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EFFECT OF GROWTH HORMONE ON THE METABOLISM OF THYMUS AND ON THE IMMUNE RESPONSE AGAINST SHEEP ERYTHROCYTES

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A number of observations indicate that hormones have an important influence on immune processes. Androgens, when administered to developing chick embryos, cause a deletion of bursa (hormonal bursectomy) (1, 2). Corticoids have long been known to be immunodepressants (3, 4), even though more recent studies show that only a population of lymphocytes are sensitive to this hormone (5). Cortisol in high concentrations markedly inhibits the synthesis of RNA, DNA, and proteins in thymocytes both in vivo (6) and in vitro (7, 8). However, at low concentrations this steroid hormone has a stimulating effect on the mitotic activity of thymocytes (9). It has also been shown to be essential for the induction of antibody synthesis in an in vitro system (10). Estrogens are reported to improve the clearance rate and phagocytosis of bacteria, viruses, and particulate matter (11). In short, the steroid hormones in general and glucocorticoids in particular have an action on the lymphoid tissue, the type of effect varying with the dose of the hormone and the stage of differentiation of the target cells.

The action of pituitary somatotropin and thyroid hormones on lymphoid tissue have also been investigated. The relevance of pituitary to the development of the lymphoid organs became first evident with the experiments on Snell-Bagg strain of dwarf mice. Hypopituitary dwarf mice were found to be immunologically deficient as judged by the number of plaque-forming cells (PFC)[1] to sheep erythrocytes (SRBC), hemagglutination titers (HAT), and graft-*versus*-host reactions (12–14). When growth hormone was injected either alone or with thyroxine, there was an improvement in response (15). Kalden et al. (16) did not, however, find a significant difference in PFC and serum antibody titers after hypophysectomy of 21-day old rats, although hypophysectomy reduced the size of the lymphoid organs.

A reciprocal relationship between the pituitary and the thymus was suggested by Pierpaoli and Sorkin (17–19). Administration of anti-pituitary serum (17) or anti-growth hormone globulins (18) produced an atrophy of the thymus and wasting syndrome. On the other hand, neonatal thymectomy was observed to cause an early degranulation of acidophilic cells of the anterior pituitary (19, 20). In sublethally

[1] *Abbreviations used in this paper:* BGH, bovine growth hormone; GH, growth hormone; HAT, hemagglutination titers; LH, luteinizing hormone; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TSH, thyroid-stimulating hormone.

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irradiated, hypophysectomized animals, there is deficient recovery of total leukocyte counts, hemagglutination titers to SRBC, and skin allograft rejection. Suggestions have been made for the possible presence of a thymotropic hormone in the pituitary (21).

Whether the action of growth hormone is of a specific type on lymphoid tissue or is a mere potentiation of cellular metabolism in a general sense is not known. The aim of the present investigation was to elucidate further the role of pituitary growth hormone on immune processes and in particular on the metabolism of the thymus. The action of the hormone on the synthesis of a number of biopolymers in the cortical and medullary regions of the thymus has been determined. An in vitro system has been evolved that demonstrates a direct and rapid action of growth hormone on thymocytes. Some aspects of this work have been presented elsewhere (22, 23).

Materials and Methods

Animals.—Holtzman male rats were used for all experiments. Hypophysectomized rats (80–100 g) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. They were used 15 wk after hypophysectomy.

Hormones.—Bovine growth hormone (BGH) (NIH. GH. B13 and B15), bovine luteinizing hormone (NIH. LH. B7), and thyrotropic hormone (NIH. TSH. B4) were obtained from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md., (courtesy of Dr. Morris Graff and Dr. A. E. Wilhelmi). The hormones were dissolved in 0.9% NaCl adjusted to pH 8 with dilute sodium hydroxide. Insulin (24 units/mg) was obtained from Boots Pure Drug Co., Ltd., Nottingham, England.

Reagents.—Uridine, thymidine, and L-leucine were obtained from Sigma Chemical Co., St. Louis, Mo. L-Glutamine was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Vitamin mixture (100 times concentration), amino acid mixture (100 times concentration) for Eagle's basal medium, and fetal calf serum were purchased from Microbiological Associates Inc., Bethesda, Md.

Radioactive Materials.—Sodium sulfate-^{35}S (carrier-free) and thymidine-^{3}H-methyl-T (specific activity 9 Ci/mmole) were received from Bhabha Atomic Research Centre, Trombay, India. L-Leucine-U-^{14}C (344 mCi/mM) and uridine-G-^{3}H (6700 mCi/mM) were obtained from the Radiochemical Centre, Amersham, England.

Preparation of Globulins.—The globulins from rabbit anti-BGH serum and from normal rabbit serum were prepared as described (24).

Plaque-Forming Cells.—2×10^8 sheep erythrocytes were injected intraperitoneally into rats. The number of plaque-forming cells per million nucleated spleen cells were determined 102 hr after SRBC injection, as per the procedure of Jerne et al. (25). Hemagglutination titers were determined in the serum of these animals, after serial dilution with phosphate-buffered saline. 2% SRBC were used for assay.

DNA Synthesis.—DNA synthesis in vivo was determined by injection of thymidine-^{3}H (25 μCi/100 g body weight). After 30 min thymus, spleen, and lymph nodes (mediastinal and mesenteric) were removed and homogenized in cold saline containing 100 times nonradioactive thymidine. The DNA was extracted from the cold trichloroacetic acid (TCA)-insoluble pellet and radioactivity was determined as described elsewhere (26). DNA was estimated by the method of Burton (27).

