DIRECT OBSERVATION OF FETAL CEREBRAL CIRCULATION DURING MATERNAL CARBON DIOXIDE INHALATION

by

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Introduction

The 2% of man's body weight devoted to the brain requires nearly 20% of the oxygen utilized by the individual at rest. It is also estimated that, with changing metabolic needs, this may vary between 50% and nearly 200% of the resting requirement. (45) These observations have, for over half a century, fostered attempts to answer at least three related questions so aptly posed by Carl F. Schmidt, ". . . (1) What is the significance of the high metabolic requirement of the brain at rest, and of its variations directly with the functional activity of the organ? (2) How are increased oxygen requirements met? (3) What happens if they are not met?" (45)

Significant experimental evidence is accumulating to help define the theoretical concepts which will eventually become the answers to these questions. This evidence has not come rapidly, for of all organ systems of the body, the central nervous system, and its vascular support has been most resistant to analysis, largely because of the problems of developing the techniques required to observe change while maintaining homeostasis.

Answers to Schmidt's questions are important in the adult, but they become increasingly so in the child, the infant, and finally in the developing fetus. The fetus, in utero, is dependent for supply of metabolic materials

and removal of metabolic products, not only on its own circulatory system, but on that of its host. It must depend, also, upon transmission of these substances through a series of membranes in the placenta. Consider, also, that the central nervous system of the fetus at term comprises about 15% (41) of the body weight, and is the most rapidly metabolizing area of the fetal body because of its phenomonal growth rate during early development. The difficulties in obtaining the answers to Schmidt's questions, when applied to the fetus, now become legion. Not only must one be able to evaluate fetal hemodynamics, but develop methods to define placental blood flow, maternal hemodynamics, and the interaction between all three systems.

The adaptation and modification of techniques developed for microscopic examination of hemodynamics in the organs of living adult mammals have made possible direct observation of regional blood flow in the living fetus. (8) These developments have enabled me to observe the circulation to the brain of the fetal rat, in vivo, and to record the changes in vessel diameter while the mother is exposed to high concentrations of carbon dioxide. The function of this study has been to explore the possibility that this new, essentially unproved, method might be of value for investigating the effects of respiratory gas mixtures, available to the mother, upon the fetal cerebral circulation. The clinical concept of "saving neurons" in

the peri-natal period is an important one, and this method should be examined for possible usefulness in studying ways of increasing cerebral blood flow in the fetus near term when fetal anoxia often presents severe problems.

While almost nothing is known concerning the physiology of the cerebral circulation in utero, a great wealth of information has accumulated concerning cerebral hemodynamics after birth. Several reviews on this subject are available in the literature. (32, 49, 54)

Methods of Studying Cerebral Circulation

Cerebral blood flow in the mammal has been studied by a variety of techniques, each of which has advantages, but each of which is also handicapped by certain inadequacies. A short discussion of the most important methods will be presented here.

Direct Observation: Many of the early observations on cerebral hemodynamics were made by the simple process of opening the skull, removing a patch of dura mater, and observing changes in the diameter of the pial vessels on the surface of the cerebral cortex. The most sophisticated of such approaches, by Forbes (15) and Wolff, (55) incorporated a sealed glass window. This allowed for normal cerebrospinal fluid pressure and permitted observation in the unanesthetized state. As Sokoloff (49) has pointed out, these procedures provided only qualitative data, demonstrated relative vasomotor responses

rather than cerebral blood flow, and provided no information about responses of the cerebral arterioles after they submerge into the cortex. However, direct observation is the one method available which does provide direct evidence of one of the two major parameters affecting cerebral blood flow, ie., vasomotor response.

Thermoelectric Measurements: Another method utilizes a hypodermic thermocouple developed by Gibbs. (19) After insertion in the internal jugular vein, changes in quantity of blood flow can be determined by the rapidity with which the warmed thermocouple is cooled by the venous return from the brain. These devices have also been placed in various areas of the brain, and provide qualitative data on the regional changes in blood flow under various experimental conditions. (45) Changes in blood temperature, changing environmental temperature, thrombosis around the needle, and other difficulties may lead to incorrect measurements using this technique, but despite these limitations much reliable qualitative information is obtainable by this method. (49)

Artificial Perfusion: Finesinger (14) and Geiger (18) developed surgical procedures for isolation of the cerebral circulation, and were able to perfuse the brain at constant pressures, thereby observing the effects of various drugs while avoiding the effect of systemic changes in blood pressure. The difficulty of such a sur-

gical preparation, and the alterations possible produced in homeostatic mechanisms, can be envisioned.

Arteriovenous Oxygen Differences: Since the difference in oxygen content between the arterial blood perfusing the brain and the venous blood leaving the brain is dependent upon the metabolic rate of cerebral tissue and the rate of blood flow, one can get an accurate estimate of the amount of cerebral flow by a measure of the A-V oxygen difference, provided the metabolic rate is constant. Venous blood of cerebral origin and without systemic contamination is difficult to obtain in common laboratory animals, except the monkey, but is readily obtained from the jugular bulb in man. This has provided apparently accurate estimations in the hands of Myerson [31] and Gibbs. [20]

Inert Gas Method: The nitrous oxide method of Kety and Schmidt[22,24] was developed about twenty years ago. It has been widely used, and has become the standard method of obtaining quantitative data, especially in man. Evidence concerning the solubility of N_2O in brain tissue indicates that after ten minutes of inspiration of N_2O containing air, an approximate equilibruim is reached between the brain tissue N_2O and the concentration in venous blood returning from the brain. [21] It is, thus, assumed that the concentration of N_2O in the ten minute venous sample is equal to the concentration per unit weight of

brain tissue. Critical analysis of the basic concepts of this method has been presented by several authors who have suggested a number of sources of error. (1, 13, 40, 43, 49) However, it remains the single most useful and reliable method for quantitative estimation of the flow through the cerebrovascular tree. Modifications based on the original method have utilized bilateral internal jugular sampling, and radioactive Krypton to provide continuous measurement of rapid changes in flow and give a more accurate representative sample of venous return. (27, 28)

Non-Diffusible Tracer: The use of Thorium-B labeled erythrocytes injected into the internal carotid artery have provided quantitative results by dilution techniques. Bilateral internal jugular sampling and rapid evaluation provide a basis for continuous measurement of changes in cerebral blood flow. (34)

Radioactive Inert Gas Technique: Tissue uptake of an inert gas is dependent on arterial concentration, diffusibility into the tissue, rate of blood flow, and the amount of time during which the gas is available to the tissue. Sokoloff et al. (51) infused cats with I^{131} tagged trifluoroiodomethane by the intravenous route and continuously recorded the arterial concentration during a fixed time. They then removed and froze the animal's brain, and were able to calculate blood flow in twenty-eight brain areas from radioautographic analysis of brain sections.

Factors Affecting Cerebral Circulation

Investigation of cerebral hemodynamics was likely retarded by blind acceptance of the views of Munro (1873) which were later expanded by Kellie (1824) and became known as "The Munro-Kellie Doctrine". (54) Munro expressed the theory that since the skull possessed a fixed volume, the amount of blood which could pass through the cerebral contents was unchanging, unless hydrocephalus or other intracranial mass expansion reduced the amount of blood by an equal amount. (30) This remained the reigning concept until the latter part of the 19th century.

The break from the theoretical concepts of the "Munro-Kellie Doctrine" evolved slowly. In 1890, as a result of intra-cranial pressure studies, Roy and Sherrington concluded that " . . . the chemical products of cerebral metabolism contained in the lymph which bathes the walls of the arterioles of the brain can cause variations of the calibre of the cerebral vessels . . . in this reaction the brain possesses an intrinsic mechanism by which its vascular supply can be varied locally in correspondence with local variations of functional activity." (39)

Since the beginning of this century, by development and application of the methods described above, the factors controlling and altering the cerebral circulation have been defined in the adult organism. However, the

specific mechanisms controlling the cerebral vessels remain unknown. It is now generally accepted that there are two major factors which determine the amount of cerebral blood flow: (1) The pressure gradient between inflow and outflow pathways and, (2) the vascular resistance in those pathways. These factors tend to operate in such a manner that they maintain adequate homeostasis for brain metabolic functions.

The pressure gradient across the cerebral vascular bed is controlled by mechanisms outside of the brain itself. Cerebral venous pressure is so nearly atmospheric, except in severe disease states, that the perfusion pressure becomes that of the systemic blood pressure. It has been demonstrated in animals, including man, that severe changes of mean systemic arterial pressure are required before there is a significant diminution of cerebral blood flow. (26, 47) Since systemic arterial pressure is directly proportional to cardiac output, one might expect to see markedly decreased cerebral blood flow in certain cardiac conditions. However, only in low output states severe enough to reduce cardiac output by greater that 1/3, did Novack (33) find a decrease in blood flow to the brain.

Rapid changes in intracranial vessel tone very effectively maintain the cerebral blood flow over a broad range of systemic pressure. The exact mechanisms controlling changes in vessel size have not, as yet, been elucidated.

It is, however, quite clear that the control of the intracranial vasculature is jealously supervised by the brain itself. (47)

McNaughton has demonstrated nerve fibers accompanying the dural, pial, and intracerebral vessels, in man and other animals. (29) These include sympathetic postganglionic fibers from the stellate and superior cervical ganglia and parasympathetic fibers from the facial nerve, via the geniculate ganglion and the greater superficial petrosal nerve to the internal carotid plexus. (7) However, investigations concerning their effectiveness in producing either vasoconstriction or vasodilatation have either produced contradictory results or shown minimal influence. (11,16) Most investigators agree that, in contrast to the peripheral circulation, the role of neurologic control of cerebral vasomotor tone is limited. However, the actual extent to which observed nervous elements affect intracranial vascular tone remains obscure.

