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IMMUNODEPRESSION IN THYROID-DEPRIVED ANIMALS

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SUMMARY

The removal of the thyroid gland in newborn or in young adult rats causes reduction in the number of peripheral blood lymphocytes and depression of either the humoral immune reactions against sheep and chicken red blood cells or the response of spleen cells to phytohaemagglutinin. These immunological defects are fully established in perinatally thyroidectomized rats after weaning, while in animals thyroidectomized in young-adult age they are observable 45–60 days after the operation. In both cases daily injections with thyroxine can completely restore the lymphoid system of thyroid-deprived animals. These results stress the importance of thyroid hormones for lymphocyte proliferation either during the period of ontogenetic development or in adult life.

INTRODUCTION

Recent studies have shown that the ontogenetic development of lymphoid tissues is dependent on the integrity of the endocrine system (Pierpaoli, Fabris & Sorkin, 1970).

Congenital deficiency of both somatotropic hormone and thyroxine causes in Snell dwarf mice underdevelopment of the thymus and of thymus-dependent functions (Fabris, Pierpaoli & Sorkin, 1971a). Treatment of dwarf mice with somatotropic hormone and/or thyroxine results in reconstitution of the immune capacity (Fabris, Pierpaoli & Sorkin, 1971b), and prolongation of their life-span (Fabris, Pierpaoli & Sorkin, 1972).

While the direct action of somatotropic hormone on the lymphoid tissues has been proven in different experimental models, the role played by thyroxine is not clearly understood. Administration of exogenous thyroxine to experimental animals results in enlargement of both central and peripheral lymphoid organs; the outflow of small lymphocytes from the thymus is particularly increased during treatment with thyroxine (Ernström & Larsson, 1966).

Moreover, it has been shown that in hyperthyroid patients the number of peripheral blood lymphocytes is frequently increased, the lymphoid organs are enlarged and the incidence of malignant lymphomas is higher than in randomly chosen patients suffering

from diseases not involving the thyroid gland (Ultman, Hyman & Burton Calder, 1963).

On the other hand, few observations have been made on the efficiency of the lymphoid system in hypothyroid patients. It has been shown, however, that the removal of the thyroid gland in experimental animals induces hypotrophy of the whole lymphoid system (Lundin, 1958). Humoral immune responses against sheep red blood cells are greatly impaired (Pierpaoli, Fabris & Sorkin, 1971), while cell-mediated immunity, as measured by the survival of allogenic skin-grafts, remains unaffected (Pierpaoli *et al.*, 1970).

In the present paper, the immunological consequences of the removal of the thyroid gland either in newborn or in young adult rats are summarized. The effect of thyroidectomy will be discussed in relation to the ontogenetic development and the maintenance of the function of the immune system.

MATERIALS AND METHODS

Operative procedures

Charles River rats were used as experimental animals. The thyroid gland was removed in young adult rats under Thiogenal (Merck, Germany) anaesthesia according to the method of Ingle & Griffith (1962). Thyroidectomy in newborn rats was carried out under the stereo-microscope. Sham-operated littermates were used as controls. Thyroidectomized rats were kept at +26°C. Completeness of operation was judged by histological examination for thyroid remnants. Those animals which showed such remnants were discarded.

Histological and haematological tests

Peripheral white blood cells (PWBC) were counted by bleeding the animals from the tail; differential counts were performed on smears stained with May–Grünwald–Giemsa. Lymphoid organs were fixed in Carnoy's fluid and stained with Haematoxylin and Eosin.

Humoral immune responses

Primary humoral immune responses against sheep red blood cells (SRBC) or chicken red blood cells (CRBC) were measured after one i.p. injection with 0·1 ml/100 g body weight of 20% erythrocyte suspension in saline. Haemagglutinins were measured on Takatsy plates. Plaque-forming cells (PFC) against SRBC were evaluated by plating $0{\cdot}5 \times 10^6$ spleen cells with 0·1 ml of 10% SRBC suspension in agar Petri dishes, and adding, after incubation, lyophilized guinea-pig serum diluted 1 : 20.

