen wirkt, dass es aber durch Bindung an Gelatine antigene Eigenschaften gewinnt.

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### Sucrose and Starch Synthesis in Sugar Cane Plant

During the investigations on the formation of sucrose in the sugar cane plant it was observed that all parts of the plant except the top node of the matured sugar cane contained only sucrose, glucose and fructose but not starch, at all stages of the development, whereas the top node of the matured sugar cane contained starch. This led us to think that a sucrose-synthesizing enzyme system may be predominant in all parts except the top node of the mature sugar-cane in which the presence of starch-synthesizing enzyme may be a special feature. Hence studies were undertaken to detect the sucrose- and the starch-synthesizing enzyme systems in various tissues of the plant at different stages of development and the results are recorded in this communication.

Young, middle-aged and matured sugar-cane plants were taken for the experiment. Cell-free extracts of leaves, roots, nodes and internodes were prepared as previously described<sup>1</sup> and tested for the presence of sucrose-synthesizing and starch-synthesizing enzyme systems.

For estimation of sucrose-synthesizing enzyme system, 2 ml of the assay system contained citrate buffer (pH 6.5), 50  $\mu$ M; fructose, 60  $\mu$ M; glucose-1-phosphate,

<sup>1</sup> K. P. PANDYA and C. V. RAMAKRISHNAN, *Naturwissenschaften* 15, 352 (1956).

<sup>1</sup> This work was supported by research grants of the National Science Foundation and the U.S. Public Health Service, and by contracts of Indiana University with the Office of Naval Research and the Atomic Energy Commission.

<sup>2</sup> M. SELA, E. KATCHALSKI, and A. L. OLITZKI, *Science* 123, 1129 (1956).

<sup>3</sup> P. MAURER, *Arch. Biochem. Biophys.* 53, 205 (1955).

<sup>4</sup> E. KATCHALSKI and M. SELA, *J. Amer. chem. Soc.* 75, 5284 (1953).

	Young sugar cane Specific activity of		Two months old sugar cane Specific activity of		Matured sugar cane	
	Sucrose synthesizing enzyme	Starch synthesizing enzyme	Sucrose synthesizing enzyme	Starch synthesizing enzyme	Sucrose synthesizing enzyme	Starch synthesizing enzyme
Leaves . . . . .	0.30	—	0.50	—	0.35	—
Roots . . . . .	0.02	—	—	—	—	—
Nodes . . . . .	0.15	—	0.20	—	0.10	—
Internodes . . . . .	0.10	—	0.14	—	0.05	—
Top nodes* . . . . .	0.10	—	0.20	—	0.01	0.009

\* In case of the top node, the sucrose content per mg dry weight of the tissue of the matured sugar cane was only 10% of that of the young sugar cane.

20  $\mu$ M; enzyme preparation 1 ml (enzyme extracts are prepared in such a way as to contain 20 mg protein per ml in the case of leaf extract whereas about 40 mg of protein in the case of other parts of the plants) and water added to 2 ml. Additions were made in the test tubes and corked well. After addition of the enzyme, the test tubes were incubated for 1 h at 37°C. Enzyme and substrate blanks accompanied the samples. At the end of the incubation period, the contents were centrifuged. Fructose, sucrose and the inorganic phosphate were determined on the supernatant. Fructose was estimated according to HANES' method<sup>2</sup>, the inorganic phosphate according to FISKE and SUBBAROW<sup>3</sup> and total sucrose according to ROE<sup>4</sup>.

For estimation of starch-synthesizing enzyme, the method of HANES modified by MURTHY, SWAMINATHAN and SUBRAMANYAN<sup>5</sup> was used. Tests carried out on the extracts showed that they did not possess phosphatase and amylase activities.

Protein was determined by estimating mikro-kjeldahl nitrogen.

For sucrose-synthesizing enzyme, a unit of enzyme activity is defined as that causing a synthesis of  $\mu$ M of sucrose in 1 h and the specific activity as units per mg protein.

For starch-synthesizing enzyme, a unit of enzyme activity is defined as that causing a synthesis of 1 mg of starch in 1 h and the specific activity as units per mg protein.