Incorporation of Sodium Sulfate-^{35}S.—Sodium sulfate-^{35}S (200 μCi/100 g body weight) was given intraperitoneally. After 5 hr the thymus was removed and homogenized in cold saline.

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The homogenates were precipitated with cold TCA added to a final concentration of 5% and washed twice with cold TCA containing nonradioactive sodium sulfate. The precipitate was washed with alcohol and ether and dissolved in Hyamine hydroxide. The dissolved material was transferred to scintillation vials and counted using toluene-based scintillation fluid, containing 4 g of 2,5 diphenyloxazole (PPO) and 0.5 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene(dimethyl POPOP)/liter.

In Vitro System for the Action of BGH on Thymocytes.—Thymus from growing rats (40–60 g) was gently teased and thymocytes were collected by passing through stainless steel sieve (250 mesh). The cells were washed once with the medium, counted, and distributed into nylon incubation flasks. The incubation medium contained 5 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer pH. 7.2, 120 mM NaCl, 5.0 mM Na_2HPO_4, 5.0 mM KCl, 1.0 mM $MgSO_4$, 0.8 mM $CaCl_2$, and 5.5 mM D-glucose. 1 ml each of amino acid mixture (100 concentration) and vitamin mixture (100 concentration) were added to 100 ml of the medium containing 29.2 mg of L-glutamine. Fetal calf serum was present to a final concentration of 10%. The cells were incubated at 37° in a Dubnoff metabolic shaker for 10 min, at the end of which the appropriate radioactive precursor(s) was(were) added and incubation continued. Incubation was stopped at specified times by chilling the flasks in ice and by adding an excess of nonradioactive precursor in saline. The cells were centrifuged and washed with cold saline twice. They were precipitated with 5% cold TCA and washed twice with cold TCA. The TCA-soluble pool was collected. The residue was extracted with 5% TCA at 80–85°C for 15 min. Aliquots of the extract were counted as described (26). For the incorporation of amino acids into proteins, the hot TCA-insoluble precipitate was dissolved in Hyamine hydroxide and counted.

RESULTS

Effect of Hypophysectomy.—The ability of rats to respond immunologically to SRBC was significantly reduced when tested 15 wk after the removal of the pituitary. Sham-operated and normal rats of the same age and strain were used as controls for comparative study (Table I). The peak for HAT appears at a later period than PFC. Nonetheless, they were determined in the same animals to substantiate the data on PFC.

The pituitary is a source of a number of hormones. Hypophysectomy could thus lead to not only a deficiency of growth hormone but also a disturbance in homeostatic feedback regulation of a number of endocrines. In order to determine whether the fall in PFC was because of the lack of growth hormone and not of other pituitary constituents, two sets of experiments were performed. In the first set, a group of hypophysectomized rats were given 1 mg BGH/day in two equal doses for 5 days. The SRBC were injected at 24 hr after the initiation of hormone therapy. Data given in Table I show a marked recovery of the response in these animals. The animals were given thymidine-^{3}H-methyl-T 30 min before sacrifice. The rate of DNA synthesis in the thymus and spleen, as indicated by the specific radioactivity of DNA extracted from these tissues, was reduced in hypophysectomized animals, with recovery on administration of growth hormone (GH) (Fig. 1). The changes in the liver were not equally pronounced, suggesting a higher sensitivity of the lymphoid organs to growth hormone.

In the second set, the effect of the hormone on plateaued rats was investi-

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gated. The plateaued rats were initially recommended by Evans for the bioassay of growth hormone (28). They have an intact pituitary. Table II and Fig. 2 demonstrate the positive effects of growth hormone on the immune response to SRBC as well as on DNA synthesis in thymus, spleen, and lymph

TABLE I

Effect of Hypophysectomy and BGH Treatment on Immune Response

	Normal	Hypox*	Hypox + GH‡	Normal + GH
§PFC/10^6 spleen cells	328 ± 57 (163–476)	31 ± 15 (10–76)	236 ± 26 (173–274)	393 (312–474)
Hemagglutination titer	1:256–1:1024	1:8–1:32	1:128–1:1024	1:256–1:1024

* Hypophysectomized 15 wk before experiment.

‡ 1 mg of BGH given subcutaneously in two doses for 5 days.

§ 2 × 10^8 SRBC injected 102 hr before assay.

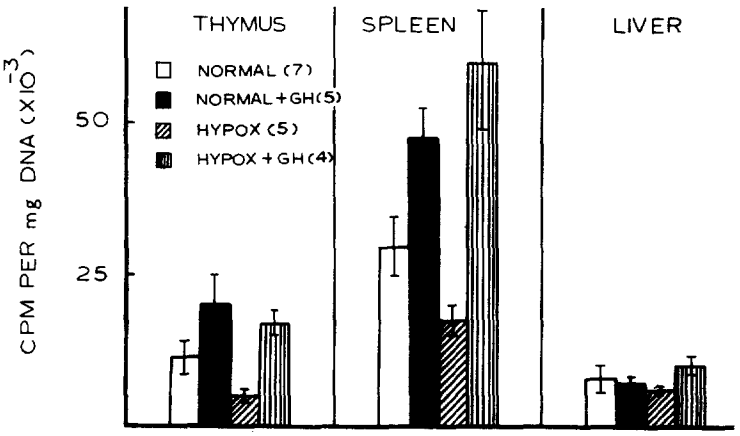

FIG. 1. Effect of hypophysectomy (HYPOX) and growth hormone (BGH) administration on the rate of DNA synthesis. Rats were given SRBC and sacrificed 102 hr later. Thymidine-^{3}H (25 μCi/100 g body weight) was given intraperitoneally 30 min before killing. DNA was extracted as described in Materials and Methods. 0.5 mg of BGH was given to experimental animals twice a day, subcutaneously for 5 days. BGH treatment started 1 day before the injection of SRBC. Mean ± standard error is given. Figures in parantheses denote the number of animals used.

