Versuche von VAN RYSELBERGE und JEENER²⁵ über die Biosynthese des TMV in einer Atmosphäre von $^{14}\text{CO}_2$ ergaben, daß dieses spezifische Protein eine Vorstufe und nicht ein Abbauprodukt des Virus ist, denn der Zuwachs an fertigem Virus in einer bestimmten Zeiteinheit entspricht der Menge an spezifischem Virusprotein, das in dieser Zeiteinheit gebildet wird.

Die RNS- und die Proteinsynthese scheinen nicht nur zeitlich, sondern auch räumlich voneinander getrennt zu sein. Nach den Untersuchungen von ZECH²⁶ ist es sehr wahrscheinlich, daß die RNS im Zellkern gebildet wird, während SCHRAMM und RÖTTGER²⁷ mit der Methode der fluoreszierenden Antikörper fanden, daß die Proteinsynthese ausschließlich im Cytoplasma abläuft, und zwar beginnt sie in einer Zone um den Zellkern herum und breitet sich dann über das ganze Cytoplasma aus.

Zusammenfassend gelangt man zu folgendem vorläufigem Bild der Synthese des TMV. Nach dem

Eindringen des TMV-Teilchens in die Zelle wird die Proteinhülle abgestreift. Die freigesetzte RNS beginnt vermutlich im Zellkern sich zu vermehren. Die gebildete RNS tritt aus dem Zellkern aus und induziert im Cytoplasma die Synthese des spezifischen Proteins. Jeweils etwa 2000 gebildete Peptidmoleküle vereinigen sich mit einem Molekül der freien RNS zum vollständigen Virusteilchen, wodurch die Menge an freier RNS nach Durchlaufen des Maximums wieder abnimmt.

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²⁵ C. VAN RYSELBERGE u. R. JEENER, *Biochim. biophysica Acta* [Amsterdam] **23**, 18 [1957].

²⁶ H. ZECH u. L. VOGT-KÖHNE, *Naturwissenschaften* **42**, 337 [1955].

²⁷ G. SCHRAMM u. B. RÖTTGER, *Z. Naturforschg.* **14b**, 510 [1959].

Gonadotrophic Hormones in Pollen Grains of the Date Palm*

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1—Gonadotrophic hormones have been identified to be present in pollen grains powder of date palm including both FSH and LH.

2—The follicle stimulating hormone (FSH) is present in pollen in approximate concentration of 100 I.U./10 grams pollen and the luteinising hormone (LH) about 3–4 I.U./10 grams pollen.

3—These findings confirm the use of pollen grain of the palm by the Bedwins for treating sterility.

In 1880 DARWIN¹ showed that when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper to the lower part. Thirty years later (1910) JENSEN² stated that this influence is a chemical substance that moves through the plant tissues. This was the first evidence that something of a hormonal nature existed in plants.

In 1933 KOGL³ described the isolation in a chemically pure state of a substance which affects curvature (cell stretching) in plants.

Using WENT's method for detecting and measuring phytohormones KOGL et al.⁴ found in human

urine a rich source of material from which they can isolate and identify chemically the active compounds. Later they used plant material such as malt, yeast, corn, peanuts, sunflower, fungi, linseed oil.

While the tips of oats, corn, fungi and yeast contain the hormone, KOGL found urine (male and female) to be the most convenient source material. He isolated three active substances, auxin A, auxin B and indole-3-acetic acid.

In (1930) JANNEY and WALKER⁵ isolated an oestrogenic like substance from catkins, willow catkins, sprouted oat and Rhubarb leaves.

* An abstract has been read before the 4th International Congress of Biochemistry, Wien, September 1958.

¹ C. DARWIN and F. DARWIN, *The Power of Movement in plants*, London 1880, p. 592.

² B. JENSEN, *Ber. dtsch. chem. Ges.* **28**, 118 [1910].

³ F. KOGL et al., *Hoppe-Seyler's Z. physiol. Chem.* **214**, 241 [1933].

⁴ F. KOGL et al., *Hoppe-Seyler's Z. physiol. Chem.* **228**, 213 [1934].

⁵ J. C. JANNEY and B. S. WALKER, *Endocrinology* **14**, 389 [1930].

In (1931) LANGE and SPHOR⁶ found also an oestrogenic substance in female flowers of catkins of *Salix* and in the yellow water lily.

