Response of the Fetus to Infection

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My presentation on this subject is drawn from the current veterinary literature, as well as my own research. The papers that you've heard so far today regard the beginning and the end of pregnancy. I want to talk about pregnancy itself, but everything has to have a beginning. I will talk about some of the responses of the individual fetus, but somewhere along the line these responses have to begin. Do we talk about the individual as beginning its life at birth or at fertilization or maybe even going back a little further to the proverbial gleam in the father's eye? The interesting thing about this is that up until a few years ago we thought about the individual's life beginning at birth but we now know that most of the reactions that we see in animals–inflammation, resistance to disease, immune reaction–all begin during uterine life, and as we continue to study this I am amazed at how young the fetus is when we first see some of these reactions. Most of what we are talking about are protective mechanisms, but some are transitory responses that are unusual because of unusual results that occur. I will just take a moment or two to cover these.

Let me give you two examples: In the intermountain west, some sheep fetuses have an aplasia of the pituitary. This is associated with severe facial deformity, and because the pituitary functions in the initiation of parturition, these ewes have prolonged gestation. Wayne Binns at Utah State University has done considerable work on this and has shown that when the ewes ingest *Veratrum californicum,* or "skunk cabbage," that this anomaly results. The interesting thing that he discovered is that he could only produce this disease on the thirteenth day of pregnancy, because this was the time in the development of this fetus when the cells that were going to form the anterior pituitary were developed sufficiently to be damaged, and yet had not developed a resistance to the toxin of this plant. We are all familiar with the results of thalidomide tranquilizer that resulted in the failure of fingers and limbs to develop. Considerable research has been done on this, and we find that the same thing occurs here. There is a very short period of time when the few cells that are going to form the legs and the arms are susceptible to this drug. If you feed the drug during this short time span, on the first day of response you destroy the legs and the arms; later you destroy only the arms, because the legs form earlier than the arms. Still later, the arms form, but the fingers may be missing. Beyond this point these cells, the nuclei of these structures, have developed to the point where they no longer are damaged by the drug. There is a natural resistance of cells as they become mature. This is one of the factors in resistance of the fetus as it develops. There is a natural or a non-specific resistance to such things as chemicals and infectious agents.

Fetuses are capable of undergoing inflammation and the protective mechanism of inflammation in the fetus the same as it is in a mature individual. Another response of the fetus that occurs late in gestation is the stress response and the response of the adrenal cortex, and the release of cortisol to the stress of infectious disease. The most interesting, and I am not sure whether it's the most important, response of the fetus is the immune response. There has been considerable work done on this. I am of the age group that when in school we were told that newborn pigs were not vaccinated at that age because they couldn't respond to the vaccine, and that you had to let them grow a little to reach immune competence. I'm sure that many of you remember that. The fact is that almost every bovine disease agent that we know of can immunize the fetus *in utero.* This immune response is both cellular and humoral. We know the plasma cells are the cells that actually produce the antibody. There has to be a proliferation of these cells and a normal chain of events, including lymphocytes and plasma cells that eventually produce antibody. We can see these changes histologically in the reticuloendothelial tissue of the fetus. If we look at the lymph nodes in a fetus that is normal and not stimulated, there is very little activity. The periphery of the lymph nodes has just a few lymphocytes. The medullary portion where the plasmocytes and the plasma cells exist is just an open network. If we look at the lymph node of the fetus that has been immunized with some agent, these lymph nodes look very much the same as you would find in a mature individual. There are follicles in the cortex of the lymph nodes. There is a thick mantle of lymphocytes. The medullary portion of the lymph node has plasma cells. All these things indicate the cellular type of reaction that has to occur before the antibody appears in the plasma, and this is exactly the way it occurs. We can find these cellular changes in the fetus before we find the appearance of antibody. Eventually, we do find antibody and we can find the specific antibody for the agent that has been introduced. In some cases when the agent is unknown, we can find

