1 Examination of the Placenta

Macroscopic Examination

Most placentas are normal, as are most babies. Therefore, an examination of all placentas may not be warranted, although this has been advocated repeatedly. Practical guidelines, including indications for the examination have been published by the College of American Pathologists (Langston et al., 1997). This reference describes in tabular form the major abnormalities and their association with clinical features. Booth et al. (1997) inquired what reasons constituted the submission of a placenta for examination and found, regrettably, that it was cesarean section delivery. This is hardly a good reason, as will be seen. A large number of surgical deliveries are repeat sections and have little impact on perinatal problems for which placental examination might be useful. Altshuler and Hyde (1996), on the other hand, found that 92% of placentas for which an examination was requested by obstetrician or neonatologist had relevant pathology. Salafia and Vintzileos (1990) made a strong plea for the study of all placentas by pathologists. We concur with this view, as the sporadic examination does not provide sufficient training for young pathologists and it does not allow the “routine” pathologist to obtain sufficient background knowledge as to what constitutes a truly normal placenta. Another reason for the examination of all placentas is today’s litigious climate; it makes study of placentas highly desirable (see Chapter 26). Furthermore, it has been shown repeatedly that a placental examination is needed to understand the causes of perinatal deaths. This was demonstrated, especially for stillbirths, by the study of Las Heras et al. (1994). The most important lesions were found in the umbilical cord (18%), with inflammatory lesions being second. Altshuler (1999) wrote a searching essay on the placenta-related epidemiology from his vast experience in these matters. Because placentas differ widely in shape, size, and in appearance, the novice must become familiar with this spectrum of placental shapes. To do so, a large number of placentas must be examined routinely. In hospitals with large numbers of deliveries, however, it may be prudent to select placentas for examination by the pathologist.

Storage

To facilitate the practice of saving placentas for a week, storage is required so that placentas are available when needed. The American College of Obstetricians and Gynecologists (ACOG), on the other hand, has suggested, surprisingly, that the routine study of the placenta is not warranted (ACOG, 1991), a decision with which we strongly disagree. Placentas should not be frozen prior to examination, as this obliterates the most useful histologic characteristics and makes even the macroscopic examination more difficult. We believe that formalin fixation has a similar unwanted effect. It is best to store the delivered placentas in containers, such as plastic jars. We have found ice cream cartons, made from Styrofoam, the most convenient and least expensive. Cardboard containers absorb the fluids, and the placentas tend to stick to them. These containers can also be readily labeled and stored in a refrigerator at 4°C. In this state, the placenta is preserved for a meaningful examination for many days. Autolysis is minimal. We cannot agree with the opinion of Naeye (1987) that this storage causes significant artifacts that render a subsequent examination difficult. Indeed, the immediate fixation of the organ in formalin, recommended by others (Bartholomew et al., 1961) as a good means to evaluate the extent of infarction, makes the placenta more difficult to evaluate critically, aside from the storage problems, expense, and odor. Prior fixation also makes tissue culture, bacteriologic examination, and other procedures more difficult or impossible. For maximal convenience, it is a good idea to have a refrigerator with seven shelves, labeled Monday through Sunday, and to discard the normal placentas from one shelf when the next similar weekday arrives. In this way, all placentas from problem births will be available for study.
The placenta loses some weight during storage. In part, the loss is due to evaporation, but most weight is lost by leakage of blood and serum occasioned by the weight of placental tissue resting on other portions. The quantity of weight loss depends on the length of storage and the degree of edema, but the edema is not great in the normal placenta. It is most significant in the edematous placentas of hydrops. We have observed a 180-g fluid extravasation from a 740-g placenta within 1 day from a hydropic placenta. The freshly examined placenta is thus softer, bloodier, and thicker than one that has been stored. On the other hand, it must be noted that the placenta gains weight when it is stored in formalin, particularly during the first day of fixation. Not all organs increase in weight uniformly after such fixation, as the detailed report by Schremmer (1967) specified. The placenta, according to this author, gains between 0.7% and 23.0%, with an average of +9.9%. It is among the organs with the largest deviations in weight gain after fixation. Our own findings are summarized in the graph shown in Figure 1.1.