Blood viscosity also affects the resistance in the cerebral vessels, but changes large enough to produce alterations in cerebral blood flow which could not be buffered by other mechanisms are rare. (49) An increase in intracranial pressure will increase the resistance to blood flow, but this is adequately buffered by a compensatory increase in systemic blood pressure, and the cerebral blood flow is not compromised in man until the intracranial pressure exceeds 450mm. of water. (47)

While each of the factors discussed has some small effect on cerebral blood flow, the extent of alterations recorded has been inadequate to account for the degree of vasomotor change which has been observed. It is now evident that the control of cerebral vascular resistance is attributable to the direct effect of carbon dioxide and oxygen on the intracranial vessels. In all animals tested, including man, cerebral blood flow is consistently, directly proportional to the arterial concentration of CO_2. Studies in animals by direct observation have demonstrated vasodilatation in response to CO_2 inhalation and vasoconstriction in response to hyperventilation. (48,55) The vasomotor action elicited by carbon dioxide has been quantitatively evaluated in man. Inhalation of 5% CO_2 produced a 50% increase in cerebral flow and 7% CO_2 led to an increase of 100%. (35) Production of hypocapnia by voluntary hyperventilation lowered the blood flow to the brain to about 60% of normal. (23) This vasomotor response is unrelated to pH changes not produced by altered PCO_2. (49)

Arterial O_2 concentration also has a significant effect on cerebral blood flow. In hypoxemia, the cerebral flow is inversely proportional to the PO_2, and if profoundly low may overcome the vasoconstriction produced by hypocapnia. The effect of increased PO_2, however, is minimal and overall the effect of carbon dioxide far surpasses that of oxygen. (44)

The cerebral circulation seems to be somewhat unusual in its susceptibility to alteration of vasomotor tone under the influence of carbon dioxide. While 7% CO_2 in the inspired gas mixture seems to produce maximal vasodilatation of cerebral vessels, it produces relatively little effect on vasomotor tone in other organs. (50) It has been shown repeatedly in adult mammals, including man, that CO_2 inhalation produces moderate changes in systemic arterial pressure and heart rate with significant increase in cardiac output. (24, 38) However, after interruption of the known vasomotor pathways to other vascular beds, they reacted to carbon dioxide concentration in the same manner as did the cerebral vessels. (50) It is, also, of interest that Assali noted an increase in uterine blood flow in the pregnant sheep in response to inhalation of 8% CO_2. (2)

Recently the effects of 5% CO_2 inhalation were studied in the rat by Takács and Kállay (52) who found a slightly increased cardiac output, accompanied by a mild drop in blood pressure and overall peripheral resistance. Results with 20% CO_2 in air were in the same direction, but more marked. However, they used pentobarbital anesthesia, which supresses respiration and the blood PCO_2 levels obtained may have been in excess of those usually obtained with 5% CO_2 inhalation. No studies were found which determined the changes in rat cerebral blood flow

during inhalation of carbon dioxide mixtures.

Fetal-Maternal Relationships

During the past several decades, there has been increasing interest in defining the relationship between the pregnant female host and the fetus. The fetus exists in a truly parasitic form, with the maternal organism providing protection, nourishment, and excretion of metabolic wastes. This relation is mediated through the temporary existence of the placenta which functions as the major lung, kidney, and gastrointestinal organ for the fetus.

The generally accepted opinion is that the placenta transfers materials between fetal and maternal circulations by both passive diffusion and active transport. It also acts as a protecting barrier against other substances. (53) Anatomical aspects of the placental circulation have been carefully studied with respect to both fetal and maternal components. While many forms of placentae exist among the mammals, one of the greatest anatomical differences concerns the proximity of fetal to maternal blood. The human placenta has been shown to be of the cotyledonous hemo-chorial type in which villi, containing trophoblast covered fetal capillary loops, are bathed in sinuses containing maternal blood. All other mammalian groups, except the higher rodents, have a greater number of tissue layers through which substances must pass to

gain access to the fetal circulatory system. The rodent has a labyrinthine, hemochorial placenta which differs only in that, rather than freely hanging villi, the fetal blood flows through an interconnected maze of trophoblast covered fetal vessels which are bathed with maternal blood. It has long been accepted, but not adequately demonstrated, that the placental anatomy of rodents and of primates should allow for optimal transfer of freely diffusible substances because of the close proximity of fetal and maternal blood. (53)

Since the basic control of cerebral blood flow is attributable to the respiratory gasses, it is of prime concern to understand the basic relationships between these gasses in the fetal-maternal relationship. From work on carbon dioxide and oxygen in the pregnant sheep, Prystowsky and others (36) have shown that both gasses are transferred across the placenta by passive diffusion. The oxygen concentration gradient between fetus and mother seems to vary considerably under different conditions and always in the direction of providing adequate fetal oxygenation, The mechanisms which control the amount of oxygen transferred from maternal to fetal blood are poorly understood at present. However, it has been shown that the gradient between fetal and maternal concentrations of carbon dioxide remain quite stable under many conditions, and that a rise or fall in maternal PCO_2 is accompanied by a similar alteration in fetal PCO_2. This indicates simple rapid diffusion. (36)

Studies have not been conducted in the rat, but in the rabbit, a closely related rodent, Barron found the transplacental oxygen pressure gradient to be of the order of 10 mm. Hg. higher on the maternal side. (5) The transplacental CO_2 gradient of the rabbit was found, by Young, to be negligible. (56) It would appear that, while the rodent fetus may live in a rather hypoxic state, CO_2 is transferred very rapidly across the placenta for excretion. The direction of transfer of CO_2 seems to depend on the relative concentration in the two circulating bloods.

Studies in profusion have appeared in recent years attempting to ascertain the effect upon the fetus produced by various factors which alter maternal placental blood flow, or have known effects on maternal circulation. Most of these studies consist of evaluation of fetal heart rate and blood pressure after introduction of drugs and hormones into the maternal circulation or production of hypoxia in the fetus. A multitude of methods have been employed to produce a hypoxic condition in the fetus, including administration of a low O_2 concentration gas mixture for maternal respiration, clamping of the uterine artery, and segmental or complete occlusion of the umbilical vessels. Most investigators found a consistent increase in systemic fetal arterial pressure. (3,6,37) The effect upon the heart rate has been variable and Born

has attributed this to the degree of hypoxia produced, claiming that moderate hypoxia leads to tachycardia, while severe hypoxia slows the fetal heart. (6) Many of these procedures, especially clamping of maternal or fetal vessels, must also affect the level of CO_2 found in the fetal circulation, but very few studies have attempted to ascertain what effect an increased PCO_2 might have on fetal circulation.

The literature is lacking in studies concerning changes produced in fetal hemodynamics during maternal CO_2 inhalation. Assali et al. (2) (1962) utilized electromagnetic flow transducers to measure the effect upon fetal carotid artery blood flow in the sheep fetus, while the mother was subjected to inhalation of 8% carbon dioxide in air. They found an increase in carotid blood flow, accompanied by an increase in systemic arterial pressure, a mild bradycardia, and an increase in femoral and umbilical blood flow. They reasoned that the increase in carotid blood flow was secondary to the rise in systemic arterial pressure since both parameters changed in the same direction. No mention is made of the possible effect of cerebral vasodilatation produced by fetal hypercapnia. It is also of interest that the rise in cerebral blood flow of nearly 70% preceded the rise in arterial pressure by about four minutes and had nearly reached its peak before the rise in arterial pressure began. If the effect

of fetal hypercapnia is contrasted to the effect of hypoxia in Assali's study, one notes that, hypoxia produces an identical increase in arterial pressure, but only a 40% increase in cerebral blood flow. More importantly, during hypoxia, the rise in cerebral blood flow and systemic arterial pressure begin coincidentally. This would lead one to assume that there were other factors affecting the increase observed in cerebral blood flow besides the systemic arterial pressure. In view of known cerebral vasomotor responses in the adult, fetal cerebral vasodilatation would provide an explanation for these differences.

There is a dearth of information concerning effects produced on fetal regional blood flow by alterations in the maternal circulation. Geber [17] (1962) measured fetal circulatory reactions to various changes in the maternal circulation in the rabbit, sheep and dog. He measured the fetal carotid artery blood flow with electromagnetic flow meters, and the carotid artery blood pressure for quantitative changes. The thermistor method was used to monitor qualitative change in cerebral blood flow. He subjected the maternal circulation to various forms of stress including vagal stimulation, pain, asphyxia, and drugs such as acetylcholine, nembutal, epinephrine, norepinephrine, and serotonin. He concluded that the fetal heart rate and systemic blood pressure were unre-

liable measurements of fetal response, and that fetal kidney or cerebral blood flow gave more constant and reliable indications that a fetal response had occurred.

When fetal responses were measured simultaneously in two fetuses from the same litter, they often exhibited variations in vasomotor tone during control observations, as well as experimental procedures. At times one fetus exhibited vasodilatation while the other demonstrated vasoconstriction, or one of the two fetuses under examination showed no reaction at all. Geber interprets this as a sign of variable physiological orientation of each fetus in utero. This physiologic orientation may be dependent upon the anatomical site occupied by each fetus in the uterus and the physiologic reactions of that uterine site. However, this variable susceptibility seems to determine individual fetal responses to maternal influences.

In summary, there are certain basic concepts available in the literature which are pertinent to this paper:

(1) Inhalation of 7% carbon dioxide in air by adult mammals results in hypercapnia.

(2) Hypercapnia produced by carbon dioxide inhalation causes cerebral vasodilatation.

(3) There is a PCO_2 gradient between maternal and fetal blood. The fetal blood maintains a slightly higher carbon dioxide concentration, and varies directly

with the maternal concentration.

(4) Carbon dioxide is transferred across the placenta passively.

(5) Cerebral vessels in the rodent, at this stage of gestation, have been shown to have vasomotor capability.

(6) There is very little known about the effect of maternal hypercapnia on the fetus, and there is no information available concerning its effect on the cerebral blood flow or vasomotor response.

On the basis of these concepts, the following study has been undertaken to determine the qualitative effect of maternal hypercapnia on the cerebral vessels of the rat fetus, and to evaluate the potential usefulness of this relatively new method for direct observation of fetal vasomotor physiology.

Materials and Methods

All observations presented in this thesis were performed on fetuses from first litters of female rats of the Sprague-Dawley strain, mated to Sprague-Dawley males. The fetuses were between 14½ and 16½ days gestational age. The mothers varied in age between three and nine months, and were maintained in standard wire cages with food and water ad-lib. Their diet consisted of Purina Lab Chow tablets.

