Humoral antibody response in the neonatal foal

by

Stephen Glen Juelsgaard

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INTRODUCTION

Immunology of the newborn and young animal has been a subject of considerable interest in the last two decades and is now being complemented by studies in the fetal animal which are receiving similar attention. A large volume of information has accumulated concerning the development of immune functions in several animal species (130, 136). This knowledge has led to a better understanding of the mechanisms involved in immune system responses to antigenic stimulation in all ages of animals and has also allowed the utilization of these immune mechanisms to provide protection to the newborn animal during this critical stage of its life.

There are, however, several animal species in which there has been only a minimal amount of study done. Extrapolations from work in other species serve as a reference in these animals. In many cases such generalizations may be extremely useful, but there are both gross and subtle differences in immunologic functions among different species. These differences have been noted, as well, in studies on neonatal animals, and point to the need for more information in each particular species.

The Equidae has been largely forgotten in immunologic studies, but this has not quelled the need for such work. With the resurgence of interest in the equine species, a desire for practical equine immunologic knowledge has surfaced.

This investigation, therefore, was conducted to study the humoral antibody response in the neonatal foal to various types of antigens. Studies were directed at determining the relationship of responses to age, to the ingestion or deprivation of colostrum in early life and to the presence or absense of maternal antibody. Also investigated were the transfer of maternal antibody to the foal and its subsequent elimination.

LITERATURE REVIEW

Factors Influencing the Immune Response

Many factors can affect the humoral antibody response including the antigen used, the methods of introducing the antigen to the animal and the animal's capacity to respond to the antigen. This review will deal with only a few such factors that were examined in the course of the experimentation, i.e., the effects of antigenicity, species and age of the animal, the presence or absence of detectable, pre-existing, circulating antibody and the effect of prior sensitization.

Antigenicity or the peculiar ability of a particular material to stimulate the body's immune system can greatly affect the response. Some important factors that can cause antigens to vary widely in this capacity include size, configuration, electrical charge, foreignness, degradability, and composition (40). In general, particulate antigens such as viruses and bacteria are more antigenic than soluble substances such as purified tetanus toxoid or human serum albumin (49). The antigenicity of such soluble materials can be enhanced by combining them with an adjuvant.

Adjuvants are materials that convert an apparently non-antigenic substance into an effective antigen or that are used to increase levels of circulating antibody (156). In the latter case the use of an intramuscular injection of adjuvanted material requires more time for antibody formation and the antibody levels increase more slowly than with intravenous

injection of nonadjuvanted material, but higher levels are attained and they decline more slowly (49). Adjuvant action is particularly valuable with highly soluble antigens of low molecular weight (49).

Some common adjuvants include Freund's complete (FCA) and incomplete adjuvant (FIA), alum and other aluminum salts including aluminum hydroxide, bentonite, and bacterial endotoxins (156). Freund's incomplete adjuvant contains a mixture of mineral oil and a detergent; killed mycobacteria are added to produce FCA (49). An aqueous antigen can be emulsified in these preparations. Although it is the most effective adjuvant, FCA produces local necrotic granulomas at the injection site, and thus its use is limited to experimental purposes (54). Aluminum hydroxide has gained wide acceptance in commercial antigenic preparations such as adjuvanted tetanus toxoid products (54). Bentonite (silica) has demonstrated an adjuvant effect in rats and rabbits (96) and in guinea pigs (158) where it was as effective as FIA in stimulating antibody production. Other adjuvants have been used less commonly and primarily for experimental purposes (49, 156).

The species of animal involved is a weighing factor in the humoral antibody response. Bacteria and viruses which are immunogenic for one animal species may produce little effect in another species. Keyhole limpet hemocyanin is a powerful antigen in rabbits, but a poor one in rats (29). Rabbits are excellent antibody producers to diphtheria toxoid while guinea

pigs are poor producers (145). Species differences can be due to a number of factors including foreignness of antigen for the species, differences in the localization, processing and metabolism of an introduced antigen and differences in the biological activities of antibodies produced to the antigen.

Age is another source of influence on the humoral antibody response. There are many reports of immunologic unresponsiveness or hyporesponsiveness in newborn and very young animals of several species (130, 136) followed by a gradual development of productive capacities to those observed in adult animals. Reports indicate that some newborn or very young animals can respond well to certain antigens, perhaps even as well as an adult (20, 72).

The presence or absense or pre-existing specific antibody appears to be involved in the regulation of humoral antibody production (43, 148). Specific passive antibodies present in sufficient levels are capable of inhibiting a primary response. This is true where antibody is already present before stimulation as well as when antibody is administered at the time of or within the first several days after inoculation (145). Less frequently, specific antibodies may block sensitization for a secondary response (146); the secondary response can also be suppressed to some degree (85, 145). It has been shown that in some cases small amounts of specific antibody injected with the antigen enhance a primary response (142, 154).

A final factor is the effect of prior sensitization on the humoral antibody response. Some doses of antigen are insufficient to stimulate detectable antibody formation but can prime animals for a secondary response upon subsequent contact with the antigen (115). Although it may appear serologically that there was a lack of stimulation by the first dose, there is a rapid increase in antibody titer to levels typical of a secondary response with a second administration. This may be the result of the production of predominantly memory cells and few or no antibody-producing cells by a small antigen dose (16).

Antibody Production in Neonates

Calves

Mitchell and Moore (84) studied the response of cattle to live Brucella abortus S19. In their study they inoculated a group of calves with no detectable anti-Brucella agglutinins and found that with increasing age during the first 200 days of life, there was an increasing responsive ability although positive responses were noted in some animals inoculated as early as 10 days of age.

In an attempt to determine the effect of colostrum deprivation on the neonatal immune response, Roberts et al. (106) administered heat-killed Salmonella dublin vaccine to colostrum-deprived and colostrum-fed calves at various intervals after birth. Calves in both groups responded from as early

as 2 days of age with the production of detectable antibody 6 to 15 days post-inoculation. No difference was noted between the 2 treatment groups.

Results somewhat contradictory to those of Roberts were obtained by Kerr (67). Heat-killed Salmonella dublin injected into calves lacking antibody to this antigen gave no detectable response to inoculations at 7 and 14 days of age. A 3rd injection at 4 months gave a good response. Seven-day-old calves immunized with live Brucella abortus S19 (2×10^{10} cells) had no detectable response. Inoculation of one animal with alcohol-precipitated B. abortus S19 at 3, 10, 15, and 30 days of age produced an agglutinating titer of 1:40 at 42 days. A 2nd inoculation at 4 months of age produced a rapid response in all animals.

Henning (50) immunized one-week-old calves having no detectable "O" or "H" antibodies to Salmonella dublin with killed S. dublin in aluminum hydroxide. Most calves produced "H" agglutinins by 7 days post-inoculation, and a 2nd dose at 30 days resulted in a marked response. No "O" agglutinins were produced following the first inoculation, while only 30-40 percent of the calves responded at 30 days and usually with low levels.

Calves inoculated before 22 days of age failed to respond to freeze-dried Trichomonas foetus bodies (68). Only weak primary responses were noted in animals inoculated during the second month of life, and responses increased with age to an adult-equivalent response at 5 months.

Smith and Ingram (128) used killed Salmonella pullorum, killed Klebsiella pneumoniae (polysaccharide antigen) and human serum albumin (HSA) in colostrum-deprived (CD) and colostrum-fed (CF) calves at one to 2 days, one and 2 weeks, and one and 2 months of age. The first group to respond to S. pullorum were those inoculated at 2 weeks, while the first response to the polysaccharide antigen was noted in the one-month-old group. No differences in onset or responsiveness were noted between CD and CF groups. Human serum albumin given intravenously or intramuscularly failed to produce detectable antibodies in 3 to 6 month old calves. When mixed with Freund's incomplete adjuvant, HSA produced responses in one week old calves, and the CD calves seemed to respond better than CF calves.

The response to a standard dose of attenuated Mycoplasma myocoides T-1 culture vaccine in calves aged one to 7 days was investigated by Stone (141). Of 5 calves so immunized, 4 had responded 10 days later and the 5th, 20 days later.

Pigs

Staub and Boguth (134) used killed Brucella abortus cells and determined that the earliest responses to this antigen in neonatal pigs occurred at 5 to 6 weeks of age in those fed colostrum, while colostrum-deprived pigs did not respond until 10 weeks of age.

Using a complicated inoculation scheme, Horlein (53) evaluated the responses of colostrum-deprived and nonimmune, colostrum-fed pigs to a Brucella abortus bacterin, ovine erythrocytes, bovine serum and a 20 per cent egg white solution. The pigs were immunized weekly with a different antigen beginning at 3 weeks of age. A 2nd dose of each agent was administered 3 weeks subsequent to the primary dose. The colostrum-deprived pigs had no detectable response to a primary inoculation with Brucella abortus but responded to a 2nd dose at 6 to 11 weeks of age. Some pigs had a weak response to the first dose of ovine erythrocytes, but a much stronger response to a 2nd dose was noted. No response to bovine serum was seen following a primary inoculation while a 2nd one produced low titers in a number of pigs. The egg-white solution gave no response. Colostrum-fed pigs from dams not immune to any of the antigens used, responded well to Brucella abortus and ovine erythrocytes from the youngest age inoculated. There was a measurable response in all to the first dose of bovine serum and about one-half responded to egg-white solution. Second doses of these antigens gave good secondary responses. There was an increased primary response as the age of inoculation increased from 3 to 6 weeks.

Pigs having no detectable maternal antibody to Salmonella paratyphi A were immunized with a formalinized preparation of this antigen (94). Those inoculated at birth produced no detectable response during the following 42-day observation

period, while of those inoculated at the 5th day of age, three-fourths had responded 9 to 11 days later. Inoculations at 7, 12, 14, and 16 days of age produced primary responses in all, but with increasing age there was a decrease in the interval from vaccination to the first detectable antibody.

Šterzl et al. (140) inoculated 6-day-old colostrum-deprived pigs with killed Brucella suis. Antibody was detected 20 days post-inoculation in this group while a CD group inoculated at one month or more of age had antibody 7 days later. Pigs removed from the uterus by hysterotomy 7 days before term and inoculated 7 days later (at normal term age) produced antibody 14 days later.

Segre and Kaeberle (119, 120) investigated the responses of newborn and 3-week-old pigs to diphtheria and tetanus toxoids injected intraperitoneally. Newborn colostrum-deprived pigs formed tetanus antibodies more rapidly and efficiently than newborn colostrum-fed pigs, while the opposite effect was observed with diphtheria toxoid to which CD pigs did not produce a detectable response. When inoculated at 3 weeks of age, colostrum-deprived pigs responded only slightly to tetanus toxoid and did not produce antibody to diphtheria toxoid. Colostrum-fed pigs, on the other hand, responded well to both antigens. The responsiveness of newborn pigs to tetanus toxoid was assumed due to some transplacental transmission of anti-tetanus antibody that stimulated antibody formation.

Pigs delivered by hysterotomy at 2 days before term were given erysipelas antigen at birth and were raised without colostrum (155). Antibody was noted 6 days later in one pig, and most responded in the 2nd week of life.

Šterzl et al. (139) measured the antibody responses to killed Salmonella paratyphi B and sheep erythrocytes injected into newborn precolostral pigs. Antibody to the sheep RBCs was detected on the 5th day post-inoculation, but only rarely had antibody to S. paratyphi been produced by this time. Antibody production to this 2nd antigen had increased by 10-15 days post-inoculation.

Lambs

Barr et al. (6) injected lambs at birth with alum-precipitated diphtheria toxoid. Within 14 days 3 of 9 lambs had responded and by 28 days, all had titers while the earlier responders had increased levels. No response was noted to purified toxoid given alone. Adult sheep produced higher levels of antibody after a first and 2nd inoculation than did the lambs.

The failure of newborn lambs to produce antibody to Salmonella typhosa, diphtheria toxoid and BCG during the first 42 days of life was reported by Silverstein (124).

Richardson et al. (103) injected lambs at birth with killed Brucella abortus cells. No antibody response was observed in lambs inoculated with 10^4, 10^6, or 10^8 organisms,

but 5 of 6 lambs receiving 10^9 or 10^{10} organisms developed titers of 1:40 by 12-15 days of age, and these persisted for one to 2 weeks. Three of 4 lambs inoculated with 10^{11} organisms responded with maximum titers of 1:160 by days 10-15. Immunization with 10^{10} organisms at one to 3 months of age produced titers of 1:640 to 1:1280 by 6 days post-inoculation; these responses were similar to those of 5-month-old and adult sheep.

Pups

One of the first reports concerning the development of humoral antibody production in young pups was that of Šterzl et al. (138). When 5-day-old pups were inoculated intraperitoneally with 5×10^7 killed Brucella suis cells, they responded with agglutinin production by 15 to 40 days of age.

Jacoby et al. (56) examined the antibody responses of neonatal and adult dogs to bovine serum albumin (BSA) and ovine erythrocytes. The neonates were stimulated within 24 hours of birth. No response was noted to a primary dose of 25 mg/kg, 8 mg/kg, or 4 mg/kg of aqueous BSA in the neonates, and a 2nd inoculation 6 weeks later also failed to elicit detectable antibody. In contrast, responses were noted in neonatal pups given a BSA-adjuvant emulsion. Adults produced antibody to both a primary and secondary stimulation with aqueous BSA. Neonatal pups responded as well as adults to ovine erythrocytes.

Newborn pups and adults were injected with sheep red blood cells and bovine γ-globulin (BGG) (72). The BGG was given either in soluble form (1 mg intravenously) or as an aggregate (10 mg intramuscularly). Adults and newborn pups responded equally well to the sheep red blood cells, but the pups failed to respond to the soluble BGG, while the adults demonstrated good primary and secondary responses. The neonatal pups gave a weak primary response to aggregated BGG and responded slowly to a 2nd inoculation.

When one-day-old pups were injected with keyhole limpet hemocyanin, they produced no detectable antibodies (123). However, a 2nd dose of this antigen at 37 days of age elicited a secondary response.

Rabbits

One of the first reports concerned with the influence of age upon antibody formation was that of Freund (35). He injected one or 2-day-old rabbits and adult rabbits with equivalent amounts of killed Bacillus typhosus. Only 2 of 10 young rabbits responded by the 5th or 6th day post-inoculation, and the highest agglutinating titer was 1:80. All the adults had responded by this time and had an average titer of 1:1280. Similar age groups were also inoculated with sheep red blood cells, horse serum, and egg white. By 5 to 6 days after inoculation all the young rabbits had responded to the sheep RBCs with an average titer of 1:103

versus 1:12,800 for the adults. The young rabbits also failed to produce precipitating antibody to horse serum and produced lower amounts of antibody to egg white than adults.

Eitzman and Smith (32) found that neonatal rabbits responded to a single injection of bovine serum albumin (BSA) at a dosage of 200 mg/kg of body weight when given at 28 or more days of age and were unresponsive to this treatment at 8 days of age or less. At 14 or 21 days of age the response varied; the majority injected at 14 days were unresponsive, while most animals produced antibody when injected at 21 days of age.

Soluble BSA injected intradermally into rabbits less than 28 days old did not induce an antibody response, while rabbits greater than 28 days of age did produce antibody (17). If BSA was incorporated into FCA and inoculated into rabbits at birth and at 7 days of age, no response was detected in the first group by 16 days of age while in the 2nd group there was a response by 19 days of age.

Říha (105) also studied responses to BSA as well as human serum albumin in young rabbits. When 5 mg was injected intraperitoneally, some animals inoculated at 3 or more days of age produced antibody 16-20 days later. Even with the incorporation of the antigens into FCA or aluminum hydroxide, no antibody formation was detected prior to the 20th day of life.

Five-day-old rabbits were injected with heat-inactivated Salmonella paratyphi B at a dosage of 10^8 organisms or 4-6 x 10^9 organisms, and newborn rabbits were inoculated with 2 x 10^9 organisms (137). The first group produced a response by 30 days of age, while in the 3rd group, antibody first appeared as early as the 14th day, and the average onset was the 24th day. The 2nd group had some responses as early as the 9th day, while the average was the 15th day of age.

Bellanti et al. (11) used killed Salmonella paratyphi B in seronegative newborn rabbits. Flagellar agglutinins appeared as early as 7 to 10 days post-inoculation, and most had agglutinins by the end of the 2nd week of life. Peak titers occurred at 14-25 days post-inoculation, and many were as high as that seen in adults. A response to the "O" antigen was found in only a few of the animals and to a much lesser degree than the response to the flagellar antigen. More frequent injections (3 times at 2-day intervals) produced higher antibody levels while increasing the number of organisms from 10^8 to 10^{10} produced earlier responses.

Killed Brucella abortus injected subcutaneously into 5-day-old rabbits (75×10^5 organisms per 100 grams body weight) gave antibody responses by 15-16 days post-inoculation (64). When injected into 10-day-old rabbits antibody was detected 10 days later.

Neonatal rabbits injected with diphtheria toxoid in silica at birth or at 3 or 6 days of age produced antibody

by 7 days post-inoculation, while those injected at the same ages with diphtheria toxoid alone produced no antibody (97).

Harris et al. (48) inoculated young rabbits with whole Shigella or Shigella-tryptose antigen at a dosage that produced good responses in adults. Rabbits 11 or 18-days-old did not respond while 2 of 3 responded when inoculated at 25 days of age although with lower levels and more slowly than adults. By 43 days of age responses were similar to those of adults. With an increase in antigen dosage there was a decreased age of responsiveness and an increased quantity of antibody produced; some one and 2-day-old rabbits produced low levels by 14 days post-inoculation. Alum-precipitated BGG inoculated into 3, 5, or 6-day-old rabbits produced responses in some that took an average of 9 days to reach peak titers, while all rabbits inoculated at greater than 6 days of age responded, and peak titers were reached at 7 days post-inoculation.

Rats

Killed Brucella abortus administered to rats at birth resulted in antibody production 7 days later (46, 52). Peak responses were observed at 10 days (46) and 2 weeks (52). By increasing the number of organisms injected into newborn or 2-day-old rats, an increase in titers at 12-15 days post-inoculation was noted (52).

Groups of rats of several neonatal ages were inoculated with 100 μg of polymerized Salmonella adelaide flagellin (159). Those injected at 16 days of age or later produced antibody after about 4 days, while those immunized at less than 2 weeks of age generally produced no antibody or only trace amounts after a few weeks. No antibody was produced before 20 days of age regardless of age at injection. A 2nd dose of 10-100 μg given at 7 or more days of age to rats inoculated at birth with 100 ng gave secondary responses that peaked 4 days later indicating sensitization to the first dose (122). The longer the period between the 2 inoculations, the greater the secondary responses.

Maternal Antibody and the Immune Response

The newborn of many of the common domestic species, including the pig, cow, sheep, and the horse, are born normally devoid of any antibody of maternal origin (14). A transfer of maternal antibody from the dam to the newborn occurs shortly after birth by means of ingestion of colostrum by the offspring. The colostrum normally contains high levels of maternal antibody, and after ingestion the newborn absorbs such antibody across its gut walls where the antibody enters the circulatory system (58). The young animal's capacity to absorb such antibodies from the gut contents is transitory, lasting only a few hours (14). The dog transmits some antibody to the fetus in utero, but the majority of transfer occurs as indicated in the species above (130).

The maximum circulating levels of maternal antibody attained in the newborn of the above species is similar to that found in the dam's serum at parturition (14). This has been demonstrated in the bovine species with antibody to Brucella abortus (74), Salmonella typhimurium (114), Salmonella dublin (51), and rinderpest virus (19) and in the ovine species with antibody to Salmonella typhosa (73), diphtheria toxin (6) and tetanus toxin (26). Similar observations have been made in the porcine species with antibody to Salmonella paratyphi (93) and in the canine species with antibody to infectious canine hepatitis virus (25) and canine distemper virus (41).

The maternal antibody levels present in the young animal decline with increasing age due to catabolism of the antibody and to ever increasing dilution as the intravascular and extravascular pools enlarge with growth of the animal (130, 14). This decline usually occurs in a linear fashion and, therefore, allows the calculation of maternal antibody half-life in the newborn. Some half-life determinations of different antibodies and immunoglobulins in the young of the various species are listed in Table 1.

There is a great diversity among species with regard to their catabolic rates for specific immunoglobulins. The fractional catabolic rate varies inversely with the size of the animal (130) and directly with oxygen consumption. This suggests that differences in the metabolic rate is one of

Table 1

Half-life Values of Various Maternal Antibodies and Immunoglobulins in Several Species

Species	Antibody (Ab) or Immunoglobulin	Half-life (days)	Reference
Bovine	FMD virus Ab	15 - 19	(44)
	rinderpest virus Ab	36.7	(19)
	diphtheria antitoxin	16	(63)
	Brucella abortus Ab	14 - 15	(68)
	Trichomonas foetus Ab	14 - 20	(68)
	IgG	20	(99)
	"fast" IgG	18	(75)
	IgM	4	(99)
	IgA	2	(99)
Porcine	diphtheria antitoxin	7	(66)
	tetanus antitoxin	9	(66)
	Serratia marcescens Ab	4.9	(18)
	Salmonella pullorum Ab	4.7	(83)
	Escherichia coli Ab	1.3	(100)
	IgG	7.5	(100)
		12	(28)
	IgM	1.3	(100)
		4.5	(28)
	γ globulin	7.5	(83)
	IgA	3.5	(100, 28)
Canine	infectious canine hepatitis virus Ab	8.6	(25)
	canine distemper virus	8.4	(41)
Ovine	diphtheria antitoxin	28	(7)

the factors producing the wide range of species differences (153). It might be expected that newborn animals show shorter half-life values than adults, but this has not been demonstrated in a number of species (130).

The various classes and subclasses of immunoglobulins also have different metabolic characteristics (153). Generally IgG is catabolized more slowly than IgM or IgA which have half-life values usually one-half or less than those of IgG. Similarly, some subclasses of IgG are catabolized more rapidly than others as has been noted in mice (131).

The presence of specific maternal antibody in the young animal can exert a significant influence of the response to many different antigens (130). High levels of maternal antibody can block or suppress to some degree antibody formation in young animals that have normal responsive abilities.

Henning (51) investigated the effect of killed Salmonella dublin administered to calves born of dams immunized during gestation with this agent. All calves received maternal anti-S. dublin "H" and "O" agglutinins after nursing, and, although the serum titer fell with increasing age, appreciable amounts of antibody remained until 2 to 3 months of age. Two inoculations one week apart were given to the calves beginning at either one to 2 days of age or one to 2 weeks of age. No response was noted after any of these inoculations.

Stone (141) immunized calves having various levels of antibody to Mycoplasma mycoides var. mycoides with the T-1 vaccine of this agent between 38 and 56 days of age. Production of complement-fixing (CF) antibody could not be demonstrated in any of the animals. Even one calf immunized 14 days after maternal CF antibodies were no longer detectable failed to produce antibody. These calves were revaccinated between 58 and 86 days of age when all were serologically negative, and all responded by 8 to 10 days post-inoculation.

Barr et al. (7) studied the response to diphtheria toxoid of lambs that received maternal antibody from their immune dams. Lambs inoculated at 14 and again at 42 days of age produced antitoxin within 4 weeks of the first inoculation if their maternal antitoxin levels at the time of immunization were sufficient to neutralize no more than 40 per cent of the injected antigen. No responses were observed with higher antitoxin levels. Good secondary responses were noted in all lambs whose antitoxin content at the first inoculation was twice that sufficient to neutralize all the injected toxoid. Poor secondary responses were observed in those lambs whose antitoxin levels at the first inoculation were 2½ to 6 times that sufficient to neutralize the toxoid. A few lambs with still higher passive titers gave no active response to the 2nd inoculation but gave good responses to a 3rd dose given 9 weeks later.

Lambs born of ewes vaccinated with alum-precipitated Clostridium welchii type D toxoid and immunized with this preparation demonstrated different responses according to the level of maternal antibody present at inoculation (10). When a primary dose was given within 3 days of birth, lambs with low passive antibody levels responded, while high levels blocked a response. Secondary responses were noted in 60 per cent of the lambs given a 2nd dose, while the remainder demonstrated a primary response. Better secondary responses were noted when the interval between inoculations was 4 or more weeks.

Responses to various antigens in the presence of maternal antibody have been studied in neonatal pigs (53). Colostrum-fed pigs from immune sows produced no antibody to killed Brucella abortus at 3 to 5 weeks of age. Responses to a 2nd dose were lower than those recorded in colostrum-fed pigs from non-immune dams. There was only a slight increase in titer after a primary inoculation with ovine erythrocytes except in 6 to 8-week-old pigs which gave a good response. No inhibition of a secondary response to a 2nd dose was noted.

Serum of newborn rabbits contains hemagglutinating antibody to erythrocytes of many animal species, but these levels decline rapidly (104). When 10 per cent suspensions of guinea pig, sheep, rat, or dog RBCs were given to 5-day-old rabbits with passive antibody, the antibody disappeared

from the circulation by the 3rd day post-inoculation; by the 5th day post-inoculation, antibody (actively produced) had appeared and peaked by 7 to 9 days post-inoculation.

Halliday (47) immunized pregnant rats with killed Brucella abortus. The offspring were then inoculated at 18 to 30 days of age with this same antigen and 4 response groups were observed. Those with no detectable antibody had a slower response than offspring of nonimmune females, and titers peaked at 2 weeks post-inoculation. With low levels present at inoculation, the response at one week was depressed while a peak response was again noted at 2 weeks post-inoculation. Peak responses in these groups were all similar. Groups with moderate or high titers had decreased responses one week later and did not reach peak titer until 3 weeks after immunization. These responses were of a lower magnitude than the previous groups.

Very low (undetectable) levels of maternal antibody have been associated with enhancement of the immune response in young animals (119, 120, 121). Segre and Kaeberle (120) noted that in newborn colostrum-deprived pigs, enhancement of responses to tetanus or diphtheria toxoid occurred when one ml of 1:10,000 diluted swine serum from pigs hyperimmunized with tetanus toxoid and tiphtheria toxoid was injected with the toxoid. Similar results were recorded in 3-week-old pigs (119, 121). The inoculation of 3-week-old colostrum-deprived pigs with tetanus and diphtheria toxoids mixed with

50 ml of swine serum from 7 to 8-week-old colostrum-deprived pigs immunized 2 weeks previously with tetanus and diphtheria toxoid resulted in enhanced antibody responses (119). Increased responsiveness was also noted when 500 mg of gamma globulin from 4 to 14-week-old CD pigs was injected with the toxoids (121).

Neonatal Immunity in the Foal

An early paper on passive immunity of domestic animals by Mason et al. (81) proposed that the foal had no immunity (i.e., antibody) prior to birth. They examined the serum of one newborn foal from a mare with a "natural" diphtheria antitoxin titer of 1.25 units/ml and found it to contain no demonstrable antitoxin. They administered sheep serum containing lamb dysentery antitoxin and equine tetanus antitoxin orally immediately after birth and noted that both antibody types were absorbed within 3 hours. In the same year Bardelli (5) also reported that the serum of a newborn foal was negative for tetanus antitoxin before nursing but became positive after nursing its dam's colostrum which had a high antitoxin titer.

Alexander and Mason (1) worked with a group of pregnant mares vaccinated with neurotropic horsesickness vaccine. They found that presuckling serum samples from foals of dams with serum antibody to the agent were negative for such antibody. In less than 30 hours after birth the serum

antibody levels in the foals had reached a concentration at least equal that of their dam's. The titer gradually decreased with age, and the duration of the decline was proportional to the original 30-hour titer. The antibody present usually persisted for about 6 months. During the period that anti-horsesickness antibody could be detected and for some unknown length of time after apparent disappearance, the inoculation of routine horsesickness vaccine had no immunizing effect. The passively acquired immunity protected foals against the virulent pantropic virus for 157 days but not for 239 days after birth.

Some slight transmission of tetanus antitoxin to the foal prior to nursing was reported by Lemétayer et al. (69, 70). These foals were offspring of mares hyperimmunized against tetanus. They found, however, that the foal acquired most tetanus antitoxin subsequent to nursing and that the serum levels of antitoxin attained by the foals approximated that of their dam's serum titer.

Bruner et al. (22) used Salmonella abortus-equi antigen to hyperimmunize mares prior to foaling. Although at parturition the mares had very high serum antibody levels against this antigen (1:5000), no antibody could be detected in the foals before nursing. At 12 hours of age the foals anti-S. abortus-equi titer was 1:1000. By one week the titer had fallen to 1:500, and it remained at this level through one month of age. At 6 months the antibody levels in the foals were undetectable.

Bruner et al. (21) attempted to determine the approximate age when the foal was no longer capable of absorbing maternal antibody. Using high titer anti-Salmonella abortivoequina serum administered orally to foals at different intervals after birth, they found that foals no longer acquired detectable serum titers at 24 to 36 hours of age.

Lemétayer et al. (71) determined that the levels of antitoxin in foals born of mares immunized against tetanus in the last weeks of gestation were protective up to 30-50 days of age. They suggested immunizing foals with alum-precipitated tetanus anatoxin at 6 weeks of age. Responses to the antigen at this age were comparable to those of the adult horse, and the maternal antitetanus antibody present did not impair the establishment or duration of immunity. They also suggested immunization of the dams routinely in late gestation with this agent.

A report (110) on the longevity of maternal tetanus antitoxin in the neonatal foal indicated that, with mares hyperimmunized with adjuvanted tetanus toxoid in late gestation, protection was afforded their foals after nursing for as long as 6 months. One foal deprived of colostrum lacked protective antitetanus levels.

In a study (23) of 18 foals born of mares seropositive to equine infectious anemia virus, almost all the foals were found to be seropositive upon initial examination after birth and remained so until 65 to 182 days of age.

Jeffcott (61) hyperimmunized 6 mares during gestation with Clostridium welchii type A toxin. Traces of antitoxin were found in prenursing sera, but the titers rose rapidly after nursing to about one-half the dam's serum titer. The levels then fell so that by 4 weeks of age they were 35 per cent of peak values. They were no longer detectable at 4 months of age.

Schützler (117, 118) reported that 5 of 10 foals born of dams hyperimmunized with aluminum hydroxide-absorbed tetanus toxoid showed an antitoxin titer of 0.01 IU/ml before nursing. The foals showed no response when immunized at one (prenursing), 4, 7, 14, or 21 days of age. However, in several of these foals, sensitization occurred because a 2nd inoculation given at 5, 6, 7, or 12 weeks of age resulted in a rapid response.

Recent work by Jeffcott (57, 59, 60) has been directed to the mechanism and duration of the transfer of maternal antibody to the foal. Intestinal absorption has been shown to be the primary if not the only transfer mechanism. A decline in absorptive ability occurs after birth, and antibody absorption ceases by 24 hours of age. Levels of maternal antibody and immunoglobulin are also greatly decreased in the maternal colostrum produced at 24 hours after parturition (59, 60).

Studies of the transfer of maternal antibody in the equine have also been made by evaluating circulating

immunoglobulins in the neonatal foal. Excellent reviews summarizing the literature on equine immunoglobulins have been prepared by McGuire et al. (77) and Montgomery (88).

The horse possesses at least 8 antigenically distinct groups of immunoglobulins (108). There are 3 subclasses of IgG - IgGa, IgGb, and IgGc. The horse also possesses IgM and IgA and 2 classes unique to the equine species, IgG(T) and aggregating immunoglobulin (AI). A summary of their properties are presented in Table 2.

IgG(T) is found in predominant levels in tetanus and diphtheria antitoxins. Aggregating immunoglobulin is found in high levels in anti-pneumococcal polysaccharide serum and is composed of noncovalently-linked, salt-dissociable aggregates. The classification and nomenclature of equine immunoglobulins are currently open to discussion (88).

In 1943 Polson (98) reported that newborn foal serum had only traces of β-globulin and an absence of γ-globulin. By 5 days of age, both levels appeared to have had greatly increased.

Rouse (111) reported the levels of IgG, IgG(T), and IgM in foals at various ages after birth. Two groups of foals were used - 9 Thoroughbreds and 9 Shetland-type ponies. No immunoglobulins were detected before colostrum ingestion, but within 12 to 24 hours after nursing the levels of all immunoglobulins studied had risen to within the adult range. For the next one to 2 months, the concentrations decreased rapidly

Table 2

Some Properties of Equine Immunoglobulins

Immunoglobulin Class	Molecular Weight	Sedimentation Coefficient	Electrophoretic Mobility	Ave. Serum Conc. in 20 Shetlands (88)
IgG a, b, c	152,000	6.7S	γ_2 γ_1	1334[a] (IgGa)
IgG (T)	152,000	7.1S	β_2	821
IgM	1,000,000	19.8S (five 6.3S monomers)	β_2 γ_1	120
IgA	150,000-700,000 Most 350,000 (dimer)	variable	γ_1	153
AI	variable 150,000 ave.	9S-15S as an aggregate (6.3S monomer)	γ_1	39

[a] Immunoglobulin levels expressed in mg/100 ml.

after which the levels remained constant for a period of time that varied with each immunoglobulin class. Immunoglobulin concentrations then increased with IgM levels rising first, followed by IgG(T) and later by IgG. Immunoglobulin concentrations increased at an earlier age in the Shetland-type pony foals than in the Thoroughbred foals.

