ultraviolet-induced erythema in albino guinea pigs. However, even in this type of assay, treatment with the anti-inflammatory agent must precede the application of the ultraviolet light and furthermore, well-known anti-inflammatory steroids such as cortisone and hydrocortisone were ineffective(3).

Our method, based on reversal of endotoxin-induced lung inflammation in mice, has proven to be a reliable and sensitive assay for testing the anti-inflammatory properties of non-steroid as well as steroid agents. Some of the characteristics which make this anti-inflammatory assay more advantageous than other methods described in the literature have been mentioned previously(1). The method has been standardized and the minimum effective doses for cortisone acetate, aspirin, and phenylbutazone have been determined.

The minimum effective dose of cortisone acetate is comparable to that required to inhibit significantly the formation of the cotton pellet-induced granuloma. The minimum effective dose of phenylbutazone is comparable to that required to produce a significant delay in the development of ultraviolet erythema, whereas that of aspirin is considerably lower. It is noteworthy to mention that by using methods other than the ultraviolet erythema or that described in this paper oral doses up to 40 times as much of aspirin and phenylbutazone are required to produce a significant anti-inflammatory effect.

Summary. An improved anti-inflammatory assay based on the reversal of endotoxin-induced lung inflammation in mice is described. Changes in design of the original apparatus have: a) increased the number of animals which can be exposed to endotoxin; b) decreased the amount of endotoxin needed for maximum inflammatory response. A direct correlation exists between concentration of endotoxin and the resulting increase in weight of the lung and degree of histopathological changes. The advantages of this method for testing anti-inflammatory activity of steroid as well as non-steroid compounds are discussed in comparison with other methods described in the literature.

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Use of Primary Human Cell Cultures in Titering Certain Human Adenoviruses for Infectivity.* (29491)

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There is a need for further development of sensitive methods for measuring the infectivity of the human adenoviruses, particularly for assays which may be used for all types. There have been frequent reports of titrations employing relatively short incubation times, although it is generally known that long periods of incubation may be required for demonstration of minute amounts of adenovirus either present in a "masked" form in the explanted tissues of the natural host or in tissue cultures as a result of inoculation.

During studies of mixed infections in tis-

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sue culture in this laboratory, it was noted that in titrations of adenoviruses types 3 and 12 in stable human cell lines, clearcut endpoints could not be obtained when 14-day periods were employed. Therefore, we undertook to devise a more sensitive procedure for detection of small amounts of infectious adenovirus. This communication reports our experiences with several cell lines and 2 methods of titering these viruses. A conventional roller tube titration in primary human amnion cells, grown and maintained in the presence of fetal bovine serum, appeared to be a highly sensitive procedure for demonstrating the presence of small amounts of adenovirus.

Materials and methods. A. *Procedures used in tube titrations and for preparation of virus stocks. Cell cultures.* HeLa and KB cells, culturably free of PPLO, were initiated in Eagle's minimal essential medium (MEM) containing 10% inactivated bovine serum (BS) and were allowed to form confluent monolayers. The monolayers were washed twice with Hanks' balanced salt solution (BSS) before being placed in Eagle's MEM with 5% fetal bovine serum (FBS) or 10% chicken serum for maintenance. Growth *in vitro* of primary human amnion cells (PHuA) was initiated in Eagle's MEM with 10% pooled inactivated human serum (HuS) and 10% inactivated BS. After approximately 4-7 days, PHuA cultures were washed twice with Hanks' BSS and placed in Eagle's MEM with 10% inactivated horse serum (HoS) or 5-10% FBS. Human fibroblastic cells (HuEF) which were derived throughout by trypsinization from the skin of fetuses were implanted in Eagle's MEM containing 10% FBS and maintained in similar medium. All cultures were refed routinely at twice weekly intervals.

Virus pools. Human type 3 and type 12 adenoviruses, obtained from Dr. Robert J. Huebner, Nat. Inst. of Health, were used to establish infections in HeLa, KB, primary human amnion and primary human fibroblastic cell tissue cultures in 8-oz. prescription bottles. When full development of cytopathogenic changes (CPC) was evident, harvests were made by scraping the cells from the surface of bottles with a rubber policeman into the maintenance medium. Cells and fluids together were frozen and thawed through 4 cycles, pooled and, after thorough pipetting, divided into 1 ml and 2 ml aliquots and stored at —20°C.