nodes. It may be mentioned that the effect of administration of BGH over 5 days is not because of its action as immunogen, since BGH is poorly antigenic in rats (29).

Effect of Anti-BGH Globulins.—The specific role of growth hormone was

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further tested by the administration of antibodies to BGH. Growing rats (6–8-wk old) were injected twice daily for 5 days with either normal rabbit serum globulins (controls) or with rabbit anti-BGH serum globulins. The treatment was started on day 0 and SRBC were given on day 1. The thymus weight was observed to decrease after administration of anti-BGH globulins

TABLE II

Effect of BGH in Plateaued Rats (7)

	Control	+ Growth hormone
PFC/10^6 spleen cells	461 ± 57.5 (358–567)	679 ± 49.0 (590–757)
Hemagglutination titer	1:256–1:512	1:256–1:2048

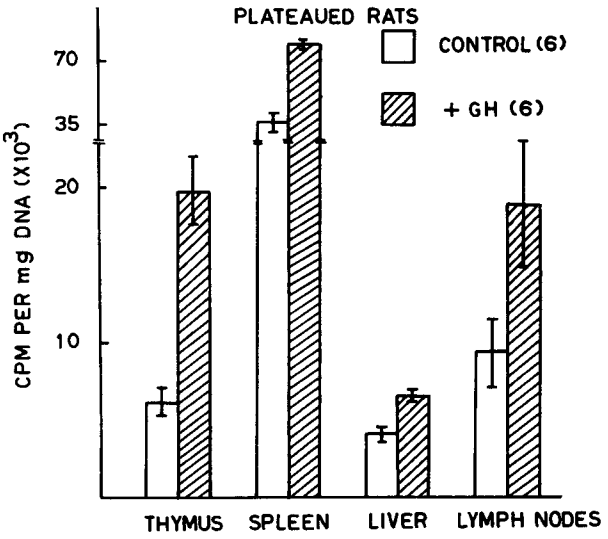

FIG. 2. Effect of BGH on plateaued rats. Plateaued rats were injected growth hormone 1 mg/day (or saline) twice a day subcutaneously for 5 days. The rest of the procedure was the same as given in Fig. 1.

(Table III). The spleen and liver were not affected to the same extent. PFC against SRBC were diminished. The rate of synthesis of DNA also decreased in lymphoid organs (Table IV).

Regeneration of Lymphoid Organs under Hormonal Influence after Starvation Stress.—In view of the fact that GH appeared to stimulate the synthesis of DNA in lymphoid organs, experiments were performed to see whether GH potentiates the reconstitution of the lymphoid organs by feeding. Rats were

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starved for 72 hr after which they were divided into two groups. Both groups were fed *ad libidum*. The experimental group also received twice daily injections of 0.5 mg of BGH. A pulse of radioactive thymidine was given to the animals in both groups at different stages and the specific radioactivities of DNA in the thymus and spleen were determined. Figs. 3 and 4 show a decline

TABLE III

Effect of Anti-BGH Globulins on Lymphoid Tissue Fresh Weight and Immune Response Against SRBC

Administration*	Tissue fresh weight‡ (mg/100 g body weight)			PFC/10^6 spleen cells§	HA titers
	Thymus	Spleen	Liver		
Normal rabbit globulins	287 (245–329)	587 (490–684)	4440 (4174–4706)	423 (373–473)	1:256–1:512
Anti-BGH globulins	180 (169–191)	480 (443–517)	4069 (4024–4116)	261 (243–328)	1:128–1:2056

* 83 mg of globulins injected in two doses each day for 5 days.

‡ Mean of three rats.

§ 2×10^8 SRBC injected on day 1. Assay on day 5.

TABLE IV

Effect of Anti-BGH Globulins on Incorporation of Thymidine-^3H in Normal Rats

	cpm in DNA/mg tissue (mean value of expts. on three rats) Treatment with	
	Normal globulins	Anti-BGH globulins
Spleen	155.9 (137–174)	85.8 (78–93)
Thymus	33.2 (26–40)	20.6 (15.7–25.5)
Liver	4.7 (4.1–5.3)	4.5 (4.2–4.8)

25 μCi of thymidine-^3H (9.0 Ci/mmole)/100 g body weight, incorporation for 30 min in vivo.

in the rate of DNA synthesis in these tissues on deprivation of the animals from food for 3 days. On refeeding, the apparent DNA synthesis is enhanced in the thymus fairly early and in the spleen after a short lag period. Administration of growth hormone activates further the regeneration process (Figs. 3 and 4).

