of these neurons in active snails do not disappear during hibernation.

We did not pay special attention to neurons other than cells of the G-group. Surprizingly, in active snails we could find only 2 H-cells in the region outlined in Figure 1; other neurons are of D types (Figure 1B). D responses are mainly associated with a conductance decrease and are similar to D responses of neurons of the G-group in these animals. To elicit such response, a relatively strong current should be applied to the 5-HT pipette. The response slowly develops and lasts sometimes for minutes after stopping the 5-HT ejection. Many prominent neurons situated in other parts of the snail CNS have 5-HT receptors of this type, e.g. the paired giant metacerebral cells and the giant bursting neuron of the right parietal ganglion.

The 2 H-cells are most probably members of a symmetric pair. Each one occupies an anterior position in the pedal ganglion close to the cerebro-pedal connective (Figure 1B) and is usually the largest neuron of the area. An H response is sometimes preceded by a short D phase indicative of a composite reception of 5-HT. The H response is associated with a conductance increase and is K^+-dependent as the value of $E_{5\text{-HT}}$ shifts to a less negative region when the external K^+ concentration is increased. So far, we could not obtain H responses from pedal neurons of this area in hibernating snails. It seems therefore that 5-HT receptors of the 2 pedal H-cells are also affected by hibernation.

It was more than 15 years ago that KOSHTOYANTZ[5] suggested seasonal behavioral changes in land pulmonates to be closely connected with changes in central 5-HT mechanisms. In fact, considerable variations in the level of 5-HT in the snail brain have been demonstrated[6]; now we present evidence that changes in composition of 5-HT receptors occur as well. The changeable neurons of the snail may provide a simple model system for studying hormonally induced modifications in neural mechanisms underlying behavior.

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A Simple Approach to the Toad's (*Bufo melanostictus*) Nerve Muscle Preparation

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Summary. A simple and modified dorsal approach method has been made in the toad's (*Bufo melanostictus*) sciatic gastrocnemius nerve-muscle preparation. This method incurs less blood loss, time consumption, nerve damage and visceral spoil compared to conventional ventral approach method.

Sciatic-gastrocnemius (SG) nerve muscle preparation (NMP) is commonly used as a basic tool for studying the proper nerve muscle function (NMF) experimentally. The previous procedure[1-3], which is generally advocated in the aforesaid NMP, has certain disadvantages over the present method.

The interrupted line shows the landmark for incision of the nerve muscle preparation. U, urostyle; F, skin fold.

Instead of ventral approach (VA) in the conventional method, the dorsal approach (DA) has been made. The object of this method is 1. minimum blood loss; 2. abdominal viscera is not disturbed and intestinal contents does not come out and spoil the nerve; 3. less time is required; 4. nerve is least damaged; 5. the same animal may be used in other experiment also; 6. study of NMF in vitro or in vivo with circulation or without circulation can be performed.

Method. The 10th vertebra, known as urostyle, was removed from pithed toad laid on the dissecting board with dorsal surface uppermost. Then the sciatic nerve (SN) on either side was exposed. A long longitudinal incision was made along the skinfold over the thigh caused by the triceps femoris and semi-membranous muscles (Figure). With the help of a glass seeker, the muscles, i.e. semi-membranous and triceps (femoral), were separated and SN along with its corresponding blood vessel was revealed. The SN was exposed by cutting through the pyriformis muscle and was traced with the help of glass seeker proximally upto their termination in the gastrocnemius muscle (GM). Then the vertebrae were bisected diagonally into 2 symmetrical halves without disturbing the root of the SN on either side. SN was

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raised with the help of a bent glass seeker and its branches were cut off.

A circular and longitudinal incision were made on the skin over the tendo-achillis (T-A) and GM respectively in order to peel off the skin over this muscle. The T-A below the ankle joint was cut off and the tendon along with the freed GM was lifted upto the knee joint. The head of the tibia and the lower end of femur were cut off. The freed SN with spinal attachment in one side and the muscle close to the knee joint on the other side were lifted from the body and placed in normal amphibian saline and used for in vitro study.