Virus titrations. Aliquots of the various virus stocks were removed from the freezer, thawed at 35°C and thoroughly mixed by pipetting. Log_{10} dilutions were made in medium sufficient to allow refeeding of all tube cultures to be infected, and 1 ml amounts were then transferred to the cultures. These inoculation fluids were not removed until the first additional feeding 48 to 72 hours later. All tube cultures were observed every 3-4 days for development of CPC.

At the end of the period of direct observation for CPC, tube cultures failing to show CPC of more than 50% degeneration and those which were "negative" were subjected to 4 cycles of freeze-thaw for cell disruption and then tested (at a dilution not greater than 1:4) for presence of virus by subculture into fresh tissue culture tubes. Long periods again were allowed for full development of CPC. Any 2 such subcultures (originating from one tube of the primary titration) which showed 50% or greater CPC were recorded as positive evidence of the presence of virus in the original culture. This constituted the "indirect assay procedure" used as a complement to the determination of titer by direct observations of CPC in the original titration. In each titration the cultures containing a given dilution of virus were fed with a separate pipette but generally at the same time and in the same area as other dilutions of the titration. Uninfected control cultures were carried throughout all experiments and subcultured. These failed to show any evidence of specific CPC or other changes interpreted as virus contamination.

All endpoints were based upon development of at least 50% CPC in a tube culture and calculated by the method of Reed and Muench.

B. *Titrations by fluorescent microscopy.* HeLa cells were grown in Eagle's MEM with 10% BS in 8-oz prescription bottles. These monolayers were trypsinized and rendered monodisperse in suspension for passage onto

TABLE I. Summary of Virus Titrations in Tube Cultures of Primary Human Cells.

Culture type	Virus lot and host cell	Adenovirus Type 3 "Titer" at 12-14 days (A)	"Titer" at 30 days (B)	"Titer" at terminal reading by direct observation* (C)	Total days in initial culture† (D)	Fold increase in "titer" from first calculation (E)
1. PHuA	Lot 10—KB	$\geqq 10^{-6.5}$		$\geqq 10^{-6.5}$	12	—
2. "	Lot A—PHuA	$\geqq 10^{-6.5}$		$\geqq 10^{-6.5}$	12	—
3. "	Lot 2—HuEF	$10^{-5.5}$		$\geqq 10^{-6.5}$	24	10
4. HuEF	Lot 1—HuEF	$10^{-2.5}$	$10^{-5.5}$	$\geqq 10^{-6.25}$	34	$\geqq$10,000
5. "	Lot 2—HuEF	$10^{-2.5}$	$10^{-5.5}$	$\geqq 10^{-6.5}$	45	$\geqq$10,000
6. "	Lot 4—HeLa	$10^{-2.5}$	$10^{-5.5}$	$\geqq 10^{-6.5}$	38	$\geqq$10,000
7. "	Lot 4—HeLa	$10^{-4.5}$	$10^{-5.2}$	$\geqq 10^{-7.33}$	35	1,000
8. PHuA	Lot 4—HeLa	$10^{-7.5}$	$10^{-8.5}$	$10^{-8.5}$	23	10

$\geqq$ At least or greater than, based on positive cultures in last dilution used.

* See text.

† This does not include sub-inoculation time.

9 × 22 mm sterile glass coverslips. At time of passage cells were washed twice in Hanks' BSS with antibiotics and resuspended in Eagle's MEM with 10% FBS at a population density of 15,000 cells/1 ml. One-tenth ml amounts of the suspension were pipetted directly onto the slips in sterile glass Petri dishes where droplets were formed which did not "flow." Incubation for 16 hours at 37°C in a sealed plastic box in presence of Pardee buffer(1) allowed growth in a monolayer over an area of approximately 1 sq cm. The slip cultures were then washed and placed in flat bottom tubes with 1 ml of maintenance fluids containing 5% FBS. Refeeding was not necessary to maintenance of the cell monolayers in good condition for 10 days.

