of Na-K-phosphate buffer (0.15 M for lower and 0.25M for higher ACh concentrations) at pH 7.5. After exposure to ACh (time and concentrations as indicated in the Figures), the cells were washed 3 times and resuspended in 0.75 ml of 0.85% NaCl solution. 0.1 ml aliquots of the cell suspension were introduced into distilled water and NaCl solutions (3 ml) at concentrations of: 0.85, 0.7, 0.6, 0.5, 0.4 and 0.3%. After 1 h at room temperature, the cell suspensions were centrifuged and the hemoglobin content in the supernatant fluids was determined using a Klett photocolorimeter (filter No. 54).

The osmotic fragility of the erythrocytes incubated for 20 h with ACh or acetylthiocholine (AThCh) at concentrations of $10^{-3}M$ to $10^{-2}M$ was definitely increased. Higher concentrations of these 2 compounds brought about a greater increase of the cell fragility up to a pronounced or a complete hemolysis, induced at the incubation stage (Figure 1). Incubation of the erythrocytes for 20 h with very high concentration ($1.4 \times 10^{-1}M$) of succinylcholine (SCh) and butyrylcholine (BCh) caused only a small increase in the osmotic fragility of the cells. The time-dependent hemolytic effect (Figure 2) was due to the production of acetic acid accompanied by a decrease in the pH of the medium, which was not prevented in isotonic conditions. Papain treatment of the red blood cells was found to abolish this hemolytic effect (Figure 3).

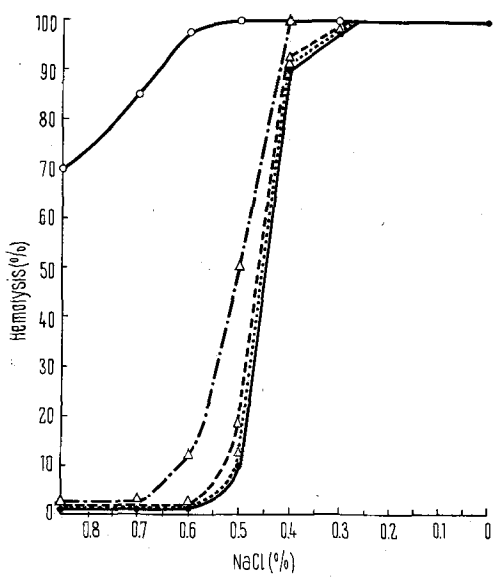

Fig. 5. The osmotic fragility of red blood cells after incubation for 20 h with 0.14 M concentrations of: sodium acetate (△....△), choline chloride (△--△), both acetate and choline (△-·-△), ACh (○—○) and without them (●—●).

External AChE from bovine erythrocytes (Sigma, type I) or from electric eel (Sigma, type V), or plasma cholinesterase, added to the incubation medium of the papain-treated cells, restored the hemolysis by ACh (Figure 4) and by AThCh. In an incubation medium contaning plasma cholinesterase, a complete hemolysis was induced by $1.4 \times 10^{-1}M$ butyrylcholine, too. As this cholinesterase catalyses a rapid hydrolysis of butyrylcholine, the butyric acid produced was involved in the hemolytic effect in this case.

Na-acetate or choline chloride at $1.4 \times 10^{-1}M$ concentration had no significant effect, but in the presence of both of them the osmotic fragility of the cells was definitely augmented (Figure 5). Addition of acetic acid at the same molar concentration, to the buffer containing incubation medium, lowered the pH of the medium to pH 4–5 and induced a complete hemolysis.

The foregoing experimental results indicate that inactivation of the erythrocyte AChE by papain protects the cells from the profound hemolytic effect of very high concentrations of ACh and AThCh.

Résumé. L'hémolyse des globules rouges, incubés avec de fortes concentrations d'acétylcholine en conditions isotoniques est due à l'acide acétique libéré dans le milieu. Cette hémolyse est complètement inhibée par l'inactivation de l'acétylcholinestérase érythrocytaire avec la papaïne.

