Immunology, 1969, **16**, 217.

Hormones and Immunological Capacity

II. RECONSTITUTION OF ANTIBODY PRODUCTION IN HORMONALLY DEFICIENT MICE BY SOMATOTROPIC HORMONE, THYROTROPIC HORMONE AND THYROXIN

W. PIERPAOLI, C. BARONI, N. FABRIS AND E. SORKIN*

Schweizerisches Forschungsinstitut, Medizinische Abteilung, 7270 Davos-Platz, Switzerland; and Department of Morbid Anatomy, University of Pavia, Italy

(*Received* 16*th July* 1968)

Summary. Hypopituitary dwarf mice are immunologically deficient. This deficiency can be overcome by injection of somatotropic hormone and thyroxin. Antibody formation in hormonally reconstituted mice as measured by the number of plaque-forming cells against sheep erythrocytes equals or surpasses that of normal mice. The number of nucleated spleen cells is increased in both normal and dwarf mice after treatment with hormones. The hypotrophic thymus and peripheral lymphoid tissue of dwarf mice can be reconstituted to normal by treatment with somatotropic hormone and thyroxin.

Anti-somatotropic hormone and anti-thyrotropic hormone antisera produce suppression of antibody formation. These effects can be reversed by somatotropic hormone and thyrotropic hormone. The anti-hormone antisera produce an involution of thymus and other lymphatic organs. A parallelism exists between involution of the lymphoid tissue, neutralization of circulating somatotropic hormone and depression of antibody production.

These results stress the importance of the thymus–hypophysis relationship for cell differentiation with particular reference to the maturation of the immunological capacity.

INTRODUCTION

Recent studies have shown that the development of the thymo-lymphatic tissue in the neonatal and perinatal life of mice is dependent on the integrity of the endocrine system (Pierpaoli and Sorkin, 1967a, 1968a, b, c; Baroni, 1967; Baroni, Fabris and Bertoli, 1967).

Young mice injected with heterologous anti-pituitary serum (Pierpaoli and Sorkin, 1967a), or mice with a hereditary hypopituitary dwarfism (Baroni, 1967) develop a syndrome similar to the wasting disease observed in some strains of neonatally thymectomized mice. Such animals also show a marked involution of the thymus and peripheral lymphatic tissue or even complete atrophy (Pierpaoli and Sorkin, 1967a; Baroni, 1967).

The remarkable modifications in the acidophilic, growth-hormone producing cells of neonatally thymectomized mice (Pierpaoli and Sorkin, 1967b) and the above mentioned observations in dwarf mice and in mice treated with anti-pituitary serum suggest a link

* Reprint requests should be sent to Davos.

between the action of certain hormones, the state of the lymphatic system and immunological capacity. Such a relationship exists in dwarf mice, which have a low or absent immunological response, an involuted central and peripheral lymphatic tissue (Baroni, 1967) and low levels of growth hormone and thyroxin (Bartke, 1964, 1965; Smith and MacDowell, 1930, 1931; Lewis, Cheever and Vander Laan, 1965). Also the action of heterologous anti-somatotropic hormone antiserum and anti-thyrotropic hormone antiserum on the lymphatic cells of thymus and spleen in normal young mice (Pierpaoli and Sorkin 1968 b, c) suggests a critical relationship between somatotropic hormone and thyroxin, the thymus and peripheral lymphatic tissue growth, and the development of the immunological capacity. We have proposed that these hormones may cause a proliferation and differentiation of cells in the thymus and of thymus-dependent precursor cells to antigen-sensitive cells (Pierpaoli and Sorkin, 1968b).

The following experiments were designed to study further the relationship between the hormones and the immunological capacity. Dwarf mice are admirably suited for this purpose since they are both immunological and hormonal cripples. If the immunological reactivity is growth hormone and possibly thyroxin-dependent, then a reconstitution of the immune response could be expected by application of these hormones. For this purpose dwarf mice were treated with somatotropic hormone or thyroxin or both together. The primary immune response to sheep red cells was measured and the effect on the morphology and repopulation of thymus and peripheral lymphatic organs was evaluated.

In other experiments normal young mice were treated with anti-somatotropic hormone (A-STH) or anti-thyrotropic hormone (A-TTH) antisera alone or together with the corresponding hormones and the formation of precipitins to the antigenic substances contained in these anti-hormone antisera was measured. A relationship was established between the action of somatotropic hormone (STH) and thyrotropic hormone (TTH), reconstitution and growth of the lymphoid tissue and the antibody-producing capacity.

MATERIALS AND METHODS

Animals

Groups of 30- and 40-day-old normal or dwarf, male and female Snell–Bagg mice (genetic symbol *dw*) raised in our laboratories were used. The dwarf mice used in the present investigation are the descendants from a mating made in 1962 between an outbred BALB/c male mouse and an inbred Snell–Bagg female, whose colony was originally obtained in 1959 from the Jackson Memorial Laboratory, Bar Harbour, Maine, U.S.A. The new colony was maintained as an inbred strain of mice and the first dwarf appeared in the F_3 generation. In our conditions of breeding and management the life span of the dwarf mice ranges on the average from 45 to 65 days. Fig. 1 shows a hereditary pituitary Snell–Bagg dwarf mouse and its normal littermate. Fig. 2 shows the spleen and thymus of both animals.

Groups of inbred, young male Charles River mice (G. Wander AG, Bern, Switzerland) were used in the experiments with anti-hormone antisera.

