mouse encephalomyelitis virus has been grown in fertile eggs and carried through nine generations.

The original FA stock virus was received in this laboratory from Dr. Max Theiler, in the form of glycerinated mouse brain, in 1941. The material used in this experiment was put through two passages in mice in our laboratory and kept frozen at −70° C. in the form of 20-per cent suspension of mouse brain. Sterile distilled water was used as diluent. This strain is nonpathogenic for monkeys but produces the usual signs of encephalomyelitis when inoculated intracerebrally in albino Swiss mice and cotton rats. A titration of mouse brain in the third New Haven passage yielded an ID_{50}[3] titer of $10^{-6.5}$ and an incubation period of 3 to 26 days.

Six-day-old fertile eggs were used, usually from Black Australorps and Rhode Island Reds. Incubation was carried out at 35°–35.5° C. before, as well as after, inoculation. A blower fan type of incubator equipped with a humidifier was used, and a reading of 85°–87° humidity was maintained. The eggs were turned five times daily, before inoculation.

An artificial air space was produced over the embryo, using the method described by Burnet and Faris (*1*). The original inoculum was prepared by grinding infected mouse brain with sterile alundum in cold, sterile distilled water sufficient to make a 10-per cent suspension. This suspension was centrifuged at 2,000 r.p.m. for 10 minutes and the supernate decanted. One-tenth milliliter of this supernate was placed on the dropped chorioallantoic membrane, using a 1-ml. tuberculin syringe and a 25-gauge needle. The opening in the shell over the embryo was then sealed with scotch tape and the small hole over the natural air sac was sealed with nail polish.

In the first three passages, four to six eggs were harvested at both 5 and 10 days after inoculation. Harvests consisted of allantoic fluid, amniotic fluid, chorioallantoic membrane, chick embryo brain, and the corpus minus the CNS. The embryo and chorioallantoic membrane were washed in three changes of cold, sterile saline. The embryo was decapitated, and the vertebral column was excised and discarded. Ten-per cent suspensions of tissues and membrane were made by grinding with sterile alundum in cold, sterile distilled water. These suspensions and the allantoic and amniotic fluids were centrifuged at 2,000 r.p.m. for 10 minutes and 0.03 ml. of the supernatant fluids were tested by intracerebral inoculation in four- to five-week-old Swiss mice. All test animals were observed daily during a period of four weeks. For passage to other eggs, 0.1 ml. of a 10-per cent suspension of chick embryo brain, harvested 10 days after inoculation, was used.

[3] ID_{50} = infective dose that produces the disease in 50 per cent of mice inoculated.

Materials harvested at both 5 and 10 days after inoculation, when tested intracerebrally in mice, showed the virus to be distributed somewhat irregularly throughout the egg. Also in our hands, the distribution differed from the results reported by Gard (see Table 1). The greatest concentration of

TABLE 1

Materials tested	1st Passage		2nd Passage		3rd Passage	
	5 days Inc.	10 days Inc.	5 days Inc.	10 days Inc.	5 days Inc.	10 days Inc.
Allantoic fluid	4/8*	0/8	8/8	5/8	1/5	5/5
Amniotic fluid	1/6	7/8	5/7	8/8	5/5	2/5
Chorioallantoic membrane	7/8	5/8	8/8	7/7	3/5	5/5
Brain	2/8	7/8	0/8	7/8	0/5	4/5
Corpus minus CNS	8/8	8/8	1/8	7/7	5/5	5/5

* Numerator represents the number of mice which became infected; denominator represents the number of mice inoculated.

virus in the egg, as judged by the incubation period and morbidity incidence in inoculated mice, appeared to be present in the corpus minus the CNS after an incubation period of 10 days. The smallest concentration appeared to be in the brain, after 5 days incubation.

A distribution study in harvests from eggs representing the fourth passage of FA virus has also been made. The results indicate that the virus is also present in the yolk sac, in a somewhat lower concentration than in the corpus minus the CNS.

Because of the similarities between the Theiler viruses and poliomyelitis virus (*5*), we are using the present results as a guide for the propagation in eggs of a rodent adapted strain of human poliomyelitis virus.

