second (B) 10 animals and the third (C) 5 animals; the latter 2 groups were controls. The animals of the former 2 groups were injected intradermally, in the 4 pads, with 0.2 ml of encephalitogenic emulsion, which consisted of equal parts of rabbit brain homogenate and Freund's complete adjuvant (5 mg B.K., H 37 Rv strain, heat-killed, per ml of adjuvant). Starting on the day of inoculation of encephalitogenic emulsion (day 0), the animals were inoculated as follows: the animals of the group A were injected 3 times a week with 1 ml of vitamin A in oil solution (300,000 U/ml), i.m. during the first 2 weeks and s.c. in the third week. The animals of group B were injected in the same way, only with oleum heliantis, the solvent of vitamin A. The animals of group C, controls, were injected only with vitamin A, 3 times a week, i.m., without being inoculated with encephalitogenic emulsion. In brief, each animal was injected 9 times with vitamin A (2,700,000 U) or with oleum heliantis. Because of the toxicity of the vitamin A administered in high doses, only the presence of definite paralysis, paresis or histological lesions were accepted as an unequivocal evidence of the disease. The animals with obvious signs of disease were killed the day of the onset of the symptoms, while the others, including all the animals of group C, were killed on the twenty-first day after inoculation with encephalitogenic emulsion. Brains of all animals were fixed in 10% formalin, embedded in paraffin and the sections stained with hematoxylin and eosin. Sections of 3 levels in brain were examined to find out the histological features of EAE.

The animals of group C exhibited signs of hypervitaminosis about 2 weeks after the beginning of the treatment; the fur was dry and rough, and the eyelids showed keratinization.

These animals became apathetic, anorexic, and lost weight; they seldom had disturbances in walking, and the bones of the cranium were very thin. Histological studies of the brain showed hyperemia of the meningeal and cerebral veins, meningeal edema and edema of the nervous matter.

Group	With lesions of EAE with symptoms	without symptoms	Without lesions or symptoms of EAE
Treated with vitamin A	2	1	10
Injected with solvent oil	9	1	0

The data obtained (Table) suggest that high doses of vitamin A inhibit EAE in guinea-pigs; the results are statistically significant ($\chi^2 = 13$). The effect of high doses of vitamin A in EAE may be due to its action upon lysosomal membranes, producing depletion of the lysosomes and therefore suppressing the delayed hypersensitivity[5]. The hydrolytic enzymes from the lysosomes might play a role in unmasking the encephalitogenic antigen from the myelin structure, that having a role in self-perpetuation of the disease. In experimental autoimmune thyroiditis in guinea-pigs, inoculated with large amounts of vitamin A, a decrease of the cellular infiltrates in thyroid has lately been shown[6].

Our results suggest a lysosome participation in the pathogenesis of EAE in guinea-pigs; the question arises which are the cells whose lysosomes play a role in the mechanism of the disease[7].

Résumé. L'encéphalomyélite allergique expérimentale n'apparaît pas chez les cobayes auxquels on a injecté de fortes doses de vitamine A.

A. VLADUTIU and N. CRINGULESCU

Department of Physiopathology and Department of Morphopathology, Institute of Medicine, Bucuresti 8 (Roumania), 9 February 1968.

[1] B. H. WAKSMAN, Int. Archs Allergy appl. Immun. *14*, suppl. (1959).
[2] M. F. KEREKES, T. FESZT and A. KOVACS, Experientia *21*, 42 (1965).
[3] M. WENDER, M. KOZIK, T. WROBLEWSKI and M. RUDNICKA, Path. europ. *2*, 135 (1966).
[4] G. WEISSMANN, J. W. UHR and L. THOMAS, Proc. Soc. exp. Biol. Med. *112*, 284 (1963).
[5] J. W. UHR, G. WEISSMANN and L. THOMAS, Proc. Soc. exp. Biol. Med. *112*, 287 (1963).
[6] A. JANUSZ, H. D. FLAD, D. KOFFLER and P. A. MIESCHER, Int. Archs Allergy appl. Immun. *31*, 69 (1967).
[7] The authors are indebted to ELISABETH MÓRE for technical assistance.

