corn oil or linolenic acid in linseed oil. The time factor in these studies is interesting in view of the report of Edwards and Marion (12) indicating that large amounts of eicosatrienoic acid (20:3) first begin to appear in the chick liver lipids of EFA-deficient chicks after they have been fed the fat-free diet for 3 weeks from time of hatch. The present studies indicate that susceptibility to *E. coli* challenge follows somewhat the same pattern. There have gradually evolved from research two general theories of function of essential fatty acids, *viz.*, (A) as precursors of the hormones prostaglandins (review 13), and (B) as integral parts of phospholipids in various coenzymes and lipoproteins of subcellular structure(14).

Unfortunately, the studies reported here do not prove which way the fats tested are functioning in offering protection against the *E. coli* infection. It might be reasoned that even the small amount of linoleic acid in coconut oil should be able to supply sufficient amounts of linoleic acid for the formation of 11a, 15-dihydroxy-9 keto prosta-5, 13 dienoic acid (PGE_2) the most active prostaglandins so far tested(15). However, this same line of reasoning acknowledges that the chicks fed the basal diet or the basal diet plus coconut oil would have very low total levels of extremely long chain polyunsaturated fatty acids that might be available as precursors of prostaglandins, while those fed corn oil, linseed oil or menhaden oil would have large amounts of long chain polyunsaturated fatty acids available. Some information(15) indicates that prostaglandins that originate from fatty acids possessing low essential fatty acid activity have some biological activity although it is much lower than the activity of prostaglandin (PGE_2) that originates from arachidonic acid. Further studies using purified fatty acid preparations are in progress to obtain information as to the mode of action of these fats in protecting the chickens from the *E. coli* infection.

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Antigen Production in Hyperoxic Organ Cultures.* (31095)

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Many current approaches aimed at establishing allograft tolerance in the adult depend upon administration of large amounts of donor antigen(1,2,3,4). Using inbred strains of mice, practically unlimited amounts of strain-specific tissue are available, and have been

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TABLE I

	Days of culture					
	1	2	3	4	5	7
No. of cells/g tissue	8,000,000	7,400,000	7,001,000	5,700,000	3,400,000	3,200,000
Viability	92%	84%	86%	78%	76%	72%

successfully used to induce specific tolerance. In the out-bred higher mammals, however, individual-specific tissue is required as a source of effective antigen and the supply of expendable donor tissue is necessarily limited. Attempts to increase the effectiveness of the available antigenic material have been described, both by extraction and solubilization(4,5) or by concurrent administration of antimetabolites(6,7). Another approach exists, namely to increase *de facto* the amount of usable antigenic material. A technique for accomplishing this in hyperbarically oxygenated organ cultures will be described.

Methods. Tissues. Lymph nodes and spleens from out-bred New Zealand white rabbits and 20-30 lb mongrel dogs were removed under nembutal anesthesia, using aseptic technique. The spleens were made to contract and emptied of excess blood by injection of 1 mg epinephrine in 10 cc normal saline into the splenic arteries. The tissues were washed in balanced salt solution (Tis-u-Sol, Travenol Laboratories, Chicago, Ill.) at 4°C, weighed, and cut into 3-4 mm thick slices.

Culture methods. Eagle's medium (Hyland Laboratories, Glendale, Calif.) containing glutamine, penicillin, streptomycin and 15% fetal calf serum was used. One-half cc of phytohemagglutinin P (PHA Difco) was added to each 100 cc when this agent was employed as a mitogen. The tissue slices were placed on 9.5 cm diameter stainless steel screens supported on 12 mm high steel rings. The rings were placed into the Petri dishes and the culture medium added until it reached the top of the screen, wetting approximately the lower half of the tissue slices. The number of slices was adjusted in each dish so as to result in 0.5-1 g of tissue per 10 cc of medium.

The dishes then were placed into a 24″ × 20″ × 6″ aluminum presure chamber. It was pressurized to 30 p.s.i.g. with 98% O_2 and 2% CO_2 four times, decompressing to ambient pressure each time. Thus nitrogen was washed out of both the chamber and the dishes contained within it (O_2 concentration in the latter was over 96% in 14 tests using this method). Finally, the chamber was pressurized to 45 p.s.i.g. and placed into an incubator at 34°C.

