Rh factor can not have children with blood lacking the corresponding Hr factor; and parents lacking any of the Hr factors can not have children lacking the corresponding Rh factors.

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EFFECT OF DDT, SULPHUR AND LETHANE DUSTS ON GERMINATION OF SUGAR-BEET AND ONION POLLENS

GERMINATION tests were made with sugar-beet pollen collected from portions of sugar-beet seed fields that had been given a single application (20 pounds per acre) of a dust containing 5 per cent. DDT and 95 per cent. pyrophyllite in comparison with similar tests with pollen from undusted portions of the field. Sugar-beet pollen throughout the entire blooming period had shown very poor germinations, making it difficult to obtain exact quantitative data. However, the results of numerous tests were appraised as showing approximately the same germination ratings for pollen from dusted plots as for pollen from undusted plots. The germination tests with sugar-beet pollen were made on an agar medium containing 40 per cent. sucrose, found to be the optimum sucrose concentration to use. Companion tests with onion pollen showed excellent germinations regardless of whether the flowers had been exposed to DDT dust or not. An agar medium containing sucrose at concentrations ranging from 15 to 32 per cent. was used. The indications are that germination of these two kinds of pollen was not adversely affected by the DDT dust. No observations were made on the effects of DDT upon the insects that frequent the sugar-beet and onion flowers. Obviously, DDT should not be used in onion-seed fields after the flowers begin to open because of its known lethal effect upon the insects that pollinate onion flowers.

Sulphur dust as a single application of superfine sulphur at the rate of 20 to 30 pounds per acre, shortly after the blooming period of sugar beets commenced, appeared in the preliminary tests to slow up or inhibit germination of sugar-beet pollen. However, if care was taken not to get sulphur particles on the agar medium along with the pollen, the rate of germination and energy of germination were not greatly affected as a result of the field having been dusted with sulphur. When sugar-beet fields are dusted heavily with sulphur, some of the dust falls upon the anthers and stigmas, so that direct inhibitory effects of sulphur comparable to those observed on artificial media may be a factor in nature.

Lethane B 71 (an organic thiocyanate dust containing beta, beta-dithiocyanodiethyl ether) used at the rate of 30 to 40 pounds per acre on onion fields during the blooming period did not adversely affect germination of onion pollen. During the first few tests agar plates receiving a considerable amount of Lethane dust together with the onion pollen showed no germinating pollen grains. To avoid these direct effects of the dust on the agar medium, both broken and intact anthers from onion flowers were removed individually and placed on the agar medium. Pollen from broken anthers germinated abundantly whether removed 2, 3 or 5 hours after exposure to the fumes of the Lethane dust. The germination energy of pollen from plants dusted with Lethane appeared to be slightly higher than that of pollen from control plants. The material for the pollen studies with onion was supplied by the Division of Horticulture of the New Mexico Agricultural Experiment Station.

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THE DEVELOPMENT OF LITOMOSOIDES CARINII FILARIID PARASITE OF THE COTTON RAT IN THE TROPICAL RAT MITE[1, 2]

VARIOUS blood-sucking arthropods have been explored as possible vectors of the cotton rat (*Sigmodon hispidus litoralis*) filariid, *Litomosoides carinii*. To date, only in the mite, *Liponyssus bacoti*, has development of the filariid been demonstrated.

In mites fed on infected rats, all stages of development have been recovered. The microfilariae grow in length from 69 μ (not including the sheath) to 105–109 μ while there is a rather gradual increase in width from 5.5 μ to 7 μ. At this point in the development there is a sudden expansion in width to 13.2 μ and a typical sausage form with a short sickle-shaped tail is formed. The width increases and individual variations ranging from 15.6 μ to 20.8 μ were found among those larvae between 125 μ and 510 μ in length. From this point on in the development of the worm the width appears to become fixed at 15.6 μ. Those forms which were presumed to be the infective stage reached a length of 800 μ. Further studies are being made on the development of the worm within the mite and its transmission to the cotton rat.

