

THE ARTIFICIAL PRODUCTION OF EYE ABNORMALITIES IN THE CHICK EMBRYO

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TWO PLATES

During the springs of 1911 and 1912 a series of experiments were conducted on hens' eggs aiming towards a definite modification of development so as to produce typical defects. A large number of eggs was used and numerous methods of treatment with various chemical stimuli were employed. The results, however, have not been of a definite nature, nevertheless they do indicate a decided tendency on the part of the developing central nervous system to respond to certain classes of stimuli in rather typical fashions. The responses are not in any sense specific for a given treatment but the same rather definite response may be obtained by a number of methods. This statement applies equally to other experiments on the artificial production of definite defects in the embryo. The earlier view that these defects were specific responses to the given chemical substance employed as has been advocated by Herbst,¹ Hertwig, O.² the writer³ and others is no doubt erroneous.

The important fact, however, is that a certain definite response on the part of the developing organism may be consistently obtained after carefully adjusted treatments with a large number of different substances. Since the response is the same in each

¹ Herbst, C. Experimentelle Untersuchungen, u. s. w., Zeitschr. f. wissensch. Zool., 4, 1892; Mitt. a. d. Zool. Staz. zu Neapel, 1893; Arch. f. Entw-Mech. 4, 1896.

² Hertwig, O. Urmund und Spina bifida. Eine vergleichende morphologische teratologische Studie an missgebildeten Froscheiern. Archiv f. Mikr. Anat. Bd. 39, 1892; Die Radiumkrankheit tierischer Keimzellen. Ein Beitrag zur experimentellen Zeugungs- und Vererbungslehre. Archiv f. Mikr. Anat. Bd. 77, Abt. 2, 1911.

³ Stockard, C. R. The artificial production of cyclopian monsters: The "Magnesium embryo." Jour. Expr. Zool., 6, 1909.

case it is very probable that the substances though widely different act similarly on the embryonic organism, for example, in certain cases they may serve simply to lower the developmental metabolism and thus prevent or arrest the formation of particular structures.

The hen's egg readily lends itself to chemical and mechanical experiments and has been largely employed in experimental teratology. It has long been known that by running the incubator at too high or too low a temperature or by reducing aeration by varnishing the shell one is able to obtain a most varied group of monsters. Féré⁴ has used a great number of methods to produce monster chick embryos. In 1899 he treated eggs with alcohol fumes before incubation and found that the fumes penetrated the shell and produced various abnormalities in the embryos. Féré⁵ also repeated Preyer's⁶ experiment of removing the egg from the shell and allowing it to develop in glass dishes. Preyer was only able to keep the eggs under observation in this manner for two or three days, while Féré devised a better means of ventilation and succeeded in keeping the eggs alive for six days. Many of the embryos developing out of the shell showed abnormalities. Féré's reports merely record the experiments and mention the types of monsters obtained but no detailed or systematic study was undertaken and his experiments have generally passed unnoticed. One must, however, appreciate the rather ingenious and various methods of treatment which Féré employed.

The experiments to be briefly described in the present communication are presented in order to show that the central nervous system and the eyes of the chick embryo become affected in a manner closely similar to that which I have recorded for the fish embryos when treated with alcohol, ether and other substances.⁷

⁴ Féré, Ch. Influence du repos, sur les effets de l'exposition préalable aux vapeurs d'alcool avant l'incubation de l'œuf de poule. *Compt. rend. Soc. de biol.* 51, 1899.

⁵ Féré, Ch. Remarques sur l'incubation des œufs de poule privés de leur coquille. *Compt. rend. Soc. de biol.* 52, 1900.

⁶ Preyer, W. *Physiologie spéciale de l'embryon.* Trad. franc, p. 16, 1887.

⁷ Stockard, C. R. The influence of alcohol and other anesthetics on embryonic development. *Am. Jour. Anat.*, 10, 1910.

Hens' eggs were exposed for different lengths of time to the fumes of alcohol and ether. The eggs used in the experiments had been laid for only two or three days. Shallow dishes were arranged with a wire screen bottom beneath which absorbant cotton soaked with 95 per cent alcohol was placed. Eggs were placed upon the wire screen and the dishes covered and left standing at the room temperature. During the two years several hundred eggs were treated in this manner.

After the eggs have been exposed to the fumes for a short while the shell becomes covered with moisture, the condensed alcohol vapour and this vapour penetrates the shell. The eggs were exposed for from twenty minutes up to thirty hours at room temperature. The shortest exposure that gave effects was three hours and forty-five minutes, though in many cases an exposure of as long as eight hours was non-effective. Exposures of from fourteen to twenty hours gave the best results. In these cases almost every embryo was abnormal yet most of them were able to continue development for several days at least. Exposures of twenty-three hours or more were usually fatal, the eggs failing to develop after being put into the incubator.

The chief point to consider in the amount of exposure is the temperature. When the temperature is high evaporation is more rapid and more alcohol enters the egg in a given time.

If eggs are placed in the incubator immediately after the treatment liquid oozes out of the pores in the shell on account of the slight expansion of the egg contents as the temperature rises. A certain amount of the alcohol is no doubt lost by this process. It is better, therefore, to allow the eggs to remain at room temperature for several hours after being removed from the fume dishes and before being placed in the incubator. Féré found that eggs put into the incubator immediately after treatment with alcohol fumes were not so decidedly affected as those treated for the same length of time but not subjected to the raised temperature until several hours after the treatment.

