mental animals. The frequently expressed wish that the course of the investigation might be expedited can not, however, be complied with, since biologic processes are involved in which any such attempts to influence matters are out of the question.

Separate from the question devolving on the Federal Health Bureau whether or not the Calmette prophylactic material as such was capable of producing the severe tuberculous infections in the infants instead of protecting them against the disease must be considered the question whether or not everything was done in Lübeck to carry out in a manner above reproach the Calmette prophylactic treatment, after it was once decided to employ it. The investigation of the manner in which the vaccine was employed is primarily the duty of the state of Lübeck. In the course of the investigations, a series of incriminatory charges developed, as was foreshadowed in the report of the referee sent to Lübeck by the Federal Ministry of the Interior, May 22. In this connection, the following points merit consideration.

1. After the federal ministry of the interior, in 1927, in the matter of protective treatment against tuberculosis by means of living bacilli, in agreement with the conclusions reached by the federal health council, had recommended a conservative policy, it would have been proper if the Lübeck centers concerned, before instituting the vaccine treatment, had inquired whether or not the federal ministry of the interior, in spite of many favorable reports from foreign countries, still preserved its waiting attitude.

2. After the original culture secured from the Pasteur Institute had been recultivated for nearly nine months in the Lübeck laboratory on various cultivating mediums, it would have been wiser, before the first application of the protective material to infants, to test its potency by animal experimentation. That was not done.

3. The surveillance of the children who were inoculated with the vaccine was not adequate.

4. The destruction by Professor Deycke, April 26 (that is, after the harmfulness of the protective material had become known), of the supply of vaccine left in his hands must be regarded as of questionable indication, irrespective of the motives that induced the act. Professor Deycke's action did not, however, militate against the clearing up of the affair, since the Federal Health Bureau was able to secure possession of entirely sufficient remnants of the protective material employed. The Federal Health Bureau was able to obtain all other research material needed.

5. It can not be justified that, after the forenoon of April 26, when the harmfulness of the protective vaccine employed had been proved by the necropsy on one of the infants who had died, several doses of the vaccine were allowed to remain in the hands of midwives. Fortunately, this remaining vaccine was not administered to any new subjects but only to such infants as, before April 26, had already received the first inoculation, which was probably decisive as regards the transmission of the infection.

6. It is subject to censure that the persons who were responsible for the application of the protective vaccine, among whom there seems, too, to have been a lack of cooperation, did not inform until a late date the center in Lübeck having first jurisdiction in such matters, of the damage that had been done. The *Reichsmedizinalverwaltung* (federal administration of medical matters) was not informed of the events until May 14.

To what extent the charges, or censures, mentioned (which do not essay to pass a judgment on the scientific merits of the Calmette procedure) should or may be considered in determining the matter of culpability, will be established by the criminal procedure, which has already been instituted.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

INTRA VITAM TECHNIQUE FOR THE STUDY OF THE LIVING CELLS OF INSECTS

THE method of studying living germ cells as practiced by Lewis and Robertson,[1] Strangeways and Canti,[2] Bélâr[3] and others shows certain lacks and deficiencies. These are evidenced by the appearance of pseudopodia, fused cells and nuclei and other abnormalities. Such irregularities do not appear in well-fixed material and we do not believe that they are a part of the behavior of germ cells in normal conditions of development.

We have been able to develop a technique for insect germ cells in which such abnormalities do not appear, due to the fact that the body pressure (follicular and cystic) and specific ferments are not disturbed. Neighboring cells are not separated from each other or from the surrounding tissues. Hence no fusion of spermatocytes or spermatids occurs, nor do

[1] M. R. Lewis and W. R. B. Robertson, (II) "The Mitochondria, etc., in Chorthippus," *Biol. Bull.*, 1916.

[2] T. S. T. Strangeways and R. G. Canti, "The Living Cells in Vitro as Shown by Dark Ground Illumination and the Changes Induced in Such Cells by Fixing Reagents," *Quart. Jour. Micr. Sci.*, 71, 1927.

[3] K. Bélâr, "Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von *Chorthippus lineatus*," *Arch. Entw. Mech.*, 118, 1929.

pseudopodia ever appear, such as all workers, using the tissue culture methods, report.

We are calling this the intra vitam method in contradistinction to the earlier in vitro method. We feel confident that it has some merits over the older ones. Because it is simple and practical we are publishing the method before the longer paper, giving the details of our observations as well as the photomicrographs and drawings, is completed. It is so easy to use that we shall employ it in the future as a part of the laboratory work in cytology.

