was 1,751. Since 44 crop years are represented, there are two 22-year periods. The average yield of this first period, ending in 1923, was 1,717 pounds/acre; that of the second period, 1924–45, 1,786 pounds/acre. Continuous cropping has therefore not resulted in any loss of yield of Lima beans in this 45-year period.

To substantiate the data from the Edwards field, interviews with officials, cooperative associations, and others familiar with local farming practices were made. These interviews indicate that continuous cropping of Lima beans for 15–25 years is rather common. The yield records of four growers who had practiced continuous cropping for a period of 16 years were obtained and are presented in Fig. 2. The 16-year period was divided into two 8-year periods. The average of the first period was 2,173 pounds/acre; that of the second, 2,224.

Prof. W. W. Mackie, emeritus agronomist of the California Experiment Station, who has been a close observer of the Lima bean industry for many years, wrote in a recent letter: "Continuous yields of large Lima beans have been observed by me many times in the Ventura area on irrigated land. On the dry mesa or hilly soils in Ventura and San Diego Counties this condition does not always follow, due to lower soil fertility of the hilly residual soils and often to an absence of nodule forming bacteria."

Some fertilizer experiments have been conducted on small

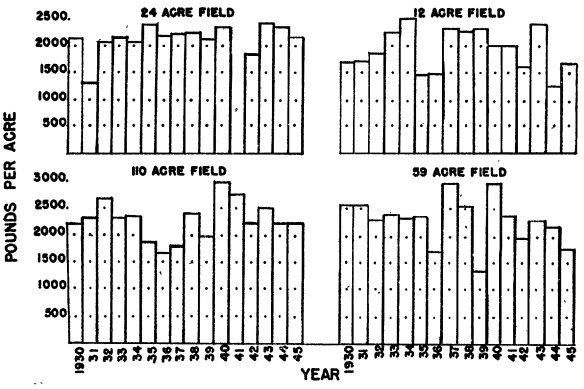

FIG. 2. Yield record of 16 years of continuous cropping with Lima beans on four independent fields in Ventura County.

plots in the Edwards field. In 1937 liquid ammonia was applied on three plots at the rate of 62 pounds of nitrogen/acre and on three at the rate of 82 pounds of nitrogen/acre. There was no difference in yield between the unfertilized check plots and those receiving the lighter application of ammonia. With the heavier application there was a barely significant increase of 117 pounds/acre (P = .05).

In 1942, 1943, and 1944 fertilizer experiments were conducted on replicated plots, 20 x 100 feet. The fertilizers were applied as side dressings 6 inches from the planted bean rows. The three treatments used were: (1) 84 pounds and (2) 168 pounds of phosphoric acid (P_2O_5), as well as (3) both 168 pounds of phosphoric acid and 100 pounds of nitrogen/acre. The phosphoric acid was supplied by treble superphosphate, and the nitrogen by ammonium sulfate. There was significant increase in yield only when both nitrogen and phosphoric acid were added. This fertilizer application gave an increase in yield of 239 pounds of beans/acre in 1942, 415 pounds in 1943, and 560 pounds in 1944. The least significant differences in pounds/acre were 198 in 1942, 287 in 1943, and 409 in 1944.

This evidence indicates that on rather fertile soil, Lima beans do not decrease in yield when continuous cropping is practiced. This may be partially explained as the replenishment of the available nitrogen supply by the Lima bean nodules. The other essential elements must be in abundance.

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Role of the Axis Cylinder in Transport of Tetanus Toxin[1]

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The purpose of this article is to show that the neurofibrillae in the axis cylinder are the agents responsible for the transport of tetanus toxin to the cell bodies of motor nerves. In this transport the rate of progression has been measured and will be stated below. The chief interest in this problem is primarily to add one more fact to our knowledge of the physical and chemical nature of the neurofibrillae. Howe and Bodian (*2*) have stated that the axis cylinder is the route taken by the virus in experimental poliomyelitis in monkeys. Their method of freezing the sciatic nerves was used in the experiments outlined below. Friedemann (*1*), in discussing the blood-brain barrier, states that the capillaries of the central nervous system are impermeable to tetanus toxin. In this article he also lists the literature dealing with the controversial subject of blood stream versus neuron routes taken by the tetanus toxin. The experiments here listed substantiate the nerve-fiber route.

After carefully exposing and severing the sciatic nerve very close to the knee joint, 8 guinea pigs were inoculated with a tetanus toxin which had 75,000 m.l.d./cc. The inoculation was made in the following manner: The central cut end of the sciatic nerve was held firmly against the end of an applicator stick which had been dipped in the tetanus toxin of the above-mentioned strength. The time of exposure in each case was 5 minutes. The end of the applicator stick did not hold a drop of the toxin but was merely moist. All of the guinea pigs were dead within 72 hours. Their death was due to tetanus, the symptoms of which were those of the local type. First to show signs of stiffness was the opposite limb, then the lower back, thoracic level, and finally the cervical and head region. The wound was closed surgically under strict aseptic conditions.