Serum concentrations of IgG, IgA, IgM, IgG(T) and aggregating immunoglobulin (AI) were measured in 3 Quarter Horse and 5 Shetland pony mares at parturition and in their foals at various ages after birth (76). Some IgM was found in the prenursing sera of 4 foals and some IgG was also found in one of these. All 5 immunoglobulins were absorbed and reached maximum levels at 24 hours. IgG was absorbed more rapidly and to a concentration more nearly equal the dam's levels than the other classes. Concentrations began to decrease after this time with minimum levels of IgG and IgG(T) occurring at 30 to 60 days of age. It was concluded that synthesis of IgG, IgG(T), and IgA began within 24 days of birth, and that synthesis of IgM probably began before birth. No differences were noted between the Quarter Horse and Shetland pony foals.

Jeffcott (61) followed the γ-globulin levels in 3 groups of foals from birth to one year of age. One group nursed normally from birth, a 2nd received supplementary colostrum and hyperimmune serum during the first 30 hours of life, and a 3rd group was deprived of colostrum. All foals were

agammaglobulinemic at birth, and in both colostrum-fed groups, the γ-globulin levels peaked 12-18 hours later. The peak values were a little lower than those of their dams. The levels in the first 2 groups then decreased to lowest values at 4 to 5 weeks after which they again rose to plateau at 6 to 8 months of age. The colostrum-deprived foals remained agammaglobulinemic until 2 weeks of age. The levels then rose steeply until 7 to 8 weeks of age after which they remained steady and were similar to values seen in the other 2 groups at 6 to 8 months.

Recently there have been 2 reports of equine fetal responses to antigenic stimulation. Martin and Larson (79) injected 5 equine fetuses with T2 coliphage at 200 days gestation. The fetuses were then delivered by hysterotomy 10 days later, and fetal serum samples were taken. All 5 fetuses had developed viral neutralizing titers by the time of delivery. No neutralizing antibody was detectable in the mares at this time. Fetuses of 9 mares at 8 to 9 months gestation were given injections of Venezuelan equine encephalomyelitis (VEE) virus vaccine (89). Serum from the 9 foals before nursing had VEE-serum neutralizing (SN) titers that were 3 to 10 times greater than those found in mature horses similarly vaccinated. No effect was noted on the mares' VEE-SN titers.

Reilly and MacDougall (102) studied the metabolism of ^{125}I-labelled equine IgG in foals beginning at 3 days of age.

The apparent plasma half-life of the labeled immunoglobulin was 23 days ± 4.5 days. Half-life values of 23 days for gamma-globulin and 20 days for Clostridium welchii type A antitoxin were calculated by Jeffcott (61) in 8 foals from birth to 4 weeks of age. He also reported a shorter half-life (18 days) for gamma-globulin in foals fed supplementary colostrum at birth and, therefore, possessing higher gamma globulin levels.

Half-life determinations in adult horses have included diphtheria antitoxin (19-26 days) (42), ^{131}I-labelled "gamma globulin" (11.0 days) (100) and I^{131} labelled IgG (14.3 days) (92). In addition, the latter study by Nansen and Riising (92) indicated that the catabolic rate of IgG was directly proportional to the plasma level of IgG.

EXPERIMENTAL PROCEDURE

Animals

This experimentation, conducted over a 2 year period, involved the use of a large group of Shetland pony mares and their offspring and a small group of Quarter Horse mares and their offspring.

In 1973 a group of 29 pregnant Shetland pony mares was obtained and individually identified by freeze branding. This group was kept on pasture isolated from contact with other horses. Three pregnant Quarter Horse mares owned by the Department of Veterinary Clinical Sciences, Iowa State University, Ames, Iowa were also utilized. They were housed during gestation with other mares owned by the Department.

When the mares appeared to be near parturition, they were transferred to a separate facility for observation and foaling. Thirty-one live foals and one dead foal were born without complications. The foals were given the same number as that of their dam for identification purposes.

At birth the Shetland foals were placed into one of 2 groups -- those allowed to nurse their dams in a normal fashion (colostrum-fed or CF) or those not allowed to nurse (colostrum-deprived or CD). Colostrum deprivation was accomplished by placing a square piece of rubber sheeting over the mare's udder and securing it to her by means of a harness. Parturition of the mares was attended whenever

possible. Seventeen of the 28 live Shetland foals were deprived of colostrum for the first 72 hours after birth. None of the foals of the Quarter Horse mares were deprived of colostrum.

The colostrum-deprived foals were fed a commercial equine milk replacer diet[1] every 6 hours until they were 72 hours of age, following which they were allowed to nurse. The dams of the CD foals were milked out by hand every 6 hours during the 72 hour period after parturition.

The CD foals were put on antimicrobial prophylactic therapy at 6 hours of age, and this was continued until they were one week old. At the beginning of the project the regimen included oral tetracycline[2] and oral sulfamethazine[3] and a parenteral penicillin-dihydrostreptomycin combination[4]. Loss of 5 foals (one from Actinobacillus equuli septicemia and 4 from Escherichia coli septicemia) prompted a change in the antimicrobial regimen. Subsequently CD foals received oral chloromycetin[5] and oral neomycin[6] and a parenteral

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[1] Foal-Lac, Borden Chemical, Norfolk, Virginia.

[2] Cosa-Terramycin, Pfizer, Inc., New York, New York.

[3] Sulfamethazine U.S.P. Powder, Veterinarian Specialties, Inc., Cedar Rapids, Iowa.

[4] Pen-Di-Strep, Diamond Laboratories, Des Moines, Iowa.

[5] Tevcocin Oral, Tevcon Industries, Inc., Omaha, Nebraska.

[6] Biosol Liquid, The Upjohn Co., Kalamazoo, Michigan.

combination of trimethoprim-sulfadiazine[1] as well as the penicillin-dihydrostreptomycin combination. The CD foals seemed much more susceptible to infectious agents, especially E. coli during the first days of life, but the second described prophylactic treatment regimen appeared to effectively prevent severe disease in the remaining foals. Of the 17 foals deprived of colostrum, 12 survived and were used in later experimentation.

When the foals were one to 3 weeks of age, they were moved back to pasture along with their dams. They were held together in one group for the duration of the project. The Quarter Horse mares and their foals were held in a separate lot isolated from other horses during the project.

In 1974 a group of 33 pregnant Shetland pony mares was obtained. Eighteen of these mares had been used in the 1973 group. The remainder were identified as described previously. These mares were kept on pasture isolated from contact with other horses.

Upon observation of impending parturition, the mares were moved to another facility for observation and foaling. Thirty-three live foals were born with no noted complications.

All foals were allowed to nurse normally after birth, and no antimicrobial agents were administered. At about one week of age the foals and their dams were moved back to

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[1] Tribrissen, Burroughs, Wellcome and Co., London, England.

pasture where all were kept in one group. One foal died at 7 weeks of age from acute Actinobacillus equuli nephritis.

Antigens

Brucella - HSA

An antigen containing at least 10×10^9 killed Brucella abortus cells, 10 mg of human serum albumin and 0.04 ml of a 50 per cent bentonite suspension per ml was employed. This antigen was identified as Brucella-HSA. To produce this antigen a commercial preparation of lyophilized live Brucella abortus Strain 19 cells[1] was reconstituted with 19 ml of the sterile diluent supplied with the preparation. The bacteria were killed by heating in a 65°C water bath for one hour. Five ml of human serum albumin (Cohn's fraction V)[2] mixed to a concentration of 50 mg/ml in normal saline solution (NSS) were added to this suspension. Also added was a fraction of sterile, washed bentonite[3] prepared by differential centrifugation and resuspended in an equal volume of NSS.

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[1] Brucella Abortus Vaccine, Jensen Salsbery Laboratories, Kansas City, Missouri.

[2] Miles Laboratories, Inc., Kankakee, Illinois.

[3] Fisher Scientific Co., Fair Lawn, New Jersey.

Tetanus toxoid, killed equine encephalomyelitis virus

A commercial product composed of tetanus toxoid and inactivated avian tissue culture origin Western and Eastern equine encephalomyelitis virus (Equiloid[1]) was used as supplied by the manufacturer.

Immunization

In 1973 the pregnant Shetland pony mares were divided into 3 groups. One group of 16 mares was immunized with Brucella-HSA. Each mare received a dose of 2.5 ml subcutaneously and 2.5 ml intramuscularly in the neck region. The 2nd group of 9 mares was inoculated with Equiloid given intramuscularly in the neck region. A 3rd group of 4 mares was not immunized.

Most mares in the Brucella-HSA immunized group were given at least 3 inoculations before parturition. The intervals between Brucella-HSA inoculations were approximately 4 months between the first and 2nd doses and one month between the 2nd and 3rd doses. Additionally, 4 mares were given a 4th inoculation about 5 weeks after their 3rd dose. Two mares received a single inoculation at 2 to 3 weeks before parturition.

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[1] Equiloid, Fort Dodge Laboratories, Fort Dodge, Iowa.

Most mares in the Equiloid immunized group received 4 injections before foaling. The interval between the first and 2nd doses was approximately 7 weeks and the 3rd dose followed the 2nd by approximately 3 months. The 4th injection was given about 5 weeks after the 3rd. Four mares were given only 2 inoculations before parturition.

The Quarter Horse mares were given a single injection of Equiloid prior to parturition.

An immunization format for the foals of these mares is presented in Table 3. Immunizations with either Brucella-HSA or Equiloid were made at one, 2, 4, 8, or 12 weeks of age. Those animals immunized one, 2, 4, or 8 weeks of age were given a 2nd dose at 12 weeks of age. Most foals were immunized with both products, but the primary immunizations with different products were always separated by at least 2 week intervals except in 2 foals immunized at 12 weeks of age.

Table 4 indicates the number of foals in each age group immunized with the various antigens.

Blood samples were collected from the mares at parturition and from their foals at birth and before nursing whenever possible. Foals were bled at 24 hours, 72 hours and one week of age and weekly thereafter until the foals were 15 weeks old. A 20 ml sample of blood was collected in an evacuated[1]

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[1] B-D Vacutainer Tubes, Becton, Dickinson and Co., Rutherford, New Jersey.

Table 3

Immunizations of 1973 Foals

Foal Number	Colostrum fed (CF) or Colostrum deprived (CD)	Age at Primary & Secondary Immunizations (weeks)			
		Brucella-HSA		Equiloid	
1	CD	2	12	4	12
2	CF	8	12	1	12
3	CF	4	12	2	12
4	stillborn	---		---	
5	CD	1	12	8	12
6	CD	1	12	8	12
8	CF	8	12	1	12
9	CF	1	12	8	12
10	CD	8	12	1	12
11	CD, died	---		---	
13	CD	4	12	2	12
15	CD, died	---		---	
16	CD	12	-	4	12
17	CD, died	---		---	
18	CD	12	-	12	-
19	CD	8	12	1	12
20	CD	4	12	2	12
21	CF	2	12	4	12
23	CD, died	---		---	
28	CF	8	12	1	12
29	CF	-	-	8	-
30	CF	-	-	2	12
33	CD, died	---		---	
41	CD	2	12	1	12
42	CF	1	12	4	12
43	CF	2	12	4	12
44	CD	1	12	8	12
45	CD	2	12	4	12
46	CF	2	12	1	12
B. Quarter Horse Foals					
101	CF	12	-	12	-
102	CF	4	12	2	12
103	CF	2	12	4	12

Table 4

Number of Foals in Immunization Groups (1973)

Immunizations	Equiloid Immunized Dams		Brucella-HSA Immunized Dams	
	CF Foals	CD Foals	CF Foals	CD Foals
I. Equiloid				
A. One week	1	2	3	1
B. 2 weeks	1	1	2	1
C. 4 weeks	2	1	2	2
D. 8 weeks	1	1	1	2
E. 12 weeks				
1. primary immunization	1	0	0	1
2. secondary immunization	5	5	7	6
II. Brucella-HSA				
A. One week	2	1	0	2
B. 2 weeks	1	2	3	1
C. 4 weeks	1	1	1	1
D. 8 weeks	1	1	1	1
E. 12 weeks				
1. primary immunization	1	0	0	2
2. secondary immunization	5	5	5	7

tube from the jugular vein with a one and one-half inch 20 gauge needle. About 4 hours after collection the tubes were centrifuged at 1000 g for 10 minutes. Serum was harvested, divided into 5 aliquots and stored at -20C.

The 1974 pregnant Shetland pony mare group was immunized in the last half of gestation with one or more of the antigens employed. The immunization schedule was designed to produce groups of mares with different specific antibody levels at parturition. An attempt was made by this means to create groups of foals with different specific maternal antibody titers. Thirteen mares received one injection, 14 mares received 2 injections, and one mare received 3 injections of Equiloid prior to parturition. Eleven mares received one injection, and 4 mares received 2 injections of Brucella-HSA before foaling. The intervals between inoculations with these products were at least one month.

The foals were immunized at various ages with the 3 antigens as indicated in Table 5. Primary immunizations with Brucella-HSA were conducted at the ages of 2 weeks (2 foals), 4 weeks (6 foals), 8 weeks (one foal), 10 weeks (19 foals), 11 weeks (2 foals) and 12 weeks (2 foals). Secondary immunizations were usually given 4 weeks later. Primary immunizations with Equiloid were administered at 2 weeks (one foal) and 8 weeks (31 foals). Secondary immunizations were made 4 weeks later.

Table 5

Immunizations of 1974 Foals

	Age at Primary and Secondary Immunizations (weeks)			
Foal Number	Brucella-HSA		Equiloid	
1	10	14	8	12
2	10	14	8	12
3	10	14	8	12
4	11	15	8	12
5	10	14	8	12
6	10	14	8	12
8	10	14	8	12
9	4	9	8	12
10	10	14	8	12
11	10	14	8	12
13 died	--	--	-	--
14	10	14	8	12
15	10	14	8	12
17	4	9	8	12
18	8	12	8	12
19	4	8,12	8	12
20	12	16	8	12
23	10	14	8	12
24	2	7	8	12
25	4	8,12	8	12
26	10	14	8	12
29	2	6	8	12
31	10	14	8	12
32	10	14	8	12
33	4	8	8	--
34	10	14	8	12
35	10	14	8	12
37	10	14	8	12
38	4	9	8	12
52	10	14	2	6
54	12	--	8	12
56	10	14	8	12
58	11	15	8	12

Blood samples were collected from the mares at parturition and their foals at birth; foals were bled prior to nursing when possible. Subsequent samples were taken at 72 hours and one week of age and weekly thereafter until the foals were 17 weeks old. A 20 ml sample of blood was taken as described previously. Centrifugation and storage of the serum was also done as described above.

Serological Testing

The macroscopic tube agglutination test (133) was used to determine the presence of agglutinating antibody to Brucella abortus. Brucella antigen[1] was diluted 1:100 in 0.5 per cent phenolized NSS. The serum samples were thawed in a 37C water bath and inactivated in a 56C water bath for 30 minutes. Serial 2-fold dilutions of the sera were made in 0.5 ml volumes of NSS starting with a 1:10 dilution. An equal quantity of the prepared tube antigen was added and mixed by gentle shaking. Tests were incubated overnight in a 37C waterbath and read the next morning. A positive test was indicated by a visible agglutinate present at the bottom of the tube. The titer was expressed as the reciprocal of the highest dilution giving a positive reaction.

A passive hemagglutination test (33) utilizing the

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[1] Brucella Antigen (tube), provided by USDA, ARS, Animal Health Division, Diagnostic Services - Diagnostic Reagents, NADC, Ames, Iowa.

microtiter technique[1] was used for the determination of antibodies to human serum albumin. Human serum albumin (Cohn's fraction V) was dissolved in NSS at a concentration of 25 mg/ml. Sheep erythrocytes were washed 3 times in NSS. An aliquot of 0.1 ml of a 50% glutaraldehyde solution[2] was added to 4.9 ml of NSS. To 0.25 ml of washed, packed sheep erythrocytes was added 0.4 ml of the human serum albumin solution and 0.8 ml of the glutaraldehyde preparation. This mixture was allowed to incubate at room temperature for one hour and was then washed 3 times in NSS. It was resuspended in 45 ml of normal rabbit serum diluent (one per cent inactivated, sheep red blood cell-absorbed rabbit serum in NSS). A 0.1 ml aliquot of this suspension was diluted with 4.9 ml of normal rabbit serum diluent. The optical density of this suspension was measured in a spectrophotometer[3] at 540 millimicrons using tubes with a 10.0 mm internal diameter and the optical density was adjusted to 0.120 by the addition to or removal of normal rabbit serum diluent from the original suspension. Sensitized sheep RBCs were prepared fresh each day on which the tests were conducted.

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[1] Microtiter, Cooke Engineering Co., Alexandria, Virginia.

[2] 50% Glutaraldehyde Solution W/W, Fisher Scientific Co., Fair Lawn, New Jersey.

[3] Spectronic 20, Bausch and Lomb, Inc., Rochester, New York.

The sera to be tested were thawed in a 37C water bath and inactivated in a 56C water bath for 30 minutes. A 0.5 ml aliquot of each serum sample was absorbed with 2.5 ml of a 20 per cent washed sheep erythrocyte suspension for one hour. Serial 2-fold dilutions were made in microtiter plates using one per cent normal rabbit serum diluent and starting with a 1:10 dilution. One drop (0.05 ml) of the sensitized erythrocyte suspension was added to each serum dilution. A positive serum of known titer and a known negative serum were included as controls. The sera and sensitized RBCs were mixed by scratching the bottom of the microtiter plates. The sensitized cells were allowed to settle at room temperature, and the test read in 4 to 6 hours. A positive test was indicated by a mat of cells covering the bottom of a microtiter plate well and a negative test by collection of the cells in a button at the bottom of the well. The titer was expressed as the reciprocal of the highest dilution giving a positive reaction.

A passive hemagglutination test (33) utilizing the microtiter technique was used for the determination of antibodies to tetanus toxoid. A concentrated fluid tetanus toxoid, L-111[1], containing 400 LF/ml, was used as the antigen. The techniques used in this test were the same as those described in the previous procedure for HSA except for the

[1] Provided courtesy of Dr. Donald Baldwin, Fort Dodge Laboratories, Fort Dodge, Iowa.

quantity of tetanus toxoid (1.6 ml) used as antigen in the sensitization of the sheep RBCs. The titers were expressed as the reciprocal of the highest dilution giving a positive reaction.

A hemagglutination inhibition test (27) utilizing the microtiter technique was used to detect antibody to both Eastern and Western equine encephalomyelitis virus. The test was essentially identical for both antibody types with the only difference being in the antigen used in the particular test. Each test employed killed equine encephalomyelitis virus harvested from suckling mouse brains.

Adult male white goose red blood cells (RBCs) were washed 4 times in Dextrose-Gelatin-Veronal solution (DGV), resuspended with 3 volumes of DGV and held overnight at 4C. A 0.5 ml aliquot of this suspension was diluted with 19.5 ml of NSS. The optical density of this suspension was measured in a spectrophotomer[1] at 490 millimicrons using tubes with a 10.0 mm. internal diameter, and the optical density was adjusted to 0.450 by the addition to or removal of DGV from the suspension. This standardized suspension was stored at 4C until use. For use in the tests, one ml of the standardized RBC suspension was mixed with 23 ml of DGV.

The lyophilized antigens were rehydrated in distilled water, diluted 1:10 in chilled 0.4 per cent bovalbumin-borate-

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[1] Coleman Jr., Coleman Instruments Corp., Maywood, Illinois.

saline, pH 9.0 (BABS) and allowed to dissociate for one hour. The antigen solutions were titrated in 0.4 per cent BABS to determine the highest dilution causing complete or partial hemmagglutination of 0.05 ml of the goose RBC preparation. This represented one unit of hemagglutinin (HA). The stock antigen solution was then diluted with 0.4 per cent BABS to give a final concentration of 4 to 8 HA units in 0.025 ml of the solution.

Before each group of tests were run, the antigen solution was titrated to determine its hemagglutinating activity. If there was a decrease in activity to less than 4 units of HA, more stock solution was added to increase the HA units to the appropriate level.

Sera were thawed in a 37C water bath and inactivated in a 56C water bath for 30 minutes. A 0.2 ml aliquot of each serum sample was diluted with 0.8 ml of borate saline (pH 9.0). One ml of a 25% suspension of acid washed kaolin[1] in borate saline (pH 9.0) was added. The serum-kaolin mixture was vigorously shaken at intervals during a 20 minute incubation period at room temperature. The mixture was then centrifuged and the supernatant fluid removed. This supernatant fluid was considered to be a 1:10 dilution of the sera and was then absorbed with one drop (approximately 0.05 ml) of a 50 per cent washed goose RBC suspension in DGV. This combination was

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[1] Fisher Scientific Co., Fair Lawn, New Jersey.

mixed well and allowed to stand 20 minutes at 4C with occasional shaking. It was then centrifuged, and the 1:10 diluted serum was removed for testing.

Two-fold serial dilutions of the diluted sera were made in microtiter plates using 0.025 ml of 0.4 per cent BABS per microtiter well as diluent. To each well was added 0.025 ml of the appropriate antigen. A control row containing the first 2 dilutions of each serum was included for detecting any nonspecific agglutination of the cells by the serum. To each of these wells 0.025 ml of 0.4 per cent BABS was added in place of the antigen. Also included as controls were a positive serum of known titer and a known negative serum. The serum-antigen mixtures were incubated overnight at 4C. To each well was added 0.05 ml of the goose RBC suspension, and the bottom of the microtiter plates were scratched to mix the contents. The plates were incubated for 30 minutes at 37C and read. Hemagglutination was indicated by a mat of cells covering the bottom of a microtiter plate well. Hemagglutination inhibition was noted where a button of RBCs had formed at the bottom of a well. Incomplete hemagglutination was read as a ring of RBCs around a partially formed mat of RBCs. The titer was expressed as the reciprocal of the highest dilution of serum inhibiting hemagglutination except where incomplete hemagglutination occurred at the endpoint region. In this case the reciprocal of the first

dilution to cause incomplete hemagglutination was considered to be the titer.

RESULTS

Transfer of Maternal Antibody

Serum samples taken from foals before nursing were assayed for the presence of antibodies to Brucella abortus S19, tetanus toxoid, human serum albumin and Eastern and Western equine encephalomyelitis virus. Table 6 illustrates the serum titers for each antibody type in the mares at parturition versus those in the foals prior to nursing.

In no instance were antibodies to Brucella abortus S19, tetanus, or HSA found by the serological tests employed in foal pre-nursing sera. In the case of WEE and EEE a number of animals demonstrated titers at 1:10 and a very few at 1:20 or 1:40. These were considered to be nonspecific in nature and related to the specific serological procedure used, i.e., hemagglutination inhibition.

The average of the foals' passive antibody titers to the above antigens at 24 hours and 72 hours of age were compared to those of the dams at the time of parturition. These results are illustrated in Figure 1. A somewhat similar comparison summarizing larger groups of animals is presented in Figure 2.

Colostrum-deprived foals were allowed to nurse their dams beginning at approximately 72 hours of age. None of the CD foals exhibited detectable antibody to any of the 5 antigens at one week of age.

Table 6

Titers of Mares at Parturition and Foals Before Nursing

	Antibody to:				
Titers	B. abortus	Tetanus	HSA	EEE	WEE
0	1/33[a]	9/29	7/32	10/11	5/10
1:10	0/0	0/0	1/0	10/16	5/17
1:20	1/0	1/0	3/0	2/2	0/1
1:40	6/0	1/0	1/0	2/2	2/0
1:80	4/0	1/0	1/0	2/0	1/0
1:160	1/0	1/0	2/0	3/0	2/0
1:320	2/0	0/0	0/0	0/0	3/0
1:640	4/0	2/0	2/0	2/0	1/0
1:1280	6/0	1/0	0/0	0/0	1/0
1:2560	3/0	2/0	0/0	0/0	2/0
1:5120	3/0	2/0	5/0	0/0	1/0
1:10240	0/0	5/0	3/0	0/0	2/0
1:20480	2/0	4/0	7/0	0/0	3/0

[a] Number of mares with indicated titer / Number of foals with indicated titer

Figure 1: A bar graph illustrating the average serum titer of the dams at parturition and the corresponding average serum titers of colostrum-fed foals at 24 and 72 hours of age. Titers compared include antibody to tetanus toxoid (17 pairs of dams and foals), Western and Eastern equine encephalomyelitis virus (14 pairs), Brucella abortus S19 (13 pairs), and human serum albumin (12 pairs).

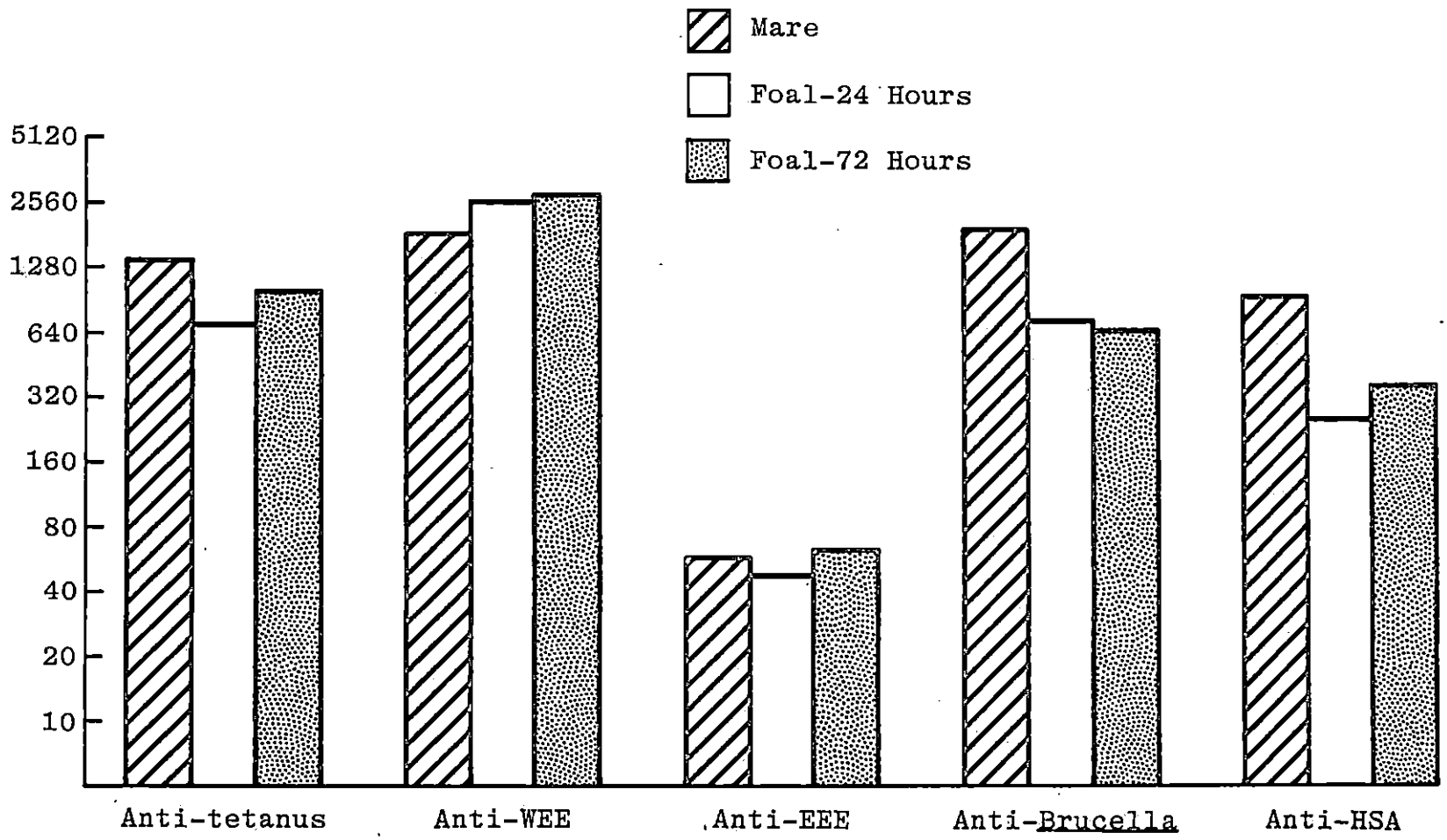
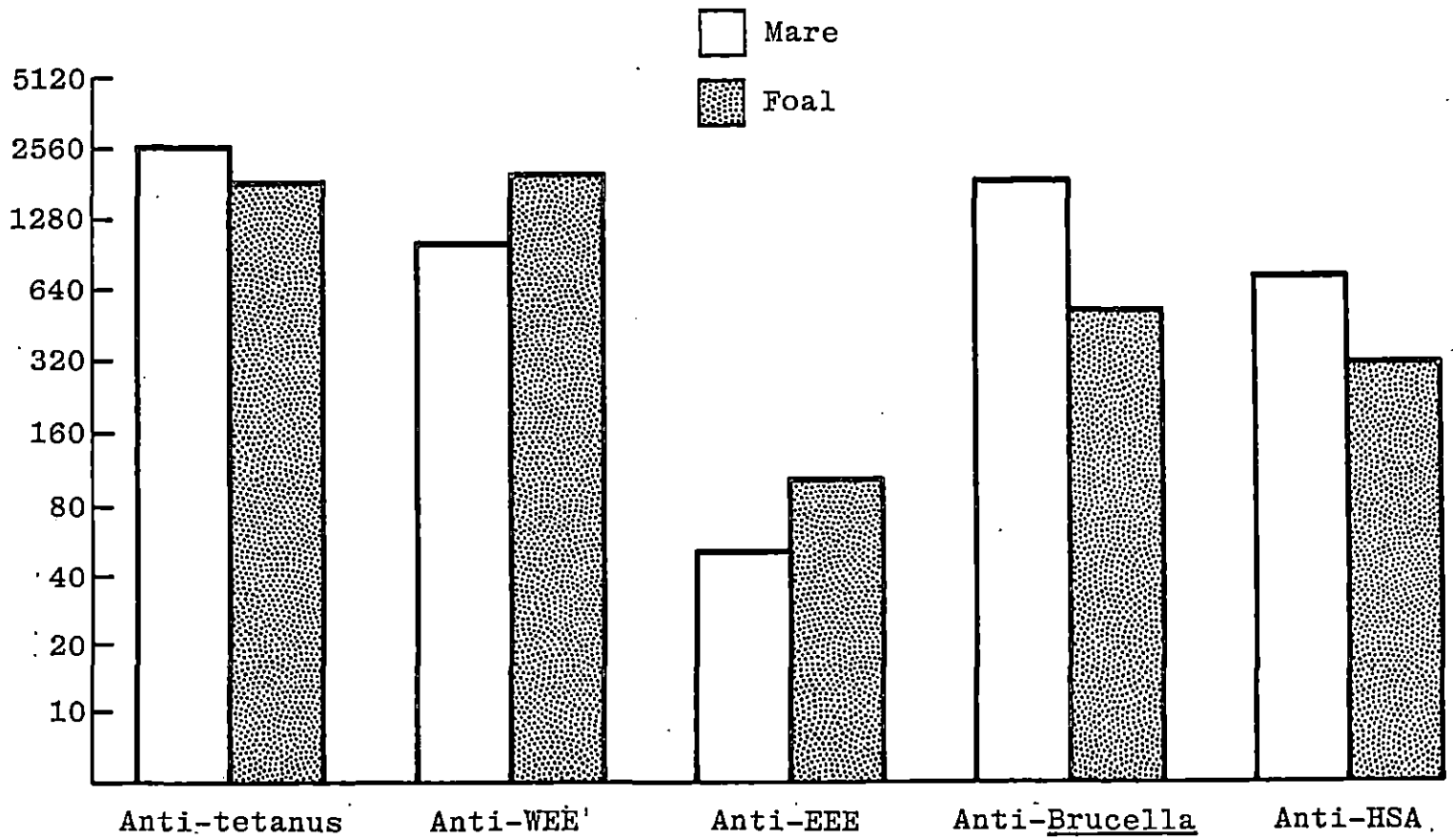

Mare
Foal-24 Hours
Foal-72 Hours
5120
2560
1280
640
320
160
80
40
20
10
Anti-tetanus
Anti-WEE
Anti-EEE
Anti-Brucella
Anti-HSA

Figure 2: A bar graph illustrating the average serum titer of the dams at parturition and the corresponding average serum titers of colostrum-fed foals at 72 hours of age. Titers compared include antibody to tetanus toxoid (38 pairs of dams and foals), Western equine encephalomyelitis virus (40 pairs), Eastern equine encephalomyelitis virus (33 pairs), Brucella abortus S19 (27 pairs) and human serum albumin (23 pairs).