non-specific antibody, i.e., immunoglobulin G or immunoglobulin M. We look for IgG, since this is the long-lasting antibody. We can find this by immunodiffusion techniques, indicating that there has been a response; and then on the other hand, we can be more specific and look for the actual antibody. The interesting thing about this is that for each different antigen there is a specific time in the development of the individual when it becomes competent to produce that kind of antibody. I will show you when I get to some examples of fetal response that you can immunize a fetus with inactivated IBR virus when it is three months or 90 days along in pregnancy. With the bovine viral diarrhea virus you can't immunize it until it is 180 days, and there are certain salmonella to which the individual will not respond until several weeks after parturition. But once this fetus or individual can respond it responds *in toto,* and just the way it is going to respond for the rest of its life. In other words, if you actively immunize the fetus *in utero,* that is the same as an active immunity that you might produce in a two-year-old heifer. It lasts essentially for the rest of the life of the animal. When you immunize with live viruses, either modified or in the course of the natural disease, you usually have a lifelong immunity of some degree, and this is the case even though immunity occurs *in utero.* One way of stating this is that once immunity occurs it's an all-or-none reaction.

What are some of the practical values of this? I think the most practical value of understanding the response of the fetus to chemicals and to disease is that it has helped us a great deal in understanding the cause of abortion and in the diagnosis of abortion. We now know when we are dealing with abortion in cattle that we are dealing primarily with a disease of the fetus and not with the disease of the cow. One of the things that you need to think about under this circumstance is that the examination of the fetus is very important. You can examine it for specific lesions. You can examine it for non-specific lesions, such as the immune response of lymph nodes, to indicate whether you have an infectious problem or a toxic problem. When you're thinking about incubation period, you need to remember that in many of the diseases causing abortion you have consecutive incubation periods. First, the disease occurs in the cow, and then the disease crosses the placenta to the fetus and it occurs in the fetus. You then have a second course of the disease, and perhaps the fetus isn't aborted until it dies. This takes several more days, so that by the time the cow drops an aborted fetus on the ground, she may have recovered from the disease several weeks, or even several months, before the problem is brought to your attention.

I think one of the reasons that our efforts in diagnosing bovine abortion in the past were so poorly rewarded is that we were looking at the cow long after whatever disease she had had passed and she had recovered. It is important in another way, because very often we are told that the way to diagnose it as an infectious disease is to take consecutive blood samples and look for a rising titer. In many infectious causes of abortion the titer has already risen by the time the abortion occurs, so it is too late to do this.

One thing I would like to say in general about fetal disease, and in line with the study of abortion, is that the placenta is also an organ. Now, we are not going to talk about this today, but any contact between a disease agent that the cow has, and eventually the fetus has, must pass through the placenta, and this is a very unique organ. Remember that the fetus is genetically different from the dam or the sire. You can't transplant tissues from the fetus to the mother, or vice versa, because they are entirely different individuals. And yet, this fetus and its placenta which is genetically the same as the fetus, do not have an immunologic antagonism. The placenta in some way can function without stimulating the immune reaction of the cow, and thus you have this special kind of transplant and a special kind of tissue. We know that there are other special things about the placenta because sometimes, in addition to there being a maternal disease and a fetal disease, there is a placental disease. There may be three separate disease entities occurring when you're dealing with an infectious disease that cause fetal disease and abortion. In the case of the placenta, it may be resistant to disease at certain times of pregnancy and thus protect the fetus, and then let the agent across at a later time. Or, there may be a long incubation period in the placenta, thus delaying the time between introduction of the infection in the dam and the abortion.

What I would like to do now that I have touched on the placenta, which is very interesting in itself, is to come a little closer to some practical things, so I will talk about three infectious diseases that illustrate some of the things I have said about fetal disease.

First, I want to talk about bovine viral diarrhea, a mild acute disease that immunizes the fetus. Then I'll discuss an acute, peracute disease that destroys the fetus in a short period of time–the IBR virus. Finally, I would like to talk about a chronic disease of the fetus which is an ongoing disease that lasts over a period of several months, a disease that we see probably only in California–the foothill abortion, or epizootic bovine abortion. I have some slides to illustrate the course of these diseases, and as we go through these you will see that these diseases illustrate some of the points that I have just made. (So, if we could have the lights and go to the slides.)