Suppression of Immunization to Tuberculosis and Diphtheria by an Extract of Lymphoid Tissue

ACTH and cortisone given in small doses tend to increase resistance to infection[1]. It has been established that these hormones have a dramatic favorable effect in allergic states and in the 'collagen' diseases. ACTH and cortisone cause a decrease and dissolution of lymphocytes with release of lymphocytic cellular constituents[2-3]. It is possible that the favorable effect of ACTH and cortisone in hypersensitivity and 'collagen' diseases is mediated by some of the components of the lymphoid tissue.

It may be possible to isolate a substance or substances from lymphoid tissue which has the therapeutic properties of the corticosteroids and hopefully, without the unfavorable side effects of these hormones. Thus, lymphoid tissue might represent a target organ in a hypothalamic anterior pituitary-adrenal cortical-lymphopoietic system, which would be responsible for an organism's reaction to stress. In such a system, the cellular components of lymphoid tissue may exercise a feedback effect on the anterior pituitary-adrenal cortical-endocrine axis. In addition, the lymphocyte components may possibly be re-

[1] G. W. THORN, New Engl. J. Med. *274*, 775 (1966).
[2] T. F. DOUGHERTY, in *The Kinetics of Cellular Proliferation* (Grune-Stratton, New York 1959), p. 264.
[3] J. H. HUMPHREY and R. G. WHITE, in *Immunology*, 2nd edn (F. A. Davis Co., Philadelphia 1964), p. 373.

sponsible for the physiological effects of the adrenal cortical hormones.

To test our hypothesis, we studied the effect of lymphoid tissue on immunity and hypersensitivity.

A sterile, aqueous bovine lymph node extract was used in these experiments. We studied diphtheria immunization in guinea-pigs, horse serum-induced immediate hypersensitivity in rabbits; and BCG-induced delayed type of hypersensitivity in guinea-pigs with and without pretreatment with lymph node extract.

Methods. Beef mesenteric lymph nodes were removed within 10 min after the animals were killed and preserved in dry ice until the time of extraction. The lymph nodes were ground in a Fitzmill with an equal weight of dry ice. The frozen lymph node powder of suspended in 6 volumes of a 0.15*M* NaCl solution. This suspension was stirred until ice had melted (temperature 1 °C). The pH of the solution was adjusted to 7.4. The precipitate was then stored in the cold room for 1 h. And then, it was centrifuged at 0 °C for 30 min at 2000 rpm. The solids at the bottom were discarded. The supernatant was filtered through a cheese cloth to remove fat particles floating on top. Then it was filtered through a celite bed on a tabletop Buchner. Finally, the solution was lyophilized and desalted. The lyophilized material was used to make a 2 g/100 ml distilled water solution. This solution was filtered through Whatman unwashed filter papers No. 1 and No. 3, and a Seitz bacterial filter. The final extract was proven sterile by bacterial culture methods and could be used parenterally. Bovine kidney extract was made utilizing the same procedure and was used in the control groups of animals.

Diphtheria toxoid, aluminum phosphate absorbed, ultrafined®, made by Wyeth Laboratories, was used for diphtheria immunization. Diphtheria toxin for Schick test (diluted), made by the same laboratory, was used for skin testing of the animals immunized to diphtheria toxoid.

Fifty guinea-pigs, averaging 300 g, were divided into 2 groups: one group was given diphtheria toxoid with pre-treatment with lymph node extract; the other, diphtheria toxoid and saline. Each animal was given diphtheria toxoid 0.3 ml i.m., twice at 4-week intervals. The animals which received diphtheria toxoid and lymph node extract also received 2 ml of a 2 g/100 ml aqueous solution of this extract/kg body weight, i.m. every 6 h, starting 12 h before and continuing for 7 days after the administration of each dose of diphtheria toxoid. Saline was substituted for lymph node extract in control animals. The results of diphtheria immunization were assessed and compared in 2 groups by Schick skin test, which was performed 12 days after the last dose of diphtheria toxoid.