Mare
Foal
5120
2560
1280
640
320
160
80
40
20
10
Anti-tetanus
Anti-WEE
Anti-EEE
Anti-Brucella
Anti-HSA

Response to Immunization

Effect of colostrum deprivation

A comparative study using 2 groups of foals was made to evaluate the effect of colostrum deprivation on the humoral antibody response. One group was colostrum-deprived; the other was composed of foals that had nursed but were offspring of dams that lacked detectable circulating antibody to the particular antigen. This 2nd group was seronegative for such antibody from birth to the first immunization. Comparisons were made between foal groups of different ages at the time of primary immunization.

Comparative responses to Brucella abortus Strain 19 at one, 2, 4, 8, and 12 weeks of age (Figures 3-7) demonstrated that agglutinating antibody to Brucella abortus was detectable by 7 days after inoculation. Peak titers were noted one or 2 weeks after immunization and levels subsequently declined.

In the one-week-old foals a 2nd immunization was given at 12 weeks of age. This resulted in an immediate rise in titer which was highest one week post-inoculation (Figure 3).

In the foal groups that were 2 or 4 weeks old at the first immunization, a 2nd immunization was given 4 to 10 weeks later. The graphs indicate the titers for the 3 weeks following the primary inoculation and the titer at the time of the 2nd inoculation (Figures 4 and 5). Again, an immediate rise in titer to the 2nd immunization was demonstrated.

Figure 3: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed Brucella abortus S19. These foals were injected at one week of age and again at 12 weeks. ↑ = first injection. ↑ = 2nd injection.

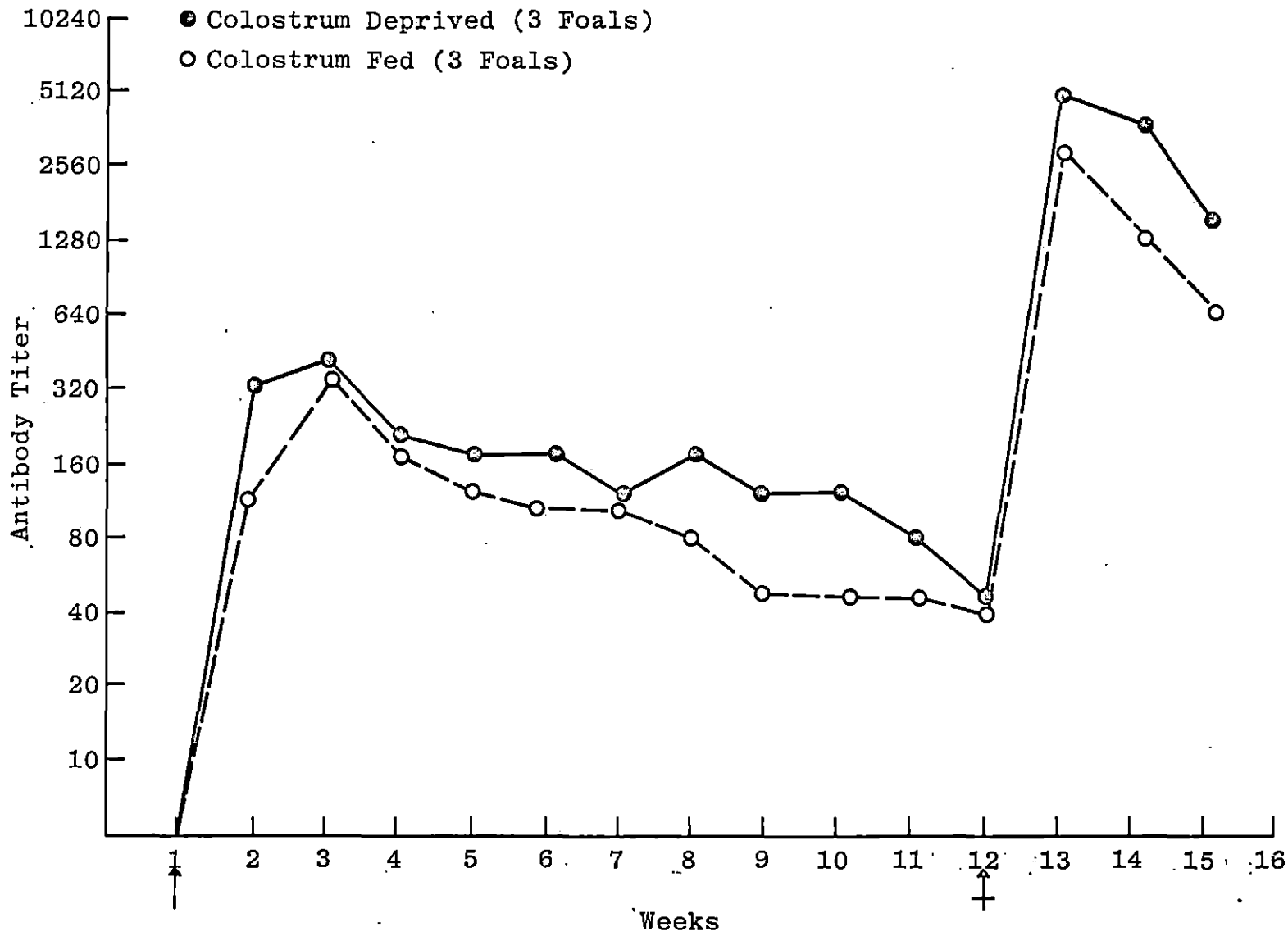

Colostrum Deprived (3 Foals)
Colostrum Fed (3 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 4: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed Brucella abortus S19. These foals were injected at 2 weeks of age, and a 2nd dose was given 4 to 10 weeks later. ↑ = first injection, ↑ = 2nd injection.

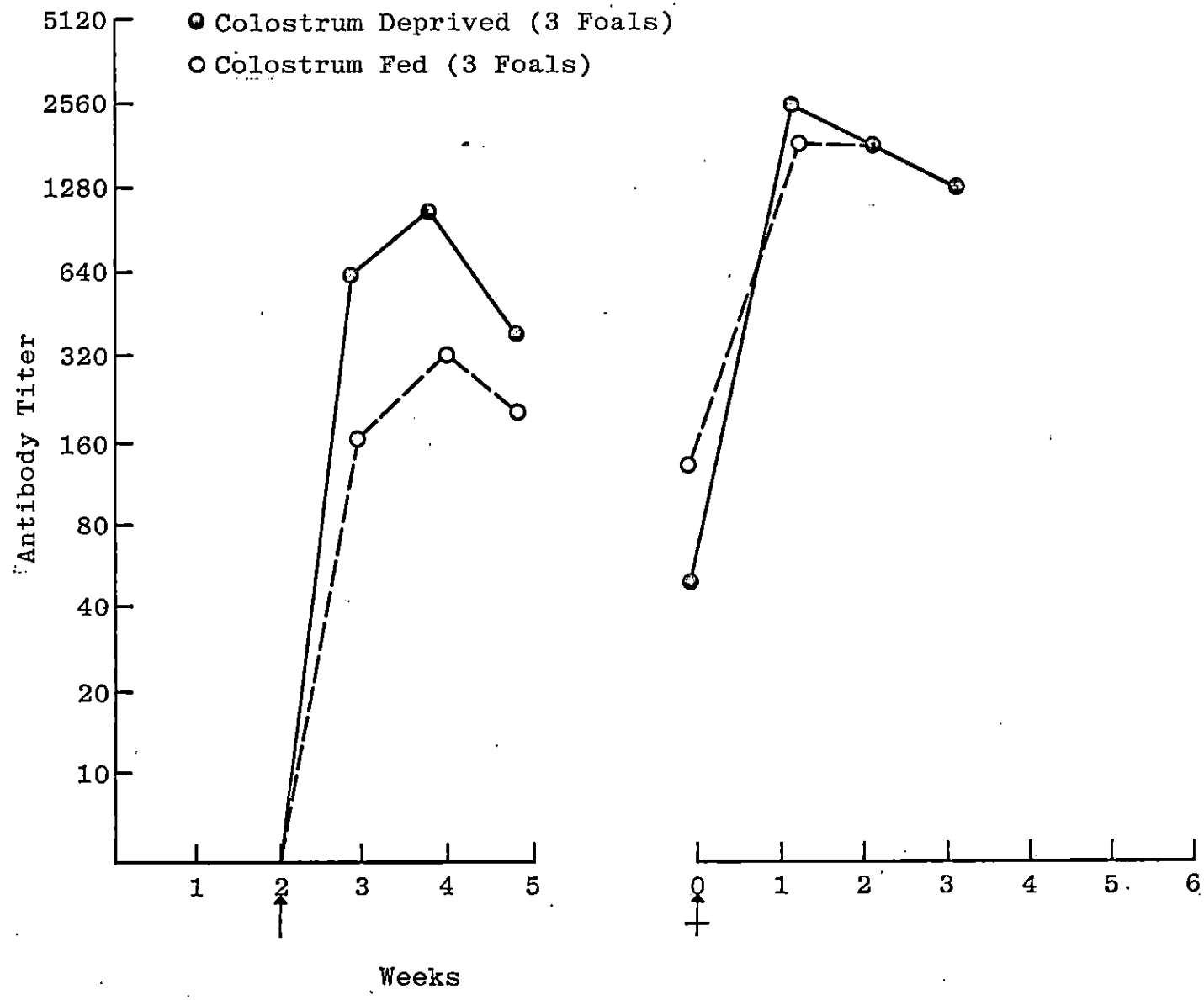

Colostrum Deprived (3 Foals)
Colostrum Fed (3 Foals)
Antibody Titer
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
0
1
2
3
4
5
6
Weeks

Figure 5: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed Brucella abortus S19. These foals were injected at 4 weeks of age, and a 2nd dose was given 4 to 8 weeks later. ↑ = first injection, ↑ =2nd injection.

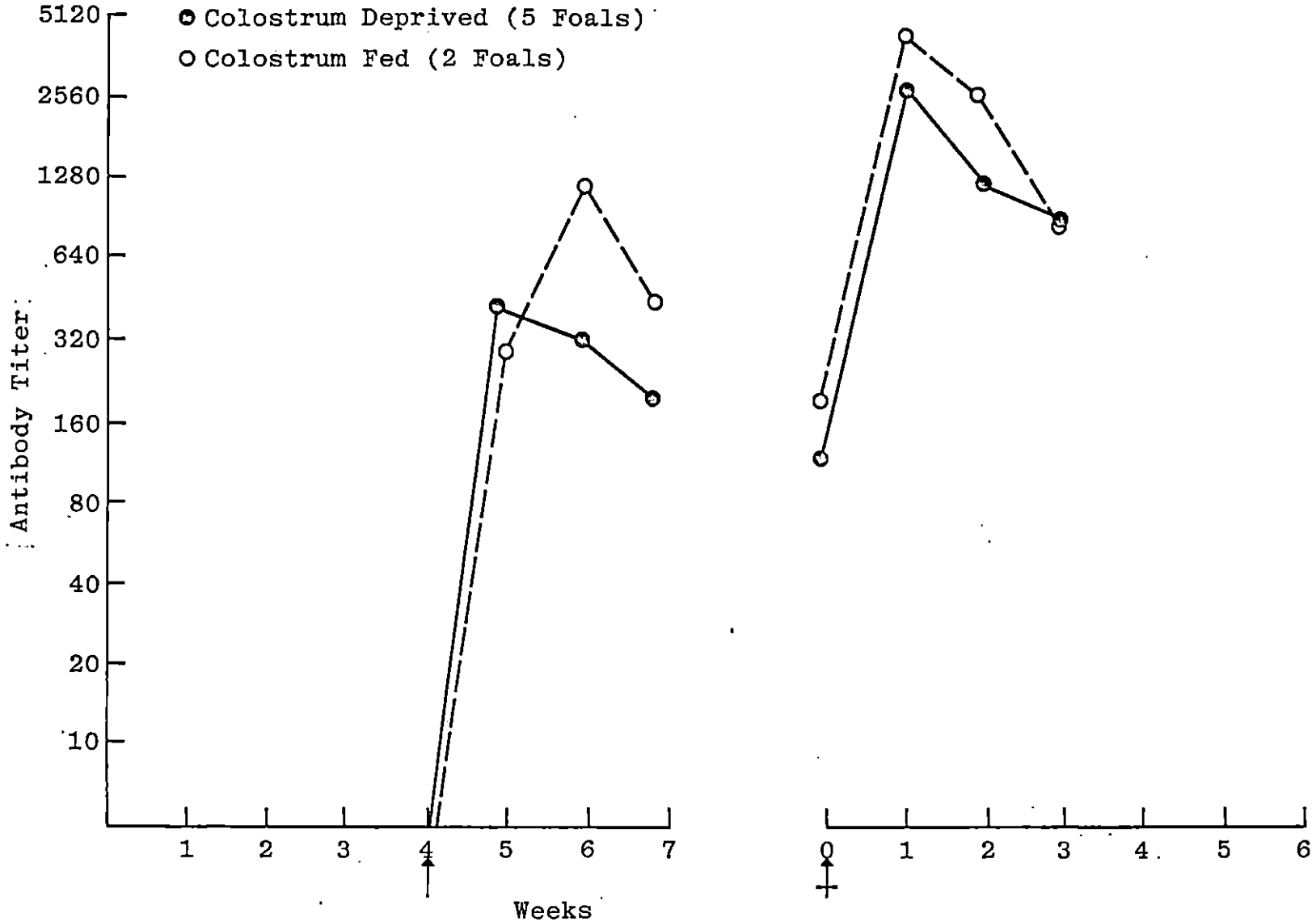

Colostrum Deprived (5 Foals)
Colostrum Fed (2 Foals)
Antibody Titer
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
Weeks
0
1
2
3
4
5
6

Figure 6: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed Brucella abortus S19. These foals were injected at 8 weeks of age and again at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

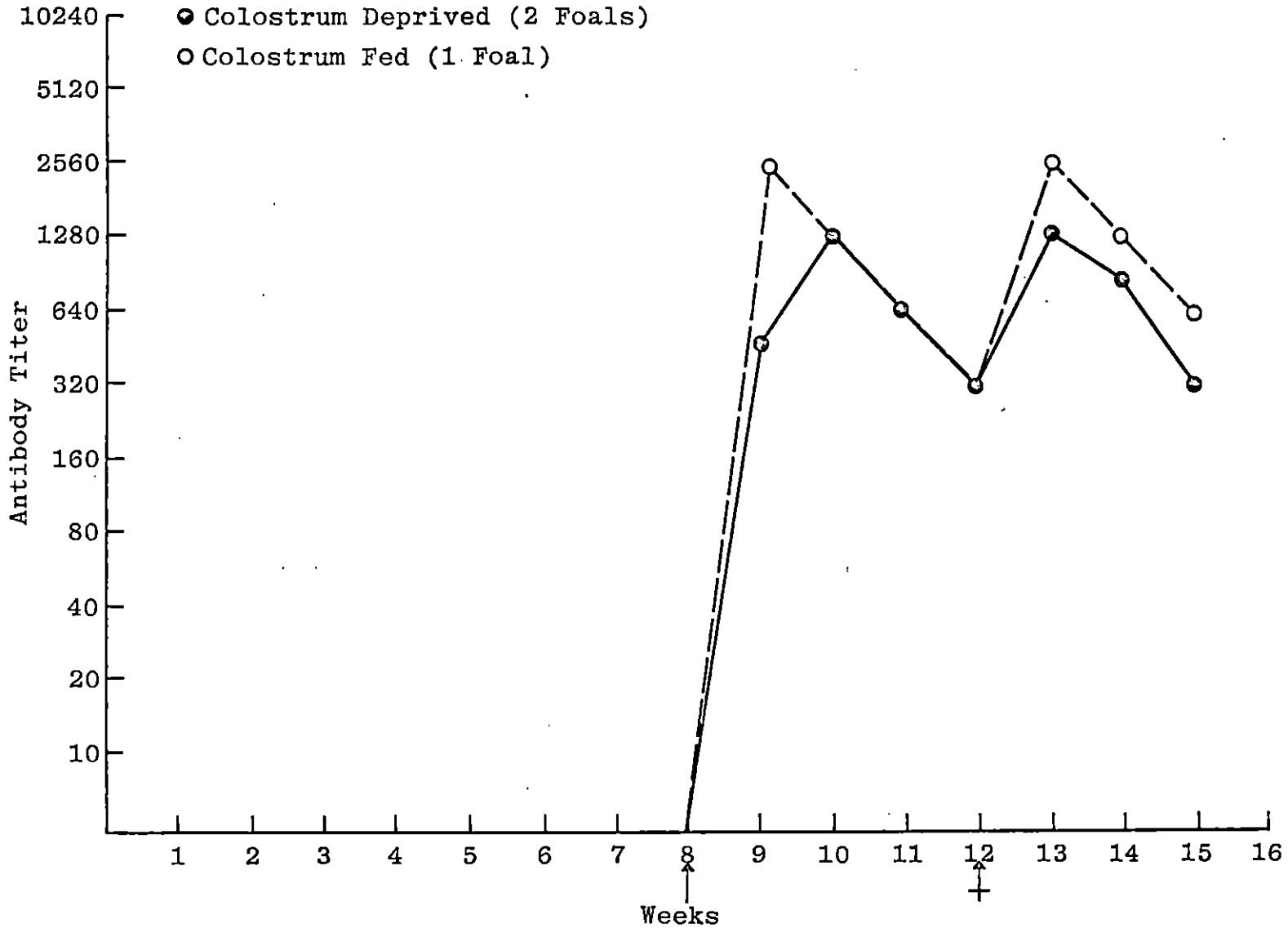

Colostrum Deprived (2 Foals)
Colostrum Fed (1 Foal)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 7: A graph illustrating the comparative primary responses of CF and CD foals following administration of killed Brucella abortus S19. These foals were injected at 12 weeks of age. ↑ = injection.

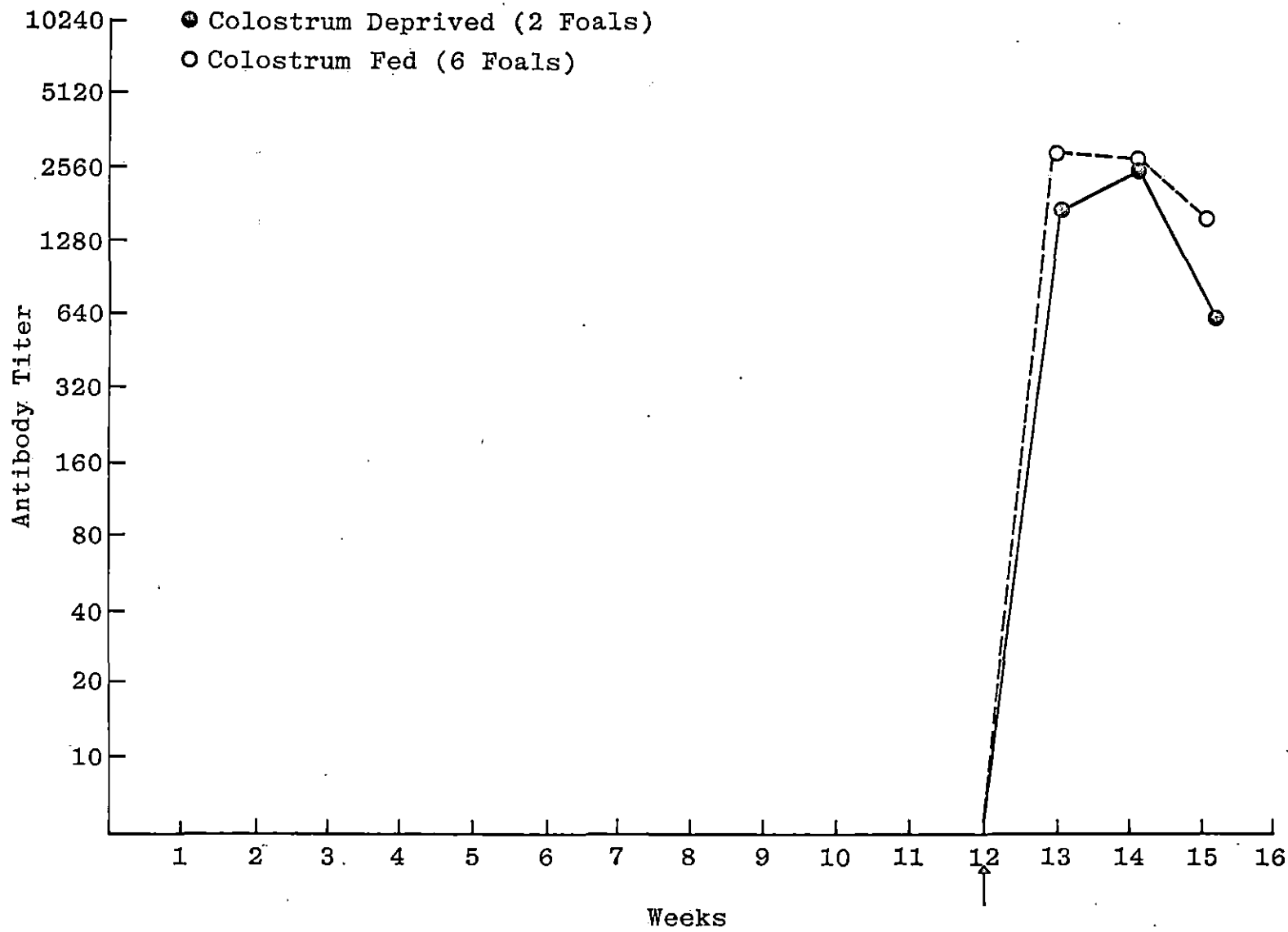

Colostrum Deprived (2 Foals)
Colostrum Fed (6 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Table 7

Some Comparative Responses of CF and CD Foals to Killed *Brucella abortus* S19

Foal age (weeks)	Peak response to first immun.	Titer at 2nd immun.	Peak response to 2nd immun.
1 CF (3)[a]	320[b]	40	3225
CD (3)	403	50	5120
2 CF (3)	320	127	2031
CD (3)	1016	50	2560
4 CF (5)	1280	184	4457
CD (2)	453	113	2560
8 CF (1)	2560	320	2560
CD (2)	1280	320	1280
12 CF (6)	3225	---	----
CD (2)	2560	---	----

[a] Indicates foals' status, i.e., colostrum-fed (CF) or colostrum-deprived (CD) and the number of foals in each group. See also Table 8.

[b] Titers expressed as the reciprocal of the average for the group. See also Table 8.

Table 8

Some Comparative Responses of CD and CF Foals to Tetanus Toxoid

Foal age (weeks)	Peak response to first immun.	Titer at 2nd immun.	Peak response to 2nd immun.
1 & 2 CF (5)[a]	845[b]	320	13512
CD (5)	381	160	11763
4 CF (2)	226	113	14482
CD (3)	1016	453	25803
8 CF (3)	320	320	5120
CD (3)	508	508	8127

Foals inoculated at 8 weeks of age with Brucella were reinoculated 4 weeks later. Although a rapid titer was noted following the 2nd injection, the levels attained were lower relative to the primary responses than those seen in previous groups (Figure 6).

Foals immunized at 12 weeks of age did not receive a 2nd immunization (Figure 7).

In all age groups evaluated, there were no appreciable differences between foals receiving colostrum and colostrum-deprived foals with regard to either primary or secondary antibody responses to Brucella abortus Strain 19 (Table 7).

Graphs of the comparative results using tetanus toxoid are shown in Figures 8-10. Results from one and 2-week-old foals in each of the 2 groups compared were combined for data presentation purposes (Figure 8). No antibody was detected at one week post-inoculation, but measurable levels were noted at the 2nd week. Titers to tetanus toxoid increased over a longer period post-inoculation than those seen previously to killed Brucella abortus. Peak titers were not reached until about 5 weeks after primary immunization of one, 2, and 4 week-old foals. The decline in anti-tetanus antibody levels after peak titers were reached was more gradual in these groups than was the decrease in anti-Brucella levels seen previously.

In the one and 2-week-old foal groups, the responses to a primary immunization were similar (Table 8). A 2nd

Figure 8: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of tetanus toxoid. These foals were injected at one or 2 weeks of age and again at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

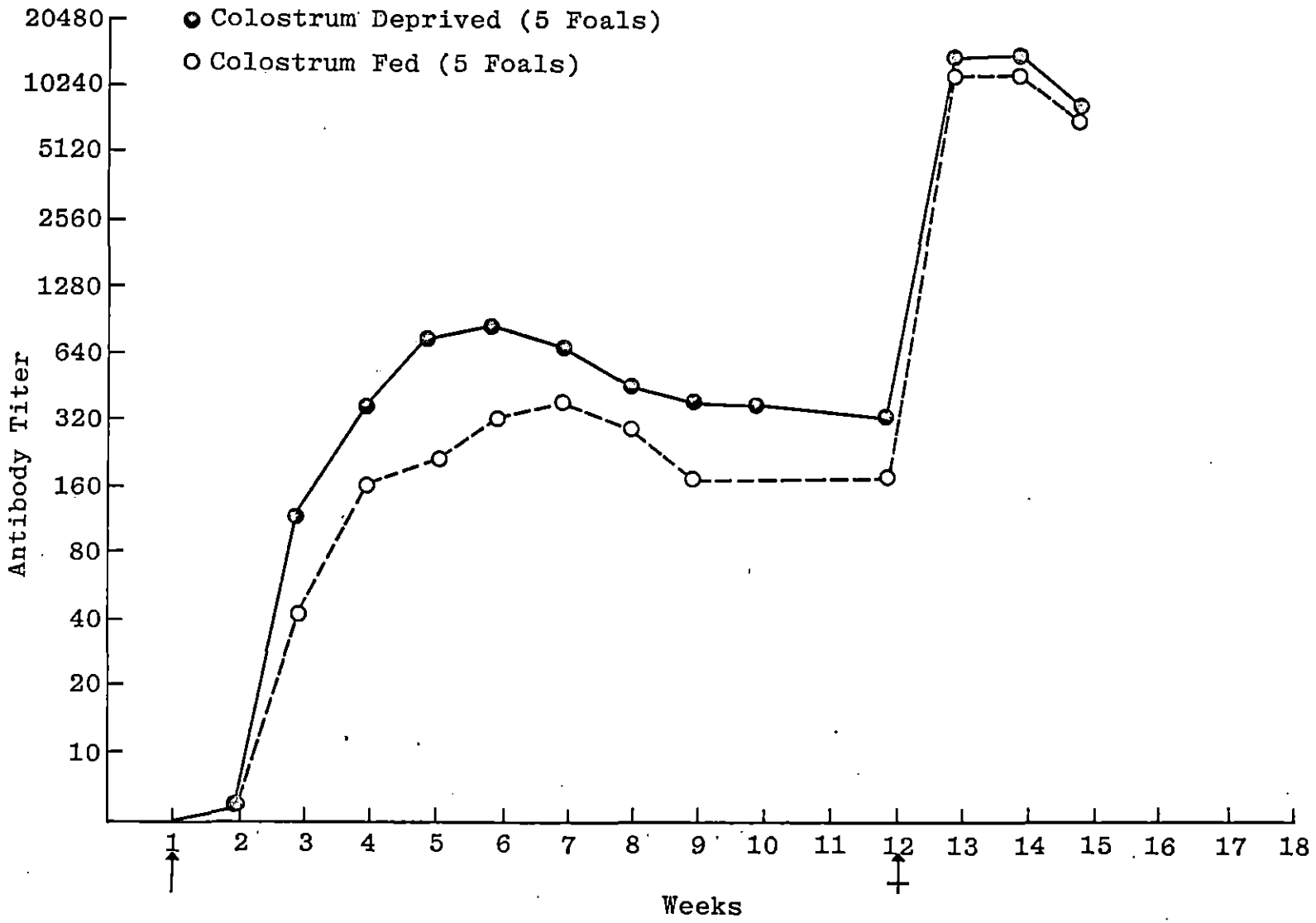

Colostrum Deprived (5 Foals)
Colostrum Fed (5 Foals)
Antibody Titer
20480
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

Figure 9: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of tetanus toxoid. These foals were injected at 4 weeks of age, and a 2nd dose was given at 12 weeks. ↑ = first injection, ↕ = 2nd injection.

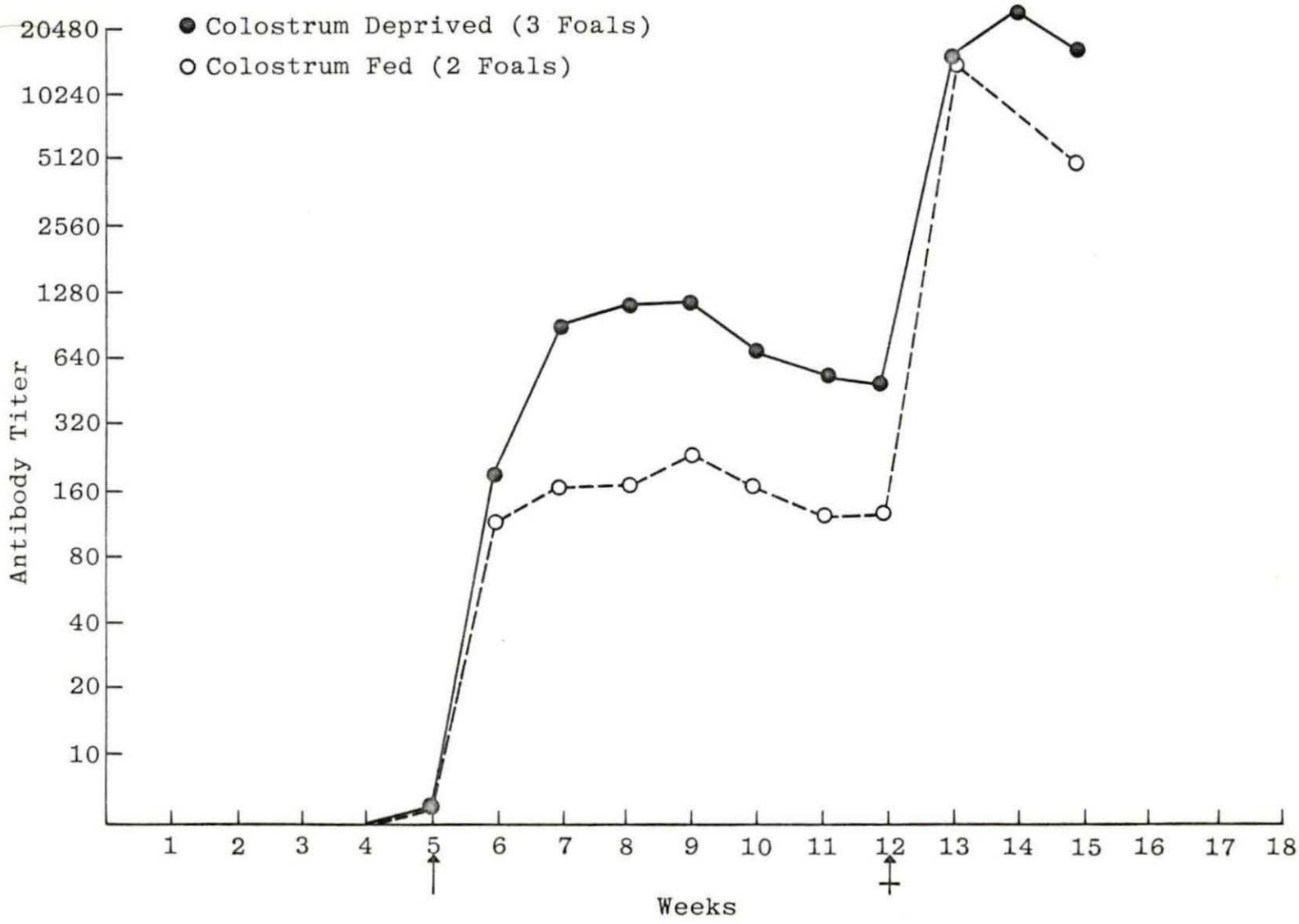
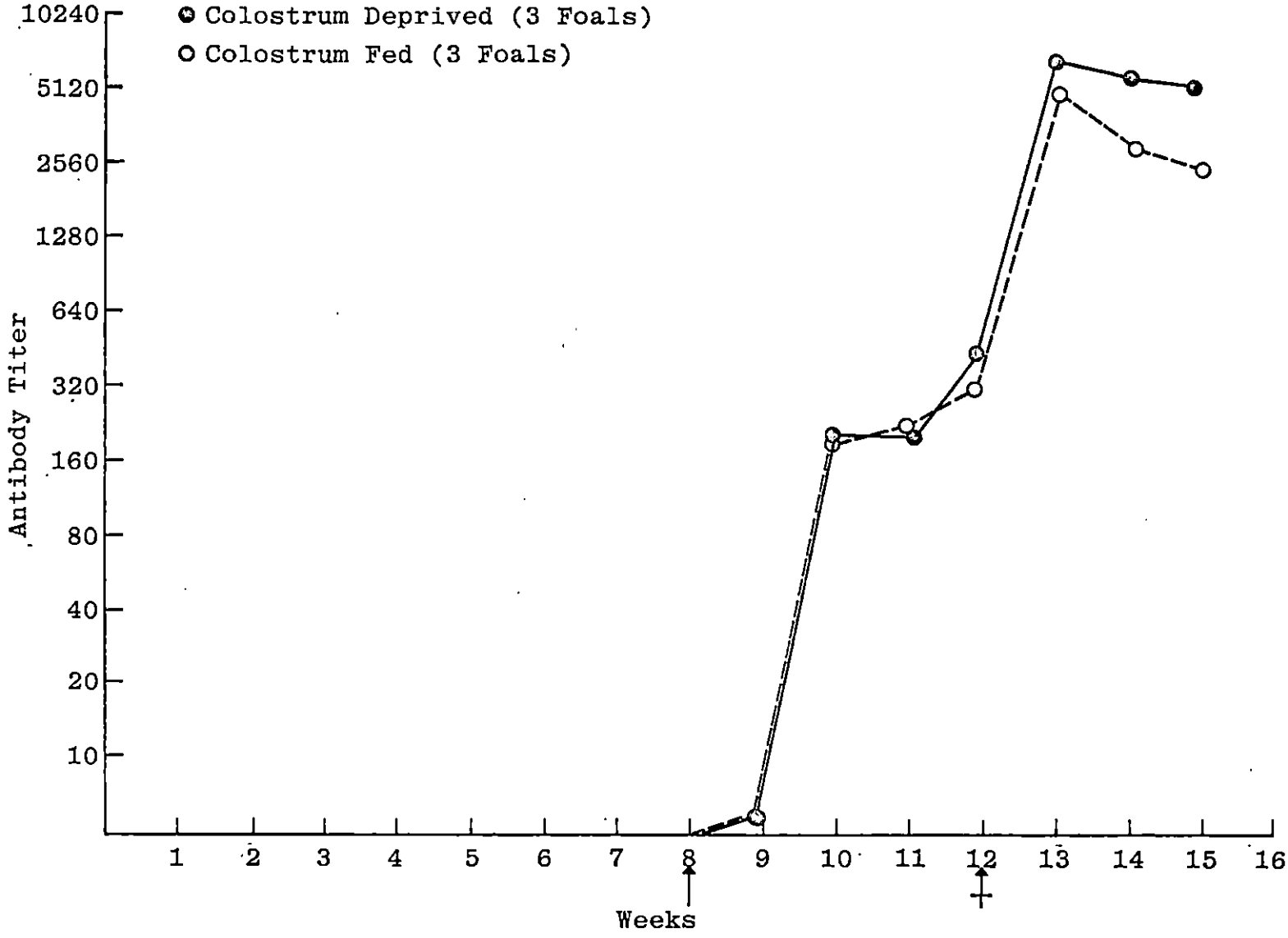

Colostrum Deprived (3 Foals)
Colostrum Fed (2 Foals)
Antibody Titer
20480
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

Figure 10: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of tetanus toxoid. These foals were injected at 8 weeks of age, and a 2nd dose was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.


