# THE PATHWAY OF SODIUM FROM AMNIOTIC FLUID TO FETAL BLOOD*

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According to currently accepted theories, amniotic fluid is produced by the fetal excretory organs,[1,2] lungs[3,4] and the amniotic membrane.[5] Its disposal, on the other hand, is effected through the fetal lungs, gastrointestinal system, and amnion. Except for the role of the kidneys, evidence supporting these theories is mostly indirect and inadequate.[6,7]

The predominant reason for ambiguity in the experimental data of this field is the multiplicity of organs responsible for the function under consideration. Furthermore most of the investigations carried out on this subject do not permit clear-cut conclusions in reference to the part played by each individual organ.

In a recent series of experiments, we endeavored to specify the role of the amniotic membrane in the circulation of amniotic fluid.[8,9] The possible contribution of other organs in this process was entirely eliminated by the following experimental arrangement. In anesthetized pregnant ewes, with the umbilical circulation unhindered, the fetus was removed from the uterus and placed in a plastic bag containing Ringer's solution. A tracer ion, $Na^{22}$, was added to the amniotic fluid. It was found that radiosodium attains detectable concentrations in the fetal blood in less than four minutes. Since the fetus could not aspirate, swallow or absorb the tracer through its skin, it was concluded that the pathway would have to be either or both of the following: 1) Amnion, interstitial tissues of fetal membranes and the capillaries within them, or 2) Amnion covering the umbilical cord, Wharton's jelly and the tributaries of the umbilical vessels. In fact, the highest concentrations of radiosodium were recovered in samples taken from these areas.[8,9,10]

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In order to confirm these results, we undertook the investigations reported here in which the tracer is identified and visually demonstrated in each step of the two pathways considered above.

*Methods*

Each of eight pregnant ewes in the last month of confinement was anesthetized with Nembutal (25 mg/Kg) and atropine sulfate (0.30 mg). After tracheal intubation, the abdomen was opened under sterile conditions in the midline and the uterus exposed. Its vessels were identified on both sides and prepared for instant interruption of circulation. At a relatively avascular area, away from the easily identifiable cotyledons, a transverse incision was made. The serosa, myometrium, endometrium, chorion and amnion, in that order, were identified, dissected and incised. When the chorion was visualized, the position of the fetus was determined and the largest pocket of amniotic fluid located. Over this area a small incision was made and approximately 300 ml of amniotic fluid was drained into a container. The incision in the uterine wall and fetal membranes was then carefully extended to prevent loss of amniotic fluid or blood. The head of the fetus was slowly delivered, its muzzle enclosed in a tightly fitting rubber glove, and the entire fetus was placed in a plastic bag containing Ringer's solution at 37 °C (Figure 1).

