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The Effects of Changes in Thyroid Function on Testis ^{32}P Uptake and the Response to Gonadotropin in the Chick

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Propylthiouracil (PTU)-induced hypothroidism has been shown to produce a significant increase in the uptake of radiophosphorus (^{32}P) by the testes of young chicks. Continued investigation of this effect demonstrated that thyroidectomy of young birds resulted in elevations in testis ^{32}P uptake comparable to those observed following the administration of PTU. It would thus appear that the increased levels of ^{32}P in the testes of PTU-treated chicks resulted from the hypothyroid condition itself rather than from some other pharmacological action of PTU. A time course study indicated that there were no differences in the turnover rates of labeled testicular phosphorus in hypo-, hyper-, and euthyroid birds which might account for the above observations.

Sensitivity of the chick testis to pregnant mare's serum gonadotropin (PMSG) was unaffected by changes in thyroid activity, and the combined effects of PMSG and hypothyroidism on the testis were seemingly additive. Alternative possibilities to explain the apparent indirect effect of subnormal thyroid activity on the testis were therefore discussed.

Alteration in thyroid activity results in morphological and physiological changes in the gonads and the accessory reproductive structures (for reviews see Myant, 1964; Van Tienhoven, 1968; Thapliyal, 1969). Firm generalizations about the nature of the thyroid–gonad relationship are precluded because of the many different species and experimental conditions used in the reported studies. Nevertheless it appears that sexual maturation, gametogenesis, ovulation, and gestation are a few of the reproductive processes which are affected by changes in thyroid function. Most work in this field has been done on mammals, but studies with the chicken have demonstrated that gonadal size, spermatogenesis, and egg production are affected by changes in the level of thyroid activity. In nondomesticated birds, such as the starling (Wieselthier and Van Tienhoven, 1971) and certain tropical finches (Thapliyal and Panda, 1965; 1967), the thyroid gland may affect cyclic changes in gonadal size. Hypothyroidism in these animals has been shown to prevent the regression of the testes which normally occurs at the end of the breeding season.

Investigation of thyroid–gonad relationships in 15-day-old white leghorn cockerels has demonstrated that propylthiouracil-induced hypothyroidism significantly increases the uptake of radioactive phosphorus (^{32}P) by the testes (Lehman, 1970). Thyroxine administration alone has been shown to have no effect on isotope uptake, although physiological doses of thyroxine, when administered simultaneously with propylthiouracil (PTU), abolish the stimulatory effect of goitrogen treatment on the testes. In this paper results are presented that support three additional conclusions concerning the effect of propylthiouracil-induced hypothyroidism on phosphorus uptake by the young chick testis: (1) thyroidectomy induces the same increase in ^{32}P uptake as does PTU—therefore the effect of PTU is clearly due to hypothyroidism rather than some other action of the drug; (2) a study of the time course of ^{32}P uptake in hypothyroid animals demonstrates that

an increase in phosphorus uptake is observed at all intervals studied, and is thus not simply an effect of hypothyroidism on the rate of phosphorus turnover in the testes; (3) sensitivity of the chick gonad to pregnant mare's serum gonadotropin (PMSG) is unaltered by hypothyroidism, and the effect of PMSG and hypothyroidism appears to be additive. This leads to the conclusion that the stimulatory effect of hypothyroidism is not due to an alteration in testicular sensitivity to gonad stimulating hormones, and suggests the possibility that the effect of hypothyroidism on the testes is an indirect one, perhaps involving an increase in the effective circulating levels of gonad stimulating hormones.

MATERIALS AND METHODS

A. General Care and Treatment

One-day-old white leghorn cockerels of the DeKalb strain were obtained from a commercial hatchery and maintained in battery brooders with food (Purina Chick Startena) and water ad lib. for the duration of the experiment. Hypothyroidism was produced by adding PTU to the feed in mixtures of 0.1 or 0.2%. The thyroid glands of animals maintained on these concentrations were characterized by extensive hypertrophy and hyperemia. Solutions of L-thyroxine were prepared by dissolving the powder in a minimal amount of 0.1 *N* NaOH and diluting to the desired concentration with distilled water. Injections were given subcutaneously in volumes of 0.1 cc/bird/day. Dosages of thyroid hormone used in these experiments were based on a previously determined average daily secretion rate of 0.65 μg/cockerel/day (Lehman, 1970). A dose of 2.5 μg of thyroxine/day was thus used to achieve a hyperthyroid state, and various degrees of hypothyroidism were produced by feeding the chicks 0.2% PTU and simultaneously administering thyroxine in doses of 0.15 and 0.30 μg/bird/day. Control birds were injected daily with 0.1 cc of distilled water.

