Table 1. EFFECT OF CHILLING ON IMAGINAL DIFFERENTIATION OF DAUER PUPÆ

Groups of exp.	Duration of chilling (days)	No. of specimens	No. of moths emerged* (days to emergence following chilling)	No. of undeveloped pupæ
1	20	25		25
2	40	25	1 (23), 1 (36)	23
3	60	25	2 (23)	23

* During 30 days at 25° C (following chilling).

Fukaya and Mitsuhashi's work[9], most dauer pupæ emerged as moths in 120–300 days following extirpation of brains under the same conditions.

Because of the reasons mentioned here, and the results presented, it is not likely that low temperature affects the induction of imaginal differentiation of dauer pupæ, that is, the prothoracic glands in dauer pupæ are not usually activated by treatment with low temperature, although a few animals did emerge as moths.

We thank Mr. Koji Nomura, of the Laboratory of Clinical Biology, Salt Lake City, for assistance during the course of this experiment, and Mr. Y. Ishitoya and Miss K. Tani, of the Sericultural Experiment Station, Tokyo, for help with the rearing of the silkworms.

This work was aided by grants from the National Institutes of Health, U.S. Department of Health, Education, and Welfare and the American Cancer Society.

MASATOSHI KOBAYASHI*
WALTER J. BURDETTE

Laboratory of Clinical Biology,
Department of Surgery,
University of Utah College of Medicine,
Salt Lake City.

* Present address: Sericultural Experiment Station, Suginami-ku, Tokyo.

[1] Williams, C. M., *Biol. Bull.*, **90**, 234 (1946).
[2] Williams, C. M., *Biol. Bull.*, **93**, 89 (1947).
[3] Williams, C. M., *Biol. Bull.*, **103**, 120 (1952).
[4] Williams, C. M., *Biol. Bull.*, **110**, 201 (1956).
[5] Van der Kloot, W. G., *Biol. Bull.*, **109**, 276 (1955).
[6] Kobayashi, M., and Yamashita, Y., *J. Sericul. Sci. Japan*, **28**, 335 (1959).
[7] Kobayashi, M., and Nakasone, S., *Bull. Sericul. Exp. Sta.*, **16**, 100 (1960).
[8] Kobayashi, M., *Recent Advances in Experimental Morphology*, 167 (1959).
[9] Kobayashi, M., Fukaya, M., and Mitsuhashi, J., *J. Sericul. Sci. Japan*, **29**, 337 (1960).

Feulgen – Schiff's Technique for Examination of the Distribution of the Intracellular *Rickettsia*-like Micro-organisms in Whole Mounts of Ticks, or their Tissues

INTRACELLULAR *Rickettsia*-like micro-organisms are known to exist naturally in ticks, usually occurring in the Malpighian tubules and oocytes[1,2]. Their distribution in the organs of the ticks has been, in the past, studied by various investigators[3,4] from conventionally stained smears and sections of tick tissues.

Histochemical methods for nucleic acids, including the Feulgen technique, have recently been used in this laboratory with sectioned material of ticks[5,6] to demonstrate in virtue of the persisting DNA content of the micro-organisms, their occurrence and distribution in the tick tissues. This type of work, however, entails dissection and serial sectioning of a considerable number of ticks.

Recently, Newcomer[7] has introduced for serial-section studies a histochemical technique, in which tissues are fixed in isopropanol fixative[8], then treated with Feulgen–Schiff's reagent before embedding. However, by modifying Newcomer's technique, I was able to overcome this tedious matter of surveying ticks in serial section and to observe directly in whole dissected ticks under the binocular microscope the occurrence and distribution of the intracellular *Rickettsia*-like micro-organisms which are to be found in their Malpighian tubules and developing oocytes. The modified technique, depending on selective staining of the DNA of the micro-organisms, is as follows.

Ticks are dissected in 0·9 per cent saline. The dorsal integument is gently separated from underlying tissues, and the alimentary canal removed. The organs thus exposed are then covered *in situ* with the fixative, isopropanol mixture, and kept thus for 1–2 days. Hydrolysis of the dissected ticks follows in N hydrochloric acid at 60° C for 10–20 min. The ticks are then treated with Schiff's solution, prepared according to the method of Newcomer[7], for 20–30 min, and washed in sulphurous solution several times. After washing in running water, they are passed through graded alcohols up to 70 per cent. The treated ticks can then be fixed in a dissecting dish and examined in 70 per cent alcohol for the micro-organisms under the binocular microscope. Moreover, pieces of Malpighian tubules and oocytes could also be removed at this stage from the ticks, dehydrated, cleared in xylene and mounted for microscopic examination in D.P.X.

As would be expected with a DNA-selective stain, tissue nuclei persisted and were stained, in this technique, purplish-red. In addition the DNA-positive *Rickettsia*-like micro-organisms were also seen in the cytoplasm of Malpighian tubule cells and of oocytes as purplish-red aggregates. Their distribution was readily determined, and confirmed by microscopic examination of mounted pieces of these tissues.

Several species of argasid and ixodid ticks have been investigated in this way, and different patterns of distribution of the micro-organisms observed particularly in the Malpighian tubules. The results of these investigations using this and other techniques are in preparation for publication.

I thank Prof. D. S. Bertram, director of this Department, for his interest.

M. A. ROSHDY

Department of Entomology,
London School of Hygiene and Tropical Medicine,
Keppel Street, W.C.1.

[1] Steinhaus, E. A., *Insect Microbiology* (Comstock, Ithaca, N.Y., 1946).
[2] Buchner, P., *Endosymbiose der Tiere mit pflanzlichen Mikro-organismen* (Birkhauser, Basel, Switzerland, 1953).
[3] Cowdry, E. V., *J. Exp. Med.*, **41**, 817 (1925).
[4] Mudrow, E., *Z. Parasitenk.*, **5**, 138 (1932).
[5] Roshdy, M. A., *J. Insect Path.*, **3**, (2), 148 (1961).
[6] Roshdy, M. A., *Nature*, **192**, 186 (1961).
[7] Newcomer, E. H., *Stain Tech.*, **34** (6), 349 (1959).
[8] Newcomer, E. H., *Science*, **118**, 161 (1953).

MICROBIOLOGY

Host-range of *Proteus morganii* Bacteriophages

COLICINE *H*, which kills many *Escherichia*, *Shigella*, *Salmonella* and a few *Proteus hauseri*[1] also attacks many strains of *P. morganii*[2]. The relationship between *P. morganii* and the other genera mentioned has recently been strengthened by the finding[3] that the guanidine + cytosine content of their deoxyribonucleic acids are very similar and differ from *P. hauseri*, *P. rettgeri* and *Providence* strains. On the other hand, it has been shown[4,5] that although *P. morganii* strains are not susceptible to *P. rettgeri* phages, many *P. morganii* organisms are attacked by *P. hauseri* and *Providence* phages. There is also a well-known antigenic relationship between *Proteus morganii* and *P. hauseri*. Work on *P. morganii* phages has been confined to temperate varieties, and host-range determinations were limited to the *Proteus* group of organisms[2,6]. The phages were found to be species specific. In view of the uncertainty[7] about the taxonomic position of *Proteus morganii*, it was decided to investigate the action of *P. morganii* phages derived from both lysogenic strains and sewage on members of the family Enterobacteriaceae.

The 17 strains of *Proteus morganii*, 28 strains of *P. hauseri*, 22 strains of *P. rettgeri* and the 24 *Providence* strains used have been described[5]. The other Enterobacteriaceae comprised 21 *Salmonella* serological varieties,

© 1963 Nature Publishing Group