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.

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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).

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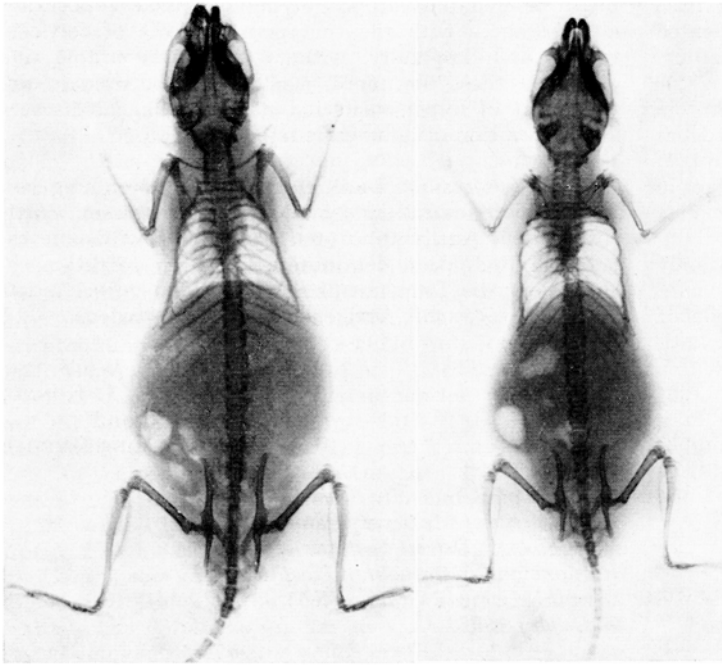

a b

Fig. 1. Roentgenogram of a neonatally thymectomized mouse with wasting syndrome (b), and a sham-operated littermate (a).

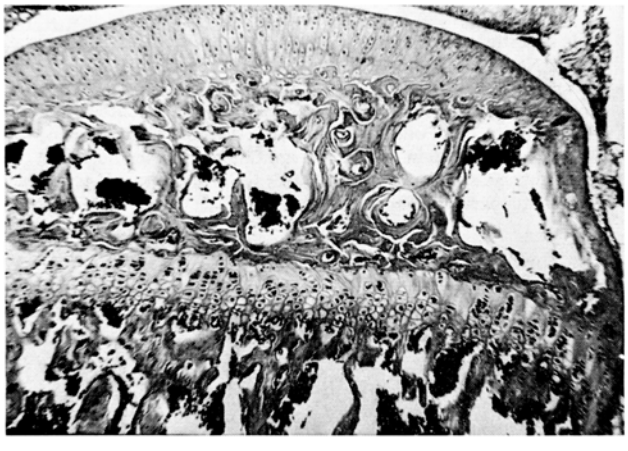

Fig. 2. Control microphotograph of the distal end of the femur of a sham-operated mouse. Hematoxylin-eosin staining. × 25.

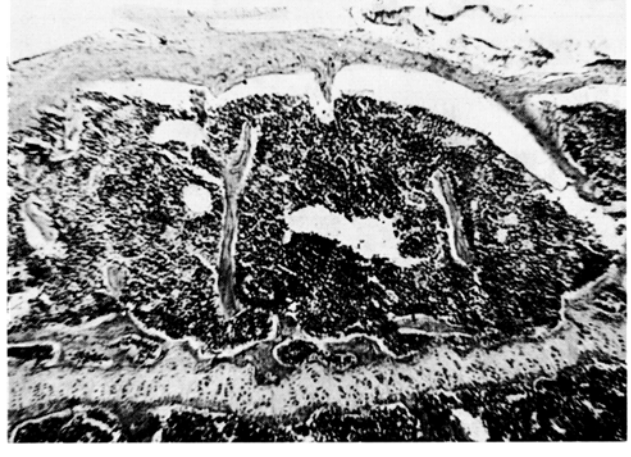

Fig. 3. Microphotograph of distal end of femur of a neonatally thymectomized mouse with wasting syndrome (littermate of mouse in Figure 2). Hematoxylin-eosin staining. × 25.

The sections reveal a remarkable thickness and extreme sparseness of osseal trabeculae in the metaphyses also. The hyaline pillars remain cartilaginous, with little or no sign of osteoblast activity at their sides. All diaphyseal cortices are strikingly thin. The observed alterations were demonstrable in all animals with the wasting syndrome and the roentgenographic findings and histology were in good agreement with the serious clinical conditions.

In an earlier publication we suggested an analogy between the pathogenesis of the wasting syndrome following neonatal thymectomy and of the wasting in premature and new-born infants due to infectious diseases[3]. Thymic regression is well known in atrophia infantum[4]. GEFFERTH found osseal changes in babies suffering from infantile atrophy[5]. Two thirds of the wasted babies showed abnormalities of osseal structure and mineral content. His findings were in short as follows: the tubular bones were shortened, the spongiosal lamina were irregularly outlined and blurred, the corticales became thin, the periosteum thickened, calcification was suppressed, the epiphyseal plate became irregular and wider and the ossification centres appeared later. The severity of these changes seemed to depend on the duration of the illness.