Hormone preparations

Raben-type bovine somatotropic hormone (STH) containing 1 USP unit/mg and bovine thyrotropic hormone (TTH) in 10 i.u. vials were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A. For injection into mice, fresh solutions of STH

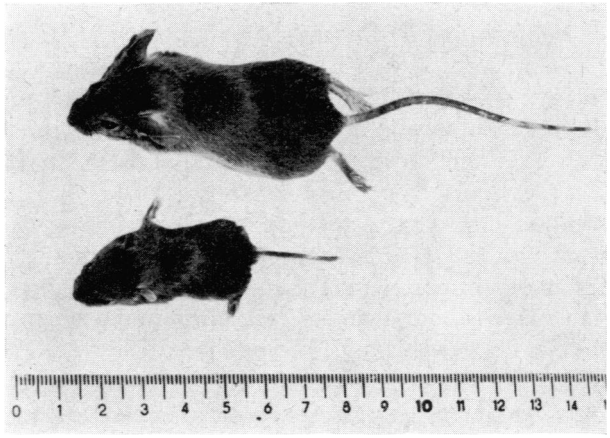

FIG. 1. Hereditary pituitary Snell–Bagg dwarf mouse, 40 days old, and the normal littermate.

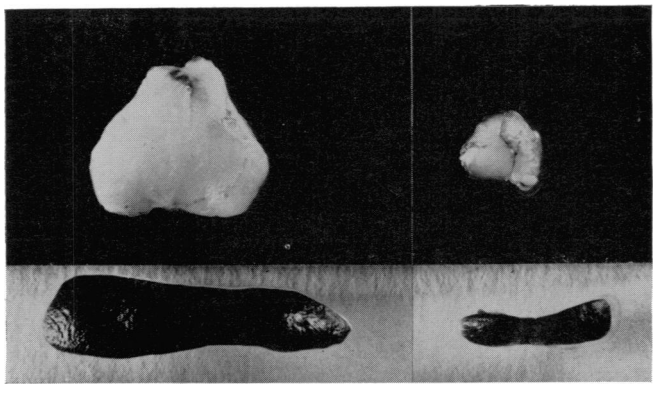

FIG. 2. Spleen and thymus of the dwarf mouse (right) and of the normal littermate (left) shown in Fig. 1.

were prepared daily by dissolving it in distilled water at alkaline pH and bringing the pH to about 7·8 by addition of phosphate-buffer, pH 7·2. Thyrotropic hormone solutions containing 2 i.u./ml were prepared by dissolving the substance in 0·9 per cent NaCl. TTH solutions were stored at +5° and used within 2–3 days. A preparation of bovine STH containing 0·95 USP unit/mg (NIH-GH-B6) was kindly supplied by the Endocrinology Study Section, National Institutes of Health, Bethesda, Maryland, U.S.A., through the courtesy of Dr A. E. Wilhelmi of Emory University, Atlanta, Georgia, U.S.A. DL-Sodium thyroxin (Tx) purchased from Hoffmann-La Roche, Basle, Switzerland, was used in the experiments with normal and dwarf Snell–Bagg mice. Fresh solutions were prepared by dissolving it with distilled water at alkaline pH.

Preparation and titration of anti-somatotropic and anti-thyrotropic hormone antisera and globulins

These methods were described elsewhere (Pierpaoli and Sorkin, 1968c).

Haemagglutination inhibition test

A test for the measurement of circulating growth hormone in mice was developed. Sera from groups of mice treated with A-STH or normal rabbit serum (NRS) globulins were pooled separately. A volume of 0·9 ml of mouse serum was mixed with 0·1 ml of a high titre preparation of rabbit anti-Raben-type STH antiserum. This antiserum was prepared and titrated as described previously (Pierpaoli and Sorkin, 1968c). The mixtures were incubated at 37° for 2 hours, kept for a few hours at +4°, then centrifuged at +4° to eliminate the precipitate. The sera were then inactivated at +56° for 30 minutes and absorbed with fresh washed and packed sheep erythrocytes. Serial dilutions of the supernatant up to 1 : 20,480 were prepared in Takatsy plates with phosphate-buffer, pH 7·3. One drop of a 5 per cent suspension of formalinized and tanned sheep erythrocytes, coated with Raben-type bovine STH was added (Pierpaoli and Sorkin, 1968c) and the plates were left 2 hours at room temperature and overnight at +5° and finally read. Positive controls were included using the same high titre A-STH antiserum utilized for incubation with mouse sera.

Injection schedule for normal and dwarf Snell–Bagg mice

Groups of 30- to 40-day-old dwarf or normal mice were injected daily by the subcutaneous route with 2·5 or 5·0 μg, respectively, of sodium thyroxin (Tx) alone. Other groups of dwarf mice and normal mice received 100 or 200 μg, respectively, of either the Wilhelmi or Raben-type STH alone by intraperitoneal injection. Still other groups received simultaneously the corresponding doses of both hormones. Uninjected normal and dwarf mice served as controls.

Injection schedule for normal Charles River mice treated with A-STH, A-TTH and NRS globulins alone or in combination with STH or TTH

Groups of four to five young male mice were injected intraperitoneally for 6 consecutive days with 0·4–0·6 ml of the concentrated globulin preparations containing 7·0–11·0 g of protein per 100 ml. The dose injected was modified according to the capacity of the A-STH and A-TTH globulin preparations to inhibit body growth. On day 7 the animals were bled and killed. Some groups of mice injected with A-STH or A-TTH globulins were simultaneously injected subcutaneously with 0·5–1·0 units of Raben-type STH or 0·5–1·0 units of TTH. The dose of the hormones was varied according to their capacity to counteract the inhibitory effect of A-STH or A-TTH globulins on body growth.

Body and organ weights

Body weights were measured daily. The thymus and spleen of Snell–Bagg mice were weighed after killing the animals. To compare the lymphoid tissues with a non-lymphoid organ the hearts were also weighed. Some of the organs and lymph nodes were fixed in 5 per cent neutralized formalin, or in Carnoy fixative and kept for histological examination.

Evaluation of number of plaque forming cells (PFC) and of nucleated spleen cells in normal and dwarf Snell–Bagg mice

In the experiments on the primary immune response to sheep red cells, groups of normal or dwarf mice were injected for 10 or 20 days with the hormones at the doses indicated above. On day 6 or day 16 after the beginning of hormone treatment, the treated dwarf mice and the untreated dwarf mice were inoculated intraperitoneally with 2×10^8 sheep

erythrocytes. Normal hormone treated or untreated Snell–Bagg mice also received 2×10^8 sheep red cells. The hormone treatment was continued for another 4 days after the injection of red cells. The mice were then killed, the spleens were removed and weighed and cell suspensions prepared from each spleen. The number of nucleated spleen cells was counted and the number of plaque-forming cells was determined by the Jerne technique (Jerne, Nordin and Henry, 1963).