Colostrum Deprived (3 Foals)
Colostrum Fed (3 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

immunization at 12 weeks of age resulted in a rapid response in both groups with approximately equal peak titers one to 2 weeks after the inoculation (Figure 8).

In 4-week-old foals there was a noticeable difference between the groups (Table 8). The colostrum-deprived group reached higher peak antibody levels after primary immunization than did the colostrum-fed group. Responses to a 2nd immunization were again rapid and more equal than the primary responses. Peak titers were noted at one week post-inoculation in the colostrum-fed group and at 2 weeks post-inoculation in the colostrum-deprived group (Figure 9).

Primary responses were similar in 8-week-old foals (Table 5). Titers were still increasing in both groups when a 2nd immunization was given 4 weeks later. Similar rapid secondary responses were observed in both groups with peak titers at one week post-inoculation followed by a decline in anti-tetanus levels (Figure 10).

Responses of one and 2-week-old foals to human serum albumin in colostrum-deprived and colostrum-fed groups were combined for data presentation purposes (Figure 11) as was done with the previous antigen. Another comparison was made of responses of foals given an inoculation of HSA at 4 weeks of age (Figure 12). No antibody was detected at one week post-immunization, but by 14 days after immunization, anti-HSA antibody had appeared. Titers increased to peak levels seen 4 weeks after immunization and then slowly declined.

Figure 11: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of HSA. These foals were injected at one or 2 weeks of age and again at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

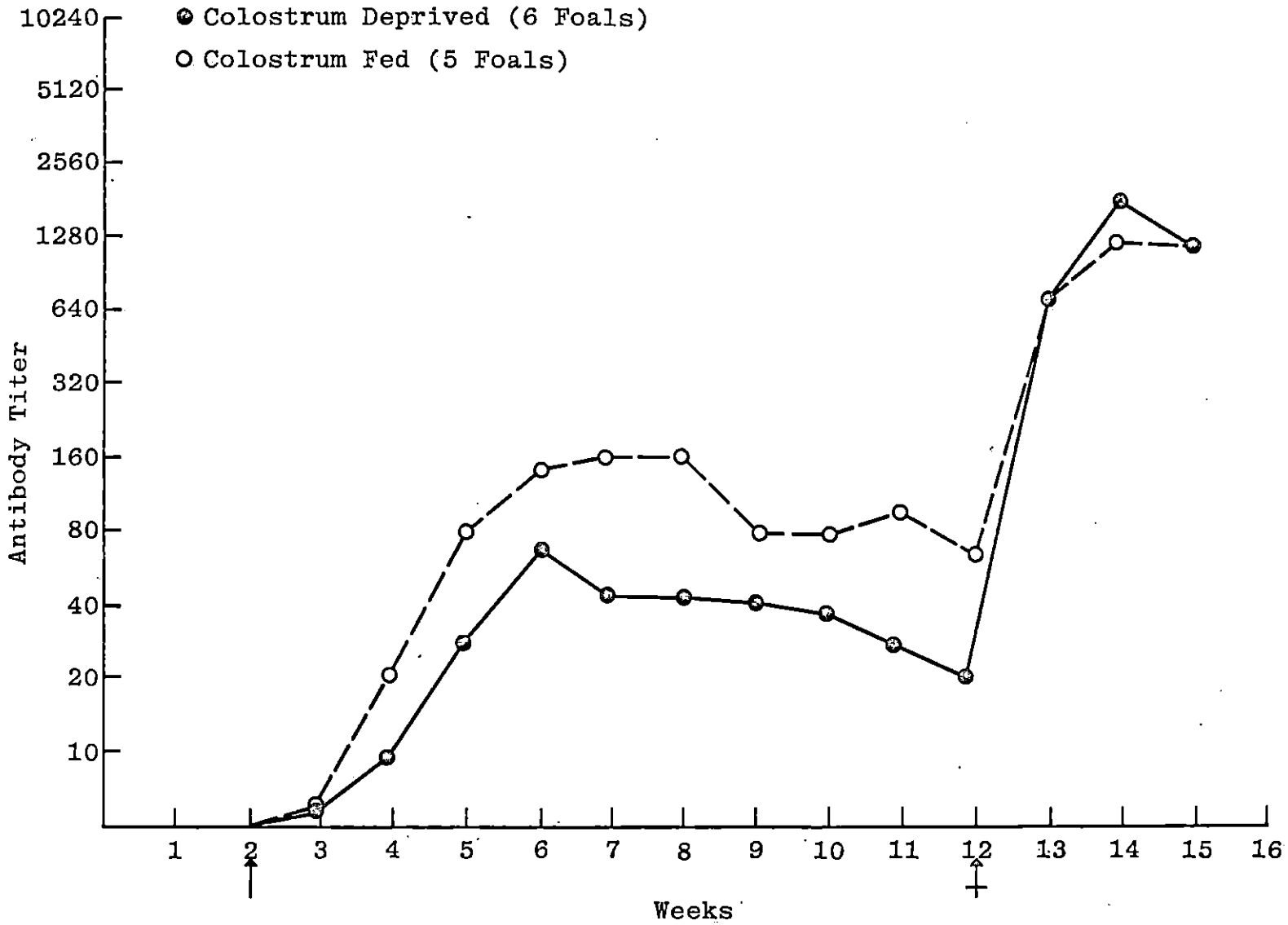

Colostrum Deprived (6 Foals)
Colostrum Fed (5 Foals)
10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 12: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of HSA. These foals were injected at 4 weeks of age, and a 2nd dose was given 4 to 8 weeks later. ↑ = first injection, ↑ = 2nd injection.

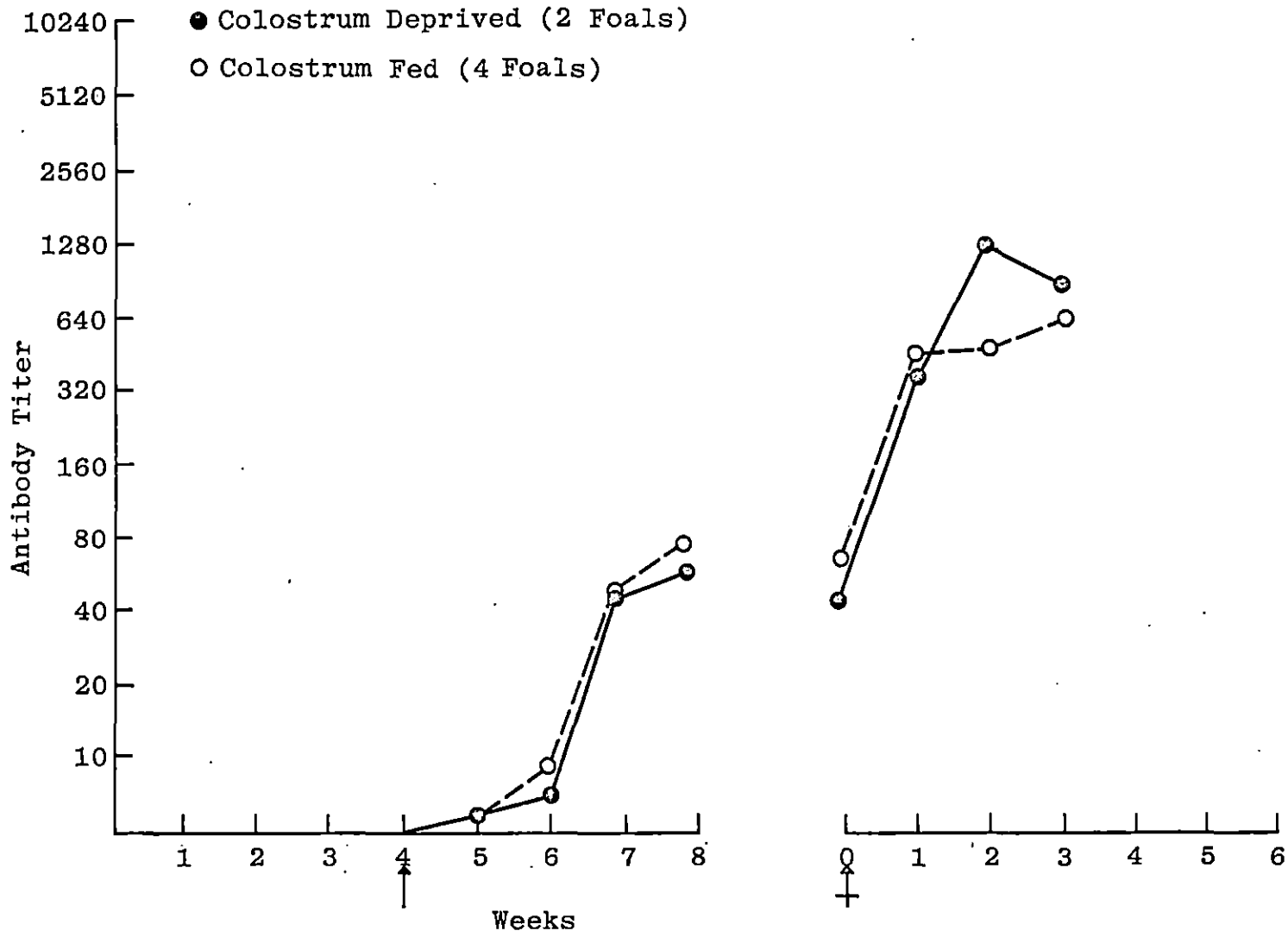

● Colostrum Deprived (2 Foals)
○ Colostrum Fed (4 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1 2 3 4 5 6 7 8
Weeks
0 1 2 3 4 5 6

Table 9

Some Comparative Responses of CD and CF Foals to HSA

Foals age (weeks)		Peak response to first immun.	Titer at 2nd immun.	Peak response to 2nd immun.
1 & 2	CF (5)[a]	160[b]	70	1522
	CD (6)	69	22	1810
4	CF (4)	80	40	1280
	CD (2)	80	67	538

[a] Indicates foals' status, i.e., colostrum-fed (CF) or colostrum-deprived (CD) and the number of foals in each group. See also Table 10.

[b] Titers expressed as the reciprocal of the average for the group. See also Table 10.

Table 10

Some Comparative Responses of CD and CF Foals to Killed WEE and EEE Virus

Foals age (weeks)		Peak response to first immun.		Titer at 2nd immun.		Peak response to 2nd immun.	
		WEE	EEE	WEE	EEE	WEE	EEE
1 & 2	CF (5)[a]	30[b]	20	17	6	3377	211
	CD (5)	63	30	63	10	2941	279
4	CF (2)	28	6	28	6	3620	320
	CD (3)	63	7	25	5	3225	63
8	CF (1)	80	0	20	0	640	160
	CD (3)	32	9	32	9	508	101

Responses to primary immunizations in one and 2-week-old foals were similar in both CD and CF groups (Table 9). After a 2nd inoculation given at 12 weeks of age, a rapid secondary response was noted with the highest titers recorded 2 weeks later followed by decreased levels (Figure 11).

Four-week-old foals showed similar responses to the first HSA inoculation (Table 9). A 2nd immunization was given 4 to 8 weeks later. The graph indicates the titers for 4 weeks following the first immunization and the titer at the time of the 2nd immunization (Figure 12). Again, a rapid rise in titer occurred with the secondary response in both groups. Peak levels were found at the 2nd week post-inoculation in the colostrum-deprived group while the titers at 3 weeks after immunization in the colostrum-fed group were increased over 2nd week levels.

Measureable antibody to killed Western and Eastern equine encephalomyelitis virus was usually produced by the first post-inoculation week especially with WEE virus; the production of detectable antibody to EEE virus at this time was extremely variable (Figures 13-18). A period of several weeks after the first inoculation was usually required to attain peak titers. A 2nd inoculation produced a rapid secondary response with peak titers noted one or 2 weeks later.

The primary responses of foals immunized at one or 2 weeks of age with killed WEE virus (Figure 13) or killed EEE virus (Figure 14) were quite similar. Secondary responses

Figure 13: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed WEE virus. These foals were injected at one or 2 weeks of age and again at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

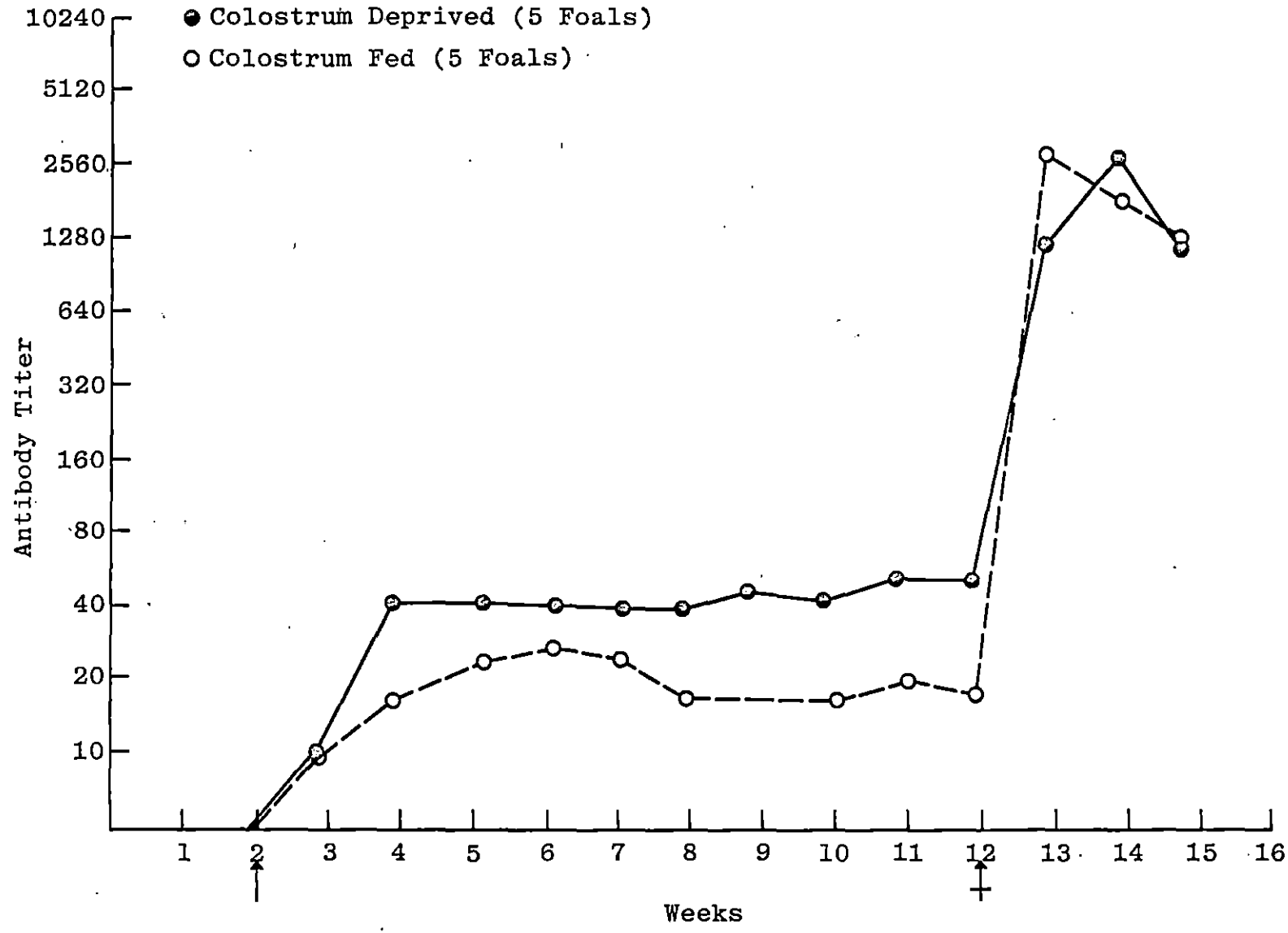

Colostrum Deprived (5 Foals)
Colostrum Fed (5 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 14: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed EEE virus. These foals were injected at one or 2 weeks of age and again at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

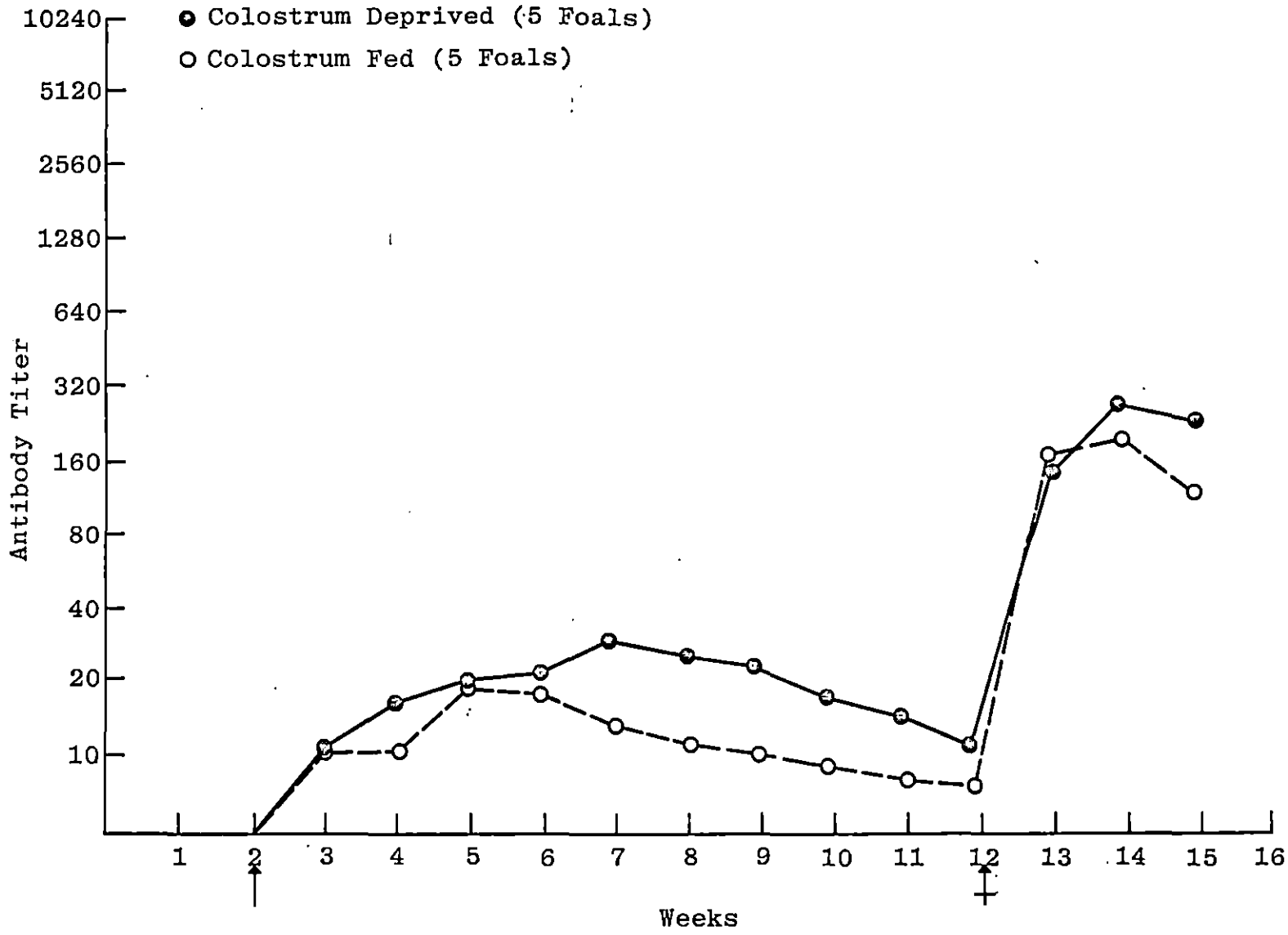

Colostrum Deprived (5 Foals)
Colostrum Fed (5 Foals)
10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 15: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed WEE virus. These foals were injected at 4 weeks of age, and a 2nd dose was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

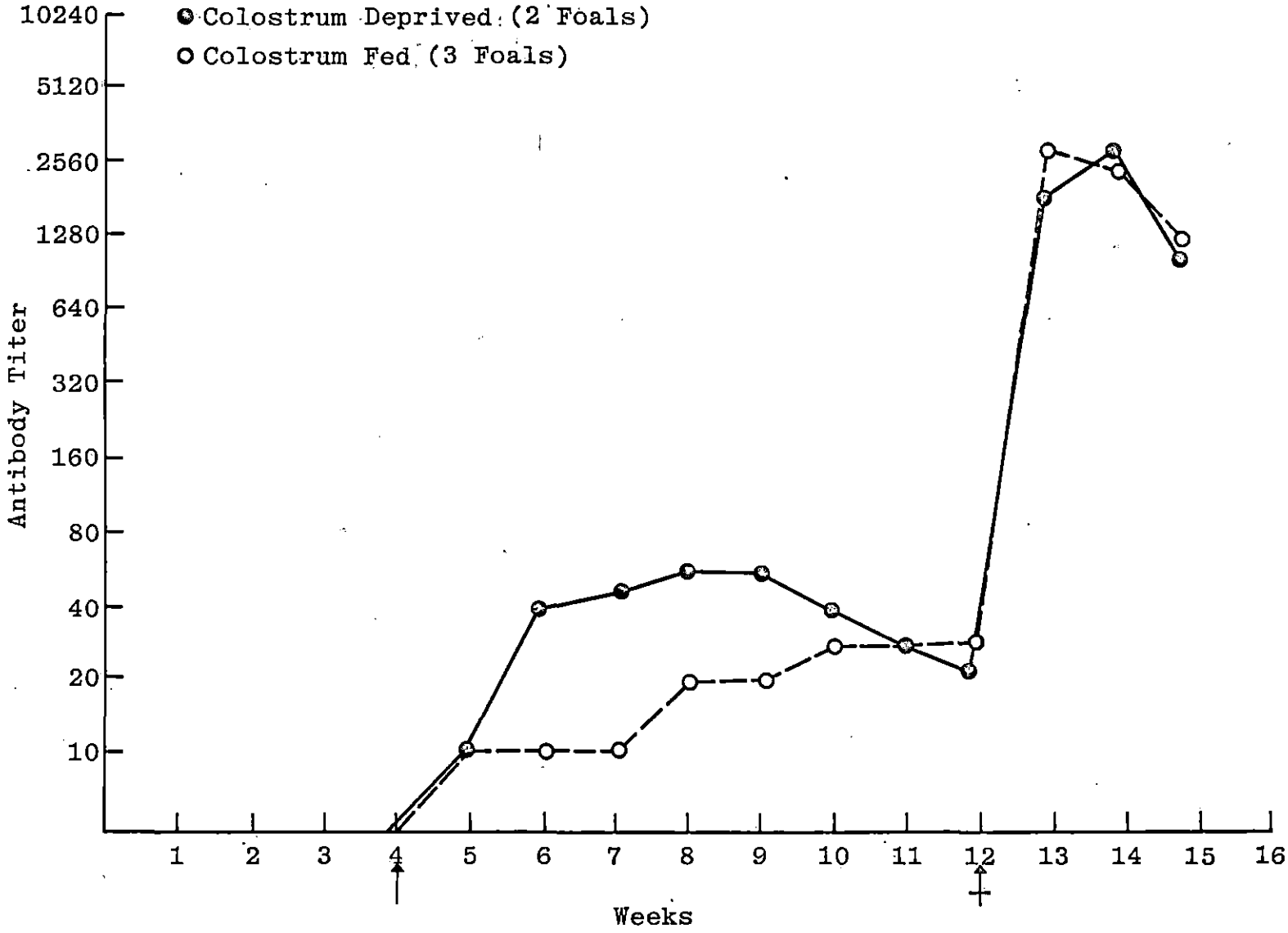

Colostrum Deprived (2 Foals)
Colostrum Fed (3 Foals)
10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 16: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed EEE virus. These foals were injected at 4 weeks of age, and a 2nd dose was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

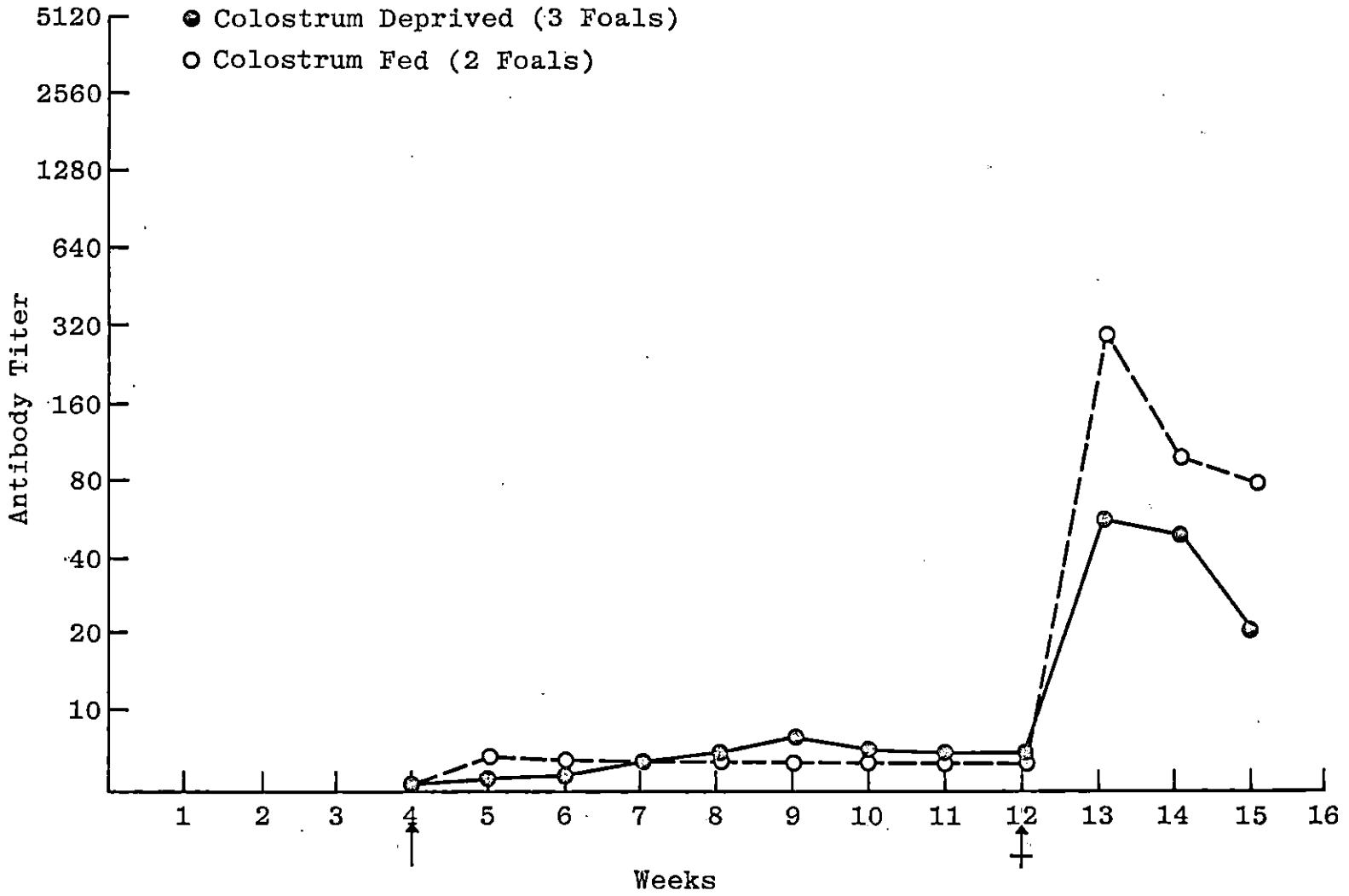

Colostrum Deprived (3 Foals)
Colostrum Fed (2 Foals)
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 17: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of WEE virus. These foals were injected at 8 weeks of age, and a 2nd dose was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

