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THE EXPERIMENTAL TERATOROGICAL METHODS OF DIRECT INTERFERENCE OF THE FETAL BRAIN IN UTERO

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A considerable amount of research has been reported on the congenital malformations of the brain which are derived from the teratologen in the fetus through the maternal body, but little work has been done to study its experimental teratological methods of direct interference of the fetus brain in utero, because the mechanical or operative techniques in teratology have never been widely adopted. Hitherto, the investigators have been considered that the mechanical or operative techniques in teratology are not potent teratogenic agents, or they have only minimal application to the congenital malformations.

The pioneer work of the direct interference with the surgical method of the fetus on the mammalia in utero was done by Wallace (1917)[17]. In 1925, Nicholas[13] published an application of the experimental method upon mammalian embryos in utero. Nicholas (1942)[14] also published results of similar experiments on the rat embryos. Several years later, Wells (1946, 1950)[18][20] reported a method for subjecting fetal rats to laparectomy by surgery. After that, the effects of the interference with the fetal environment have been reported by Walker[16] (1959), Jackson et al (1960)[10], Knese(1960)[12], Skreb et al (1960)[15] and Brent et al (1960)[1].

About the decapitation of the fetus for the hypophysectomy, a few research has been reported, that is, in 1943 Raynaud[5] succeeded in provoking lesions in the pituitary of mouse fetuses by X-ray and a few years later Wells (1947)[19] surgically decapitated rat fetuses, Jost (1947)[11], rabbit fetuses, Foote and Foote (1949)[7], hamster fetuses, Case (1951)[3], chick embryos, Domm and Leroy (1951, 1955)[4][5], rat fetuses, Noumura (1960), rat fetuses, and Eguchi (1960)[6], mouse fetuses respectively. But the studies of the malformations of the brain as the result of the direct interference in utero are only Hall (1950)[8] who reported effects of the experimental incision on the brain in the rat.

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Among these published results, a method of the uterine vascular clamping by Brent (1960)[1] is very interesting report for me, but in generally, they have limited to general field of the congenital malformations.

It is very important to research the agents which occur the congenital malformation of the central nervous system resmbling the clinical syndrom.

For this purpose, the following operative procedures have been found and are widely used in experimental teratology.

MATERIALS

An inbred strain of pathogen-free rat with an extremely low rate of random malformations was used in this study. The Wistar's strain is fit for this experiment.

The rats were subjected to a 24 hour mating period, and vaginal smears were used to determine pregnancy.

From the 15th day to 20th day of pregnancy, the experiment was performed as following procedures.

PROCEDURE

1. *The Anesthesia*

Sodium nembutal (20 mg in 1 cm^3 sterile water per kilogram body weight) is generally injected intra-muscularly. The complete anesthesia, however, cannot be established sometimes by this quantity, so that the ether is used helpfully during the operation.

Avoiding the anesthesia of the fetus itself, the anesthesia by sodium nembutal seems to be more suitable than that by ether only. Besides, it is not necessary to administer an antispasmodic to the pregnant rat because of few abortion in my experiments, which stands for the fact that the surgical manipulations for the uterus hardly cause the contraction of the uterus.

2. *Exposure of the uterus*

After the above anesthesia, the pregnant rat (from the 15th to 20th day of pregnancy) is prepared on supine position on an operating board. The abdomen is shaved widely and carefully, not injuring the nipple, and sterilized with the tincture of iodine or the mercurochrome.

A median abdominal incision, approximately 3 cm in length, is made through skin and muscles, and held open by means of the hemostatic forceps, holding the sterilized covers for operation on the incised area. After the median incision of the peritoneum, the desired portion of the uterine horn is exposed from the abdominal cavity.

The exposure, in this procedure, of whole uterine horn on the sterilized covers (above mentioned) in order to select the aimed fetus is so convenient that the place and

number of the operating fetus is checked easily. It is preferable that the fetus for operation is selected as distal to the ostium uteri as possible, so as to prevent the abortion. When the fetus adjecent to the ostium uteri is operated, the abortion occurs in almost all of the cases.

Unless the fetuses are operated less than half of the total numbers in the uterine horn, the abortion occurs in high incidence.