Precipitin formation in Charles River mice treated with A-STH-, A-TTH-globulins alone or together with STH or TTH

The mice were treated for 6 days and killed on the 7th day. Sera were obtained from individual mice. Equal amounts of serum and phosphate-buffer, pH 7·3 (0·45 ml each), were mixed and serial dilutions up to 1 : 256 were prepared. A volume of 0·1 ml of concentrated NRS-globulins (= antigen) was added to the mouse serum dilutions, the tubes were shaken and incubated for 2 hours at +37°. The tubes were then left overnight at +5°, centrifuged in the cold and the results were read. The last tube showing a visible precipitate was taken as the end-point.

Histology

Histological examination was carried out on thymus, spleen and lymph nodes of the dwarf mice and normal Snell–Bagg mice. Sections were stained with methyl-green–pyronin. Sections of lymphatic organs of Charles River mice were also prepared, and stained with haemotoxylin and eosin.

RESULTS

EFFECTS OF HORMONE TREATMENT IN NORMAL AND DWARF SNELL–BAGG MICE

(a) Effect of somatotropic hormone and thyroxin on antibody formation (PFC) and number of nucleated spleen cells

Fig. 3(a) and (b) shows the effect of 10 or 20 days treatment with somatotropic hormone and thyroxin alone or in combination. In both groups of dwarf mice injection of STH increases significantly the number of plaque forming cells, while treatment with Tx alone does not increase the number of PFC as compared with the untreated dwarf mice. In contrast, treatment with Tx alone produces an increase in number of nucleated cells in the spleen. This effect on numbers of cells in the spleen is also evident after 20 days treatment with STH alone (Fig. 3a and b). Simultaneous injection of both hormones completely restores the number of plaque-forming cells of dwarf mice to the level observed in normal mice (Fig. 3a and b). The synergistic effect of STH and Tx on the restoration of the primary immune response in dwarf mice is particularly striking in dwarf mice treated for 20 days, when the number of PFC reaches an even higher value than normal hormone treated or untreated mice. It is also evident that the number of nucleated spleen cells is lower in the STH plus Tx-treated dwarf mice than in the group receiving Tx alone.

Hormone treatment of normal Snell–Bagg mice for 10 or 20 days shows that STH or Tx alone results in an evident increase of the number of nucleated spleen cells in both groups. Combined treatment with both hormones produces effects similar to the individual hormones alone. STH or thyroxin treatment increases the number of PFC, however thyroxin is more potent. Combined treatment with both hormones produces a slight increment in number of PFC in animals treated for 10 days, while in animals treated for 20 days the number of PFC is decreased if compared with normal untreated mice (Fig. 3a and b).

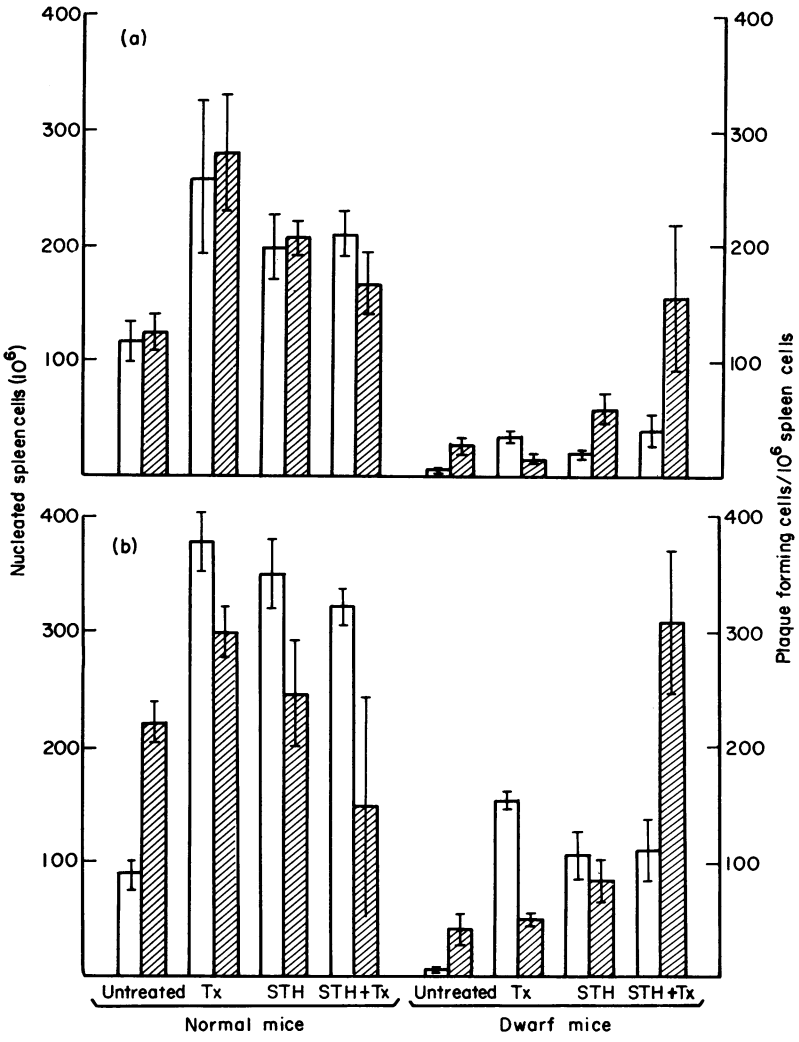

FIG. 3. (a) Effect of somatotropic hormone (STH) alone, thyroxine (Tx) alone or somatotropic hormone and thyroxin together on restoration of antibody formation (PFC, hatched columns) and number of nucleated spleen cells (open columns) in dwarf Snell–Bagg mice. Dwarf mice, 20 days old, were injected subcutaneously daily for 10 days with 100 μg STH and/or 2·5 μg Tx. Sheep erythrocytes (2×10^8) were injected intraperitoneally on day 6 after the beginning of the hormone treatment. The number of plaque forming cells (PFC) in spleen was determined 4 days later. (b) as (a) except that 20-days-old mice were treated for 20 days with the hormones and sheep red cells were injected 16 days after the beginning of the hormone treatment. The number of nucleated spleen cells was also determined. Identically treated normal Snell–Bagg mice and untreated dwarf and normal mice served as controls.