In studies of testicular responses to gonadotropin, the animals were treated with PTU and thyroxine for 14 days and injected subcutaneously with 30 IU of PMSG (Sigma) per day for the last 4 days of the 14-day experimental period. Dose responses to gonadotropin were also studied by this method using doses of PMSG ranging from 40–160 IU/day.

In general, treatment with PTU or thyroxine was continued for 14 days. The animals were taken off feed 20 hr before autopsy and were killed by cervical fracture when 15 days of age. An exception to this procedure was made during the age study, at which time the birds were treated for 7 or 21 days and then killed on days 8 and 22, respectively.

At autopsy, the testes were removed, blotted to remove excess moisture, weighed, and placed in liquid scintillation vials for counting. The procedure was performed as rapidly as possible between the hours of 9:30 and 11:30 AM in order to insure that differences in ^{32}P uptake reflected treatment effects rather than the effects of possible circadian rhythms.

B. Isotope Administration

Carrier-free radioactive phosphorus ($H_3{}^{32}PO_4$; New England Nuclear Corp.) was diluted to the desired concentration with distilled water and administered subcutaneously in an injection volume of 0.1 cc. In general, each chick received 4 μCi of ^{32}P 18 hr before it was killed. However, in determining the rate of ^{32}P uptake by the testes of hypo- and hyperthyroid cockerels, the birds were killed at various intervals after isotope administration (see Fig. 2). At autopsy the testes were removed, weighed, and counted immediately in a Nuclear Chicago liquid scintillation counter. Differences in isotope uptake levels implied changes in testicular metabolism because of the demonstrated incorporation of ^{32}P into the various chemical fractions and subcellular components of the chick testis and the general distribution of the isotope throughout the metabolic pool of phosphorus (Connell, 1964).

C. Thyroidectomy

One-day-old cockerels were obtained from the hatchery and thyroidectomized when 6 days of age. Chicks undergoing surgery were taken off both food and water 18 hr before the operation.

Each chick received an initial intraperitoneal injection of 0.075 cc of Equi-Thesin (Jensen-Salsbery Laboratories) and a very small booster injection (0.025–0.05 cc) if necessary during the course of the operation. A single midline incision was made slightly anterior to the sternum, and the thyroids were gently teased out using watchmakers' forceps. In sham-operated controls the thyroids were exposed but not removed. After the incision had been sutured and swabbed with Zephiran, the birds were placed in straw-lined chick pullmans and kept warm under a lamp for 12–15 hr. Thereafter they were kept in battery brooders with food and water ad lib. for the next 14 days.

Eighteen hours before autopsy the birds were taken off feed and injected subcutaneously with 4 μCi of ^{32}P. On day 15 after surgery, the birds were killed by decapitation and the thyroid area was examined with the aid of a dissecting microscope to determine if thyroid remnants were present. Thyroid remnants remaining after surgery were found to hypertrophy rapidly during the 14-day experimental

period into visible thyroid tissue showing extensive hyperemia and a ^{32}P uptake significantly greater (on a cpm/mg basis) than that of normal thyroids. Only those animals showing no trace of thyroid tissue were used. The testes were removed as described previously and counted to determine the ^{32}P uptake.

D. Statistical Methods

The group comparison *t* test and the two-way analysis of variance were used to determine the existence of significant differences between the means of experimental groups. When *F* values were found to be significant at the 0.05 level, specific treatment comparisons were performed using the Student–Newman–Keuls' multiple range test (Steel and Torrie, 1960) with the α level set at 0.05.