The conversion of the tuberculin reaction was studied in guinea-pigs with and without pre-treatment of lymph node extract.

103 PPD-negative guinea-pigs averaging 500 g, were divided into 3 groups: one group received BCG vaccine with pre-treatment with lymph node extract; the second group BCG vaccine with pre-treatment with kidney extract, and the third group BCG vaccine and saline. Each animal was given the BCG vaccine i.c., 0.1 mg in 0.05 ml water solution. The animals of the group treated with BCG and lymph node or kidney extract were given 2 ml of a 2 g/100 ml aqueous solution of these extracts/kg body weight, i.m. every 6 h, starting 12 h before and continuing for 7 days after the administration of BCG vaccine. Saline was substituted for the extracts in one of the control groups. All animals were PPD-tested 6 weeks after the administration of BCG vaccine and the results compared in the 3 groups. Mantoux i.c. method was used for PPD-testing (0.005 mg PPD/dose). The test was read 48 h later and was considered positive if both erythema and an induration, of not less than 5 mm in diameter, were present.

Eight tuberculin positive animals were given a week-treatment with lymph node extract, as indicated above. At the end of this treatment, the PPD-test was repeated.

Of the 50 guinea-pigs used for testing the effect of lymph node extract on diphtheria immunization, 2 died in the course of the experiment; 17 (77.5%) of the 22 animals given diphtheria toxoid and saline became Schick negative, and 5 (22.5%) remained Schick positive. Twenty (72%) of the 26 animals given diphtheria toxoid with pre-treatment with lymph node extract remained Schick positive and only 6 (28%) became Schick negative (Table I).

39 (73%) of 53 guinea-pigs given BCG and lymph node extract remained tuberculin-negative; 12 (22.5%) became tuberculin-positive, and 2 died during the experiment. 12 (48%) of 25 guinea-pigs which received BCG and kidney extract remained tuberculin-negative, and 13 (52%) became tuberculin-positive. Of the 25 guinea-pigs which received BCG vaccine and saline 4 (16%) remained tuberculin-negative and 21 (84%) became tuberculin-positive (Table II).

All of the 8 tuberculin-positive guinea-pigs which received a week treatment with lymph node extract, remained tuberculin-positive following this treatment, indicating that lymphoid extract had no effect on the already-developed hypersensitivity (Table III).

Discussion. We previously had shown an inhibition of induced serum sickness by bovine lymph node extract[4]. An inhibitory effect on diphtheria immunization and on

Table I. Effect of lymph node extract on diphtheria immunization in guinea-pigs

Animals	Schick-positive	Schick-negative
Diphtheria toxoid and lymph node extract	20	6
Diphtheria toxoid and Saline	5	17

Table II. Effect of lymph node extract on BCG-induced hypersensitivity in guinea-pigs

Animals	PPD-negative	PPD-positive
BCG and lymph node extract	39	12
BCG and Saline	4	11
BCG and kidney extract	12	13

[4] N. RADOIU, R. W. LONG, G. DUBOFF, J. VAZQUEZ and P. L. WOLF J. Retic. Soc. *2*, 199 (1965).

BCG induced delayed hypersensitivity has now been demonstrated. Although other authors have demonstrated immunological paralysis[5,6] of the 'protein overloading' type[7], it is unlikely that the inhibitory effect of the lymphoid extract is of that nature because far less protein 20–30 mg/day was used. In addition, it was pointed out in our earlier work that in the control animals when beef kidney extract was substituted for lymph node extract in the same amount, no inhibitory effect on serum sickness or anaphylactic shock could be demonstrated.

The interference of lymphoid tissue with antibody formation could conceivably be the result of a non-specific neutralization of antigens, by a cellular component (s) of the lymphopoietic tissue, before they reach the antibody cells.

This study tends to confirm our hypothesis that lymphoid tissue might represent a target organ in a hypothalamis-anterior pituitary-adrenal cortical-lymphopoietic system which would be responsible for the organism's reaction to stress. It is conceivable that one might be able to isolate from lymphoid tissue a substance or substances with the therapeutic effects of corticosteroids and, hopefully, without the unfavourable side effects of these hormones, which could be used in the treatment of hypersensitivity states, 'collagen' diseases and prevention of homograft rejection[8,9].

