

	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 μ 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' method², the inorganic phosphate according to FISKE and SUBBAROW³ and total sucrose according to ROE⁴.

For estimation of starch-synthesizing enzyme, the method of HANES modified by MURTHY, SWAMINATHAN and SUBRAMANYAN⁵ 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 μ 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.

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² C. S. HANES, *Biochem. J.* **23**, 99 (1929).
³ C. H. FISKE and Y. SUBBAROW, *J. biol. Chem.* **66**, 375 (1925).
⁴ J. H. ROE, *J. biol. Chem.* **107**, 15 (1934).
⁵ 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 sukroshaltigen 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 JACOBI¹, HASSAN and WAF² and EL-RIDI and WAF³). 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 SOLIMAN⁴). 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 gonadotrophic principles were then extracted, using the method of McSHAN and MEYER⁵. 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 SOLIMAN⁶ was used. The plant hormone was compared with a standard preparation of HCG (Physex, Leo). The slope-ratio method of FINNEY *et al.*⁷, 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

¹ A. BUTENANDT and H. JACOBI, *Hoppe-Seyl. Z.* **218**, 104 (1933).
² A. HASSAN and H. ABOU EL WAF, *Nature* **159**, 409 (1947).
³ M. S. EL-RIDI and M. ABOU EL WAF, *Roy. Egypt. Med. Assoc.* **30**, 124 (1947).
⁴ F. A. SOLIMAN and L. SOLIMAN, *Exper.*, in press (1957).
⁵ W. H. Mc SHAN and R. K. MEYER, *J. biol. Chem.* **135**, 473 (1939).
⁶ F. A. SOLIMAN, *Egypt. Med. Assoc.*, in press (1957).
⁷ D. J. FINNEY, J. H. BURNS, and L. G. GOODWIN, *Biological standardization*, 2nd edition (Oxford Univ. Press 1957).

Table I.—LH Content of Date Palm Pollen Grains

Treatment	Body Weight	Average No. of Corpora Hemorrhagica
20 I. U. PMS . . .	8.80	0.40±0.11
1 I. U. HCG . . .	9.80	0.60±0.03
10 I. U. HCG . . .	8.50	4.00±0.35
100 I. U. HCG . . .	9.00	7.60±0.06
1 g Pollen	11.50	1.33±0.09
2 g Pollen	8.80	1.67±0.60

Each group contained 5 animals. ± Standard error.
* All mice given HCG were previously treated with 20 I. U. PMS.
b = 3.50.

follicles become mature, the mice were divided into six groups of five animals each. Three groups were injected with 1, 10, and 100 I.U. of HCG. One group was injected with extract from 1 g pollen, another with extract from 2 g pollen. The last group was left without any further treatment. Two days later the animals were killed with ether and the number of corpora hemorrhagica formed were used as an index of LH content.

The follicle-stimulating activity of pollens was compared with a standard curve established under the same conditions. The LH augmentation method of STEELMAN and POHLY⁸ was used. Twenty-eight immature female rats were divided into 7 groups and injected with graded doses of PMS to which 20 I.U. of HCG were added. A group of rats was injected with extract from 2 g pollen, and another group with extract from 2 g pollen plus 20 I.U. of HCG. The animals were killed with ether on the fourth day and their body weights and ovary weights were recorded. The data were analyzed statistically.

Table II.—FSH Content of Date Palm Pollen Grains

Treatment	Body Weight	Ovary Weight in mg/100 g Body Weight
Control.	30.25	24.90±1.43
20 I. U. HCG. . .	27.00	41.62±0.50
1 I. U. PMS*. . .	24.67	44.62±0.03
10 I. U. PMS . . .	27.67	241.90±4.98
100 I. U. PMS*. .	25.00	412.03±7.80
2 g Pollen	29.25	45.15±1.76
2 g Pollen*	32.00	60.22±3.10

Each group contained 4 rats. ± Standard error.
* Twenty I. U. were added to the injected material.
b = 218.