Other ectoparasites of rats that were examined, including fleas, lice and ticks, did not harbor developing filariae. In five species of mosquitoes (*Aedes aegypti, A. taeniorhynchus, A. sollicitans, A. albo-*

[1] This study was made possible through the financial support of the John and Mary R. Markle Foundation.
[2] The authors wish to express their gratitude to the Hegener Research Supply Company of Sarasota, Florida, for the helpful assistance rendered.

pictus and *Mansonia perturbans*) no development of the microfilariae took place and three days after a blood meal all microfilariae appeared to be digested. Similar results were obtained with the bedbug, *Cimex lectularius*. Three species of sandflies (*Culicoides*) showed no interest in feeding on the cotton rats.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID STAINING METHOD FOR RICKETTSIA ORIENTALIS[1]

THE difficulties encountered in staining *Rickettsia orientalis*, the causative agent of Tsutsugamushi disease (scrub typhus or mite-borne typhus fever), in smears from infected yolk sac membranes and animal tissues are well known. In smears stained by Machiavello's method[2] or with 1 per cent. methylene blue the rickettsiae are stained deeply enough to be recognized, but the lack of contrast with the tissue background makes differentiation of the organisms difficult.

Recently Syverton and Thomas[3] reported that preliminary treatment of smears with lipid solvents followed by Giemsa stain gave uniformly satisfactory results in the staining of *R. orientalis*. However, their technique is somewhat more time-consuming than the method, used in this laboratory for the past eight months, described below.

In 1944, Dr. T. J. Kurotchkin[4] of this laboratory used a dilute aqueous solution containing both methylene blue and basic fuchsin to good advantage in staining *Rickettsia prowazeki* and *Rickettsia mooseri*, the causative agents of louse-borne (epidemic) typhus and murine (endemic) typhus respectively. In yolk sac smears thus treated the rickettsiae stained blue while the tissue background assumed a pinkish-purple color.

The present authors found the dilute stain solution to give nearly identical results with smears of *R. orientalis*, providing the smears were first treated with a lipid solvent such as xylol, ether or chloroform, to remove excess fatty substances. Xylol was found to be most convenient to use since it is less volatile and is readily kept in a glass-stoppered dropping bottle.

The method of staining is as follows: Smears of infected yolk sac membranes or other tissues are prepared, air dried and fixed by heat. The slide is flooded with xylol, drained and after drying in a current of air is immersed for five minutes in a distilled water solution containing methylene blue 1:5,000 and basic fuchsin 1:5,000. The preparation is then washed in tap water, dried and examined.

The dilute stain is readily made from 1 per cent. stock solutions of each of the two dyes and should be prepared daily to obtain best results.

SUMMARY

A rapid method for staining *R. orientalis* is described. Smears of infected tissues are defatted and stained in a dilute aqueous solution of methylene blue and basic fuchsin. Smears thus stained show *R. orientalis* as blue organisms on a pinkish-purple background.

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A METHOD OF OPENING VACUUM DESICCATORS

WHEN analytical samples are dried in vacuum desiccators, the covers often "freeze" and it becomes almost impossible to remove them manually, particularly when certain high-vacuum type greases are used. In some cases it has been necessary to resort to the dangerous practise of putting air under pressure into the desiccator, generally with loss of the sample being dried.

A simple and successful method, which is new to us, for opening such desiccators has been used in this laboratory for some months. A single-edged and sharp razor blade, such as the "Gem," which has a metal backing strip, is placed between the edges of the top and body of the desiccator and gently tapped with a small block of wood. The extremely thin wedge thus attained forces the lid off. Even under full vacuum, lids may be removed; thus the method is useful for removing the lids of ordinary desiccators when a vacuum is inadvertently obtained by allowing hot samples to cool therein with the lid on. Thin double-edged blades are not very satisfactory, since they are too flexible for use.

The precaution of wearing goggles should be observed to avoid possibility of injury by a flying particle of glass or steel. Careful cleaning of the ground glass surface before the cover is replaced is necessary to remove particles which might lodge in the grease and cause injury to the surface.

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[2] H. Zinsser and S. Bayne-Jones, "A Textbook of Bacteriology." 8th edition. pp. 654–655. New York: D. Appleton-Century Company, 1939.

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[4] Timothy J. Kurotchkin, personal communication.

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