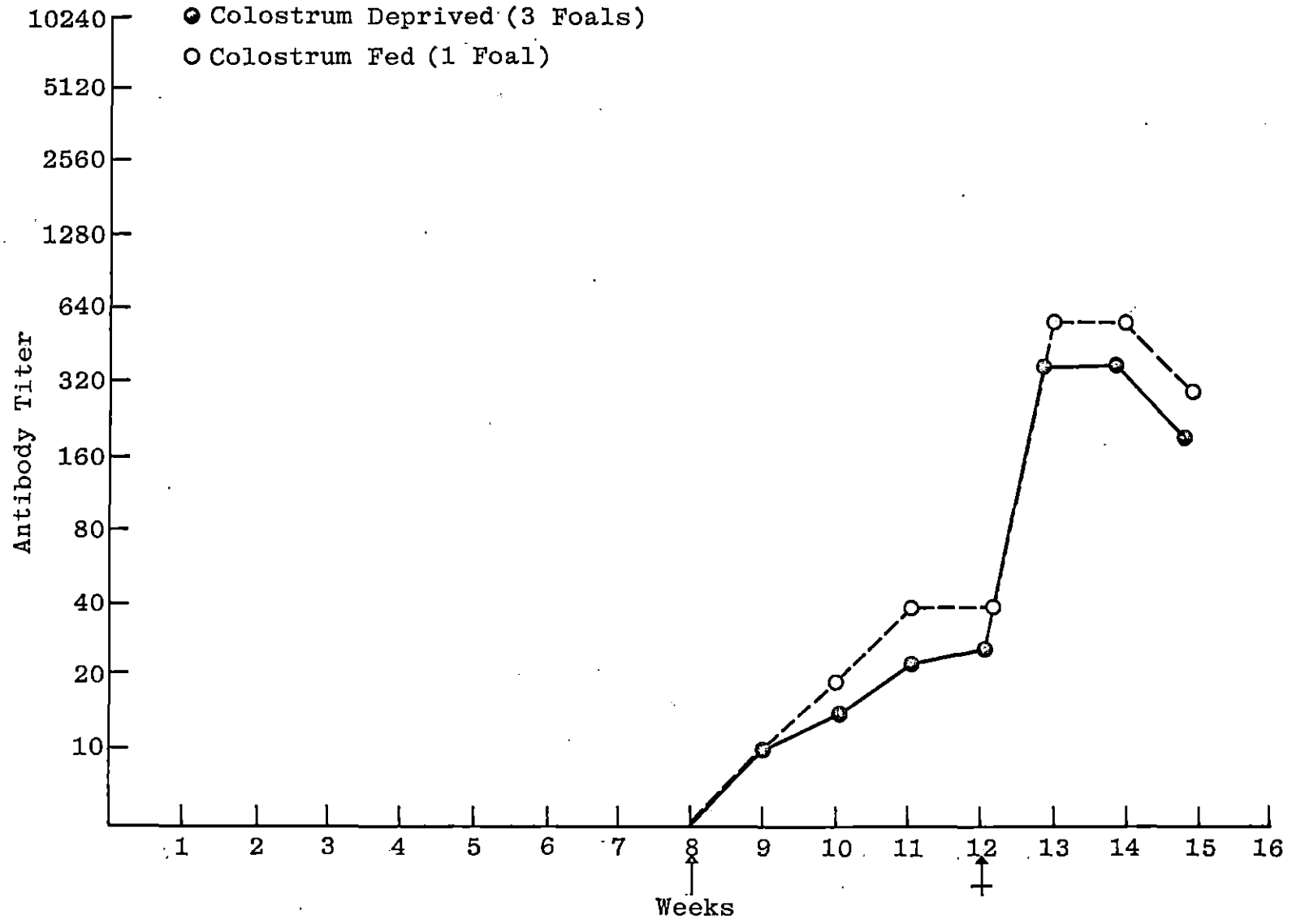
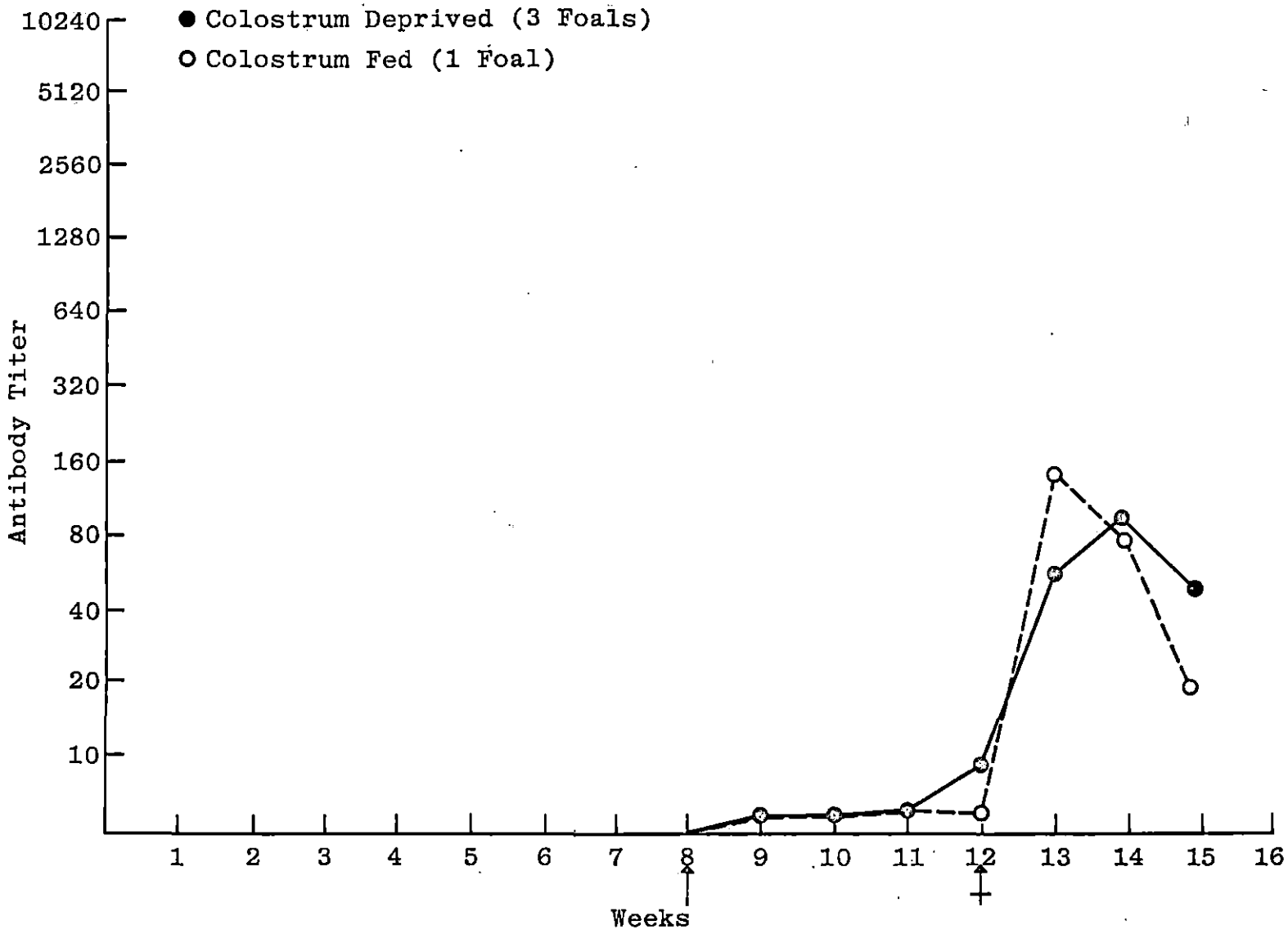

Colostrum Deprived (3 Foals)
Colostrum Fed (1 Foal)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 18: A graph illustrating the comparative primary and secondary responses of CF and CD foals following administration of killed EEE virus. These foals were injected at 8 weeks of age, and a 2nd dose was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.


● Colostrum Deprived (3 Foals)
○ Colostrum Fed (1 Foal)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

to a 2nd dose given at 12 weeks of age were also quite comparable for CD and CF foals (Table 10).

The primary response of 4-week-old foals to killed WEE virus reached peak levels more rapidly in the CD group (Figure 15), but such a difference was not noted with killed EEE virus (Figure 16). The secondary response of both groups to a 2nd inoculation at 12 weeks was again similar with killed WEE virus (Table 10), and, although there seemed to be a greater response in colostrum-fed foals to killed EEE virus, the size of the group (2 foals) may have accounted for the difference.

Eight-week-old foals showed similar primary responses and also secondary responses 4 weeks later to a 2nd injection of killed WEE virus (Figure 17) and EEE virus (Figure 18).

Effect of age

To evaluate the effect of age on the humoral antibody response, foals were grouped according to their age at the time of the first immunization with a particular antigen. Each age group was composed of colostrum-deprived foals and foals seronegative to the specific antigen used from birth until their first immunization.

Antibody titers after inoculation with killed Brucella abortus S19 reached their highest levels at the 2nd week post-inoculation in the one, 2, 4, (Figure 19) and 8-week-old groups. The highest recorded titer in the 10 and 12-week-old

Figure 19: A graph illustrating the primary and secondary responses of anti-Brucella abortus seronegative foals (CD and CF) to killed Brucella abortus S19. The first dose was administered at one, 2 or 4 weeks of age, and a 2nd was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

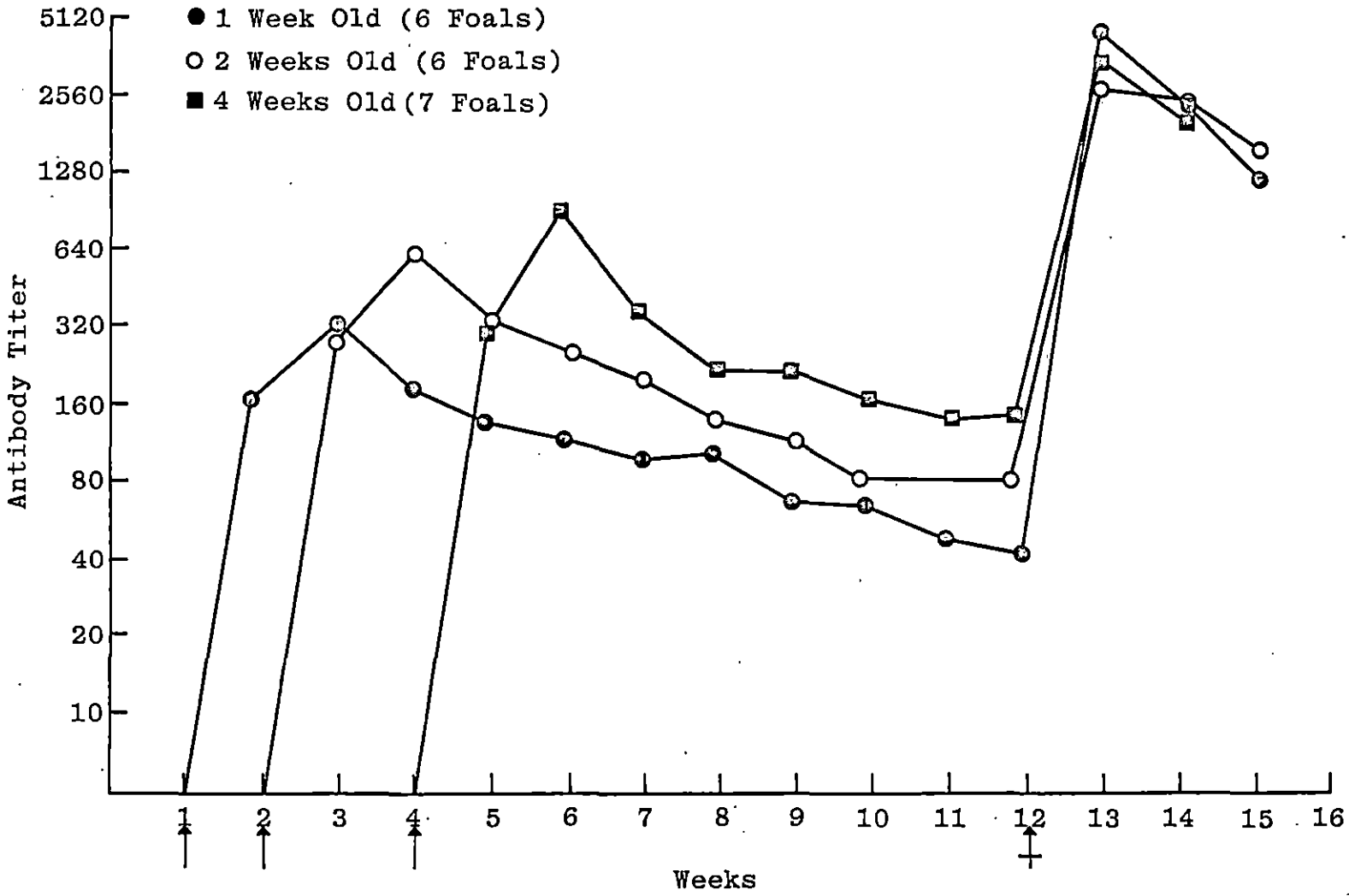

● 1 Week Old (6 Foals)
○ 2 Weeks Old (6 Foals)
■ 4 Weeks Old (7 Foals)
Antibody Titer
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 20: A graph illustrating the primary and secondary responses of anti-Brucella abortus seronegative foals (CD and CF) to killed Brucella abortus S19. The first dose was administered at 8, 10, or 12 weeks of age, and a 2nd inoculation was given 4 weeks after the first. ↑ = first injection, ↑ = 2nd injection.

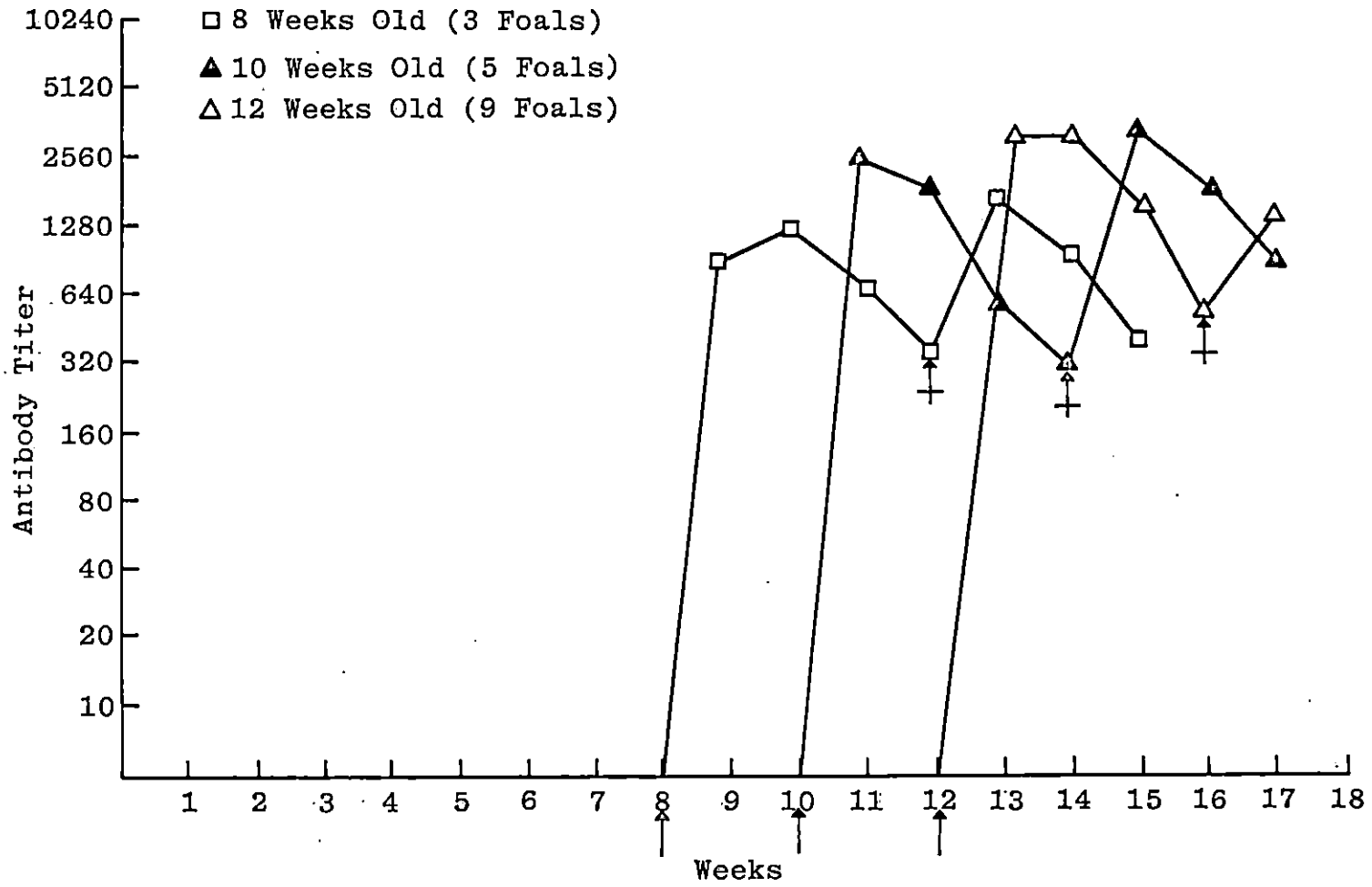

□ 8 Weeks Old (3 Foals)
▲ 10 Weeks Old (5 Foals)
△ 12 Weeks Old (9 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Weeks

Table 11

Some Comparative Responses of Various Foal Age Groups to Killed *Brucella abortus* S19

Foal age (weeks)	Peak response to first immun.	Titer at 2nd immun.	Peak response to 2nd immun.
1 (6)[a]	320[b]	45	4561
2 (6)	570	80	2560
4 (7)	861	144	3804
8 (3)	1280	320	1613
10 (5)	2560	279	2941
12 (9)	2792	538	1280

[a] Number of foals in each group. See also Table 12.

[b] Titers expressed as the reciprocal of the average for the group. See also Table 12.

Table 12

Some Comparative Responses of Various Foal Age Groups to Tetanus Toxoid

Foal age (weeks)	Peak response to first immun.	Titer at 2nd immun.	Peak response to 2nd immun.
1 (5)[a]	485[b]	190	13512
2 (5)	640	211	11763
4 (5)	557	279	13512
8 (7)	390	390	6451

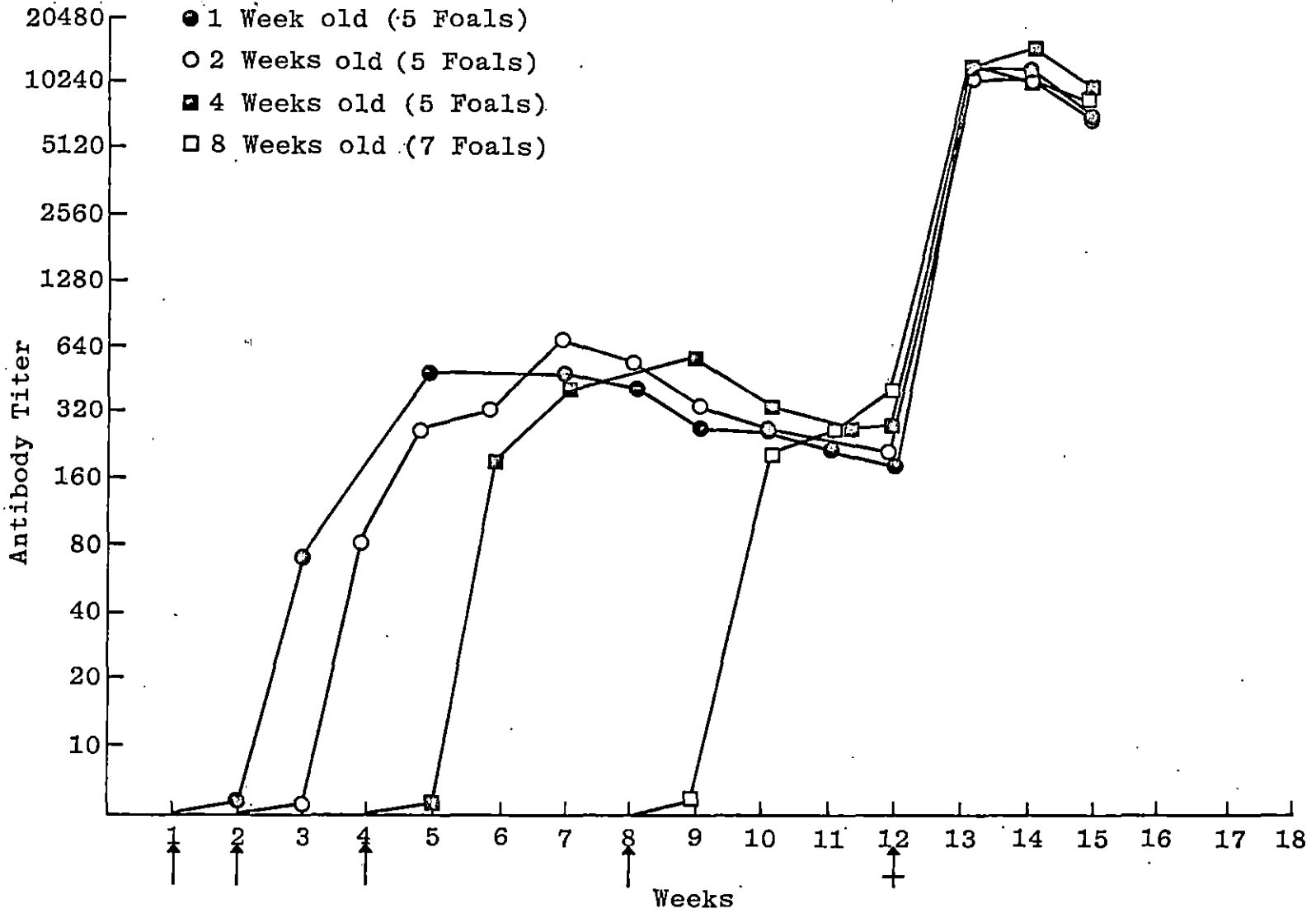
foals' primary responses occurred at one week post-inoculation (Figure 20). With increasing age, there was an increase in the amount of antibody produced during the primary response (Table 11).

A 2nd immunization produced a rapid increase in circulating anti-Brucella antibody with peak titers in this secondary response occurring one week later. Generally, the secondary responses were similar in all foal groups, but the ratio of the secondary response peak titer to that of the preceding primary response decreased with increasing levels of antibody present at the time of second immunization (Table 11).

The response to tetanus toxoid was compared among 4 foal age groups. As noted previously, anti-tetanus antibody was not detectable at one week after primary immunization but had appeared by 2 weeks post-inoculation (Figure 21). A period of 4 to 5 weeks was required before peak levels of antibody were produced. There appeared to be an increase in level of antibody present by 2 weeks post-inoculation with increasing foal age. Peak titers reached following primary stimulation, however, were similar for all age groups (Table 12). The peak of this response in the 8-week-old group could not be determined because a 2nd immunization was given while the titer was still rising.

When a 2nd dose of tetanus toxoid was given, a rapid response was observed with production of high levels of

Figure 21: A graph illustrating the primary and secondary responses of anti-tetanus seronegative foals (CD and CF) to tetanus toxoid. The first dose was administered at one, 2, 4, or 8 weeks of age, and a 2nd was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.


1 Week old (5 Foals)
2 Weeks old (5 Foals)
4 Weeks old (5 Foals)
8 Weeks old (7 Foals)
Antibody Titer
20480
10240
5120
2560
1280
640
320
160
80
40
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Weeks

antitetanus antibody (Figure 21), and the responses of all the groups were nearly identical.

Responses to human serum albumin were compared in one, 2, 4, 8, and 10-week-old foal groups. One week after the first inoculation, no antibody could be detected in the one, 2, or 4-week-old groups (Figure 22). Low levels were detected in the 8-week-old foals and higher levels were found in the 10-week-old group (Figure 23). Peak titers were observed 4 weeks after the first inoculation and then started to decline in the one, 2, and 4-week-old foals. It could not be determined if peak titers were reached in the 8 and 10 week-old groups before a 2nd HSA dose was given. Increasing age did not appear to influence the eventual peak anti-HSA titer (Table 13), but it did shorten the time required for the appearance of detectable anti-HSA antibody after inoculation.

The response to a 2nd inoculation was rapid with peak antibody levels attained usually at the 2nd week after inoculation. Some differences were noted among groups in the height of secondary response and were apparently related to the degree of primary response, i.e., the greater the primary response the greater the secondary response.

The response to killed Western and Eastern equine encephalomyelitis virus was compared among 4 foal groups (Figures 24 and 25). A period of 3 or more weeks was required to reach peak titers after inoculation with both antigens.

Figure 22: A graph illustrating the primary and secondary responses of anti-HSA seronegative foals (CD and CF) to human serum albumin. The first dose was administered at one, 2, or 4 weeks of age, and a 2nd inoculation was given 4 to 11 weeks after the first. ↑ = first injection, ↑ = 2nd injection.

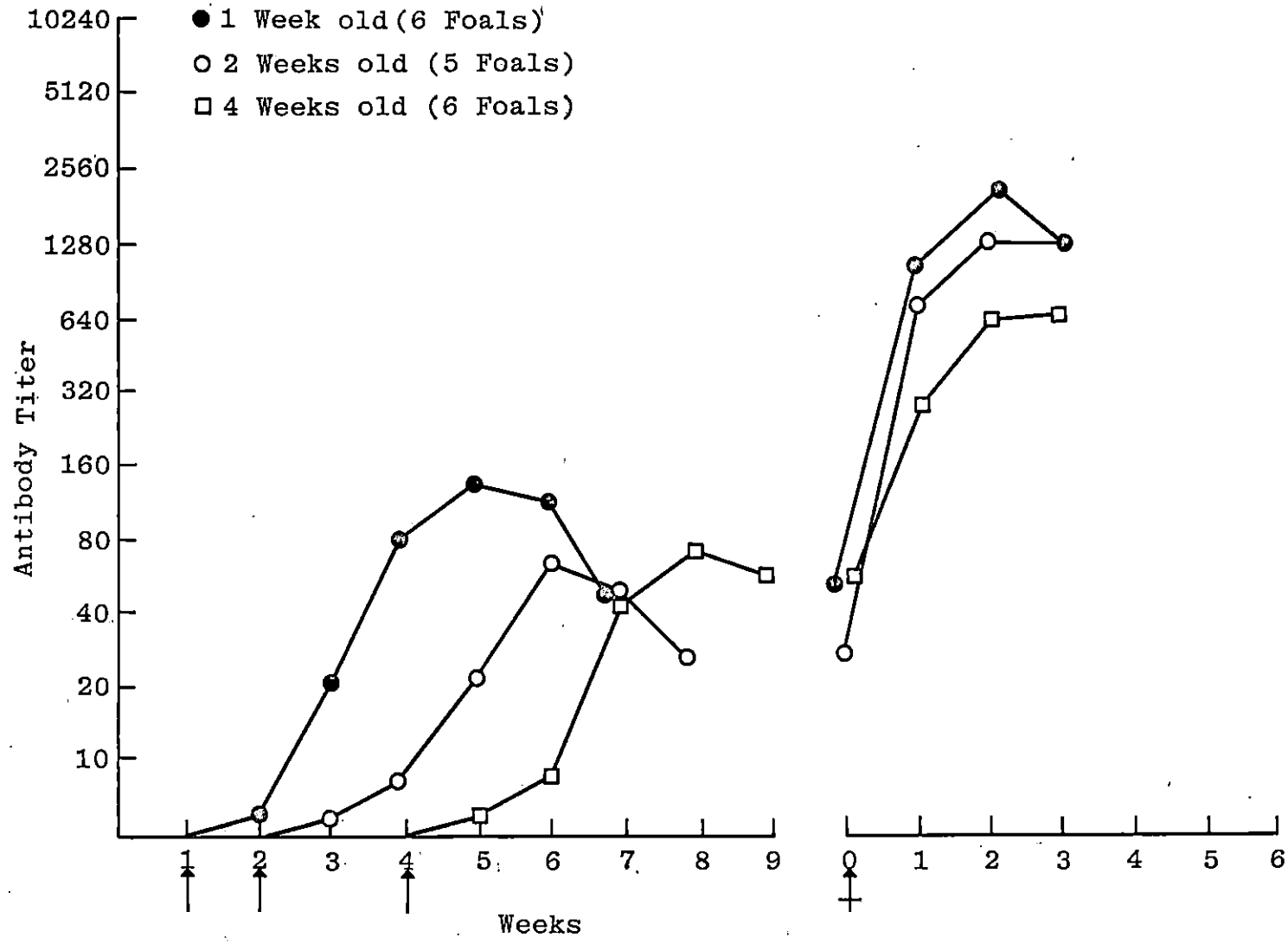

● 1 Week old (6 Foals)
○ 2 Weeks old (5 Foals)
□ 4 Weeks old (6 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6
Weeks

Figure 23: A graph illustrating the primary and secondary responses of anti-HSA seronegative foals (CD and CF) to human serum albumin. The first dose was administered at 8 or 10 weeks of age, and a 2nd inoculation was given 4 weeks after the first. ↑ = first injection, ↑ = 2nd injection.

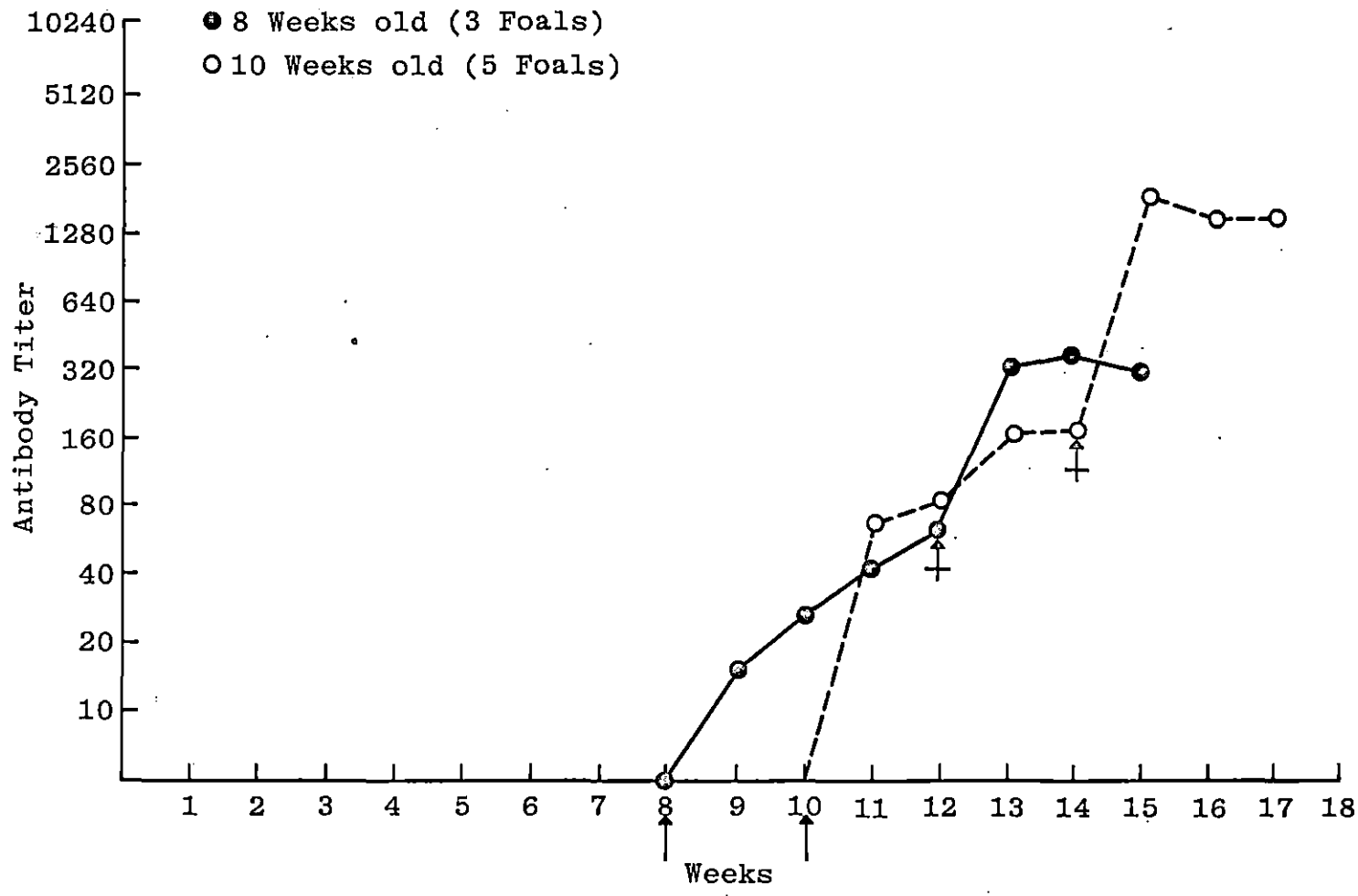

● 8 Weeks old (3 Foals)
○ 10 Weeks old (5 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Weeks

Figure 24: A graph illustrating the primary and secondary responses of anti-WEE seronegative foals (CD and CF) to killed Western equine encephalomyelitis virus. The first dose was administered at one, 2, 4, or 8 weeks of age, and a 2nd inoculation was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

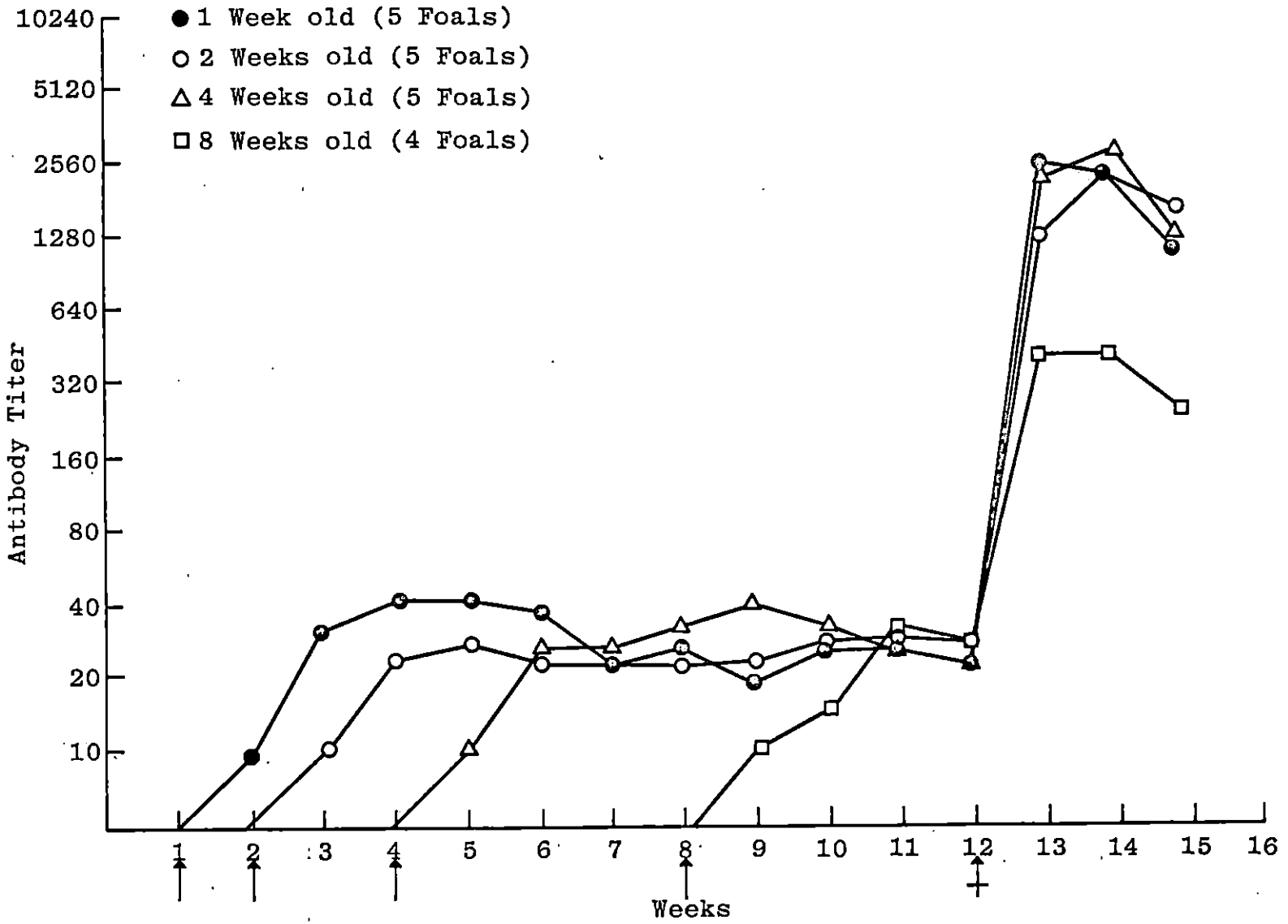

● 1 Week old (5 Foals)
○ 2 Weeks old (5 Foals)
△ 4 Weeks old (5 Foals)
□ 8 Weeks old (4 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 25: A graph illustrating the primary and secondary responses of anti-EEE seronegative foals (CD and CF) to killed Eastern equine encephalomyelitis virus. The first dose was administered at one, 2, 4, or 8 weeks of age, and a 2nd inoculation was given at 12 weeks. ↑ = first injection, ↑ = 2nd injection.