DORN, FAURE, POLL and BLOTEVOGEL⁷ (1926) worked on the ovules and fruits of numerous plant extracts and found a substance similar to the follicular hormone and called it tokokinine. They found this substance in the beet root seeds, potatoe tubers as well as in yeast.

BUTENANDT⁸ (1933) describes the isolation of a crystalline product from the expressed residue of palm leaves which is in every way identical with theelin. He found it to give the same physiological action as the female hormone specially the alpha follicular hormone. EL RIDI et al.⁹ (1947) identified also an oestrogen like substance in the pollen grain of date palm. In 1938 FEVOLD, HISAW¹⁰ and GREEP found in yeast extract some gonadotropic effect. They isolated from yeast a water soluble substance capable of causing enlargement of the testicles of both hypophysectomised and immature pigeons.

While plant hormones belong more specially to plant biochemistry and not to animal biochemistry, plant and animal hormones may be related and since the female hormone can be obtained from plant kingdom and the auxin which is a phytohormone from human urine, the distinction need not be pressed too closely.

Materials and Methods

Extraction of the gonadotrophins from pollen was carried out with glacial acetic acid by the aid of heat on a steam bath, using defatted pollen grain powder of Date palm, according to the method of PAYNE, RABEN and ASTWOOD¹¹ (1950) for the preparation of crude extract of corticotrophin, FSH and LH from the anterior pituitary gland.

The crude gonadotrophins so obtained were purified by the addition of tricalcium phosphate suspension to their solution in phosphate buffer after adjusting the pH to 7 as mentioned by CROOKE et al.¹² (1954).

The purified fractions (FSH fraction and LH fraction) were biologically assayed by injecting their saline

solutions to intact immature male and female rats 21 days old and weighing from 35–45 grams as described by LORAINÉ et al.¹³ (1955) and HAMBURGER¹⁴ 1941 and FAVOLD et al.¹⁵ 1937 and 1939.

Procedure

About 350 grams of defatted pollen grain powder (defatted by ether for about 7 hours using continuous hot extraction) were extracted with about 1500 cc. glacial acetic acid heated on water bath up to 70 °C. (for about 1/2 hour) with shaking, then left to cool to about 35 °C. The supernatant yellow liquid decanted then filtered through sintered glass funnel. The residue (yellow in colour) washed with three successive quantities of glacial acetic acid filtering each portion through the same sintered funnel. The combined dark yellow liquid (about 1650 cc.) treated with 30 cc. of 5 M sodium chloride solution and 3500 cc. acetone well shaken and left overnight in the refrigerator. The precipitate was filtered through sintered glass funnel (crude gonadotrophin) (FSH and LH), (yield about 3%).

The crude gonadotrophic hormones were purified by dissolving in about 100 cc. of 0.002 M Na_2HPO_4 buffer solution and the pH adjusted to 7, using pH meter (Radiometer type) then the solution was centrifuged. To the clear supernatant liquid a suspension of tricalcium phosphate added (50 cc. of 8% suspension in water) and the mixture shaken for 10 minutes then centrifuged. To the clear supernatant liquid 750 cc. of acetone added the mixture well shaken then left overnight in refrigerator to precipitate the FSH fraction which was removed by centrifugation then dissolved in normal saline for injection. The residue obtained by centrifugation after dissolving in the phosphate buffer of pH 7 together with that obtained after treating with tricalcium phosphate, were dissolved in a known volume of 0.02 M Na_3PO_4 then centrifuged. The supernatant liquid containing the LH fraction was tested by injecting the test animals. The animals used were the Albino Wester rats. They were intact immature male and female rats varying in age from 19–23 days and weighing 35–45 grams. The female rats were injected subcutaneously twice per day on three consecutive days for 5 injections. They were killed on the 4th day and the reproductive organs carefully dissected out from the surrounding structures and fixed in Bouin's solution for 24 hours. On the 5th day the organs were freed from fat, dried between pieces of filter paper and weighed on a torsion balance. The male rats were injected subcutaneously once per day on 8 consecutive days at approximately

⁶ F. LANGE and E. SPHOR, *Biochem. Z.* **180**, 1 [1927].

⁷ M. DORN, W. FAURE, H. POLL and W. BLOTEVOGEL, *Med. Klin.* **22**, 1886 [1926].

⁸ A. BUTENANDT and H. JACOBI, *Hoppe-Seyler's Z. physiol. Chem.* **218**, 104 [1933].

⁹ M. S. EL RIDI and M. H. ABOULWAFIA, *J. Roy. Egypt. med. Assoc.* **XXX**, No. 3, 124 [1947].

