bers after a period of 12 hours. When oxygen was admitted into the bowel containing the culture for a period of 90 minutes no *Bact. necrophorum* organisms were recovered at the end of that time. Nitrogen similarly injected into the bowel was without effect.

9017 C

Absence of Vitamin E in the Royal Jelly of Bees.*

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In 1925, one of the writers (K.E.M.) working under the direction of the late Drs. T. B. Osborne and L. B. Mendel at the Connecticut Agricultural Experiment Station, attempted to assay the vitamin E-content of the royal jelly of the honey bee, which is the substance necessary for transformation of worker larvae into the queen or sexually productive form. Due to the relatively small amount of material procurable at the time, and to the negative data obtained, the results of these studies were not published. Since that time, Hill and Burdett[1] in England, claim to have demonstrated the presence of appreciable amounts of vitamin E in this interesting substance. However, there are many obvious objections to the method of assay used, and to the interpretations of results obtained by the latter investigators. They assume in the first place that, in normal stock females placed upon an E-deficient diet at the time of parturition, the process of suckling the litter would completely remove the stores of vitamin E in the maternal tissues. The fallacy of such an assumption is clear to all those who have had experience in the experimental production of vitamin E-deficiency. Furthermore, they state that out of 3 rats receiving a daily supplement of 50 mg. of royal jelly over a period of 37 days, 2 females were able to deliver litters of fully developed young. Out of 4 other rats, 3 of which received 2 gm. supplements of honey and pollen and 1 of which received 2 gm. of worker larvae brood comb, daily, only 1

* This investigation was aided by a grant to Vanderbilt University from the Division of Medical Sciences of the Rockefeller Foundation. The royal jelly was furnished by the Southern States Bee Culture Field Laboratory, Baton Rouge, Louisiana.

1 Hill, L., and Burdett, E. F., *Nature*, 1932, **130**, 540.

rat was able to come to term with delivery of 1 dead fetus. The 3 control rats, fed the E-deficient diet only, failed to conceive. They conclude from their experiments that daily additions of approximately 50 mg. of royal jelly, during the period of one month, are capable of preventing sterility in E-deficient rats. It had previously been shown by Taylor and Nelson[2] that honey is devoid of vitamin E.

In view of the unsatisfactory nature of the experiments of Hill and Burdett, and in view of the frequent reference in the literature to royal jelly as a source of vitamin E, it seemed advisable to reinvestigate this whole question more extensively. To date we have tested varying amounts of royal jelly, using 5 female rats in which from 1 to 2 proven resorptions had occurred. That the sterility in these animals was typical of E-deficiency is adequately demonstrated by (1) the appearance of typical E-deficiency paralysis, as first described by Evans and Burr,[3] at the end of the suckling period in the young delivered by 3 of these rats at the pregnancy period immediately preceding the one in which first resorption occurred, and (2) by the repeated repair of sterility in a large number of rats from the same experimental group after administration of various substances which were being simultaneously tested for their vitamin E-content.

The royal jelly was stored below freezing temperature. The daily supplements were accurately weighed, mixed with yeast to form a pellet and fed to the experimental animal. The animals were closely observed until they had completely consumed the entire pellet. The reproductive history of all the rats was followed by daily vaginal smears from the beginning of sexual maturity to the termination of the experiment. The results obtained from 5 tests with royal jelly, together with that from a few typical tests with other substances, are presented in Table I.

From these data it is quite obvious that as much as one gm. of royal jelly fed daily throughout the period of gestation does not convey sufficient vitamin E to permit the completion of gestation in E-deficient female rats. Possible criticism that administration of the substance as late as the 3rd or 4th day of gestation (rats 597 and 590) might not permit sufficiently early storage of vitamin E to protect against the early resorptive changes of E-deficiency is negated by the fact that several rats (606 and 597) received appreciable amounts of royal jelly prior to beginning of the pregnancy

2 Taylor, M. W., and Nelson, V. E., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 521.

3 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

TABLE I.

Rat	No. of successive resorptions prior to the "test" pregnancy	Type of supplement tested	Daily dose Mg.	Total dose Gm.	Days of gestation period when daily supplement was given	Results and general remarks.
607	1*	Royal jelly	50	1.1	1-21	Complete resorption.
597	1	" "	100	2.1	4-21	Delivered 1 dead and 2 living fetuses on 21st day.†
590	1	" "	300	3.6	3-14	Killed on 17th day pregnancy. Six fetal sites present, all in advanced stages of resorption.
549	1	" "	400	4.0	1-9	Killed on 13th day pregnancy. Five fetal sites, all in early stages of resorption.
606	2	" "	1000	19.0	1-20	Killed on 20th day pregnancy. Five fetal sites, all very advanced stages of resorption.‡
592	2	Ether extract wheat germ oil (6 mo. old)	100	2.0	1-20	Killed on 20th day pregnancy. Five normal fetal sites containing normal, living fetuses.
658	2	Cold-pressed wheat germ oil	50	1.05	1-21	Delivered 6 living young on 22nd day.
654	1	Dried spinach	500	10.0	2-21	Delivered 9 living young on 21st day.
668	1	Cold-pressed wheat germ oil	Single intraperitoneal injection of 500 mg. on the 5th day of pregnancy.			Delivered 5 living young on 21st day.

*In the pregnancy prior to the "test" pregnancy, laparotomy was performed on the 22nd day of gestation, 8 advanced resorptions were present and a single living fetus found was removed at the time of operation.