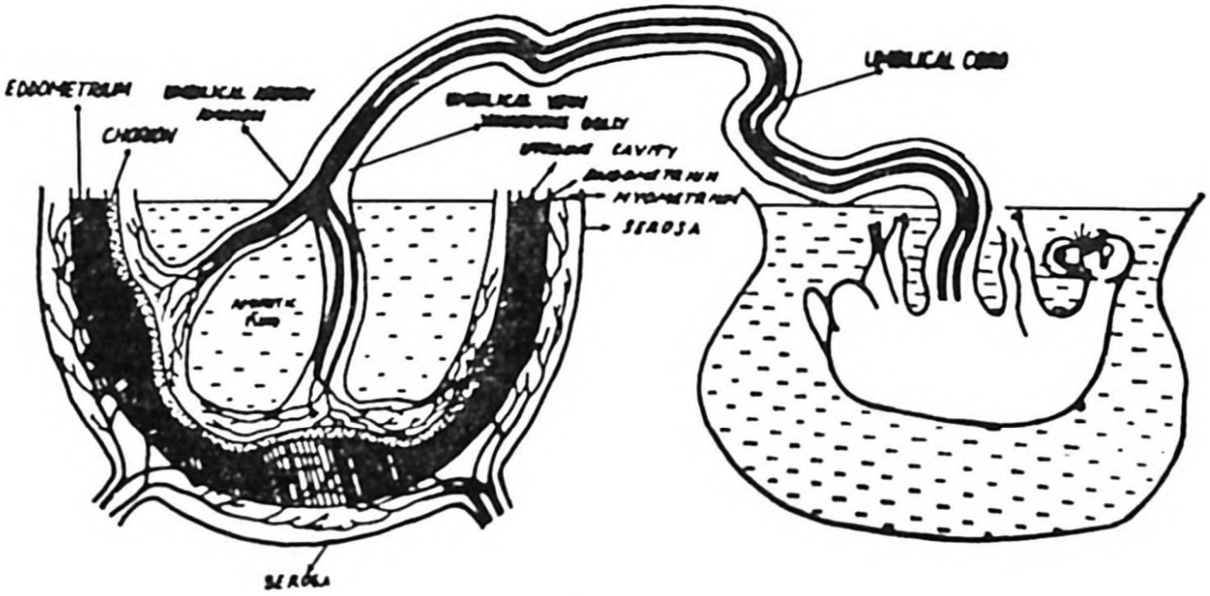


**Figure 1.**

The evacuated uterine cavity was examined for defects and the correct anatomical relationship of the fetal membranes. The previously removed amniotic fluid was then replaced. The pulsations in the umbilical cord were examined to be sure that they were vigorous

and regular. One to two ml of sodium fluorescein was then added to the amniotic fluid in the uterus and stirred. In this experimental preparation, the amniotic fluid with sodium fluorescein was in contact only with the amnion covering the cord and lining the amniotic cavity. Four to ten minutes were allowed for the tracer to reach the fetal blood stream and at the end of this period the uterine vessels and the umbilical cord were simultaneously clamped and severed. Hysterectomy was then completed, the abdominal incision repaired, and post-operative care was given to the mother sheep.

Multiple samples were taken in duplicate from the cord, membranes and the uterus. Care was taken to preserve the anatomical relationships of the membranes. One set of samples was fixed in formaldehyde solution and each specimen of the second set was separately wrapped in waxed paper, properly identified and immediately placed in deep freeze. A total of four slides was made from each tissue, two frozen and two paraffin fixed specimens. One of each was stained with hematoxylin and eosin, and the other was examined unstained. The slides were studied microscopically under an incandescent light and by ultraviolet spectrum. Photographs were made of the ultraviolet spectrum studies.

## *Results*

*1. The fetal membranes lining the wall of the amniotic cavity as a pathway of sodium fluorescein:* Figure 2 shows the amnion and the chorion in

**Figure 2.**

their anatomical relationship. The amniotic epithelium and the compact and fibroblast layers underneath fluoresce as irregular waves in the upper part of the photograph. Spongy layers, made up of remnants of extra-embryonic coelomic reticulum, appear as a loose network of fibers. There are brilliantly fluorescing particles in the lumina and wall of many blood vessels in this field. The reticular layer of the chorion appears brighter than the chorionic trophoblasts.

