

that have been decerebrated by the method outlined have survived indefinitely, presenting typical pictures of decerebrate birds.

A preliminary fasting period of from eighteen to twenty-four hours was imposed upon the bird previous to operation.

The hen was secured in a cloth sack, the head and neck protruding, and anesthetized with ether. Feathers were plucked from the operative area from ear to ear and from the comb posteriorly to the first cervical vertebra. A transverse incision was made through the skin from ear to ear and the periosteum removed from the bones covering the cerebral hemispheres. Two holes were trephined over the center of each hemisphere, and with small bone forceps, the openings were enlarged, care being taken not to injure the dura mater, also carefully avoiding the longitudinal sinus. The dura mater was slit antero-posteriorly, after applying a solution of codrenin (cocaine 2 per cent. solution, with adrenalin 1:15000) to control hemorrhage. Using a small spatula the cerebral hemispheres were lifted, care being taken not to injure the brain stem. After the hemispheres were removed, hemorrhage was checked by using pledgets of cotton moistened with codrenin. Because of the larger blood supply, the control of hemorrhage is more of a problem in fowls than in pigeons. The usual procedure in decerebrating pigeons is to suture the dura mater and skin. This procedure was followed upon the first four birds operated, all these birds dying immediately following the operation. Autopsies of these birds revealed that death resulted from the pressure of blood clot upon the vital centers. Therefore, in all succeeding operations no attempt was made to suture the dura mater and skin. Extended over a period of two years fifty-four birds have been operated, the cerebral hemispheres being removed partially or completely. No ill results oc-

curred from blood clot or from infection of operated areas, all birds surviving for indefinite periods.

D. W. ASHCRAFT

OHIO STATE UNIVERSITY

MARKING GEOLOGICAL SPECIMENS

DURING the past year we have very successfully used quick-drying lacquer for marking specimens both for use in elementary laboratory and for numbering working and museum collections. The process is simple, consisting only of placing a drop of lacquer on the specimen and writing upon it the significant letters or figures with steel pen and drawing ink.

The advantages in using lacquer over many other methods of specimen marking are several. It is permanent; it provides a smooth writing surface, and it can be secured in colors which may be used to indicate different sets, classes, groups or other divisions. The method is quick, drying after spotting, and marking being accomplished within half an hour. The colors may be contrasting with the specimens marked or closely matched to them. We have found a contrasting color ordinarily the most satisfactory, in most cases using white or orange with lettering in black ink.

The use of lacquer makes possible the marking of specimens which would otherwise be difficult. A deep drop may be used to cover granular or rough surfaces, and even when applied in considerable depth there does not appear the wrinkling or furrowing such as is common with the usual enamels. A single application has served to form a satisfactory writing surface on coarsely granular pyrite. The size of the lacquer spot commonly used is about three sixteenths of an inch in diameter, and is applied with a small brush. A spot of this size holds conveniently a letter and two figures, or two pairs of figures, one above the other.

EARL T. APPEL

SYRACUSE UNIVERSITY

SPECIAL ARTICLES

ON THE NATURE OF GENE ACTION

THAT the structural units which give form to life, as we know it, are the inherited units called genes is becoming increasingly clear. But what these genes are and when and how they act are yet problematical. Experiments of the last two years have, however, furnished certain facts suggesting the answers to these questions. These facts resulted from treating the larvae of *Drosophila* with X-rays at timed stages in their development in much the same manner as that used by Patterson.¹ The larvae for the treatments

were in groups less than twenty-four hours old, one to two days, two to three days, three to four days—seven to eight days. Flies showing changes were obtained only in the three-day-old group. The four pairs of chromosomes present in the individuals treated were made to have the following known genes: One sex chromosome had the genes for white eye color, miniature wings and beadex wings, the other X-chromosome was wild type; one second chromosome had the wing gene for curly, its mate was wild type; one third chromosome had the genes for the eye colors scarlet and claret, and gene mutomat causing greatly reduced crossing-over in all chromosomes,

¹ J. T. Patterson, "The Effects of X-rays in Producing Mutation in the Somatic Cells of *Drosophila melanogaster*," *SCIENCE*, 68: 41-43, 1928.

non-disjunction in one or all chromosomes, etc.; the other chromosome had the gene *mutomat*.