PHA response

Spleen cells were obtained by teasing the spleen with fine forceps in cold Eagle's minimum essential medium (MEM). Further disruption was achieved by gentle aspiration with a Pasteur pipette. After sedimentation for 10 min the supernatant was centrifuged and the cells washed with Eagle's solution. Cells were finally resuspended and the number of viable ones determined by Trypan Blue exclusion.

The cell suspension was diluted to 1×10^6 viable cells/ml with Eagle's minimum essential medium supplemented with vitamins (Gibco, Grand Island, N.Y., U.S.A.), essential and non-essential amino acids (Gibco), glutamine (Gibco) and 10% foetal calf serum (Wellcome batch No. 4251). The cultures were incubated in 12×50 mm glass tubes at 2 ml/tube. Unless otherwise stated, 50 μl of PHA (Difco) was finally added. [^{3}H]Thymidine (^{3}H-TD)

(Amersham, specific activity 5000 mCi mM) was added in the amount of 0·5 μCi/tube at 48 hr of culture. The amount of tritiated thymidine incorporated by the cells was evaluated in a Tri-Carb (Packard) liquid scintillation counter.

Hormone preparation

L-Thyroxine (Hoffman–La Roche, Basel, Switzerland) was dissolved in alkalinized physiological saline and injected subcutaneously at the daily dose of 1 μg/10 g body weight.

RESULTS

(A) *Effect of perinatal thyroidectomy*

The removal of the thyroid gland in perinatal age does not affect body growth within the first 3 weeks of life, e.g. before weaning; in the following periods of life, however, the rate of body growth is progressively reduced until complete arrest within 3 weeks of weaning (Table 1). At this time many thyroidectomized animals show symptoms similar to those observed in 'wasting' rats after neonatal thymectomy; they lose weight and die within a few weeks.

TABLE 1. Absolute and relative weights of lymphoid organs in perinatally thyroidectomized or sham-operated rats

Animals	Age at killing (days)	Body weight (g) at killing	Thymus weight		Spleen weight	
			(mg)	(%)	(mg)	(%)
Thyroid	30	35	88	0·251	124	0·354
Sham-operated	30	67	275	0·410	416	0·621
Thyroid	60	37	43	0·116	75	0·203
Sham-operated	60	247	475	0·192	884	0·357

Some thyroidectomized animals, however, recover the normal growth rate without showing any sign of wasting. Such a recovery is due to a compensatory hyperplasia of thyroid remnants which can be detected by histological examination.

(a) *Size and structure of lymphoid organs.* In perinatally thyroidectomized rats both the absolute and the relative weight of the thymus are significantly reduced when compared to normal values (Table 1). Such an involution is progressive and reaches its maximum at about 40–60 days of age in those thyroidectomized animals which show symptoms of wasting. Morphologically, the thickness of the thymus cortex is reduced in thyroidectomized rats (Fig. 1a) when compared to that of sham-operated controls (Fig. 1b).

Spleen and lymph nodes are also reduced in size and weight. The degree of hypotrophy of peripheral lymphoid tissues is more pronounced than that observed on the thymus of the same animal (Table 1). Histologically, the spleen of thyroidectomized animals shows a clear-cut reduction of cellularity, both in the white and in the red pulp.

(b) *Peripheral white blood cells* (PWBC). The number of PWBC is reduced in thyroidectomized animals. In particular, the progressive increase in the number of PWBC in the post-weaning period is absent. The low PWBC count is due to the reduction of the number