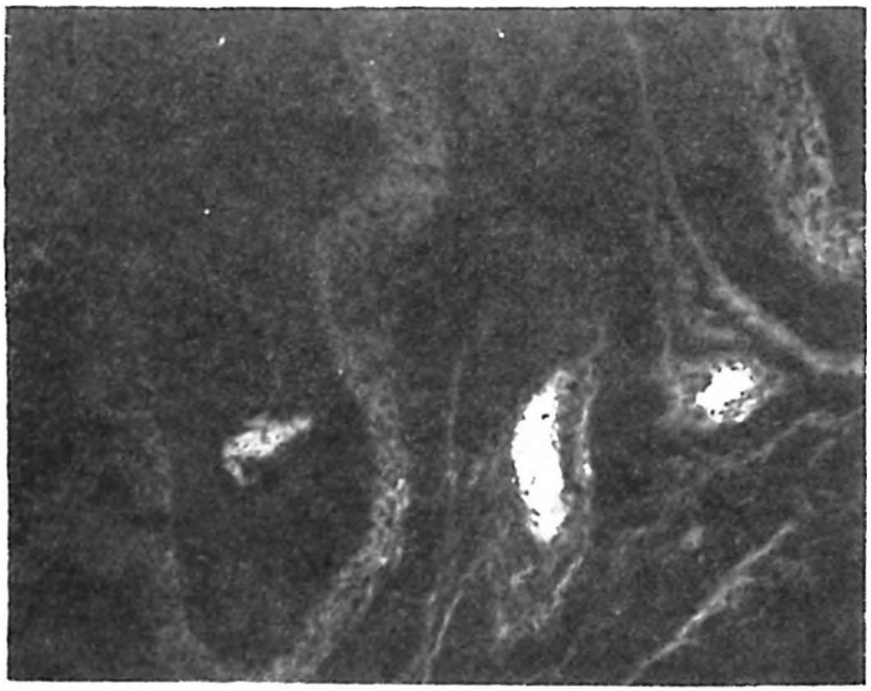

**Figure 3.**

Figure 3 shows a higher magnification of the amnion and a capillary in the spongy layer.

Figure 4 shows the two fetal membranes with the connective tissue between them. There are no vessels in this area and the tracer, as in Figure 1, is unequally distributed. Here the amnion is seen in the upper part and the chorionic villi are in the lower half of the picture. Note the very markedly fluorescent, round or oval spots in the villi representing the capillaries with blood in them.

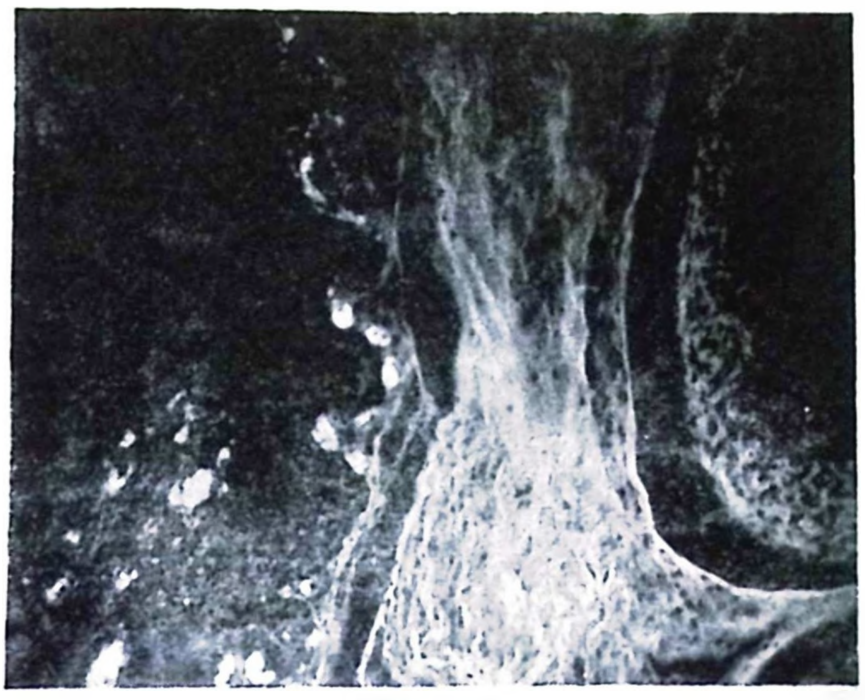

**Figure 4.**

Figures 5 and 6 show the chorionic villi. Strands of the spongy layers are seen in the upper third of Figure 5. The reticular layer