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Seed Pitting of the Lima Bean by Lygus Bugs in California

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Lima beans grown in California for dry food, seed, the fresh market, and freezing have for some years shown a pitting of the seeds indistinguishable from that attributed to yeast (Nematospora) in the southeastern United States (*10*). However, no Nematospora or other pathogen has ever been found in lesions

of California material. The injury may be a discolored spot centering on a minute feeding puncture, but in more severe cases or when several such spots coalesce, an open corroded pit is evident in the seed coat and cotyledon. Such injuries have been called aphid spot, yeast spot, or seed pitting by growers and shippers. Accompanying this condiiton is the shedding of blossoms and of pods up to two inches or more in length. The pods which wilt, turn yellow, and abscise are termed "buckskins" by growers and have been attributed to other causes, such as heat injury, or to steaming from irrigation after the vines have covered the ground. Pods containing pitted seeds cannot be detected positively by external examination alone. However, they frequently exhibit internal proliferations and brown areas, some of which contact the pits of the seeds.

These several types of injuries have now been demonstrated to result from the toxic feeding of Lygus bugs (*Lygus hesperus* Knight, *L. elisus* V.D., and perhaps others) on the developing fruiting structures of the Lima bean plant without the involvement of pathogenic microorganisms. It seems desirable at this time to clarify the situation in California, the leading Lima-bean-producing area of the country, and to indicate the impossibility of distinguishing this trouble, solely on the basis of symptoms, from that attributed to yeast.

Pitting of field-bean seeds apparently was first reported (*2*) in Michigan in 1895 and shown to be a result of Lygus feeding. This relationship has been confirmed in New York (*3*) and Idaho (*5*) and has been shown to occur also on the baby Lima bean in the latter state. Specimens of injured California beans submitted to W. E. Shull were said to be identical with those which resulted from Lygus feeding in Idaho. Pitting was reported (*1*) on Lima beans from California in 1922 and was attributed, apparently on the basis of symptoms, to yeast spot. That the seed pitting, and blossom and pod drop of Lima beans in California actually resulted from Lygus feeding was first suspected by the writers following visits to fields in Ventura County in August 1944, and subsequent work has confirmed this relationship. Such injury has been observed on large and baby Lima bean and black-eyed cowpea, but not on common bean.

Fields with severe blossom and pod drop tend also to have abundant seed pitting and, in Ventura County, a high incidence of Lygus bugs. In some areas, however, the Lygus populations late in the season may become too low to account for the evident damage initiated earlier. The injury has been worst in fields adjacent to seed beets and to alfalfa, particularly after it is cut. Damage also has been worst in parts of Lima bean fields closest to these established, favorable hosts of the insects, and within such areas the loss commonly diminishes with distance. The first crop of pods are not usually injured, but with the high Lygus populations of midsummer, much pod shedding and seed pitting occurs.

Lygus bugs commonly feed on flowers and fruits or seeds and are known to cause severe blossom drop on alfalfa (*8, 9*) and cotton (*6*) and to reduce germination of beet seed (*4*); they have been shown (*7*) to be highly toxicogenic. The observed facts in California are in accord with published data on the injuries produced by these insects on various plants, including common and Lima beans.

Experiments in which Lygus bugs were caged on plants in the field and on individual pods in the greenhouse fully confirmed the fact that they produced the various types of injury. Dropping of blossoms and young pods always resulted, and on older pods the internal proliferations and typical pitting of the seed occurred within seven days. Checks remained uninjured. There was no evidence of yeast or other microorganisms in the tissues.

The results establish the fact that the toxic feeding of Lygus bugs is responsible in the California Lima bean crop for a seed spotting and pitting, and for some of the dropping of blossoms and pods. It is possible that other insects also may be involved. Details are being published elsewhere.

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Oral Penicillin X [1]

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A brief resumé of some of the properties of penicillin X and the results of a clinical trial in gonorrhea were recently published by workers of the Food and Drug Administration (*4*). It was shown that, unit for unit, penicillin X is superior to the usual commercial preparations of penicillin in acute gonorrhea and in protecting mice against pneumococcal infec-

[1] Accepted for publication on 12 September 1945.