Selection

Placentas from all prematurely delivered infants and all twins should be examined routinely, at least macroscopically, and many of them require histologic study as well. In addition, many circumstances arise during the first few days of life of an infant where the neonatologist is interested in placental findings. These often help to clarify whether a particular disease had a prenatal onset. Furthermore, there are some maternal conditions that warrant placental examination, for example preeclampsia, the condition known as lupus anticoagulant, diabetes, fever, and many more. In our routine study of placentas, the obstetricians and neonatologists alert us as to which placentas they believe warrant more scrutiny, and thus perhaps 5% to 10% of all placentas undergo histologic examination in our hands.

Photography

A photographic record is often desirable and is useful for many purposes. Most pathology laboratories are equipped with cameras that can take Kodachrome color pictures, which are then valuable for teaching purposes. It has been our experience, however, that colors tend to disappear, and certainly these photographs cannot generally be retrieved years later when they may be of interest in litigation or review of material. Nowadays, digital photography has become such a routine procedure and storage of the digital images has become so easy that much more photography of specimens is desirable and practiced. The photographic task is generally quickly accomplished. Colleagues have often been amused by this recommendation, but they agree that a good picture is worth a lot of words, especially when it comes to litigation.

Examination

Detailed protocols for the examination of the placenta have been presented in the past (Snoeck, 1958; Benirschke, 1961a,b; Gruenwald, 1964; Fox, 1997). Some protocols were designed to allow an unbiased examination of the placenta and record keeping by the many different medical centers of the Collaborative Study so that, ultimately, correlations could be made regarding fetal outcome. Our routine procedure now is to select for histologic study those that appear abnormal or whose perinatal circumstances demand such an examination. The selection process just outlined has been helpful, and we have rarely missed a placenta that was of importance. Other recommendations for a “triage” of placental study and other

Figure 1.1. Weight gain of placenta (trimmed, without cord or membranes) after formalin fixation.
ramifications come from a joint conference held in 1990 (Travers & Schmidt, 1991). That volume provides useful information on many aspects of placental pathology.

The tools for the examination are simple (Fig. 1.2). They consist of a ruler, a long and sharp knife, a toothed forceps, a pair of scissors, and a scale. Our ruler is permanently mounted over the cutting board, thus enabling rapid measurement of the length of the umbilical cord and the placenta’s diameter. A butcher’s scale with removable bucket that weighs items up to 2 kg is also available. The long knife, best obtained from a butcher supply house, is sharpened just before examination.

The placenta is removed from its container. At this time one often perceives rather characteristic odors. For instance, when a mother has recently eaten garlic, the intense smell of its diallyl sulfides is readily apparent. Also, in infected placentas the fetid smell of *Escherichia coli* and the rather sweeter smell of *Listeria monocytogenes* can be distinguished by an experienced pathologist. Storage in the refrigerator enhances the growth and hence the recognition of these organisms.

The shape of the placenta is then ascertained by stretching it flat on the cutting board. Is it normally round or oval? Are there accessory (succenturiate) lobes? Is a question now answered? One finds that, during the delivery, the membranes have generally inverted over the maternal surface (Schultze procedure) and rarely are they found in the position they held in utero (Duncan) (Pritchard et al., 1985). They are then inverted by the examiner so that they assume the in utero configuration, and one next ascertains the completeness of the membranes. It is also noted at this time if the tear that allowed the infant to escape from its membranous enclosure extends to the edge of the placenta or if free membranes extend beyond the edge. If there is any margin of intact membranes, this placenta could not have been a placenta previa, provided it was from a vaginal delivery. If the edge of the membranous tear is far from the placental border (often the case with circumvallate placentas), a fundal position can be deduced. Torpin and Hart (1941) made the point that when the minimally disturbed sac is immersed in a bucket of water the sac assumes the configuration of the uterus, and that its position before birth can be reasonably accurately determined by this study. At this time it is prudent to inspect the color and appearance of the fetal surface of the placenta. Normally it is shiny, and the subjacent blood is seen as a clear blue hue, particularly in the immature organ. When chorioamnionitis is present, the membranes become opaque by the interposition of leukocytes, and the surface usually loses its sheen. Greenish discoloration betrays either meconium (slimy) or hemosiderin deposition.