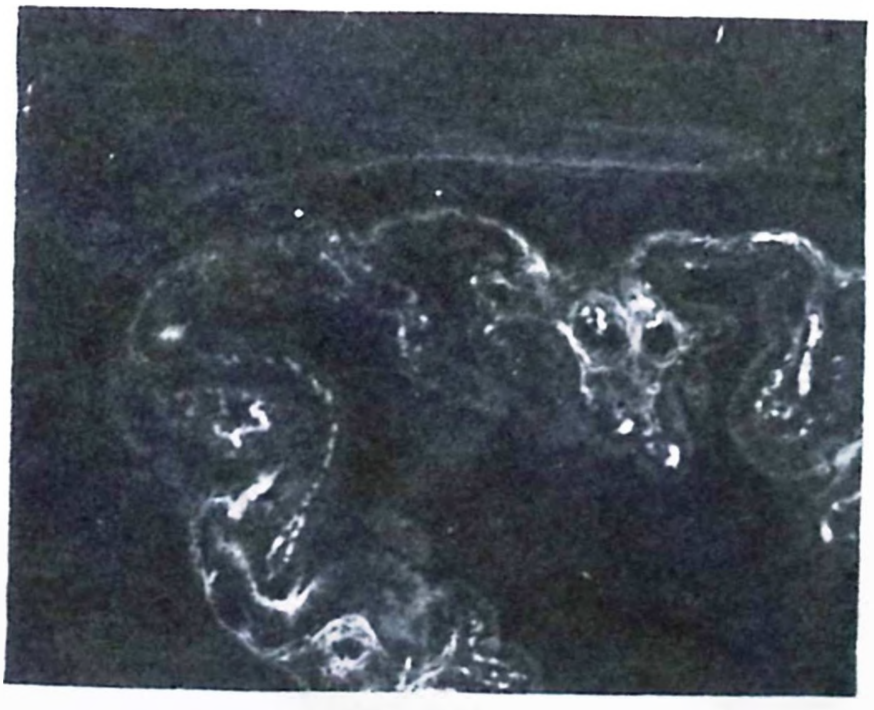

**Figure 5.**

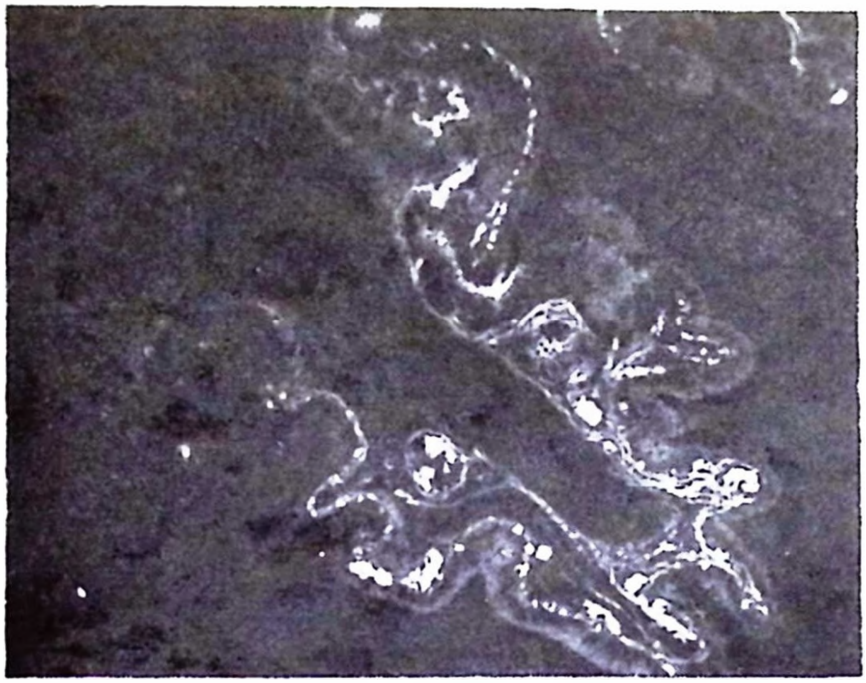

**Figure 6.**

of the chorion is very brightly fluorescent as are the capillaries. This rich vascular network is also seen in Figure 7.