The chamber was decompressed every 24 hours, the slices and screens washed with balanced salt solution and the slices transferred to new screens inverting (turning) them in the process. The cells adhering to the bottom of the Petri dish were flushed away with a stream of balanced salt solution. The cells were counted in a Coulter counter after being trypsinized for 5 minutes in order to break up clumps. Viability was determined using eosin exclusion(8) .

Skin grafts were performed and evaluated by methods previously described(9,10).

Results. Lymph nodes. Daily yields from canine nodes are shown in Table I.

The antigenicity of these cultured cells was compared with that of parent cells. The dose of fresh lymph node cells required to induce accelerated skin allograft rejection by subcutaneous injection 14 days prior to transplantation was determined by the use of graduated doses of cells in 25-30 lb dogs.

No. of cells injected	Rejection (days)	MST (days)
1×10^5	9, 8, 11, 11, 8	9.4 ± 1.4
1×10^6	8, 11, 10, 7, 10	9.2 ± 1.5
1×10^7	7, 10, 8, 7, 9	8.2 ± 1.2
3×10^7	6, 6, 5, 3, 7	5.4 ± 1.4
6×10^7	5, 6, 5, 7, 4	5.4 ± 1.0

The same studies were repeated using cultured lymph node cells. The results suggest that antigenicity, at least in terms of ability to induce allograft rejection, is unaltered.

No. of cells injected	Rejection (days)	MST (days)
1×10^6	11, 8, 9, 9, 8	9.0 ± 1.1
1×10^7	8, 9, 8, 11, 7	8.6 ± 1.4
3×10^7	7, 6, 4, 8, 4	5.8 ± 1.6
6×10^7	6, 4, 4, 6, 5	5.0 ± .9

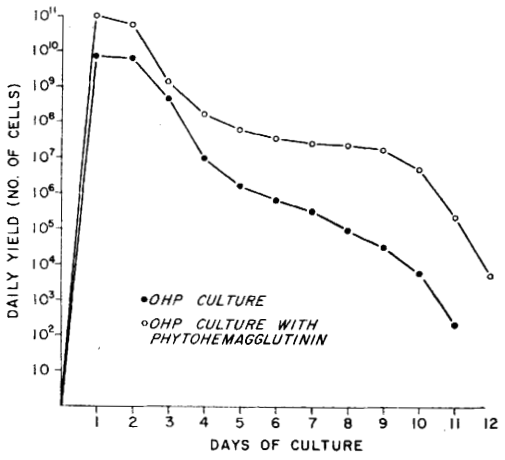

FIG. 1. Cell yield from hyperoxic culture of dog spleen slices.

Spleen cells. The yield of eleven 20-25 g canine spleens (wet weight after contraction with epinephrine) over 12-day culture periods was determined. An average of 2.1×10^{10} cells (range $0.9\text{-}3.5 \times 10^{10}$) were obtained with overall viability of 87%. Eighty to 85% of the cells were of the lymphocytic-monocytic series. Half of the remainder were other leukocytes (with a few myelocytes and blast forms) and half appeared to be reticuloendothelial cells.

Ten similar studies were then carried out adding 0.05 ml phytohemagglutinin P (PHA) to each 10 cc of culture media. The yields in these studies averaged 3.7×10^{11} cells (range: 2×10^{10}-6.5×10^{11}) with 84% overall viability (Fig. 1). About 75% of the cells were of the lymphocytic-mononuclear type. Pretreatment of 10 spleen donors with PHA (0.1 cc/kg s.c. 20 and 5 days prior to splenectomy) did not alter this yield quantitatively (mean 4.5×10^{11}, range: 3.5×10^{10}-5.1×10^{11}). However, when using spleens from donors pretreated with PHA 15-20% of the cells produced in culture were plasma cells.