The first slide goes back to an earlier point in my talk and lists some of the antigens, or some of the disease agents, which will immunize the fetus. You don't have time to count all these agents, but there are approximately 30 different agents there. Beginning at the bottom, you will see *E. coli,* bluetongue, vibrio, eggalbumen, enterovirus, bovine viral diarrhea, IBR, and a number of other agents. They are placed on the scale on the left which indicates time of gestation. This slide comes from an article by Schultz in *The*

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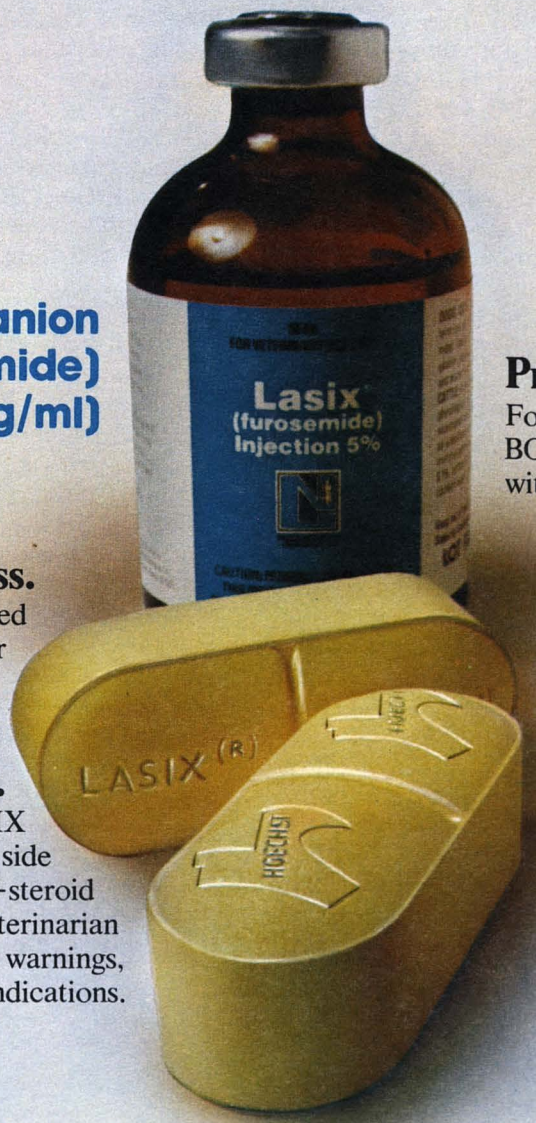

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A diuretic-saluretic for prompt relief of edema.

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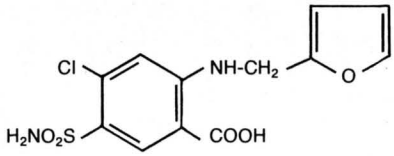
DESCRIPTION

Lasix® (furosemide) is a chemically distinct diuretic and saluretic pharmacodynamically characterized by the following:

1) A high degree of efficacy, low-inherent toxicity and a high therapeutic index.
2) A rapid onset of action and of comparatively short duration.[1,2]
3) A pharmacologic action in the functional area of the nephron, i.e., proximal and distal tubules and the ascending limb of the loop of Henle.[2-4]
4) A dose-response relationship and a ratio of minimum to maximum effective dose range greater than tenfold.[1,2]
5) It may be administered orally or parenterally. It is readily absorbed from the intestinal tract and well tolerated. The intravenous route produces the most rapid diuretic response.

Lasix® (furosemide), a diuretic, is an anthranilic acid derivative with the following structural formula:



Generic name: Furosemide (except in United Kingdom – frusemide). Chemical name: 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid.

ACTIONS

The therapeutic efficacy of Lasix® (furosemide) is from the activity of the intact and unaltered molecule throughout the nephron, inhibiting the reabsorption of sodium not only in the proximal and distal tubule but also in the ascending limb of the loop of Henle. The prompt onset of action is a result of the drug's rapid absorption and a poor lipid solubility. The low lipid solubility and a rapid renal excretion minimize the possibility of its accumulation in tissues and organs or crystalluria. Lasix® (furosemide) has no inhibitory effect on carbonic anhydrase or aldosterone activity in the distal tubule. The drug possesses diuretic activity in presence of either acidosis or alkalosis.[1-7]

INDICATIONS

Cattle:
Lasix® (furosemide) is indicated for the treatment of physiologic parturient edema of the mammary gland and associated structures.