**Figure 7.**

Figure 8 shows a chorionic villus in higher magnification with two allantochorionic blood vessels. Trophoblastic cells reveal little fluorescence in their cytoplasm and none in their nuclei. It is clearly seen in this photograph that the connective tissue is markedly fluorescent. Whether the tracer is in the fibers themselves or is contained within the interstices cannot be differentiated in this image.

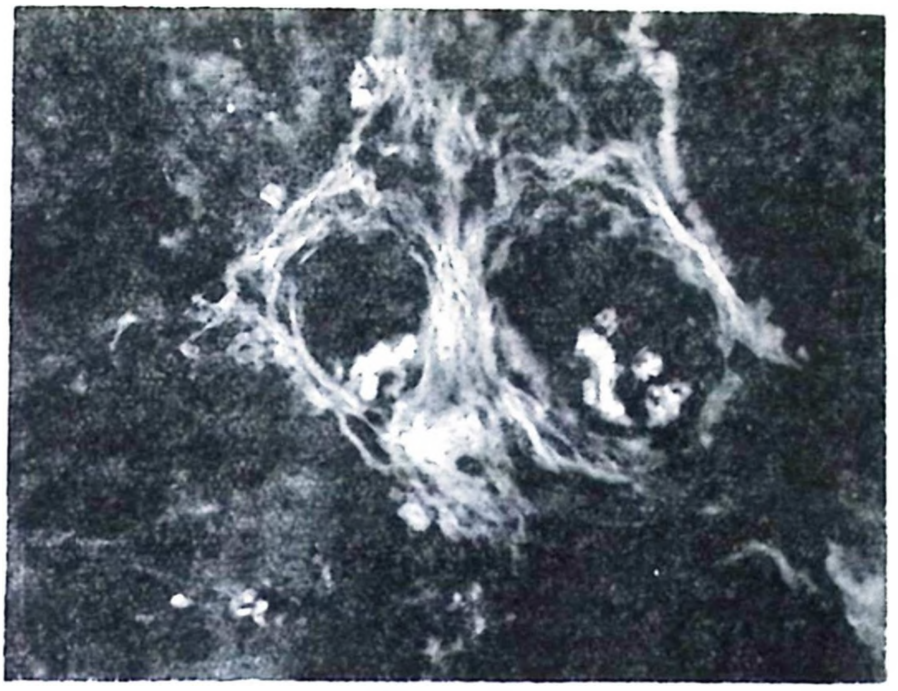

**Figure 8.**

Figure 9 shows a chorionic villus with its central blood vessel and the capillary network immediately under the trophoblastic layer.

**Figure 9.**

Figure 10 shows the relationship of maternal and fetal layers at a site away from the cotyledons. Here the chorionic villi are at the upper left corner beneath which lies the decidual epithelium. Between these two layers, the brightly fluorescent necrotic substance of "no man's land" is observed. Next come the silhouettes of maternal uterine glands, and the blood vessels of the mucosa; in the right lower corner is the myometrium.