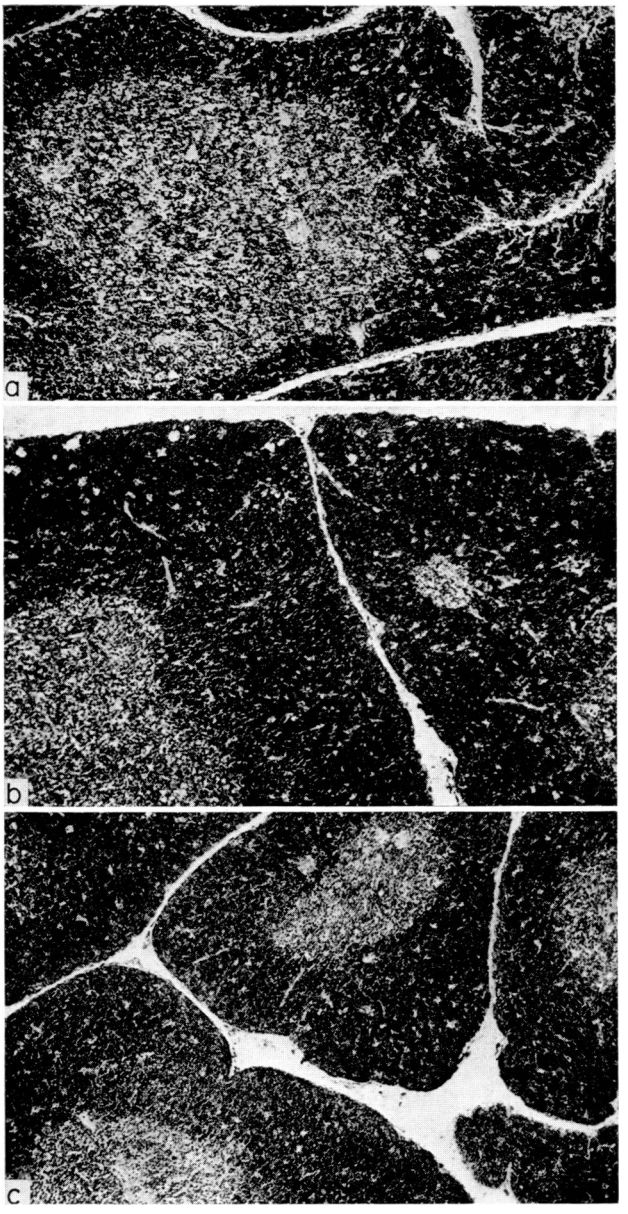

FIG. 1. Effect of perinatal thyroidectomy on thymus structure. Note the reduction of thickness of the thymus cortex in (a) thyroid-deprived animals when compared to (b) sham-operated littermates. Such a reduction is prevented by daily treatment with (c) thyroxine (10 μg/100 g body weight). (H & E, × 126.)

of peripheral lymphocytes. Polymorphs are not significantly affected by thyroidectomy (Fig. 2).

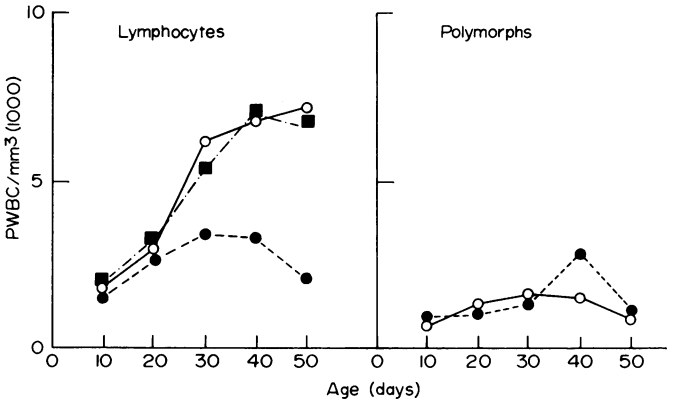

FIG. 2. Effect of perinatal thyroidectomy on the number of peripheral white blood cells (PWBC) evaluated at different times after birth. (●- - ●) Thyroidectomized rats; (○) sham-operated controls; (■- · - ■) thyroidectomized rats daily injected with thyroxine (10 μg/100 g body weight).

