arrive at random in a skin test site as the cells recycle from the blood. Interaction of these lymphocytes with antigen triggers secondary events involving the participation of blood monocytes and macrophages (10). This notion is in accord with the fact that local accumulation of mononuclear cells (lymphocytes and macrophages) is a rate-limiting step in the evolution of the delayed inflammatory reaction in guinea pig skin (11). In the foregoing speculations we have assumed that the inflammation elicited by casein is similar to that induced by specific antigen in individuals with delayed-type hypersensitivity. However, it should be emphasized that this analogy may hold for only a component of the delayed hypersensitivity reaction, namely, for the inflammatory events that occur in irritated tissue irrespective of the underlying stimulus.

Our results do not support the view that thoracic duct lymph contains macrophage precursors. Thus, radioactively labeled macrophages were not found in the peritoneal cavities of rats injected with labeled thoracic duct cells. All of the labeled exudate cells were scored as "small lymphocytes," although two of them had a larger than usual cuff of basophilic cytoplasm. The possibility cannot be excluded that these atypical small lymphocytes were macrophage antecedents.

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Fetal Response to Viral Infection: Interferon Production in Sheep

Abstract. *When virus was inoculated intravenously during the third trimester, the gestating ewe produced only low amounts of serum interferon, whereas the fetal lamb had the capacity to produce extremely high amounts. There was no evidence of transplacental transfer of interferon between mother and fetus in either direction.*

Enhanced susceptibility of fetuses and newborns has been described in many viral infections of man and of experimental animals. Although the development of the immune response in the fetal animal has been well documented (1), relatively little data has been reported concerning the host response to viral infection. Interferon is an antiviral protein produced by the host during the course of viral infections, and it has been shown to be important in recovery from the infection (2). Tissues from younger chick embryos produced less interferon and were less sensitive to the antiviral effect of interferon when compared to tissues from older embryos (3). In studies of Coxsackie B1 virus infection of suckling and adult mice, Heineberg *et al.* found that adult mice produced interferon, had only moderate titers of virus in infected tissues, and recovered from the infection, whereas suckling mice produced little or no interferon, had high titers of virus in the tissues, and died from the disease (4). On the basis of these data it has been postulated that one aspect of enhanced susceptibility of the fetal and newborn animal to virus infection might be an immature or inefficient system of interferon production and action. However, this report and another recent study (5) have shown that the fetal animal is capable of producing interferon.

Pregnant ewes of known gestational age were obtained from Stumbo Farms, Lima, New York. An indwelling catheter was inserted in a placental vessel of the fetus by sterile surgical procedures after spinal anesthesia (6). An indwelling catheter was placed in the external jugular vein of the adult ewe. Stock pools of Chikungunya virus (CV) were prepared in brains of suckling mice. They had titers of 2×10^7 plaque-forming units (PFU) per milliliter in rat embryo fibroblast tissue. Virus was inoculated intravenously into the fetuses or ewes through the catheters, which were then flushed out with phosphate-

buffered saline. Serial blood samples were obtained through the same catheters after inoculation. Heparinized saline was left in the catheters between sampling times. Blood specimens were allowed to clot overnight at 4°C; the serum was separated and stored at −20°C until it was assayed for interferon. Because of clot formation in fetal catheters, some animals had to be killed in order to obtain a specimen from the fetus at 18 to 24 hours after inoculation. Preparations of sheep interferon were made by challenging sheep kidney tissue cultures with Newcastle disease virus (NDV). The sheep interferon was assayed in fetal lamb kidney tissue culture by the plaque-reduction technique with vesicular stomatitis virus (VSV) as the challenge virus (7).

An equal inoculum of Chikungunya virus (6×10^7 PFU) was used in all animals so that an equivalent number of cells could potentially be infected and stimulated to produce interferon in both the adult and the fetus. The fetal lambs produced higher amounts of serum interferon than the adult sheep— as high as 250,000 unit/ml, whereas the highest amount produced by an adult was 300 unit/ml (Fig. 1, Table 1). The estimated blood volume for fetuses (fetal plus placental blood) of this age is 300 to 500 ml (8) and that of adult sheep is 2300 to 2700 ml (9). Although the fetal lambs had a greater initial concentration of virus in the blood, the total number of infected cells should be similar to that in the adult. If the same total amount of interferon were produced in both the adult and the fetus, there would be a lower amount of interferon in the serum in the adult because of dilution in a larger blood volume. However, it seems unlikely that these differences, either alone or in combination, would account for the 100- to 1000-fold higher amounts of interferon in the serum of the fetus. There was no detectable transplacental transfer of interferon from the inoculated fetuses 1, 2, and 3 to their uninoculated mothers, nor from the inoculated mothers 4, 5, and 6 to their uninoculated fetuses (Table 1). Procedures for characterization of interferon were carried out on serum samples from each of the fetuses and adults as well as on the standard sheep interferon prepared in tissue culture (Table 2). In addition, the antiviral effect was not removed by washing the cell sheet after it was incubated overnight at 37°C with the interferon-containing serum.