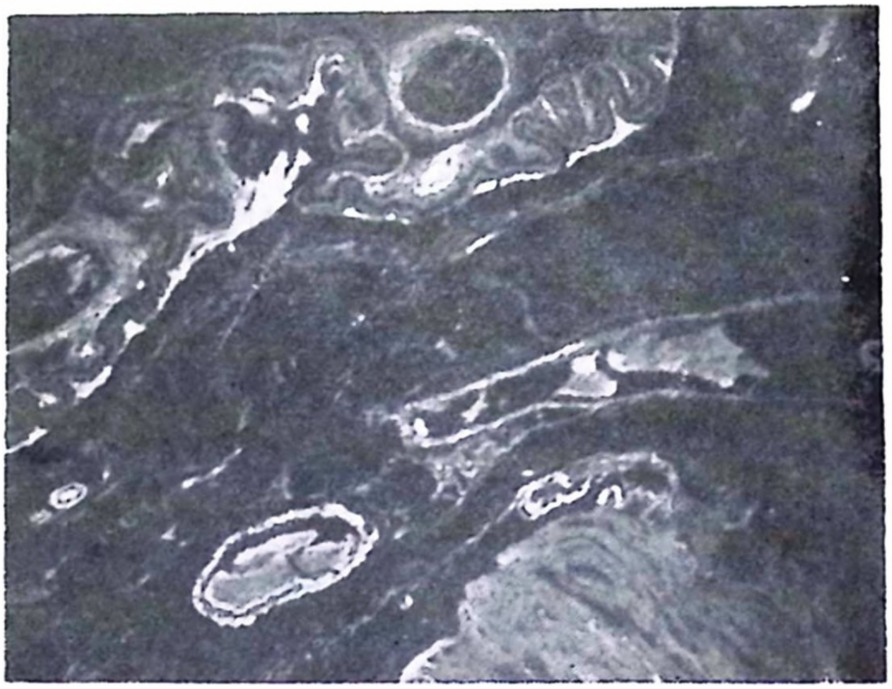

**Figure 10.**

Figure 11 is another example of the same area where fetal and maternal membranes meet. The maternal capillaries immediately beneath the endometrial epithelium are markedly fluorescent.

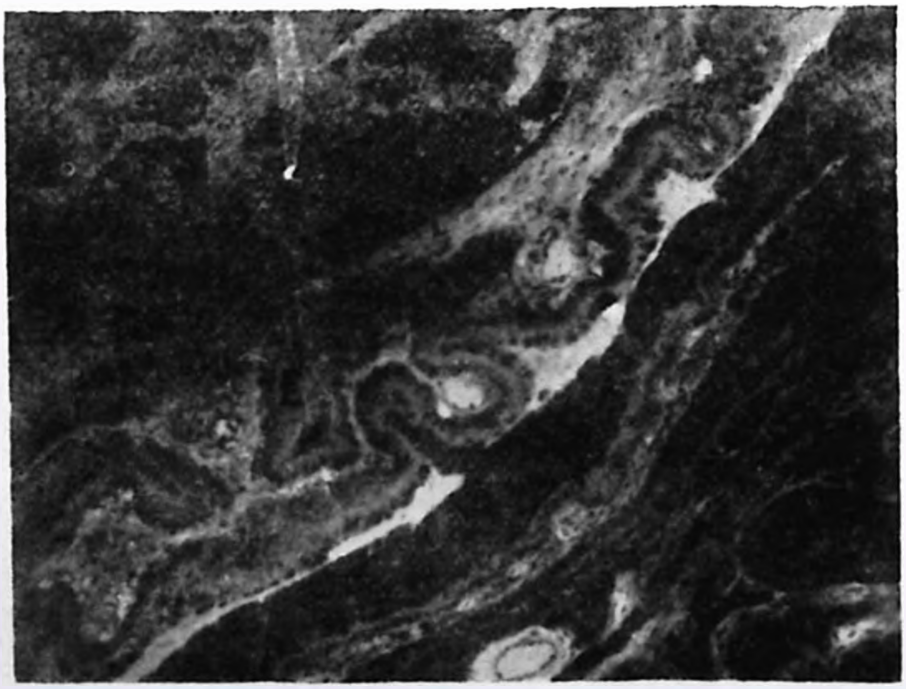

**Figure 11.**

*2. Umbilical cord as the pathway of sodium fluorescein:* Figure 12 shows a part of the cord. The outer layer of the amnion is seen above a rich capillary network within the Wharton's jelly. These blood vessels and the blood contained in them are viewed under higher magnification in Figure 13.

