moved in the usual manner using a dental engine with a drill or an abrasive disc. Care is taken to avoid damaging the eggshell membrane. A drop of sterile liquid paraffin placed on the exposed area makes the membrane transparent. The egg is placed in the holder with the air-sac toward the light source (15) and with the selected vein on top. The long axis of the blood vessel is set approximately parallel to the work-table and in line with the syringe. The syringe-carrier is adjusted so that the needle forms an angle with the blood vessel of about 15°, and rests on the surface of the eggshell membrane over the blood vessel. The needle is then driven forward, and into the vein, by manipulating the screw (10).

Injection is always made in the direction of the blood flow, and withdrawal of samples preferably in the opposite direction. The needle is withdrawn slowly; but in cases following injection, only after a pause of several seconds. In our experience bleeding after removal of the needle, even from 10–12-day-old chicks rarely occurred. It was not necessary to reseal the egg in the course of these studies even though incubation was carried forward for several days after veno puncture.

In our experiments on 10- to 14-day-old chick embryos, we have injected over 2500 eggs, using the apparatus described, with a casualty rate of less than 3%, and more than 250 eggs have had blood withdrawn from 2–4 times, with less than 10% loss. Routine bleedings of 0.1–0.2 ml of blood at 1–2-hr intervals can be carried out with ease for 4–6 bleedings (up to 7 or 8 with increasing difficulty). In one series, 15 chick embryos were bled every 2 hr for 8 hr, withdrawing 0.2 ml of blood each time without any casualty.

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Manuscript received December 12, 1952.

A Mechanical Fly-Tagging Device

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A mechanical device has been developed for tagging flies that are to be used as experimental free-flying insects. The tagging consists of gluing 1-in. lengths of brightly colored nylon thread to the thoraces of flies anesthetized with carbon dioxide. This procedure permits suitable test insects to be more readily located, recovered, and identified, and is particularly useful

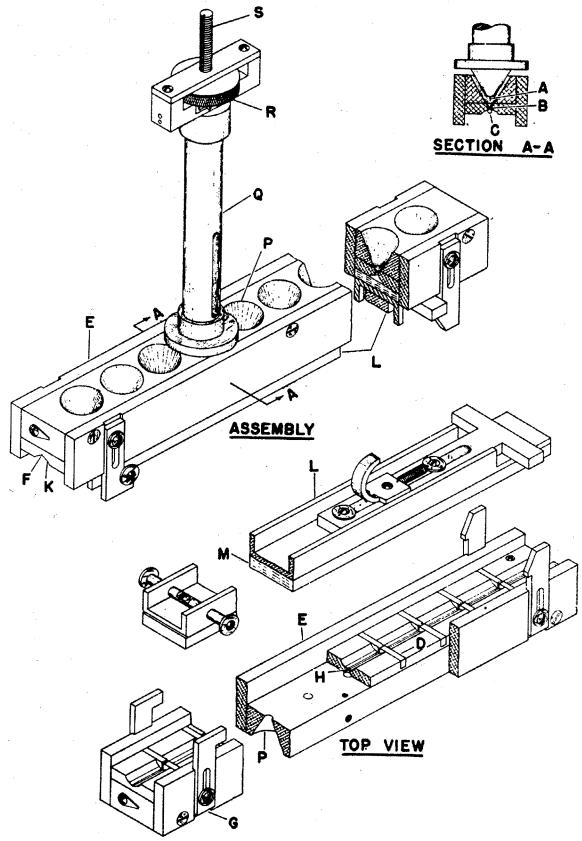

FIG. 1. Drawing of a fly-tagging device.

for aircraft-disinsectization studies. The bright colors are used to aid in locating the flies and to distinguish between different series of releases made in the same enclosure. The 1-in. length of thread extending directly back from the thorax of each tagged fly is readily grasped with forceps without agitating the fly into flight. The captured insects are then transferred to holding cages. When properly attached, the threads do not interfere with the normal activities of the flies.

Originally a manual method was used to attach threads to flies' thoraces, using high-melting-temperature paraffin. A small amount of the paraffin was put on the end of a piece of thread and this in turn was placed on the dorsum of the thorax. A warm metal rod was used to keep the paraffin melted and to hold the thread in contact with the anesthetized fly until the paraffin hardened. In about an hour the flies became accustomed to the attached threads and resumed normal activity. No fly injury has been observed, but proper precautions must be taken to prevent inactivation of the wings. Using this method, a highly skilled worker has sexed and attached threads to as many as 300 female flies an hour for short periods. The procedure is quite tiresome when large numbers of flies are to be tagged, and relatively unskilled workers have been able to produce only about 800 tagged flies per day. With the mechanical device, workers

with comparable skill can tag from 1200 to 1500 flies per day without undue fatigue.

