L-Ascorbic acid obtained in trial runs was 99.7% pure by indophenol titration and had a melting point of 189–191° C (cor.). Analysis of the inactive L-ascorbic acid gave the data:

Calculated:	C	40.91,	H	4.55;
Found:	C	41.06,	H	4.61.

Additional details regarding the preparation, administration, and end products of the substance will be given in another paper.

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Sterile Pieces of Chick Embryo as a Medium for the Indefinite Axenic Cultivation of *Rhabditis briggsae* Dougherty and Nigon, 1949 (Nematoda: Rhabditidae)

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During the past eight months (i.e., since December, 1948) it has been possible to rear successive generations of a rhabditid nematode, *Rhabditis briggsae*,[2] on sterile pieces of chick embryo. This medium may be termed "axenic"[3] (without strange [life]), inasmuch as the pieces of chick embryo are nonliving. Moreover, in some cases the pieces have been frozen, thawed, and then used; in others they have undergone a certain amount of autolysis before use by reason of being stored at 4° C for up to a month's time. To date the cultures, which were originated in triplicate with streptomycin-sterilized larvae (from two-membered cultures with *Escherichia coli*) according to the technique of Dougherty and Calhoun (*4*, *5*), have passed through four transplants and are now in their fifth; at least 12 successive generations of *R. briggsae* are thereby represented. In addition, from the third and fourth transplants larvae have been used for experimental purposes; these have passed through two additional axenic generations. Each transplant is routinely tested for sterility by inoculation of a loopful of the old culture into nutrient broth at the time of transfer. Whenever the appearance or odor of a culture has suggested contamination, further tests have been done by inoculation into 0.1% thioglycollate-0.1% dextrose-0.05% agar-peptone broth and 1% dextrose-0.5% yeast extract medium, or by Gram stain.

Although the ultimate nematode yield in chick embryo cultures is excellent, the growth rate of *R. briggsae* is definitely slower than that in two-membered cultures or in cultures with a mixed microbial flora. Nigon and Dougherty (*8*) have found that this species requires 4 days from egg to egg at 16° C with a mixed bacterial flora present, whereas I have observed that, at least in the initial stages of the culture, maturation takes not less than 6 days at a similar temperature on chick embryo. In the latter case, either growth factors are not available in optimal amounts, or inhibitory substances are present; or perhaps both situations obtain. Indefinite maintenance of *R. briggsae* on media containing unheated, sterile liver extract as the sole source of the essential heat-labile factor or factors, such as has been attempted for *R. pellio* (*5*), has not yet been tried.

At present studies are being conducted in an effort to develop a chemically defined medium for *R. briggsae*. So far it has not been possible to obtain indefinite growth on any such medium. These studies will be reported in later publications.

The function of a defined medium in the case of rhabditid nematodes would be to permit, among other things, biochemical genetic studies. This problem has been discussed by Dougherty and Calhoun (*3*), and the advantages that these organisms offer have been stressed (*2*, *3*). The particular importance of demonstrating that *R. briggsae* is capable of indefinite axenic growth lies in the fact that this self-fertilizing, hermaphroditic species is admirably suited on theoretical grounds to the detection of recessive autosomal mutants, particularly those involving nutritional defects. The recent production of a morphological mutant of *R. briggsae* (*9*), the first to be described in the Nematoda, provides evidence of the practical mutability of these forms. With the development of a completely synthetic medium, biochemical genetic studies should become possible. The problems remaining in the realization of this end are unquestionably numerous and difficult. Nevertheless, the fact that chick embryo permits indefinite axenic growth offers valuable leads toward the ultimate solution of these problems.

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[2] This species has been incorrectly referred to as *Rhabditis elegans* Maupas, 1900, in recent publications (*5*, *7*); it is actually a new species, the description of which is in press as this is written (*6*).

[3] A term proposed in 1942 by Baker and Ferguson (*1*).

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