in January and flooded after they had been kept at room temperature for 10 days to bring them out of their winter dormant condition. These eggs gave maximum hatches with infusions made from 10 milligrams of dry willow leaves per cubic centimeter of water. With infusions of this strength 80 per cent. of the eggs of both species hatched. The percentages of hatch for the two species showed close correlation at all strengths tested.

Preliminary tests with the eggs of *Aedes dorsalis* Meig. showed that these eggs also require the hatching stimulant supplied by leaves and grass. In tests with *Theobaldia incidens* Thomson, *Culex pipiens* L. and *Anopheles punctipennis* Say the eggs of all three species hatched readily in unmodified tap water. The eggs of *Theobaldia, Culex* and *Anopheles* are laid directly on the water, whereas those of the three species of *Aedes* tested are laid on the soil and hatch when flooded by rising rivers, high tides, etc.

In an effort to determine the nature of the egg-hatching stimulant, a number of organic chemicals and inorganic chemical elements necessary for plant and animal growth were tested individually and in combination. These did not cause hatching. However, it was shown that the stimulant was present in oats, milk and beef liver.

Pantothenic acid,[1] a recently discovered growth stimulant which is believed to be present in all living cells, occurs abundantly in liver, milk and yeast. The addition of infinitesimal amounts of the acid produces a remarkably stimulating effect on the growth of various types of plants and single-celled animals. The effects of this acid were therefore tested on the eggs in a medium composed of several chemicals in which the acid was produced by yeast. It was found that asparagine, one of the ingredients of the medium, caused the eggs to hatch and that potassium phosphate, another ingredient, acted to increase this hatch. As pantothenic acid was not available except in solution with these two chemicals, its effect could not be definitely determined, but it appeared to have no stimulating action. Solutions containing about 1 milligram of asparagine and 0.5 milligram of potassium phosphate per cubic centimeter of tap water produced the largest hatches, 77 per cent. of the eggs of *Aedes vexans* and 42 per cent. of those of *Aedes aldrichi* eggs.

Six amino acids were found which were stimulating to the eggs. These are asparagine, glycocoll, alanine, cystine, leucine and aspartic acid. The first two were most effective. Hatches were increased with both these materials when calcium or sodium phosphate was added. Putrecine, urea and potassium acetate also caused hatching.

It seems probable that the amino acids and proteins present in vegetation may be the stimulants which cause the eggs to hatch when flooded in nature. Further research work on the composition and action of the egg-hatching stimulant, the species affected by it and its relation to mosquito egg hatching in nature are under way.

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[1] These tests were made possible through the cooperation of Dr. Roger Williams, of the Chemistry Department of Oregon State College, who kindly supplied the acid and assisted with the tests.

AN OBSERVATION SUGGESTING THE PRESENCE OF A GONADOTROPIC HORMONE IN ROYAL JELLY[1]

THIS preliminary note presents evidence suggesting that extracts of royal jelly injected subcutaneously into immature female rats produce precocious development of the ovaries.

Royal jelly is an essential factor in the life of a colony of bees. It is secreted by glands in the mouth of the worker bee. It is fed to the queen while she is laying eggs and to all new-born larvae for the first three days of their lives; from then on only those larvae destined to become queens receive this special diet; queen larvae receive this food during their whole larval stage. All other female larvae, that is, those destined to become workers, are fed on nectar and pollen, after the first three days. These two types of females are strikingly different. The worker takes twenty-one days to develop from egg to adult, the queen sixteen; yet the queen bee is nearly twice as large as the worker. The worker can lay eggs only under extraordinary circumstances; the queen lays eggs steadily for as long as three or four years. The ovaries of the worker are infantile, while those of the queen are large. The worker exhibits many maternal instincts, the queen none. The fact that all these differences apparently could be produced simply by the giving or withholding of royal jelly led to the deduction that royal jelly might contain an active principle that would behave like a gonadotropic or growth hormone.

Hill and Burdett,[2] working on rats kept on a "vitamin E free diet," reported correction of this deficiency by the feeding of royal jelly. Mason and Melampy[3] later repeated this work and were unable to verify it

[1] From the Departments of Pediatrics and Surgery, Harvard Medical School, and The Children's Hospital, Boston.

[2] L. Hill and F. F. Burdett, *Nature*, 130: 540, 1932.

[3] H. K. Mason and R. M. Melampy, *Proc. Soc. Exp. Biol. and Med.*, 35: 459, 1936.

when an actual vitamin E deficiency was established. Investigation of hormones in insects has only recently been undertaken. In this regard the contributions of Wigglesworth[4] and Weed[5] on the corpora allata and its relation to ovulation in insects are intensely interesting.