Next the membranes are cut off the edge of the placenta with the knife. If one anticipates making sections of the placenta for histologic study, it is wise to follow a routine protocol for doing it, as it enhances subsequent interpretation. Therefore, it is recommended that one get used to doing it one way, once and for all. It is preferable to cut the membranes off in such a manner that one knows the point of rupture; then, when sections are made, the membrane roll is prepared in such a fashion that one knows the point of rupture; then, when sections are made, the membrane roll is prepared in such a fashion that the point of rupture is in the center of the roll with the amnion inward (Fig. 1.3). This method of preparing a roll of membranes (the “jelly roll”), in order to obtain a maximum amount of membranes with decidua capsularis, was first described by Zeek and Assali (1950). In immature placentas, there may be a large amount of decidua, and it is often ragged. In more mature organs the decidua atrophies and often it degenerates. Occasionally, one finds an intrauterine device in this decidua capsularis, usually at the edge of the placenta and associated with old clot and debris (Figs. 1.4 and 1.5). Frequently, there are areas of brown to green discoloration in the membranes that are from former hemorrhages, or they may have been induced by amniocentesis.

In many placentas that come from patients after labor, in contrast to those after cesarean section, the amnion is disrupted or sheared off the underlying chorion. In fact,
1. Examination of the Placenta

Figure 1.3. Rolling of membranes for fixation and later sectioning. It is best to prepare them in a standardized fashion, for example, amnion inside, starting at the site of rupture and proceeding toward the edge of the placenta, as shown at left. A segment is then taken from a well-rolled portion (center) and is fixed for a day (right) before trimming.

Figure 1.4. Edge of the placenta (right) with an intrauterine device embedded in degenerating decidua (partly removed) and old blood clot.

Figure 1.5. Intrauterine devices at the placental margin at term (left) and in a slightly immature (right) pregnancy. Note the attending hemorrhagic degeneration of the adjacent tissues.
the amnion may be totally detached. Often, though, there is milky, white vernix caseosa that has dissected underneath the amnion; it is readily moved about by pressure. It has no significance. Moreover, the membranes near the edge of the placenta frequently contain the remnant of the yolk sac, a small white to yellow oval disk that is located underneath the amnion. The yolk sac of early stages of development can now be visualized ultrasonographically. Measurements have shown that the size of the yolk sac is variable, and that it is not a useful prognosticator of fetal well-being (Reece et al., 1988). Occasionally, one sees remnants of tiny vessels traversing from it to the insertion of the cord, or even within the cord.

The color of the membranes is noted, as are the surface characteristics. A slimy feeling is often the result of meconium discharge, as is a green color. The length of time of meconium discharge can be estimated by the presence of green discoloration in different layers. When it is only in the amnion, this suggests a short time interval; when meconium is found also in the chorion after the amnion is stripped off, a longer interval has passed since discharge (Miller et al., 1985). We found that after 1 hour the meconium macrophages are visible within the amnion; after 3 hours they may be seen in the chorionic membrane. At even later times it reaches the decidua capsularis. Greenish or brownish discolorations in immature placentas are more often due to blood breakdown products (hematoxilin, hemosiderin) following hemolysis, rather than due to meconium. Hemosiderin can be stained with the Prussian blue method for iron, and the bilirubin of meconium stains (poorly) with bile stains. The very immature fetus cannot discharge meconium, lacking the hormonal maturation for intestinal propulsion (Lucas et al., 1979). The surface of the membranes, the amnion, is normally shiny. Around the insertion of the cord, one may find squamous metaplasia in the form of concentric nodules that are hydrophobic (Fig. 1.6). They are normal features. Amnion nodosum, usually represented by a finely granular, dull appearance of the amnionic surface, correlates with oligohydramnios. One must be cognizant of whether the amnion is present at all and, if not, whether amnionic bands exist. Also, often some blood has dissected underneath the amnion during delivery or especially when fetal blood has been aspirated for diagnostic tests from the fetal surface blood vessels.