The present data revealed histological and roentgenographic manifestations of severe osseal changes appearing

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in the experimental atrophy ('wasting syndrome') following neonatal thymectomy.

Concerning the pathological mechanism of these severe disturbances in the ossification process, the experimental data of PIERPAOLI and SORKIN are of interest[6]. They observed a marked degranulation of acidophilic cells in the anterior lobe of the pituitary after neonatal thymectomy in mice and postulated the existence of a thymus-hypophysis axis, possibly involving growth hormone. LAW et al. reported that in neonatally thymectomized and wasting mice the cells of the anterior pituitary exhibited a reduction in nuclear and cytoplasmic volume in comparison with control mice[7]. The thymic regression and the count and the nuclear volume of the acidophilic cells in the anterior lobe of the pituitary in atrophia infantum[4], and the control of enchondral ossification by somatotropic hormone are well known[8,9].

Taken together these data indicate the importance of the thymus and hypophysis for these osseal alterations. It appears a plausible supposition that the osseal changes are dependent on alterations produced by neonatal thymectomy and resulting in changes in the somatotropic hormone producing cells[10].

Zusammenfassung. In neonatal thymektomierten Mäusen wurden nach 6–7 Wochen röntgenologisch sowie histologisch das «wasting syndrome» und schwere Knochenatrophien beobachtet. Der Mechanismus der beobachteten enchondralen Ossifikationsstörung wurde diskutiert.

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Adrenal Lipid and Plasma Corticosterone Depletion after 7,12-Dimethylbenz(a)anthracene Administration to the Albino Rat

Mammary carcinoma may be induced in rats by the single feeding of the polynuclear aromatic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA)[1]. Following administration of this compound necrotic changes occur in steroid hormone producing cells[2,3]. WONG et al.[3] have interpreted their results relative to tumor formation in terms of steric competition between carcinogen and steroid hormone. Thus, a depletion of steroid hormones brought about as a result of cellular necrosis has an enhancing effect on carcinogenesis by reducing the competitive inhibition on DMBA which would normally be exerted by the presence of these hormones. In order to test a portion of this hypothesis, i.e. depletion of steroid hormones we have measured the concentration of plasma corticosterone (CCS) in DMBA-treated animals. In this communication we wish to present the results of our investigations.

Materials and methods. Female Sprague-Dawley rats, 140–160 g, were obtained from a commercial supply house and maintained on laboratory chow and water ad libitum in appropriate animal quarters. At age 50 days, termed day 0, the experimental animals received, via stomach tube, 20 mg of purified DMBA dissolved in 1.0 ml of sesame oil. Control animals were given an equivalent amount of sesame oil in a similar manner.

On day 0, and for 6 consecutive days, blood for CCS determination was obtained by cardiac puncture and the plasma separated immediately by centrifugation. Plasma CCS was determined by a modification of the procedures of SILBER et al.[4] and GUILLEMIN et al.[5]. Plasma samples were pre-extracted with 3–5 volumes of petroleum ether and measured 1.0–3.0 ml aliquots were taken for analysis. The aliquots were extracted with 10.0 ml of dichloromethane. Following plasma aspiration the dichloromethane was extracted with the fluorescent reagent, sulfuric acid-ethanol, 3:1, V/V. This solution was transferred to matched cuvettes and read 30 min later in a Turner Fluorometer, Model 111, with a primary filter No. 47B and secondary filters Nos. 2A12 and 58. In order to be certain CCS was the steroid being measured the procedure was checked by various controls. Addition of known amounts of CCS to plasma was measured by the same technique as well as measurements of recovery from saline samples. Additionally, approximately 50,000 dpm of radioactive CCS was added to plasma samples, extracted, chromatographed in the Bush B_5 system[6] and scanned with a Vanguard Radiochromatogram Scanner for localization on the paper strips. Cold standards of several adrenal steroids were detected on these and parallel strips by their absorbance under UV-light at 240 μm.

Results. The data for plasma CCS levels of control and experimental animals are shown in the Table. Average values for the control animals, 15 μg/100 ml, remained unchanged throughout the period of the experiment. In the experimental series there was a rapid depletion of plasma CCS during the initial part of the experimental period. Recovery experiments with standard CCS showed our technique to be 85–92% efficient for the recovery of known amounts of crystalline steroid from rat plasma. Additionally, by the use of the ^{14}C-labeled CCS we found we could recover 90–94% of our starting material. Radiochromatographic analysis of the dichloromethane extracts

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