TABLE I. Renal Artery Perfusion—2 hr.

No. of animals	HCG injected	% of animals ovulating
1	50 IU	100
9	25 IU	91
2	10 IU	100

TABLE II. Portal Vein Perfusion—2 hr.

No. of animals	HCG injected	% of animals ovulating
5	50 IU	100
17	25 IU	75
6	20 IU	100
4	10 IU	100

(Table II). The failure of ovulation would have served to indicate the ability of the kidney and liver to inactivate HCG. Ovulation occurred in 86.3% of the cases, indicating that HCG remained physiologically active following renal and hepatic perfusion.

Controls were run on 20 animals by injecting 10 IU of HCG into the marginal ear vein over a period of 2 hours. Ovulation was observed in all animals.

Discussion. Determination of the quantity of HCG excreted by the kidneys was achieved by using anti-HCG serum. This test was chosen because of its sensitivity, specificity, and accuracy of quantitation.

When 10,000 IU of HCG were employed as the test dose, over 75% of the HCG was recovered in the urine in a period of 23.5 days. The test dose was reduced to 1000 IU of HCG. In 18 animals injected with 1000 IU of HCG an average of 71.8% of HCG was recovered in the urine in a period of 4.2 days. Subsequent studies involving the isolated organ perfusion (kidney and liver) of 44 animals with varying physiologic amounts of HCG over a period of 2 hours demonstrated that little if any HCG was altered biologically by these organs. The results of this follow-up study along with the data offered in the present study suggest that a large amount of HCG is excreted unchanged by the kidney in the rabbit.

Summary. Approximately 75% of HCG injected intravenously into female rabbits was recovered in the urine over a period of 4 to 24 days. The duration of excretion was directly proportional to the amount of HCG administered. Perfusion of the liver and kidney, *in vivo,* with HCG did not cause biologic inactivation of the hormone.

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Human Thymus Cell Cultures—Evidence for Two Functional Populations.* (30746)

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Cells morphologically classified as small lymphocytes were long thought to be biologically inactive. These cells are now known to be immunocompetent since they may initi-

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ate graft-*vs*-host reactions, and participate in delayed hypersensitivity reactions(1). It is uncertain how much they are involved in production of gamma globulin although some experiments have shown that small lymphocytes stain with anti-γM fluorescent antibodies(2).

In vitro, such cells from peripheral blood appear to be "resting" metabolically, but in the presence of phytohemagglutinin they synthesize DNA, RNA and protein, and some of them enter mitosis. These changes accompany a morphological transformation from a small cell with scanty cytoplasm and a compact nucleus to a much larger cell with a larger nucleus and more abundant cytoplasm. These large cells have been termed "blast cells"(3-6). Similar changes have not been seen in cultures of human tonsillar tissue(7).

The thymus is concerned in the development and maintenance of the peripheral lymphoid tissue of the spleen and lymph nodes. There is evidence that this influence occurs both by seeding of thymus cells to the periphery(8), and by production of a diffusible substance which maintains lymphoid tissue stimulation(9). There are also data showing that cells enter the thymus, but the nature of these cells is unknown(10).

Because most of the cells of the thymus are morphologically similar to the small lymphocytes of the peripheral blood, and because of the evidence that thymic cells are also immunocompetent in that they can mount weak graft-*vs*-host reactions and under special circumstances manufacture antibody (11), it is of interest to determine if they behave *in vitro* the way peripheral small lymphocytes do. Published data are discordant. In one laboratory, rat thymus cells were stimulated by phytohemagglutinin (PHA)(12) while other workers found only slight stimulation of DNA synthesis in rabbit thymus cells exposed to PHA(13). No previous data are available for human cells.

Materials and methods. Human thymus cells were removed in the operating room from patients undergoing surgery for cardiac disease, both congenital and acquired. The tissue was pushed through a stainless steel screen, and the cells were washed once and suspended in Eagle's minimal essential medium for spinner cultures containing 20% fetal calf serum and 100 units penicillin G and 100 μg streptomycin per ml. The cells were cultured at 37°C in duplicate test tubes containing 5 ml of suspension with 10×10^6 thymus cells per tube. Phytohemagglutinin M (PHA) (Difco) (0.1 cc per tube) was added at the beginning of culture in most experiments. After varying periods of time, cells were harvested by centrifugation, suspension in 1.1% sodium citrate, fixation with ethanol-acetic acid and staining with aceto-orscein. Some tubes had 0.037 μg of vinblastine sulfate added five hours before harvest. Cells were examined by phase contrast microscopy and cell size was estimated using an eyepiece micrometer. Three hundred cells per tube were classified by nuclear diameter either as small (under 10 μ) or large (over 10 μ), and the mean for each pair of tubes was calculated. Because of the clumping of cells in the presence of PHA, cell counts were not done.