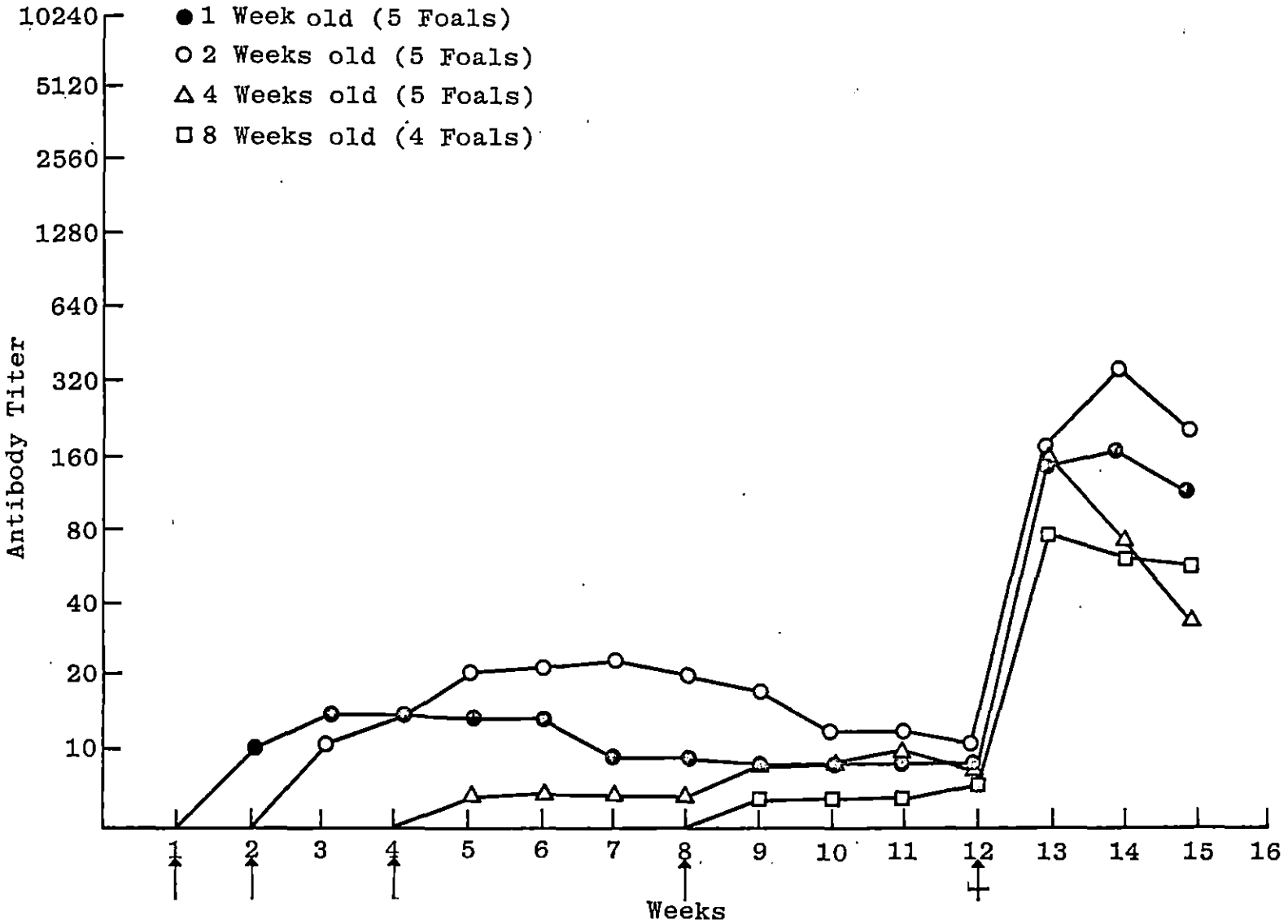

● 1 Week old (5 Foals)
○ 2 Weeks old (5 Foals)
△ 4 Weeks old (5 Foals)
□ 8 Weeks old (4 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Table 13

Some Comparative Responses of Various Foal Age Groups to HSA

Foal age (weeks)	Peak response to first immun.	Titer at 2nd immun.	Peak response to 2nd immun.
1 (6)[a]	137[b]	50	2281
2 (5)	69	26	1470
4 (6)	71	57	718
8 (3)	64	64	403
10 (5)	211	183	1940

[a] Number of foals in each group.

[b] Titers expressed as the reciprocal of the average for each group.

Table 14

Some Comparative Responses of Various Foal Age Groups to Killed WEE and EEE Virus

Foal age (weeks)	Peak response to first immun.		Titer at 2nd immun.		Peak response to 2nd immun.	
	WEE	EEE	WEE	EEE	WEE	EEE
1 (5)[a]	48[b]	14	25	7	2940	160
2 (5)	28	24	28	10	2560	368
4 (5)	40	9	24	7	2941	160
8 (4)	34	5	28	5	452	80

Peak titers reached following the first injection were similar for all age groups (Table 14).

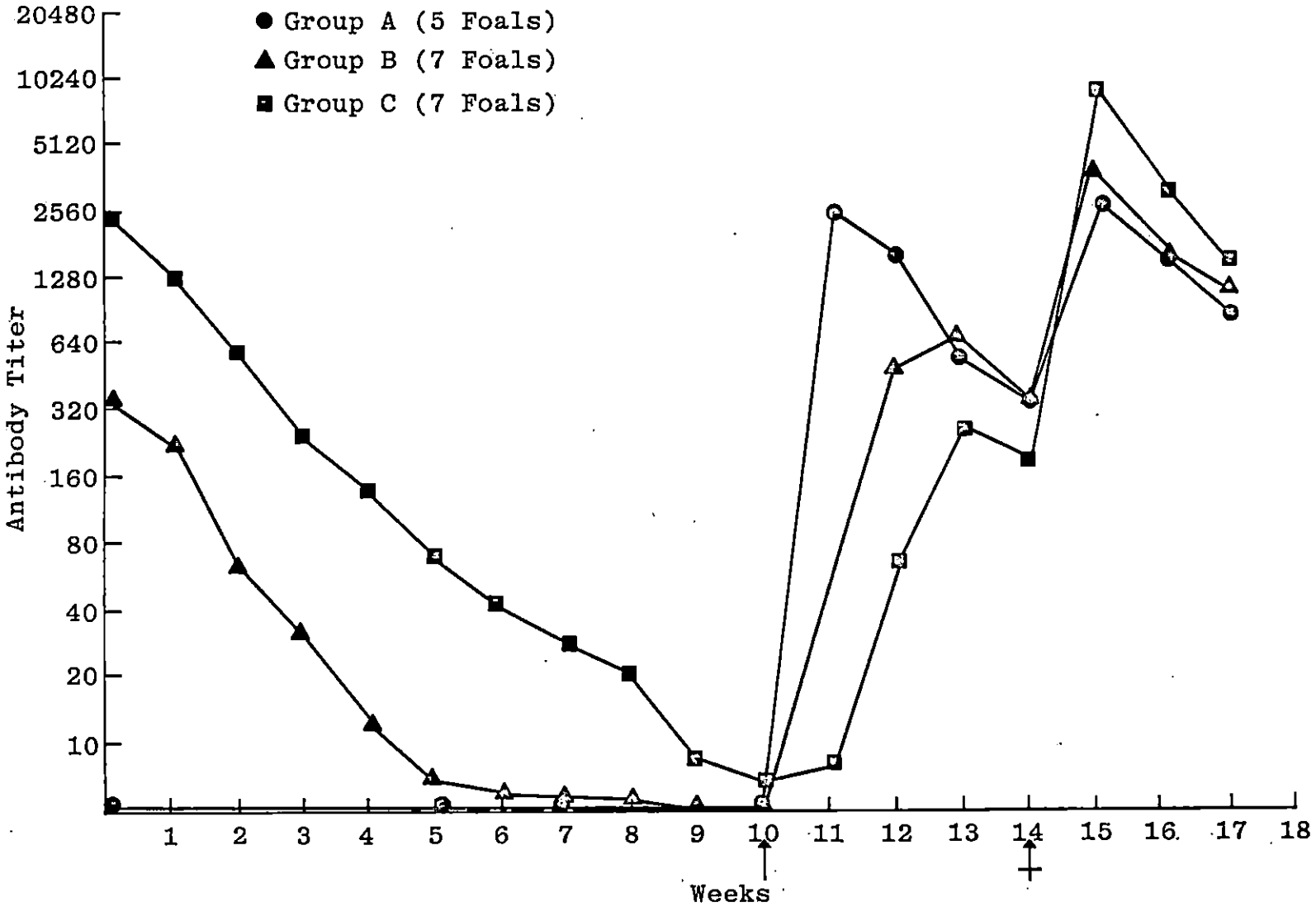
Response to a 2nd inoculation produced a rapid secondary response with peak titers reached one or 2 weeks later. These responses were alike in all groups except those given their first dose at 8 weeks of age. In this latter case the response, especially to WEE, was less than that observed in the other groups (Table 14).

Effect of maternal antibody

To evaluate the effect of the presence of maternal antibody on the humoral antibody response, foals were grouped according to their titers of a particular antibody at 72 hours of age. The foals received their first inoculation at a selected age for each antigen. A 2nd dose was given 4 weeks after the first.

Foals were divided into 3 groups on the basis of their titers against Brucella abortus at 72 hours of age (Figure 26). One group had no detectable antibody at this time and remained negative until immunization at 10 weeks of age. A 2nd group had low levels at 72 hours of age. The average titer of this group was 1:353 and had decreased to nondetectable levels at the first immunization. The 3rd group had high maternal anti-Brucella levels (1:2100) at 72 hours of age, and they had declined to very low levels ($<$1:10) at 10 weeks of age when the first immunization was given.

Figure 26: A graph illustrating the comparative responses of 10-week-old foals to killed Brucella abortus S19 at 10 and 14 weeks of age. Shown are foals with no (Group A), low levels (Group B) and high levels (Group C) of maternal anti-Brucella antibody at 72 hours of age. ↑ = first injection, ↑ = 2nd injection.


Group A (5 Foals)
Group B (7 Foals)
Group C (7 Foals)
Antibody Titer
20480
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

The foals with no detectable anti-Brucella antibody from birth until the first inoculation gave an immediate primary response to an average peak titer of 1:2560 at one week after immunization. Antibody levels declined thereafter to an average level of 1:320 when a 2nd inoculation was given 4 weeks after the first. Titers increased to an average of 1:2940 at one week post-immunization. This was only slightly higher than the peak titer during the primary response. Levels had declined again at the 2nd post-inoculation sampling.

When given the first inoculation with killed Brucella abortus, the foals with low anti-Brucella maternal levels responded more slowly than the previous group. Peak titers were not noted until the 3rd post-inoculation sampling at which time they averaged 1:707. When a 2nd dose was given, a good secondary response was noted with a peak response of 1:4200 reached one week post-inoculation. This was considerably greater than the primary response peak titer.

The foals with high anti-Brucella abortus maternal levels at 72 hours of age responded more slowly and with less antibody production than the 2 previous groups to an inoculation with killed Brucella abortus. At one week post-inoculation there was only a very slight rise in average titer (from 1:4 to 1:8). The peak response (1:262) was noted at the 3rd week post-inoculation. A 2nd dose given 4 weeks after the first resulted in a strong, rapid secondary response with an average peak titer of 1:6241 occurring at one week post-inoculation.

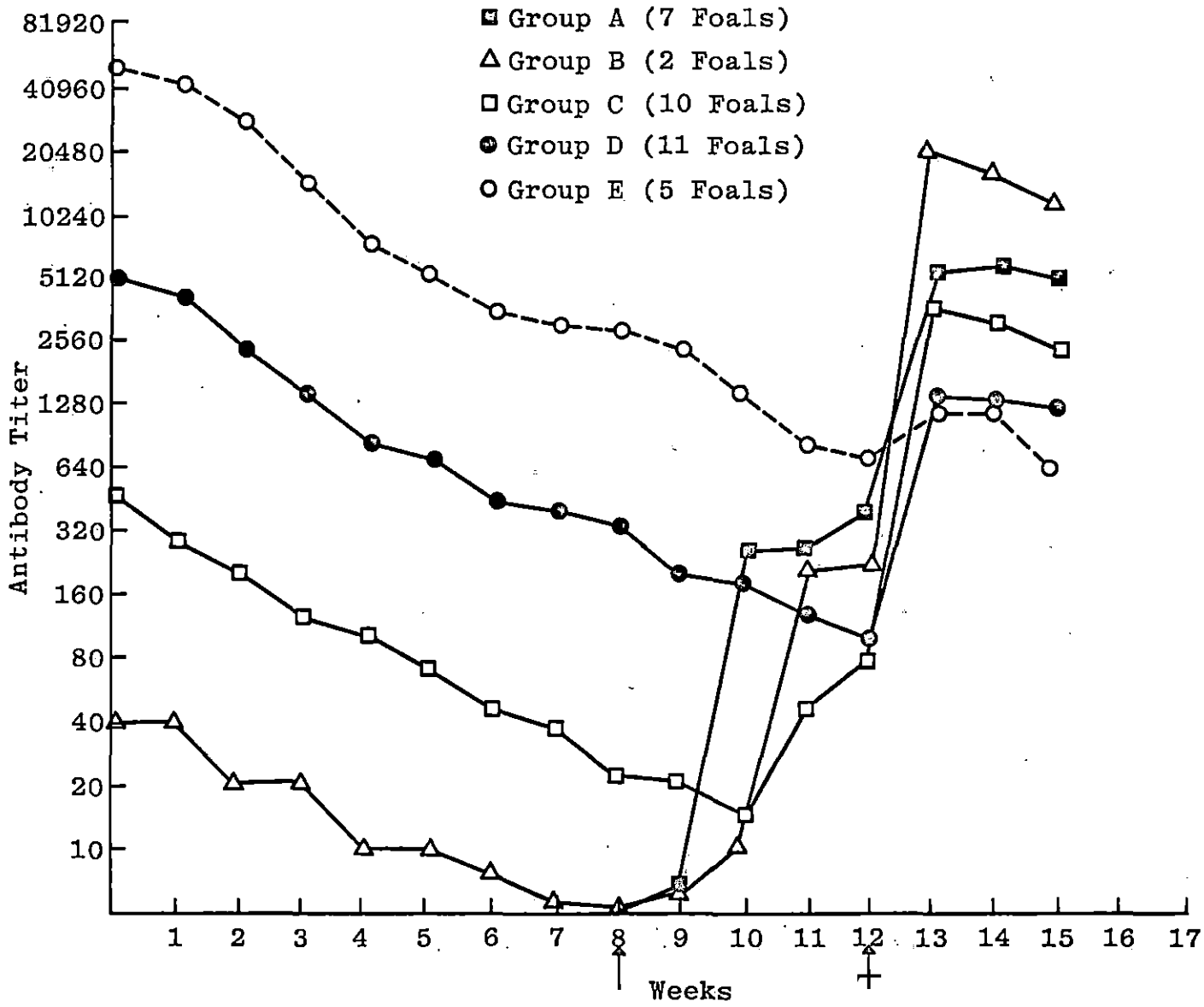
The foals were divided into 5 groups based on their anti-tetanus titer at 72 hours of age--no detectable circulating antibody, low levels, moderate levels, high levels, and very high levels (Figure 27). All foals were given their first immunization at 8 weeks of age and a 2nd dose 4 weeks later.

The first foal group, which had no detectable circulating anti-tetanus antibody at 72 hours of age, remained negative until the 2nd week post-inoculation at which time the levels were 1:285. Titers had increased to 1:453 at 12 weeks of age when the 2nd immunization was administered. This resulted in another rapid increase in titer which peaked at 14 weeks at 1:7241.

The foal group with low maternal anti-tetanus levels at 72 hours of age (1:40) had nondetectable levels by 7 weeks. Response to the first immunization was not noted until the 2nd post-inoculation week when the levels had risen to 1:10. At the time of the 2nd immunization, the titer was 1:226. There was a rapid, strong response with titers peaking at 1:20480 at one week after this 2nd inoculation.

The anti-tetanus levels had decreased to 1:26 at 8 weeks of age in the 3rd group which had moderate antibody levels at 72 hours of age (1:485). Levels continued to decline for the 2 weeks after the first immunization to a titer of 1:14. Titers rose to 1:75 by the time a 2nd dose was given and

Figure 27: A graph illustrating the comparative responses of 8-week-old foals to tetanus toxoid at 8 and 12 weeks of age. Shown are foals with no (Group A), low levels (Group B), moderate levels (Group C), high levels (Group D) and very high levels (Group E) of maternal anti-tetanus antibody at 72 hours of age. ↑ = first injection, ↑ = 2nd injection.


Group A (7 Foals)
Group B (2 Foals)
Group C (10 Foals)
Group D (11 Foals)
Group E (5 Foals)
Antibody Titer
81920
40960
20480
10240
5120
2560
1280
640
320
160
80
40
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Weeks

then increased again to a peak of 1:3880 at the first week after this inoculation.

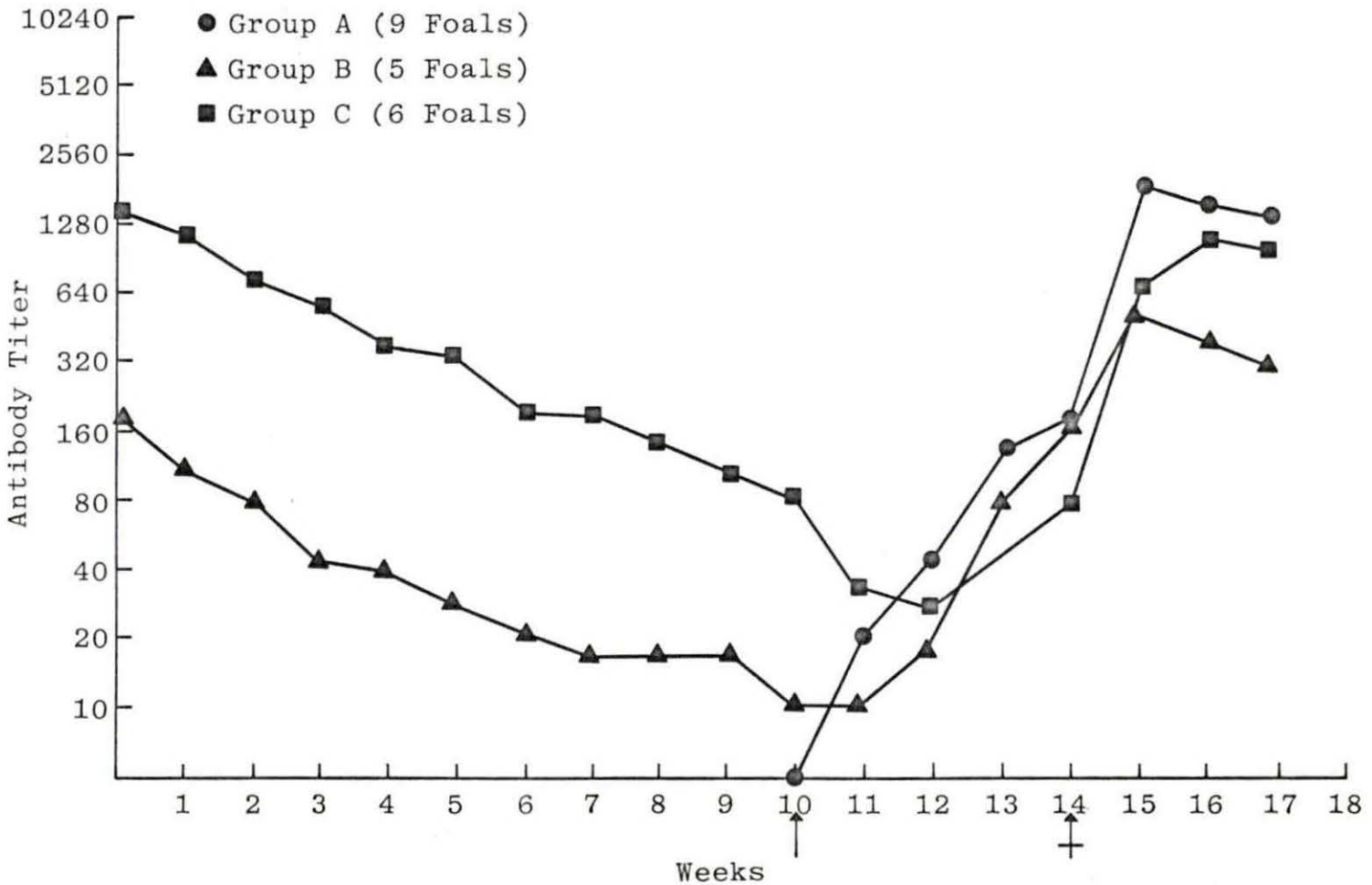
The 4th foal group had high anti-tetanus levels at 72 hours of age (1:5120). By 8 weeks of age the titer had decreased to 1:363. No detectable response to the first immunization was noted in this group, and levels continued to decline to 1:117 at 12 weeks of age. A 2nd dose produced a rise in antibody titers to 1:1451 one week post-inoculation.

The last group had very high levels of anti-tetanus antibody at 72 hours of age (1:54047). At the time of the first immunization, the titer had decreased to 1:2940, and at the 2nd immunization they had decreased further to 1:735. A slight response to the 2nd immunization was noted with antibody levels rising to 1:1174 by the 2nd week post-inoculation.

Three groups were compared based on their circulating anti-human serum albumin levels at 72 hours of age (Figure 28). One group had no detectable anti-HSA antibody from 72 hours until the first inoculation. The 2nd group had low levels at 72 hours of age (1:184) while the 3rd group had high levels (1:1437). All foals received a first immunization at 10 weeks of age, and a 2nd dose was given 4 weeks later.

The first group of foals had a rise in anti-HSA titer to 1:23 at one week post-inoculation. The titer continued

Figure 28: A graph illustrating the comparative responses of 10-week-old foals to human serum albumin at 10 and 14 weeks of age. Shown are foals with no (Group A), low levels (Group B) and high levels (Group C) of maternal anti-HSA antibody at 72 hours of age. ↑ = first injection, ↑ = 2nd injection.


Group A (9 Foals)
Group B (5 Foals)
Group C (6 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

to rise and by the time of the 2nd inoculation it was 1:172. The 2nd dose produced a rapid rise in titer with a peak response of 1:2032 one week later after which titers again decreased.

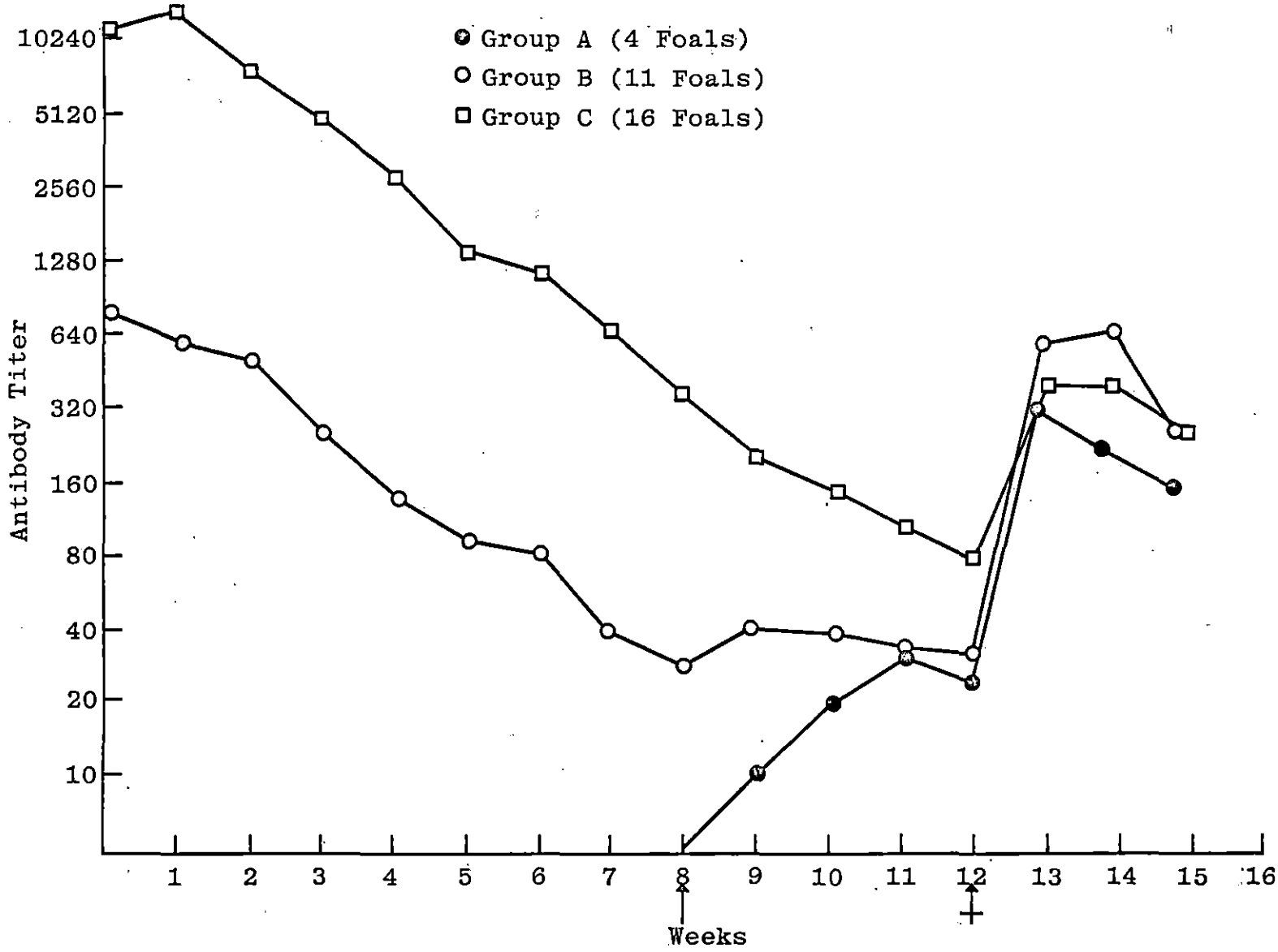
Foals with low titers at 72 hours of age had an average anti-HSA titer of 1:13 at 10 weeks. The titer was the same at one week post-inoculation and rose to 1:20 at the 2nd week. By 14 weeks of age, the titer had increased to 1:160. A 2nd dose resulted in a rise in titer that peaked at 1:557 one week post-inoculation and then declined.

The 3rd group had a titer of 1:100 when the first HSA immunization was given. The titer declined to 1:30 at 2 weeks post-immunization and then rose again to 1:80 by the 4th post-inoculation week. The 2nd dose resulted in another rise in titer that peaked at 1:1140 by the 2nd week after inoculation.

Three groups of foals divided according to their titers against Western equine encephalomyelitis virus at 72 hours of age were compared with regard to their responses to killed WEE virus at 8 and 12 weeks of age (Figure 29). The first group had no detectable antibody at this time and remained negative until immunization at 8 weeks of age. The 2nd group had low titers (1:836) at this age, while the 3rd group had high titers (1:13,278).

The first group produced a peak primary response of 1:34 at 3 weeks post-inoculation. A 2nd dose, 4 weeks

Figure 29: A graph illustrating the comparative responses of 8-week-old foals to killed Western equine encephalomyelitis virus at 8 and 12 weeks of age. Shown are foals with no (Group A), low levels (Group B) and high levels (Group C) of maternal anti-WEE antibody at 72 hours of age. ↑ = first injection, ↑ = 2nd injection.


Group A (4 Foals)
Group B (11 Foals)
Group C (16 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

after the first, produced a rapid secondary response which peaked one week later at 1:342.

Titers in the 2nd group had decreased to an average of 1:33 at the time of the first immunization. One week later the titer had increased to 1:45 but then decreased again to 1:33 at the 2nd immunization. The 2nd dose produced a secondary response which peaked at 1:726 2 weeks later.

The 3rd group had an average titer of 1:450 at the first inoculation, and the titer continued to decrease subsequently to 1:92 at the 2nd inoculation. The 2nd dose produced a peak response of 1:369 one week later.

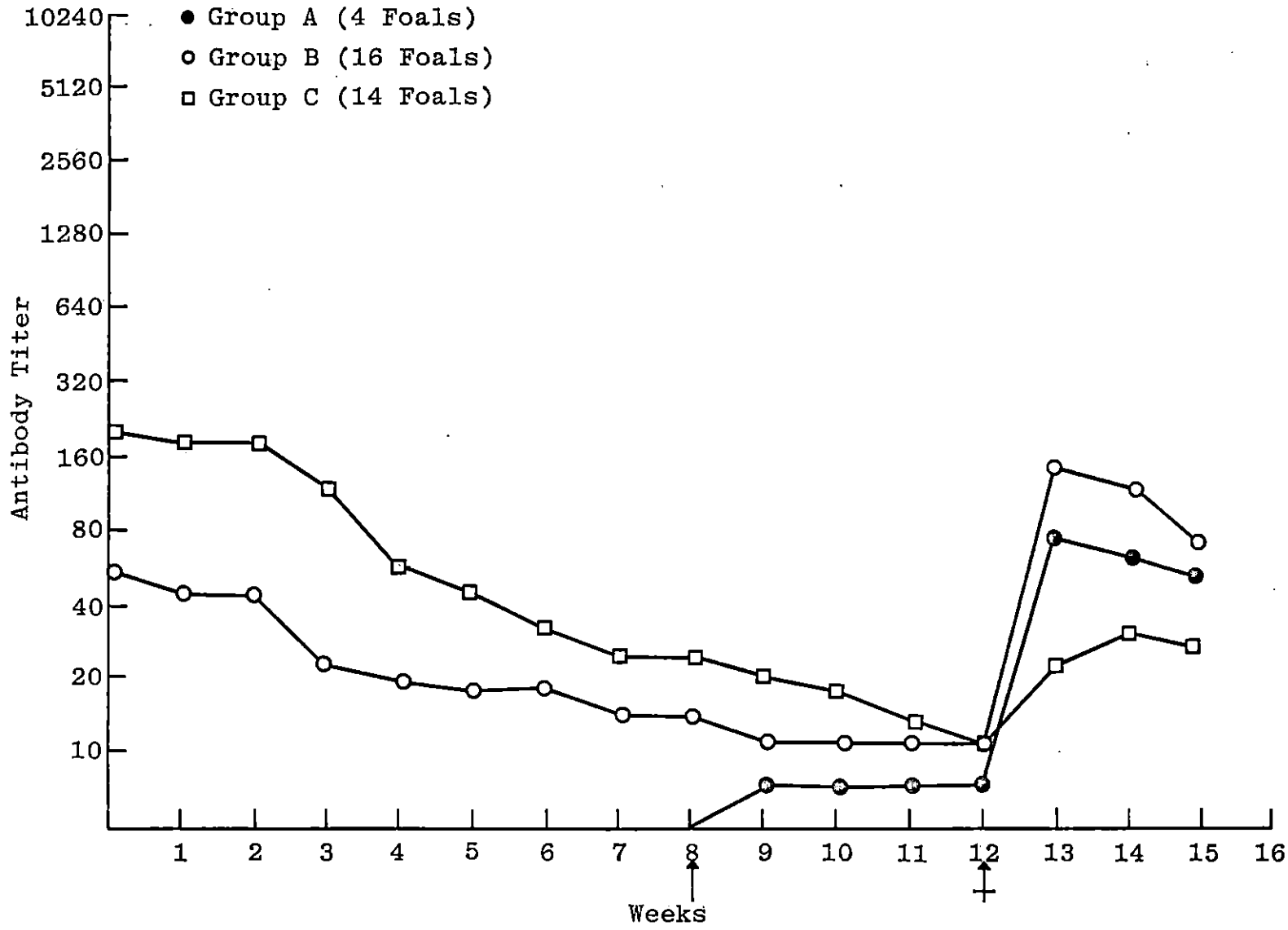
Comparisons were made in 3 groups of foals with regard to their anti-Eastern equine encephalomyelitis virus titers at 72 hours of age (Figure 30). Foals were immunized with killed EEE virus at 8 and 12 weeks of age. Groups compared had undetectable levels, low levels (1:63) and high levels (1:200) of maternal anti-EEE antibody at 72 hours of age.