For the work reported in this communication, two extracts of royal jelly[6] were prepared with dilute NaOH and one with aqueous pyridine (Fevold[7]). Twenty immature female rats, twenty-one days old, were used. These had been raised and weaned under our observation and were all fed the same standard diet during the experimental period.

Ten rats of this experimental group were kept as controls. The remaining ten were injected with extracts of royal jelly. Amounts varying from 60 to 700 mgm of natural royal jelly equivalent were injected subcutaneously on each of five days; the average amount of extract in each injection was 0.3 cc. On the twenty-sixth day of life, the ovaries were removed from all twenty animals. The ovaries of the control group, as would be expected, showed primary follicles that were small and relatively inactive. The ovaries of the animals that had received royal jelly were moderately enlarged, with the Graafian follicles in varying stages of rapid maturation. There was little evidence of luteinization. The twenty control ovaries (two from each animal) averaged 9 mgm in weight; the twenty stimulated ovaries averaged 15 mgm. However, even the smallest of the stimulated glands showed unusual follicular activity. The vagina did not open in any of the controls or treated animals. The degree of the response was directly proportional to the strength and amount of the extract used. The pyridine extract was the most potent of those tried. All gained weight steadily, with the treated animals tending to be slightly heavier than the controls at the end of the experiment.

It is obviously too early in this investigation to draw final conclusions. It seems particularly interesting, however, that this material produced by an insect apparently contains a principle which behaves like a hormone when injected into an animal. The results presented in this communication prompt speculation as to the possibility of an anlage of Rathke's pouch, present in the bee and acting as the functional evolutionary fore-bear of the pituitary gland.

[4] V. B. Wigglesworth, *Quart. Jour. Micr. Sc.*, 79: 91, 1936.

[5] I. G. Weed, *Proc. Soc. Exp. Biol. and Med.*, 34: 883, 1936.

[6] The author is grateful for the cooperation of Dr. R. M. Melampy, the A. I. Root Company of Medina, Ohio, Mr. Allan Latham, of Norwichtown, Conn., Mr. C. C. Ellison, of Belton, S. C., and Dr. Ouida Abbott, of Gainesville, Fla., who supplied the royal jelly used in these experiments.

[7] H. L. Fevold, F. L. Hisaw and S. L. Leonard, *Am. Jour. Physiol.*, 97: 291, 1931.

Summary: The injection of extracts of royal jelly into immature female rats for five days is attended by precocious development of the Graafian follicles.

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RECOVERY OF THE VIRUS OF EQUINE ENCEPHALOMYELITIS (WESTERN TYPE) FROM HUMAN BLOOD SERUM

ON August 7, 1938, through the courtesy of Dr. Ellis Sox blood was received from a man at the Tulare County Hospital in the San Joaquin Valley, California. He had been admitted to the hospital on August 2 in a state of coma with a rectal temperature of 108° F. His earlier symptoms were those of severe headache, stiff neck and general malaise. The blood was taken on August 6, or not long before death from what was considered an acute encephalitis.

At the time this blood was received, the virus of equine encephalomyelitis had not as yet been recovered from a human case and had not been considered a possibility as the etiological agent of the encephalitic cases occasionally occurring in this region. However, neutralizing antibodies for the St. Louis encephalitic virus[1] had been found in many serums of the recovered patients so that this S serum was tested for antiviral properties. It was negative and the serum was stored in the refrigerator for future reference. After the later recovery of the equine virus from a human case,[2] this serum was remembered and on October 8, two months after collection, it was inoculated intracerebrally into two mice. Two weeks later one mouse became paralyzed in the hind legs. Both animals were killed, and a 10 per cent. suspension of their brains in Ringer's solution was passed on to other young Swiss mice.

After several serial passages a virus was established that killed mice in 4 days. Berkefeld filtrates of the brain material were also infective for mice. The virus was found to be infectious for monkeys, guinea pigs and rabbits with a four- to five-day incubation period, and typical symptoms for the virus of equine encephalomyelitis of the western type.

From the clinical picture in guinea pigs, a weak prostration, usually with a dragging of the hind legs, and a typical temperature curve rising to a maximum on the third day, the virus seemed to more closely resemble the original equine western type than did the other recently recovered human or Br strain previously described.[2] The latter was more virulent, being infective in a 1–10,000,000 dilution in mice, had a 60-hour duration accompanied by more spasticity

[1] B. F. Howitt, *Proc. Soc. Exp. Biol. and Med.*, 38: 334, 1938.

[2] *Ibid.*, SCIENCE, 88: 455, 1938.