On 8 other guinea pigs the sciatic nerve was exposed and frozen with CO_2 snow as far centrally as was conveniently possible. This technique produces the degeneration of the axis cylinder but does not alter the blood supply or the neurilemma sheath. The frozen area is about 2 mm. in length. Sixteen days

[1] Present address of author: University of Louisville. Tetanus toxin supplied by courtesy of Eli Lilly and Company, Indianapolis.

later the same sciatic nerve in each animal was inoculated as described above. Two of the animals died on the fourth day of undetermined cause, showing no signs of tetanus. The other six were sacrificed on the sixth day after inoculation. The toxin failed to reach the central nervous system because of the degeneration of the axis cylinder.

Another series of 8 guinea pigs was inoculated in like manner without freezing. Four of these received .5 cc. of tetanus antitoxin subcutaneously 10 minutes previous to the inoculation. The other four were given 0.1 cc. of the antitoxin into the sciatic nerve central to the cut end immediately after inoculation. All 8 of these animals were sacrificed 14 days later without any signs of tetanus. No attempt has been made to freeze the nerve and then allow for regeneration of the axis cylinder before inoculation.

The average length of the central stump of the sciatic nerve was 57 mm. The average time from inoculation until the opposite limb began to show tetany was 17 hours. Assuming that the tetanus toxin travels uniformly up the axis cylinder, the rate of progression then is 3.35 mm./hour. No attempt has been made to determine the method by which the toxin ascends the nerve fiber.

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Therapy of Experimental Tsutsugamushi Disease (Scrub Typhus)[1]

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Tsutsugamushi disease (scrub typhus), an acute febrile disease of the Asiatic areas, became of major military importance during the war in the Pacific theater. It was late in 1943 that the first strain of the etiological agent, *Rickettsia tsutsugamushi*[3] (Karp), was obtained by the U. S. Navy[4] for study in this country. At that time an investigation was begun to determine possible therapeutic agents that might be used in combating the experimental infection in Swiss mice.

It was observed during the course of these experiments that mice inoculated intraperitoneally with the Karp strain of *R. tsutsugamushi* and subjected to elevated oxygen tension, whether at sea level or increased pressure, showed a decreased mortality.

In a typical experiment 90 mice were inoculated intraperitoneally with 1,000 m.l.d. of the Karp strain. Immediately after inoculation, one group of 30 mice was placed in a standard oxygen tent and subjected to an oxygen tension of 50 per cent

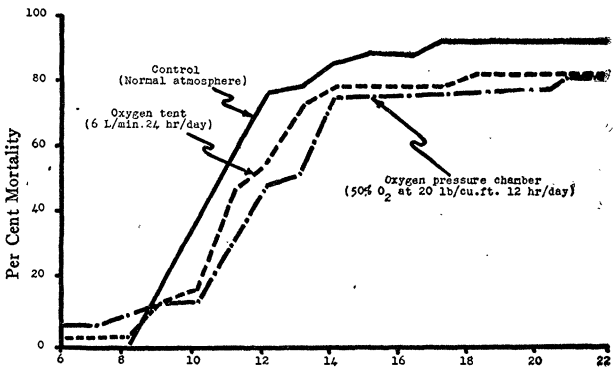

FIG. 1. Effect of oxygen tension upon the survival time and mortality of *R. tsutsugamushi* (Karp) infected mice.

maintained by a flow rate of 6 l./minute; a second group of 30 mice was placed in a pressure chamber containing 50 per cent oxygen in nitrogen at 20 pounds pressure; and a third group of 30 mice was kept under normal atmospheric conditions. It will be noted that increased oxygen tensions had a slight yet consistently beneficial effect on the survival time and the mortality of infected mice (Fig. 1).

On the basis of the action of this physiological factor it appeared advisable to test a series of agents for therapeutic activity under both environmental conditions—normal atmosphere and 50 per cent oxygen.

The institution of therapy was always delayed for at least 96 hours following inoculation of the mice. This period represented, as previously shown (*3*), the time usually required for the blood of mice inoculated with 1,000 m.l.d. of the Karp strain to become infective in sufficient titer to kill passage mice. It was thus assumed to be that period in the course of the infection which closely represented the end of the incubation period. Therefore, agents which proved to be efficacious when treatment was delayed for at least 96 hours could be classed as therapeutic rather than prophylactic.

The therapeutic agents were administered orally by mixing them intimately with the normal diet of ground fox chow.

One agent, methylthionine chloride (methylene blue), in contrast to the others, proved to be remarkably effective in combating the infection in Swiss mice under these conditions. It was observed that 0.2 per cent methylthionine chloride (MTC), added to the diet 96 hours following inoculation of the mice, reduced the mortality to 30–40 per cent, as contrasted to a 90–100 per cent mortality for the untreated controls. When oxygen therapy was instituted in conjunction with MTC, the mortality of the treated mice was further reduced to 20–30 per cent.

MTC has been used as a therapeutic agent in such diverse human infections as gonorrhea, tympanosomiasis, malaria, etc. (*1*). It has also been reported as an active agent in the

[1] The opinions or assertions contained herein are the authors' and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. This paper is based on a report of the authors entitled "Studies in tsutsugamushi disease. II: Experimental therapy of the infection in Swiss mice" (U. S. Naval Medical Research Institute, Research Project X-222, Rep. No. 2, November 9, 1944).

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[3] R. Lewthwaite has made a comprehensive survey of the literature regarding the correct nomenclature for the etiological agent of this disease and has recommended *R. tsutsugamushi*. Personal communication.

[4] This strain was isolated by F. M. Burnett, Royal Melbourne Hospital, Victoria, Australia.