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Aberrant Thymus Tissue in Rat and Mouse Thyroid

One of the most important discoveries of modern immunology is the role of the thymus in the control of the immune response. Many studies have shown the function of this gland by removing it, especially from new-born animals, where its role is much more prominent than in adults. We want now to report aberrant thymus tissue located near and in the thyroid gland in mice and rats. In these animals the thymus is in the thoracic cavity, ventral to the base of the heart and aortic arch.

The thymus is a lymphoid organ, differing from lymph nodes in being epithelial in origin and character and in having no sinusoids. The thymic lobes are epithelial thickenings in the region of the 3rd and 4th pharyngeal pouches in 11-day-old mouse embryos. During the 15th day these epithelial vesicles separate from the pharyngeal epithelium and come to lie anterolateral to the heart, and during the subsequent 4 days they grow, migrate posteromedially and become lymphoidal[1]. The rodent thymus has a dense cortex, surrounding a pale, irregularly arranged medulla. The cortex is composed of densely packed masses of small lymphocytes called 'thymocytes'.

More than 40 years ago, DE WINIWARTER[2] described the possibility that thyroid and parathyroid glands include thymic tissue, which might be confused with tangential sections of the thyroid follicles. He thought that the thyroid and parathyroid cells could be locally

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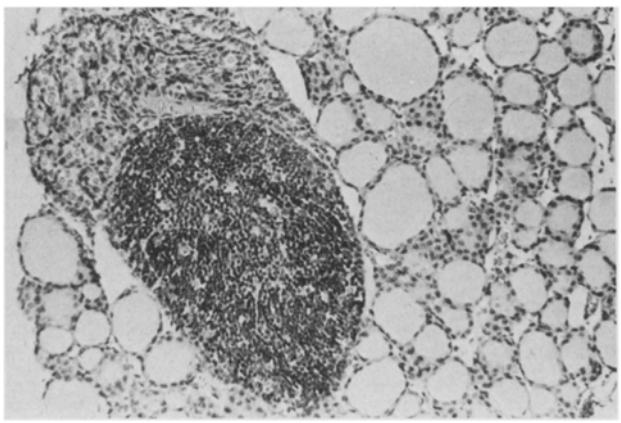

Fig. 1. Thymus tissue under parathyroid. The tissue is separated from the thyroid follicles. H and E, ×30.

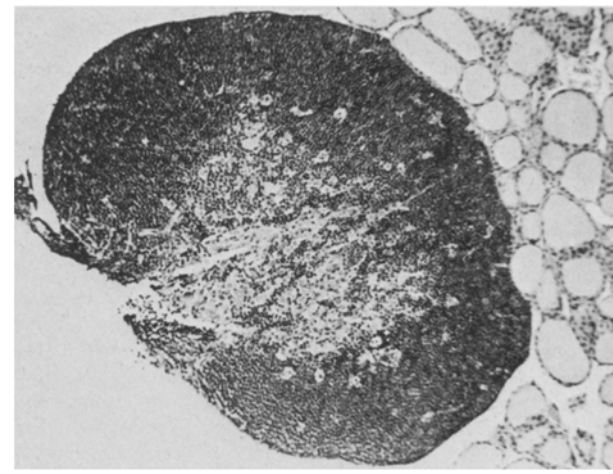

Fig. 2. Thymus tissue with cortex and medulla near the thyroid H and E, ×35.

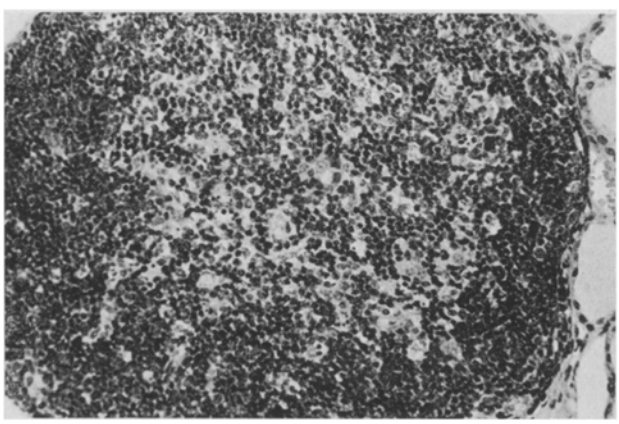

Fig. 3. Aberrant thymus tissue near the thyroid. The cortex is composed of small lymphocytes and the medulla has large cells, the equivalent of Hassal bodies. H and E, ×88.