¹⁰ F. L. HISAW, H. L. FAVOLD and R. O. GREEP, *Pituitary Gonadotr. Hormone; Pit. Research gland, Research Laborat. Vol. XVII, Assoc. Research, (Neov. and Meet. dis. chapt. 10) William and Wilkus Co., Baltimore 1938.*

¹¹ R. W. PAYNE, M. S. RABEN and E. B. ASTWOOD, *J. biol. Chemistry* **187**, 723 [1950].

¹² A. C. CROOKE et al., *Lancet* **1**, 379 [1954].

¹³ J. A. LORAINÉ et al., *J. clin. Endocrinol.* **XV**, No. 4, 424 [1955].

¹⁴ C. H. HAMBURGER, *Acta pathol. microbiol. scand.* **18**, 459 [1941].

¹⁵ H. L. FAVOLD, *Cold Spring Harbor Sympos. quantit. Biol.* **5**, 93 [1937]; *J. biol. Chemistry* **128**, 83 [1939].

Fig. 1. a) Ovary of immature rat injected with gonadotrophins from pollen grains of date palm (Graafian follicles large and numerous).

Fig. 1. b) Ovary of control rat injected with saline (Graafian follicles are small and immature) (same magnification as Fig. 1 a).

Fig. 2. Ovary of immature rat injected with gonadotrophins from pollen, showing an early corpora lutea.

Fig. 3. a) Testis of immature rat injected with gonadotrophins from pollen grain of date palm. The tubules are bigger and contain some spermatozoa.

24 hours intervals. They were killed on the 9th day and treated exactly like the female animals. Another group of each of male and female animals were injected with the standard gonadotrophic hormone obtained from Roussel Laboratories, for comparison. At the same time a group of each of the male and the female animals were injected with saline as a control. Each group consisted of at least 5 animals used for the test. The results were expressed as milligram organ weight/100 grams rat weight.

The procedure depends on the increase in ovarian weight in female rat and the increase in seminal vesicle weight and the weight of the testes, in the male rat together with the histological examination.

The results are shown in the following tables and the accompanied photographs.

Discussion

As seen from tables 1 and 2 there is an increase in the ovarian weight nearly about 100% over the control ovary which indicates the presence of follicle stimulating substance in the pollen grain powder used. This increase compared with standard gonadotrophic hormone shows the presence of the FSH in pollen in nearly 100 I.U./10 grams pollen.

Fig. 3. b) Testis of immature rat injected with saline (control). The tubules are smaller. No Spermatozoa (same magnification as Fig. 3 a).

Fig. 4. Section in seminal vesicle of immature rat injected with gonadotrophins from pollen grains of date palm. Note the glandular tissues.

Gonadotrophic hormones extracted from pollen			Standard Gonadotrophins	
Equivalent amount of pollen used [gm.]	Fraction injected	Average increase of ovary wt.percent.	Material used [I.U.]	Average increase of ovary wt.percent.
5	FSH	38	FSH 20	59
10	FSH	109	FSH 100	82
15	FSH	45	FSH 300	218
5	FSH + LH	44	FSH 50 + LH 20	136
10	FSH + LH	125	FSH 100 + LH 20	339
15	FSH + LH	92	FSH 300 + LH 20	371

Table 1. Shows the effect of gonadotrophic hormones (FSH and LH) extracted from pollen grains of date palm, on the female intact immature rat ovary compared with standard FSH and LH.

Gonadotrophic hormones extracted from pollen				Standard gonadotrophins		
Equivalent amount of pollen used [gm.]	Fraction injected	Average increase [%]		Material used [I.U.]	Average increase [%]	
		Seminal vesicle	Testes		Seminal vesicle	Testes
10	LH	91	62	LH 2	46	27
20	LH	60	40	LH 4	110	91
10	LH + FSH	31	44	LH 20	471	42
				FSH 100		

Table 2. Shows the effect of gonadotrophic hormones (FSH+LH) extracted from pollen grains of date palm on the male intact immature rat seminal vesicle and testes compared with standard FSH & LH.

The presence of FSH in pollen is further confirmed by histological examination as shown in Fig. (1: a and b) in which the graffian follicles compared with the control, are bigger and more numerous.

Fig. (2) shows an early corpora lutea formation

indicating the presence of LH in the pollen extract. The data in table 2 shows an increase in the weight of seminal vesicle equivalent to the same increase obtained by nearly 3–4 I.U. of standard LH per 10 grams pollen.