Acute Actions of Growth Hormone.—In experiments described above, mul-

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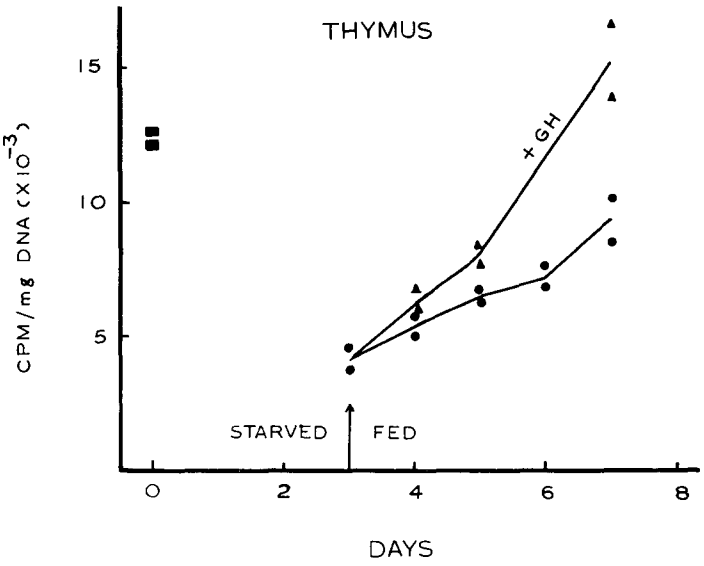

FIG. 3.

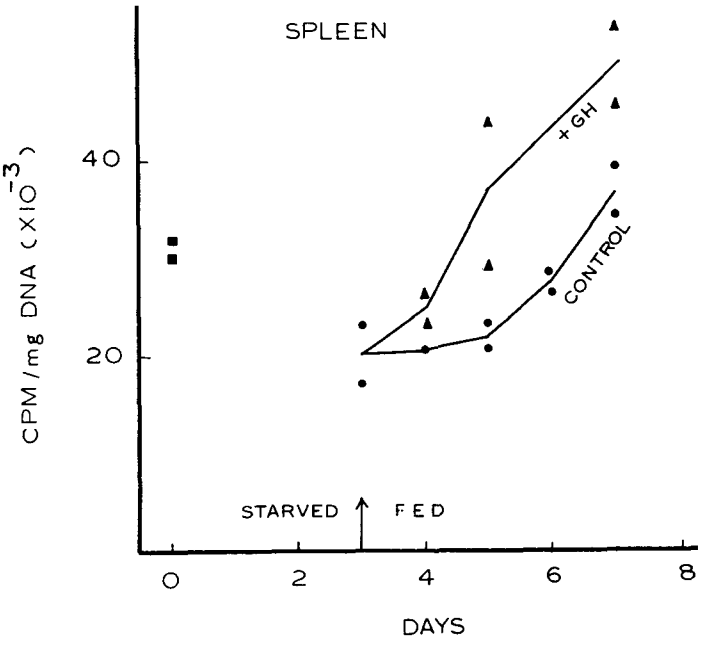

FIG. 4.

FIGS. 3 and 4. The influence of growth hormone on the regeneration of lymphoid organs in starved rats. Normal rats 6–8 wk of age were starved for 3 days and then fed *ad libidum*. Littermates were divided into two groups. The control group received saline and the experimental group were given BGH (500 μg/day per rat) daily, twice a day intraperitoneally. Rats were sacrificed on days specified after a 30 min pulse of thymidine-^{3}H (25 μCi/100 g body weight). ■, controls; fed normally; no growth hormone. ▲—▲, starved for 72 hr; fed *ad libidum* after 72 hr; growth hormone given every day. ●—●, starved for 72 hr; fed *ad libidum* after 72 hr of starvation.

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tiple injections of growth hormone were given and the effects observed after several days. It can be argued that the observed effects of the hormone may be secondary and a cumulative result of several events. To analyze the early effects, a single injection of BGH was given to growing rats (4-wk old). At 5½ hr a pulse of thymidine-^{3}H was given and the animals were sacrificed at the 6th hr. The controls received saline. DNA synthesis was measured in thymus, spleen, liver, and lymph nodes. There was a consistent and statistically significant increase of DNA synthesis in the thymus by treatment with growth hormone (Fig. 5). On the other hand, the spleen, lymph nodes, or liver did not show notable changes at this time period. These results can be interpreted to

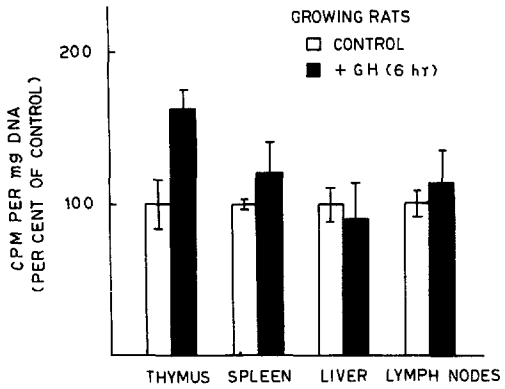

FIG. 5. Acute action of growth hormone. Growing rats (4–6-wk old) were given BGH (200 μg/rat) intraperitoneally. At 5½ hr, thymidine-^{3}H (25 μCi/100 g body weight) was injected. At the 6th hr the animals were sacrificed and DNA extracted from the thymus, spleen, liver, and lymph nodes. The specific radioactivity in DNA is expressed with respect to control values taken as 100. Mean ± standard error is given. Figures in parantheses represent the number of animals used in the experiment.

indicate a primary preferential action of the hormone on the thymus. The apparent differential effect on various lymphoid organs could also be because of a higher turnover-rate of DNA in the thymus than in the spleen and lymph nodes and the likely presence of more cells in the thymus at a given time in S phase.

