necessary to wash the test cultures thoroughly if a "glutamine free" experiment is anticipated. This is especially true if the cultures were grown or maintained in a medium containing glutamine prior to inoculation. Our findings, however, do not necessarily rule out the possibility that another factor or factors may be involved in the development of RS virus syncytia, especially when different cell lines are used.

Because of the instability of glutamine in the balanced salt solution, even at refrigerator temperature, glutamine was not generally included in the ready made commercially available Eagle's medium, or in the essential amino acids concentrates. However, precautions should be taken that the culture medium used contains appropriate amount of glutamine, since the deteriation of this amino acid may have occurred during storage.

Summary. Glutamine was found to be an essential component in Eagle's medium for the maximal multiplication of RS virus and for the development of syncytia cytopathic effect in Hep-2 cell cultures. The minimal amount of glutamine needed for syncytium formation was only 18 μg/ml. It is suggested that it be ascertained that the culture medium used contains glutamine when Hep-2 cell cultures are used for the cultivation and recognition of RS virus.

1. Chanock, R. M., Finberg, L., Am. J. Hyg., 1957, v66, 291.
2. Jordan, W. S., J. Immunol., 1962, v88, 581.
3. Tyrrell, D. A., Am. Rev. Resp. Dis., 1963, v88, 77.
4. Eagle, H., Science, 1959, v130, 432.
5. ———, ibid., 1955, v122, 501.
6. Reed, J., Muench, H., Am. J. Hyg., 1938, v27, 493.
7. Hsiung, G. D., Diagnostic Virology, Yale Univ. Press, New Haven, 1964, 103.
8. Eagle, H., Habel, K., J. Exp. Med., 1956, v104, 271.
9. Morgan, H. R., ibid., 1956, v103, 37.
10. Jensen, E. M., Liu, O. C., Proc. Soc. Exp. Biol. and Med., 1961, v107, 834.
11. Reissig, M., Black, F. L., Melnick, J. L., Virology, 1956, v2, 836.

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Migration of Cells to the Thymus Demonstrated by Parabiosis.* (31675)

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Lymphocytes are known to recirculate between the lymph nodes, spleen and blood(1) but the thymus is generally considered not to be involved in this recirculation. Cell injection studies using labelled thymus and lymph node cells have shown cells to lodge only rarely in the thymus(2,3). However, the lymphoid population of thymus grafts is repopulated completely by host cells(4,5) and parabiotic studies suggest the occurrence of continuous repopulation of the lymphoid cortex of the thymus by blood-borne cells(6). Further, spleen and bone marrow cells are capable of repopulating the thymus after whole body irradiation(3).

In the present studies one partner of parabiotic pairs of mice was labeled with tritiated thymidine and the unlabeled partner monitored for the presence of labeled migrant cells, to determine the frequency of cell migration to the thymus and the location of migrant cells in the thymus.

Materials and methods. Pairs of inbred C57Bl or AKR strain mice, maintained in this Institute, were united in parabiosis using silk sutures between opposing scapulae and glutei and the skin wounds united with skin clips. One member of each pair was always a 2 months old animal and the other partner was

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aged either 2 or 7 months. Four weeks after parabiotic union, a rubber-covered bowel clamp was applied to the parabiotic union and the 3 months old partner injected intravenously with 5 μc/g tritiated thymidine (specific activity 14.8 c/mM; Radiochemical Centre, Amersham, England) the clamp being released one hour later. Previous observations (7) on C57Bl mice given a single injection of tritiated thymidine indicated that by day 3, approximately 10% of blood lymphocytes were labeled. In the present studies the parabiotic mice were killed 3 days after restoration of the blood flow across the parabiotic union to allow time for a reasonable percentage of cells migrating across the union to become labeled. Portions were taken of the thymus, lymph nodes, spleen, kidney, liver and bowel of both the labeled and unlabeled partners, fixed in 10% formol saline, embedded in paraffin and sectioned at 5 μ. Sections were floated on gelatin coated slides and dipped in NTB2 autoradiographic emulsion (Eastman Kodak Co. Rochester, N.Y.). These slides were exposed at 4°C for 80-90 days, developed and stained with methyl green-pyronin. Sections of the various organs of the unlabeled partner were scored for evidence of leakage of label across the parabiotic union, the bowel being used as the primary index organ. Drawings were made using a camera lucida apparatus of the sections of thymus and lymph nodes and the relative areas of the component tissues in these organs (*thymus,* cortex and medulla; *lymph node,* follicles, loose cortex and medulla) were determined by weighing the cut-out outlines of the drawings of the organs. The sections were then surveyed at $\times$ 1000 magnifications and the number and location of migrant cells scored. Background grain counts of the unlabeled partners varied from one pair to another due possibly to varying degrees of leakage of label across the clamped union or to variation in reutilisation of label. In the pairs accepted for final analysis, the background nuclear counts in the organs surveyed varied from 0.1 to 0.6 grains per nucleus and these cells were sharply distinct from all cells scored as labeled migrant cells which had at least 25 grains over the nucleus. A number of the migrant cells were so heavily labeled that the morphology of the cell could not be determined.