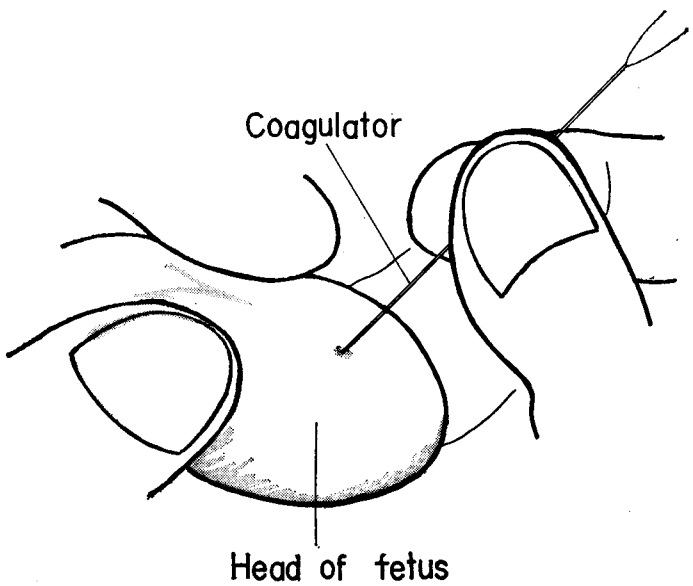
3. *Location of the head and interference of the brain*

The uterin wall is so thin and translucent that it is relatively easy to detect the outline of the fetus in the uterus and to locate its head. The head is then grasped gently and held between thumb and indexfinger of the operator's left hand.

The interferance of the brain is performed in two ways, one method is destruction of the brain with a small electro-coagurating needle, the other is ligation of the uterine vessels for a while, making the lesion in the brain due to anoxia.

The former method is to fix the electro-coagulating needle of 0.3~0.5 mm in diameter with operator's right hand into the aimed portion of the fetal brain between thumb and index-finger of the operator's left hand. Then the brain is destroyed by the coagulating needle with the continuous current of 0.2~0.5 mA, 10 V for 5 to 10 seconds. (Fig. 1)

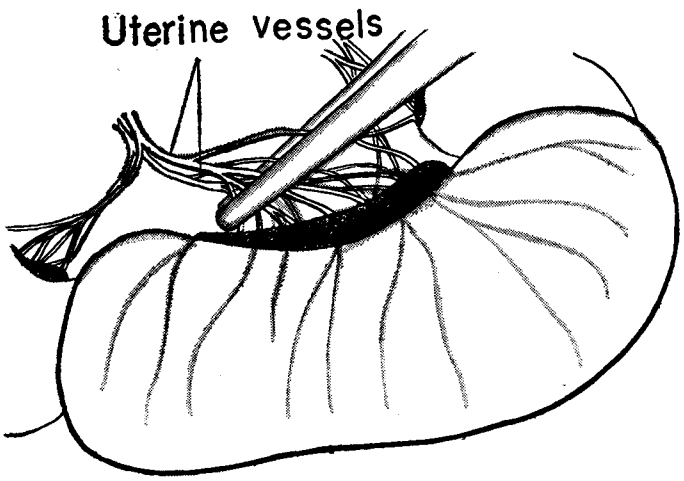
Fig. 1



The electro-coagulating needle is taken off from the brain later. Since the amnion fluid flows scarecely, the uterine wall is not necessarily sutured.

The latter method is to clamp the uterine vessels distributed to each fetus with hemostatic forceps or operator's fingers continuously for 5 to 10 minutes or in the repeated about ten times clamp of 2 minutes. (Fig. 2) The fetus, which becomes pale due to

Fig. 2



inadequate blood supply during the clamp, returns normal color when the clamp is stopped. As it ts impossible to clamp the only uterine arteries or veins, both vessels are clamped together. Almost all of the fetuses die, if the clamp is continued more than 30 minutes. The uterine vessels may be rather better to be clamped in proximal part than in distal to the placenta.

4. *Return the Uterus into the Abdominal Cavity.*

The exposed uterine horn of the uterus is returned into the abdominal cavity. The peritoneum is closed with interruped suture of fine (no. 3) catgut. After the wound has been cleaned, muscles and skin are sutured in the customary manner.

I have employed the procedure outlined above. Then these operated rats are placed into small individual cages with food and water. They are kept in a comfortable warmth and humidity for following 2 or 3 days. It is not necessary to administer a glucose nor an antispasmodic, but is better to inject antibiotics, for example a penicillin, to prevent the infection.