TECHNIQUE

A male grasshopper of suitable age is anesthetized for several seconds. As soon as the ether has taken effect, the hind legs are severed at the autonomous joint. The wings are cut off behind the pronotum, and a rectangular opening, about 2 mm by 1 mm, is cut through the chitinous wall of the second, third and fourth abdominal segments, just left of the mid-dorsal line. The insect is then placed on its right side, parallel to the width of a sterile glass slide, to which it is firmly attached by means of melted paraffin. The paraffin is drawn into a pipette and is then carefully dropped onto the forelegs and antennae of the grasshopper. The paraffin is run around the head of the insect and along the ventral side of the body, which faces the right-hand side of the glass slide. A narrow ribbon of paraffin is continued around the last abdominal segment and on out toward the left side of the slide for about 15 mm. Next, the paraffin is led up and back to the anterior end of the grasshopper (see figure). These three paraffin walls, ap-

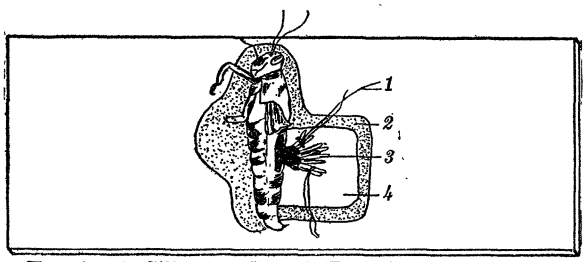

FIG. 1. 1. Silk threads. 2. Paraffin. 3. Follicles. 4. Lake of medium.

proximately 15 mm by 10 mm, together with the body of the grasshopper and the enclosed surface of the medium (Bélâr, '29), convert this basin into a nutrient lake. A hot needle applied to the outer rim of the paraffin wall insures close adherence to the glass slide and prevents leaking.

Care must be taken, when affixing the grasshopper to the slide, that the spiracles in the abdominal wall are not flooded with paraffin. The anal aperture is also left free so that normal evacuation can take place in preparations which are to be maintained for several hours.

Before the grasshopper recovers from the effects of the anesthesia, the testes are drawn out through the aperture in the abdominal wall. The yellowish connective tissue membrane, which encloses the tightly packed follicles, is carefully torn away with a sharpened needle point and the follicles float out into the lake of medium. They remain attached, of course, at the proximal end, to the vasa efferentia. Several of the upper ones, as well as two or three of the lower follicles, are secured with a loop of silk thread. The loop is drawn tight and firmly fastened to the wall of paraffin with a drop of melted wax. This attachment of some of the free follicles prevents the withdrawal of the testes into the body cavity and it lessens the movement of the intervening follicles.

The eight or ten free follicles, exposed along nearly their entire length, may be studied for hours, at intervals or continuously. In time the culture medium evaporates slightly and must be renewed. It is best to draw off the used medium and fill the lake with fresh fluid.

OBSERVATIONS UNDER THE MICROSCOPE

Some of the interesting things that we have observed with the 16 mm objective are the tridimensional or tubular nature of the follicle; the shape of the cysts with their walls; the variations in the shape of the cysts. These increase greatly in size and the walls thicken as they grow. In the spermatid region the cysts elongate greatly, the conical structure reaching down toward the center and proximal end of the follicle. The stages of germ cell development can also be recognized and their place of occurrence in the follicles fixed. The bundle of sperms in the grasshopper as they move and turn (Landrum, work unpublished) can be followed. The turning of the sperm in the crickets (Baumgartner, work unpublished) can be recognized.

With a 1/12 water immersion objective and good illumination (a bright substage lamp) practically every structure previously described in fixed material may be studied without the aid of intravital staining.

The walls of the follicles are thick and most probably muscular. Cysts of spermatogonia are readily distinguished from spermatocytes by their locus, size and cellular inclusions. Spermatocytes go through the various stages of cell division while under observation. Spermatogonia also divide. Chromosomes can be watched as they migrate from the equatorial plate to the poles. Here a very small refractive spherical centrosome is visible. Astral rays may be seen radiating from this point or body. Numerous threads of mitochondria are distributed to the daugh-

ter cells and form the nebenkern. Telokinetic movements can be followed. The young spermatid cells have short axial filaments. These can be seen to elongate and become the tails of the more mature spermatids. The long tails can be traced from the heads down toward the open end of the follicle. The tails are in groups and are more or less intertwined. The aggregation of the sperm into bundles can be studied, as well as the movement of the bundle to the open end of the follicle.