Vaginal smears were examined to determine the stage of the estrous cycle. Animals found to be in estrous or late pro-estrous were placed with males for periods ranging from six to twenty-four hours. Vaginal smears were repeated at the end of the mating period, and those found to have sperm in the vagina were placed in dated separate cages. The midpoint of the period during which they had been with the males was considered as the beginning of the gestation period. The animals were housed in an inverted light cycle room which was dark from ten A.M. until ten P.M. Since the optimum mating period for rats is considered to be near midnight, the light cycle room made it possible to mate the animals for short periods of time in mid-day.

Anesthesia

Light anesthesia of the pregnant rats was

attained by intraperitoneal injection of 50% urethane in 10% ethanol. The initial dosage was 0.2 cc./100 grams body weight. The response to this anesthetic was found to be quite variable and occasionally additional small doses were required as determined by response to manipulation or pain. If anesthesia was considered sufficient to maintain the animal in a motionless state, but not sufficient for surgical procedures, they were exposed to ether inhalation for short periods. Depth of ether anesthesia was determined by response to pain and depth of respiration. At no other time was ether used as an anesthetic.

Surgical Preparation

After the anterior neck region was shaved, a midline longitudinal skin incision was made from the mandible to the sternum with lateral extensions for about one centimeter along the clavicles. This type of incision insures adequate room for insertion of the tracheal cannula. By blunt dissection, the two large anterior lymph glands were separated in the midline, and the sternomastoid muscles dissected free from surrounding fascial and areolar tissue. The sternohyoid muscles lie superficial to the trachea, and their fibers were split longitudinally from origin to insertion, reflected laterally, and held in place by bulldog clamps. The trachea was then dissected free, with care being taken to avoid disruption

of the fragile thyroid and inferior thyroid artery which lies infero-lateral to the trachea. A 3/0 silk suture was passed under the trachea. A horizontal incision between two of the tracheal cartilage rings, so as to leave only the posterior wall of the trachea intact, allowed ample room to insert the cannula, which was fashioned from the mouthpiece of a hematology pipette. The cannula was then made fast with the silk suture. (Figure 1)

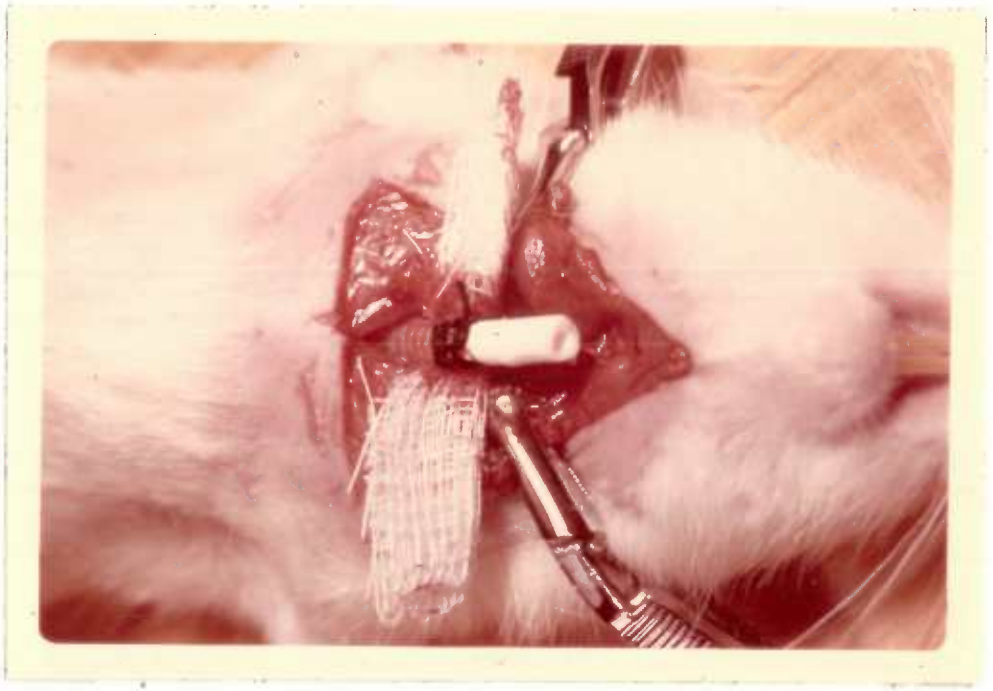
After shaving the lower abdomen, a four centimeter midline skin incision exposed the linea alba, through which entrance to the peritoneal cavity was obtained. Almost no bleeding was encountered. Without unnecessary manipulation, one uterine horn was located, and a segment containing one or two implantation sites was delivered through the incision. This segment of uterus was then externalized by placement of a 3/0 silk suture through the abdominal wall on one side, through the uterus, and then through the abdominal wall on the opposite side of the incision. After accomplishing this at both ends of the uterine segment, a piece of transparent cellulose film, four inches square, was placed over the implantation site and an incision about two cm. long made through the film and through the antimesometrial uterine wall. Such an incision avoids the placenta site which normally lies on the mesometrial aspect of the uterus. This also is the least vascular area of the uterus, and there was very little maternal blood loss.

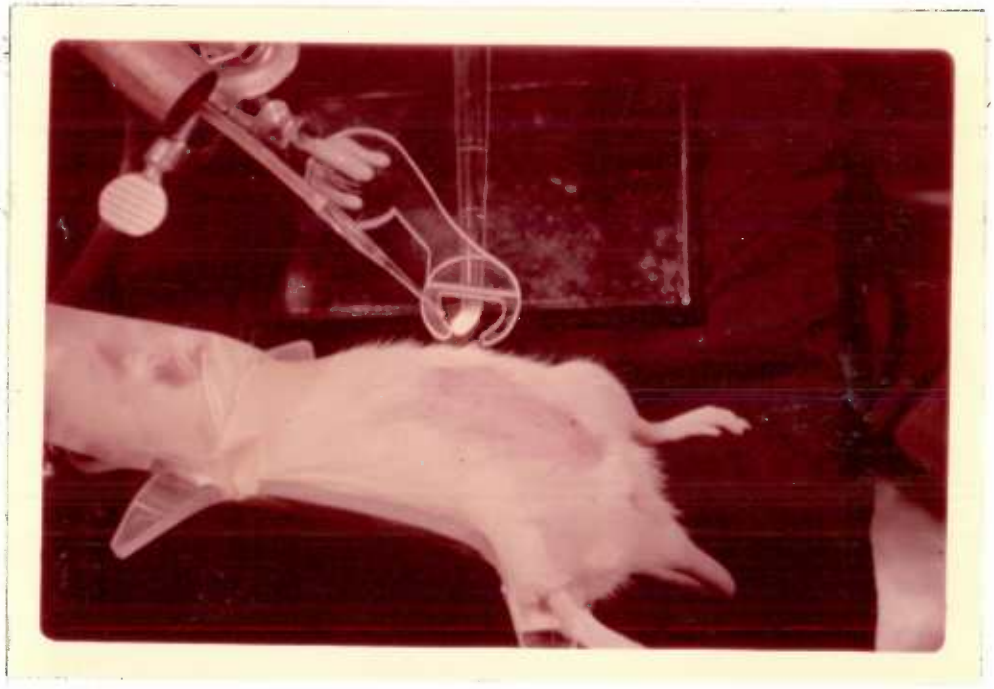
FIGURE I

The tracheal cannula in place in the anesthetized mother. Bulldog clamps hold the anterior neck muscles and lymphatic tissue at the sides of the incision facilitating insertion of the cannula. The small gauze sponge absorbs tissue fluids and blood from the incision preventing aspiration while the cannula is inserted.

FIGURE II

The anesthetized mother is supported by a contoured lucite frame. The polyethylene bag, through which passes a continuous flow of the respiratory gasses, is seen over the animal's head on the left. The lucite chamber form is shown in position over the transilluminating rod. The portion of the ring closest to the animal is discontinuous allowing the umbilical cord to be supported only by the cellulose film which is draped over this form. This method avoids stress on the umbilical vessels. The conduit bringing the warmed Ringer's solution to the chamber is positioned just over the chamber form.





Upon incision of the uterus in this manner, the fetus, enveloped by its yolk sac and amnion, was delivered to the superior surface of the cellulose film drape. This drape was then positioned directly over a small lucite chamber form. (Figure 2) By indenting the surface of the drape, a transparent chamber was formed which was of adequate size to contain the fetus and its surrounding membranes. (Figure 3) The placenta and uterus remained covered by the drape, which prevented evaporation and cooling of its surface. The circulation in the highly vascular yolk sac is confluent with that of the fetus. It is important that the incision through the yolk sac be carried out with great care, since hemorrhage from yolk sac vessels may cause marked change in fetal hemodynamics. It was not necessary to remove the fetus entirely from the enveloping yolk sac. By grasping the sac with pointed forceps, an incision was made near the base, which passed through one of several relatively avascular areas. (Figure 4) This allowed the amnion-covered cranial structures of the fetus to be clearly visible. While the amnion is a relatively transparent avascular membrane, vascular details of the type studied herein could not be adequately observed and recorded without its removal from the fetal head. This was accomplished by grasping the amnion with sharp forceps and incising it with small opthalmologic scissors, after which it was readily pulled back to expose the cranial structures of the fetus to

FIGURE III

The fetus in its enveloping yolk sac is positioned within the chamber formed by indenting the cellulose film through the center of the chamber form. The chamber is being perfused with warmed Ringer's solution and the uterus, placenta site and fetal structures are covered by a small piece of the transparent film to prevent surface evaporation. Light from the tip of the lucite rod illuminates the fetus.