†Examination of the vaginal smear indicated that other fetuses were resorbed during this pregnancy. The next gestation in this rat was not established until 67 days later. Between the 9th and 20th, and the 29th and 36th days of this 67-day period, the rat received a total of 2.6 gm. of royal jelly, administered after several positive matings which later proved, from the study of the vaginal smears, to have been infertile. If royal jelly contains a significant amount of vitamin E there should have been sufficient storage from the jelly supplied during and subsequent to the first "test" pregnancy to prevent resorption during the following pregnancy. However, this rat when autopsied on the 10th day of pregnancy presented 6 placental sites in early stages of resorption. The latter was confirmed by histologic examination.

‡ This rat also received a total of one gm. of royal jelly, following infertile copulation, 3 weeks previous to the experimental test.

period. The record of rat 597 might lead one to conclude that royal jelly contains significant amounts of vitamin E were it not for the convincing results obtained from rats on higher doses of the jelly, and for the fact that, very occasionally, one encounters such instances of an apparent resorption in an E-deficient rat followed by the delivery of a litter in a succeeding pregnancy. It should also be mentioned that this rat was the first, out of a group of 24 rats, to show a resorption, and that one may occasionally mistake a resorption for a pregnancy terminated by delivery of young which are eaten by the mother soon after birth.

Certain of the rats were autopsied during pregnancy in order to conserve upon the supply of royal jelly and in order to assure ourselves, by histologic study of the fetal sites, that we were dealing with typical vitamin E-deficiency. Both gross and microscopic study of the placental sites have adequately confirmed this assumption. It should also be mentioned that 12 littermate sisters of the test rats used, with the same dietary and reproductive records, were permitted to continue pregnancies without the addition of any "test" substances. In all cases these pregnancies resulted in resorptions.

Certain suggestions and claims relating to oestrogenic and gonadotropic actions of vitamin E, and to a specific rôle of vitamin E in the endocrine activity of the sex glands and pituitary,[4, 5] have not been substantiated by more careful experimental studies.[6-9] The majority of experimental data available indicate that vitamin E is specifically needed for nuclear activity and function of cells in general, its lack becoming first manifest in those tissues such as the germinal epithelium of the testis and the developing fetus, where cellular proliferation and differentiation are especially rapid.[6, 7, 10, 11]

One might expect vitamin E to play an important rôle in the rapid cellular proliferation involved rather than in the bestowing of fertility upon what would otherwise be a non-functional form, as newly hatched larvae weigh about 0.1 mg. while 7-day larvae attain a weight of nearly 300 mg. The results of the present study

4 Verzár, F., and coworkers, *Proc. Staff Meet. Mayo Clinic*, 1929, **4**, 351; *Arch. f. d. ges. Physiol.*, 1931, **227**, 499, 511; *Biochem. Z.*, 1931, **240**, 19.

5 Szarka, A., *Arch. f. d. ges. Physiol.*, 1929, **223**, 657.

6 Juhász-Schäffer, A., *Klin. Wchnschr.*, 1931, **10**, 1364; *Ergeb. d. inn. Med.*, 1933, **45**, 129.

7 Mason, K. E., *Am. J. Anat.*, 1933, **52**, 153.

8 Olcott, H. S., and Mattill, H. A., *J. Biol. Chem.*, 1934, **104**, 423.

9 Saphir, W., *Endocrinology*, 1936, **20**, 107.

10 Adamstone, F. B., and Card, L. E., *J. Morphol.*, 1934, **56**, 325, 339.

11 Adamstone, F. B., *Science*, 1934, **80**, 450.

indicate that vitamin E is not concerned in either of the above phenomena and that as much as one gm. of royal jelly of the honey bee is insufficient to cure the sterility of E-deficiency in the female rat.

9018 C

Antigenic Differences in Strains of Human Influenza Virus.

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On the basis of earlier studies with mouse and ferret passage virus,[1] it was concluded that the Puerto Rico (PR8) and the Philadelphia (Phila) strains of human influenza virus were immunologically identical, while the swine influenza virus was serologically distinct. The 2 strains of human virus were also indistinguishable from the WS strain of the English workers.[1, 2] After repeated inoculation of ferrets with human influenza virus, however, it was noted that the serum of an animal so treated developed the capacity of neutralizing the swine virus as well.[3] Moreover, the serum of rabbits (a non-susceptible animal species) vaccinated with ferret-passage human influenza virus developed antibodies against both the human and swine viruses, whereas rabbits vaccinated with swine influenza virus produced antibodies which neutralized only the swine virus.[3] Identical results were obtained with horse sera prepared by Laidlaw, Smith, Andrewes and Dunkin.[3, 4] It was suggested, therefore, that the human and swine viruses, while immunologically distinct contained common antigens and that the swine antigenic components were present in the human virus as secondary antigens.[3, 5]

Since it seemed likely that the antibody response of an insusceptible animal might reflect the secondary antigens of the virus more completely than that of the susceptible animal in which antibodies to the primary antigen rather than to the secondary antigen

1 Francis, T., Jr., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1172.

2 Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Brit. J. Exp. Path.*, 1935, **16**, 566.

3 Francis, T., Jr., and Shope, R. E., *J. Exp. Med.*, 1936, **63**, 645.

4 Laidlaw, P. P., Smith, W., Andrewes, C. H., and Dunkin, G. W., *Brit. J. Exp. Path.*, 1935, **16**, 275.

5 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1936, **63**, 655.