In other cases eggs were exposed to the alcohol fumes while in the incubator. Weak alcohol solutions were placed below the egg tray and evaporated slowly. This treatment was also

continued for different lengths of time and in many cases gave more decided effects than those obtained by the treatments before incubation.

Ether fumes were also employed in the above manner. These fumes induce the same general types of developmental abnormalities though they are more decided in action than alcohol fumes and kill the embryos much more readily.

Several injection methods were used and a number of substances were injected into the egg but the results were indefinite and often negative. In many cases the injection was a failure in that it either coagulated the albumen in the region, or injured the egg so that it did not develop.

Following effective treatments with the fumes of alcohol or ether the embryos were found to be small and behind the control in their rate of development. The abnormalities most abundant were of a general nature, in some cases the entire body of the embryo was absent while the area vasculosa was present containing blood islands and embryonic vessels. Other cases showed small embryos with the brain portion of the neural tube poorly developed. The circulation in many of the embryos was slow and sluggish and in such cases hydramnious conditions were present and the blood sinuses were also distended.

A number of the embryos showed various abnormal eye conditions and these are the defects of particular interest since exactly similar abnormalities have been gotten in abundance when developing fish eggs are subjected to the actions of ether and alcohol. In several experiments embryos occurred with small poorly formed eyes which closely resembled the minute defective eyes most commonly found in alcoholized fish embryos.

A few typical cyclopean conditions were obtained showing different degrees of the defect. However, never more than three or four per cent of the embryos showed cyclopia even in the most successful experiments and in most instances cyclopia did not occur at all. Nevertheless, it is of importance to find that these treatments do occasionally induce the same variety of defects in the chick as was so abundant in many of the fish experiments.

The monster monophthalmicum asymmetricum, that is, an individual with one eye of the normal pair perfectly developed and the other eye either absent or defective to a marked degree, was commonly seen in the different groups of embryos, plates 1 and 2. This condition was more often found than cyclopia, yet it also was not as abundant as in fish embryos developing in solutions of alcohol or ether.

The failure to obtain definite defects in large numbers in the chick embryos is no doubt due to the fact that the amount of treatment is much more difficult to regulate than in such an egg as that of the fish. The great variation in the size of hens' eggs, the amount of albumen as well as yolk, the thickness of the shell, etc., makes it almost impossible to treat a number of eggs to the same degree. The treatment must of course be delicately balanced in order to obtain such typical defects as cyclopia and monophthalmica since they only occur as responses to a certain injury or arrest at a critical developmental stage.

It has also been found in a series of experiments which is being conducted to test the effects of alcohol and ether on the structure of the offspring from guinea-pigs that a completely eyeless young animal was produced and the nervous systems of almost all the offspring show some defects due to the treatment.⁸

During the winter of 1912 one of the incubators in the laboratory was placed in a room into which a ventilation system opened. The same system communicated with rooms in the chemical laboratory and fumes conveyed by the ventilator although rarely noticeable in odor were sufficient to injure the developing chicks. Many of the embryos died during early stages. The eggs were being used in tissue culture experiments by Dr. Burrows and were usually opened after having developed for about twelve to eighteen days. Several of these large chicks were found to have only one lateral eye. They were similar to the early embryos formed in the above experiments and were asymmetrical monophthalmic monsters identical with those I have described in

⁸ Stockard, C. R. The effect on the offspring of intoxicating the male parent and the transmission of the defects to subsequent generations. *Am. Naturalist*, vol. 47, 1913.

fish embryos. Photographs of three of these large chick embryos are figured in plates 1 and 2, since they illustrate the defect far better than the young three and four day twisted embryos. The fumes injured the eggs and caused the same types of developmental arrests or suppression as are obtained with the other substances discussed above. After the incubator was removed from this room the eggs in it developed in a perfectly normal manner.

These structural deformities and their experimental production are recorded to emphasize the general nature of such defects and their wide occurrence among different types of embryos when treated with any substance which tends to arrest development or lower their developmental rate and vigor. Elsewhere⁹ I have attempted to show how all abnormalities such as these eye structures may be explained merely as developmental arrests. Thus their wide occurrence in spite of their typical appearance.

PLATE 1

EXPLANATION OF FIGURES

Three views of an asymmetrical one-eyed chick monster which occurred in Dr. Burrow's incubator. The upper photograph shows the eyeless side, a small nodule of skin in the orbital depression represents an abortive eye-lid formation. The lower left figure represents the opposite side with a perfect eye, the fully developed lids are closed. The lower right figure giving a dorsal view of the head emphasizes the general asymmetry due to the absence of the one eye. The beak is permanently crossed since the upper jaw is forced to incline towards the eyeless side while the lower jaw remains in a normal position. It is thus impossible to close the beak as all the figures show.

⁹ Am. Journ. Anat., vol. 15, no. 3, 1913.



PLATE 2

EXPLANATION OF FIGURES

Two other specimens of monster monophthalmicum asymmetricum. The huge eye of the embryo chick is seen on one side of the head while the other side is eyeless. Both of these embryos also show the twisted upper jaw and the permanently open condition of the mouth.