In the normal course of events, since each known gene has a wild type gene in the chromosome which is its mate, the fly will show only the dominant factors *Beadex* and *Curly*, the other factors being completely submerged so far as recognizing their presence is concerned. By producing a change in the wild type gene opposite any of these recessives, however, they may be made to become visible as the character, eye color or wing shape. X-ray is known to be an agent which produces such changes. The treated flies in these experiments were exposed to 2,500 Roentgen units of X-ray.

A single-celled fertilized egg divides into two cells which in turn divide into four cells, etc., each cell containing a complete set of chromosomes similar, so far as we now know, to those which were contained in the original egg. In the course of the cell divisions, however, cells are separated from the rest which have definitive objectives, like the formation of the eye, which on further division form these parts as they are seen in the adult. The time element in this question of this divergence of the cells to form only organs like the eye from those to form other organs may be approached by the use of X-ray and stock like that indicated above. The very fact that such an approach is successful is attendant upon the equally important fact that the theory of the nature of gene action involved has some measure of truth.

The facts of the experiment are as follows. First, all observed changes occurred in the flies treated at three days old. They involved only the eye colors, although changes in wing and sexual characters were equally closely watched. The changes involved all three of the recessive eye colors present. The amount of the organ affected was apparently essentially the same. The gene in the X-chromosome was changed as frequently as those in the third chromosome. Individually considered the changes were as follows: culture 11774 showed a fly with a white V-shaped segment on dorsal side of left eye of twenty-nine cells or ommatidia. A fly from culture 11775 showed two sharply differentiated groups of scarlet cells on the right eye, one group, extending from the anterior margin posteriorly, of twenty-seven ommatidia and another, V-shaped on the ventral side, of thirty-one ommatidia. In the middle portion of the left eye of a fly from 13113 there was an oval spot of thirty-six ommatidia claret in color; 13152 had a fly both eyes of which were affected, the left eye showing ten white ommatidia in a V on the anteroventral edge of the eye, and the right a V of twenty-five ommatidia on the anterior edge; 14146 had a fly whose right eye

showed a double V-shaped patch of thirty-two white ommatidia on the ventral edge and another fly whose right eye had an oval spot of claret in the mid-position involving twenty-eight ommatidia. In each case the rest of the eye was wild type, normal in appearance. The line of demarcation between the changed area and the wild type was abrupt. There was no intergrading. The changes affect only the soma of the fly, the germ-cells showing no change.

So far as the time relations are concerned these results agree with those on the white eye factor obtained by Patterson, although the areas affected are slightly larger, due possibly to the fact that our larvae were kept at a lower temperature than his, thus retarding development somewhat.

Considering for a moment the divergent paths which the cells of the developing embryo take, we note certain critical conclusions to be derived from the facts. As the heavy majority of the effects of X-ray in producing changes in eye color change only a small portion of the cells of one eye it follows that the cell or cells forming the right eye are early separated from the cells forming the left eye. From the fact that only a small portion of the eye cells was changed it would seem that at the time of treatment the eye had developed to a number of cells stage, perhaps the thirty-two-celled, since the original one had left the rest of the soma tract. As the frequency of change is greatest where only one area was present on an eye, it would seem that each area represents a separate original cell in which the chromatin material was altered. The fact that flies showing two cell areas affected have these areas of about equal size points strongly to the conclusion that daughter cells of the eye anlage at the same stage of division tend to form an equal proportion of the final organ. The cells forming the eyes on either side of the body appear to be equally susceptible to X-ray effects. It is worthy of note that when two changes in the same organism are seen it is the same gene which is involved.

Genes altering organs so similar as those presumably responsible for the fly's eye color would be expected to travel along the same path in cell division and to be acted upon similarly. All these genes showed changes by X-ray which later developed into areas containing essentially the same number of cells. Thus proof is furnished for the hypothesis that the eye anlage has left the other soma tract four to five cell generations earlier than the time when the X-ray was applied.

The fly larvae exposed to X-ray all carried six marked genes. Since in each case only one of the six genes was affected, the conclusion appears sound

that the X-ray changes were distinctly local in character. It further is clear that the effect in the case of the third chromosome was less than a chromosome in length, since had it not been so both characters scarlet and claret would have appeared as the eye color orange instead of only one of the factors showing.