The placenta is next measured, and then the cord is examined. Is it central, eccentric, marginal, or membranous (velamentous) in its insertion? What is its length, and is it spiraled? We now believe that the length of the umbilical cord is determined primarily by fetal movements and that excessive spiraling implies unusual fetal motions (Moessinger et al., 1982). There may be a genetic component to the spiraling and the length, as the umbilical cords of some animals have different and consistent lengths; but this characteristic is so far unknown for human umbilical cords. Are there knots, thrombi, or discolorations? Can any other unusual features be detected? The cord is then severed from the bulk of the placenta, and its cut surface is studied at several locations. The most important observation to be made here is whether there are three vessels and if other unusual features are present.

A single umbilical artery (SUA) is the commonest abnormality. One must also appreciate that there is almost always an anastomosis (Hyrtl’s anastomosis) between the two umbilical arteries, which is usually found near the point of insertion on the placental surface (Priman, 1959). Thus, counting the number of vessels is best done farther away from the insertion. When a velamentous insertion of the cord is found, the examiner must pursue the ramifications of the fetal vessels after they leave the site of cord insertion, at times finding thrombi, and particularly in membranous vessels. These vessels may be disrupted, as in vasa previa, and acute exsanguination of the fetus is common in such circumstances.

Figure 1.6. Squamous metaplasia of amnion in concentric patches, usually found near the insertion of the umbilical cord. The plaques are water-repellent.
The weight of the remaining disk is ascertained. It is generally useless to know the weight of the entire organ, including cord and membranes. Correlations with fetal weight and development can be made only by knowing the “net” weight of placental tissue (Walker, 1954; Gruenwald & Minh, 1961). Excessive amounts of maternal, retroplacental clots must also have been removed before weighing. Note again that the weight of formalin-fixed placentas is greater than that of fresh organs (Fig. 1.1) (Schremmer, 1967). Variations in normal placental weight are common. They reflect mostly the length of storage and the amount of fetal blood content.

When studying the fetal surface of the placenta, one notes its color and the possible presence of granular excrescences. Most importantly, however, one must carefully inspect the fetal vessels, which are carried in the chorion; the amnion has no blood vessels. In nearly all placentas, one can recognize the fetal arteries as those vessels that cross over the veins (Hyrtl, 1870; Bacsich & Smout, 1938; Boe, 1953; Crawford, 1962). It will be observed that the terminal branches of arteries dip singly into a cotyledon; and next to it, a vein emerges to return the blood to the cord (Fig. 1.7). One often finds thrombi in these vessels in placentas of abnormal newborns. They appear as white-yellow streaks on the vessel’s surface and are usually not completely occlusive. When they are, an area of hemolysis is often seen adjacent to the thrombosis. Thrombi may also calcify. They must be sampled for histologic study.

The fetal surface of the mature placenta is often described as being “bosselated” or “tessellated,” meaning that tiny white elevations are present underneath the chorion, giving the surface a mosaic, irregular pattern. These protrusions represent accumulations of fibrin in the intervillous space and they increase in number with advancing maturity. Larger patches of fibrin also exist; at times they have a liquefied center, but they are assumed to be of little significance (Geller, 1959). In our experience, however, larger subchorionic thrombi are abnormal and occasionally associated with fetal growth restriction. Cysts from the subchorionic extravillous trophoblast cells (“X-cells”) may bulge on the surface and contain a clear, slightly viscid mucoid substance. At times it is discolored with blood. Finally, the insertion of the membranes is observed. Was it at the edge, or was there a ring of “circumvallation”?

