Sixty-four chemical analyses were made, and 138 rats were used in the biological assays. Eighteen different lots of kale, purchased on the Washington market, have been used in this study, one bushel being obtained for analysis weekly. Since kale is commonly eaten by human beings in the cooked form, the analyses were made on cooked kale. In addition, cooking the kale served to inactivate the enzymes destructive of carotene, thus eliminating one factor that is impossible to control in bio-assays. The kale was thoroughly washed with distilled water and steam cooked. It was then spread on trays, cooled, and a representative sample of appropriate size withdrawn. The leaves were stripped along the midrib so that none of either the midrib or petiole was included. One-half of each leaf blade was removed for the biological assays, and the corresponding half was used in preparing the extract which was analyzed biologically and chemically. The chlorophylls and xanthophylls were removed by saponification with potassium hydroxide, leaving the carotenoid pigments in the solution. Aliquots were taken from the extract for chromatographing and determining β-carotene spectrophotometrically; the remaining extract was concentrated in cottonseed oil containing hydroquinone and was analyzed biologically.

The measurement of β-carotene was accomplished by a procedure devised in our laboratories based on the methods of Moore and Ely (*1*), using acetone, absolute methanol, and Skellysolve F as extractants and magnesium oxide and magnesium carbonate (1:9) as adsorbents.

The biological assays were performed essentially according to the *U. S. Pharmacopoeia* (11th ed.) method using as the standard of reference pure β-carotene in cottonseed oil prepared in our laboratories.

The data shown in Table 1 represent the average vitamin A values of cooked kale as determined on the vegetable itself and on an extract of the kale.

TABLE 1

Description of sample	Chemical assay*	Bio-assay
	IU/gram	IU/gram
Kale, cooked	90	60
Extract of cooked kale	90	84

* Determined as carotene and converted to vitamin A value, using 0.6 μg. carotene as equivalent to 1 international unit.

From these figures it appears that the vitamin A value of kale as determined by bio-assay is about two-thirds of the value measured spectrophotometrically after chromatographing. On the other hand, when the carotenoid pigments are completely extracted from kale and this extract is used as the vitamin A supplement instead of kale itself, the bio-assay gives a vitamin A value of only 6 per cent less than that obtained by the chemical method. This is well within the experimental error of the bio-assay method and near the upper range of error of the chemical method. This evidence suggests that incomplete digestion of the kale, and therefore incomplete absorption of the vegetable carotene in the intestinal tract of the animal, may be an important factor causing the difference between the vitamin A values of kale obtained by chemical and biological methods.

Reference

1. Moore, L. A., and Ely, R. *Ind. eng. Chem.*, 1941, **12**, 600; 1942, **13**, 726.

The Growth and Distribution of Murine Encephalomyelitis Virus in the Developing Chick Embryo[1]

John T. Riordan and M. José Sá-Fleitas[2]
Section of Preventive Medicine, Yale University School of Medicine

Gard (*2*) has reported the transfer of Theiler's (*5*) FA "mouse poliomyelitis" virus to the developing chick embryo and propagation through four passages. Using the chorioallantoic technique, Gard demonstrated the presence of virus only in the central nervous system (CNS) of embryos which had been inoculated at the age of five, six, and seven days. In eggs inoculated at eight days of age, no virus was recovered from any site. In no instance was virus discovered in membranes or in the corpus minus the brain and vertebral column. Also, CNS harvested 10 days after inoculation was infective for mice inoculated intracerebrally, but CNS harvested 5 days after inoculation was noninfective when tested in the same manner. In Gard's experiments, all eggs were incubated at 37° C. before, as well as after, inoculation. The inoculum consisted of 0.05 ml. of bacteriologically sterile 10-per cent suspension of infected mouse brain. Gard also was successful in transferring the UFI strain of "mouse poliomyelitis" to eggs.

Jungeblut and Sanders (*3*) reported the successful propagation of a murine neurotropic virus in embryonic mouse brain and embryonic guinea pig brain cultures. Using similar tissue cultures, Parker and Hollender (*4*) also were recently successful in propagating the GD VII strain of Theiler's virus through 20 passages.

In the present work the FA strain of Theiler's

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.
[2] Alfredo Hirsch Foundation Fellow of the National Academy of Medicine, Buenos Aires, Argentina.

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.

References

1. Burnet, F. M., and Faris, D. D. *J. Bact.*, 1942, **44**, 241.
2. Gard, S. *Acta Med. Scand.* (Suppl.), 1943, 143.
3. Jungeblut, C. W., and Sanders, M. *J. exp. Med.*, 1940, **72**, 407.
4. Parker, R. C., and Hollender, A. *Proc. Soc. exp. Biol. Med.*, 1945, **60**, 88.
5. Theiler, M., and Gard, S. *J. exp. Med.*, 1940, **72**, 49.

Seed Pitting of the Lima Bean by Lygus Bugs in California

Kenneth F. Baker and William C. Snyder
Division of Plant Pathology, University of California Los Angeles and Berkeley

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