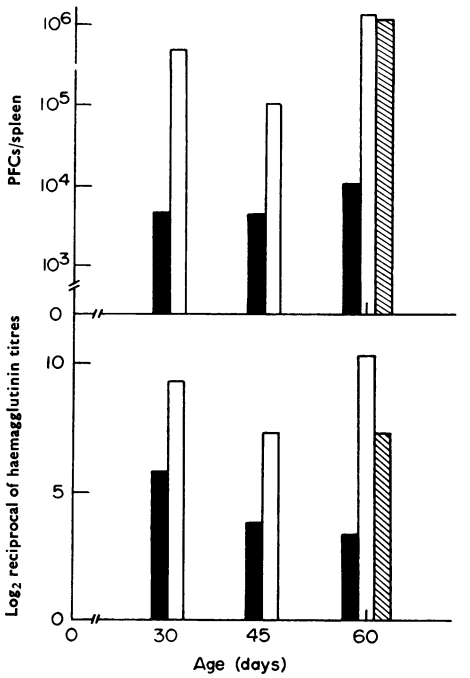

FIG. 3. Effect of perinatal thyroidectomy on the number of PFCs/spleen and on the haemagglutinin titres, evaluated at different times after birth. Rats were immunized i.p. with 0·1 ml of 20% sheep red blood cells suspension/100 g body weight 4 days before killing. (■) Thyroidectomized rats; (□) sham-operated controls; (▨) thyroidectomized rats daily injected with thyroxine 10 μg/100 g body weight.

(c) *Humoral immune response to SRBC.* The number of PFCs per spleen 4 days after immunization with SRBC is reduced in animals thyroidectomized at birth and killed at different ages after weaning (Fig. 3). Also the number of PFCs per million nucleated spleen cells is reduced (Fig. 4), indicating that the generation of PFCs is more sensitive to thyroxine deficiency than the maintenance of spleen cells. Nevertheless, it is to be noted that the total number of nucleated cells in the spleen of thyroidectomized rats is generally reduced to a greater extent than would be expected from the impairment of body growth (Fig. 4).

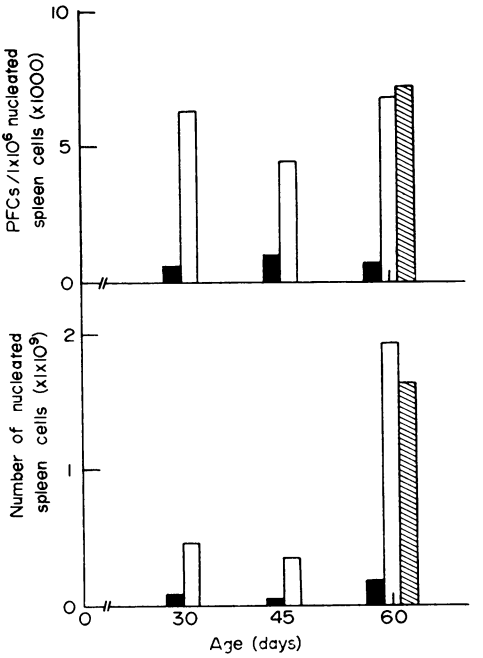

FIG. 4. Effect of perinatal thyroidectomy on the number of nucleated cells in the spleen and on the number of PFC/1 × 10^6 spleen cells, evaluated at different times after birth. Rats were immunized i.p. with 0·1 ml of 20% sheep red blood cells suspension/100 g body weight 4 days before killing. (■) Thyroidectomized rats, (□) sham-operated controls; (▨) thyroidectomized rats daily injected with thyroxine (10 μg/100 g body weight).

The antibody titres present in the blood of thyroidectomized rats are lower than those of sham-operated controls (Fig. 3). The difference between the two experimental groups is particularly significant at 60 days of age.

(d) *PHA response.* Spleen cells from perinatally thyroidectomized rats react to PHA *in vitro* to a lesser extent than spleen cells from sham-operated controls. DNA synthesis, as measured by ^{3}H-TD uptake, is reduced in stimulated cultures when donors of cells have been thyroidectomized in perinatal age and killed in the post-weaning period, while during lactation there are no differences between thyroidectomized and sham-operated controls (Fig. 5).