Table 1. Serum interferon after intravenous inoculation with Chikungunya virus (6 × 10⁷ PFU) in: fetuses 1, 2, and 3 with simultaneous samples being obtained from their respective uninoculated mothers; in pregnant ewes 4, 5, and 6 with simultaneous samples being obtained from their respective uninoculated fetuses; and in ewes 7 and 8 (adult nonpregnant ewes). Values are expressed as units of interferon per milliliter of serum.

Animal	Gestational age (days)	Hours after inoculation with CV										
		0	2	4	8	12	16	18	20	24	48	72
Fetus 1	140	<20	15,000	27,000	10,000		2,000		500	500	100	20
Ewe 1		<20	<20	<20	<20		<20		<20	<20		
Fetus 2	131	<20	250,000	60,000	40,000					3,000		
Ewe 2		<20	<20	<20	<20				<20	<20		
Fetus 3	117	<20	200,000	40,000	8,000					3,000		
Ewe 3		<20	<20	<20	<20		<20			<20		
Ewe 4		<20	120	80	40		20			20		
Fetus 4	133	<20	<20	<20	<20					<20		
Ewe 5		<20	200	100	50		20	20				
Fetus 5	126	<20	<20					<20				
Ewe 6		<20	250	150	150		50	30				
Fetus 6	119	<20	<20	<20	<20			<20				
Ewe 7		<20	180	100	30	30				<20	<20	
Ewe 8		<20	300	180	60	40				<20	<20	

There was some evidence of inactivation of VSV (60 to 80 percent of the control value) by a serum from ewes 6 and 7; however, the base-line serum from each animal resulted in the same degree of inactivation. We interpret the above data to indicate that, during its third trimester, the fetal lamb can produce great amounts of serum interferon after it is inoculated intravenously with CV.

There was no evidence of replication of CV in any of the animals. A late (18 to 24 hours after inoculation) serum sample from each of the inoculated animals was assayed for CV; none was detectable at a 1 : 10 dilution. In addition, samples of brain, liver, spleen, and lung obtained from the fetuses at the time of death were assayed for virus; none was detectable at a 1 : 10 dilution of a 10 percent suspension.

There are several possible explanations for the differences in amounts of serum interferon between fetal lambs and adult sheep. There could be circulating antibody to CV present in the adult but not the fetal serums. The presence of such neutralizing antibody could result in much less virus reaching the interferon-producing cells in the adult. However, there was no evidence of neutralizing or cross-reacting antibody to CV in any of the adult or fetal base-line serums assayed at a 1 : 20 dilution by the plaque-reduction technique in rat embryo fibroblasts. Alternatively, the virus could be adsorbed or inactivated by a serum or cellular component present in the whole blood of the adult but not of the fetus. However, no evidence of such antiviral activity could be demonstrated. A portion of virus was mixed with (i) whole blood from ewe 7, (ii) an equal volume of tissue culture medium containing 10 percent fetal calf serum. The samples were stored overnight at 4°C, the serum was separated from the clot, and the serum and the medium control were assayed for virus. The serum specimen had a titer of 1 × 10⁵ PFU/ml, and the medium control had a titer of 1.8 × 10⁵ PFU/ml. Thus, there was no evidence that whole blood of adult sheep inactivated the virus.

The curves of amount of interferon production plotted against time, for both the fetal and the adult animals, resemble those of release of preformed interferon rather than those of newly synthesized interferon (10). There might be greater stores of preformed interferon in the fetus because of efficient placental filtration mechanisms which would prevent interferon inducers such as endotoxin or viruses from reaching the fetus. Adult sheep, on the other hand, would likely receive periodic stimulation from endotoxin absorbed from the gastrointestinal tract, subclinical virus infection, or other inducers which could deplete preformed interferon. Evidence against this is found in studies on the induction of interferon in germ-free mice and normally maintained mice that had been inoculated with NDV (11). Similar amounts of interferon were formed in serum from both groups of animals.

Other possibilities which might account for the difference between the adult and fetal response include: (i) enhanced production of interferon by fetal cells; (ii) decreased renal clearance of interferon in the fetus; (iii) decreased uptake of interferon by fetal cells or tissues; (iv) decreased degradation of interferon by the fetus; (v) presence of a population of cells in the fetus, which are not yet committed to a particular function and still are capable of being "recruited" to produce interferon; (vi) distribution of virus to a larger number of interferon-producing cells in the fetus as a result of an altered pattern of virus uptake and inactivation; and (vii) presence of cells capable of producing interferon in the fetal placenta which are not available to the adult or newborn animal.

One might predict that there would be little or no interferon produced by
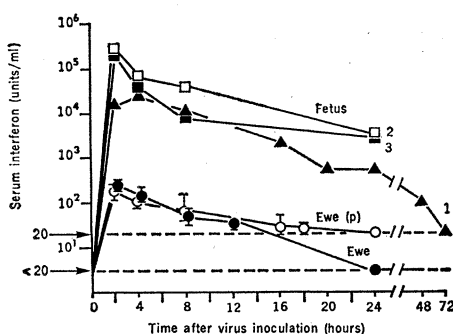

Fig. 1. Serum interferon after intravenous inoculation with Chikungunya virus (6 × 10⁷ PFU). Fetus 1, 140 days gestational age; fetus 2, 131 days; fetus 3, 117 days; ewe(p), mean derived from three pregnant ewes; ewe, mean derived from two adult nonpregnant ewes.