For study in vivo, the pithed toad was placed on myographic board with dorsal surface up and the muscles and nerves were exposed as previously. Freed tendon of the muscle was hooked by the pins attached to the short arm of the lever and the knee joint was fixed to the myographic board by the pin. The head of the tibia and lower end of femur were not cut off as done in isolated NMP.

Results and discussion. A simple-routine method developed for the NMP was experimented in our laboratory during the last 10 years. This preparation was found to be more adventageous than other existing methods[1-3] in certain respects. Since the approach was made on the dorsal surface of the animal, there was minimal chance of blood loss. In the previous method VA was made. As a result, the abdominal viscera came out with risk of damage. The contents of the rectum and urinary bladder might come out while bisecting the pelvic girdle. These waste products may disturb the sensitivity of the SN. This risk is overcome in the present method, and the time required for complete preparation is not more than 5 min. Study of NMP can be made in vitro and in vivo with and without circulation. Moreover, the same animal can be used for other purpose also (dissection of digestive system, urinogenital system etc.) as the abdominal viscera was not disturbed. These advantages are not feasible in the previous methods[1-3].

Gibberellin and Nucleic Acid Metabolism during *Zea mays* Fertilization

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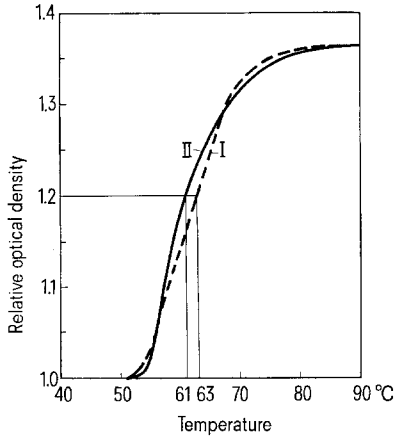
Summary. Either by direct GA supply or by release of glycosidic bound GA, pollination causes partial opening of double-stranded DNA in somatic maize kernel tissues, as evidenced by increased Tm profiles. This phenomenon is associated with enhanced RNA and protein production.

In lower organisms the act of fertilization triggers RNA activity and enhances amino acid incorporation, while in higher mammalian eukaryotes these promotive effects are experienced somewhat earlier in the sexual cycle, i.e. upon ovulation[2]. The effect reported here indicates that in higher plants the act of fertilization and/or pollination induces similar changes and that the process may be triggered by the phytohormone gibberellic acid (GA).

Methods. Cell experiments were carried out upon *Zea mays cv* Jubilee kernels from plants growing in sandy loam soil at the Beit Dagan Agricultural Experimental Station in central Israel. The two developmental stages compared (referred to henceforth as stage I and stage II) were just prior to pollination when the silk stigmata were about to reach the receptive stage to pollen, and subsequently 36–40 h later. This period is the maximal time reported[3] for the pollen tube to extend and fertilize the ovule.



Comparison of Tm profiles of DNA extracted from *Zea mays cv* Jubilee kernels at 2 successive stages. I, just prior to pollination; II, upon fertilization.

Biochemical parameters measured were thermal denaturation (Tm) profiles[4] of extracted DNA[5] (indicating extent of single stranded regions); extraction[6] and bioassay[7] of endogenous GA-like activity, and determination of RNA[8] and protein[9] content. All extractions were performed on 200 g (fresh weight) samples of whole kernels. Each extraction was performed in 4–6 replicates and results statistically assessed by analysis of variance: least significant differences (LSD) are expressed at the $p < 0.05$ level.

Results. The Figure compares Tm profiles of the 2 developmental stages. From this figure it is apparent that in stage II following ovule fertilization the Tm profile is 2 °C lower than in stage I. This indicates increment of single stranded DNA regions.

The Table provides a comparison of other measured parameters. As seen from this table, in stage II there is a significant rise in free-GA as well as considerable increase in RNA and protein.

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