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.

when large doses were given and showed a more abrupt temperature curve in guinea pigs. The new S strain was infective to mice intracerebrally in a dilution of 1–100,000 and occasionally 1 to 1,000,000 and was also fatal by intranasal, subcutaneous and intracutaneous routes of injection.

Wild mice, *Peromyscus maniculatus,* could be infected intracerebrally with 0.03 cc of a 1–100,000 dilution of the virus. Young puppies also succumbed after injection by the same route. The virus could easily be grown on the chorioallantoic membranes of the developing chick, and is now in the 40th passage. The embryo dies in about 15 to 18 hours and the virus may be recovered from the membranes, the amniotic fluid and the tissues of the chick. The Br strain could likewise be grown in the developing egg.

Immune serums of the S strain gave positive neutralization and complement fixation tests against the Br and the western equine types of virus, but occasionally there was a slight crossing with the eastern variety by the first method. Eastern immune serums, however, failed to neutralize the S virus, as did also the immune serums of the Moscow 2 equine type and the Japanese B virus. Both tests were negative against the St. Louis encephalitic strain. There was no crossing with the eastern variety in the complement fixation test, although occasionally the S antigens were weak when used against the Br and the western equine serums. The S serums usually were positive against the other two western antigens. It was also found feasible to use as antigen the supernatant fluid after centrifugation of the ground membranes of eggs infected with either of the two strains.

In cross tissue immunity experiments, 3 out of 4 guinea pigs immune to the S strain succumbed after intracerebral injection of the eastern type, while the fourth became sick and weak but recovered. One old guinea pig immune to the latter virus, after inoculation with the S strain, ran a temperature and became ill but recovered.

From the general characteristics of this S strain, the incubation period, temperature curve, clinical picture in animals and from the serological and immunological reactions, it is apparent that the western variety of equine encephalomyelitic virus may be recovered from adult human blood serum even after prolonged storage in the refrigerator.

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ENCEPHALOMYELITIS IN MONKEYS

DURING the last few years equine encephalomyelitis has been spreading widely among the horses of the United States. Though the possibility of its infectiousness for man had been realized,[1] it was first recognized[2] last year as the cause of serious disease in man. Innumerable people are exposed during an epidemic in horses; and now many laboratory workers have intimate contact with the disease and its causative viruses while making the embryo vaccine[3] being used to control the disease. We have been carrying out experiments with monkeys to throw light on probable modes of human infection and to indicate what might be done to control or check the disease in man. These experiments have been designed to determine (1) the sensitivity of monkeys to infection by different routes; (2) the influence of hyperimmune serum on the course of the disease; and (3) the possibility of protecting by vaccination.

It has been known[4] that monkeys are susceptible to encephalomyelitis virus injected into the brain and that they can in some instances be infected by virus introduced into the peripheral circulation. We have found that when a massive dose of either eastern or western virus is instilled intranasally into a young rhesus monkey, it will in most instances succumb to a fatal infection. The first symptom of this infection has been fever. The animal has been mildly excitable during this febrile stage, after which it has become paralyzed, has sunk into coma and has died. The symptoms of the two American diseases have been similar, except that as in other animals, the eastern has run a shorter course. We have never observed the recovery of any young monkey infected with either strain if it became paralyzed. A few of the animals receiving intranasal virus have remained healthy; we have found that at least some of these monkeys developed a high content of neutralizing antibodies after exposure. They have therefore suffered non-clinical infections. In our experiments disease has not been produced by very large doses of virus injected subcutaneously or intravenously, though these inoculations have been followed by the appearance of circulating antibodies. Dropping virus into the eye has not resulted in either disease or measurable antibodies. Eastern virus injected intralingually and western virus introduced by stomach tube have proved fatal, but we have not diseased healthy animals by keeping them caged with sick ones.

Hyperimmune horse serum has provided passive protection against nasally instilled virus. Incomplete protection has been furnished by serum administered within three hours of infection. In numerous trials we have never seen any beneficial effect from such a serum given at and after the time of first temperature

[1] K. F. Meyer, *Ann. Int. Med.,* 6: 645, 1932.
[2] L. D. Fothergill, J. H. Dingle, S. Farber and M. L. Connerly, New England Jour. Med., 219: 411, 1938; L. T. Webster and F. H. Wright, SCIENCE, 88: 305, 1938; B. F. Howitt, SCIENCE, 88: 455, 1938.
[3] J. W. Beard, H. Finkelstein, W. C. Sealy and R. W. G. Wyckoff, SCIENCE, 87: 89, 490, 1938.
[4] E. W. Hurst, *Jour. Path. Bact.,* 42: 271, 1936.