The data furnish some information on the mechanism by which the gene acts to produce its end product. As it is possible to change the gene in a cell before this gene has manifested its appearance, it is evident that the gene is potentially present in every cell which eventually forms the eye during the entire embryology of the animal, governing its destiny throughout development. The patches of cells which result from the cell in which the mutation was originally brought about are absolutely sharp and distinct from those not so changed. There is no intergrading of the two areas of cells in color. The action of the gene must therefore be within the cell in which it is contained and on no others. On this view the classic reaction, gene $\longrightarrow$ enzyme $\longrightarrow$ end product, must be all cell-contained, or an all-or-none reaction. Such a view presents difficulties which, without any definite proof for the enzyme stage, would seem to be best treated as still a gene-to-end-product reaction taking place within each cell. The facts thus point to the gene as present at each embryological stage in an organ's development and capable of changing this organ at any stage at which the gene itself is changed.

JOHN W. GOWEN

DEPARTMENT OF ANIMAL PATHOLOGY,
THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH

THE FUNCTION OF THE FERTILIZATION MEMBRANE IN THE DEVELOPMENT OF THE LARVA OF THE SEA URCHIN

It is possible to secure fertilization and development of the egg of the sea urchin without the formation of either the fertilization or the hyaline membrane. If unfertilized eggs of *Strongylocentrotus purpuratus* are put into an isosmotic solution of urea for two minutes and then transferred to sea-water containing sperm, the eggs are fertilized, but no membranes of any sort appear on them or subsequently on the blastomeres. Nuclear and cytoplasmic division go on, but the blastomeres are loosely strung together, resembling colonies of yeast cells. The cells usually lie in one—not more than two layers. In twenty-four hours the cells are quite small and have largely lost contact with each other as they lie on the bottom of the dish. The loss of the membrane precursor in the solution of non-electrolyte is irre-

versible, since if unfertilized eggs, after being kept in the solution of urea, are transferred to sea-water for thirty minutes and then fertilized, they develop exactly as do those eggs which are fertilized immediately after removal from the solution of non-electrolyte. There is no regeneration of membrane precursor.

The addition of Na or K ions does not alter the effect of the solution of non-electrolyte, except that eggs which had been kept in a solution consisting of 50 cc urea M/1 + 1 cc K Cl M/2 before fertilization, during development show ameboid forms. On the other hand, Mg, Ca, or Sr ions when added to the urea solution protect the egg by preventing the outward diffusion of the membrane precursor. If the eggs are kept two minutes in a solution consisting of $\frac{1}{2}$ cc of $\frac{3}{8}$ M solution of either $MgCl_2$, $CaCl_2$ or $SrCl_2$ + 50 cc urea M/1, and are then fertilized in sea-water, they form a comparatively inelastic membrane which closely invests the egg. This is membrane formation without elevation. As a result, nuclear division occurs without immediate cytoplasmic division, and the larva is a solid blastula not larger than the unfertilized egg since it is kept from swelling by the closely investing membrane. The addition of 2 cc of a solution of either $MgCl_2$ or $CaCl_2$ results in very nearly complete protection of the eggs, so that when they are fertilized, the fertilization membrane is formed and also elevated as in normal eggs. As a result, division is approximately normal and blastulae with cavities are formed. It is of interest to note that in this reaction Mg ion is equal to Ca ion in protective action, indicating a valence effect. In other physiological processes Ca ion is five to ten times more powerful in toxic or antagonistic action, than Mg ion. Sr ion is approximately twice as effective as either Mg or Ca. This shows a qualitative action in addition to the valence effect.

The results prove that membrane formation is of fundamental importance to the development of organisms consisting of closely associated groups of cells, i.e., metazoa. Furthermore, the results show the difference between membranes which are definite anatomical structures, i.e., the fertilization membranes and plasma membranes. In the typical case of division without membrane formation the cells are well rounded and separate, each cell has a globular form indicating the presence of a plasma membrane, but if the eggs have previously been treated with a solution of urea containing K ion then the plasma membranes are weakened to such an extent that the cells resulting from division may coalesce and show ameboid forms.

A. R. MOORE

UNIVERSITY OF OREGON