The deficient PHA response of spleen cells from thyroidectomized animals is independent of the concentration of PHA in the culture (Fig. 6).

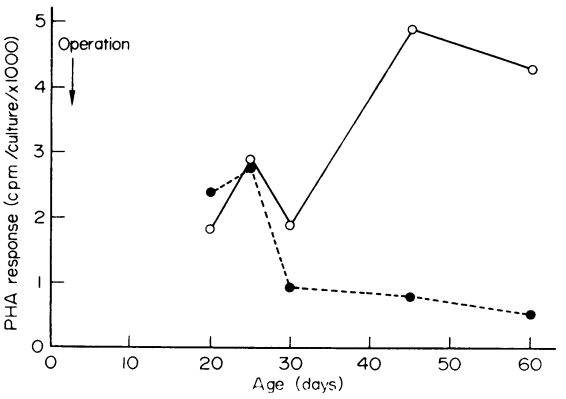

FIG. 5. Effect of perinatal thyroidectomy on the PHA response of 1×10^6 nucleated spleen cells at different times after birth. The reaction has been measured by ^{3}H-TD uptake during a 22-hr period, in 3-day cultures. Unstimulated cultures showed amounts of incorporated radioactivity below 100 cpm/culture. (●) Thyroidectomized rats; (○) sham-operated control.

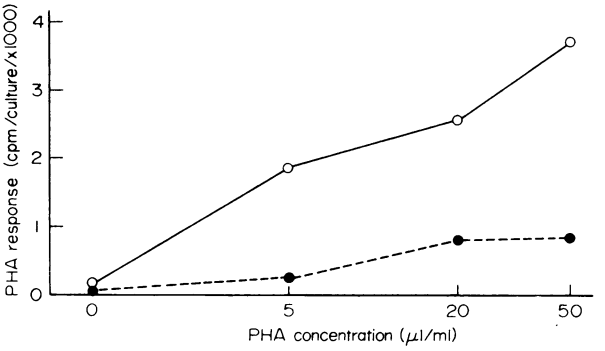

FIG. 6. PHA response of 1×10^6 spleen cells from perinatally (●) thyroidectomized or (○) sham-operated rats at different PHA concentrations.

(e) *Recovery of immunodepression by thyroxine.* The immunological defects in perinatally thyroidectomized rats can be prevented by daily injections with thyroxine starting from the day of operation. As shown in Figs 1c, 2, 3 and 4, treatment with thyroxine prevents the morphological alterations of thymus structure, the reduction of the number of peripheral lymphocytes and the depression of the humoral immune responses. Recent findings not included in the present paper, have also shown that the reduced PHA response of spleen cells from thyroidectomized rats is fully restored by administration of exogenous thyroxine. Moreover, it has been found that treatment of sham-operated rats with thyroxine increases their usual responsiveness either to SRBC or to PHA.

(B) *Effect of thyroidectomy in young-adult age*

Rats thyroidectomized at 60 days of age show a great impairment of body growth, starting from the day of operation. The diminished growth is not due to reduction of food consumption. Unpublished experiments have shown that the quantity of food eaten

per day per 100 g body weight by thyroidectomized animals is comparable to that taken up by sham-operated controls.

The mean life-span of the two experimental groups is slightly different: 26 months for sham-operated and 22 months for thyroidectomized rats. The body weight of thyroidectomized rats in adult age is only 30–40% of that of sham-operated controls.

(a) *Size and structure of lymphoid organs.* The absolute weight of the thymus in animals thyroidectomized at 60 days of age and killed 45 or 60 days after the operation is reduced as compared to that of the thymus of sham-operated controls. The relative weights, however, are similar in the two experimental groups. The histological structure of the thymus in thyroidectomized animals appears normal. Both the absolute and relative weights of the spleen are reduced in thyroidectomized animals when compared to those of sham-operated littermates. The histological examination of the spleen and lymphnodes, however, does not show significant alterations.