Autoradiographs were made by adding 1.0 μc H^3-thymidine to each tube 5 hours before harvest. The cells were fixed and stained as above, dipped in Kodak NTB-3 emulsion and developed after one week. Two to three hundred cells per tube were classified as labelled or not labelled, and the mean for each pair of tubes was calculated. Virtually all labelled cells were heavily labelled.

Results. Morphological studies (Table I). Stained specimens of cell suspensions examined after 5 hours of culture showed predominantly small round cells with a deeply-staining nucleus and scanty or invisible cytoplasm. Some of the cells were larger and had more cytoplasm and less darkly staining nuclei, but the nuclei were still under 10 μ in diameter. In cultures not containing PHA the cell population (except for patient #6) changed to one composed almost entirely of these small cells after 48-72 hours of culture. In contrast, suspensions exposed to PHA showed increasing percentages of large cells after longer incubation periods. These large cells had abundant cytoplasm, large nuclei and often prominent nucleoli. After 72 hours, 35.8% to 45% of the cells were large (nuclei

TABLE I. Morphologic Studies on Human Thymus Cells After Various Periods of Incubation and Effects of Phytohemagglutinin (PHA).

Patient No.	Age, years	Hr of culture	% of cells of different types					
			–PHA			+PHA		
			Small	Large	Mitosis	Small	Large	Mitosis
3	46	72	96.4	3.3	.3*	49.0	45.0	6*
6	36	72	87.5	12.5	0*	63.8	35.8	.4*
8	4.5	48	98.5	1.5	0*	83.3	16.5	.2*
11	5.5	24	97.3	2.7		86.3	13.7	
		48	93.2	6.8		79.0	21.0	
12	11	24	95.7	4.3	0	83.8	16.2	0
		48	97.0	3.0	0	63.4	36.2	.4
		72	98.7	1.3	0	64.0	36.0	0
14	4	5	98.0	2.0		97.7	2.3	
		24	98.7	1.3		98.7	1.3	
		48	97.0	3.0		92.5	7.5	
15	39	5	97.9	2.1		97.9	2.1	
		24	99.3	.7		96.5	3.5	
		48	98.8	1.2		93.0	7.0	
		72	98.8	1.2	0*	49.7	36.0	13.3*

* Vinblastine sulfate added before harvest.

over 10 μ in diameter), and mitoses occurred in cultures with vinblastine, and occasionally in tubes without vinblastine.

Autoradiographs. Tables II and III show the percentage of labelled cells after exposure to H^3T. At 5 hours, 2.5-11.75% of the cells were labelled and there was no significant difference between tubes with and without PHA. The degree of labelling was not correlated with the patient's age. Thereafter, those tubes without PHA showed a progressive decrease in labelling while those tubes with PHA showed a progressive rise in percentage of labelled cells up to 72 hours. Some cultures which had a high initial rate of labelling (cases #14 and #19) showed a transient dip in labelling at 24 hours. Mean labelling percentages at different periods from the pooled experiments (Table III) emphasize both the progressive drop in percentage of labelled cells in the tubes without PHA, and the transient fall and subsequent rise in percentage labelling in the tubes with PHA. In a subsequent experiment, PHA was added to a suspension 48 hours after culture began, at a time when labelling was very infrequent. These cells were harvested 3 days later (120 hours after the culture began) and showed 16.5% labelling while a control culture to

TABLE II. Autoradiographic Studies After Varying Periods of Incubation.

Patient No.	Age, years	Hr of culture	% of cells labelled	
			–PHA	+PHA
11	5.5	24	6.0	6.25
		48	1.0	5.5
12	11	24	.5	.5
		48	.0	19.75
		72	.5	19.5
14	4	5	7.0	8.25
		24	2.5	4.2
		48	1.75	5.75
15	39	5	5.0	3.75
		24	3.0	4.2
		48	.5	7.7
		72	.75	17.75
16	4	5	2.8	2.8
		24	4.8	4.8
		48	.25	6.2
17	35	5	2.5*	9.5*
		72	0*	20.5*
19	14	5	10.5	11.75
		24	4.75	5.25
		48	.75	3.9

* Only a single tube was available for each determination.

TABLE III. Autoradiographic Studies After Various Periods of Incubation, Both With and Without PHA.

Hr of culture	% of cells labelled with H^3T*	
	–PHA	+PHA
5	5.6	7.2
24	3.6	4.2
48	.7	8.1
72	.3	19.2

* These figures are derived from pooling the results shown in Table II.

which no PHA was added showed 0% labelling.

A comparison between experiments in which *both* morphological and autoradiographic data are available (patients 11, 12, 14, and 15) shows that the increase in percentage of labelling in cultures with PHA correlates with the development of large cells.