RESULTS

A. The Effect of Hypothyroidism on Testis ^{32}P Uptake in Young Chicks

An increase in testis ^{32}P uptake over control values was observed repeatedly in 15-day-old PTU-treated cockerels (Lehman, 1970, and present paper). This effect was also present in 8- and 22-day-old chicks following goitrogen administration periods of 7 and 21 days duration. Testis ^{32}P uptake values ($\bar{X} \pm s_{\bar{x}}$) for 8-day-old control and PTU–treated birds were 27.9 ± 6.6 and 44.4 ± 9.0 cpm/mg, respectively. In 22-day-old cockerels the respective levels of isotope uptake for control and treated individuals were 10.1 ± 2.0 and 34.6 ± 7.1 cpm/mg. No attempt has been made to compare the magnitude of the goitrogen effect at the two different ages because the experiments were not performed simultaneously. However, the pattern of response was the same at both ages, and a significant response ($P < 0.05$) to PTU occurred as early as the first week of age and was evident for at least 2 weeks thereafter. Testis weights at these times did not differ significantly in treated and untreated animals. Mean and standard error values were 15.7 ± 1.4 and 15.5 ± 1.8 mg for 8-day-old control and goitrogen-treated cockerels, and 45.3 ± 2.3 and 44.0 ± 3.0 mg for 22-day-old control and treated individuals, respectively.

B. The Effects of Thyroidectomy on Testis ^{32}P Uptake

As a result of thyroidectomy, ^{32}P uptake by the testes was increased significantly ($P < 0.05$) over sham-operated control values (Fig. 1). The response qualitatively mimicked that observed when the animals were treated with 0.1% PTU. Absolute uptake values were similar to those obtained when higher concentrations of the goitrogen (0.2 and 0.5% PTU) were employed in preliminary experiments. A PTU concentration of 0.2% was thus used in the remainder of the study. Testis weights of thyroidectomized animals ($\bar{X}$ = 25.4 mg; $s_{\bar{x}}$ = 1.7) were not significantly different from those of sham-operated controls ($\bar{X}$ = 31.6 mg; $s_{\bar{x}}$ = 3.4).

C. The Uptake of ^{32}P by the Testes of Hypo- and Hyperthyroid Cockerels at Various Intervals after Isotope Injection

Testes ^{32}P uptake was essentially maximal in all groups 1 hr after injection (Fig. 2). Although a slight rise in absolute uptake values was observed 2 to 4 hr after injection, this increase was not statistically different from values obtained 1 hr after the injection of isotope. Maximal uptake values were maintained in all groups for the remainder of the 24-hr period under study. Testis ^{32}P uptake of hypothyroid cockerels was significantly higher at all points than that of either control or thyroxine-treated

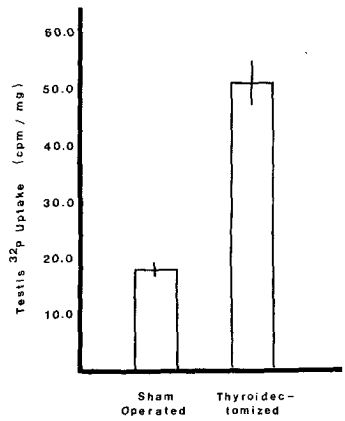

FIG. 1. The effect of thyroidectomy on testis ^{32}P uptake. Data are presented as mean ± standard error. Values for 3-week-old control and thyroidectomized animals are significantly different at a P level < 0.05. N = 12.

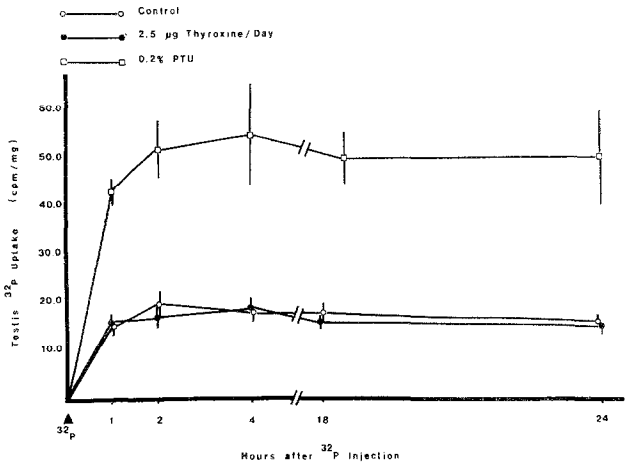

FIG. 2. Twenty-four hour uptake of ^{32}P by the testes of hypo- and hyperthroid chicks. The isotope was injected at time 0 and the animals were killed at the intervals indicated. N = 6 at each sample interval.

animals. At no time were there any significant differences in testis uptake when control and thyroxine-treated animals were compared.