The results are given in the Table.

From the table, it can be seen that in two months old sugar cane the activity of sucrose synthesizing enzyme system is high and it is less in matured sugar cane. Starch-synthesizing system could not be detected in any other part of the plant except the top node in matured sugar cane where alone the starch was detected. This shows the possibility that, in top node of the matured sugar cane, starch may be synthesized.

The authors thanks are due to Dr. BALAKRISHNA PATEL and RANCHODBAI PATEL of Institute of Agriculture, Anand, for the generous supply of sugar cane plant.

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Biochemistry Department, Faculty of Science, M. S. University of Baroda (India), November 7, 1957.

<sup>2</sup> C. S. HANES, *Biochem. J.* 23, 99 (1929).

<sup>3</sup> C. H. FISKE and Y. SUBBAROW, *J. biol. Chem.* 66, 375 (1925).

<sup>4</sup> J. H. ROE, *J. biol. Chem.* 107, 15 (1934).

<sup>5</sup> H. B. N. MURTHY, M. SWAMINATHAN and V. SUBRAMANYAN, *J. sci. industr. Res.* 13, 223 (1954).

### Zusammenfassung

Im Gipfelknoten des ausgereiften Zuckerrohrs liess sich die Anwesenheit eines stärkebildenden Enzyms nachweisen, während die anderen stärkefreien Teile dieser Pflanze ein sukrosebildendes Enzymsystem enthalten. Diese Befunde stehen im Einklang mit der Speicherung von Stärke im Gipfelknoten und dem Fehlen von Stärke in den übrigen sukrosehaltigen Pflanzenteilen des Zuckerrohrs.

### The Gonad Stimulating Potency of Date Palm Pollen Grains

Several investigators were able to extract estrogenic materials from palm kernels and date palm pollen grains (BUTENANDT and JACOBI<sup>1</sup>, HASSAN and WAF<sup>2</sup> and EL-RIDI and WAF<sup>3</sup>). Recently a gonad-stimulating principle was extracted from pollens. When injected into immature male and female rats, it increased the weights of their gonads and activated them (SOLIMAN and SOLIMAN<sup>4</sup>). Preliminary experiments also indicated that this effect is obtained if defatted pollen grains are fed to immature animals.

In the present investigation, it was planned to determine quantitatively the follicle-stimulating and lutenizing potency of date palm pollen grains (*Phoenix dactylifera* L.). 100 g of pollen grains were freshly obtained. They were defatted with three changes of 200 ml of petroleum ether, then washed with acetone and left to dry at room temperature. The gonadotropic principles were then extracted, using the method of McSHAN and MEYER<sup>5</sup>. The extract was then dissolved in 10 ml of distilled water and stored at the temperature of 5°C.

For determination of lutenizing activity, the method adopted by SOLIMAN<sup>6</sup> was used. The plant hormone was compared with a standard preparation of HCG (Physex, Leo). The slope-ratio method of FINNEY *et al.*<sup>7</sup>, was used to evaluate the relative activity. Thirty immature female mice were injected subcutaneously with 20 I.U. of PMS (Antex, Leo). Four days later when the graafian

<sup>1</sup> A. BUTENANDT and H. JACOBI, *Hoppe-Seyl. Z.* 218, 104 (1933).

<sup>2</sup> A. HASSAN and H. ABOU EL WAF, *Nature* 159, 409 (1947).

<sup>3</sup> M. S. EL-RIDI and M. ABOU EL WAF, *Roy. Egypt. Med. Assoc.* 30, 124 (1947).

<sup>4</sup> F. A. SOLIMAN and L. SOLIMAN, *Exper.*, in press (1957).

<sup>5</sup> W. H. Mc SHAN and R. K. MEYER, *J. biol. Chem.* 135, 473 (1939).

<sup>6</sup> F. A. SOLIMAN, *Egypt. Med. Assoc.*, in press (1957).

<sup>7</sup> D. J. FINNEY, J. H. BURNS, and L. G. GOODWIN, *Biological standardization*, 2nd edition (Oxford Univ. Press 1957).