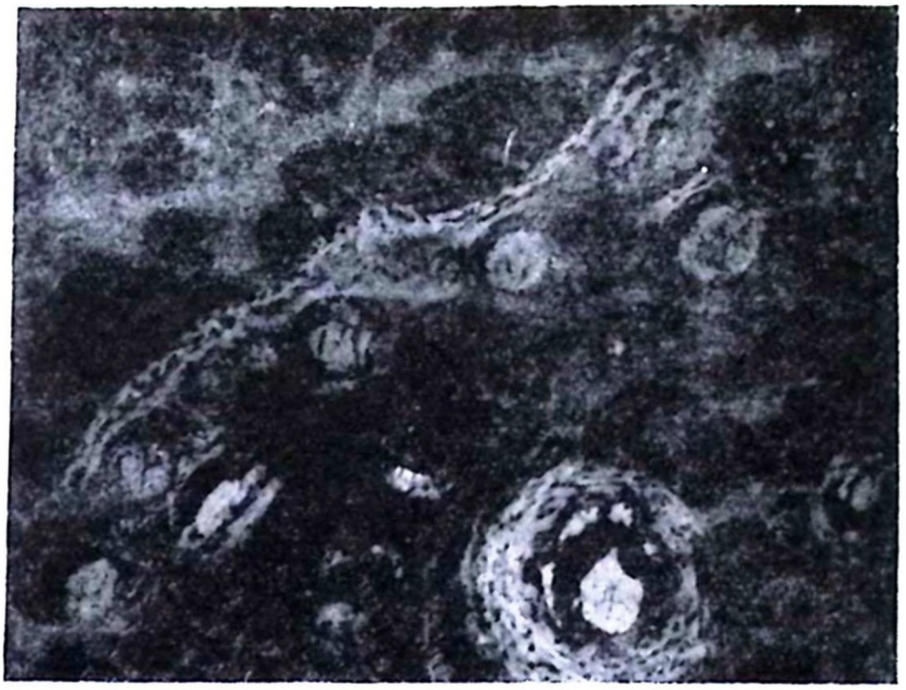

**Figure 12.**

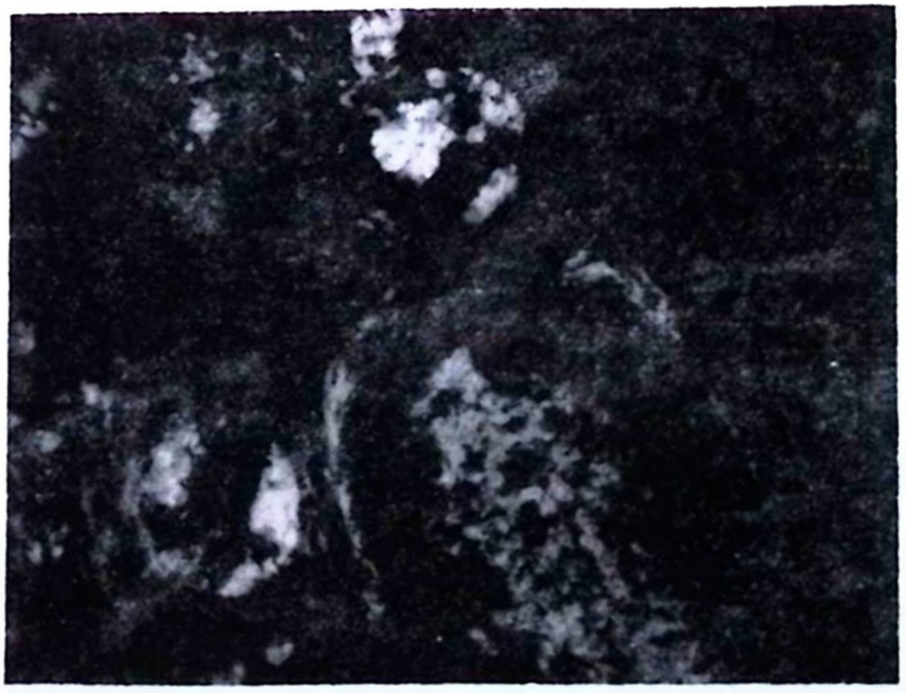

**Figure 13.**

*Discussion*

In the foregoing experiments, the tracer added to the amniotic fluid reached the fetal membranes as well as the maternal endometrium and myometrium. The question is by what route the dye arrived at these organs. In the experimental arrangement used here, in order that the tracer may reach the fetal interstitial space, it would first have to go through the amnion. This means that it has arrived at the interstitial space of the fetal membranes and from there it can, theoretically, follow either or both of these pathways: 1. It can diffuse through the walls of the tributaries of the cord vessels and reach the fetal blood stream. It can then gain access to all the fetal organs and, through the exchange in the cotyledons, to the maternal blood stream. 2. After penetrating the amnion, it can then go through the chorion and decidua and then enter the maternal circulation.

The photographic evidence presented here identifies the tracer within the amnion and in every step of the pathways from the amniotic cavity to the fetal and maternal blood streams.

In our former communications we presented evidence specifying the role of the fetal membranes in the transfer of radiosodium. This evidence is in complete agreement with the conclusions derived from the present studies which show that the transfer of sodium via fetal membranes is rapid and provides high concentrations in the fetal blood within 1.5 to 4 minutes.[10]

In the light of these results, it appears very unlikely that the fetal digestive tract or the lungs play a role in this very rapid process. Whether they contribute to the circulation of amniotic fluid at a slower rate cannot be inferred from the available data. It is still possible that either or both of these systems may be responsible for the transfer of certain particles with specific characteristics.