(b) *PWBC number.* The number of PWBC is reduced in thyroidectomized animals to about 50 % of normal levels. Such a reduction is mainly due to lymphocytes, while polymorphs are quantitatively unaffected. Eosinophils are, however, increased over the usual percentage.

(c) *Humoral immune response.* Plaque-forming capacity is impaired in thyroidectomized animals tested 45–60 days after the operation (Pierpaoli *et al.*, 1971). Similarly, the haemagglutination titres are reduced when compared to normal values (Fig. 7).

The deficiency of antibody synthesis is present only when animals are tested 45 or 60 days after the operation. As reported in Fig. 7, thyroidectomized animals challenged with SRBC shortly after the operation react with a consistent antibody production. The same animals, however, challenged with CRBC 45 days after the operation show deficient haemagglutinin formation. Similar results have been obtained in thyroidectomized animals tested shortly after the operation with CRBC and later on with SRBC.

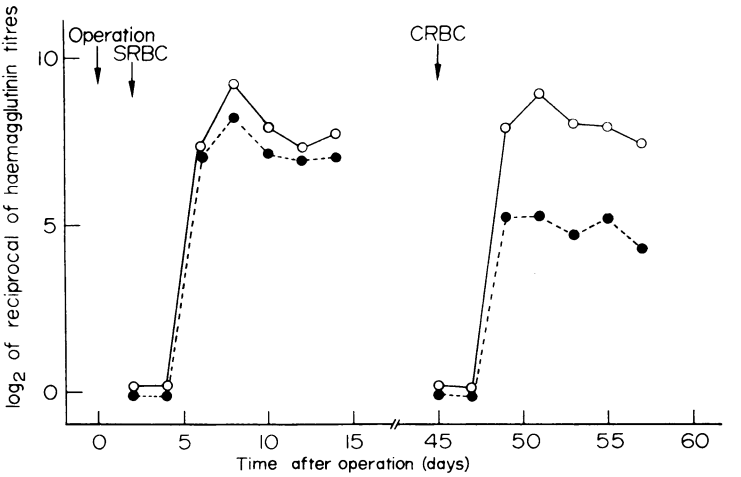

FIG. 7. Temporal relationship between thyroidectomy and decreased haemagglutinin production in young adult rats. (●) Adult thyroidectomized or (○) sham-operated rats were injected i.p. with 0·1 ml of 20% SRBC suspension/100 g body weight, 2 days after the operation. The same animals were immunized with 0·1 ml of 20% chicken red blood cells (CRBC) suspension, 45 days after the operation.

(d) *PHA response.* Thyroidectomy in young to adult age reduces the responsiveness of spleen cells to PHA. Such a deficiency is already established 2 months after the operation, and it is constantly observable throughout the residual life of the animals (Fig. 8). It is of interest to note, however, that the PHA response of spleen cells decreases progressively with advancing age both in thyroidectomized and in sham-operated animals.

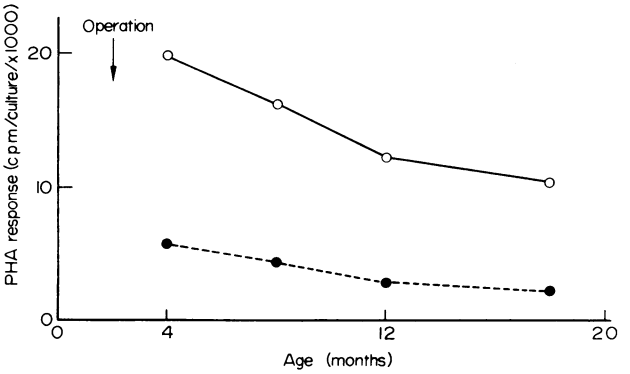

FIG. 8. PHA response of 2×10^6 nucleated spleen cells from (●) adult thyroidectomized or (○) sham-operated rats at different times after the operation. The reaction has been measured by ^{3}H-TD uptake during a 22-hr period, in 3-day cultures. Unstimulated cultures showed amounts of incorporated radioactivity below 300 cpm/culture.