The mechanical unit consists of a fly-holding frame and glue dispenser, both made of aluminum, and an ordinary ¼-cc hypodermic syringe. The holding frame shown in Fig. 1 (top view) is a troughed bar divided into 25 compartments by means of notched ribs (D), and a foam-rubber-padded cover (L). A nylon thread (C in section A-A) is held in the bottom of the groove (F) by being stretched between jam cleats at both ends of the frame. Anesthetized flies are placed upside down in the trough (K) with their heads toward the holes (H) centered about 3/16 in. from the ribs. The frame is tilted slightly upward from the right end and tapped lightly causing the flies to slide down until their heads rest against the ribs. They are now in such a position that a conical hole (P and H), extending from the under side of the frame, is centered under the thorax of each fly. The cover (L) padded with sponge rubber (M) is snapped into place to hold the flies securely and the whole unit is inverted to expose the thoraces of the flies through the conical holes.

Glue is dispensed with a ¼-cc syringe held in the dispenser (Q). A micrometer screw (S), attached to the end of the dispenser barrel, moves the syringe plunger forward. The nose of the dispenser is placed in each hole and the knurled wheel (R) turned 1 notch clockwise. This procedure placed 1/1600 ml of Duco cement, thinned with 50% acetone, on each fly at the point where the thorax touches the nylon thread. The holding frame is now turned right side up, the cover removed, and the chain of 25 flies is taken out. The string is cut in front of the head of each fly and the separated flies are then placed in holding cages for later release tests.

Manuscript received December 22, 1952.

A Quantitative Analysis of the Factors Involved in the Variations in Virulence of Rickettsiae[1]

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Previous investigators have observed that strains of *Rickettsia rickettsii,* the causative agent of Rocky Mountain spotted fever, recovered from ticks in nature differed widely in their apparent pathogenicity for guinea pigs (*1*). Furthermore, Spencer and Parker (*2*) found that, if *Dermacentor andersoni* ticks infected with virulent spotted fever were left in the icebox for several months, a ground suspension of these ticks injected into guinea pigs produced fewer, if any, spotted fever symptoms, yet immunized the animal against subsequent infection by the virulent agent. If such ticks were given a blood meal and then injected into guinea pigs, these animals came down with virulent spotted fever. This was termed a reactivation phenomenon. Unfortunately, the above observations are open to some criticism since little attention was paid to the quantitative relationships. However, this system suggested an intensive study of the factors involved in the variation in virulence of an intracellular microparasite.

The series of experiments here reported was undertaken to establish more precisely by quantitative methods the differences in virulence of the representative strains of *R. rickettsii,* the possibility of modifying strain virulence by animal passage and by tick passage, the nature of the reactivation phenomenon, and the identity of the enzyme systems principally involved in these changes of virulence.

Characterization of Strain Variation in Virulence.[3] The first series of experiments was planned to establish by quantitative methods the range in virulence of four representative strains of *R. rickettsii.* The following procedures were adopted. The strains were isolated from yolk sacs by a procedure similar to that of Wisseman *et al.* (*3*). The number of rickettsiae in the suspension was determined by the electron scope method described in Table 1. Each strain was titered in guinea pigs and chick embryos as described in Table 1. This experiment was repeated three times with similar findings. The more virulent organisms grow better in the guinea pig than the less virulent strains, the major differences being particularly noticeable in the heart, brain, and blood. Thus strain R reaches a maximum concentration of 300 and 100 LD_{50} in the brain and heart, respectively, and of 300 LD_{50} in the blood. Strains S and T show a maximum of only 1 to 3 LD_{50} in the brain and heart, and only about 10 LD_{50} in the blood.

Guinea pigs injected with 10,000 LD_{50} of one strain are immune to the three other strains. Furthermore, strains T and U will completely protect guinea pigs from strain R when given in 100 times the concentration (10^6 LD_{50} of T or U and 10^4 LD_{50} of R) with both weakly virulent and virulent strains injected intraperitoneally simultaneously.

All strains shown in Table 1 possess a toxin[4] similar

[1] This work was supported by a grant from the National Institutes of Health, United States Public Health Service.

[2] I wish to express my sincere appreciation to Carl L. Larson, Director of the Rocky Mountain Laboratory, National Microbiological Institute, National Institutes of Health, Hamilton, Mont., for placing the facilities of the laboratory at my disposal. A few of the experiments reported in this paper were carried out at that laboratory. I wish also to thank David Lackman for carrying out many of the complement fixation determinations. Acknowledgment is also due to H. Sargent, L. Hughes, Mrs. M. Smith, Miss H. Emerson, C. H. Sawyer, and E. G. Pickens for valuable technical assistance in various phases of this work.

[3] All LD_{50} determinations refer to egg titrations. Using the R strain, 1 LD_{50} is equivalent to minimal infectious dose (ID_{50}) for guinea pigs. In all instances where eggs and tissues are titrated, the LD_{50} refers to the number of LD_{50} in 0.5 ml of a 10% suspension.

[4] E. J. Bell and E. G. Pickens of the Rocky Mountain Laboratory, in an independent series of investigations, have found that *R. rickettsii* produce a toxin.