Fluorescein-tagged antibody (FTA) preparations. Rabbits were injected 4 times intravenously with 5 ml of type 3 adenovirus tissue-culture fluids (prepared in PHuA cell cultures) which had been frozen and thawed 4 times for cell disruption. Serum was collected 10 days after the last injection and was found (in a dilution of 1:640) to neutralize 5 logs of homotypic virus. Guinea pigs were used as a source of antibody for production of antiserum to type 12 virus. Two groups of animals were used; one group received concentrated adenovirus intramuscularly twice, the second dose being given with Freund's adjuvant one week after the first dose. The other group received an initial dose of concentrated virus with 4% sodium alginate and a second dose of concentrated virus one week later. Sera were harvested at approximately 21 days after the first injection and those found to have CF antibody in a dilution of 1:1024 or greater were combined for use. Antibody globulin conjugates were prepared by a standard method(2) and then separated through a DEAE column(3). The specificity of the fractions thus collected was analyzed using infected and uninfected cells and observed in the fluorescence microscope in the blind. Only those fractions inducing intense specific intranuclear fluorescence and a very low degree of non-specific cytoplasmic fluorescence were used in titration experiments.

The slip cultures used for titrations were removed from tubes, washed in Hanks' BSS, fixed in absolute ethanol, washed, and then stained with the conjugate. After a second wash the slips were mounted in a polyvinyl alcohol (Elvanol) and observed for fluorescence under a Leitz Ortholux microscope with a fluorescent attachment. All cultures were searched thoroughly by one of us (JDC) at a magnification of 125× and then at 660× for final determination of positive or negative results. A positive slide was determined to be one that contained at least 2 cells with aggregates of intensely fluorescent antigen inside the nuclear membrane. All infected and uninfected control cultures were coded and the readings were made without knowledge as to identity of preparation.

Results. A. *Tube titrations in primary cell lines. Type 3 virus.* Fifteen titrations of 6

TABLE II. Summary of Virus Titrations in Tube Cultures of Primary Human Cells.

Culture type	Virus lot and host cell	Adenovirus Type 12 "Titer" at 12-14 days (A)	"Titer" at 30 days (B)	"Titer" at terminal reading by direct observation* (C)	Total days in initial culture† (D)	Fold increase in "titer" from first calculation (E)
1. HuEF	Lot A—HeLa	$10^{-2.5}$	$10^{-3.5}$	$\geqq 10^{-5.24}$	43	$\geqq$ 1,000
2. "	Lot A—HeLa	$10^{-1.5}$	$10^{-2.5}$	$\geqq 10^{-5.24}$	59	$\geqq$10,000
3. "	Lot A—KB	$10^{-2.5}$	$10^{-4.5}$	$10^{-5.5}$	62	10,000
4. "	Lot A—HeLa	$10^{-1.5}$	$10^{-3.2}$	$10^{-5.5}$	60	1,000
5. PHuA	Lot A—HeLa	$10^{-4.0}$	$10^{-5.6}$	$10^{-5.5}$	37	30

$\geqq$ At least or greater than, based on positive cultures in last dilution used.

* See text.

† This does not include sub-inoculation time.

lots of type 3 virus were carried out over incubation periods ranging from 12 to 45 days. Table I summarizes the results of representative titrations of certain stocks in the 2 types of primary cell cultures. It may be seen that there are comparisons of 2 separate lots (Lot 2-HuEF and Lot 4-HeLa) in the 2 cell types. In comparative titrations of the Lot 2-HuEF (Lines 3 & 5), a "titer" of $\geqq 10^{-6.5}$ was reached in amnion cells in less than 24 days, based upon >50% CPC in tubes of the limiting dilution, whereas in the fibroblastic cultures, a "titer" of $<10^{-5.5}$ had developed in a similar period. All of the titrations carried out in amnion cells up to this point (Lines 1, 2 & 3) had been in the presence of 10% HoS used in the maintenance medium. Since equine serum is known to be inhibitory to replication of adenoviruses(4) and for strict comparisons with the fibroblastic cell cultures maintained in medium with 10% FBS, additional comparisons were made of the Lot 4 virus preparation (Lines 6, 7 & 8) using 10% FBS in maintenance fluids of both cell types. When comparisons were drawn at 12-14 days after inoculation (column C), the differences in development of CPC in the 2 cell types were accentuated, a "titer" of $10^{-7.5}$ in the amnion cells and $10^{-2.5}$ to $10^{-4.5}$ in the fibroblastic cells. The overall results of all the titrations, compared for "titer" at 12-14 days, were similar to the actual comparisons. The results indicated also that prolongation of cultivation (over the periods indicated) in either cell line invariably resulted in observation of viral activity in tubes of higher dilutions, except in the last titrations in PHuA cells, in which limiting dilutions used were 10^{-10}. An additional titration carried out with the Lot 4 virus in amnion cells with 10% FBS has confirmed the "titer" of $10^{-8.5}$ per 0.1 ml inoculum. A period of incubation satisfactory for full development of CPC in titration of the type 3 virus in PHuA cells with FBS seemed to be approximately 25-30 days. Titrations in the fibroblastic cells required periods longer on the average by 10-15 days.