(b) Effect of somatotropic hormone and thyroxin on weight increase of lymphatic tissues and heart

Table 1 shows the effects induced by 10 or 20 days treatment with hormones on body weight increment and on spleen, thymus and heart weights of dwarf mice. It can be seen that lymphatic tissues of dwarf mice are far more sensitive to growth hormone and thyroxin than heart tissue. The most pronounced effect on spleen weight was produced in the dwarfs after 20 days of hormone treatment. It seems that this effect is dependent on

TABLE 1

EFFECT OF SOMATOTROPIC HORMONE AND THYROXIN ON BODY WEIGHT INCREMENT AND ON WEIGHT INCREASE OF LYMPHATIC TISSUE AND HEART OF HEREDITARY PITUITARY DWARF SNELL–BAGG MICE

Treatment of mice	Age at killing (days)	No. of animals	Days of treatment	Body weight		Thymus weight		Spleen weight		Heart weight	
				Initial	Final	mg	% body weight	mg	% body weight	mg	% body weight
Normal untreated	30	13	—	8·2±1·1	13·2±1·3	63±7	0·48±0·08	129±11	0·98±0·05	91±10	0·71±0·08
Dwarf untreated	30	5	—	4·1±0·6	3·9±0·9	8±2	0·19±0·05	13±2	0·31±0·03	34±5	0·84±0·11
Dwarf+Tx	30	8	10	5·1±0·3	6·8±0·6	22±4	0·29±0·04	45±5	0·65±0·06	68±6	1·02±0·06
Dwarf+STH	30	11	10	5·2±1·5	5·9±2·4	11±1	0·19±0·02	37±5	0·63±0·08	45±3	0·75±0·09
Dwarf+STH+Tx	30	5	10	4·4±0·4	6·4±1·3	22±4	0·37±0·07	55±10	0·86±0·11	64±7	1·03±0·09
Normal untreated	40	7	—	9·3±1·1	15·8±1·2	63±4	0·40±0·05	134±14	0·89±0·09	89±9	0·56±0·05
Dwarf untreated	40	7	—	4·2±0·4	4·6±0·6	7±1	0·15±0·02	12±2	0·26±0·03	24±4	0·50±0·04
Dwarf+Tx	40	4	20	4·7±0·5	10·0±0·8	38±4	0·38±0·07	105±8	1·05±0·10	97+9	0·97±0·12
Dwarf+STH	40	4	20	3·7±0·5	9·3±1·0	39±6	0·44±0·08	72±10	0·82±0·17	57±4	0·64±0·08
Dwarf+STH+Tx	40	5	20	5·4±0·3	9·8±0·9	34±5	0·32±0·07	103±12	1·05±0·14	109±9	1·13±0·11

The 20- or 30-day-old mice were injected daily subcutaneously for 10 or 20 days with 2·5 μg of DL-sodium thyroxin (Tx) and /or 100 μg of Wilhelmi bovine somatotropic hormone (STH).