Culture media. Culture media were injected subcutaneously in an attempt to demonstrate antigenicity. Using 3 groups of 5 rabbits each 1, 2 and 10 cc aliquots of micropore filtered culture media (pore size 0.5 μ) were injected subcutaneously 15 and 5 days prior to skin allografting. The skin was taken from the donor of the spleen, the culture of which yielded the media injected. Graft survival in the 3 groups was comparable and no different from the control.

Amt of media injected (cc)	Rejection (days)	MST (days)
Control	11, 9, 8, 11, 9	9.6 ± 1.2
1	9, 10, 12, 9, 10	10 ± 1.1
2	11, 10, 11, 12, 9	10 ± 1.0
10	10, 9, 9, 12, 8	9.6 ± 1.3

Contamination occurred in 2/374 cultures, representing 0.53%.

Discussion. The number of lymphoid-mononuclear cells originally contained in the spleen (averaging 4.5×10^9 in 8 spleens) is increased by the use of this hyperoxic culture method to a mean of 3.7×10^{11} cells. The bulk of this yield is produced in the first 48 hours, thus allowing use of large numbers of cells in the early period, yet multiplication decreases gradually enough to make viable cells available for repeated use over a 12-day period.

PHA effectively increases this yield. The effect of PHA on the cell yield is quantitatively unaffected by sensitizing the donor of the lymphoid tissue to it. This fact suggests that the increased yield is the result of specific stimulation of mitotic activity by PHA, rather than its antigenicity alone, as suggested by Hirschhorn *et al*(11). The latter does probably account for the increased plasma cell production by sensitized lymphoid tissue.

Hyperoxic cultures avoid the two main problems of lymphocyte cultures by standard techniques, namely high cell mortality(12) and fibroblastic transformation(13). In addition, several technical advantages are offered. Tissue slices can be used instead of dissociated cells or fine suspensions. Cultures in simple large dishes are entirely satisfactory, doing away with the need for special containers, stirrers, feeders, etc. Also, contamination is exceedingly rare, partly at least because of the bacteriostatic effects of hyperoxia (14).

Specific antigenic material has been found in and extracted from rabbit spleen culture media by Mannick *et al*(15). The absence of such antigenic activity in the hyperoxic cul-

ture media is of interest. It suggests that such antigens are released by the cell damage and breakdown seen in routinely cultured spleen suspensions(12) and that, with anoxic necrosis avoided in hyperoxic cultures, antigen is not released.

Summary. In hyperbarically oxygenated cultures, using phytohemagglutinin as a mitogen, spleen and lymph node slices showed great proliferative activity for a 12-day period. Nine-tenths of the yield was obtained in the first two days. Seventy-five to 80% of the cells produced were lymphocytes. The antigenicity of these culture-grown cells did not appear to be diminished as tested by their ability to produce second-set skin graft rejection. Culture media contained no demonstrable antigen.

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Methods for Obtaining the Venous Outflow from the Hypothalamus and Hypophysis.* (31096)

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Control of the anterior pituitary by neurohormones from the hypothalamus is well established. The mechanisms by which the control centers are regulated, however, are quite obscure. While there is general agreement that target-organ hormone feedback (*i.e.*, steroids on gonadotropin production and/or release) is important, some authors(1) visualize an "internal feedback" loop whereby the hormones of the anterior pituitary modify hypothalamic activity. Obviously a vascular link from the pituitary to the hypothalamus is a vital part of such a concept. Anatomical evidence for proximally direct (*i.e.*, toward the hypothalamus) venous drainage of pituitary blood was reported by Popa and Fielding(2). Later studies have concentrated mainly upon the well-known distal drainage of the hypophyseal portal system(3). Recent observations in the living cat and dog(4) have verified proximal blood flow and give indirect support for an internal feedback theory.

According to Peele(5) the major portion of venous blood draining the hypothalamus empties into the great vein of Galen (*V. Cerebri magna*). Demonstration of higher titers of pituitary hormones in the effluent of this vessel, compared with peripheral blood, would give further support for proximal transfer.

The present study deals with: (1) a method for intradural sinus cannulation of the great

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