Results. The present observations are restricted to 8 pairs of either C57Bl or AKR parabiotic mice, examined 3 days after labeling and restoration of the blood flow across the parabiotic union. Thymus and lymphoid organ weights of both members of all pairs of mice were within the normal limits for mice of the same age, sex and strain, suggesting the absence of serious stress in the animals under study.

Labeled cells, presumably derived by blood-borne spread from the labeled partner, were found in all lymphoid organs examined and occasional labeled cells were observed in the intestinal villi, the liver sinusoids and the glomerular tufts of the kidney. In the spleens, large numbers of migrant cells were present in the red pulp and in many cases such cells were identifiable as lymphoid, erythroid or reticulum cells. No example of a labeled megakaryocyte was observed in the spleens examined.

Migrant cells were extremely numerous in spleen lymphoid follicles and in the subcutaneous and mesenteric lymph nodes. The subcutaneous lymph nodes were examined in detail and used as control tissues for the thymus. Migrant cells in the lymph nodes were classified according to location—follicles, loose cortex and medullary region—and the frequency of migrants in these regions is shown in Tables I and II. Most migrant cells were classifiable as medium or small lymphocytes although in the medullary region occasional cells had the morphology of plasma cells. Many migrant cells were seen to be in transit through post-capillary venules or to be in the collar of cells surrounding such venules.

Migrant cells were observed in every thymus examined in the present study. The frequency and location of these migrants are shown in Tables I and II. Migrant cells were most frequent in the medulla (Fig. 1) and at the junction of the medulla and the cortex. Migrant cells were much less frequent in the cortex and approximately half of those which were present in the cortex were located in the perivascular spaces surrounding the radial

vessels of the cortex (Fig. 2). Many labeled cells were seen also in the spaces around vessels in the medulla. In most cases, the labeled migrant cells had the morphology of small or medium lymphocytes. Mitotic figures were not always clearly visible in these preparations but in the present material no labeled mitotic cells were observed in either the thymus medulla or cortex. Several labeled cells in the medulla had large oval, vesicular nuclei and voluminous pyroninophilic cytoplasm. These cells on morphological grounds would possibly have been classified as epithelial cells, although they were not part of Hassall's corpuscles or lining cystic spaces. The nature of these occasional cells remains

TABLE I. Migration of Labeled Cells into Subcutaneous Lymph Nodes and Thymus of Unlabeled C57Bl Parabiotic Partners.

Age of unlabeled partner in mo	Subcutaneous lymph node						Thymus				
	No. of migrants			Migrants/unit area			No. of migrants			Migrants/unit area	
	Follicles	Loose cortex	Medulla	Follicles	Loose cortex	Medulla	Cortex	Junctional area	Medulla	Cortex	Medulla
2	—	—	—	—	—	—	15	28	92	.04	.5
7	7	70	67	.5	.8	1.5	7	3	36	.01	.2
7	22	293	107	1.2	3.4	2.0	6	4	39	.01	.5
7	100	571	443	1.5	2.4	3.2	13	16	49	.09	.9

TABLE II. Migration of Labeled Cells into Subcutaneous Lymph Nodes and Thymus of Unlabeled AKR Parabiotic Partners.