Type 12 virus. In Table II results are tabulated of titrations of 2 lots of adenovirus type 12. The findings indicate a very slow rate of development of CPC for adenovirus type 12. This may be related in part to the fact that the infectivity "titer" here was lower than that of the adenovirus type 3 stocks. It is obvious that prolonged periods of cultivation were necessary to allow development of CPC in higher dilutions. Whereas latent periods for the 10^{-1} dilutions were 3-5 days, as much as 48 days elapsed at 10^{-5} dilutions in HuEF cultures before viral CPC became apparent. A selected stock (Lot A) was titrated also in PHuA cells maintained in 5% FBS and the $10^{-5.5}$ per 0.1 ml endpoint was confirmed (Line 5). The "titer" at 12-14 days was significantly higher than in HuEF cells. Comparative titrations of this virus stock and another one of higher "titer" were also carried out in HoS. The HoS was found to be strongly inhibitory and this effect was particularly evident during the shorter period of observation (12-14 days). As with the

TABLE III. Summary of Titrations of Adenoviruses Types 3 and 12 in Established and Primary Human Tissue Cultures.

Virus	KB		HeLa			Primary human amnion cells
	Direct observations*	Indirect assay†	Direct observations*	Indirect assay†	FTA‡	Direct observation
Adenovirus Type 3§	$10^{-4.3}$	$10^{-5.5}$	$10^{-4.5}$	$10^{-6.5}$	$10^{-6.5}$	$10^{-8.5}$
Adenovirus Type 12‖	$10^{-1.5}$	$10^{-3.5}$	$10^{-2.5}$	$10^{-4.5}$	$10^{-3.5}$	$10^{-5.5}$

Infectivity endpoints

* Based on development of CPC with 50% or greater cell involvement in 12 to 14 days after inoculation.

† Based on test for virus in higher dilutions displaying minimal or no CPC, by passage into fresh cultures (see text).

‡ See text. § Lot 4—HeLa (Table I). ‖ Lot A—HeLa (Table II).

type 3 virus, 25-30 days in PHuA cells with FBS seemed to be sufficient for full development of CPC in all dilutions.

B. *Tube titrations in established cell lines.* The selected lots of type 3 and type 12 viruses were further assayed for infectivity as measured by titrations carried out in the stable cell lines, KB and HeLa. Table III summarizes the results of these titrations based upon direct observation and upon the indirect assay procedure and compares the results with "titers" reached in the primary cell cultures. The relative inefficiency of titration in the two established cell lines is apparent, particularly when based upon direct observations of CPC over 12-14 day periods. After this period, non-specific cell degeneration generally became evident and tended to obscure viral CPC. Demonstration (by the indirect assay procedure) of "masked" virus present in tubes inoculated with the higher virus dilutions was common in the established cell cultures; whereas, after prolonged incubation periods in primary human cell strains, 99% of tube cultures showed no augmentation of "titer" by the indirect method. Type 3 virus used in these titrations induced cell clumping and greater generalized cell destruction than type 12 virus, which tended to produce monocellular degenerative effects.

Type 12 virus was harvested from certain terminal dilution HuEF cultures (10^{-5}) and passaged once in HuEF cells grown and maintained in medium with 10% FBS. This stock has been shown to have an infectivity endpoint of $\geqq 10^{-7.5}/0.1$ ml and to induce tumors in hamsters with a high level of efficiency.

Since fetal bovine serum was used in some of the titrations, comparisons were made of FBS and chicken serum for determination of presence of viral inhibitor(4). The FBS was found to be equally as efficient in allowing rapid development of CPC as chicken serum and in addition, seemed to offer greater sustenance to the established cell cultures (KB and HeLa) such that infectivity endpoints reached over the longer periods were actually higher than those reached in cultures when maintained in chicken serum. It was concluded, therefore, that FBS did not inhibit the growth of adenovirus in these experiments.

