prevention effect of NaI may demonstrate that Cereal-G is deficient in iodide; however, these data could result if NaI reduced the effectiveness of a goitrogenic mechanism (*12*) other than iodine deficiency.

References and Notes

1. Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.
2. R. E. Remington, *J. Nutrition* **13**, 223 (1937). Remington diet No. 347 contained wheat gluten 18 percent, brewer's yeast 2 percent, corn meal (obtained from "iodide-deficient" areas) 78 percent, calcium carbonate 1 percent, sodium chloride 1 percent.
3. Composition: casein (0.11 μg of iodide per gram, vitamin free) (*1*) 13 or 26 percent, sucrose (Domino) 68 or 55 percent, vegetable fat (Crisco) 10 percent, purified salts, and crystalline vitamins.
4. H. Eartly and C. P. Leblond, private communication.
5. Produced by Mead Johnson and Company, Evansville, Ind., and marketed under the trade name Pablum Mixed Cereal. This cereal is stated to contain "wheat meal (farina), oatmeal, yellow corn, wheat germ, tribasic calcium phosphate, powdered alfalfa leaf, dried yeast, sodium chloride, thiamine hydrochloride, riboflavin, and reduced iron." The mixture is "precooked" and dried to a moisture content of 7 percent. This cereal was purchased on the open market, 1-mo supply at a time, from July 1953 through January 1955.
6. This investigation was supported by grants from the U.S. Atomic Energy Commission and U.S. Public Health Service. Such support should not be construed as endorsement of, or concurrence in, the findings and opinions expressed herein, which are solely my own.
7. Analysis for total iodine in dietary materials are subject to many serious errors (*8*). The values reported were the best analytic results obtained by the methods of Chaney (*9*), Barker (*10*), and Zak (*11*).
8. L. Van Middlesworth and J. Truemper, *Federation Proc.* **12**, 147 (1953).
9. A. L. Chaney, *Ind. Eng. Chem., Anal. Ed.* **12**, 179 (1940).
10. S. B. Barker *et al.*, *J. Clin. Invest.* **30**, 55 (1951).
11. B. Zak *et al.*, *Anal. Chem.* **24**, 1345 (1952).
12. L. Van Middlesworth, *Federation Proc.* **11**, 166 (1952).

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Production of Milky-Disease Spores (*Bacillus popilliae* Dutky and *Bacillus lentimorbus* Dutky) on Artificial Media

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The larvae of two destructive soil-inhabiting insects, the Japanese beetle, *Popillia japonica* Newm., and the European chafer, *Amphimallon majalis* (Razoum.), are susceptible to milky disease (*1*). Dutky (*2*) described two species of spore-forming bacilli, *Bacillus popilliae* and *B. lentimorbus* that cause milky disease. These two species are able to invade the blood of larvae and produce large numbers of spores. After the larvae die, the spores are liberated in the soil, where they may infect other larvae.

To facilitate the natural spread of the disease, it has been feasible to inoculate the soil with milky-disease spore dust produced by a patented process (*3*) described by White and Dutky (*4*). Living larvae are used as the substrate. Dutky (*2, 5*) reported cultivation of both species of bacilli on artificial media in the vegetative state; however, they did not sporulate. The biological control of these insects could be considerably facilitated if a practical method for the production of milky-disease spores on artificial media were developed.

This article (*6*) presents the results of a project that was set up to study the *in vitro* characteristics of these bacteria with the objective of duplicating, as far as possible, the *in vivo* reactions, particularly sporulation.

A detailed study was undertaken of the growth of these organisms in the vegetative state on various types of media (*7*). Particular attention was given to *p*H, oxygen tension, growth factor, and the carbohydrate and nitrogen requirements of the organisms. Excellent vegetative growth was obtained, and spores formed occasionally. However, the spores were not typical of those formed within the living larvae.

Sporulation in a diseased larva occurs after the vegetative cells have become very numerous in the blood. This suggested that the supply of an essential nutrient might become deficient preceding sporulation. A nutrient deficiency might be a factor inducing sporulation (*8*). This possibility was investigated by growing the vegetative cells on a complete medium and then transferring them to a starvation medium on which further growth was impossible. Spores formed that were indistinguishable from those produced within the living larva. Details of the method follow.

An inoculum was prepared by sterilizing the surface of a diseased larva in 0.5-percent sodium hypochlorite solution, puncturing the hemacoele through the dorsal body region, and suspending the spores in sterile water. The spore suspension was heated at 70°C for 15 min. to destroy the vegetative cells. The spores were inoculated onto the surface of petri-dish cultures containing a complete medium made with the following ingredients: tryptone, 5 g; yeast extract, 3 g; K_2HPO_4, 3 g; glucose, 1 g; maltose. 1 g; soluble starch, 10 g; agar, 15 g; and distilled water, 1000 ml. The cultures were incubated at 32°C for approximately 4 days in order to insure good growth and germination of all spores.