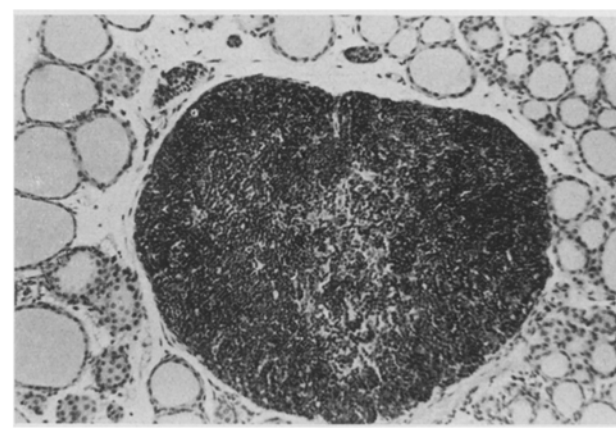

Fig. 4. Thymus tissue within the thyroid. Note the dense accumulation of small lymphocytes of the thymus, well separated from the thyroid. H and E, ×28.

transformed into thymocytes, because he saw many mitoses in these thyroids. Later, DUNN[3,4] described aberrant thymus tissue in almost all mice of BALB/c strain studied. This tissue was often on one side, and sometimes seen as only a collection of lymphocytes. The association with the parathyroid is important in recognizing it[5].

In our studies on autoimmune thyroiditis in mice and rats we used several inbred strains of mice, as AKR/J, BALB/cJ, BRVR, C_3H/HeJ, CBA/J, C_{57}BL/KsJ, DBA/2J, and SJL/J, randomly bred CF-1 mice as well as the inbred rat strains: Buffalo, Lewis and the randomly bred Sprague-Dawley rat strain. Thyroids attached to the trachea were removed from the mice and rats, cleared of the adjacent esophagus and laryngeal muscles, and fixed in buffered formalin. After processing, serial sections 6 μm thick were stained with hematoxylin and eosin.

We found aberrant thymus tissue in 20 to 60% of animals in the various strains studied. The thymus tissue is often close to the parathyroids: sometimes the parathyroid seems to be molded onto the thymus tissue (Figure 1). In many cases the tissue has an easily recognizable cortex and medulla (Figure 2). The medulla has large cells, the equivalent of human's Hassal bodies (Figure 3). The aspect is quite similar to that of the normal (intrathoracic) thymus. In some instances the aberrant thymus is many times larger than the parathyroid gland. In other cases the thymus tissue is located within the thyroid itself as a well-defined accumulation of small lymphocytes, surrounded by normal thyroid follicles (Figure 4). This may be confused with a mononuclear cell accumulation seen in autoimmune thyroiditis but does not have the diffuse infiltrative character, with disruption of thyroid follicles, as is seen in that disease. In such cases serial sections are required for a better recognition of tissue. Electron micrographs might be helpful in detecting myeloid cells.

In chickens, where the thyroid is normally very close to the thymic lobes, JANKOVIĆ et al.[6] considered that the thymus is a source of mononuclear cells in autoimmune

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thyroiditis. Sometimes we found in mice and rats with this disease a spreading of the lymphocytes from the aberrant thymus tissue into adjacent thyroid tissue, but this may well be an artifact.

The thymic tissue in the thyroid gland reflects the close embryological evolution of these 2 glands. The aberrant thymus tissue does not seem to be derived from the thyroid. It looks quite normal and we have no reason to believe that the aberrant thymus tissue in the thyroid is not functioning. It was shown that aberrant parathyroid tissue in the neck and thymus in rats is normally functioning and can explain why many parathyroidectomized rats do not develop hypocalcemia[7]. Therefore, histological examination of the thyroid area is important in all thymectomized animals, in order to exclude the possibility of additional thymus tissue[8].