CONTRAINDICATIONS – PRECAUTIONS

Lasix® (furosemide) is a highly effective diuretic-saluretic which, if given in excessive amounts, may result in dehydration and electrolyte imbalance. Therefore, the dosage and schedule may have to be adjusted to the patient's needs. The animal should be observed for early signs of electrolyte imbalance, and corrective measures administered. Early signs of electrolyte imbalance are increased thirst, lethargy, drowsiness or restlessness, fatigue, oliguria, gastrointestinal disturbances and tachycardia. Special attention should be given to potassium levels. Lasix® (furosemide) may lower serum calcium levels and cause tetany in rare cases of animals having an existing hypocalcemic tendency.[10-14]

Although diabetes mellitus is a rarely reported disease in animals, active or latent diabetes mellitus may on rare occasions be exacerbated by Lasix® (furosemide).

Electrolyte balance should be monitored prior to surgery in patients receiving Lasix® (furosemide). Imbalances must be corrected by administration of suitable fluid therapy.

Lasix® (furosemide) is contraindicated in anuria. Therapy should be discontinued in cases of progressive renal disease if increasing azotemia and oliguria occur during the treatment. Sudden alterations of fluid and electrolyte imbalance in an animal with cirrhosis may precipitate hepatic coma; therefore, observation during period of therapy is necessary. In hepatic coma and in states of electrolyte depletion, therapy should not be instituted until the basic condition is improved or corrected. Potassium supplementation may be necessary in cases routinely treated with potassium-depleting steroids.

WARNINGS

Lasix® (furosemide) is a highly effective diuretic and, as with any diuretic, if given in excessive amounts may lead to excessive diuresis that could result in electrolyte imbalance, dehydration and reduction of plasma volume, enhancing the risk of circulatory collapse, thrombosis and embolism. Therefore, the animal should be observed for early signs of fluid depletion with electrolyte imbalance, and corrective measures administered. Excessive loss of potassium in patients receiving digitalis or its glycosides may precipitate digitalis toxicity. Caution should be exercised in animals administered potassium-depleting steroids.

Sulfonamide diuretics have been reported to decrease arterial responsiveness to pressor amines and to enhance the effect of tubocurarine. Caution should be exercised in administering curare or its derivatives to patients undergoing therapy with Lasix® (furosemide) and it is advisable to discontinue Lasix® (furosemide) for one day prior to any elective surgery.

CATTLE: Milk taken from animals during treatment and for 48 hours (four milkings) after the last treatment must not be used for food. Cattle must not be slaughtered for food within 48 hours following last treatment.

DOSAGE AND ADMINISTRATION

The usual dose of Lasix® (furosemide) is 1 to 2 mg/lb body weight (approximately 2.5 to 5 mg/kg). A prompt diuresis usually ensues from the initial treatment. Diuresis may be initiated with Lasix® (furosemide) Injection 5% and maintained by oral treatment following a 12-hour interval.

DOSAGE:
Oral: CATTLE
One 2 g bolus daily.
Treatment not to exceed 48 hours postparturition.

Parenteral: CATTLE
The individual dose administered intramuscularly or intravenously is 500 mg (10 ml) once daily or 250 mg (5 ml) twice daily at 12-hour intervals. **Treatment not to exceed 48 hours postparturition.**

Milk taken from animals during treatment and for 48 hours (four milkings) after the last treatment must not be used for food. Cattle must not be slaughtered for food within 48 hours following last treatment.

Instructions for use of Lasix® (furosemide) in other animal species are included with dosage forms designed for that purpose.

HOW SUPPLIED

Parenteral:
Lasix® (furosemide) Injection 5% (50 mg/ml)
Each ml contains: 50 mg furosemide as a diethanolamine salt preserved and stabilized with myristylgamma-picolinium chloride 0.02%, EDTA sodium 0.1%, sodium sulfite 0.1% with sodium chloride 0.2% in distilled water, pH adjusted with sodium hydroxide.
Available in 50 ml multidose vials.

Oral:
Lasix® (furosemide) 2 g Bol-O-Tabs®
Each bolus contains 2 g of furosemide: 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid.

Available in boxes with 12 Bol-O-Tabs® each.
Store at controlled room temperature (59°-86°F).
Avoid exposure to light.
Tablets with 50 mg or 12.5 mg each are available for use in small animals.