Preparations of crickets and beetles have been set up, with slight modifications of the technique. It is best to mount a cricket with the back flat on the slide. The follicles are shorter and the cells are smaller, but they may be studied by using the above-described method.

We have been able in a few months to see most of the characteristic structures and to follow many of the activities of the *living* germ cell, in a state that very nearly approaches the normal condition. We anticipate that our continued efforts will bring out other important data. It may not be too optimistic a viewpoint to expect the solution of some knotty problems in chromosome behavior and spermatid transformation by the use of this method. With such a hope we are offering the details of the method to other investigators.

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A RAPID POSITIVE CONTROL METHOD OF HANDLING SMALL QUANTITIES OF LIQUIDS

FOR a certain experiment here there was needed a means of adding small quantities of liquids that would make it possible to secure more positive and satisfactory control than is afforded by any ordinary pipette or burette. The final design of the special tube or pipette for this purpose is shown in Fig. 1. As is at once apparent, the pipette substitutes a mercury plunger or piston for the usual rubber bulb or the rubber tube connected with the mouth. By inclining the pipette at an angle such as indicated and rotating it until the mercury has filled bulb N the air on the N side is crowded out at the tip. If now the tube, still at the same angle, is inserted into the liquid supply and rotated about its own axis until the mercury runs from N into the bulb M the liquid will be drawn up into the pipette to a height depending on both the angle of inclination and the amount of rotation, each of these conditions being subject to definite control individually. Scratches on the tube corresponding to volumes desired for particular work make the tube suitable for quantitative work. To expel any quantity of the liquid from one drop to the entire quantity contained one has but to rotate the pipette in the reverse direction, the mercury flowing back into N and crowding out the liquid to an extent positively controlled by either the inclination or the rotation, or both.

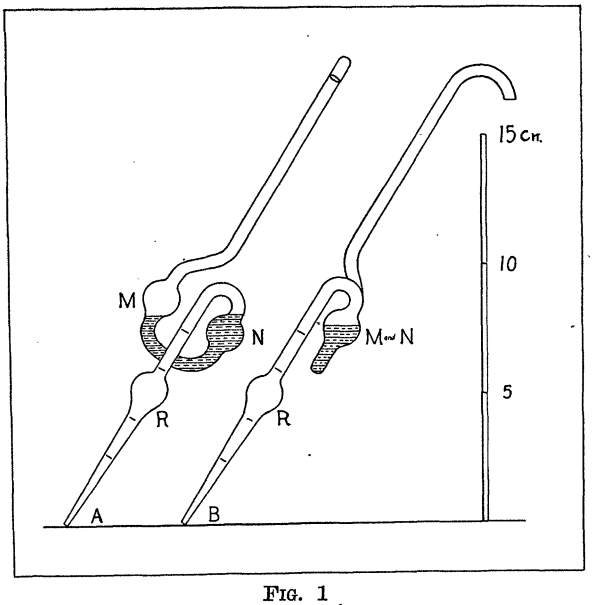

FIG. 1.

Compared to a burette in handling small quantities of liquids it is much more quickly filled and emptied and involves none of the uncertainties of either a stopcock or a pinch-cock. It is superior to any type of pipette operated by suction from the mouth, directly or through a short length of rubber tubing, for it eliminates any chance of fumes or liquids being inadvertently drawn into the mouth, as well as all danger of mouth or breath moisture contaminating the pipette, and relieves the user of the rather unpleasant and often inconvenient, if not unsanitary, use of his mouth.

On many grounds it is to be preferred to a pipette using a rubber bulb, particularly where definite quantities are required. When using a rubber bulb one is never sure just how much it should be squeezed in order to get the precise quantity needed—if too little, one has to begin over; if too much, one has to remove it from the liquid before it is fully distended and then allow air to bubble through the liquid, spraying the same into the bulb, or else must maintain the pressure on the bulb just so until the liquid is ejected. Rubber bulbs become contaminated and the contamination is invisible. They are flabby, often leaky, and offer uncertain control of the position of the tip of the pipette, in contrast with the rigidity of the new type. In using ordinary pipettes the heat of the hand is likely in the case of volatile liquids to cause a vapor