Résumé. Dans différentes lignées de souris et de rats, on a souvent trouvé du tissu thymique d'aspect normal au voisinage ou dans la thyroide même. Les animaux thymectomisés doivent être examinés pour contrôler la présence d'un tissu thymique aberrant.

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[9] Research assistant professor, supported by Bertha H. and Henry Buswell Research Fellowship.

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∅-Isoantigenic Marker in Phytohemagglutinin-Responding Mouse Blood Lymphocytes

Studies based on thymectomy and/or bursectomy[1,2] and on thymus/bone marrow grafting in karyologically distinguishable syngeneic strain combinations[3-5] indicate that the proliferative response induced in vitro by phytohemagglutinin (PHA) and some other mitogenic agents is under thymic control, and that the cells participating in the response are predominantly T-(thymus dependent) lymphocytes. The introduction of the ∅-isoantigen[6], as a marker for thymus-dependent lymphocyte in mouse[7,8], has made it possible to analyze the participation of T-cells in immune responses by using an alternative experimental design. In this communication we wish to report that depletion of ∅-bearing cells from mouse blood lymphocyte population abolishes the PHA-response, and that all but a minor fraction of the blast cells transformed by PHA in culture contain the ∅-antigen on their surface.

Materials and methods. Blood was collected from adult male CBA/T_6T_6 mice, which received intravenously 0.15 ml of 1:3 diluted *B. pertussis* culture eluate 3 days prior to the bleeding. The pertussis treatment, which was done to increase the cell yield[9,10], slightly decreased the number of ∅-positive cells in the blood (Table I). Optimum conditions for culture have been described before[10,11]. Blast cell counts and uptake of 3H-thymidine (New England Nuclear Corporation, Boston, Mass, – Sp. act. 6.7 mCi/m*M*) were used for the evaluation of the response[10]. ∅-isoantiserum was produced in AKR/Jax mice by 5 weekly injections of CBA/Jax thymus cells as described by RAFF[7,12]. Fresh hamster and guinea-pig sera were used as sources of complement[12]. The cytotoxic titer of the antiserum against CBA/T_6T_6 and C3H/HeJ thymus cells was 1:64–128. Normal AKR/Jax serum was used as control. The cytotoxicity tests were performed according to RAFF[12] at 1:2 and 1:4 dilutions of antiserum using 2 different methods: by the trypan blue dye exclusion test with purified lymphocytes populations, or by preparing cell smears after treatment with antiserum and complement using a Shandon cytocentrifuge (Shandon Scientific Co. Ltd., London NW 10)[13]. The latter of the 2 methods also permitted morphological examination of surviving cells. In cases where lymphocytes were cultured after the antiserum and complement treatment, the sera were sterilized by Millipore filtration (Millipore Filter Corporation, Medford, Mass. – pore size 0.22 μm), and the cell densities were readjusted prior to culture.

Results and discussion. The elimination of ∅-bearing cells prior to the culture nearly completely abolished the PHA-response (Table II). These cultures, when harvested on the 3rd day, contained primarily small lymphocytes, usually in aggregates around macrophages, some eosinophilic granulocytes and few degenerating cells with distorted morphology (Figure). The occasional blast cells seen in these cultures were within the aggregates and

Table I. Cytotoxic effect of anti-∅ and complement on mouse blood lymphocytes before and after *B. Pertussis* treatment[a]

Treatment	Dead cells (%) Blood lymphocytes (no pertussis)	Blood lymphocytes (pertussis)	Thymus cells
None	2	6	9
Anti-∅ + C′	78	70	94
AKR serum + C′	5	4	8

[a] Target cell population purified from red cells and granulocytes by NH_4Cl lysis and short term incubation on plastic. Antiserum dilution 1:4. Trypan blue (0.1%) dye exclusion test.

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