The group with no antibody at 72 hours of age produced a peak response of 1:8 at 3 weeks post-inoculation. The 2nd dose resulted in a response of 1:80 one week later.

Titers in the 2nd group had decreased to 1:16 at 8 weeks of age and continued to decrease to 1:11 at 12 weeks of age. A secondary response of 1:160 was noted one week after the 2nd dose.

The group with high levels of antibody at 72 hours of age had a titer of 1:28 at the first immunization, and this

Figure 30: A graph illustrating the comparative responses of 8-week-old foals to killed Eastern equine encephalomyelitis virus at 8 and 12 weeks of age. Shown are foals with no (Group A), low levels (Group B) and high levels (Group C) of maternal anti-EEE antibody at 72 hours of age. ↑ = first injection, ↑ = 2nd injection.


● Group A (4 Foals)
○ Group B (16 Foals)
□ Group C (14 Foals)
Antibody Titer
10240
5120
2560
1280
640
320
160
80
40
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

subsequently decreased to 1:12 at the 2nd immunization. The 2nd dose produced an increase in the anti-EEE titer to 1:34 2 weeks later.

Persistence of Maternal Antibody

The disappearance of maternal antibody from the circulation of the foals was calculated from the groups of animals used previously in the study on the effect of passive antibody on immune responsiveness. The rate of elimination was determined from the antibody titers, relative to foal age. The initial titer used (Week 0) in all calculations was that present in the foal at 72 hours of age. The number of weeks used for this measurement varied among the antigens used, but at least 6 points were used for each calculation. Comparisons were made among groups of foals with different initial (72 hour) antibody concentrations to each antigen. A pooled group average determination was then made for each antibody type. Comparisons using pooled group determinations were also made among the 5 different antibodies to study their relative rates of disappearance.

The reciprocals of the titers were converted to the corresponding logarithm and used to calculate a regression equation and regression line for each group (135). The regression coefficients were used to calculate antibody half-life for each group of titers evaluated (116).

Maternal antibody half-life ($T\frac{1}{2}$) calculations for anti-Brucella abortus antibody indicated that the antibody was catabolized at a constant rate regardless of initial concentration (Figure 31). With maternal anti-tetanus antibody, however, there was a decrease in half-life time with an increase in initial antibody concentration (Figure 32). This same relationship was noted with maternal antibody to HSA (Figure 33) and WEE virus (Figure 34) but not to EEE virus (Figure 35).

A comparison of pooled groups of each antibody type showed a variability in disappearance rates among the different antibodies (Figure 36). When regression lines were drawn from a single reference titer (1:640) to further compare the elimination rates, the differences between antibody types was clearly noted (Figure 37). Maternal antibody to Brucella abortus S19 with a half-life of 7.6 days was eliminated the most rapidly. Anti-WEE and anti-tetanus antibody appeared to decrease at a similar rate with half-lives of 10.8 and 12.0 days respectively which was noteably slower than that for anti-Brucella antibody. Yet slower disappearance times were recorded for antibody to EEE ($T\frac{1}{2}$=18.2 days) and to HSA ($T\frac{1}{2}$=18.7 days).

Figure 31: A graph illustrating the elimination of maternal anti-*Brucella abortus* antibody from the circulation of colostrum-fed neonatal foals. Shown are foals with low (Group A) and high (Group B) levels of antibody at 72 hours of age and a combination of the 2 groups (pooled). $T\frac{1}{2}$=antibody half-life in days.

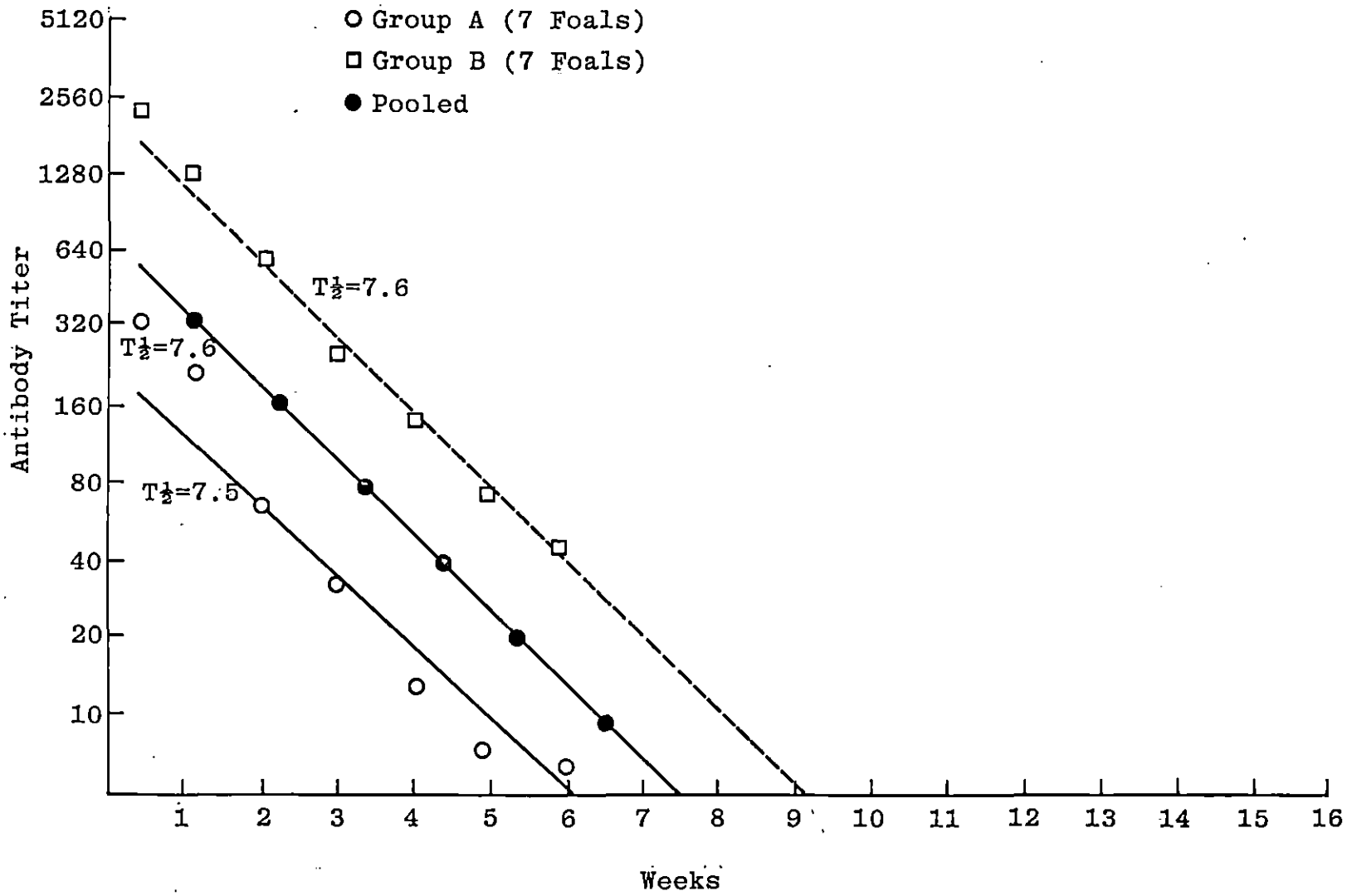

Group A (7 Foals)
Group B (7 Foals)
Pooled
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
T½=7.6
T½=7.6
T½=7.5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 32: A graph illustrating the elimination of maternal anti-tetanus antibody from the circulation of colostrum-fed neonatal foals. Shown are foals with low (Group A), moderate (Group B), high (Group C) and very high (Group D) levels of antibody at 72 hours of age and a combination of the 4 groups (pooled). $T_{\frac{1}{2}}$=antibody half-life in days.

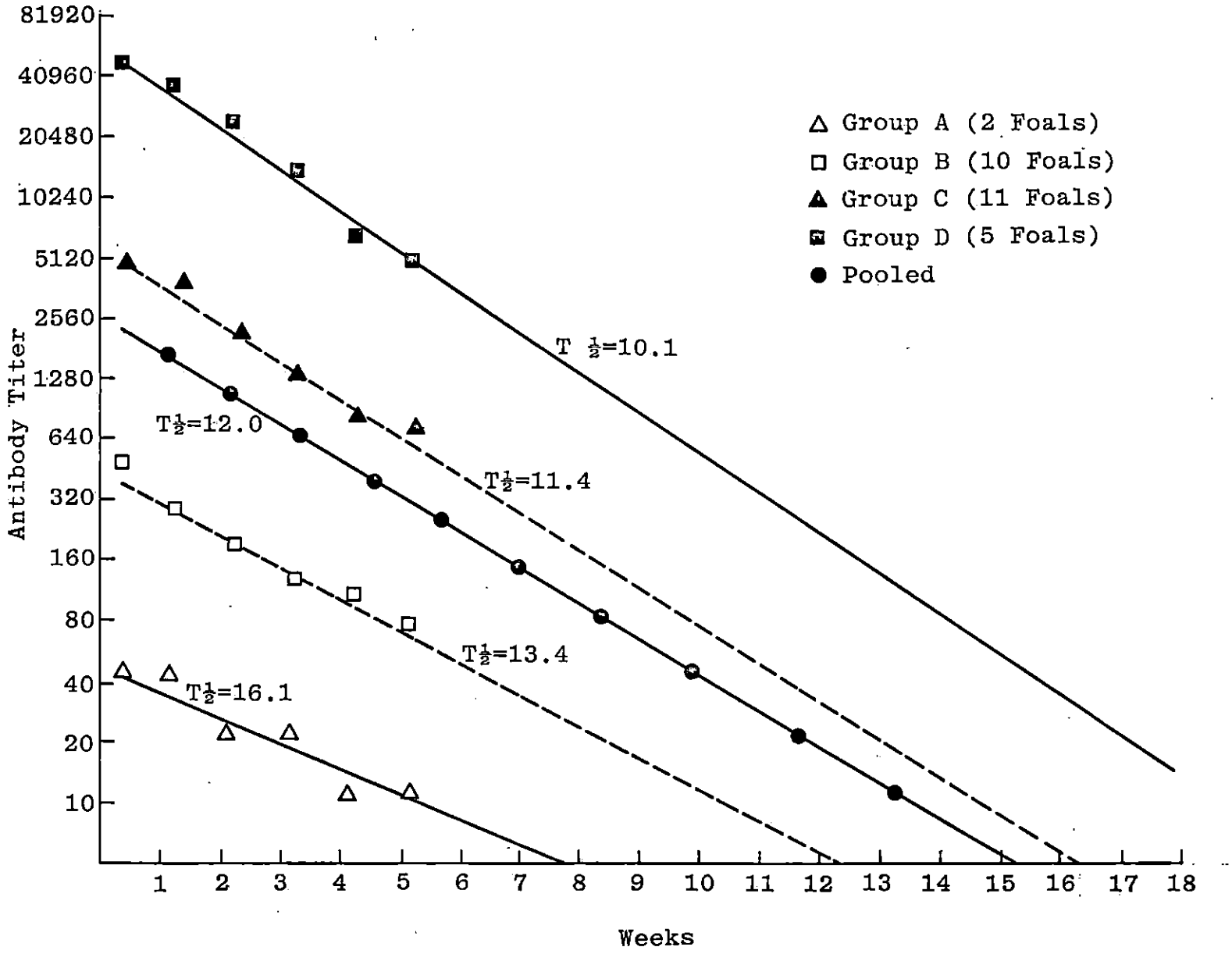

81920
40960
20480
10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks
Group A (2 Foals)
Group B (10 Foals)
Group C (11 Foals)
Group D (5 Foals)
Pooled
T ½=10.1
T½=12.0
T½=11.4
T½=13.4
T½=16.1

Figure 33: A graph illustrating the elimination of maternal anti-HSA antibody from the circulation of colostrum-fed neonatal foals. Shown are foals with low (Group A) and high (Group B) levels of antibody at 72 hours of age and a combination of the 2 groups (pooled). $T_{\frac{1}{2}}$=antibody half-life in days.

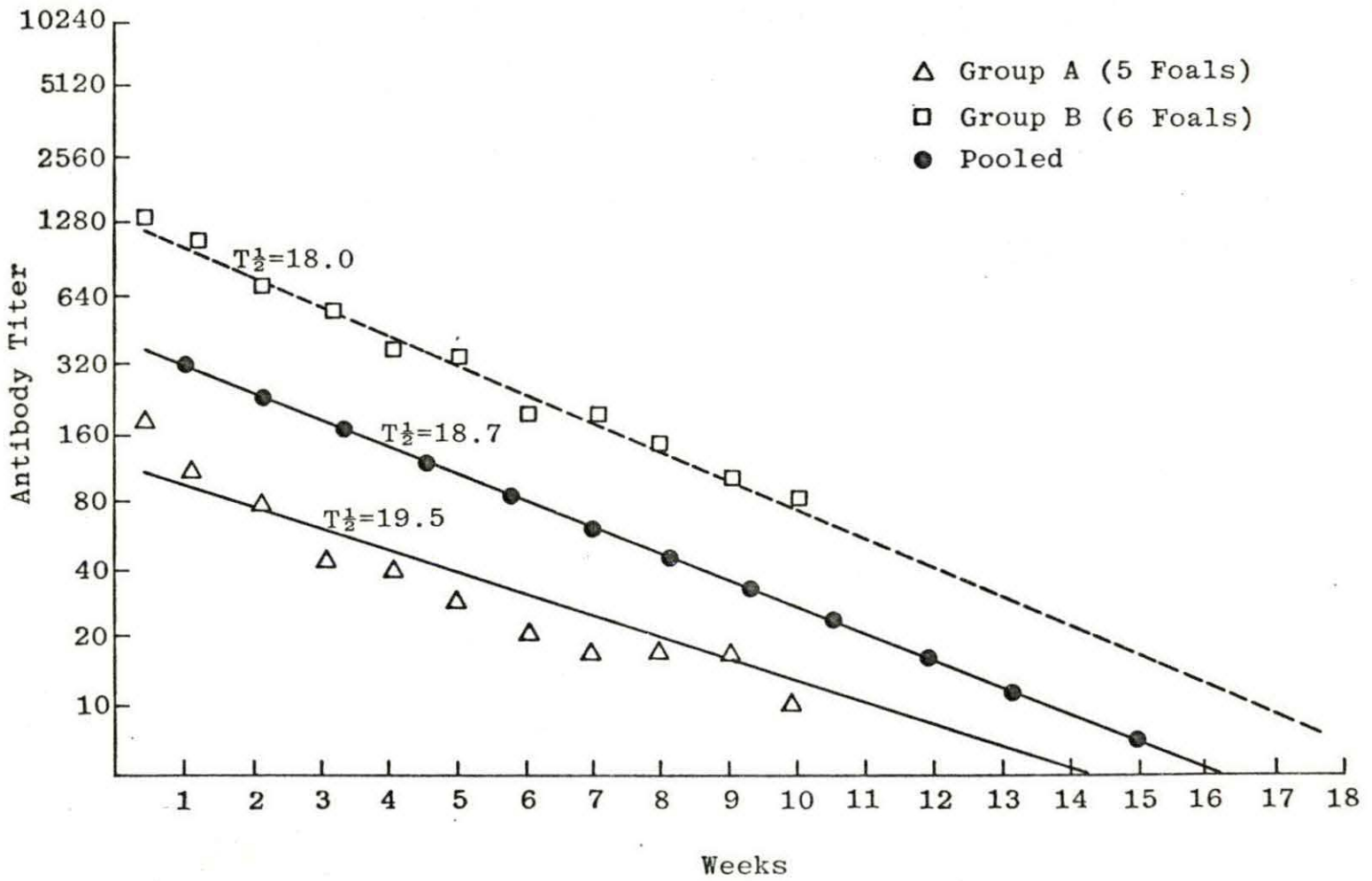

10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
Group A (5 Foals)
Group B (6 Foals)
Pooled
T½=18.0
T½=18.7
T½=19.5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks

Figure 34: A graph illustrating the elimination of maternal anti-Western equine encephalomyelitis virus antibody from the circulation of colostrum-fed neonatal foals. Shown are foals with low (Group A) and high (Group B) levels of antibody at 72 hours of age and a combination of the 2 groups (pooled). $T_{\frac{1}{2}}$=antibody half-life in days.

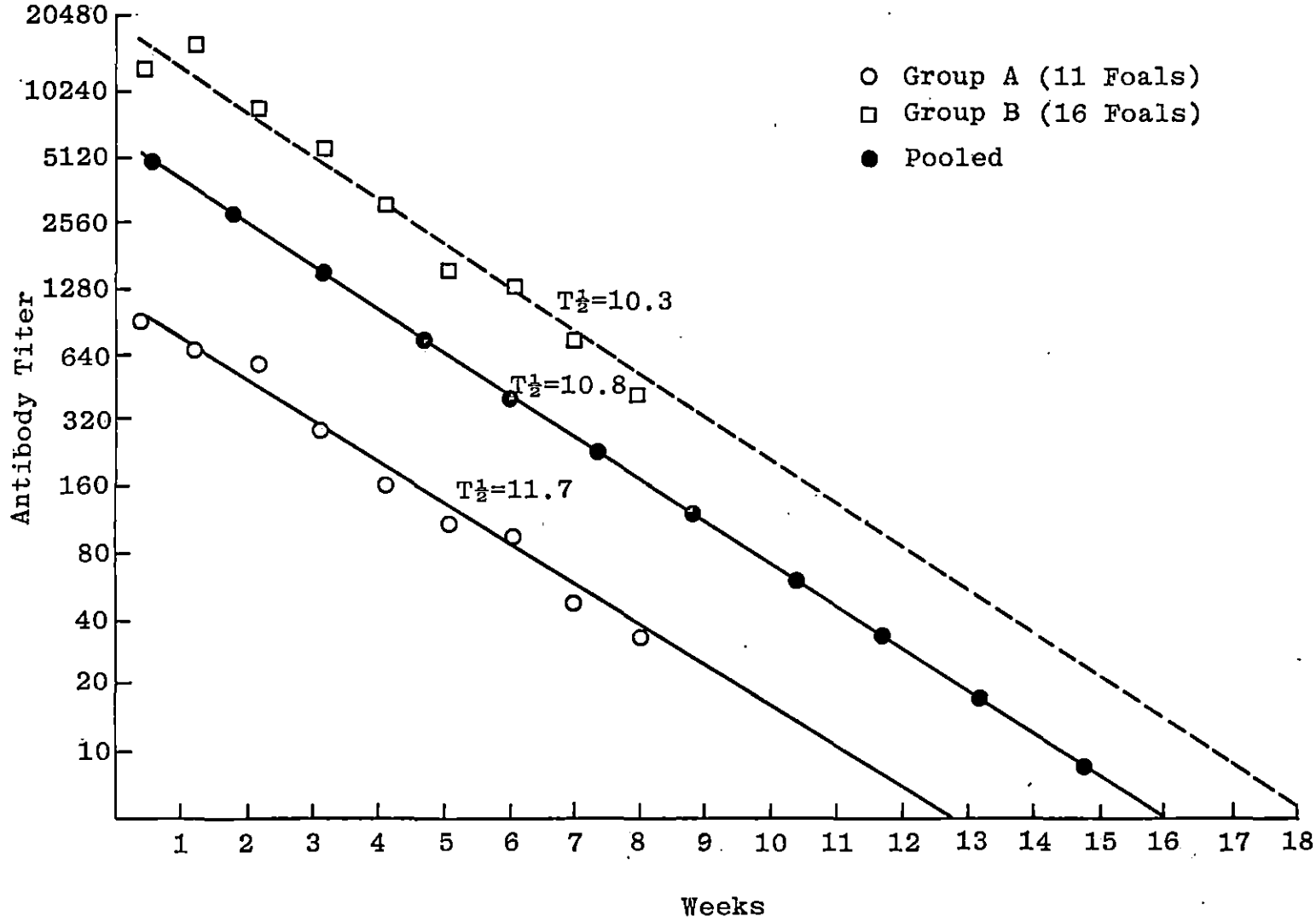

20480
10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks
Group A (11 Foals)
Group B (16 Foals)
Pooled
T½=10.3
T½=10.8
T½=11.7

Figure 35: A graph illustrating the elimination of maternal anti-Eastern equine encephalomyelitis virus antibody from the circulation of colostrum-fed neonatal foals. Shown are foals with low (Group A) and high (Group B) levels of antibody at 72 hours of age and a combination of the 2 groups (pooled). $T_{\frac{1}{2}}$=antibody half-life in days.

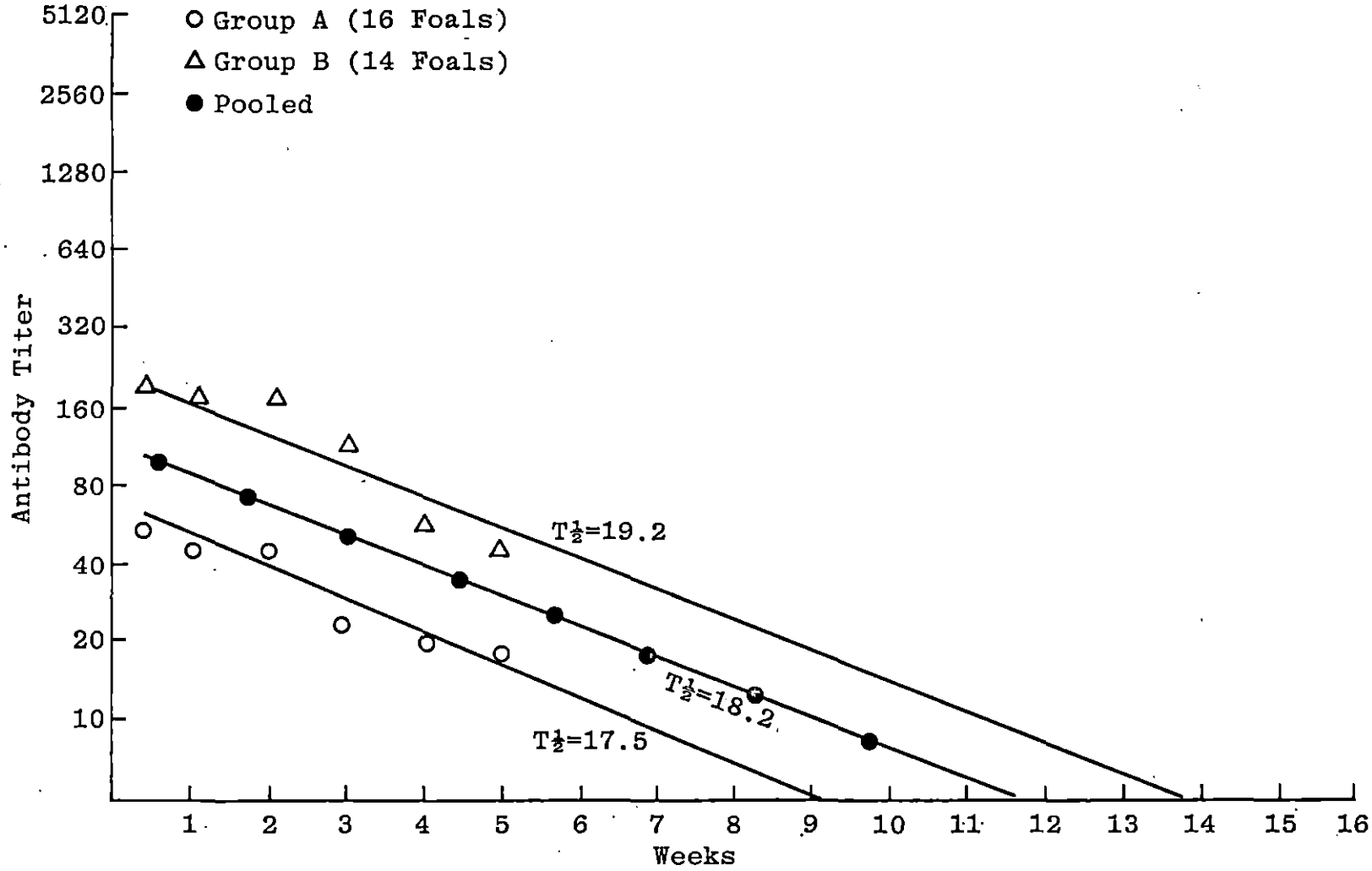

Group A (16 Foals)
Group B (14 Foals)
Pooled
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
T½=19.2
T½=18.2
T½=17.5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Weeks

Figure 36: A graph illustrating a comparison of previous pooled antibody elimination rates for maternal antibody to Brucella abortus, tetanus toxoid, human serum albumin and Western and Eastern equine encephalomyelitis virus in neonatal colostrum-fed foals. $T_{\frac{1}{2}}$=antibody half-life in days.

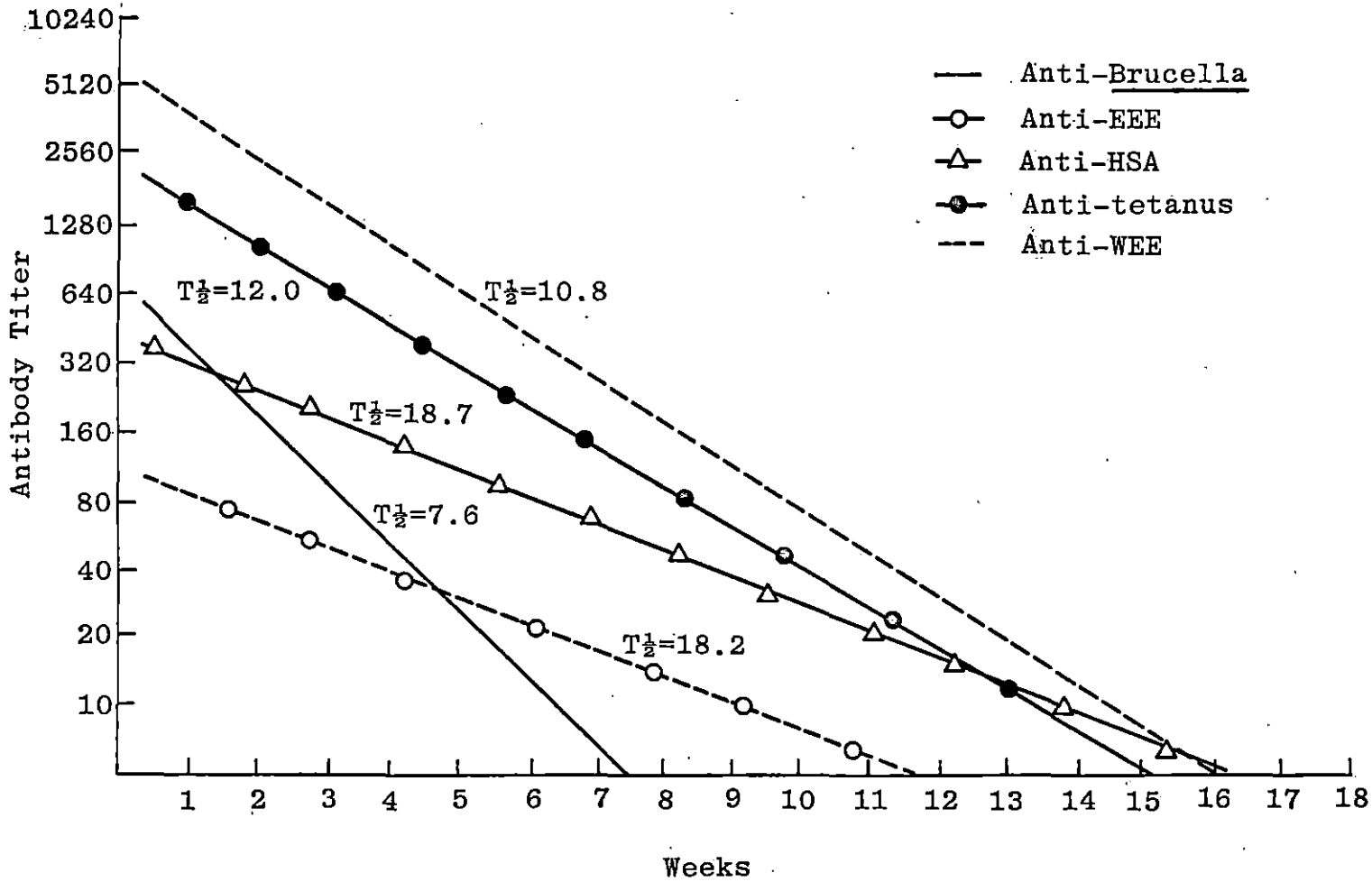
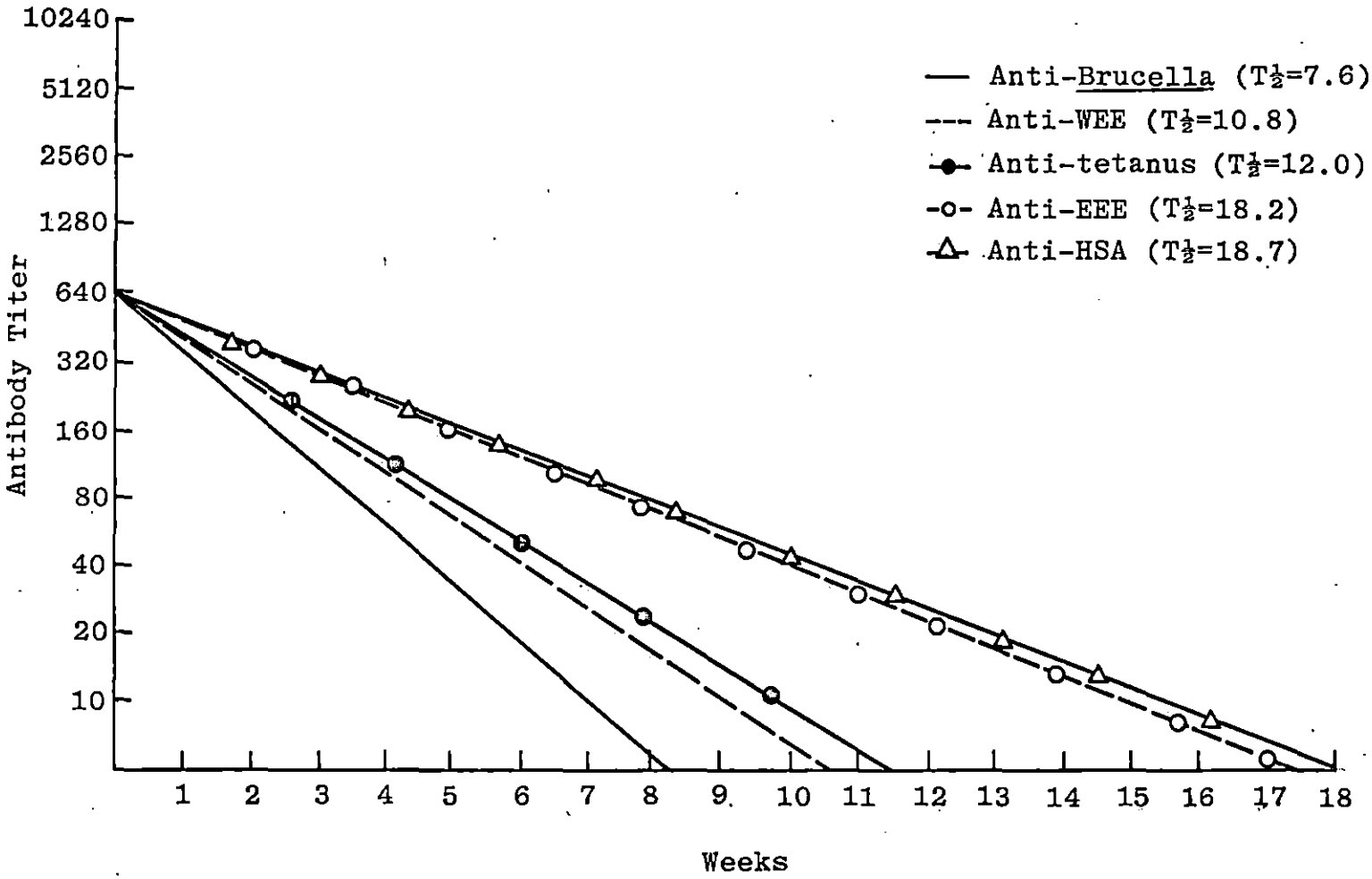

10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks
Anti-Brucella
Anti-EEE
Anti-HSA
Anti-tetanus
Anti-WEE
T½=12.0
T½=10.8
T½=18.7
T½=7.6
T½=18.2

Figure 37: A graph illustrating a comparison of elimination rates for maternal antibody to Brucella abortus, tetanus toxoid, human serum albumin and Western and Eastern equine encephalomyelitis virus in neonatal colostrum-fed foals utilizing a single initial reference titer of 1:640. $T^{1}/_{2}$=antibody half-life in days.