TOXICOLOGY

Acute Toxicity:
The following table illustrates low acute toxicity of Lasix® (furosemide) in three different species. (Two values indicate two different studies.)

LD_{50} of Lasix® (furosemide) in mg/kg body weight

SPECIES	ORAL	INTRAVENOUS
Mouse	1050-1500	308
Rat	2650-4600*	680
Dog	>1000 and >4640	>300 and >464

*NOTE: The lower value for the rat oral LD_{50} was obtained in a group of fasted animals; the higher figure is from a study performed in fed rats.

Toxic doses lead to convulsions, ataxia, paralysis and collapse. Animals surviving toxic dosages may become dehydrated and depleted of electrolytes due to the massive diuresis and saluresis.

Chronic Toxicity:
Chronic toxicity studies with Lasix* (furosemide) were done in a one-year study in rats and dogs. In a one-year study in rats, renal tubular degeneration occurred with all doses higher than 50 mg/kg. A six-month study in dogs revealed calcification and scarring of the renal parenchyma at all doses above 10 mg/kg.

Reproductive Studies:
Reproductive studies were conducted in mice, rats and rabbits. Only in rabbits administered high doses (equivalent to 10 to 25 times the recommended average dose of 2 mg/kg for dogs, cats, horses, and cattle) of furosemide during the second trimester period did unexplained maternal deaths and abortions occur. The administration of Lasix® (furosemide) is not recommended during the second trimester of pregnancy.

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Cornell Veterinarian of a couple of years ago, and I know that there are a number of additional agents that have since been identified. So, there are certainly now more than 30, and there are very few infectious agents that do not immunize the fetus.

We are now talking about bovine viral diarrhea, and this virus is a mild virus for the fetus. The fetus survives the disease and is immunized *in utero* by the disease. We infected 11 cows in late gestation and their calves were examined for specific antibody before they had colostrum. Nine out of the 11 had antibody, indicating they had experienced the disease *in utero* and had recovered. Notice that these are all in late pregnancy, that in no case did abortion occur, and that there were no abnormalities in these fetuses. They all survived the disease, and as far as we could tell there was nothing wrong with them. It was just as though they were the same as a two-year-old heifer that had gone through some disease and recovered completely. The slides of the studies that I am reporting here are from experimental studies; none of these is based on field observations of the disease. There are two reasons for this: One is that it's very hard to make observations on fetal disease in the field because you cannot look at the fetus and you don't know when the disease is occurring. Second, wherever we have been able to make these observations experimentally, we have found that we can introduce the disease by inoculating the fetus directly through laparotomy, and that this is the same as the disease that occurs when the virus, or whatever agent it is, crosses the placenta and infects the fetus. If you'll stop and think about it a minute, we do quite a bit of work with SPF animals. The fetus is the ideal SPF animal. We wanted to produce antibody to a specific agent and we wanted to isolate the animal while we were producing the antibody. We found the easiest way to do this was to inoculate a fetus, then recover the fetus and collect our antibody. That way we were probably more sure of our isolation than if we'd built a 5-million-dollar maximum isolation building with graded ventilation, etc. This is a practical point for the infectious disease researcher.

The disease we produce experimentally is very similar, and so I feel that this relates to the kinds of things that you see in the field. All of this work was done by laparotomy and by direct injection at the University of California in association with A.P.E. Casaro.

Let us look at the differences in response of the fetus at different stages of pregnancy. These fetuses were inoculated at 80-120 days of gestation and were recovered and examined six or seven days after inoculation. They were all alive. We isolated a large quantity of virus. They showed some signs of disease, primarily hemorrhage and edema, but they survived at least six or seven days after inoculation of the virus. In another group they were inoculated at the same age but we waited longer to examine them. We wanted to see what the chronic disease would be. We found there was no chronic disease, because sometime after six or seven days and before 14 or 21 days, these fetuses died. In every case the fetus died as a result of inoculation with the bovine viral diarrhea virus. So, at this stage this virus is lethal to the fetus. I will tell you now that this is the only stage of pregnancy in which this virus will kill the fetus, and at all the later stages of pregnancy the fetus survives.

