Proteins were demonstrated also by simple staining after isotachophoresis on the strip of film. For this purpose the films were first fixed and rinsed to remove ampholytes in a 10% solution of TCA and stained with Amido black or with Coomassie R-250. In this way the optimal regime for fractionation of a test mixture of antigens can be quickly selected.

The variant of immunoisotachophoresis described above has special advantages for the analysis of micro quantities of antigens on account of their preliminary concentration and the absence of "smudging" of the zones during fractionation. It is also useful for the study of electrophoretic microheterogeneity of individual proteins.

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CHEMICAL MYELOTOMY IN GUINEA PIG FETUSES

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A method of immobilizing the fetuses of laboratory animals by chemical myelotomy is suggested: 96% ethanol is injected into the spinal canal.

KEY WORDS: chemical myelotomy; spinal cord.

Investigations on fetuses of animals, when removed by caesarian section into a thermostatically controlled bath, retaining their placental connection with the mother, are often complicated by the considerable mobility of the fetuses. Difficulties also arise during catheterization of the umbilical vessels (separation of the layers of the umbilical vessels, their detachment from the amnion), in the recording of brain electrical activity, in the maintenance of an artificial placental circulation, and during other procedures. Work with young fetuses of animals whose motor activity is continuous is particularly difficult in this respect. Immobilization of the fetuses, on the other hand, by means of curare-like drugs completely prevents observation on their behavior.

To limit the movements of fetuses the writers have used a method of chemical myelotomy (complete blocking of the spinal cord by means of chemical substances injected into the spinal canal). Chemical myelotomy (by the injection of 96% ethanol into the spinal canal) was first used with the aim of immobilization and anesthesia instead of division of the spinal cord in pregnant rabbits, as a more sparing operation [1]. Furthermore, classical anesthesia in very young experimental animals is a difficult procedure because of the limited volume of their subarachnoid space. Schwartze et al. [2], when using this method on pregnant guinea pigs, inject 96% ethanol in a dose of 0.1-0.2 ml intradurally at the level of the 1st and 2nd lumbar vertebrae. The only complication of the method of chemical myelotomy in adult animals, according to these workers, is the possibility of respiratory arrest. However, this complication, associated with the interruption of respiratory movements, is no threat to the fetus, whose gas exchange is maintained by the placental circulation.

EXPERIMENTAL METHOD

Chemical myelotomy was performed on 25 guinea pig fetuses at the 6th-10th weeks of intrauterine life. The pregnant guinea pig likewise underwent chemical myelotomy before the caesarian section operation. For the operation of chemical myelotomy an insulin syringe was used; this is convenient because it enables the

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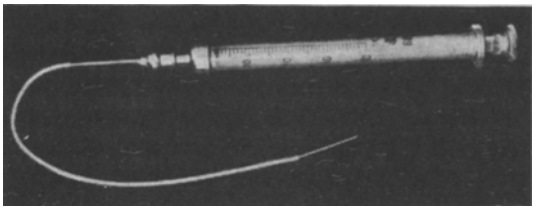

Fig. 1. Syringe for chemical myelotomy.

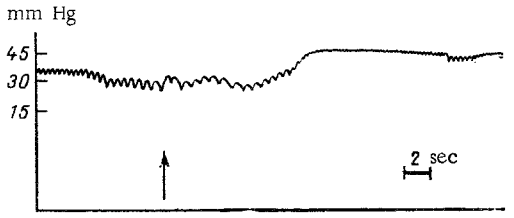

Fig. 2. Blood pressure curve of 9-week guinea pig fetus during chemical myelotomy. Arrow marks time of injection of 96% ethanol into spinal canal. Ordinate, blood pressure, in mm Hg.

dose of the substance injected to be accurately measured (Fig. 1). A thin polyvinyl chloride catheter was fitted on the needle of the syringe. An injection needle with sawn-off cannula was fitted into the free end of the catheter. The catheter between the needle and syringe facilitates entry of the needle into the spinal canal and giving the correct dose of ethanol. The needle end was inserted into the spinal canal of the fetus at the level of the 1st and 2nd lumbar vertebrae after palpation of the interval between the spines by means of the thumbnail. The needle was inserted between the spinous processes of the vertebrae to a depth of 2-3 mm, and 0.05 ml of 96% ethanol was expressed from the syringe. During this procedure the fetuses rested on a gauze towel stretched below the surface of the fluid, and only the region of the spine into which the injection was given projected from the fluid into the air. The head of the fetus remained in the fluid in order to prevent breathing. Chemical myelotomy was performed at different levels of the vertebral column. In one experiment chemical myelotomy was carried out on a fetus in the region of the foramen magnum. After injection of 96% ethanol into the spinal canal, the fetus was instantaneously immobilized. The state of shock lasted 1-1.5 min. Recording the arterial pressure of the fetus revealed a transient fall of pressure by 10-20 mm Hg, lasting 10-14 sec, followed by a return to the original level (Fig. 2). The duration of the fall of blood pressure was shorter than the duration of loss of all reflexes. As the signs of spinal shock disappeared, the reflexes from the upper part of the trunk, upper limbs, head, and ears were restored. Injection of ethanol into the spinal canal in a dose of 0.05 ml at the level of the 1st or 2nd lumbar vertebrae was followed by loss of spontaneous and reflex movements of the lower limbs. By increasing the dose of ethanol injected, immobilization of higher regions can be obtained: the upper limbs, neck muscles, and so on.

The results of these experiments showed that if ethanol in a dose of 0.05 ml is injected into the spinal canal of a guinea pig fetus at the level of the lumbar vertebrae the motor activity of the fetus is partially blocked, so that it becomes possible to carry out manipulations with the fetus freely without appreciably affecting its state.

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