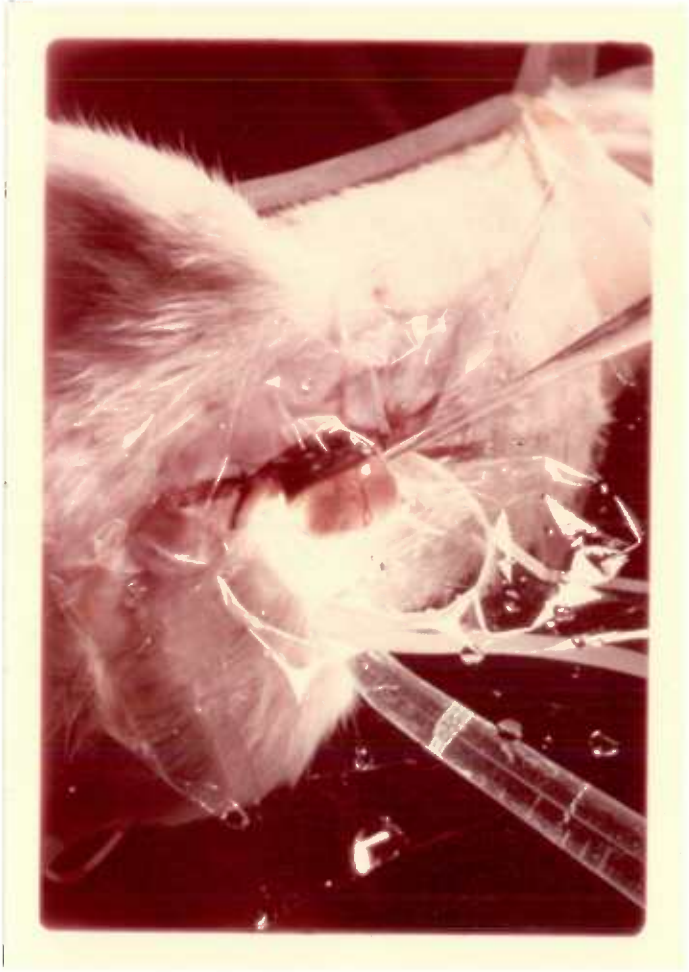
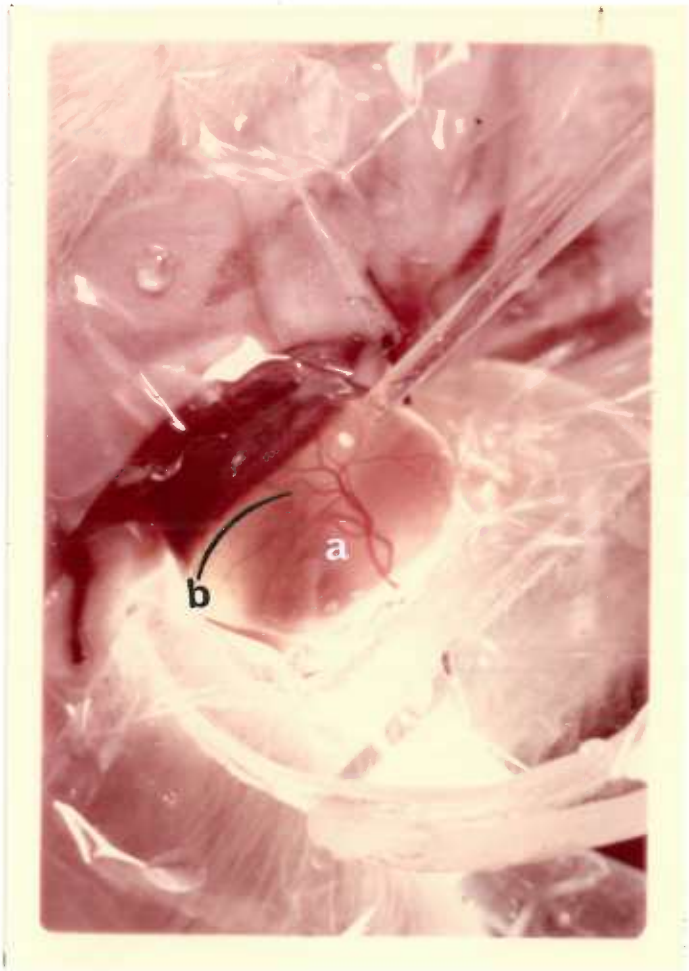


FIGURE IV

The circulation in the yolk sac can be seen in this photograph. The larger vessels at point (a) lying within the yolk sac cavity are confluent with the fetal circulation and carry blood to and from the yolk sac. In order to avoid hemorrhage from the yolk sac circulation in exposing the fetus, an incision is made through the relatively bloodless area shown by line (b). The fetus is delivered through this incision into the chamber.


a
b

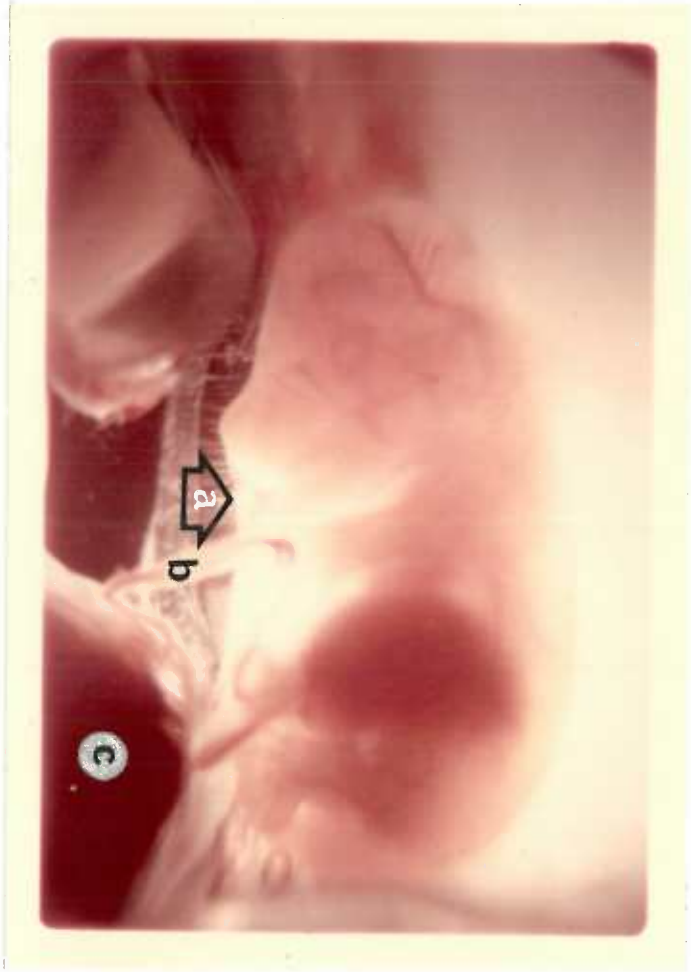
direct observation. (Figure 5) While it is usually possible to accomplish the exposure of the fetus from the yolk sac and amnion without a lens, it was, at times, necessary to utilize a dissecting microscope (approximately 30X magnification) if the vascular pattern over the fetal head was such as to make the dissection difficult.

Homeostasis

Every effort was made to maintain the fetus in an environment closely resembling that of intrauterine life. Mammalian Ringer's solution was utilized to moisten all exposed tissues. This solution contained 160 meq/L of sodium, 6 meq/L of bicarbonate, 4.3 meq/L of calcium, 164 meq/L of chloride, and 5.6 meq/L of potassium. Early in the procedure, maternal structures exposed to the atmosphere were protected with moistened gauze sponges, and during the final stages, maternal structures were under a cellulose film. Fetal structures, including the yolk sac, were placed in the chamber already described, and a continuous flow of the Ringer's solution was directed over them. To maintain this solution at 38.5° C., the polyethelene conduit delivering it to the chamber was passed through a large constant temperature water bath. During the experiment, the warmed Ringer's solution was directed into the chamber in such a fashion that the fetus was completely immersed, thus maintaining temperature and

FIGURE V

The fetus enveloped in the amnion (a) is seen in this view. The umbilical cord (b) can be seen running from the placenta (c) to the fetus across the transparent drape.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hydration, as near as possible, to that found in utero. The umbilical cord, passing from the placenta to the fetus along the drape, was also covered by a thin layer of the circulating Ringer's solution. As an added precaution against cooling by surface evaporation, a small piece of cellulose film was placed over the umbilical cord and all of the fetus, except that portion being studied. Because of the water tight drape over the anesthetized mother's abdomen, very little solution came in contact with her body, thus cooling via evaporation from her body surface was prevented.

Of major importance, was prevention of manipulation or stress on the fragile umbilical structures. Several different chambers were designed to support the fetus in a bath of amniotic fluid obtained from other fetuses. Basically, each was composed of a metal sleeve which had been coated with an inert plastic. Warmed water was circulated around the sleeve to maintain the amniotic fluid at body temperature. It was discovered that with use of such chambers, the fetal circulation deteriorated rather rapidly. As the cord had to be draped over the edge of the metal sleeve to allow the fetus to lie within the chamber, it was noted that there was occlusion and spasm of the umbilical vessels. The chamber used in all of the reported observations was specifically designed to put a minimum of stress on the umbilical cord. The blood

flow in the umbilical vessels was periodically inspected and if any alteration was noted, observations on that fetus were discarded, even though flow in fetal vessels appeared normal.

The projection lamp, used as a light source in these observations, produced a considerable amount of infra-red radiation, particularly when operated at low voltages. Since heat transmitted to the fetus could have a great effect on the fetal circulation, every precaution was taken to prevent such transmission. A Corning 3965 infra-red filter was inserted between the projector and the rod which eliminated about 80% of the infra-red waves while allowing most visible light to pass. The light was only used for short periods of observation, usually less than 30 seconds. Heat emitted by transformation of radiant energy within the fetal tissue was absorbed rapidly by the Ringer's solution because of its high specific heat, and was rapidly dissipated by the constant renewal of the circulating fluid. Heat emitted at the tip of the rod by transformation of radiant energy was not transmitted to the fetus since at no time was the rod in contact with the fetal surface. With these precautions, I noted no changes in the fetal microcirculation which I could attribute to the effect of heat, either transmitted or produced by transformation of radiant energy.

Methods of Observation

Of primary importance in making direct observations on the living fetus have been the principles involved in the techniques of transillumination developed by Knisley. (25) By this method, high concentrations of light may be directed from a distant source to a small area beneath the tissues to be examined. It is, thus, possible to provide sufficient light for microscopic observation and photographic recording. With a fused quartz rod, Knisley was able to transmit light over a curved pathway utilizing the property of internal reflection. Other substances, besides quartz, exhibit this property, and I chose to use a lucite rod which was readily obtained, less expensive, and easily shaped to fit any specific need. A sixteen inch length, of one inch diameter, molded, lucite cylinder was turned on a lathe so that there was a gradually diminishing diameter from the middle of the rod to a tip diameter of one-quarter inch. This was then sanded and fire-polished so that there would be no "light leaks" from translucent surface abrasions. With a bunsen burner, the tip of the rod was heated until it became pliable and a 75° bend was made. The tip was then polished so that its surface was parallel with the axis of the main body of the rod. This provided a rod with in-put surface one inch in diameter and an outlet surface of about one-fourth inch diameter.