The cells were then transferred as a paste from the surface of the complete medium to the surface of a starvation medium containing the following ingredients: $(NH_4)_2HPO_4$, 1 g; KCl, 0.2 g; $MgSO_4$, 0.2 g; yeast extract, 0.2 g; agar, 15 g; and distilled water, 1000 ml. Cells were incubated on the starvation medium at 32°, 37°, and 45°C. Although no further growth took place under these conditions, sporulation occurred within 24 to 72 hr at all three temperatures; the highest yield of spores was obtained at 37°C.

Table 1. Comparative virulence of artificially produced and natural spores to European chafer larvae.

Method of testing	Source of spores	Percentage of larvae diseased after		
		7 days	14 days	21 days
Injection	Artificial	98	98	98
	Natural	100	100	100
Feeding	Artificial	1	10	16
	Natural	3	81	92
Checks		0	0	0

This indicated that temperature was a factor influencing sporulation. In order to test the effect of temperature alone on sporulation, vegetative cells were grown on the complete medium and incubated at 32°C and at 37°C. No spores formed. However, when the cultures grown at 32°C were reincubated at 37°C and those grown at 37°C were reincubated at 45°C, spores formed in both sets of culture plates. This indicated that it was not essential to remove the cells from the complete medium in order to secure sporulation. The heaviest yield of spores was obtained when both factors—that is, starvation and higher incubation temperature—were combined to stimulate spore formation.

Two lots of *B. popilliae* spores that had been produced by the starvation method were tested for virulence against third-instar European chafer larvae. Larvae were incubated at 26.7°C in trays of moist soil. The first lot showed a high order of virulence in a preliminary injection test.

The second lot of spores was used to compare its virulence with that of natural type-A spores through injection and feeding. A diseased European chafer larva was used as a source of natural type-A spores for injection, and type-A spore dust produced from diseased Japanese beetle larvae was used for the feeding test. Larvae were injected with each group of spores at the rate of 500,000 spores each. Others were incubated in soil containing 1 billion spores per kilogram of soil. Duplicate trays of 50 larvae each constituted a treatment. Checks included a tray of 50 larvae injected with sterile distilled water and another tray of noninjected individuals. The incidence of disease is summarized in Table 1 and is based on the average numbers of living larvae in each treatment.

The artificially produced spores showed a high order of virulence when injected into the larvae but much less virulence when ingested. The blood of larvae infected with artificially produced spores contained rods and spores typical of larvae infected with natural spores. It can be concluded that spores capable of causing milky disease in European chafer can be produced on artificial media.

References and Notes

* Attached on a cooperative basis to the Department of Entomology, New York State Agricultural Experiment Station.

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2. S. R. Dutky, *J. Agr. Research* **61**, 57 (1940).
3. U.S. Patent No. 2,258,319 (1940). Assigned to Secretary of Agriculture.
4. R. T. White and S. R. Dutky, *J. Econ. Entomol.* **35**, 679 (1942) ; S. R. Dutky, *U.S. Dept. Agr. Bur. Entomol. Plant Quarantine, E.T.* No. ET-192 (1942).
5. S. R. Dutky, *J. Bacteriol.* **54**, 267 (1947).
6. Approved by the director of the New York State Agricultural Experiment Station for publication as journal paper No. 998.
7. K. H. Steinkraus, unpublished experiments.
8. G. Knaysi, *Bacteriol. Revs.* **12**, 63 (1948).

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Communications

Charr or Char—History of a Common Name for *Salvelinus*

The increasingly popular usage of the term *char* or *charr* as a distinctive and universal English name for those fishes belonging to the genus *Salvelinus* is very encouraging. Such increased usage tends to restrict the term *trout* to the genus *Salmo* where it rightfully belongs. During a rather intensive study of the literature on this interesting group of fishes over the past decade, I found the differential spelling of the term puzzling. Although both spellings commonly appear in fishery literature, with few exceptions individual ichthyologists have confined themselves to consistent use of one form or the other. I have been unable to find any published reason for such individual preference, although there is considerable evidence to show that since the turn of the century most American writers have followed Jordan's preference for *charr*, and most British and European writers have followed Regan's preference for *char*.

I have become aware of a steadily increasing usage of the single *r* by a rather militant group of fishery writers in both scientific and popular publications. This trend probably reached its climax in 1951 when the Committee on Common Names of the American Fisheries Society reversed its 1948 approval of *charr* and favored *char* [*Trans. Am. Fisheries Soc.* **81**, 326 (1952)]. It is unfortunate that the committee took a definite stand in favor of one form over the other, because this action, in effect, made it practically impossible to publish in an American fishery periodical a manuscript using the double *r*. In my opinion, which I submitted to the committee in writing, if the committee felt it must take a stand, the bulk of evidence seemed to indicate that *charr* was the better spelling. Correct spelling in English is based upon common or popular use over a long period of time, and many