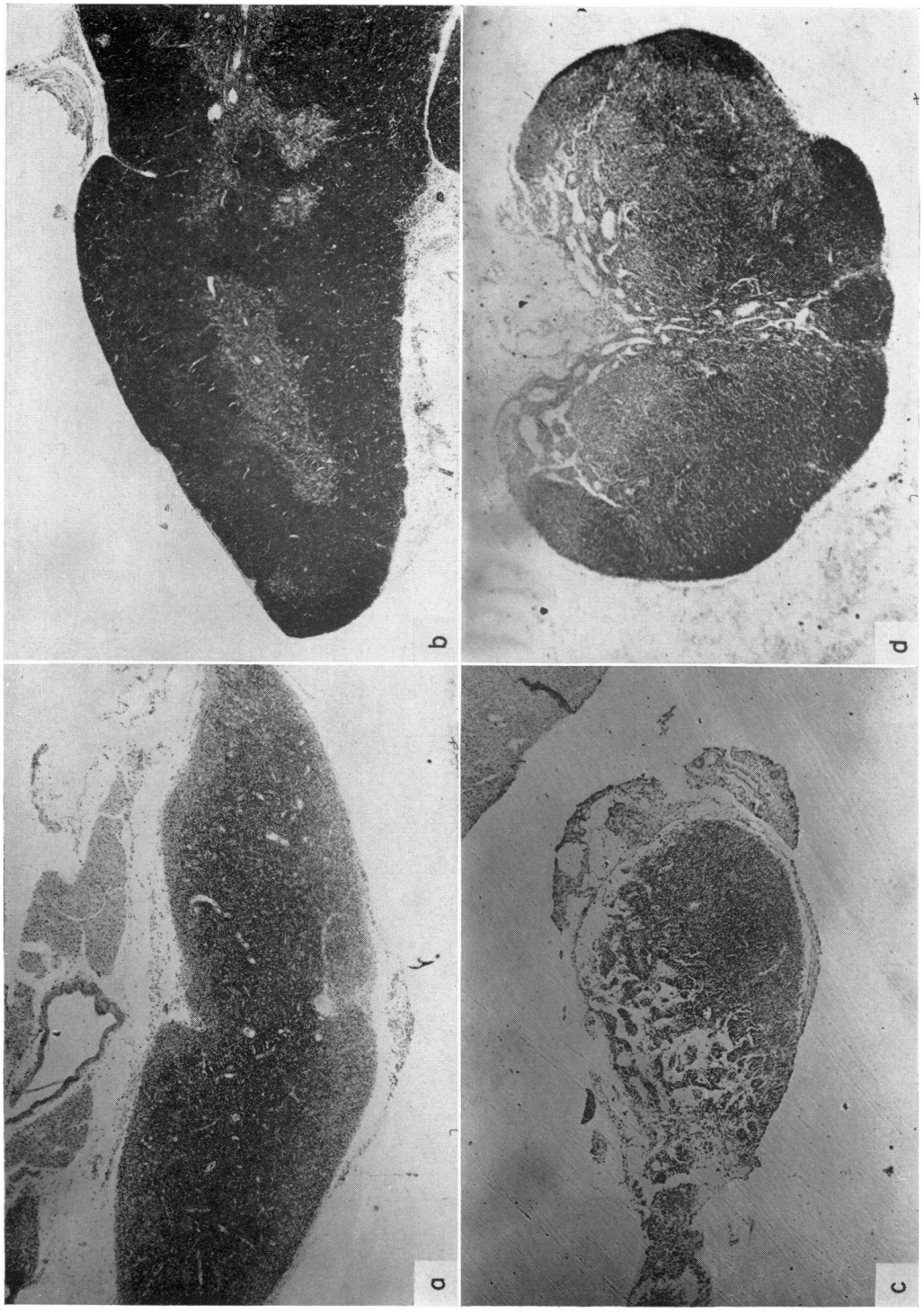

FIG. 4. (a) Section of thymus lobe of an untreated 30-days-old dwarf Snell–Bagg mouse. Note complete atrophy and absence of lymphoid cells in the cortex. (b) Section of thymus of a 30-day-old dwarf Snell–Bagg mouse after 10 days treatment with 100 μg bovine somatotropic hormone and 2·5 μg thyroxin. Note complete reconstitution of the thymus size, structure and repopulation of thymus cortex. (c) Section of a lymph node of the untreated dwarf mouse described in (a). Note absence of lymphoid cells and germinal centres. (d) Section of a lymph node of the hormone-treated dwarf mouse described in (b). Note repopulation of cortical and subcortical areas with lymphoid cells and numerous germinal follicles. Methyl-green–pyronin, × 32.

the proliferation of nucleated cells in the spleen (Table 1 and Fig. 3a and b). Groups of hormone-treated and untreated normal mice did not show significant differences in body growth and in lymphatic tissue and heart weight increment. Therefore, only results on untreated mice are reported in Table 1.

(c) Histology of thymus and peripheral lymphoid tissue

Fig. 4 (a) and (b) shows that in dwarf mice combined treatment with STH and Tx together for 10 days produces a repopulation of the thymus cortex and reconstitution to a normal structure. Combined hormone treatment of dwarf mice for 10 days shows that the peripheral lymphoid tissue is also responding to these hormones but lags behind the thymus reconstitution (Fig. 4c and d). If the treatment is prolonged, however, then full reconstitution of the lymphoid tissue is also obtained. Germinal centres in lymph nodes are increased in number and size and the medulla shows appearance of numerous pyroninophilic cells. Detailed results will be reported elsewhere (Baroni, Fabris and Bertoli, 1969).

EFFECT OF A-STH AND A-TTH GLOBULINS ON ANTIBODY FORMATION AND ON CENTRAL AND PERIPHERAL LYMPHOID ORGANS

Fig. 5 shows that injection of A-STH or A-TTH globulins into young male Charles River mice for 6 consecutive days completely inhibits the formation of precipitins against antigenic substances contained in these heterologous globulin preparations. Simultaneous inoculation of STH and A-STH globulins or of TTH and A-TTH globulins prevents the inhibition of the antibody forming capacity brought about by the anti-hormone globulins alone (Fig. 5). Surprisingly, injection of STH to A-TTH-treated mice also produces complete recovery of the immunological capacity. In the animals with an immune response inhibited by anti-hormone globulins a marked thymus and/or spleen involution was also observed. This was particularly evident in the thymus cortex, in the thymus-dependent areas of the spleen and in the perifollicular mantles of the splenic lymphoid follicles (Fig. 6a and b). Some of these results have been recently described (Pierpaoli and Sorkin, 1968b, c).

TABLE 2

RELATIONSHIP BETWEEN SERUM LEVELS OF SOMATOTROPIC HORMONE (STH), SPLEEN WEIGHTS AND PRECIPITIN FORMATION IN MICE

Test for serum STH in mice treated with:	Passive haemagglutination test (reciprocal of sera dilutions)	STH in mouse serum	Spleen weight (mg) (average of thirty mice)	Precipitin formation
Rabbit A-STH globulins (A)	1280	Absent	203 ± 15	Negative
Rabbit A-STH + STH (B)	20	Present	332 ± 18	+ + +
Normal rabbit serum globulins (C)	20–40	Present	267 ± 12	+ + +
Untreated	20–40	Present	153 ± 8	Negative
Rabbit A-STH serum control	1280			

Results of haemagglutination-inhibition test for measuring circulating STH in serum of mice. The animals had been treated for 6 consecutive days with rabbit-anti-Raben-type bovine somatotropic hormone (A-STH) globulins alone (A) or together with STH (B), or with the same quantity of normal rabbit serum globulins (C). The mouse sera were tested for their capacity to bind antibody against Raben-type STH by first incubating them with a high titre rabbit anti-Raben-type bovine STH serum. After centrifugation the supernatants were serially diluted and tested with STH-coated sheep erythrocytes for residual antibody against STH. The spleen weights are in mg ± standard error. For further details on techniques see under 'Materials and methods'.