When the placenta is turned over then, thus exposing the maternal surface, the first need is to identify possible areas of abruptio placentae. When an abruption is fresh, one may not be able to differentiate it from the normally present postpartum maternal blood clot that adheres. Within a few hours, though, the blood dries, becomes firmer and stringy, and then changes color to brown and eventually it may become greenish. In such cases, the placenta underneath the clot is usually infarcted or it is at least compressed. Abruptios are common, and most are clinically silent. Most are located at the margin of the placenta; and on occasion one finds old clot behind the membranes. Calcification on the maternal surface is then sought: small yellow-white granules in the decidua basalis and septa. Calcifications vary a good deal in quantity; they are usually found only in mature organs. The quantity has no clinically important correlations (Fujikura, 1963a,b; Jeacock et al., 1963), but clinicians have paid much attention to the recognition of calcification. It may be detected by sonography and has served as a method to “grade” (age) the placenta (Fisher et al., 1976). This is rarely done now as it has not been found to be helpful. At this point one also observes the cotyledons, the major subdivisions of the placental tissue. They increase in size and differentiation with advancing gestation, being absent early. One needs to ascertain now whether all of the cotyledons are present.
placental “floor” is present or whether there are missing cotyledons. If no cotyledonary subdivisions exist in the mature placenta, then the floor is often too thick and it may be infiltrated with an excess amount of fibrin. This condition is known as maternal floor infarction (MFI) and is best noted at this time (Naeye, 1985).

Long, parallel cuts are now made with the long knife and, most importantly, the color of the villous tissue is observed. The red color of the villous tissue is almost wholly determined by its content of fetal blood. Thus, a congested placenta (as in maternal diabetes, for instance) is dark, and that of an anemic, hydropic, exsanguinated, or erythroblastic fetus is pale, and it is usually also much more friable. Such a placenta is also commonly thicker, 3 to 5 cm, in contrast to the normal placenta, which averages 2.0 to 2.5 cm at term.

It is normal to find “holes” in the center of many placental cotyledons (Fritschek, 1927). Such holes were filled, in vivo, with maternal blood and represent the areas of first blood distribution into the intervillous space from the maternal injection jet. Intervillous thrombi, often located in these spaces, may be dark when fresh; alternatively, they are composed of layered white fibrin when older. The intervillous thrombi differ from infarcts in that they displace villous tissue. Furthermore, infarcts are granular in contrast, because they are composed of dead villous tissue. Fresh infarcts are red, and older ones are yellow to white. When sectioning the placenta, one also finds that the intercotyledonary septa contain some calcium, and often they contain some cystic spaces filled with trophoblastic secretions, the same clear mucoid material as contained in surface cysts. They too arise from extravillous trophoblast cells. Occasionally, one encounters round tumors of a solid nature, chorioangiomas. “Gitterinfaracts” or “Netzinfaracts,” because they appear to form a network of fibrin, accompany maternal floor infarction. They may be prominent, appear as dense fibrin patches throughout the organ, and have great clinical significance. It is a good practice to estimate the total amount of infarction and to record it; in fact, it is ultimately of some importance and may have medicolegal implications in infants with growth retardation. Single marginal infarcts are common and do not correlate with either fetal or maternal conditions. Other lesions are occasionally seen. Thus, some lesions that appear grossly as “infarcts” may turn out to be choriocarcinoma on histologic study (Driscoll, 1963).