Instead of the elaborate light source and cooling devices utilized by Knisley, I found it adequate, and more portable, to utilize as a light source a 500 watt 35mm slide projector containing a cooling system with very low vibration. When it was necessary to further reduce the vibration, this light source could be mounted on another small table or stool, thus, dissociating it from the table bearing the animal and microscope. The use of the projector as a light source further offered the advantage of the condensing lens system, which provided maximal light from the lamp to an area roughly the same size as the end of the transilluminating rod.

After exposure of the fetus and yolk sac from the uterus, the lucite rod was positioned under the fetus and the lamp turned on at low voltage to provide minimal lighting for gross observation. The yolk sac was then incised, as described, and the fetus delivered into the chamber. The rod was repositioned and gross examination was made of the umbilical blood flow and the placental attachment site to observe, as far as possible, any abnormalities in exchange between maternal and fetal circulation. If no abnormalities were discovered, then a microscopic evaluation of cord circulation and the general fetal circulation was carried out to determine if there was impingement of tension on the umbilical structures. Examination was also made to detect abnormality in fetal heart rate or rhythm,

obvious fetal trauma, or any change from the usual steady flow pattern in the fetal circulatory system. When all of these parameters were felt to be within normal limits, the middle cerebral artery was located as it emerges just posterior and superior to the fetal eye and the pattern of its trifurcation noted. (Figure 6) A suitable area of one of these branches, or a subsequent branch, was chosen for the observation and the transilluminating rod positioned so as to provide maximum light in the microscopic field.

Observations were made using a trinocular Bausch and Lomb Zoom microscope, a 3.5X objective and 15X wide field occulars. The xoom scope provides continuously variable magnification between 1X and 2X so that observations were conducted with magnifications of 52.5X to 105X. The magnification was chosen which gave the greatest clarity to the structures being observed. Because of the amount of equipment centered around the area of the fetus, the microscope was mounted on a specially constructed and weighted drill press stand to which a movable carriage had been attached. This allowed the scope to be positioned by movements in the horizontal and vertical planes, as well as to and fro radially around the drill press post. The mass of this stand also aided in reducing vibrations inherent in the photographic equipment. (Figure 7)

For accumulation of data by direct observation of change in vascular diameter, reliance on memory of the

FIGURE VI

This photomicrograph shows the anterior portion of the fetal head in lateral projection. The developing fetal eye (a) is seen in the lower left corner, and the snout (b) in the lower right corner. Just superior and posterior to the eye, the middle cerebral artery (c) emerges and assumes its superficial position over the cerebral cortex and undergoes its characteristic trifurcation. The area of the vessel chosen for observation in each case presented in this paper was chosen from that portion of the vessel lying between the two arrows.

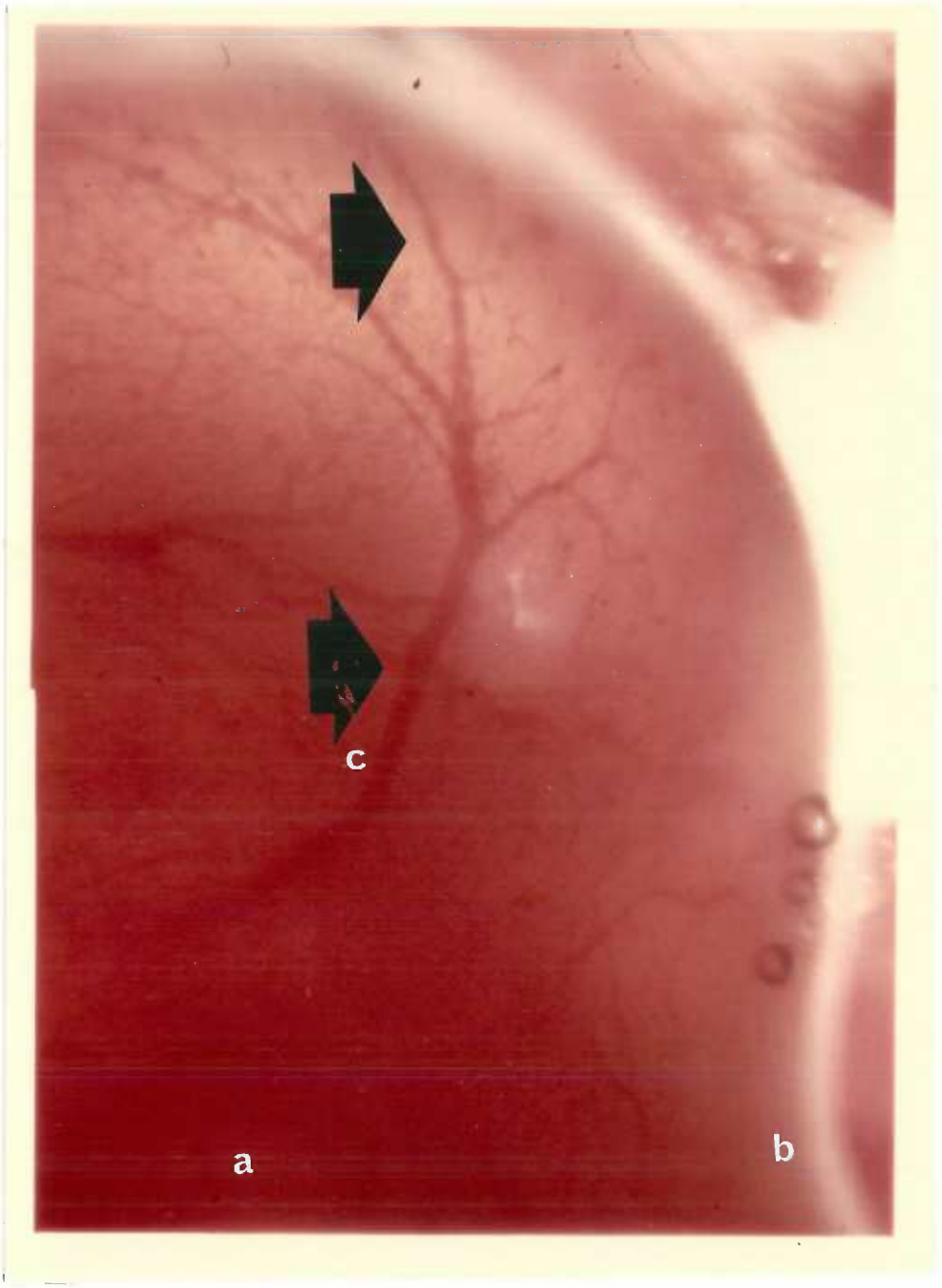
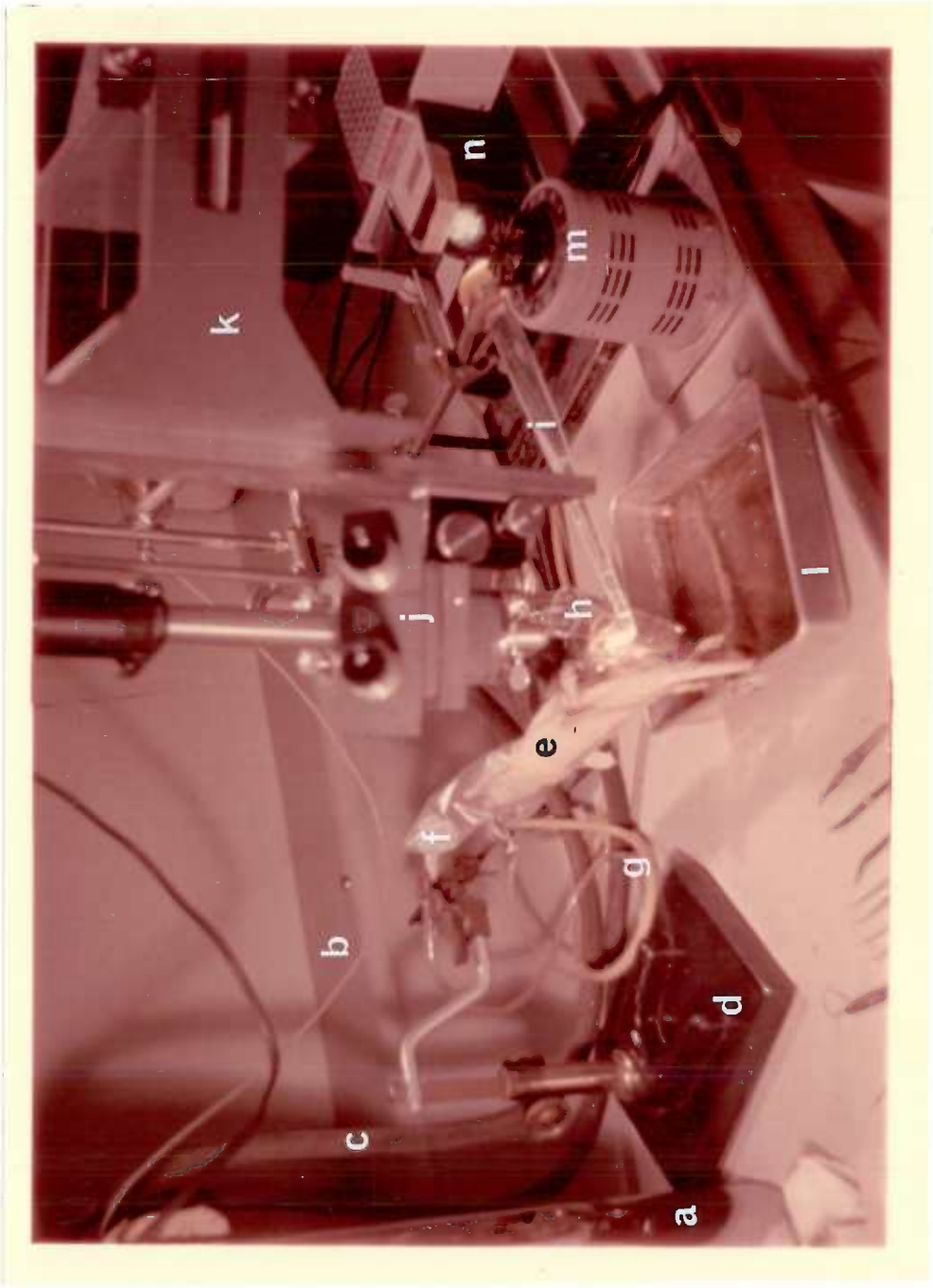