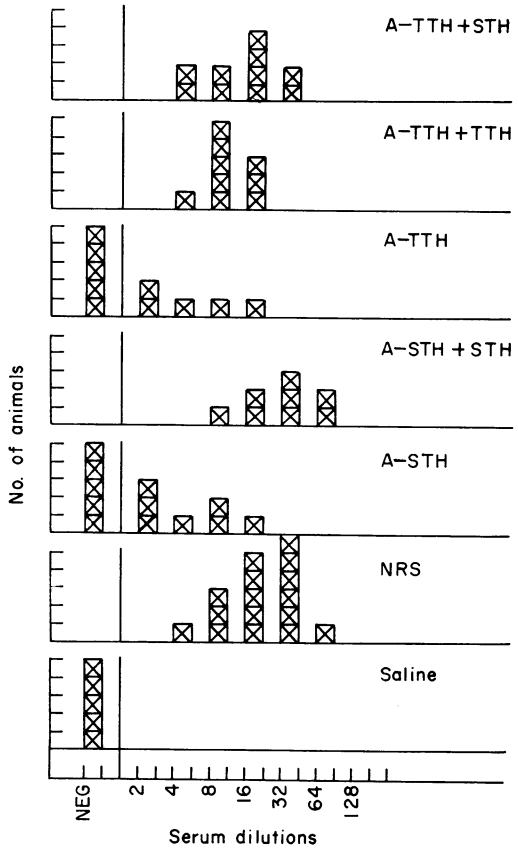

FIG. 5. Effect of injections of rabbit anti-Raben-type somatotropic hormone (A-STH) globulins or rabbit–anti-bovine thyrotropic hormone (A-TTH) globulins alone or together with somatotropic hormone (STH) or thyrotropic hormone (TTH) on the production of precipitating antibody in individual young male Charles River mice. Volumes of 0·4–0·6 ml of globulins (= 70–110 mg of protein per ml) were injected intraperitoneally for 6 consecutive days and the mice were killed on the 7th day. The control mice received the same quantity of NRS globulins. For reversal of the anti-hormone activity, 0·5–1·0 unit of STH or 0·5–1·0 unit of TTH was injected subcutaneously daily. The precipitin test was performed using normal rabbit serum globulins as antigen. Each cross in a square represents one animal and the reciprocal of the serum dilution at which the precipitin reaction was still positive.

HISTOLOGY OF A-STH-TREATED MICE

Fig. 6 (a), (b), (c) and (d) illustrates the appearance of the thymus and spleen of untreated normal Charles River mice and of mice injected with A-STH globulins, showing involution of thymus and splenic lymphoid tissue. These A-STH-treated mice showed inhibition of precipitin formation (Fig. 5).

ABSENCE OF CIRCULATING STH IN MICE INJECTED WITH A-STH GLOBULINS, AS SHOWN BY THE HAEMAGGLUTINATION INHIBITION TEST

The haemagglutination inhibition test indicates that mice injected with A-STH globulins fail to bind antibodies against Raben-type bovine STH, while the reaction was strongly inhibited by serum of untreated mice or of mice injected with the same quantities