Discussion. Taking the morphologic and autoradiographic data together, these experiments indicate the following points. The thymus contains a morphologically heterogeneous population of cells, some of which incorporate H^3T and presumably synthesize DNA soon after removal of the cells into tissue culture. Approximately 6% of the cells were labelled after a 5-hour exposure to H^3T. This correlates well with the high mitotic rate known to exist in the thymus *in vivo*(14). In the absence of PHA, this ability to incorporate H^3T wanes with time, and the cell population becomes a homogeneous collection of small cells. This behavior resembles that reported by Gowans in which thoracic duct cells lost the ability to incorporate H^3-adenosine after standing in culture at room temperature overnight(15). In the presence of PHA, however, the thymus cells become transformed into a population containing about 40% large cells with abundant cytoplasm. About 20% of the cells take up H^3T after 72 hours.

These data are best interpreted as demonstrating two populations of cells in the thymus. One population contains the cells seen at 5 hours and sometimes at 24 hours, but not after 48 hours, which are somewhat larger than the majority of thymic cells. These cells take up H^3T spontaneously, without PHA, but by 48 hours they have either died or have stopped incorporating H^3T and are indistinguishable from the rest of the small thymocytes. The other population contains the bulk of the thymus cells which morphologically resemble the small lymphocytes of the peripheral blood. These cells do not incorporate H^3T but are capable of becoming transformed into larger "blast" cells which do incorporate H^3T. The evidence for this is that virtually no H^3T uptake occurs at 48 hours without PHA when almost the entire population consists of these small cells, but when PHA was added at that time and the cells harvested 72 hours later, large transformed cells appeared, and 16.5% of all the cells seen took up H^3T.

The data showing stimulation of small thymocytes by PHA indicate that they behave similarly to the small lymphocytes of peripheral blood(3-6).

Summary. Human thymus cells were cultured *in vitro* with and without phytohemagglutinin (PHA). At the time of culture, the cells were morphologically heterogeneous with respect to size, and 5-7% took up H^3-thymidine (H^3T). After longer periods of incubation, the labelling decreased and the cell population was composed almost solely of small cells. In the presence of PHA, large cells appeared and up to 20% of the cell population took up H^3T at 72 hours. The data are interpreted to show 2 populations of cells in the human thymus—one synthesizing DNA spontaneously for a short period of time and the other a "resting" population of small cells many of which can be transformed into large cells capable of manufacturing DNA in the presence of PHA.

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Persistence of Neutralizing Antibody in Adult Volunteers Immunized With Adenovirus Soluble Antigens. (30747)

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A previous report has described the antigenicity and protective effects of 2 vaccines prepared from non-infectious soluble antigens of adenovirus type 1(1). Vaccine effectiveness was evaluated by homotypic virus challenge of adult volunteers early in the post-immunization period.

An important consideration in the use of such inactivated vaccines is the duration of neutralizing antibody elicited by these preparations. The following investigation was therefore undertaken to determine the persistence of neutralizing antibody in adults immunized with the type-specific and group-specific soluble antigens of adenovirus types 1 and 2.

Materials and methods. Volunteer selection and clinical procedures. The study was conducted at the Clinical Center of the National Institutes of Health with volunteers from Federal correctional institutions. Criteria of selection and clinical evaluation of the men have been previously described(1).

Antigen preparation. The type-specific antigens employed in this study correspond to the C(2) and E(3) antigens; the group-specific antigens correspond to the A(2) and L(3) antigens.

Adenovirus type 1 and type 2 strains obtained from throat specimens* of children were employed to prepare stock virus suspensions. The materials used to obtain soluble antigens were the fourth (type 1) and third (type 2) passages in primary human embryonic kidney (HEK).† Except as noted below, procedures for the preparation and safety-testing of soluble antigen vaccines were identical to those previously described(1,4). Fractions containing the type-specific and group-specific antigens of adenovirus type 1 were rechromatographed twice, as was the group-specific antigen of adenovirus type 2. The fraction containing the type-specific antigen of the latter serotype was rechromatographed a single time. Protein determinations were done by the Biuret technique(5). The type-specific antigen preparation of adenovirus 1 contained 200 μg of protein per milliliter. The other antigen preparations had less than 100 μg of protein per milliliter. The type-specific antigen vaccine of adenovirus 1 had a complement-fixing titer of 1:512 and the three other vaccine preparations had titers of 1:128, respectively, when tested by a previously described procedure(6). The absence of DNA (less than 0.5 μg per ml) was determined by Burton's modification(7) of the diphenylamine method of Dische.

Isolation of virus. Virus isolation studies of throat and rectal specimens obtained prior to and twice weekly for 5 weeks after immunization were done employing HEK cell cultures using two cultures per specimen. Each culture was examined at weekly intervals for four weeks.

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