Histological examination shows spermatogenesis

with formation of spermatozoa in the testes, Fig. (3: a and b) confirming the presence of FSH in pollen. The microscopic examination of seminal vesicle shows that they are well developed and contain glandular tissues confirming the presence of LH in pollen grains, Fig. 4.

The optimum increase in ovarian weight occurred by the use of 10 grams of pollen. By increasing the amount of pollen, the response decreases. This may be due to the formation of antigenadotrophic¹⁶ like substance in the injected animal.

¹⁶ C. E. FRIEDGOOD, Bull. Johns Hopkins Hosp. 54, 48 [1954].

Über den Einfluß von monochromatischem Licht auf die Darstellbarkeit der Ektodesmen

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Die Wirkung von monochromatischem Licht (Interferenzfilter) auf die Darstellbarkeit der Ektodesmen wurde untersucht (2400 erg/cm²·sec). Blaulicht (430 mμ) und Rotlicht (655 mμ) hemmen die Darstellbarkeit, während grünes (540 mμ) und gelbes (572 mμ) Licht nur wenig wirken. Die Ergebnisse werden als weiterer Beweis für die Steuerung der Variabilität der Ektodesmen durch das lebende Plasma gewertet.

Unter den Außenfaktoren, die das Leben der Pflanze beeinflussen, ist seit geraumer Zeit das Licht als ein sehr wesentlicher erkannt worden. Bei der Kausalanalyse wurde es hierbei unvermeidbar, auch die Wirkung der verschiedenen Spektralbereiche auf die mannigfaltigen photobiologischen Prozesse zu untersuchen. Dieser Fragestellung sind in den letzten Jahren viele Arbeiten gewidmet worden (Zusammenfassungen u. a. bei BÜNNING¹, SIMONIS² und VAN DER VEEN und MEIJER³, weitere Lit. bei MOHR⁴).

Auch für die Wechselhaftigkeit in der Darstellung von Ektodesmen konnten eine Reihe von äußeren Faktoren, darunter das Licht, verantwortlich gemacht werden (SCHNEFF⁵, SIEVERS⁶). Die Zahl und Form der Ektodesmen wird zweifellos durch „Licht“ gehemmt und durch Dunkelheit gefördert (SIEVERS⁶). Um den Versuch zu machen, einen Einblick in den Mechanismus dieses Vorganges zu erhalten, wurden daher genauere Untersuchungen mit definierten Spektralbereichen erforderlich, wie sie von mir bereits in einer früheren Arbeit mehr orientierend angestellt worden waren⁶ (S. 301 f.).

Methode

Als Lichtquelle diente die Osram-Quecksilber-Hochdrucklampe HQL 400 W. Sie wurde so über den Objek-

ten angebracht, daß gemäß der Lichtverteilungskurve der Herstellerfirma eine optimale Lichtausbeute möglich wurde (s. Abb. 1). Im Abstand von 10 cm von der Lampe befand sich ein 3 mm starkes Wärmeschutzfilter (KG 3 von Schott & Gen., Mainz), das die gesamte Wärmestrahlung > 775 mμ praktisch wegfiltert. Unterhalb dieses Filters waren im Abstand von 3 cm vier Interferenzfilter von 50 mm Ø in einer Ebene angebracht, die ihr Durchlässigkeitsmaximum im blauen, grünen, gelben und roten Spektralbereich haben (vgl. Tab. 1). Die Wellenlängen der Durchlässigkeitsmaxima

Abb. 1. Schematischer Längsschnitt durch die Versuchsanordnung (ca. 1 : 5). 1. Lichtquelle, 2. Wärmeschutzfilter, 3. Interferenzfilter, 4. Schälchen mit den auf Wasser schwimmenden Blättchen. Zwei weitere Interferenzfilter und Schälchen befinden sich hinter den hier eingezeichneten.

¹ E. BÜNNING, Entwicklungs- und Bewegungsphysiologie der Pflanze. 3. Aufl., Berlin-Göttingen-Heidelberg 1953.

² W. SIMONIS, Handb. d. Pflanzenphysiol., Bd. 2, Berlin-Göttingen-Heidelberg 1956.

³ R. VAN DER VEEN u. G. MEIJER, Licht u. Pflanzen, Eindhoven 1958.

⁴ H. MOHR, Planta 53, 219 [1959].

⁵ E. SCHNEFF, Planta 52, 644 [1959].

⁶ A. SIEVERS, Flora [Jena] 147, 263 [1959].