Action of BGH on Metabolic Activities of Medullary and Cortical Regions of the Thymus.—Clark (30) has shown radioautographically that thymidine-^{3}H is incorporated mainly in the cortex of the thymus and sodium sulfate-^{35}S in the medulla. Medullary epithelial cells are not dividing as fast as the lymphocytes in the cortex of the thymus (hence lower incorporation of thymidine) but secrete sulphur-containing mucopolysaccharides (30, 31). The measure of these two metabolic activities can therefore serve as an index of the action of

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GH on the two areas of the thymus. 4-wk old rats were divided into four groups. The first group received saline at 0 hour and thymidine-^{3}H at 5½ hr. The second group received BGH (200 μg/rat) at 0 hr and thymidine-^{3}H at 5½ hr. The third group was given saline at 0 hr and sodium sulfate-^{35}S at 1 hr. The last group was given BGH at 0 hr and sodium sulfate-^{35}S at 1 hr. The animals were sacrificed at 6 hr. Thymus tissue was homogenized and DNA extracted as described in Materials and Methods. The residual pellet was dissolved in hyamine hydroxide and radioactivity was determined. BGH increased the

TABLE V

Effect of BGH on Incorporation of Thymidine-^{3}H and Sodium Sulfate-^{35}S in Thymus

	cpm/mg DNA	
	Control	+ Growth hormone
Thymidine into DNA (7)	4960 ± 767	7688 ± 548
^{35}S into TCA-insoluble pellet (7)	30,670 ± 4661	109,958 ± 4742

Thymidine-^{3}H, 25 μCi/100 g body weight. Incorporation time 30 min. Sodium sulfate-^{35}S, 200 μCi/100 g body weight, given at 1 hr after BGH thymus removed at 6 hr. Experimental details given in the text.

TABLE VI

Effect of In Vivo Administration of BGH on In Vitro Incorporation of Thymidine-^{3}H and Uridine-^{3}H in Thymocytes

	cpm/mg DNA		*P*
	Control	+BGH	
Uridine-^{3}H into RNA	45,770 ± 6230	66,475 ± 5300	<0.01
Thymidine-^{3}H into DNA	13,265 ± 1907	18,479 ± 884	<0.05

200 μg of BGH (or saline) were injected intraperitoneally to 4-wk old rats. The thymus was removed after 5½ hr and thymocytes were incubated in the medium containing 0.5 μCi/ml of uridine-^{3}H or thymidine-^{3}H. Results are the mean of four experiments ± standard error of the mean.

rate of DNA synthesis as well as the incorporation of sulfate-^{35}S into the TCA-precipitable mucoproteins (Table V). It is likely that BGH may stimulate both the cortical and the medullary activities. It remains however to be demonstrated whether the action is exercised independently on both regions or is first on the medulla and then on the cortex (or vice versa).

Effect of BGH In Vivo on Incorporation of Uridine-^{3}H and Thymidine-^{3}H In Vitro.—The apparent stimulation of DNA synthesis in thymocytes on administration of BGH to animals can be because of several reasons among which would be the possibility that the hormone makes more of the radioactive precursors available to the tissue. To bypass vascular considerations, an ex-

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periment was performed where the hormone was given intraperitoneally to 4-wk old (40–60 g) rats. At 5½ hr, after the administration of the hormone, the thymus was removed and thymocytes were prepared as described in Materials and Methods. The cells from control and hormone-treated animals were incubated in vitro for 30 min with either uridine-^{3}H or thymidine-^{3}H in the medium. Table VI shows that the thymocytes from hormone-treated animals incorporate these radioactive precursors better than controls even when the same concentration of radioactive precursor is available in the medium to both.

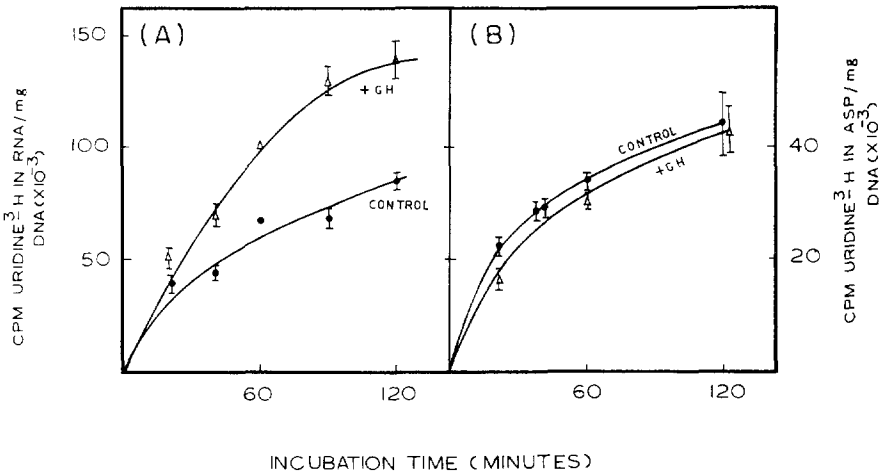

FIG. 6. Effect of BGH in vitro on thymocytes. Thymocytes from 4-wk old normal growing rats were prepared as described in Materials and Methods. They were incubated for 10 min at 37°C. BGH (15 μg/ml) was added to the experimental flask, the control tube receiving an equal volume of saline. 0.5 μCi/ml of uridine-^{3}H (1.0 μM) was added and incubation continued. Aliquots were taken at each time point. Cold TCA-soluble and hot TCA extracts were collected as described. Each point is the mean of 2–6 experiments. Mean ± standard error is given. 6 *A* gives the cpm of uridine in the nucleic acid fraction. 6 *B* gives the cpm of uridine in the cold TCA-soluble fraction. ASP, acid-soluble pool.