We inoculated some fetuses 150-170 at days of gestation. After six or seven days, again the fetuses were alive when we took them out by cesarean section or sent the cow to the slaughterhouse. They did have some edema and some hemorrhage, indicating that there was a disease, and we did find moderate amounts of virus in these fetuses. We looked for antibody as an indication of immune response and we did not find it. There are two reasons we did not find it in this case: Seven days is too short a time for an antibody response, and later on we learned that these fetuses were too young to respond immunologically. Fetuses of the same age were left in the uterus for a longer period of time, and up to 28 days we had fetuses that survived. These fetuses all looked normal at this stage, and we were able to isolate virus in a low amount. It is an important point that even though these fetuses had recovered from the disease, the virus persisted in the fetus and/or the placenta. In this case it probably was the placenta and not the fetus that remained infected, but since the fetus is bathed in placental fluid we couldn't absolutely differentiate this point. All fetuses remained in the uterus more than 14 days after inoculation, the usual period of time in which you can get an antibody formation and no antibody formed. In this case, the reason it did not form is that the fetus was too young. On the other hand, when we examined the lymph nodes in these fetuses histologically, they all showed changes that indicated an immune response, so they were ready to produce humoral antibody but had not yet reached an age when they could do so.

The fetuses inoculated at 180-225 days of gestation all survived, and in this case showed no evidence of disease. We isolated virus but in low level. A group of fetuses inoculated in late gestation were allowed to remain *in utero* from 22 up to 30 days after inoculation. These fetuses were all alive and normal. They had no abnormalities, either grossly or histologically, because some of them were necropsied for that purpose. Again, we found a low level of virus, which indicated that even after the usual recovery time this fetus or placenta could maintain the virus. Four of the five fetuses had specific antibody to bovine viral diarrhea virus. This experiment only included five fetuses. We've repeated this on a number of others and allowed these calves to survive for at least a year. During the period of up to a year of age there was essentially no change in the antibody level in these calves. This is the basis on which I make the statement that this immune response or immunization that occurs in the fetus is an active immune response, which is in every way the same as in a postnatal in-

dividual and will last for a long period of time.

I told you that this disease is a mild disease. The disease is primarily a vasculitis, and even in the blood vessels there are just little placques in the vessel wall. This is the primary lesion of this disease, and it is probably the reason why we have edema, because of the disruption of the vasculature. We did see some diffuse inflammation characterized by mononuclear infiltration. This was mild in the fetus. This was more severe in the fetal membranes. In addition to this mild inflammatory response, this virus causes necrosis of three specific tissues in the fetus. Because it can cause necrosis of specific tissues, particularly during the developmental stage–and the fetuses survive–then you have the potential for teratological defects. If you can, as I have mentioned earlier, destroy the cells which were going to develop into an organ or structure during that stage, then you have a defective development of that structure.

The nervous system develops more slowly than other organ systems, and it is still developing at the fifth month of pregnancy. (Here we have a histologic section of the cerebellum.) A fetus infected with the bovine viral diarrhea virus at approximately the fifth month of pregnancy may have necrosis of the external granular layer of the cerebellum. There is a mild inflammatory reaction around the area of necrosis. What has happened here is that the virus destroyed this tissue, which is a part of a developmental sequence in the development of the cerebellum, and the result is cerebellar hyperplasia. This is probably the most common teratologic defect caused by this virus. It has been seen in a number of instances all across the country, and we are able to say fairly definitely that this is bovine viral diarrhea, because with cerebellar hyperplasia like this the calves can't get up. Consequently, you know that they do not get colostrum unless somebody gives it to them. We can take a blood sample from these calves, and almost invariably they have the specific antibody for bovine viral diarrhea, indicating that they have had this disease *in utero*. Experimentally, we have produced this lesion in the cerebellum, and that's the basis for saying that this cerebellar hypoplasia is due to BVD. To have this type of defect you need to have a mild viral disease; the fetus has to survive so that it can live to have whatever defect it's going to have.

Another defect that is much more rare is partial alopecia, which occurs when the BVD virus causes dermatitis. Necrosis of the dermis in a 90-day fetus will cause partial alopecia. The hair on the body is quite rough and the haircoat is very thin. If you wet this hair down, you can see the skin through it, and it's obvious that there are fewer functional hair follicles. A biopsy of the skin reveals fewer hair follicles than you would find normally, and the sebaccious glands that you see just below the dermis are cystic.