Placentas of Multiple Births

Placentas of multiple births are important records for infants and pediatricians alike, and they are routinely examined. A recording of the membrane relation between twins, triplets, and so on is mandatory. For meaningful analysis it is necessary that the umbilical cords be labeled with sutures or clamps by the obstetrician, in the order of births. The most important decisions to be made in examining placentas of multiple births are (1) the number of membranes that divide the sacs (two or four) and (2) the types of vascular anastomoses that are generally present only in monochorionic twin placentas (Schatz, 1886). Fraternal (dizygotic) twins essentially always have diamnionic/dichorionic (DiDi) placentas. Fused placentas, however, are not always monochorionic. They may be DiDi and they may be diamnionic/monochorionic (DiMo). Finally, there may be no “dividing membranes” between the fetuses, as in the monoamnionic/monochorionic twin placenta (MoMo). All monochorionic twin placentas belong to monozygotic (MZ; identical) twins. The time at which MZ twins separated one from another during the early embryonic stages presumably determines the type of placentation that ultimately develops, and this can thus be estimated from an examination of the membrane relation. It is easiest, but not necessarily best, to separate the dividing membranes from each other. If there are four distinctive leaves, it is a DiDi placenta, whereas if only two membranes are apposed it is a DiMo placenta. Equally readily, the diagnosis of a DiDi twin placenta is made by ascertaining that the dividing membranes are opaque and contain remnants of old vessels or other debris (old decidua, degenerated villi). Also, in DiDi placentas one usually finds a ridge at the site where the membranes meet over the placenta. It is caused by the buckling of tissues from the collision of the two expanding placental tissue masses. The dividing membranes of DiMo placentas, in contrast, are transparent. The diagnosis of membrane relationship is easiest and most permanently established by a histologic section of a membrane roll of this tissue, or by a T-shaped section that includes this area (Fig. 1.8).

The location of the cord insertion is especially important in twin placentas, as it is much more frequently marginal or membranous than in singletons; this may reflect some problems in early placental development. Moreover, the absence of one umbilical artery (single umbilical artery, SUA) is more common in multiple births (Heifetz, 1984).

After the membrane relation is established, the “vascular equator,” that is, the area where the two chorionic vascular districts meet, is examined. In DiDi placentas, there is never a confluence of fetal blood vessels; if one were found, it would be exceptional and would be the basis for the exceedingly rare blood chimerism in fraternal twins. It must be cautioned here that ascertainment of a DiDi relation does not make the diagnosis of fraternal twins. Approximately one third of identical twins have this placentation (discussed in greater detail in Chapter 25). In monochorionic placentas (DiMo, MoMo), there are almost always some anastomoses, particularly in the prematurely delivered placentas. These anastomoses have
a great influence on the well-being of the developing fetus (Benirschke, 1961b; Bejar et al., 1988). They take three forms: artery to artery (AA), vein to vein (VV), and artery to vein (AV). The latter is doubtless the most important and is the basis for the twin-to-twin transfusion syndrome (TTTS). It must here be remembered that arteries lie on top of veins and that they are thus readily identified macroscopically. An arteriovenous (AV) anastomosis carries the blood of one twin, through a cotyledon in a one-way direction, from one twin to the other. Often the various types of anastomoses coexist, and the consequences for fetal development may be different depending on the arrangements that are present. When in doubt, one injects the vessels in question with colored water or milk, all being readily available in obstetrical suites. Only the most sophisticated studies require injection with plastics (Panigel, 1962). Injection of vessels has presented some problems for novices. First it must be remembered that most placentas have suffered some disruption during delivery, especially the immature twin placentas. Thus, only small, selected districts should be injected, and this should be done only after the umbilical cords have been cut off in order to reduce resistance. Before injecting one should seek to identify by careful inspection those areas that seem most profitable for the injection study. Large interarterial anastomoses (common) may be identified by one’s ability to push blood back and forth from one side to the other. When one attempts to demonstrate the areas that reflect shared cotyledons, as in the transfusion syndrome, one best uses a 20-mL syringe and a large (15-gauge) blunt needle. This is inserted into an arterial branch a short distance away from the prospective site and gradually one then fills the area with water or milk. The cotyledon will first rise and, when completely filled, it will empty into the vein that drains the cotyledon. It is also advisable to make a drawing or photograph of the anastomotic arrangements among multiple placental vascular districts, just to have them available for the record.