When compared with the respective standard curves and by using the formula $\bar{Y}_x = \bar{Y} - b(X - \bar{X})$, where $\bar{Y}_x$ represents the average value of response, $\bar{Y}$ the total average response values, *b* the measure of slope and $\bar{X}$ the total average of the logarithm of the doses used. It was found that 1 g pollen contains 1.05 and 2 g contained 2.06 I.U. of LH activity. The FSH activity was calculated by the same method. It was found that 2 g pollen contained about 1.61 I.U. of PMS. A look at the ovarian weights of rats treated with 2 g pollen (Table II) shows that it is comparable with that of rats treated with 20 I.U. of HCG alone. This shows that the two fractions of LH and FSH

⁸ S. L. STEELMAN and F. M. POHLY, *Endocrinology* 53, 604 (1953).

are present in a ratio which resulted in the augmentation of their effects. The combined activity of the two fractions of hormones present in 1 g pollen is close from 10 I.U. of HCG.

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Résumé

La principale substance gonadotrophique des grains de pollen des palmiers-dattiers est extraite avec de l'acétone. On a constaté qu'un g de pollen contient 1,05 I.U. de LH et 0,80 I.U. de FSH. L'activité combinée des deux fractions augmente leur effet qui atteint jusqu'à 10 I.U. HCG par g.

DISPUTANDA

Der respiratorische Quotient von Tabakblättern

Der Atmungsquotient (RQ) von grünen Tabakblättern ist bis heute schon von verschiedenen Autoren untersucht worden. So hat EICHENBERGER¹ gefunden, dass die Blätter der schweizerischen Tabaksorte Mt. Calme brun während des Tages einen RQ aufweisen, der zwischen 1,15 und 1,35 liegt. FREY-WYSSLING und WALTZ² stellten daraufhin eine umfassende Studie über dieses Problem an und untersuchten alle in der Schweiz angebauten Tabaksorten. Sie fanden dabei zum Beispiel für die Sorte Paesana einen RQ von 1,26. Da der Tabak eine Stärkepflanze ist³, sind vor allem Kohlehydrate als Atmungssubstrate zu erwarten. Das würde einen RQ von ungefähr 1 bedingen. Die beiden Tatsachen, dass das Tabakblatt reich an organischen Säuren ist³ und dass der RQ auch während der Nacht nie unter 1 sinkt, bewogen FREY-WYSSLING und WALTZ, eine vom Sukkulenitentypus⁴ verschiedene Atmung für den Tabak zu postulieren. WALTZ⁵ versuchte darauf, eine Korrelation dieses neuen Atmungstypus mit dem Nitratstoffwechsel erwachsener Pflanzen nachzuweisen. Es gelang ihm dies aber nicht. Auf der andern Seite hatte ROBERTS⁶ schon 1941 den Tabak-RQ untersucht. Er fand ebenfalls hohe Werte bei Luftfüllung des Gasraumes der Warburggefässe; bei Sauerstofffüllung hingegen erhielt er einen RQ von 1,01. Wir untersuchten die Sorte Paesana, bei der WALTZ⁵ nach sechsständigem Aufenthalt im Dunkeln noch einen RQ von etwa 1,27 fand. Die Messungen wurden am Blatt Nr. 18 (vom untersten sichtbaren Blatt an gezählt) von Freilandpflanzen ausgeführt. Um eine Kohlendioxid-Dunkelfixierung⁷, die die Messung eventuell hätte stören können, abklingen zu lassen, wurden die Blätter nach dem Pflücken vorerst 6 h im Dunkeln gelassen. Nachher gingen wir nach der Methode von WALTZ⁵ vor.

¹ E. EICHENBERGER, *Ber. Schweiz. bot. Ges.* 62, 123 (1952).
² A. FREY-WYSSLING und P. WALTZ, *Exper.* 11, 178 (1955).
³ A. I. SMIRNOW, *Biochemie des Tabaks* (Den Haag 1940).
⁴ T. A. BENNET-CLARK, *Sci. Proc. R. Dublin Soc.* 20, 293 (1932).
⁵ P. WALTZ, *Ber. Schweiz. bot. Ges.* 67, 55 (1957).
⁶ E. A. H. ROBERTS, *Biochem. J.* 35, 1289 (1941).
⁷ R. E. STUTZ und R. H. BURRIS, *Plant Physiol.* 26, 226 (1951).