I said earlier that there were three different tissues that became necrotic. I don't have an example of the third; the pulmonary bronchials do become necrotic in this disease. Whether this causes atalectasis in newborn calves or not, I don't know, but certainly the potential is there.

We inoculated fetuses at 125, 110, 115 and 100 days, very early in gestation at a time when they could not respond immunologically. We left them in the uterus until almost term. All fetuses had specific BVD antibody. The virus apparently remains in the fetus until it matures to the point where it can become immune (180 days) and then responds and forms antibody. The virus is eliminated from the fetus by the time parturition occurs because, although it is there throughout pregnancy, we have never been able to find it at the time of parturition. Some other maturing effect must occur, or maybe just the death of the placenta at the time of parturition eventually eliminates the virus. Some of these things that I'm telling you are probably more interesting than practical, but they do again illustrate the complexity of some of the development of these protective mechanisms in the fetus.

Let's go quickly now to an acute disease. This is IBR or infectious bovine rhinotracheitis. We know from a number of studies that from the time of infection to death of the fetus is always less than 48 hours. Once infection of the fetus occurs, every fetus dies within this period of time. After the virus gets into the fetus, death is very rapid. Abortion does not occur for several days after death, because abortion is a result of cessation of function of the placenta. It may be as long as five or six days after death of the fetus that abortion finally occurs. So this dead fetus lies in the uterus during this time and autolysis takes place. That is why you see this very brown tissue here. It is autolyzed hemoglobin that diffuses through the tissues. Also, fluids accumulate in the peritoneal and plural cavities, and you see dark red fluid in the body cavity. All of this is only post-mortem change. The only specific change that we see grossly in the fetus is a greatly enlarged kidney capsule. The kidney is very susceptible to this virus and there is massive necrosis of the kidney. Within 24 hours after infection you begin to get necrosis of the kidney and hemorrhage in the kidney. There is a viremia with a very high level of virus, so that hemorrhage spreads the virus very quickly through the kidney and you very soon begin to see cavities forming as the virus kills the cells. Eventually, almost the entire kidney cortex is destroyed, so that as you cut through the capsule you end up with only a thin layer (1 mm) of cortex attached to the capsule. All of the rest of the kidney, with the exception of the medulla, is destroyed by this virus which is so virulent and so destructive to fetal tissue. In other tissues of the fetus the lesions are focal necroses, which can only be seen microscopically.

In the case of IBR, because of the rapid death of the fetus, we never see antibody as a result of the naturally-occurring infection, or even the experimental infection. We were curious to know whether the fetus was capable of producing antibody, so we used

some inactivated IBR vaccine and immunized the fetus *in utero* with this inactivated virus and found that we could immunize these fetuses by inoculating as early as the third month of gestation. We consulted with the manufacturer of the vaccine (Elanco) and he informed us that the titers we got in fetuses were similar to the titers that were achieved with this vaccine when used in yearlings and two-year-olds. With the inactivated vaccine we were able to get essentially the same response in the fetus as you would get in a mature individual.

We will now take about two minutes to go through this chronic fetal disease. This is foothill abortion, or epizootic bovine abortion, a disease that we see quite frequently in California, but I doubt if it is seen outside this state. We have worked on this disease at the University of California for many years. I want to use it to illustrate a chronic disease. We now know that the incubation period from the time this agent is introduced into the cow until abortion occurs is two to three months. When abortion does occur, the fetus remains alive up until the time of abortion. The disease doesn't kill the fetus but it dies when aborted. There are hemorrhages in the mouth and on the conjunctiva. These fetuses have a dermatitis. When you necropsy them, some have ascites and necrosis of the liver. There is enlargement of the mesenteric lymph nodes and other lymph nodes. Chronic disease is indicated by the chronic stimulation of these lymph nodes. They are four or five times larger than normal. Abortion from this disease occurs when the stress of the disease causes adrenocortical hyperplasia.

I have given examples of the effects of mild acute, virulent acute and chronic disease of the bovine fetus. Knowledge of fetal disease is important to the diagnosis of bovine abortion.