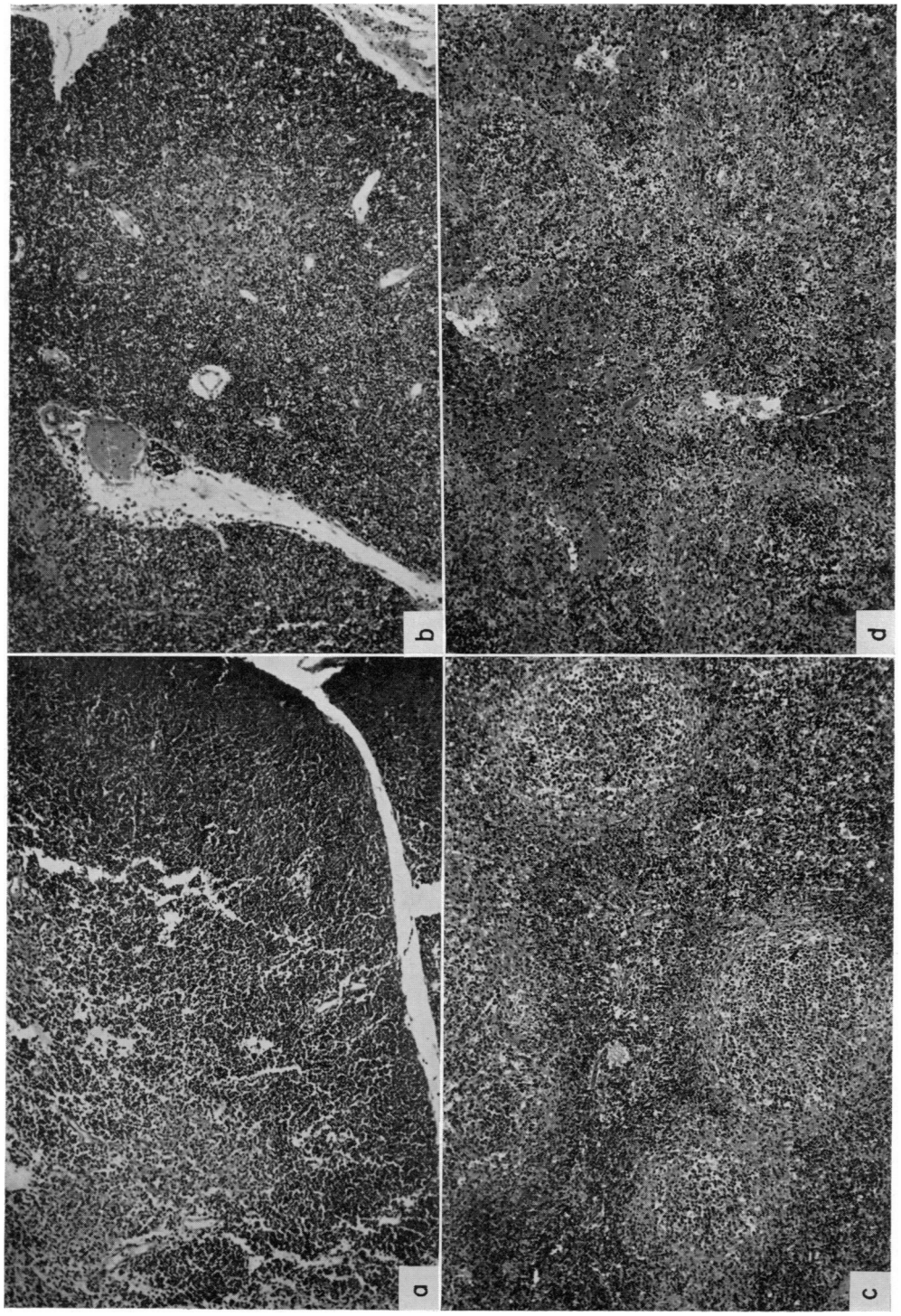

FIG. 6 (a) Section of the thymus of a 22-day-old Charles River mouse after 8 days of daily treatment with normal rabbit serum globulins. (b) Section of the thymus of a 22-day-old Charles River mouse, after 8 days of daily treatment with rabbit anti-bovine somatotropic hormone globulins. Note involution of the organ and loss of thymocytes in the cortex of this wasting mouse. (c) Section of spleen of mouse described under (a). Note presence of large lymphoid follicles. (d) Section of spleen of mouse described under (b). Note involution of the structure with loss of lymphoid cells and disappearance of lymphoid follicles after anti-somatotropic hormone globulins treatment. H & E, × 45.

of NRS globulins. Table 2 shows the results. These data also indicate the existence of a cross-reactivity between mouse STH and the Raben-type bovine STH preparation used for immunization of rabbits and reconstitution of dwarf mice. Mice injected with low titre A-STH globulin preparations had a normal immune reactivity and showed a normal splenic cellular reaction following the injection of these globulins. They also had circulating growth hormone as shown by the capacity of their sera to bind antibodies to STH (Table 2).

DISCUSSION

The data presented above indicate that both somatotropic and thyrotropic hormone and thyroxin are required for the development of central and peripheral lymphatic tissue and for the maturation of the immunological capacity. This is well illustrated by the fact that immunologically deficient dwarf mice can be fully reconstituted to normal by giving somatotropic hormone and thyroxin (Fig. 3a and b). Whereas untreated dwarf mice usually die presumably of infection between 45 and 65 days after birth, it was noted that hormonally reconstituted dwarf mice can live up to 1 year or even longer. This fact in itself suggests that the reconstitution leads to a permanent restoration of the immunological capacity. Preliminary experiments have also indicated that after 20 days of hormone treatment reconstituted dwarf mice do not lose their antibody forming capacity (PFC) for at least 30 days. The findings on the reconstitution of dwarf mice also agrees well with indications obtained earlier on the hormone-dependence of the development of thymus and peripheral lymphoid tissue. (Pierpaoli and Sorkin 1967a, b; Baroni, 1967; Pierpaoli and Sorkin 1968 b, c).

In addition the function of the immunological system in normal mice can be suppressed by giving anti-somatotropic hormone or anti-thyrotropic hormone antisera (Fig. 5). These sera or their globulins have no cytotoxic effect on thymocytes or lymph node cells as measured by the uptake of Trypan Blue by these cells.

The cells which seem to respond first to the hormone treatment in dwarf mice or to specific hormone inhibition in normal mice are the dividing small lymphoid cells in the thymus subcortical area, in the thymus-dependent areas of the spleen and in the perifollicular mantles in the spleen and lymph node germinal centres.

A previous attempt by Hayashida and Li (1957) to establish the significance of growth hormone for antibody production in normal rats did not succeed but they showed that STH can counteract the immunosuppressive effect of ACTH.

The recovery of the ability of immunologically deficient mice to produce antibody after STH and Tx treatment and challenge with antigen, and the inhibition or reconstitution of the precipitin-forming capacity in normal mice treated with A-STH or A-TTH globulins alone or together with STH or TTH are good evidence for this view. It is noteworthy that a complete reconstitution of the immune capacity in dwarf mice needs the presence of both hormones. In contrast complete inhibition of precipitin formation in normal mice can be obtained by interference with growth hormone activity alone. Surprisingly, it was found that STH alone can fully reconstitute the antibody-forming capacity in mice whose immune response has been completely inhibited by A-TTH globulins treatment (Fig. 5). The findings with dwarf mice that thyroxin increases the number of nucleated spleen cells (Table 1, Fig. 3a and b) can be interpreted that thyroxin is required for active reproduction of lymphoid cells and repopulation of thymus and peripheral lymphoid organs. Somatotropic hormone on the other hand seems to be needed for differentiation of lym-

phoid cells to antibody-producing plasma cells. Indeed it can be seen that while thyroxin alone is unable to increase the number of plaque forming cells as compared to untreated dwarf mice, STH alone induces a definite increase of PFC. The simultaneous administration of STH and Tx does not further increase the number of nucleated spleen cells but only the number of plaque forming cells. It can therefore be argued that there is a limit to the thyroxin-induced reproduction of lymphoid and other cells in the spleen beyond which antigen and STH are needed for further maturation to antibody-producing cells. At which stage STH is needed for the transformation of precursor cells into plasma cells is now being studied by us also in relation to the well known differentiating function of perinatal thymus (Doria and Agarossi, 1967; Miller and Mitchell, 1967; Mitchell and Miller, 1968).

The importance of thymus reconstitution by hormones in dwarf mice or of hormones in neonatally thymectomized mice for the recovery of the immediate and delayed-type hypersensitivity needs also be clarified.

The histological examination of lymphoid tissues of dwarf and normal mice shows that somatotropic hormone and thyroxin produce a reconstitution of the lymphoid organs (Figs. 4 and 6). This fact illustrates the parallelism between the cellular multiplication and differentiation of the lymphatic organs and the recovery of the antibody-producing capacity. We may suppose that the lymphoid cells are the substrate on which the effect of hormones or of anti-hormones is enacted.

From our own data and the results of other investigators on the pituitary-dependence of thymus growth and reproductive activity (Lundin, 1958; Takemoto, Yokoro, Furth and Cohen, 1962; Dougherty, Berliner, Schneebeli and Berliner, 1964; Ernström, 1965; Bearn, 1966, 1968; Pierpaoli and Sorkin, 1967a, b, 1968) it appears that the problem of thymus development and maturation of the immunological capacity should be studied in foetal stages of life, when the hormone-dependence of cell differentiation is of especially critical importance (Triplett, 1962; Yatvin, 1966).

