when obtained give exceedingly poor hatches, so that many fruitless attempts preceded the obtaining of the desired hybrids.

Forty hybrids have been or are now being studied and some data obtained on the inheritance of the two mutant characters involved in the stock used in the crosses. The characters involved are sex-intergradedness and "excavated" head. Twenty-nine of these hybrids are from wild type ♀ × mutant ♂, and 11 are from the reciprocal cross. Nineteen of these hybrids genetically possess the mutant character, excavated head, and 19 show sex intergradedness. It seems probable, therefore, that each of these characters is a dominant mendelian character and is heterozygous in the mutant clones used in the crosses. Further breeding and study will be necessary before the full extent of the inheritance of these characters is determined.

The absence of both these characters in all except the mutant laboratory strains, in much wild stock examined, and in sexual offspring from wild type parents, their presence and heritability in parthenogenesis in the mutant strains, and in sexual offspring from these strains, indicate that these characters are definitely heritable and behave in bi-parental inheritance like characters in bi-parental inheritance in other organisms.

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A method for preserving and counterstaining vitally-stained cells.*

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Recently, in the course of some experiments on the viability of monocytes,[1] it became necessary to preserve, for further study, cells which had been vitally stained with neutral red. The cells

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1 Sabin, F. R., Doan, C. A., and Cunningham, R. S., Contributions to Embryology No. 82, Carnegie Inst. of Wash. Pub., No. 361, 125-162.

consisted of various types of white blood cells present in the peritoneal exudate of the guinea pig. These cells had been under observation in hanging drop preparations such as are commonly used in tissue culture work,[2] and since the usual methods of preservation seriously affected or entirely removed the vital stain, it was necessary to apply a new method. The one finally devised, as described below, not only retains the vital dye but permits other staining methods, such as Wright's method for differentiating blood cells, to be superimposed upon the vitally-stained cells.

In brief, the method consists in taking the cover glass from a hanging drop preparation in which the cells have been well stained vitally, either by direct contact with neutral red on the cover slip,[3] or by exposure to a dye bath consisting of a few crystals of neutral red dissolved in unbuffered Ringer's solution in the incubator at 38° to 40° C., and immersing the preparation for one half to one hour at 38° to 40° C. in the Ringer-formalin fixative, suggested by Fischer,[4] which is buffered as noted below. The tissue is then washed in running water for three hours, rinsed with distilled water and permitted to dry. After drying, the preparation may be counterstained with a one-tenth of one per cent aqueous solution of methyl green, as a nuclear stain, or with Wright's blood stain. The preparation is again dried and may then be mounted in damar, or, if necessary, it may be cleared according to Fischer's[4] method, by subjecting it, before mounting, to passage for two minutes each through a graded series of acetone-xylol mixtures consisting of 5 per cent, 30 per cent, 70 per cent, xylol, and two changes of pure xylol.

In order to obtain uniform results with this method and to insure the retention of the pseudopodial, granular, and vacuolar structures of the cells in the forms and relationships obtaining immediately before fixation, several precautions are necessary. First, all glassware used is chemically clean. Second, the granules within the cells are heavily stained with neutral red before fixation, otherwise, when counterstained with Wright's stain,

[2] Harrison, R. G., *J. Exp. Zool.*, 1911, ix, 787. Baitsell, G. A., and Sherwood, M. B., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 96.

[3] Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277-88.

[4] Fischer, A., Tissue Culture, Studies in Experimental Morphology and General Physiology of Tissue Cells *in vitro*. Levin and Munksgaard, Copenhagen, 1925, 98-100.

they will appear bluish or black instead of red, making classification more difficult. Cells in which the granules are heavily stained from a deep scarlet through a cerise red even to an orange red, and in which the nuclei show no tinting with the dye, lend themselves most readily to permanent preservation and counterstaining by the present method. Third, in order to avoid the shrinkage or swelling or even the premature death of the cells, the variations in hydrogen ion concentration of the various reagents should not be great. It is our custom to use Ringer's solution from the same lot for the dye bath, for any necessary washing of the culture, and for the fixative. In all cases, the Ringer's solution is permitted to reach an equilibrium in hydrogen ion concentration by standing in the incubator at 38° to 40° C. for several days before use. In non-sterile Ringer's solution, whether made with distilled water or tap water, this equilibrium usually varies in pH value from 7.3 to 7.6. The formalin used is either acid formalin 37 per cent, or the same formalin kept over magnesium oxide. The buffers employed are either a phosphate-hydroxide mixture, pH 7.4; or primary and secondary M/15 phosphate mixtures, usually pH 7.4. The Ringer-formalin fixative consists of 20 cc. of Ringer's solution, 1.8 cc. of formalin and 2 cc. of buffer mixture. For general use, a final reaction from pH 7.3 to 7.6 is satisfactory, although, when simple fixation without the use of a dye bath is employed, a pH value as low as 6.8 gives as good results. The dilution of the Ringer's solution by the addition of formalin and buffer mixtures is negligible. Fourth, the use of the incubator during the dyeing and fixation processes insures a more rapid penetration of the cells by the reagents employed. Fifth, drying, instead of dehydrating by means of alcohol, avoids the loss of the alcohol-soluble neutral red and apparently has no ill effects upon the cells.

When Wright's method is employed to counterstain there is no confusion of the eosinophilic with the vitally stained granules; the former range from a bright pink to yellow, while the latter are red to reddish brown. Cells which did not show vitally stained granules before fixation do not present a granular appearance following the above treatment. Phagocytosis of red blood cells, eosinophilic and polynuclear leucocytes is beautifully demonstrated in the preserved preparations. On degeneration, there appears to be a tendency for the cytoplasm of the cells to assume acidophilic properties when subjected to Wright's stain, while

fragmentation of the nuclei is seen in those cells which were regarded as dead before fixation.

Further information as to the technical details, will be available later.

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Pseudobacteriophage of Bacillus anthracis.

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During the examination of some old stock cultures in November, 1924, there was found an agar slant of *Bacillus anthracis* which had the appearance of undergoing lysis by bacteriophage. The film of growth was interrupted by well defined partly confluent denuded areas, many of them containing small centrally located secondary colonies. Transplants of the culture to agar gave similar appearances after incubation for two days at 37° C. and one or more days at room temperature. Transplants to broth did not show clearing but the plaques again appeared when the broth cultures were transplanted to agar. An older agar slant culture from which the culture first mentioned above had been inoculated did not show plaques nor did fresh transplants of this culture, but when this "negative" strain was inoculated with material from one of the plaques of the "positive" strain plaques appeared in subsequent transplants. Filtrates of broth cultures of the positive strain were inactive when added to the negative strain.

Plaques on agar plates examined under the microscope were found to contain a nucleus of free spores, the remainder to the plaque being made up of pale remnants of bacilli and amorphous granular detritus. The matrix of growth surrounding the plaques was composed of typically curled but apparently spore-free anthrax filaments. Fishings from the centers of plaques yielded cultures of typical sporulating *Bacillus anthracis*. Fishings from the matrix yielded cultures of non-sporulating bacilli