Table 2. Characterization of sheep interferon. Plus, characteristic present; minus, characteristic absent; plus-minus (±), see text.

Characteristic	Tissue culture	Adult serum	Fetal serum
Inhibition of heterologous viruses (VSV, Sindbis)	+	+	+
Species specificity (no activity in mouse L cells)	+	+	+
Inhibition of activity by actinomycin D	+	+	+
Trypsin sensitivity	+	+	+
Stability at pH 2	+	+	+
Direct virus inactivation (VSV)	−	±	−
Inactivation by antibody to inducing virus		−	−
Sedimentation at 100,000g	−	−	−

very young fetuses when they are most susceptible to many virus infections. The normal gestational age of the fetal lamb is 150 days. We have shown that fetuses as young as 117 days can produce interferon. Mendelson (5) has shown that older fetal rats can produce interferon after virus challenge whereas younger fetal rats do not.

One must not conclude that, because the fetal lamb can produce great amounts of interferon after inoculation with CV, this will obtain for all viruses. It is still possible that diminished production of interferon after infection with a particular virus in the fetus near term is one of the factors underlying enhanced susceptibility to that virus.

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of hydrocarbons to foods and animal feeds (3). Hybridization of selected lines and production of large-celled diploids, characterized by more rapid growth and greater ease of harvesting the larger cells, may improve the economics of the process. Examples of similar improvements are the production of soy sauce and of the Japanese fermented food, miso, through diploid hybridization of selected strains of the heterothallic haploid yeast *Saccharomyces rouxii* Boutroux (4).

A Latin description validating the perfect form of the species as *Endomycopsis lipolytica* will be published elsewhere.

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1 December 1969

Sexual Reproduction in Candida lipolytica

Abstract. Candida lipolytica *is a rather common yeast isolated more frequently from substrates containing lipids or proteins, such as dairy products, than from substrates rich in sugars. This species assimilates hydrocarbons and is currently being studied for its potential to convert petroleum into yeast cells for use in feeds and foods. We have found* C. lipolytica *to exist in nature primarily in the heterothallic haploid state. When appropriate strains of opposite sex are mixed on a suitable sporulation medium, conjugation occurs followed by the production of ascospores. Since heterothallism permits laboratory control of hybridization, this characteristic of* C. lipolytica *enhances the possibility of improving its strains for technological uses.*

So far as we are aware, strains of Candida lipolytica (Harrison) Diddens et Lodder are isolated from nature primarily in the haploid state. Because haploid strains apparently are strongly heterothallic, they do not sporulate when grown individually on sporulation media. When cells of opposite sex are mixed on yeast extract–malt extract medium (1) and incubated at 25°C, mating occurs followed by the production of ascospores. Although diploid cultures may be isolated from nature, the occurrence of ascosporogenous diploids is rare. Such a culture of *C. lipolytica*, NRRL YB-423, was isolated from material obtained from a corn processing plant in Illinois. The culture produces one to four ascospores per ascus with an unusually wide range of spore shapes, including spheroidal, angular, hemispheroidal, hat-shaped, and shallow bowl–shaped. The asci are produced either laterally or at the tips of hyphal cells, rarely from conjugated blastospores. The asci commonly rupture when mature.

Asexually reproducing clones were obtained from ascospores isolated from intact asci of strain YB-423 by micromanipulation. When these ascosporic isolates were mated, the same variety of ascospore shapes was noted. However, when ascosporic strain YB-423-12 was mated with a haploid (YB-421) isolated from the same material that supplied strain YB-423, the pair produced an abundance of ascospores all, or nearly all, shaped like a shallow bowl. Their germinability was approximately 12 percent as compared with approximately 0.1 percent for ascospores of strain YB-423.

Candida lipolytica is outstanding among yeasts for its ability to produce lipase (2) and extracellular protease. It has potential for utilization of industrial wastes containing sugars, lipids, proteins, and hydrocarbons; currently, its greatest potential is the conversion

Isolation and Zonal Fractionation of Metaphase Chromosomes from Human Diploid Cells

Abstract. A simple method for isolating large quantities of human metaphase chromosomes has been developed. Fractionation of chromosomes from peripheral lymphocytes on a large scale is accomplished by velocity sedimentation in an A-XII zonal rotor.

There have been several reports on the isolation and fractionation of mammalian metaphase chromosomes (1). For chemical examination of isolated chromosomes large quantities of cells in metaphase are needed; for this reason aneuploid cell lines such as mouse L cell, mouse lymphoblast tumor, Chinese hamster, and HeLa cells have been used. Fractionation of chromosomes into groups of distinct size has been achieved by sucrose gradient sedimentation. Dependence on this technique has severely restricted the quantity of