c
a
b

FIGURE VII

This figure shows most of the equipment involved during a typical observation. (a) Water bath for warming Ringer's solution is carried in the conduit (b) and through the tubing (c) containing circulating water to maintain the Ringer's solution at body temperature until it reaches the fetus. The adjustable stand (d) supports the animal (e) and allows for adjustment of her position. The polyethelene bag (f) provides a breathing chamber for respiratory gas mixtures carried through the tubing (g). The exposed fetus is positioned in the chamber (h) over the tip of the transilluminating rod (i). Observations were made through the trinocular microscope (j) supported by the movable carriage of the modified drill press stand (k). The basin (l) drains away the excess Ringer's solution. The rheostat (m) is used to vary the intensity of light from the projector (n) used as a light source.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visual image of a vessel at a prior time was not considered adequate for several reasons: (1) Known effects of carbon dioxide on the adult cerebral circulation reach a maximum only after several minutes time. It is difficult to retain for that period a visual image of the original vessel diameter when gradual change occurs continuously in the visual field. (2) The magnitude of change in vessel diameter required to allow twice the blood flow is very small (less than a 50% increase). (3) There is, also, the effect of the investigator's knowledge of the expected reaction, which makes scientific bias probable. It was, thus, decided to record observations photographically.

Although some 16mm motion pictures have been attempted, time lapse cinephotomicrography has not proved successful because of fetal movement which requires frequent readjustment of the microscopic field and light source. Therefore, all observations reported have been made by sequential 35mm still shots. A Bessler Topcon 35mm single lens reflex camera with focal plane light meter was used. After initial centering of the microscopic field, all light was shunted through the vertical tube by means of the built-in prism and further focusing and field adjustment, as well as light meter readings, were performed via the reflex mirror of the camera. The ground glass focusing screen of the camera was found to be too

coarse for fine focusing. This was modified by mounting a large glass cover slip with Permount on the ground glass surface of the screen. As soon as possible after the fetus was exposed, draped, and examined for defective circulation, an electric stopwatch was started and the interval, in seconds, between photographic observations was recorded.

Respiratory gas mixtures were bubbled through a water trap to provide adequate moisture, and then were conducted through tubing to a polyethelene bag placed over the upper half of the mother's body to provide a breathing chamber. The gas was allowed to flow at a rate sufficient to keep the bag distended and the excess permitted to escape through an outlet on the side opposite the inflow.

With the mother breathing room air, at least two initial control observations were made on each fetus; the second, a minute or more after the first. Then the gas mixture supplied to the mother was changed by opening the valve supplying 7% CO_2 in air and then closing the valve supplying room air. The time that the CO_2 mixture was begun was recorded. Subsequent observations were made at intervals as close as 30 seconds apart during the early stages and as infrequently as 3 minutes apart during extended observations. The usual interval between observations was 60 to 100 seconds. If any change in the normal characteristics of fetal blood flow, heart rate or

rhythm, umbilical circulation, or placental function was noted during the course of the experiment, the data were discarded. All observations reported were evaluated for a period of about ten minutes. Some animals were used as controls and all steps of the procedure were performed as described for the experimental animals, but the mother was allowed to breathe only room air for the duration of the observation.

Various photographic films were tried, but Kodak Tri-X provided the best results. Recording high magnification photomicrographs of a relatively thick translucent structure, such as the fetal cranium, with added motion from fetal muscular contractions and maternal respiration, presented severe difficulties. It was, therefore, necessary to use shutter speeds between 1/60th and 1/250th of a second. Use of the high speed film permitted these shutter speeds, but it was necessary to sacrifice the contrast and fine grain available in slower films. Negatives were developed with Kodak D-76 in a small daylight tank for eleven minutes and, after fixing and washing, were rinsed in Kodak Photoflo solution and dried.

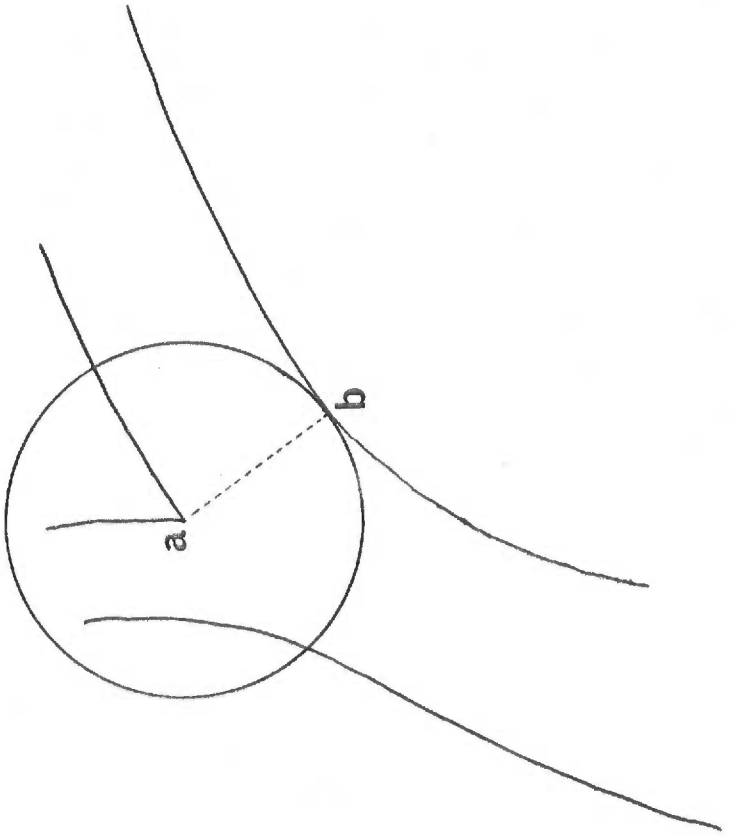
Evaluation of the negatives presented a number of difficulties. This was finally felt to be best achieved by projection from an Omega photographic enlarger which was set at a fixed distance. The enlarged vessel image was traced on paper with the greatest possible accuracy.

Special care was taken to establish the apex of the "V" formed by a constantly present bifurcation. This fixed point was established in each subsequent tracing and all measurements of the vessel diameter were made with reference to it. Three tracings of each negative were made and each tracing was numbered with respect to experimental animal and its order in the experimental sequence. Those negatives which were considered to be of inferior quality, due to improper focus or lack of sufficient contrast to provide good determination of vessel margins, were not utilized in the study.

The vessel diameter was measured by drawing a circle with its center at the bifurcation reference point and tangent to the opposite vessel wall. This distance was determined with dividers and measured on a millimeter scale. (Figure 8) This was done for each of the three tracings and the mean value was used. The data obtained was tabulated and subjected to statistical evaluation.

FIGURE VIII

This drawing of one of the tracings made of vessel 12-A demonstrates the method used to determine the vessel diameter. The bifurcation reference point (a) was established in each tracing and it was from this point that all measurements were made. The circle with (a) as its center was drawn so that the opposite vessel wall is tangent to it at point (b). The radius of this circle (ab) is the measured diameter of the vessel on the tracing and is proportional to the actual vessel diameter.



VESSEL 12-A

Results

The mean vessel diameter was calculated from measurements of the three tracings of the artery in each fetus observed. This measurement is proportional to the actual vessel diameter. Since the volume of blood flow through a vessel is directly proportional to the cross sectional area of the vessel, rather than the diameter, each of the measurements was squared so as to provide a more adequate estimation of actual blood flow. The control and experimental groups each contained nine fetuses and were subjected to identical statistical analysis.

Table I presents an analysis of the data obtained on the animals used for control observations. The mean of two observations made during the first minutes of the procedure was used to establish a baseline measurement of cross sectional area at a particular point on each vessel. These baseline measurements are presented in Column A of Table I and Column B contains the mean vessel size during an eight to twelve minute observation period under standard conditions with the mother breathing air. Comparison of these observations, using the Student t test, demonstrates that there is no significant change in the size of the observed fetal cerebral arteries during the observation period under standard conditions ($p > 0.05$).

Table II presents an analysis of the data ob-

tained on animals subjected to maternal inhalation of 7% carbon dioxide. Column A contains observations during the first two to three minutes before beginning the CO_2 and was used as the baseline vessel size. Column B contains the mean for each vessel, of observations over the next eight to twelve minutes. Analysis with the Student t test demonstrates a significant change in vessel size in the direction of dilatation during maternal inhalation of 7% CO_2 in air ($p < 0.001$).

The procedures utilized in this thesis have been designed to lead only to decisions about qualitative changes in the middle cerebral arterial tree. However, some insight into the degree of this change can be gleaned from comparison of the extent of deviation from the mean baseline vessel size in the control and experimental animals. Vasodilatation induced by increasing carbon dioxide levels would be expected to increase with time to a maximum value. Thus, the mean change in vessel size, as presented in the qualitative analysis would represent a rather subdued measure of the actual extent of vasodilatation. However, it would not be proper to select the largest measurement in the series to represent the maximum dilatation, since there is the possibility of obtaining an erroneously large value due to chance errors in measurement.

It was finally decided that the best estimation of the total amount of dilatation could be obtained by subtracting each observation from the one directly following it in time, with careful attention to the sign of the value obtained (ie., positive or negative). The simple sum of the interval change between each observation thus provided a rather quantitative estimate of the total dilatation. This sum was computed as a percentage of the baseline cross sectional area for each vessel, and the mean value calculated for the control and experimental groups.

Table III shows a comparison of these values and it will be noted that the mean change observed, in per cent of cross sectional area of the cerebral vessels in the experimental fetuses, is roughly four times as great as in control fetuses. It will be noted that, with the exception of three fetuses, these changes are substantial and always in the direction of vasodilatation. In Table IV these three fetuses (12-A, 24-A, and 27) are compared to the control animals with respect to dilatation from the baseline value in per cent of cross sectional area. Again using the Student t test it is demonstrated that there was no significant vasodilatation in the cerebral arteries of these animals even though they were exposed to maternal hypercapnia. More will be said in the discussion concerning possible explanations for the lack of response in these three fetuses.