10240
5120
2560
1280
640
320
160
80
40
20
10
Antibody Titer
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Weeks
Anti-Brucella (T½=7.6)
Anti-WEE (T½=10.8)
Anti-tetanus (T½=12.0)
Anti-EEE (T½=18.2)
Anti-HSA (T½=18.7)

DISCUSSION

No detectable serum antibody was found in the prenursing sera of any foal by the serological techniques used. This was true even though the circulating antibody titers in the dams to some of the antigens were 1:20480. Some foals had prenursing titers of 1:20 to WEE virus and 1:40 to EEE virus, but these titers were considered to be nonspecific and a function of the hemagglutination-inhibition test. The lack of detectable serum antibody at birth confirms similar reports of others (1, 22, 81).

By 24 hours of age substantial antibody had appeared in the foals' sera due to intestinal absorption of maternal antibody present in the ingested colostrum. Serum titers in the foals at this time closely approximated that of their dams; this was especially true of antibody to tetanus toxoid and WEE and EEE virus, while foal titers to Brucella abortus S19 and HSA were lower than their dams'. The presence of two groups of comparative titers may have been related to quantitative differences in the amount of antibody present in the dam's colostrum and/or to differences in the immunoglobulin types(s) composing the colostral antibody to each antigen and the foal's ability to absorb these different immunoglobulins. The newborn calf nonselectively absorbs all immunoglobulin types (15, 99), while the pig shows the ability to absorb all immunoglobulin

classes although some may be absorbed more readily than others (28, 100). The newborn foal appears to have an enhanced absorption of IgG as compared to other equine immunoglobulin classes, i.e., IgG(T), IgM, IgA, and AI (76). The antibody present in the colostrum, therefore, to tetanus toxoid and WEE and EEE virus may have been predominantly IgG in nature, while colostral antibody to Brucella abortus and HSA may have contained a higher proportion of the other immunoglobulin classes. It is of interest to note that the tetanus toxoid and killed WEE and EEE virus used as antigens were incorporated into aluminum hydroxide, while the other 2 antigens were not.

At 72 hours of age the foals' serum titers had either remained equivalent to the 24-hour-old titers or had risen slightly. By 24 hours of age the foal had acquired almost all of its circulating maternal antibody, and only a slight amount was absorbed subsequent to that. This is in agreement with the findings of Jeffcott (57, 60) and Bruner et al. (21). A comparison of mare titers at parturition and foal titers at 72 hours with a larger number of animals demonstrated results similar to those seen at 24 hours, i.e., nearly equivalent titers of anti-tetanus and anti-WEE and anti-EEE virus antibody and somewhat lower foal titers of anti-HSA and especially anti-Brucella abortus antibody.

Foals deprived of colostrum and fed a milk replacer product for the first 72 hours of life did not acquire any

detectable circulating antibody when allowed to nurse their dams after this period. After 72 hours of age, therefore, only nondetectable amounts of colostral antibody, if any, were absorbed by the neonatal foals.

Decline in foal maternal antibody levels from 72 hours of age showed some variability among the different antibody types as well as among the different initial concentrations of a particular antibody. This was to be expected given a heterogeneity of immunoglobulin classes representative of antibody.

The most rapid decrease was noted with maternal anti-Brucella abortus agglutinating antibody which had a half-life of 7.5 to 7.6 days. This was most likely due to a high proportion of IgM composing this antibody. Brucella as a primary antigen induces the formation of IgM antibody followed by some IgG antibody, but in some species even hyperimmunization fails to produce very much IgG antibody (14). The half-time of survival of IgM is one-tenth to one-half that of IgG in man, rabbits, guinea pigs, and mice (153), one-fourth to one-fifth that of IgG in pigs (28, 100) and one-fifth that of IgG in calves (99).

The similarity of the half-life values for the groups with initial low and high levels of anti-Brucella abortus antibody also reflected the probable high percentage of IgM antibody. The catabolic rate of IgG is in direct proportion to the plasma concentration (153), but the

catabolic rate of IgM is independent of the serum concentration (153, 8). An increase or decrease in IgM serum concentration does not lead to a concomitant change in the speed of catabolism.

Antibody to tetanus toxoid and Western equine encephalomyelitis virus showed similar average half-life values, 12.0 vs 10.8 days. This may have indicated that the proportion of immunoglobulin types present in these two antibody populations were very similar. A point strengthening such a conclusion is that both antigens were incorporated in the same adjuvant, aluminum hydroxide, and administered together. Antibody to Eastern equine encephalomyelitis virus did not yield an equivalent half-life value, but this was considered to be associated with the serological procedure used to detect the antibody. Nonspecific titers of 1:10 and 1:20 were common and tended to increase the average titers especially at the latter points along the regression line. This decreased the slope of the line and increased half-life values, so it is not certain that the calculated values are a true representation of the half-life of this antibody.

The half-life value for antibody to human serum albumin (18.7 days) exhibited a slower catabolic rate than for the others discussed above. Again this probably reflected a heterogeneity of the immunoglobulin classes composing this antibody which were in apparently different proportion than in previous antibody populations. No effort was made to

calculate the relative amounts of IgG_a, IgG_b, IgG_c, IgM, IgG(T), IgA, or aggregating immunoglobulin (AI) composing these antibodies. There are large differences in the relative catabolic rates of the different immunoglobulins in most species (153). Differences among IgG subclasses also exist in man (IgG_3 is catabolized more rapidly than IgG_1, IgG_2, or IgG_4) (132) and mice (131).

As the maternal antibody titers to tetanus toxoid increased, the antibody half-life decreased (16.1 days for the lowest to 10.1 days for the highest titer) indicating an elevation in the antibody catabolic rate with increased levels. Similar results were obtained with antibody to WEE virus and HSA. Two plausible explanations can be offered to account for this phenomenon, and both may play some major or minor role.

It is known in man that the catabolic rate of IgG is directly proportional to the serum concentration of IgG (153). An increase, therefore, in IgG levels leads to increased IgG catabolism and subsequently a shorter half-life. This same situation exists in mice (34) and rabbits (153). It should be noted that such a relationship does not exist for IgM and IgA in man (153). A similar situation may be inferred for the horse with IgG and possible with IgG(T) and AI. Increased levels of these immunoglobulins could result in their being more rapidly catabolized.

Another possibility is that as maternal antibody levels increase, so do the proportions of various immunoglobulin class constituents. An increased percentage of a more rapidly metabolized immunoglobulin class would, therefore, lead to a shorter half-life for that antibody. Hyperimmunization of horses with tetanus or diphtheria toxoid results in a large relative increase in the production of IgG(T) type antibodies (151, 152), so this represents a possible cause of anti-tetanus antibody catabolic rate changes if IgG(T) half-life is shorter than that for IgG.

When compared to other half-life determinations for the young foal (61, 102), the values presented here are notably lower. The only similar half-life values are those of 20 days for Clostridium welchii type A antitoxin and 18 days for gamma globulin in foals with greater than average levels (61) and 18.7 days for anti-HSA antibody in this study. In fact, the values calculated in this study, especially with antibody to tetanus toxoid and WEE virus, more nearly reflected those found in the adult horse, i.e., 11.0 days for gamma globulin (82) and 14.3 days for IgG (92). It should be noted that the calculations presented here not only include the effects of catabolism but also antibody dilution due to increasing animal size and, subsequently, increasing size of intra and extra-vascular compartments. The estimations, therefore, of Reilly and MacDougall (102) of a

half-life of 23±4.5 days of IgG in the neonatal foal may be more accurate since corrections were made for increases in body weight.

The primary response to Brucella abortus S19 observed in these neonatal foals exhibited a distinctly different pattern than the responses to the other antigens. This difference was probably due to the fact that tetanus toxoid, WEE and EEE virus, and HSA were all administered in an adjuvanted form while Brucella abortus was not. Some difference was likely due, also, to the differences in antigenicity among the agents used.

Primary responses to antigenic stimulation are usually characterized by a lag period of variable length during which there is no detectable serum antibody followed by the appearance of antibody of the IgM class (147). Particulate antigens lead to a more rapid onset of antibody production than soluble antigens partly because they are more rapidly cleared from the circulation and presented to the immune system (49). Secondary or anamnestic responses are characterized by the almost immediate production of high levels of predominantly IgG antibody, although some antigens may stimulate considerable IgM antibody (65).

Certain roles for macrophages, T lymphocytes and B lymphocytes in antibody response have been described (13, 45, 150). The macrophage may bind antigen to its surface and interact with a T lymphocyte (thymus-dependent lymphocyte,

"antigen-reactive" cell) having a specific receptor for that antigen. The T lymphocyte presents the antigen to a B lymphocyte (bursa equivalent-derived lymphocyte, "antigen-sensitive" cell) and also stimulates the B cell to undergo proliferation and differentiation that leads to the production of antibody and immunologic memory. "Processing" of the antigen may occur during the time it is in contact with the macrophage and T lymphocyte. Some antigens (thymus-independent) do not require T lymphocyte involvement, and many of these are also macrophage independent.

The primary response to Brucella abortus was characterized by a rapid increase in agglutinating antibody production during the first one to 2 weeks post-inoculation followed by a decline in levels. Brucella abortus S19 has been shown to produce a greater predominance of IgM antibodies during the primary response than other antigens, at least in some species (109), and may be the case in this study.

Antigens which induce primarily IgM antibody production are, for the most part, thymus-independent (45). They are large polymeric molecules with a high density of repeating identical determinants and are not easily metabolized by the body. The lack of a need for T lymphocyte and macrophage cooperation may lead to a more rapid appearance of antibody to these antigens than to thymus-dependent antigens since less steps would be required to activate B lymphocytes. The absence of T lymphocyte cooperation also prevents a shift to

predominantly IgG antibody production, possibly because T lymphocyte activity may be needed to turn on IgG precursor cells to switch from IgM to IgG production (45).

When a second inoculation of killed Brucella abortus was given, there was a rapid production of antibody with peak levels noted by one week post-inoculation suggesting a typical secondary response. Although variability was noted at times among the levels of primary responses, secondary responses were usually similar indicating an equal state of recall preparation in each group. There was a tendency for the secondary responses to be lowered with increasing levels of antibody from the primary response present at inoculation. This indicated some antibody suppression of the immune response which will be discussed in more detail later.

In contrast to the response to Brucella, the primary responses to tetanus toxoid and HSA showed a more delayed onset, a slower increase in levels to more of a plateau than a peak and a subsequent slower decrease in titer. This pattern probably reflects both the nature of the antigens (soluble proteins) and their combination with an adjuvant, i.e., the use of aluminum hydroxide in the preparation of tetanus toxoid and bentonite with the HSA. The slower primary response to a soluble protein was noted when nonadjuvanted tetanus toxoid was administered to sheep (107). The first detectable antibody appeared at 9 days post-inoculation, and peak titers were exhibited 12 to 18 days post-inoculation.

With adjuvants typically more time is required for antibody formation, and antibody levels increase more slowly (49).

Several mechanisms have been proposed to explain the effects of an adjuvant on the immune response (2, 30, 36, 37, 156) including: 1) a protracted antigenic stimulus resulting from the slow release of antigen, 2) denaturation or precipitation (in the case of soluble proteins) of the antigen, 3) stimulation of macrophages to more effectively process the antigen, 4) the recruitment of reactive cells by a "trapping" mechanism whereby macrophages and T and B lymphocytes are brought together in increased numbers in lymphoid tissue draining a site of adjuvant inoculation, 5) stimulation of the proliferation of T lymphocytes and 6) stimulation of the proliferation and differentiation of B lymphocytes.

Different adjuvants may work by one or more of the above mechanisms. Aluminum hydroxide may cause the slow release of antigen from a depot at the site of injection. A smaller amount of antigen would, therefore, be available initially and only after a sufficient quantity of antigen contacted the involved immune system cells, would antibody become detectable. This mechanism may be involved in the late onset of detectable antibody production and slow rise of serum titer. Aluminum hydroxide also causes the precipitation of tetanus toxoid thus changing its structural nature to a more immunogenic one.

Bentonite absorbs soluble proteins onto its particulate structure (158) and may, therefore, change the structural nature of a soluble protein such as HSA. Bentonite is phagocytized by macrophages (156) and may affect their ability to present antigen to T lymphocytes or to process antigen.

Bentonite also causes "trapping" or sequestration of circulating lymphoid cells in lymphoid tissue as do several other adjuvants (37). Trapping refers to a shutting-off of the outflow of lymphocytes from lymphoid tissue; it brings macrophages and T and B lymphocytes into closer contact in larger numbers. It is interesting to note that particulate antigens also cause trapping, while soluble proteins do not, which may explain some of the difference in the pattern of primary response to each.

The stimulation of T and/or B lymphocytes may be due to some specific action of an adjuvant on the particular cell population involved (30). Some adjuvants affect only T lymphocytes, others affect only B lymphocytes, and some involve both. The effect of aluminum hydroxide and bentonite in this regard is unknown.

The primary response to killed EEE and WEE virus was detectable at one week post-inoculation but exhibited the same characteristics of the other responses to adjuvanted materials. The particulate nature of the viral antigen induced an earlier formation of antibody than would be seen

with soluble proteins, but the adjuvanted nature created a more prolonged response.

The prolonged stimulation of the immune response by an adjuvanted product would necessarily lead to features of a secondary response incorporated into the primary response, i.e., the shift in production of antibody to predominantly an IgG type. Thus, additional exposures to the adjuvanted immunogen would create an almost exclusive IgG type response leading to an increasingly greater predominance of this serum antibody. The slower decline in these antibody titers after peak levels were reached as compared to those to Brucella abortus may reflect a greater proportion of IgG in this antibody which is catabolized more slowly.

Responses to a 2nd inoculation (secondary response) were rapid and peak titers were attained in one to 2 weeks. Immune memory had been well-established, and only small amounts of antigen were required to trigger subsequent antibody production. There is some indication that antigen-antibody complexes may also initiate the trapping mechanism (37) which, combined with immunologic memory, would lead to a rapid, strong secondary response.

Hyperimmunization of the horse with toxoids, e.g., tetanus or diphtheria toxoid, leads to the production of large proportions of specific IgG(T) antibody (151, 152). Although not studied in this work, a similar response probably occurred to some extent in the foals immunized with tetanus toxoid.

The shift to IgG(T) production and memory during the response to the first immunization with the adjuvanted tetanus toxoid probably contributed significantly to the ability of a 2nd inoculation to produce a rapid, strong response.

The age of the foal at the time of administration of antigen had a demonstrable effect upon the primary response to some of the antigens used. The effect noted varied somewhat between antigens, but it was apparent that the ageing process played a similar role for the various responses to the antigens.

Some apparent maturation of the humoral antibody response occurred with increasing foal age. Such maturation led to the production of increasing levels of antibody during the primary response to Brucella abortus and shortened the post-inoculation interval required for peak antibody production. This activity occurred within the first 2 weeks following immunization. In this same response period the effect of maturation on antibody production to tetanus toxoid and human serum albumin was also noted, i.e., the shortening of the interval between inoculation and detectable antibody production. After this initial period there was an apparent "leveling" of response to stimulation; the maturation process during these first 2 weeks appeared to have progressed to a similar extent in all age groups. No differences in peak titers or the time required to attain them, therefore, could be discerned.

The effects of increasing age to raise peak titers to killed Brucella abortus and to shorten the time required to produce detectable antibody to tetanus toxoid and human serum albumin were similar in that their influence was noted primarily in the first 2 weeks subsequent to inoculation. This may suggest a similar underlying mechanism(s) operating on 2 fundamentally different types of antigen.

Several possibilities exist to explain the phenomena that ageing produces: 1) an increase in the number of cells capable of responding to antigen, 2) an increase in the ability to process antigen or 3) an increase in the ability to produce IgG. One, a combination, or all of these mechanisms may play a role.

An increase in the population of "antigen-sensitive" cells (B lymphocytes, bursa equivalent-derived lymphocytes) after birth has been advocated by some (78, 143) as the cause of an increase in antibody-forming potential during the first months of postnatal life. The mechanism responsible for the increase could be an adaptive one, i.e., repeated contacts with antigens (bacterial antigens, antigens from food), especially via the gut tract. These antigens have a variety of determinants, some which may invoke antibodies that cross-react with other antigens (143). Evidence for such a development has been acquired by raising pigs delivered by hysterectomy in a germ-free environment with a low-antigenic diet and subsequently exposing them to antigens (143). In such

pigs, the ability to produce antibody does not change quantitatively during the first 60 days after birth, while pigs raised under conventional conditions have a considerable increase in immunologic capacity during the first month.

Arguments against such a theory have been raised (126, 149) based on work in the fetal lamb and fetal monkey. In these animals the fetus gives a very competent adult-type response on the first introduction of some antigens which could indicate that an adult level of "antigen-sensitive" cells were already present (109). There appears to be a hierarchy of development of responses to antigens during fetal development with some strong antigens stimulating antibody production at various stages of fetal development, while weak antigens may not induce any antibody response, even for some time after birth (136).

Such a mechanism may be operational in the foal. It was noted during the experimentation that many mares carried low titers (1:20 to 1:40) of antibody that agglutinated Brucella abortus S19 cells, although they had no history of exposure to this agent. Similar findings in the horse have been reported by others (39, 80), and the antibody involved has been considered to be a nonspecific, cross-reacting type. Contact with agents that stimulate the production of this antibody could conceiveably enlarge the number of "antigen-sensitive" cells to Brucella abortus in the developing foal. By a similar mechanism an increased number of "antigen-

sensitive" cells to tetanus toxoid or human serum albumin would allow the earlier production of detectable levels of antibody during the primary response.

The possible increase in the ability to process antigen for antibody production during neonatal life suggests a change in the abilities of macrophages and/or T lymphocytes (thymus-dependent lymphocytes). Both of these cells appear to play an important role in humoral antibody responses (13) especially to thymus-dependent antigens such as soluble proteins.

Evidence of differences in macrophage function and morphology between very young and adult animals has been presented for several animal species (3, 130) and has been suggested as a possible source of delayed responsiveness to sheep red blood cells in newborn mice (4). Development of T lymphocyte function after birth has also been suggested as an event in postnatal immunologic maturation of mice (9). Gradual development of one or both of these cell types in neonatal foals may lead to an age-related increase in the ability to produce antibody.

The young animal's earliest antibodies formed under controlled antigenic stimulation have been found to be of an IgM type in most species (101). In the neonatal foal the IgM class has been shown to be the first whose levels increase due to autogenous production (76, 111). As development proceeds, the ability to produce IgG becomes

increasingly apparent. The addition of this second immunoglobulin class to antibody production would lead to increased antibody producing capabilities with ageing.

The delayed onset of IgG synthesis may by closely related to the first proposed mechanism, i.e., an increase in "antigen-sensitive" cells with ageing. A late maturation of IgG-producing cells would add to the population involved; evidence exists that the switch to increased IgG synthesis is an ontogenic development (95).

Although there was some age-related difference in the foal's ability to produce antibody during a primary response, considerable immunologic capacity existed from the earliest inoculation age, one week. Many of the reports dealing with other species presented previously in the review of literature have demonstrated a lack of similar ability in these animals at an equivalent age. The fact that the foal is born at a more advanced stage of physiological development than many other animals may be responsible to a large degree for the noted difference in immune capabilities (130).

The neonatal foal is, therefore, fully capable of responding to antigens similar to the ones used in this study from one week of age, providing no inhibiting influences such as passive serum antibody were present. No particular advantage would be gained by waiting until an older age to begin a foalhood vaccination program and, indeed, would leave the foal unprotected until such measures were taken.

Colostrum deprivation had generally little effect on the immune response to the antigens employed. Colostrum-deprived foals at one and 2 weeks of age had slightly greater primary responses to killed Brucella abortus S19, tetanus toxoid, and killed WEE and EEE virus than did CF foals, although this was not true of the response to human serum albumin. Four-week-old, colostrum-deprived foals also responded better to tetanus toxoid than did colostrum-fed foals. Responses to other antigens at 4 weeks of age and to all antigens in the other older groups exhibited no notable differences between colostrum-fed and colostrum-deprived groups.

A deficiency in humoral antibody responsiveness has been noted in neonatal, colostrum-deprived pigs (53, 119, 120). This same effect was not observed in neonatal calves (106, 128) and was not noted in this study.

The lack of responsive ability in the CD neonatal pigs was attributed to a lack of a factor normally supplied in the colostrum which participated in antibody production, i.e., small amounts of specific antibody (119). The reported requirement for the specific antibody was based upon Jerne's natural selection hypothesis of antibody formation (62, 63) which postulates that preformed antibody is needed for antibody formation. Mechanistically, antibody would interact with antigen aiding in the transport of antigen to antibody-forming cells.

Comparisons between this study and those with CD pigs (119, 120) are difficult. The CD pigs were obtained by hysterotomy, raised in a sterile environment and fed a diet of known composition. The CD foals in this study were born normally, raised in a normal environment and fed a diet of unknown composition. Such differences could easily introduce factors to the foals which would cause them to respond in a manner unlike that which might be seen if they were handled similar to the CD pigs.

The equine and porcine species have similar placental structures, i.e., they are both epitheliochorial in nature (14). It has been reported that some transplacental transmission of antibody may occur in pigs before birth (91), although this has not been conclusively determined. Such a situation could exist in the equine, especially since in several reports (61, 69, 117) some slight precolostral transmission of antitoxins was observed. If this is the case, small amounts of specific preformed antibody would already be present at birth, and colostrum would not be needed to supply them. The serological techniques used in this study did not demonstrate the presence of any detectable specific antibody before nursing nor at any subsequent pre-immunization time in CD foals and so cannot confirm such reports. It is possible, however, that specific preformed antibody was present in the foals below the level of detection by the procedures used.

There appeared to be a slightly greater responsive ability in the colostrum-deprived foals. This could have been due to a general immune system stimulation provided in the first week of life by the presence of clinical illness which was noted in all CD foals. Such a stimulation could act upon lymphoid tissue, especially cell types such as the macrophage, T lymphocyte, and/or B lymphocyte to hasten their maturation, especially at a young age. Colostrum-deprived foals did appear to have normal capacity to produce humoral antibody responses to a variety of antigens from as early as one week of age.

The failure of foals to receive colostrum led to severe problems with infectious diseases during the first week of age, especially with Escherichia coli septicemias. Similar problems have been seen in CD animals of other species (38, 55). Even with high levels of broad-spectrum antibiotics the foals exhibited signs of clinical illness (elevated body temperature, depression, anorexia) during the first few days of life. None of these problems were noted in colostrum-fed foals, and they required no antimicrobial therapy. The effects of the deprivation of colostrum gave dramatic evidence of the importance of colostrum as regards maintenance of good health and survivability in the young foal.

The presence of maternal antibody had a demonstrable effect on the primary immune response to all the antigens

used. Generally, the more maternal antibody present at the time of the first inoculation, the slower and weaker was the ensuing response.

The response to killed Brucella abortus demonstrated just that effect. While a peak response in seronegative, 10-week-old foals was noted at one week post-inoculation, the presence of maternal antibody delayed the noted peak response until the 3rd post-inoculation week. The presence of increasing amounts of maternal anti-Brucella antibody in the foals at the time of injection resulted in lower peak titers during the primary response.

The response to tetanus toxoid exhibited a lack of active antibody production following the primary immunization in 2 groups with high levels of maternal antibody present; titers continued to decrease in a linear fashion after inoculation. Some sensitization had apparently occurred, however, because a 2nd injection resulted in an increased titer one week later in both groups. (Primary responses to tetanus were not detectable until the 2nd week post-inoculation.) There was a suppression of the secondary response in these groups because they had noteably lower peak titers following a 2nd injection than did groups with lower maternal antibody levels. This was especially true of the group with the highest maternal antibody levels; a 2nd dose produced only a slight increase in titer. Maternal antibody, therefore,

demonstrated its capacity to inhibit a primary response and also a secondary response as previously reported (148).

Primary responses to human serum albumin and killed WEE and EEE virus all showed the same general effect. No apparent influence was noted on secondary responses except in the group with the highest levels of maternal anti-EEE antibody in which some decreased production was observed.

The ability of passive antibody to affect the primary and secondary response to its respective antigen is of considerable interest in the study of the normal regulatory mechanisms that limit the extent of these responses. Such mechanisms restrict the amount of antibody formed to an antigenic stimulus preventing the body from uncontrolled synthesis of unnecessary and potentially harmful levels, especially to nonmetabolizable antigens.

The post-inoculation suppression of measurable antibody formation in a primary response by the presence of pre-existing antibody has been observed to be highly specific for such diverse antigens as H-2 isoantigens in mice (85), sheep and chicken red blood cells (157) and picrylated bovine γ-globulin (12). Only maternal antibody to Brucella abortus would, therefore, affect the response to this antigen. Such specificity implies that interaction of passive antibody with the antigen is the first step in suppression since the only mechanism known at present by which an antibody molecule can recognize antigenic specificity is by combining with specific

antigen (144). An alternate mechanism suggests that specifically competent lymphoid cells have antigen-like structures on their surface, and that antibody interaction with such receptors can inhibit active antibody production (113). Such receptors have not been demonstrated.

Not all antigenic sites on a molecule have to be bound by antibody for suppression to occur (148). Less than half of the antigenic sites on a single molecule of diphtheria toxoid can be bound to antitoxin and cause suppression of the antibody response (145). Some inhibition of the primary response was noted in the face of low and even undetectable levels of antibody to Brucella abortus, tetanus toxoid and HSA, although probably only enough antibody to cover some fraction of the antigenic determinants was present.

The ability of passive antibody to interfere with sensitization or a secondary response is less effective than with a primary response (90, 112, 146), although it has been demonstrated (86, 87, 145). It is probable that less antigen is required for sensitization or priming for a secondary response than is required for an active primary response. Similarly, less antigen is required to elicit a secondary response. The effect of high levels of maternal anti-tetanus antibody, therefore, to prevent an observable primary response but not completely block sensitization may

have been due to a slight amount of antigen yet available for stimulation of immunocompetent cells.

The smaller dosage requirement for a detectable secondary response and the difficulty in inhibiting it are probably related to the increase in the average binding affinity that occurs after immunization as demonstrated by Eisen and Siskind (31). Such a change likely reflects similar changes in the cell population producing the antibody. These cells have specific cellular receptors which are considered to be antibody ("cell associated" antibody) identical to that they produce, and so, as there is a heterogeneity of serum antibody, such also exists for the antibody-forming cells (127, 148). It is theorized that antigen interacts with its complementary "cell associated" antibody during some step in the process of antibody formation. With a limited amount of antigen or a decreasing amount of antigen after immunization, only cells having "cell associated" antibody of the highest binding affinity can capture antigen and be stimulated (148). With increased receptor avidity the immune mechanism becomes less suppressible by serum antibody (unless it is of greater avidity). The anti-tetanus levels that were able to block a primary response were thus unable to completely block a secondary response, although they severely restricted it.

The combining of passive antibody to antigen may be able to block an immune response by rendering the antigenic determinants sterically unavailable to immune system cells (148). Alternatively, the combination may affect antigen metabolism, and the antigen may be more rapidly broken down and eliminated, or it may be removed from the circulation at a site that precludes significant antibody formation (148).

It is interesting that passive antibody produced a delayed but demonstrable primary response to the antigens studied here. The delay may have been due to the eventual availability of more antigen to the immune system to stimulate such an event. Some dissociation of antigen from antigen-antibody complexes in vivo has been demonstrated using diphtheria toxoid in rabbits and guinea pigs (145). This freeing of antigen from antibody may be a simple "disattachment", especially with antibody of low avidity, or may be due to metabolism of some of the antibody. Such "free" antigen then becomes available to the appropriate cells to initiate an antibody response, albeit weaker due to a less than optimal amount of antigen.

The site in the body of antigen-antibody interaction in suppression of the antibody response is yet unknown. One possible site is in the circulation, but this is not necessary because antibody given subsequent to the clearing

of antigen from the circulation is still effective in blocking a response (148). Inhibiting activity may, therefore, occur after the antigen has contacted immune system cells. Evidence has accumulated that suggests that there are at least 2 antigen-dependent steps in the progression of events from initial cell contact with antigen to production of antibody (16, 24). The first step may involve contact with an "antigen-reactive cell" which may be a macrophage or T lymphocyte. The 2nd contact may be with an "antigen-sensitive" cell which is a B lymphocyte and which has "cell associated" antibody on its surface. The first step may involve antigen processing and the production and/or release of an activator complexed with the antigen (16). This complex could then be introduced to the appropriate "antigen-sensitive" cell where the antigen attaches to the "cell-associated" antibody and the activator stimulates the cell to eventually produce antibody or to proliferate leading to production of memory cells and more antibody-forming cells. Memory cells would also require a subsequent antigen interaction to be transformed into antibody-producing cells. Lower quantities of antigen induce primarily memory cells and few antibody-forming cells, i.e., sensitization (16). The fact that sensitization occurred with tetanus toxoid indicates that at least the first and probably, to some extent, the 2nd antigen-dependent step had taken place with some memory cell production the primary result.

The relationship of maternal antibody to the active immune response also has much practical application. The effectiveness of an immunization in a young animal depends largely on the amount of passive antibody present at the time of injection and the ability of the antibody to suppress the immune response.

The level of maternal antibody acquired by the newborn is related closely to the serum titer of its dam at parturition as has been discussed previously. The antibody levels in the dam are a reflection of her past disease and immunization history, especially that during the period of time the foal was residing in utero. Such history is, therefore, very important in estimating the amount of antibody that the newborn foal would receive subsequent to nursing and antibody absorption. The half-life values calculated previously may be used to estimate an age at which the antibody levels should be low enough so they interfere only minimally with active immunization. Some antibody may be present and inhibit to some extent a primary response and, yet a 2nd immunization will produce a normal anamnestic response.

A more desirable method of determining antibody levels in the foal would be to measure serum antibody levels in the dam at parturition or in the foal at 72 hours of age. From this information calculations using the antibody half-life values would give an estimate of passive titers at various

ages. From the data on the effect of various titers on the responses to different antigens presented in this report, one should be able to determine quite accurately an immunization age for each antigen. Such a system called a nomograph has been devised for use in pup immunization against canine distemper virus (41). The relatively high value of many newborn foals would make this a feasible and valuable tool to the veterinary practitioner and horseowner.

SUMMARY

The ability of the neonatal foal to produce humoral antibody was studied in relation to the effects of colostrum-deprivation, age at immunization and the presence or absence of maternal antibody at immunization. Also investigated were the transfer of maternal antibody to the newborn foal and its subsequent rate of elimination.

Pregnant Shetland pony and Quarter Horse mares were immunized at various stages of gestation with tetanus toxoid-killed Western and Eastern equine encephalomyelitis virus in aluminum hydroxide or killed Brucella abortus S19 and human serum albumin in bentonite. Colostrum-fed foals and foals deprived of colostrum for 72 hours after birth were inoculated with these same antigens at one, 2, 4, 8, 10, or 12 weeks of age and a 2nd immunization was given 4 to 11 weeks later. Blood samples were collected before nursing, at 24 and 72 hours and one week of age and weekly thereafter for 15-17 weeks.

Colostrum-deprived and colostrum-fed foals responded with equal ability to the antigens studied. There was some enhancement of the primary response to the antigens used as age increased, but secondary responses were similar, and the foals were able to respond well to the antigens employed from as early as one week of age. Depending on the particular levels at the time of immunization, maternal antibody

exhibited an ability to suppress or block a primary response, inhibit sensitization and depress the secondary response.

Maternal antibody was not present in detectable levels in the foal before nursing. After nursing the serum levels increased rapidly to approximate those of the dam at 24 and 72 hours of age, although there was some variation in the comparative serum levels between antibody types. A heterogeneity of elimination rates of the various antibodies was noted, and with some antibodies an increase in catabolism was noted as initial levels increased. Average half-life values of specific antibodies were: 1) B. abortus = 7.6 days, 2) tetanus toxoid = 12.0 days, 3) WEE = 10.8 days, and 4) HSA = 18.7 days.

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