Examination of the maternal surface and of other parameters of the placentas of twins follows that of the regular protocol. It must be borne in mind that when the blood content of twins differs considerably it may be reflected in the macroscopic placental examination as well. One portion of such a twin placenta may be severely congested and larger, with the other being pale and smaller. This condition is present when only one AV anastomosis exists. Here, in the classical mechanism of the transfusion syndrome, one twin constantly loses blood through this one-way AV shunt, whereas the other becomes pletoric. Usually, it leads to hydramnios, premature birth, and disparate birth weights of these “identical twins.” It must be recognized, however, that differences in neonatal hemoglobin content of monochorionic twins may also occur acutely, when large AA and VV anastomoses exist. Thus, after the delivery of one twin, the other twin may “bleed” through anastomoses if the cord of the delivered twin is not promptly clamped. Likewise, when one such twin dies in utero, significant shifts of blood may occur from the live twin through such large anastomoses into the relaxed vascular bed of the deceased twin. Finally, it is our practice to dissect the two halves of the twin placenta at the site of the vascular equator in order to determine the placental weight of each twin. Higher multiple births are handled the same way.

Fixation

The pathologist is used to fixing tissues for histologic study in 10% formalin solution (a 1:10 dilution of the commercial 40% formaldehyde) and there is no need to make an exception with the placenta. For routine histopathology we prefer Bouin’s solution, however, because it makes embryonic and placental tissue considerably

Figure 1.8. Preparation of a “T section” of the meeting point of the dividing membranes in twin placentas.
Macroscopic Examination

harder and allows one to trim the tissue more readily before embedding. After a membrane roll is made with the help of the forceps, it is also much easier to trim this jelly roll when Bouin’s solution, rather than formalin, has been used. Bouin’s solution is made by preparing a saturated solution (1.2%) of picric acid in water and adding 40% formaldehyde solution and glacial acetic acid in proportions of 15:5:1. After overnight fixation, the tissue is ready to be trimmed. The solution has the additional benefit of decalcifying fetal tissues. Prolonged storage of tissues in Bouin’s solution makes them very hard.

Carnoy solution is a useful alternative if immediate fixation is required directly after delivery and if obstetricians refuse the use of formaldehyde in the vicinity of the delivery room. This fixative is composed of 60mL absolute ethanol, 30mL chloroform, and 10mL glacial acetic acid. It guarantees good structural preservation, provided that the thickness of the tissue blocks does not exceed 3 to 4 mm.

Many other fixatives have been used. Jiricka and Preslickova (1974) made a detailed study of seven solutions and evaluated the effect for the staining characteristics with different dyes. They found that none is ideal for all purposes, so the fixative must be chosen that gives the best results for a specific reason. The authors presented this information in tabular form, and the article must be consulted if optimal results are to be obtained.

Ideally, the sectioned slices, when Bouin-fixed, are immersed in a saturated lithium carbonate solution before embedding. This step is not absolutely required, but it helps to remove extraneous pigments. Moreover, some intervillous blood is lysed, and pigments derived from blood (“formalin pigment,” acid hematin) are more frequently present when lithium carbonate is omitted. Note, however, that occasionally the use of Bouin’s fixation is disadvantageous. For instance, Altshuler and Hyde (1985) reported that infection with fusobacteria was less readily appreciated after Bouin’s fixation than when formalin was used. Furthermore, Bouin’s solution is not useful for fixation when the purpose is to conduct immunohistochemical or in situ hybridization studies (Gleich, personal communication, 1989). Today many immunohistochemical studies can be carried out on paraffin sections, such as the demonstration of most cytoskeletal proteins, extracellular matrix molecules, and several proliferation markers (Frank et al., 1994). For all these purposes we suggest the fixation in 4% neutral buffered formaldehyde solution for a maximum of 24 hours followed by paraffin embedding not exceeding 60°C. Possible flow cytometry is also more readily done with such material.