Fetus 18-B in the control group is also remarkable, demonstrating a rather significant vasodilatation without being subjected to maternal CO_2 inhalation. Comments explaining this type of response will also be found in the discussion.

TABLE I

This table summarizes the measurements of vessel size obtained for each control fetus and compares the mean baseline measurement to the mean measurement obtained during extended observation under standard conditions with the maternal respiration of air.

Fetus Number	MEAN VESSEL SIZE in values proportional to vessel cross sectional area	
	A Baseline Size (air inhalation)	B Procedure (air inhalation)
17-A	3.65	3.52
17-B	3.69	4.38
18-A	3.98	3.78
18-B	2.51	3.31
19-B	5.15	5.06
20-A	3.71	3.93
20-B	2.32	2.86
23-A	2.36	2.43
26-A	7.43	7.00
	$\sum X_A = 34.80$	$\sum X_B = 36.27$
	$\overline{X}_A = 3.87$	$\overline{X}_B = 4.03$

$$H_o = \overline{X}_A = \overline{X}_B$$

$$H_e = \overline{X}_A = \overline{X}_B$$

Significance level:
t at 8 D.F. at p of 0.05 = 2.306

Calculated t at 8 D.F. = 1.05

TABLE II

This table summarizes the measurements of vessel size obtained for each experimental fetus and compares the mean baseline measurement to the mean measurement obtained during extended observation under maternal respiration limited to 7% CO_2 in air.

Fetus Number	MEAN VESSEL SIZE in values proportional to vessel cross sectional area	
	A Baseline Size (air inhalation)	B Procedure (7% CO_2 inhalation)
11-B	4.39	5.51
12-A	5.59	6.20
12-B	3.50	4.78
14-A	4.08	5.21
15-A	5.25	5.95
23-B	3.67	4.15
24-A	6.48	7.01
26-B	5.91	6.71
27	4.25	4.39
	$\Sigma X_A = 43.12$	$\Sigma X_B = 49.91$
	$\overline{X}_A = 4.79$	$\overline{X}_B = 5.55$

$$Ho = \overline{X}_A = \overline{X}_B$$

$$H_e = \overline{X}_A < \overline{X}_B$$

Significance level:
t at 8 D.F. at p of 0.001 = 5.041

Calculated t at 8 D.F. = 6.23

TABLE III

This table summarizes the total change in vessel size observed in each fetus. This is expressed in percentage of the cross sectional area of the baseline measurement, and was derived by summing the interval change between each observation. Mean change in the control group can be compared to that in the experimental group to obtain a rough idea of the extent of the dilatation observed.

Control Fetus Number	A CONTROL	B EXPERIMENTAL	Exp. Fetus Number
	Change from Baseline in % of area	Change from baseline in % of area	
17-A	- 3.29	38.95	11-B
17-B	13.82	11.81	12-A
18-A	- 7.88	53.71	12-B
18-B	35.06	33.09	14-A
19-B	- 3.50	20.95	15-A
20-A	7.82	20.16	23-B
20-B	6.93	10.80	24-A
23-A	8.47	23.35	26-B
26-A	- 1.21	9.89	27
	$\Sigma X = 56.22$	$\Sigma X = 222.71$	
	$\bar{X}_A = 6.25\%$	$\bar{X}_B = 24.75\%$	

TABLE VI

In this table the percentage change over base-line vessel size in three experimental fetuses not showing substantial vasodilatation is compared to the control group.

Control Fetus Number	A CONTROL	B EXPERIMENTAL	Exp. Fetus Number
	Change of Base-line in % of area	Change of Base-line in % of area	
17-A	- 3.29	11.81	12-A
17-B	13.82	10.80	24-A
18-A	- 7.88	9.89	27
18-B	35.06		
19-B	- 3.50		
20-A	7.82		
20-B	6.93		
23-A	8.47		
26-A	- 1.21		
	$\Sigma X = 56.22$	$\Sigma X = 32.50$	
	$\bar{X} = 6.25\%$	$\bar{X} = 10.83\%$	

Significance level:
t at 10 D.F. at p of 0.05 = 2.228

Calculated t at 10 D.F. = 0.58

Discussion

As previously mentioned, there is a wealth of evidence that the cerebral arterial system of the adult mammal is directly controlled by the carbon dioxide level of the blood perfusing the brain. Direct observation of the cerebral vessels in the adult have demonstrated vasodilatation to be the response to hypercapnia. On the basis of the results just presented, it appears that maternal hypercapnia produces a vasomotor response in the fetal cerebral vascular bed which is similar to that produced in the adult.

Studies concerning the cardiovascular reactions of the fetus to various stresses, administered either via the mother or directly to the fetus, have largely dealt with overall responses such as blood pressure, heart rate, and blood gas concentrations. Many mechanisms of the fetal-maternal relationship have thus been clarified, while many other factors have become increasingly puzzling. As Geber [17] points out, it seems at times that each fetus in an experimental animal has its individual methods, within broad limits, of maintaining homeostasis.

It is only in recent years that a few investigators have turned their attention to the physiology of regional blood flow in the fetus. The experimental method presented here will have its greatest application in this

area. Direct observation of regional circulation in the mammalian fetus has been used to define the formation of the vascular pattern in the limb bud, [8,9] to demonstrate the vasoconstrictor action on the cerebral arterial system, [10] and now to demonstrate vasodilatation in response to maternal hypercapnia. It will also be possible with these techniques to observe reactions of many regional circulatory circuits to stimuli from alterations in maternal circulation such as shock, hyperthermia, hypothermia, anaphylaxis, etc. The development of regional circulation to the eye, the brain, the skin, and bone have previously been described only by comparison of serial sections. Using this method, with improved techniques and the application of cinephotomicrography, the dynamics of development to these organs could be worked out in detail.

Since this is a relatively new procedure, which has been applied in limited fashion, this investigator feels that it deserves a rather critical analysis by one who has used the method and can see its limitations.

It will be noted from the data that, although, most of the experimental animals showed a response to maternal CO_2 inhalation, there were several fetuses in which there was no demonstrable vasodilatation. This causes one to ask why the reaction failed to appear consistently. As in many new procedures, there remain a significant number of uncontrolled variables in the experimental model

which has been presented. It is most likely that therein lies the answer to the inconsistent results obtained in some fetuses.

Several reasons for obtaining results other than that expected are possible: (1) the cerebral vessels at this stage of gestation are not uniformly able to dilate in response to carbon dioxide stimulus; (2) the methods of measurement of vessel change were inadequate to evaluate the response obtained; (3) individual fetuses react in variable manners to the same stimulus; (4) the vessels not responding were dilated maximally, and could thus show no response. These possible explanations will now be examined individually.

Assali and other [2] have demonstrated that the cardiovascular system of the fetus responds to a variety of stimuli. Christiansen et al., [10] in this laboratory, previously demonstrated in the rodent that the middle cerebral artery and its tributaries, at this stage of development and earlier, exhibit vasomotor functions similar to the adult in response to vasoconstrictor substances. The post-constriction dilatation observed by them demonstrates that the cerebral vessels are capable of dilating. While there is, as yet, no other experimental evidence to substantiate the idea, this investigator finds it highly improbable that the smooth muscle in the walls of the middle cerebral artery reacts to the presence of carbon dioxide

by vasodilatation in the adult, and some fetuses, but fails to respond to the same substance in other fetuses at identical stages of development.

While improvements in photographic technique could probably be made, it is felt that the methods of recording the data, as applied to this procedure, were generally adequate to avoid serious errors in measurement. Improvement of techniques for homeostasis and immobilization of the fetus would allow the use of slower, finer grain films with greater contrast which would improve resolution of vessel margins. It would, also, allow for recording of data at fixed intervals which would facilitate statistical analysis. While these improvements would tend to accentuate the difference between the dilated and homeostatic vascular state, I do not feel that this would be sufficient to explain the failure to demonstrate vasodilatation in the fetuses in question.

The question raised by Geber, [17] who found individual variation in fetal response among fetuses in the same mother examined simultaneously, is fascinating in its implications and could offer an explanation for the fetal reactions in question. However, this is a relatively new concept without theoretical explanation, and must await varification and further study.

In the course of each experiment, it was necessary to assume that the PCO_2 of the fetus was a direct reflection

of the maternal PCO_2. It has been shown by Barron and others [5,36] that CO_2 transfer across the placenta appears to be passive and dependent upon a fetal-maternal concentration gradient. Therefore, this assumption seems to be logical, in the event that there are no external factors affecting this transfer. Despite great care given to prevention of any procedure which might have compromised fetal-maternal gas transport, there remain several ways in which the exchange of CO_2 across the placenta could have been altered without detection.

Fetal hypercapnia, leading to maximal vasodilatation prior to onset of observation, would decrease the response seen in the experimental animals in question. Fetal hypercapnia, during the control procedure could lead to the unexpected vasodilatation in the control animal. Such an effect could have occurred in several ways: (1) any stress which would decrease fetal-maternal exchange ie., stress or spasm of the umbilical vessels, partial abruption of the placenta, compromise of uterine arterial perfusion of the placental site, etc.; (2) fetal hyperthermia (dilatation sans hypercapnia); (3) maternal hypercapnia prior to onset of 7% CO_2 inhalation from hypoventilation caused by depth of anesthesia or respiratory difficulty. (Figure 9)

While every effort was made, as described earlier, to prevent premature fetal hypercapnia, it is always possible that such a change occurred without detection by the investigator. The one critical parameter which remains

FIGURE IX

The relationship between the fetal and maternal circulations are presented here in diagramatic form. The diagram at (A) represents the maternal circulation. (a) represents the circulation in the uterine wall at the placental site, (b) the fetal side of the placenta, and (B) the fetal circulation via the umbilical cord.

This diagram is intended to present graphically the concepts outlined in the discussion in explanation of those several fetuses which, either failed to show significant vasodilatation in response to maternal inhalation of 7% CO_2, or showed a considerable vasodilatation under standard conditions. In those animals failing to respond, it was felt that fetal hypercapnia due to undetected loss of homeostasis early in the experiment, caused the vessel to be maximally dilated prior to recording the baseline vessel measurement. An abruption of homeostatic conditions during the recording period, with resultant hypercapnia, offers the best explanation for the vasodilatation observed in the fetus under standard conditions.