Studies by Wislocki and Snyder should be discussed and compared with our data. Both authors injected dyes into the amniotic fluid and after an interval of a few minutes to an hour recovered the dye material from the membranes, lungs, and gastrointestinal system of the fetus. It is important to note that in Wislocki's experiments the dye was in all fetal organs except the central nervous system. This would indicate that recirculation of the dye had occurred for a relatively long period of time. One may be misled as to the site of original entry to the blood stream because it could have come there secondarily without the fetal organ playing a role in its original uptake from the amniotic cavity.

Plentl, as a result of his experiments, has suggested that the umbilical cord acts as a pathway for the transfer of amniotic fluid. [11] A time lapse of at least 10 minutes between the removal of the umbilical cord in the delivery room and its arrival in the experimental laboratory was inherent in his experiments. Since anoxia is known to increase the permeability of the vessels, it is possible that his figures may have been somewhat high, exaggerating a process which also occurs under normal circumstances.

Enns and his collaborators demonstrated the tracer in the omphalomesenteric vessels in rabbits after its injection into the amniotic fluid. [12] His method of preventing fetal contributions in this process was rather traumatic and could easily have damaged the membranes and the capillaries which would of necessity increase their permeability. In a recent study, Scoggin et al presented evidence indicating that human fetal membranes play a role in the transfer of amniotic fluid. [13]

## *Summary*

In these experiments with pregnant sheep, the fetus was removed from the uterine cavity and with the umbilical circulation and the relationship of the fetal membranes to the decidua maintained, sodium fluorescein was added to the amniotic fluid. The possible contribution of the fetal lungs, gastrointestinal tract and the skin was therefore completely eliminated. The tracer was then identified in the fetal and maternal blood streams and its pathway from the amniotic cavity was traced step by step in each intervening organ. It is, therefore, concluded that the removal of sodium fluorescein from the amniotic cavity is affected by the fetal membranes lining the wall of the amniotic cavity and covering the umbilical cord.

It is possible that other constituents of the amniotic fluid might also use this same route in their circulation.

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