Effect of BGH on Thymocytes In Vitro.—The action of growth hormone on thymocytes may be direct or indirect as it is in bones through the mediation of sulfation factor (32). The possibility of the direct action of BGH on thymocytes was tested by exposing the thymocytes to BGH in vitro. BGH increased the incorporation of uridine-^{3}H into RNA in vitro (Fig. 6 *A*). In some other in vitro systems, such as diaphragm and adipose tissue (33, 34), growth hormone is known to facilitate the uptake of metabolites from the medium, which in turn become the causative factors for the stimulation of at least some of the metabolic activities. It was therefore considered appropriate to determine

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whether the hormone was not increasing the permeability of the tissue for uridine-^{3}H. The radioactivity in cold TCA-soluble fraction was not, however, found to be altered in the tissue incubated in presence of the hormone (Fig. 6 *B*).

Requirements for the Action of BGH In Vitro on Thymocytes.—The composition of the incubation medium in which GH activates in vitro the incorporation of radioactive precursors into biopolymers is given in the Materials and Methods section. To assess the essentiality of various constituents, experiments were performed by omission of some of these components. The presence

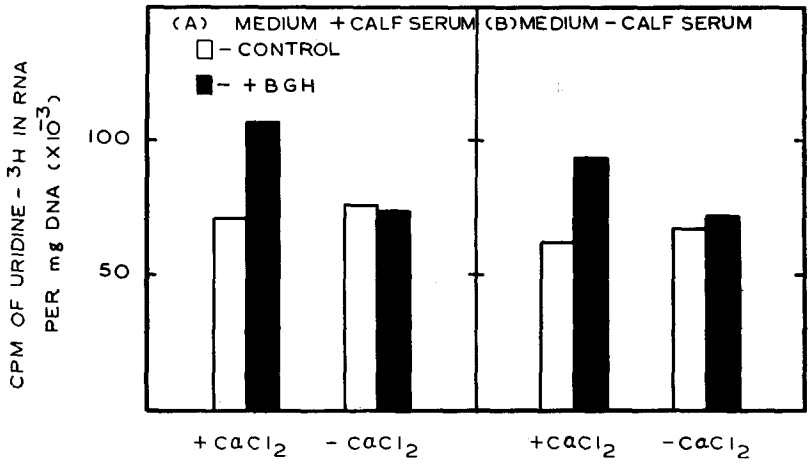

FIG. 7. Requirements for the in vitro action of BGH. Thymocytes were prepared from normal rats in calcium chloride and fetal calf serum-free medium. The cells were divided into four groups and incubated in the media with the following additions: (*a*) nil, (*b*) $CaCl_2$ alone, (*c*) fetal calf serum alone, (*d*) $CaCl_2$ + fetal calf serum. The incorporation of uridine-^{3}H into RNA for 2 hr was measured in each case. Values are the mean of two experiments.

of calcium is obligatory. If this cation is omitted, the stimulatory response to BGH is not obtained (Fig. 7 *A*). Calf serum is however not essential (Fig. 7 *B*).

Specificity of BGH Action.—The growth hormone preparation used for the studies, though highly purified, still contained traces of other pituitary hormones as contaminants. The preparation is reported to contain less than 0.05 USP units/mg of thyroid-stimulating hormone (TSH) and less than 0.025 NIH. LH. S1 units of luteinizing hormone (LH)/mg. It was therefore pertinent to inquire whether LH and TSH had any action on thymocytes in vitro. These hormones were used at the same concentrations as growth hormone but did not evoke an enhanced incorporation of uridine into RNA (Fig. 8). To further confirm that the action of growth hormone was in fact characteristic of molecular conformation of the native hormone, parallel experiments

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were done with heat-denatured growth hormone. The latter was without any effect. While testing other metabolic hormones, it was observed that insulin also stimulated the incorporation of uridine into RNA in thymocytes in vitro (Fig. 9 *A*). The sites of action of these two hormones may not, however, be identical, since when cells were incubated in the medium containing insulin, an increase in the radioactivity of the TCA-soluble pool was obtained (Fig. 9 *B*)

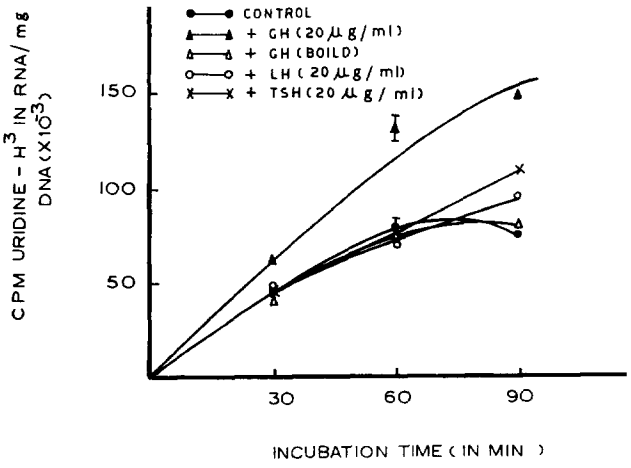

FIG. 8. Specificity of the effect of growth hormone. Thymocytes were incubated with uridine-^{3}H as in Fig. 6. Various hormones were added at the concentrations mentioned. GH was inactivated by heating in boiling water for 10 min.