DISCUSSION

The surgical removal of the thyroid gland in rats, depresses both antibody synthesis and phytohaemagglutinin response. Plaque-forming cells capacity against sheep red blood cells is significantly reduced. The production of haemagglutinins against either sheep or chicken red blood cells is lower in thyroidectomized than in sham-operated animals.

The phytohaemagglutinin response of spleen cells from thyroidectomized rats is reduced to about 25% of normal values. Moreover, the number of peripheral blood lymphocytes is quite low in thyroidectomized rats while polymorphs are not quantitatively modified. These immunological deficiencies are fully established 45–60 days after adult thyroidectomy, while in perinatally thyroidectomized rats they are observable after weaning.

In agreement with the findings obtained in hyperthyroid patients (Bogusz & Lisiewicz, 1968) or in experimental animals (Lundin, 1958; Ernström & Larsson, 1966) these data would indicate that thyroxine is required for the development and the maintenance of the efficiency of the immune system. This assumption is confirmed by the ability of thyroxine, when given daily after the operation, to prevent all the immunological defects observed in thyroidectomized rats.

It is of interest to note that a few weeks must elapse before the immunological deficiencies observed after adult thyroidectomy are fully established. The apparent delayed effect of thyroidectomy on the lymphoid system could be due to residual amounts of thyroxine present in various tissues during the first post-operative weeks. Another possible explanation for this delayed action is that the thyroid hormone has a greater effect on the number of responding cells and primarily on the number of precursor cells, rather than on their actual performance.

According to this interpretation, spleen cells from normal rats, transplanted into syngeneic lethally-irradiated thyroidectomized rats, should produce as many PFCs against SRBC as in sham-operated lethally-irradiated animals. This hypothesis is, at the present time, under investigation.

Preliminary experiments have also shown that the administration of exogenous thyroxine to normal animals significantly increases their capacity to respond either to sheep red blood cells or to phytohaemagglutinin (Fabris & Corda, unpublished experiments). This observation suggests further studies into whether or not administration of thyroxine also enhances the immunological reactivity in those pathological conditions in which such an effect could be therapeutically helpful.

Another interesting finding is that the consequences of perinatal and adult thyroidectomy are different. Many perinatally thyroidectomized animals undergo wasting-like disease, accompanied by reduction of the relative weight of the thymus and prolongation of allogeneic skin graft survival (Pierpaoli *et al.*, 1970). By contrast, adult thyroidectomized animals are relatively healthy for a long period of time, do not show alteration in thymus structure and weight and reject allogeneic skin-grafts in normal time (Pierpaoli *et al.*, 1970). Signs of wasting have never been observed.

The different consequences of perinatal and adult thyroidectomy in rats suggest that the hormonal requirements are higher during the ontogenetic development of the immune system than in adult life. The peripheral immunological deficiencies following thyroidectomy, however, are present to a similar extent in animals operated either in neonatal or in adult age. In other words the wasting-like disease observed in our animals does not seem to depend on those immunological deficiencies which can be experimentally measured by the antibody response or by the sensitivity to PHA. Only the survival of skin allograft is prolonged in perinatally operated rats, whereas it is normal in adult thyroidectomized animals (Pierpaoli *et al.*, 1970). One possible explanation for such a discrepancy is that thyroidectomy in perinatal age affects some functions of the thymus which are relevant for wasting-prevention (Pierpaoli & Sorkin, 1972a), but not necessarily linked either to the antibody synthesis or to the PHA response. Recent studies on athymic nude mice (Pierpaoli & Sorkin, 1972b) have in fact shown that the absence of the thymus during the ontogenetic development induces morphological abnormalities in the thyroid and in the adrenal gland. In our experimental model one could assume that the thyroid is acting on the lymphoid tissues and particularly on the thymus, leading on one hand to normal development of immunological functions, and on the other to physiological production of some thymus factors or cells which prevent wasting. In order to prove such an hypothesis, further experiments are needed.

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