Age of unlabeled partner in mo	Subcutaneous lymph node						Thymus				
	No. of migrants			Migrants/unit area			No. of migrants			Migrants/unit area	
	Follicles	Loose cortex	Medulla	Follicles	Loose cortex	Medulla	Cortex	Junctional area	Medulla	Cortex	Medulla
2	189	208	166	3.6	4.5	4.3	17	15	30	.03	.7
2	463	280	221	2.4	5.1	2.1	7	9	20	.02	.3
2	—	—	—	—	—	—	8	8	26	.03	.5
7	7	144	42	.2	1.0	.6	4	3	44	.006	.3

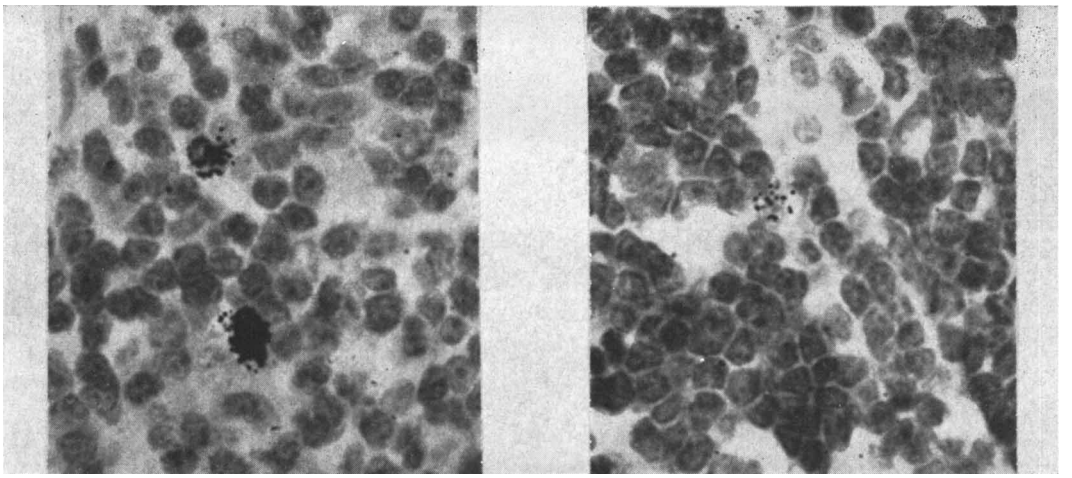

FIG. 1. Autoradiograph of thymus medulla of unlabeled AKR partner showing 2 labeled migrant cells. Exposure time 90 days. Methyl green-pyronin × 1000.

FIG. 2. Autoradiograph of thymus cortex of unlabeled AKR partner showing a labeled migrant small lymphocyte in perivascular space. Exposure time 90 days. Methyl green-pyronin × 1000.

uncertain. As may be seen from the Tables no evidence was obtained that cell migration to either the lymph nodes or the thymus was less extensive in the old partners as compared with the young partners, and no differences in the extensiveness of cell migration were observed between C57Bl pairs and AKR pairs.

Before AKR mice become leukemic, there is an extended period during which there is progressive depletion of lymphocytes from the thymus cortex(8). In 3 additional parabiotic pairs between 2 and 7 months old AKR mice, not listed in Table II, the thymus of the old unlabeled partner showed extensive lymphocyte depletion and the organ was extremely small. In these thymus glands, no migrant cells were observed in either the medulla or cortex.

Discussion. The present observations confirm that migrant cells regularly enter the normal thymus, although the numbers of these cells are much smaller than those entering the lymph nodes or spleen. The present experiments give no information on the origin of these cells or their nature, although most had the morphology of lymphoid cells.

Migrants were much more frequent in the medulla than in the cortex, suggesting that, unlike the thymus cortex, the thymus medulla may be one of the cell depots involved in lymphocyte recirculation. Alternatively, cells seeding out in the thymus medulla may move subsequently to the cortex and there may take part in the regular replacement of the dividing cell population of the cortex by blood-borne cells. If such cells in the cortex rapidly entered division their label might have been diluted by these cell divisions and their presence as migrant cells might have been undetected.