Several of the more important areas where events might disturb homeostasis leading to fetal hypercapnia are indicated by the arrows.

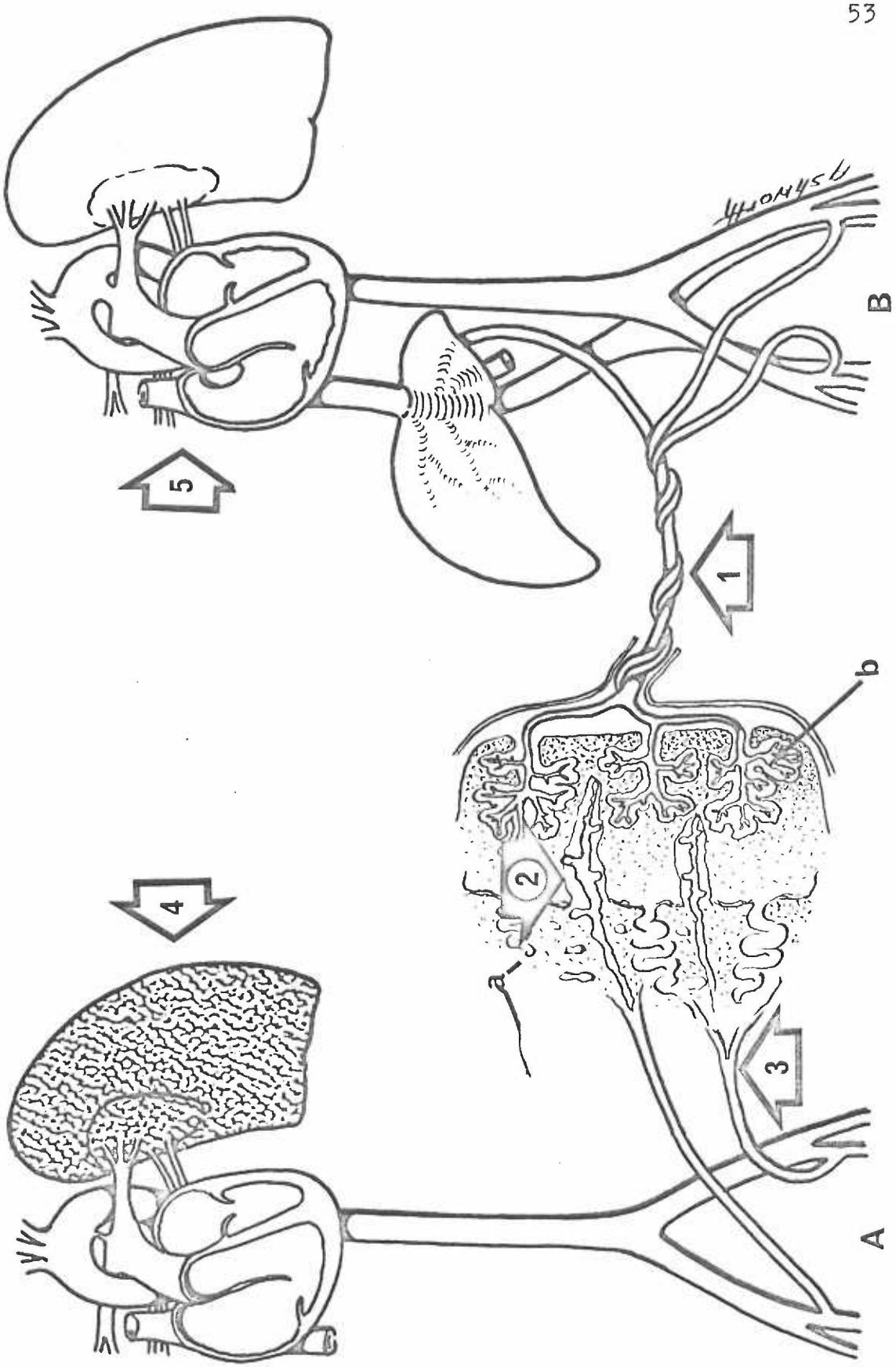
(1) Stress on the cord or spasm of the umbilical vessels.

(2) Partial abruption of the placenta undetected by the investigator.

(3) Compromised uterine blood flow to the placental site.

(4) Maternal hypercapnia secondary to hypoventilation as a result of anesthesia or respiratory distress.

(5) Physical change in the fetal environment such as hyperthermia leading to vasodilatation without fetal hypercapnia.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unknown, and would be of great value in this procedure, is the fetal PCO_2 during each experimental period. Many attempts were made to devise a method of assaying fetal blood for carbon dioxide, but due to the minute size and delicate structure of the fetal vessels in the rat there is no method known to the author of obtaining sufficient blood, even for micro assay techniques, without disruption of normal fetal hemodynamics.

In view of the above discussion, it seems to this investigator that the reason for failure to demonstrate significant vasodilatation in those few fetuses in question, is most likely a result of fetal hypercapnia prior to the onset of maternal CO_2 inhalation. The extent of this hypercapnia, or its exact cause, remains undetermined by present methods in this procedure.

Similar to the sequence of events in acquiring our knowledge of control of blood flow to the adult brain, direct observation of vasomotor response has now been demonstrated in the fetus. Although the results obtained in this experiment show a significant qualitative vasomotor change, as a result of maternal hypercapnia, it is not possible to claim that this is a direct result of carbon dioxide concentration in fetal cerebral blood. It also remains only speculation that the vasodilatation demonstrated, indicates an increase in cerebral blood flow. While both of these concepts are logical extrapolations

from known mechanisms demonstrated in adult mammals of other species and from known effects of hypercapnia on fetal circulatory responses, the final proof that fetal hypercapnia leads to increased cerebral blood flow must await further experimentation.

Evidence could be readily obtained by methods now available. Such an experiment would utilize a larger animal, such as the sheep, with fetus amenable to the necessary surgical procedures for measurement of cerebral blood flow, measurement of PCO_2, and carotid artery pressure. With utilization of the radioactive inert gas method, or tracer dilution method previously described, one could ascertain the cerebral blood flow in the fetus. If this were combined with direct observation of pial vessels in the same fetus, all important factors affecting cerebral blood flow would be known, and the effect of carbon dioxide on the fetus in utero could be compared with that of the adult.

The final answer to questions about circulatory control mechanisms of the fetal brain could have profound impact on present day management of labor and delivery. From one to two per cent of the children born in the United States suffer mental retardation severe enough to prevent them from becoming self sufficient. It is estimated that between 30% and 70% of these cases are the result of perinatal brain damage, chiefly anoxia. (42) It has

long been known that during labor the brain of the fetus, in cephalic presentation, is undergoing a great deal of trauma as the caput is forced through the avenue of the birth canal. Yet at this time the fetal brain receives a diminished amount of oxygen due to the effects of uterine contraction and impaired placental function. There is frequently maternal hyperventilation secondary to the pain and anxiety associated with labor. Hyperventilation serves to reduce the maternal PCO_2 and provide for a reduction in fetal PCO_2, and could further compromise the cerebral circulation in the fetus by production of a hypocapnic vasoconstriction.

Perhaps one method of providing the fetal brain with substantially more oxygen for maintenance of function and repair, would be to effectively increase the maternal PCO_2 and thereby provide fetal hypercapnia with subsequent cerebral vasodilatation.

Maternal hypercapnia could be achieved by inhalation of 5 to 7 per cent CO_2 in air or oxygen. However, this would be rather cumbersome during labor and delivery, and would also provide some degree of maternal discomfort. The carbonic anhydrase inhibitor, acetazolamide, was shown by Ehrenreich et al. [12] to cause an increase in cerebral blood flow in adult humans which was equal to that produced

by inhalation of 5% CO_2. * In the light of the present discussion, this might offer a practical clinical method for protecting fetal neurons from hypoxia by providing increased fetal cerebral blood flow.

This investigator hopes to be able to pursue the questions raised in this work about basic maternal-fetal relationships with reference to carbon dioxide, as well as to test the validity of some of these theoretical concepts and their clinical application. One hopes, by such strides in lower animals, to eventually understand the basic mechanisms by which the human fetus prepares itself for entry into the outside world. Truly, as Barron once said; "There can be nothing more exciting to any individual than the way by which we come into this universe. These events have been before people for generations, but for the most part the clinician has dealt mainly with the mechanics of birth and never looked into the fascinating physiology that comes before. Until this is done on a comparative basis there can be no vistas and no challenge." (4)

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* Initial observations were recently made in our laboratory of the effect which acetazolamide has on the fetus. The number of fetuses observed was too small to make a positive statement as to its effect, but initial observations indicate some degree of fetal cerebral vasodilatation in response to the drug administered intravenously to the mother. At this time there is no knowledge concerning the transmission of acetazolamide across the placenta.

Summary and Conclusions

Modifications of a technique adapted from methods used to study microcirculation in the living adult mammals, have been used to make direct observations of fetal cerebral hemodynamics.

Rat fetuses of $14\frac{1}{2}$ to $16\frac{1}{2}$ days gestation were surgically exposed and maintained under homeostatic conditions for microscopic observation. Vasomotor changes occurring in the middle cerebral arterial system were photographically recorded in control animals during maternal inhalation of room air, and in experimental animals during maternal inhalation of 7% carbon dioxide in air. The extent of variation in the functional cross sectional area of the experimental and control animals were compared statistically. It was found that significant vasodilatation occurred in the observed vessels of fetuses whose mothers inhaled the carbon dioxide mixture over a period of about ten minutes.

These results would indicate that fetal hypercapnia at this stage of gestation, as in the adult, produces cerebral vasodilatation and perhaps increased cerebral blood flow. Final proof of this concept awaits studies in larger animals which are amenable to recording of fetal PCO_2, carotid arterial pressure, and cerebral blood flow, as described herein.

A critical discussion of this method is presented with reference to several fetuses in the experimental group which showed no vasodilatation.

There are numerous possibilities for use of these techniques to observe regional fetal circulation during maternal stress and changing environmental conditions, and for study of the dynamics of regional vascular development.

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