Another important issue is the manner of tissue handling prior to fixation. The time and the mode of cord clamping (Bouw et al., 1976), the ischemic period before onset of fixation (Voigt et al., 1978; Kaufmann, 1985), as well as the composition of the fixative (Kaufmann, 1980) may well influence the distention of the fetal vascular bed and the width of the intervillous space (see tables in Chapters 15, 17, 18, and 19). We recommend particularly for all studies concerning the pathology of the feto-placental vessels (e.g., in cases with Doppler high resistance index) that a strictly standardized sampling and fixation protocol be used. If this is not done, false impressions of such conditions as chorangiosis may be obtained.

Other techniques may also require special handling, as for instance in the studies by Becker and Bleyl (1961) on toxemia. They employed fluorescence microscopy of villi to understand their different composition in this disease.

It is the recommended practice to save at least one section of umbilical cord, a membrane roll, and three pieces of placental tissue for histologic examination. Of course, having more sections of umbilical cord available for histological study is ideal, as an inflammatory response, thrombi, and other features are not always uniformly distributed throughout the length of the umbilical cord. Preparing more than one piece of placental tissue for histologic study is also desirable because so many areas of the placenta show histologic variations. Thus, one can much better determine the existence of inflammatory lesions and is less apt to overlook changes that are not ascertained macroscopically. Moreover, one must obtain sections from the more normal portions of the placenta as well. Although the pathologist is used to sampling abnormal areas for histologic study, it is not desirable to take only abnormal areas of the placenta. Indeed, almost all infarcts are histologically alike, and since they also have a typical macroscopic appearance, they are rarely worth the trouble of histologic study, except that the sections provide verifiable evidence of the existence of infarcts. It is much more important to save normal-appearing placental tissue for microscopy. One must sample both the fetal and maternal surfaces in order to include some fetal surface blood vessels. Because it is generally impossible to anticipate from macroscopic inspection whether chronic villitis and many other lesions exist, it is better to preserve too much than too little in the fixative. It goes without saying that unusual-appearing areas must also be sampled.

For histologic examination, we prefer the hematoxylin and eosin (H&E) stain. On many occasions, however, it is useful to employ special stains, such as elastica preparations, bacterial and spirochete stains, periodic acid-Schiff (PAS) preparations, and specific immunohistochemical stains that disclose the presence of viruses (e.g., cytomegalovirus and herpes antigens) as well as specific proteins [e.g., human chorionic gonadotropin (hCG), human placental lactogen (hPL), major basic protein (MBP), cytokeratin, vimentin, fibrin, proliferation markers] (see Chapter 3). These tests have given much insight into the distribution of various placental tissue components, the
sites of hormone production, and the involvement by organisms as well as other pathologic processes. The report form that we use during placental examination is reproduced in Figure 1.9.

Special Procedures

The placenta can serve as a good source of tissue for chromosome analysis. This is especially true when the fetus is macerated. One proceeds best by disinfecting the amnion with some alcohol and then stripping the amnion off a portion of placental surface. For the purpose of taking the biopsy from chorion, sterile instruments are recommended. A small piece of chorion, ideally with a bit of fetal surface vessel, is best for the purpose of establishing a tissue culture. The biopsies are placed into tissue culture medium with antibiotics and transferred to the laboratory. Touch preparations of amnionic surface or from the undersurface of the amnion may be useful for the identification of bacteria or leukocytes. It is of parenthetic interest that Jauniaux and Campbell (1990) showed that many structural abnormalities of the placenta can already be anticipated from sonography.
1. Examination of the Placenta


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