C. *Titrations by fluorescence microscopy (FTA).* Fluorescence microscopy was employed in an attempt to shorten the period of time necessary to demonstrate cellular infections in a titration procedure. If only a few cells were infected by an extremely dilute inoculum, it was theorized that the new antigens occurring in a single cell could be detected in the nucleus by fluorescence microscopy if these antigens would react with the antibody made to the complete virus. Seven such titrations of the type 3 virus and 5 titrations of the type 12 virus were performed. Representative titrations are given in Tables IV and V. For type 3 virus the endpoints reached in 5-10 days were equal to those reached by the indirect assay procedure in roller tubes in 28 days. Intranuclear fluorescence of the type 12 virus required a longer period to appear and this is reflected in the results where "titers" after 7-10 days did not equal those by indirect assay. These "titers" were, nevertheless, higher than those

TABLE IV. Summary of Virus Titrations* by Fluorescent Microscopy in HeLa Cells.

Adenovirus Type 3				
Titration	Days of growth on slip before inoculation	Medium & serum† for cell maintenance for infection	Days after infection at time of stain	Titer‡
3	72 hr	EMEM 5% FBS	4	$10^{-5.5}$
5	96 "	*Idem*	5	$10^{-5.5}$
6	48 "	"	10	$10^{-6.5}$

* All titrations are of Lot 4—HeLa (Table I).
† EMEM 5% FBS = Eagle's minimal essential medium with 5% fetal bovine serum.
‡ Calculated by method of Reed and Muench, per 0.1 ml inoculum.

TABLE V. Summary of Virus Titrations* by Fluorescent Microscopy in HeLa Cells.

Adenovirus Type 12				
Titration	Days of growth on slip before inoculation	Medium & serum† for cell maintenance for infection	Days after infection at time of stain	Titer‡
1	48 hr	EMEM 5% FBS	7	$10^{-3.5}$
2	48 "	*Idem*	10	$10^{-3.5}$
3	48 "	"	10	$10^{-3.5}$

* All titrations are of Lot A—HeLa (Table II).
† EMEM 5% FBS = Eagle's minimal essential medium with 5% fetal bovine serum.
‡ Calculated by method of Reed and Muench, per 0.1 ml inoculum.

found by direct CPC in HeLa cells (Table III).

Discussion. These data suggest that time is a factor in measurement of infectivity of human adenoviruses of the types studied. Other types probably also require prolonged incubation periods for measurement of infectivity when based upon direct observations of viral CPC(6-12). To some extent, apparently, periods as long as 28 days may be required for plaque titrations of types 4 and 5(13). Isolation of adenoviruses from human materials also is dependent upon time of incubation and types of cell cultures used (14,15).

Some factors relating to the interaction of the adenoviruses with host cells that have been observed to play a role in development of grossly observable cell damage (cytopathogenic changes) are virus input multiplicities (13), concentration of a "cytotoxin"(6), variability in susceptibility from cell to cell (in a single culture)(10,13), slow release of intracellular virus(6). Cell environmental factors also have been noted to have an effect upon virus growth(17-19).

The results of this study indicate that approximately 100 times greater amounts of virus were required to induce delayed CPC in KB or HeLa cells than to initiate an infection in these cultures, as proven by blind passage (the indirect assay procedure). Thus, when at 12-14 days after inoculation, CPC endpoints were no greater than $10^{-4.5}$, presence of virus could be detected at $10^{-6.5}$ by indirect assay.

Liminal dilutions of virus, regardless of cell line used, required prolonged periods to induce recognizable virus infections when measured by development of CPC. However, by FTA staining, "new" antigens could be detected in HeLa cells as early as 4 days after infection with multiplicities of 0.1-0.2 under which conditions no evidence of CPC was observed over a relatively prolonged period of time (12-14 days).

It had been hoped, since endpoints depended (in the FTA method) upon as few as 2 infected cells per slip culture, that over a period equal to several replication times of the virus, small amounts of virus present in dilutions higher than those in which positive cells were found, might have been demonstrated. That this was not the case may have depended to some degree upon the presence of protein inhibitor derived from bovine serum in the growth medium of the HeLa cell cultures although the cultures had been

washed twice and placed in FBS before inoculation. It is known that protein present as a constituent of culture fluids may become a part of the antigenic composition of the cell(5) and possibly impart resistance after the source protein has been removed from the cell environment. On the other hand, contact by chance, of the few infectious virus particles (in a high dilution inoculum) with insusceptible cells of a heterogeneous cell population may also explain the results(10, 13).