Table III. Tuberculin skin test in PPD-positive guinea-pigs after a 7-day treatment with lymph node extract (LNE)

Animals	PPD-positive	
	Before treatment with LNE	After treatment with LNE
1	+	+
2	+	+
3	+	±
4	+	+
5	+	+
6	+	+
7	+	±

Zusammenfassung. Dank einer neuen Anwendung von Lymphknotenextrakten (statt Röntgenextrakten, Corticosteroiden, Antilymphozytenserum usw.) wird eine erhebliche Reduktion der immunologischen Reaktion erzielt, wenn die Tiere mit diesen Extrakten vorbehandelt und gleichzeitig mit Antigenen behandelt werden.

N. Radoiu, P. L. Wolf,
F. A. Zydeck, E. T. Konno,
Janice Vazquez and
Elisabeth Von der Muehll

Wayne State University School of Medicine, Departments of Medicine, Pathology and Microbiology, Detroit Institute of Cancer Research and Metropolitan Hospital, Detroit (Michigan 48207, USA),
23 October 1967.

[5] F. J. Dixon and P. H. Maurer, J. exp. Med. *101*, 245 (1955).
[6] P. Liacopoulos and T. Neveu, Immunology *7*, 26 (1964).
[7] E. H. Kass, Ann. N.Y. Acad. Sci. *88*, 108 (1960).
[8] This investigation was supported in part by U.S.P.H.S. Research Grant No. CA-02624 from National Cancer Institute; and in part by an institutional grant to Detroit Institute of Cancer Research from United Foundation of greater Detroit allocated through Michigan Cancer Foundation and the Detroit General Hospital Research Corporation, and Newaygo County Cancer Society.
[9] We would like to express our thanks to Mrs. Connie Malacusky for her stenographic assistance.

Osseal Changes in Mice Following Neonatal Thymectomy

The decisive role of the thymus in the development of immunological competence is generally accepted. Mice thymectomized immediately after birth develop a depression of immunological capacity and, within several weeks, a wasting syndrome. This condition is characterized by a rapid weight loss, lethargy, ruffled fur, hunched posture, diarrhoea and death. Necropsy reveals characteristic atrophic changes in the spleen and lymphoid system[1,2]. Animals suffering from the wasting syndrome show a general retardation of development. The present communication discusses the nature of the osseal changes associated with this condition.

Materials and methods. Our experiments were performed on inbred C_3H mice. The animals were thymectomized within the first 24 h after delivery. The control littermates were sham-operated. Evidence for the wasting syndrome, other than the clinical symptoms, included the decline in body weight and the low absolute lymphocyte count.

Radiographs of 10 animals with the wasting syndrome and 10 control mice were taken during the 6th–7th postoperative weeks. The animals were then sacrificed and dissected. Successful thymectomy and atrophy of the spleen were confirmed macroscopically and by histology.

The distal ends of the femora were chosen for histological study. 4% formalin fixative, decalcification paraffin embedding and hematoxylin-eosin staining were used.

Results and discussion. In the radiographs, great differences between the control (a) and thymectomized (b) mice were observed. These appeared to be particularly significant in the long bones. Loss of bone minerals or bone atrophy was also remarkable (Figure 1).

The normal structure can be seen in microscopic sections from the control group. The structure and thickness of the articular cartilage are normal. The epiphyseal plate shows continuous endochondral ossification (Figure 2). Similar histological sections from the bones of the thymectomized mice show remarkable phenomena. In the distal epiphyses, there are only 2–3 irregular, very thin fragments of osseal trabeculae. The cartilaginous surface is thin and fibrous and irregular in structure. The epiphyseal plates show minimal or no sign of ossification (Figure 3).

[1] J. F. A. P. Miller, Lancet *2*, 748 (1961).
[2] J. F. A. P. Miller, Proc. R. Soc. B *156*, 415 (1962).