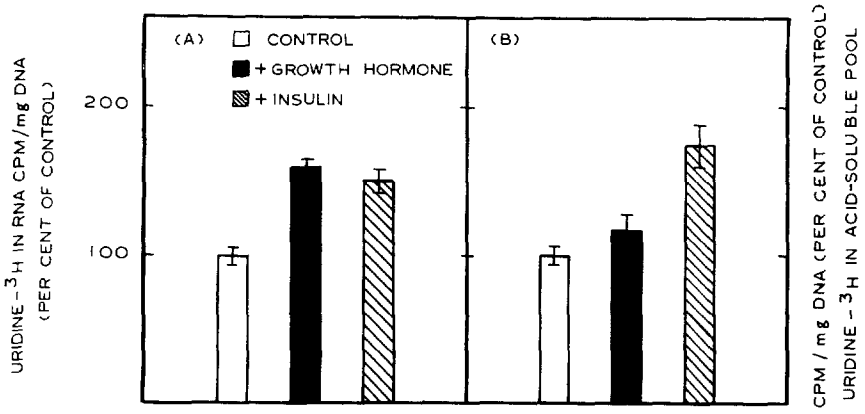

FIG. 9. Action of BGH and insulin on thymocytes in vitro. Thymocytes were prepared from normal animals. Insulin (250 μunits/ml) and growth hormone (15 μg/ml) were added to the experimental tubes. Incubation time was 2 hr. Controls are taken as 100; mean of three experiments ± standard error of the mean are given.

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in contrast to the observations with GH (Fig. 6). In fact, the increase in the radioactivity of uridine into RNA was proportional to the increase in radioactivity of acid-soluble fraction, suggesting that the action of insulin was to facilitate the uptake of the radioactive precursor.

Sequence of Metabolic Activities Stimulated by GH In Vitro.—Data given above show that the hormone activates the incorporation of uridine to RNA and thymidine to DNA in thymocytes. It was logical to seek a temporal correlation between these two activities influenced by the hormone. The incorporation of radioactive amino acids into proteins was also investigated. Thy-

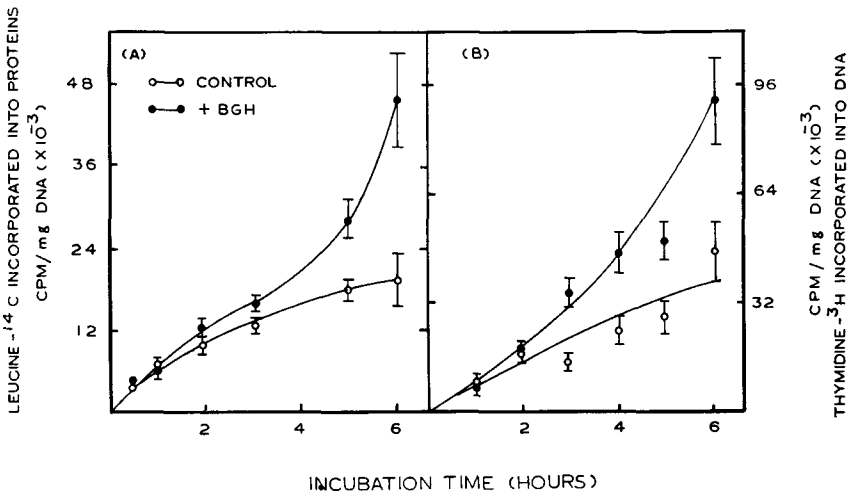

FIG. 10. Time curve of the action of growth hormone on the synthesis of DNA and proteins in thymocytes in vitro. Thymocytes were incubated in the medium containing 0.5 μCi of leucine-^{14}C (0.2 mM) and 0.5 μCi of thymidine-^{3}H (1 μM) with or without growth hormone. Aliquots were removed at the specified times and radioactivity measured as described in Materials and Methods. Results are the mean of four to six experiments ± standard error of the mean.

mocytes were exposed to BGH in a medium containing leucine-U-^{14}C and thymidine-^{3}H methyl-T. The cells were incubated for variable lengths of time and the incorporation of radioactivity into proteins and DNA was determined. While the incorporation of uridine into RNA is activated by the hormone at an early time period (Fig. 6), there was a lag period for the activation of the synthesis of proteins and DNA (Fig. 10 *A* and *B*).

DISCUSSION

The pituitary exercises directly and/or indirectly an effect on immune responses. It has probably some role in maintenance of lymphoid organs, since 15 wk after hypophysectomy of rats PFC and HAT to SRBC are significantly

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diminished. The synthesis of DNA as measured by the incorporation of radioactive thymidine in vivo is also reduced in the thymus and in the spleen. Among the pituitary hormones, the pivotal role of growth hormone is suggested by the following observations: (*a*) administration of growth hormone to hypophysectomized rats restores the immune response to SRBC; (*b*) rabbit anti-BGH globulins in contrast to normal rabbit serum globulins given over 5 days decrease the weight of lymphoid organs and the ability to form plaques; (*c*) plateaued rats with intact pituitary also respond positively to growth hormone; and (*d*) the effect of growth hormone on thymocytes is obtained in vitro.