DISCUSSION

The destruction of a part of the brain by electro-coagulation cannot occur in natural situation, of course. It is, however, possible to issue the brain defect secondarily due to hemorrhage, ischemia, tumor or trauma. The brain destruction by electrocoagulation in my procedure stands for the fundamental experiment of the brain defect which may occur clinically,

This method follows hydrocephalus frequently whose mechanism is unknown and not being researched. Clinically hydrocephalus may develop in infants or adults as the result of occlusion of the cerebrospinal fluid pathways by tumors in the third ventricle or brain stem and congenital hydrocephalus are considered elsewhere, and my present

study is concerned only with the type of the hydrocephalus which results from obstruction of the normal flow of the cerebrospinal fluid by congenital maldevelopment of the brain or intra-uterine infections of the central nervous system. In my experiment, the intra-uterine infection has been prevented very carefully.

As far as clamping of the uterine vessels is concerned, the complete ischemia of the brain for a few minutes issues the degneration of the nerve cell. In my experiment the clamp of the uterine vessels more than 30 minutes makes a fetus die surely, but the clamp for a few minutes does not make a fetus die in normal condition, which is regarded that the uterine vessels to a fetus through the placenta have collateral vessels derived from the stem vessels to other fetus. This is convenient to make anoxia in fetus for a long time without making them die.

The brain of anoxia due to clamping of the uterine vessels shows the following pictures of the nerve cells. The cell body is swollen, and the nucleus is also swollen. The Nissl granules are found to be fragmented and undergoing a process of dissolution and finally disappears. The change in the Nissl substance is chromatolysis or tigrolysis. Moreover, the severe cellular damage is found, that is, large vacuoles appear in the cytoplasm. The nucleus is elongated, often triangular, and stains darkly. Injured cells show a complete disappearance of the Nissl substance. The cytoplasm appears homogeneous. Hence these pictures are frequently referred to as ischemic cell change.

There is also found minor hemorrhages in the cerebrum especially cortical or subcortical layer which interests me and inspires me the further study in comparison with the pictures of the cerebral palsy.

SUMMARY

Two methods are described for the experimental teratological studies of direct interference of the fetal brain in the uterus, that is, one is destruction of the brain with a small electro-coagulating needle, the other is clamping of the uterine vessels for a while, making the lesion in the brain due to anoxia. The procedures stand for the fundamental experiment of the brain defect which may occur clinically, for example, the cerebral palsy.

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PLATE 1.

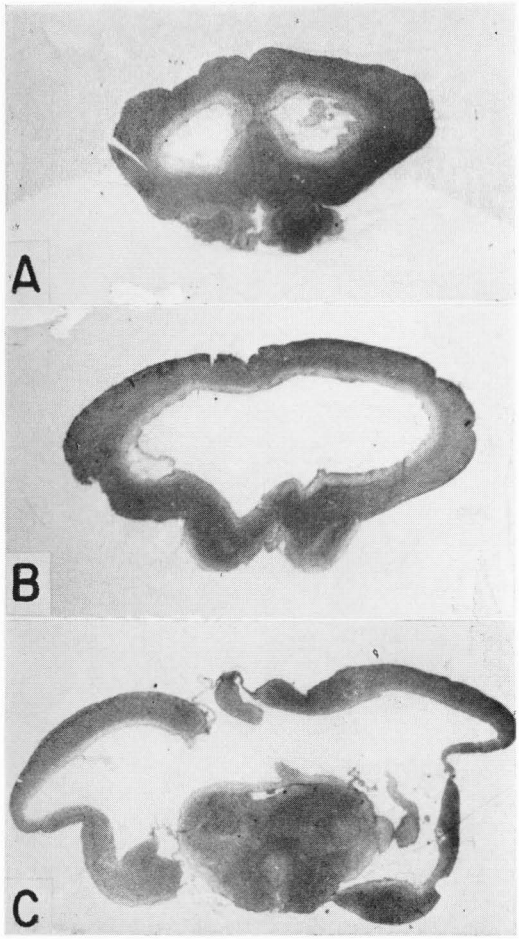

PLATE 1. Experimental hydrocephalus
Nissl stain ×5

PLATE 2. Hemorrhage in the cerebrum following anoxia (↙)
Hematoxylin-eosin stain ×170

PLATE 2.