It has been claimed that the lymphoid cells observed in the perivascular spaces around thymic vessels are cells in the process of migrating out of the thymus(9). This may be so, but the high incidence of labeled cells in the perivascular spaces compared with the small number of labeled cells within the cortex in the present material suggests that some at least of these cells are *entering*, not leaving, the thymus.

Summary. Three days after labeling one member of parabiotic pairs of C57Bl or AKR mice, labeled migrant cells were observed in the thymus in every normal unlabeled 2 or 7 months old partner. Thymic migrant cells were less frequent than in the lymph nodes and spleen and were located mainly in the medulla or in the junctional region between the cortex and the medulla. Migrant cells in the thymus cortex were commonly in the

perivascular space surrounding cortical blood vessels.

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1. Gowans, J. L., J. Physiol., 1959, v146, 54.
2. Gowans, J. L., Knight, E. J., Proc. Roy. Soc. B, 1964, v159, 257.
3. Ford, C. E., Micklem, H. S., Lancet, 1963, 359.
4. Metcalf, D., Wakonig-Vaartaja, R., Proc. Soc. Exp. Biol. and Med., 1964, v115, 731.
5. Dukor, P., Miller, J. F. A. P., House, W., Allman, V., Transplantation, 1965, v3, 639.
6. Harris, J. E., Barnes, D. W. H., Ford, C. E., Evans, E. P., Nature, 1964, v201, 884.
7. Matsuyama, M., Wiadrowski, M. N., Metcalf, D., J. Exp. Med., 1966, v123, 559.
8. Metcalf, D., J. Nat. Cancer Inst., 1966, v37, 425.
9. Sainte-Marie, G., Leblond, C. P., Proc. Soc. Exp. Biol. and Med., 1958, v98, 909.

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A Comparative Study on the Virulence of *Mycoplasma arthritidis* And "*Mycoplasma hominis*, Type 2" Strains in Rats.* (31676)

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Mycoplasma hominis, type 2, and *M. arthritidis*, the causative agent of rat polyarthritis, have many common properties. Antigenic identity has been shown by complement fixation tests(1,2) although agar-gel double diffusion studies show minor differences between established laboratory strains(3). Pease reported that *M. hominis*, type 2, was antigenically identical with a strain of *M. arthritidis* which had recently been isolated by one of us from a bronchiectatic lesion in rat lung(4). On the basis of morphology and physiology, these strains are essentially the same(5,6). Finally, the pathogenic properties of *M. hominis*, type 2, for rodents were demonstrated by the production of a local abscess in mice following subcutaneous injection of the organism with agar(5). In 1965, Edward and Freundt proposed that *M. hominis*, type 2, be reclassified as *M. arthritidis*(6).

The above observations lend strong support to the identity of these strains. Nonetheless, additional evidence is desirable before final classification is accepted. We therefore have compared the pathogenicity of *M. hominis*, type 2, with a laboratory maintained and recent isolate of *M. arthritidis* for the rat.

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Materials and methods. Strains. M. hominis, type 2, strain 14152 (Edward strain PG 27, originally obtained from Dr. L. Dienes as strain 'Campo'), and *M. arthritidis*, strain 14124, were obtained from the American Type Culture Collection (Rockville, Md.). *M. hominis*, type 2, strain 158 (cloned 3 times from Dr. Edward's† strain PG 27) was obtained from Dr. M. F. Barile (Division of Biologic Standards, Bethesda, Md.). *M. arthritidis*, strain PN, was isolated by the authors from a subcutaneous nuchal abscess in a rat.

Physiology. PPLO (Difco Laboratories) agar and PPLO (Difco) broth, supplemented with 10% (v/v) horse serum (inactivated at 56°C for 30 minutes) were used throughout as the basal media. Carbohydrate fermentation was tested in broth containing 0.005% (w/v) phenol red and 1% (w/v) carbohydrate. Glucose, maltose, starch and dextrin were added from sterile 10% (w/v) Seitz-filtered solutions. Cultures were examined at intervals for 10 days of incubation for change of phenol red to a yellow color. Ammonia production was tested by adding one drop of Nessler's reagent to one drop of a 3-5-day broth culture containing 1% (w/v) arginine monohydrochloride. Lipolysis was determined by growth on agar containing

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