ACKNOWLEDGMENTS

We thank Miss Laurence Schmocker and Mr W. Theilkäs for their excellent technical assistance. This work was supported by the Schweizerische Nationalfonds zur Förderung der Wissenschaftlichen Forschung (Gesuch Nummer 4518).

One of us (W.P.) is a Research fellow of the Italian National Research Council, Rome. C.B. is on leave of absence to the Salk Institute for Biological Studies, San Diego, California, U.S.A., and is recipient of a NATO-CNR fellowship from the Italian National Research Council, Rome.

REFERENCES

BARONI, C. (1967). 'Thymus, peripheral lymphoid tissues and immunological responsiveness of the pituitary dwarf mouse.' *Experientia*, **23**, 282.

BARONI, C., FABRIS, N. and BERTOLI, G. (1967). 'Age dependence of the primary immune response in the hereditary pituitary dwarf and normal Snell–Bagg mouse.' *Experientia*, **23**, 1059.

BARONI, C., FABRIS, N. and BERTOLI, G. (1969). 'Hormonal control of lymphoreticular tissue in Snell–Bagg pituitary dwarf mice.' *Second Conference on Germinal Centres* (Praha). Springer Verlag. (In press).

BARTKE, A. (1964). 'Histology of the anterior hypophysis, thyroid and gonads of two types of dwarf mice.' *Anat. Rec.*, **149**, 225.

BARTKE, A. (1965). 'The response of two types of dwarf-mice to growth hormone, thyrotropin and thyroxine.' *Gen. Comp. Endocrinol.*, **5**, 418.

BEARN, J. G. (1966). 'Influence of the pituitary-adrenal axis on development of the rat foetal thymus.' *Proc. Soc. exp. Biol. (N.Y.)*, **122**, 273.

BEARN, J. G. (1968). 'The thymus and the pituitary-adrenal axis in anencephaly. A correlation between experimental foetal endocrinology and human pathological observations.' *Brit. J. exp. Path.*, **49**, 136.

DORIA, G. and AGAROSSI, G. (1967). 'The effect of thymic action on the precursors of antigen-sensitive cells.' *Proc. nat. Acad. Sci. (Wash.)*, **58**, 1366.

DOUGHERTY, T. F., BERLINER, M. Z.; SCHNEEBELI, G. L. and BERLINER, D. L. (1964). 'Hormonal control of lymphatic structure and function.' *Ann. N.Y. Acad. Sci.*, **113**, 825.

ERNSTROEM, W. (1965). 'Studies on growth and cytomorphosis in the thymo-lymphatic system.' *Acta path. microbiol. scand.*, Suppl. 178.

HAYASHIDA, T. and LI, C. H. (1957). 'The influence of adrenocorticotropic and growth hormones on antibody formation.' *J. exp. Med.*, **105**, 93.

JERNE, N. K., NORDIN, A. A. and HENRY, C. (1963) *Cell-bound Antibodies* (Ed. by V. Defendi), Wistar Institute Symposium Monograph No. 3, p. 109. Wistar Institute Press, Philadelphia.

LEWIS, U. J., CHEEVER, E. V. and VANDERLAAN, W. P. (1965). 'Studies on the growth hormone of normal and dwarf mice.' *Endocrinology*, **76**, 210.

LUNDIN, P. M. (1958). 'Anterior pituitary gland and lymphoid tissue growth.' *Acta endocr.* (*Kbh.*), **28**, suppl. 40.

MILLER, J. F. A. P. and MITCHELL, G. F. (1967). 'The thymus and the precursors of antigen reactive cells.' *Nature* (*Lond.*), **216**, 659.

MITCHELL, G. F. and MILLER, J. F. A. P. (1968). 'Immunological activity of thymus and thoracic-duct lymphocytes.' *Proc. nat. Acad. Sci.* (*Wash.*), **59**, 296.

PIERPAOLI, W. and SORKIN, E. (1967a) 'Relationship between thymus and hypophysis.' *Nature* (*Lond.*), **215**, 834.

PIERPAOLI, W. and SORKIN, E. (1967). 'Cellular modifications in the hypophysis of neonatally thymectomized mice.' *Brit. J. exp. Path.*, **48**, 627.

PIERPAOLI, W. and SORKIN, E. (1968a). 'Effect of gonadectomy on the peripheral lymphatic tissue of neonatally thymectomized mice.' *Brit. J. exp. Path.*, **49**, 288.

PIERPAOLI, W. and SORKIN, E. (1968b). 'Effect of growth hormone and anti-growth hormone serum on the lymphatic tissue and the immune response.' *Symposium on The Immune Response and Its Suppression* (Ed. by E. Sorkin). Karger, Basel.

PIERPAOLI, W. and SORKIN, E. (1968c). 'Hormones and the immunological capacity. I. Effect of heterologous anti-growth hormone (ASTH) antiserum on thymus and peripheral lymphatic tissue in mice. Induction of a wasting syndrome.' *J. Immunol.*, (In press).

SMITH, P. E. and MACDOWELL, E. C. (1930). 'An hereditary anterior pituitary deficiency in the mouse.' *Anat. Rec.*, **46**, 249.

SMITH, P. E. and MACDOWELL, E. C. (1931). 'The differential effect of hereditary mouse dwarfism on the anterior pituitary hormones.' *Anat. Rec.*, **50**, 85.

TAKEMOTO, H., YOKORO, K., FURTH, J. and COHEN, A. I. (1962). 'Adrenotropic activity of mammosomatotropic tumors in rats and mice. I. Biologic aspects.' *Cancer Res.*, **22**, 917.

TRIPLETT, E. L. (1962). 'On the mechanism of immunologic selfrecognition.' *J. Immunol.*, **89**, 505.

YATVIN, M. B. (1966). 'Hypophyseal control of genetic expression during chick feather and skin differentiation.' *Science*, **153**, 184.