The stimulatory action of growth hormone on RNA and DNA synthesis is manifest more quickly on the thymus than on the spleen and lymph nodes, though the latter organs are also affected. This could imply that the sensitivity of thymus cells to this hormone may be more than the secondary lymphoid organs. The stage of differentiation of lymphoid cells may be important for target organ effects. It is also possible that the observed difference may be more apparent than real and be a mere consequence of a higher rate of turnover of DNA or a greater number of cells present in S phase at a given time in the thymus than in the spleen.

The thymus has an important role in both cell-mediated immunity as well as in the humoral response to heterologous erythrocytes, serum proteins, and to a few other antigens (35–39). Factors regulating the metabolism of the thymus are not fully understood. The stimulus regulating proliferation and differentiation of thymus lymphoid cells is intrinsic, antigen independent, and linked to the cells of the epithelial cytoreticulum (39). In this context, the responsiveness of this organ to the hormone is of interest. Administration of growth hormone influences the synthesis of biopolymers in both medullary and cortical areas of the thymus. The action may be exercised independently on the two regions or the hormone may initially stimulate the production of humoral factors like thymosin (40–42) in the medulla that activate in turn the cellular proliferation in the cortical areas. The fact that thymocytes respond to this hormone in vitro is suggestive of the direct action of the hormone on the cortical cells besides ancillary effect on the medulla of the thymus.

The mechanism of action of growth hormone on thymocytes is not known. The hormone acts on many tissues of the body. It facilitates the uptake of glucose and amino acids in the diaphragm (33) and in the adipose tissue (34, 43), suggesting that one of the sites of action of the hormone is on membrane-linked functions in these tissues. The lack of increase in the radioactivity of the TCA-soluble pool of thymocytes, incubated in a medium containing growth hormone (Fig. 6), would argue against a possible action of the hormone on permeability of thymocytes to metabolites like uridine.

Another action of the hormone is in stabilization of certain key regulatory

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enzymes such as phosphofructokinase in adipose tissue (44). It remains to be demonstrated whether any enzyme(s) is (are) activated by the hormone in thymocytes.

The general growth-promoting effect of this hormone is probably exercised by an activation of RNA and protein synthesis in target cells. An early effect of the hormone on thymocytes is also manifest in an enhanced incorporation of radioactive uridine into RNA. This is followed in time sequence by a generalized activation of the synthesis of proteins and DNA. Previous work has shown that this hormone stimulates the synthesis of RNA in the liver (45, 46) and in muscles (47, 48). There is an increased synthesis of both ribosomal and messenger RNA (49, 50). The hormone does not apparently activate expression of dormant genes in the liver as the template activity of the chromatin is not altered nor are new species of RNA detected by hybridization experiments (51, 52, 48, 53). The action is essentially on the enhancement of the rate of RNA synthesis.

The effect on the synthesis of proteins may be a direct consequence of more ribosomes and protein synthesizing units available. There could be additional effects on the translation processes. Ribosomes from hypophysectomized rats have been found deficient in the incorporation of amino acids in cell-free systems, while ribosomes prepared from animals given growth hormone showed improvement (54).

There is an obligatory requirement of calcium in manifestation of the effect of the hormone on thymocytes in vitro. In some other systems calcium has been observed to play a synergistic role with cyclic adenosine monophosphate (cyclic AMP) (55). It remains to be investigated whether growth hormone acts on thymocytes through cyclic AMP as a second messenger.

SUMMARY

The effect of pituitary growth hormone on the biosynthesis of DNA in the thymus and other lymphoid organs, as well as the ability of the rat to respond immunologically to sheep red blood cells, has been evaluated. There is a marked reduction in plaque-forming cells, hemagglutination titers, and DNA synthesis in animals when examined at 15 wk after hypophysectomy. Administration of bovine growth hormone (BGH) leads to the enhancement of DNA synthesis in lymphoid organs and recovery of the immune response. Similar effects of the hormone are observed in plateaued rats. Injection of rabbit anti-BGH globulins, in contrast to normal rabbit globulins, over 5 days causes a drop in the weight of the thymus and in the rate of DNA synthesis in this organ. The thymus is also the organ in which stimulation of DNA synthesis is observed at a time period earlier than the spleen and lymph nodes after a single injection of BGH. The hormone stimulates not only the incorporation of thymidine-^{3}H

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into DNA in the cortical cells, but also the incorporation of sodium sulfate-^{35}S into TCA-insoluble biopolymers reported to be elaborated in the medullary area of the thymus.

An in vitro system for the action of BGH on the thymus has been described. There is an obligatory requirement for calcium, but not for fetal calf serum in the medium for the hormone effect. An early action of the hormone is the enhanced incorporation of uridine-G-^{3}H into RNA in thymocytes which is followed by a stimulation of the synthesis of proteins and DNA. The stimulatory action of growth hormone on RNA synthesis is not because of a facilitated uptake of the radioactive uridine by the cells under hormonal influence, a mechanism by which insulin is observed to increase RNA synthesis in thymocytes in vitro. The action of growth hormone on thymocytes is specific, since thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and heat-inactivated growth hormone are not effective. BGH has also a beneficial action on the regeneration of the thymus and spleen in starved rats.

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