Whereas the fibroblastic cells allowed observable endpoints to develop as a response to small amounts of virus, very prolonged intervals were necessary before CPC began and was "complete" (>50%). A greater sensitivity to virus growth was found with amnion cells and equivalent endpoints were reached over shorter periods of cultivation, particularly for type 3 virus but also for type 12. Thus CPC endpoints (by direct observation; without blind passage) at 14 days were 30× to 100,000× greater in amnion cells than in the fibroblastic cells and 30× to 1000× those in established cells. At 30 days CPC endpoints in amnion were 100× to 1000× greater than in the fibroblastic cells and 10× to 100× greater than those obtained in established cells by the indirect assay procedure (blind passage).

The prolonged periods of cultivation necessary for demonstration of small amounts of virus may be likened to the phenomenon of "masked" virus in the tissue cultures of explanted human adenoid fragments as demonstrated by Rowe(8). Schlesinger(16) has observed that of 2×10^3-2×10^5 adenoidal cells (derived from surgically removed tissues) explanted in primary tissue cultures, one cell is likely to be infected with adenovirus. Arbitrarily, one might postulate that under conditions where very small numbers of virus particles infect a small number of cells within a large cell population, "masking" seems likely to occur.

Failure of small amounts of virus to induce overt cytopathogenic changes after incubation with potentially susceptible cells over periods equal to many replication cycles, must be to some degree the result of "natural resistance" of uninfected cells to spread of virus infection. This is more significant where extensive cellular infections must be established by the progeny from one or a very limited number of primary infectious centers. Since cell-to-cell spread is a prominent part of an adenovirus infection and since adenovirus infections result naturally in low extracellular virus yields, it is easy to believe that a single infected cell or a few infected cells may not result in extensive CPC except after long periods. As infected cells age, increased permeability and disruption may, by release of intracellular virus, then favor further virus infections. Effectual concentrations of cytotoxin (probably not present in the fluids of tissue cultures containing a single infected cell or a very limited number of infected cells) may also influence the course of infection; certainly, adenovirus "toxin" induces, to some degree, those changes interpreted as CPC, after infection with certain types. The manner in which such factors influence adenovirus replication under the wide-ranging conditions of a titration procedure, where cell population density and metabolic conditions particularly, vary considerably, is not known.

Others have indicated that fibroblastic cell cultures derived from kidneys of human fetuses and mixed epithelial-fibroblastic cell cultures derived from surgically removed human thyroid glands are highly efficient in detecting infectious adenovirus from clinical materials or in a titration procedure(14,20). Further experiments are being carried out in this laboratory to compare other systems, including plaque assay, to the procedure described herein.

Summary. Adequate measurement of infectivity of human adenovirus types 3 and 12 was found to depend upon time of incubation, and upon the type of *in vitro* cell culture system employed. Primary human amnion cells were found to be superior to human fibroblastic cells (derived from skin of fetuses) and to two established cell lines (HeLa and KB) for infectivity titrations of type 3 and type 12 viruses.

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Growth and Morphology of Mouse Lymphoma Cells in Diffusion Chambers.* (29492)

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Algire *et al*(1) demonstrated that mouse tumor allografts could survive in non-immune and immune hosts when placed in diffusion chambers that presumably allowed passage of fluid and not of cells through their membranes. These authors postulated that the host cells carrying the capacity to reject the allograft could not pass the membrane, gain access to the donor cells and destroy them. Shelton and Rice(2) have shown that transmitted lines of mouse ascites lymphoma cells could remain viable, *i.e.,* retain their ability to produce tumor, when left in diffusion chambers for as long as 10 months in allogeneic hosts. Lieberman(3) has described studies in which normal thymus from AKR and C57 black mice was cultivated intraperitoneally in diffusion chambers in isogeneic and allogeneic hosts. Viable cells representing the reticular-epithelial elements of the thymus were found to persist for as long as 8-10 months. Chambers containing leukemic thymus fragments showed cells which retained their distinctive appearance and ability to produce a disseminated lymphoma for as long as nine months. In all of these studies it was assumed that growth of leukemic and normal allogeneic tissues occurred because the chambers were impermeable to host cells which might cause allograft rejection. Conversely then, it has been assumed that the cells in the chamber could not escape, and lack of tumor growth in isogeneic hosts which were used in the last two of the above mentioned studies would be evidence for this.

More recent studies of Osoba and Miller (4) and Levey *et al*(5) have shown that normal thymus fragments placed in "cell tight" diffusion chambers of 0.30 and 0.45 μ mean pore size respectively restore immunologic function and lymphoid tissue to mice thymectomized when newborn. They have observed persistence only of the epithelial reticular elements of the thymus tissue within

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