D. The Effect of Various Degrees of Hypothyroidism on Testis ^{32}P Uptake and Testicular Response to Gonadotropin

1. Baseline levels in the absence of exogenous gonadotropin. The normal average daily thyroxine secretion rate for 15-day-old cockerels has been estimated by goiter-prevention assay to be approximately 0.65 μg/100 g body wt/day (Lehman, 1970). Thus different levels of hypothyroidism were produced by the several doses of thyroxine (T_4) replacement used. Figure 3 indicates that as the degree of hypothyroidism became more severe, the uptake of ^{32}P by the testes was progressively elevated. Testis ^{32}P uptake in control animals and in those which received PTU + 0.3 μg of thyroxine/day did not differ significantly, but isotope uptake in the remaining two groups was significantly elevated above the control value. Mean uptake levels in all three groups of goitrogen-treated animals were significantly different from each other. In the absence of PMSG no significant differences were found in the testis weights of the various groups.

2. Response to PMSG. Testis ^{32}P uptake was significantly elevated in all hypothyroid groups treated with PMSG. In control chicks, however, the PMSG effect was

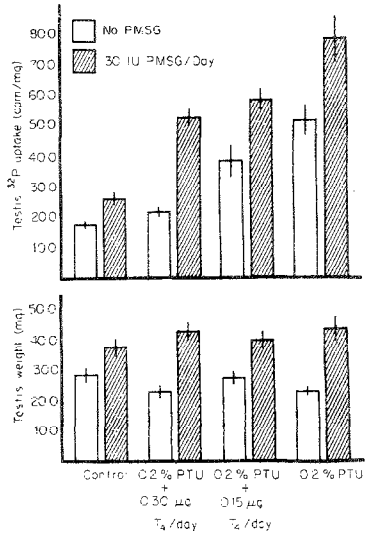

FIG. 3. The effect of various degrees of hypothyroidism on testis ^{32}P uptake and the response to gonadotropin in 2-week-old chicks. Data are presented as mean ± standard error. N = 10.

slightly below the 5% level of significance. The greatest percentage response was obtained in animals which had received PTU + 0.3 μg of thyroxine/day. Several repetitions of the experiment demonstrated, however, that the percentage response was not consistently greater in this group than in other groups of hypothyroid animals. Absolute uptake values increased as the degree of hypothyroidism became progressively more severe. The uptake of ^{32}P by the testes of gonadotropin-treated animals which received PTU alone was significantly greater than that found in both groups of cockerels which had received a combination of PTU and thyroxine.

It is interesting that testis ^{32}P uptake following goitrogen treatment alone was significantly greater than that of control animals which received PMSG, and statistically equivalent to that observed when moderately hypothyroid individuals (thyroxine plus PTU) were treated with gonadotropin. Analysis of variance (factorial treatment arrangement) indicated that while both PMSG administration and thyroid function significantly affected testis ^{32}P uptake, there was no significant interaction between the two factors on this parameter.

Mean testis weight values were significantly elevated in all groups following the administration of PMSG. Analysis of variance and multiple range tests indicated that the degree of thyroid activity had no effect on this response, and that there were no significant differences between the mean testis weights of the PMSG-treated groups.

E. The Effect of Hypo- and Hyperthyroidism on the Dose Response to PMSG

Changes in thyroid activity had no apparent effect on the shapes of the dose–response curves shown in Fig. 4. Significant elevations ($P < 0.05$) in testis ^{32}P uptake were obtained in hypo- and hyperthyroid individuals at a PMSG level of 40 IU/day. Although the curves tended to rise slightly with higher doses of PMSG, these elevations in testis ^{32}P uptake were not statistically different from responses obtained with the lowest dose. In control animals the increases in isotope uptake were below the 5% level of significance. However, uptake levels of control and thyroxine-treated birds did not differ significantly at any given dose level of PMSG, and the overall significance of the gonadotropin response in these two groups of animals must therefore be interpreted with caution. Uptake levels in hypothyroid individuals were at all times significantly greater than those found in control and hyperthyroid animals. The multiway analysis of variance procedure indicated a significant effect of both PMSG treatment and hypothyroidism on testis ^{32}P uptake, but revealed no significant factorial interaction.

Statistical analysis also indicated the absence of interaction between PMSG administration and thyroid activity on testis weight. No significant differences were found to exist between the testis weight values of any of the three groups, regardless of the level of gonadotropin administered (40–160 IU). A dose of 40 IU of PMSG/day produced a significant increase in this parameter in all groups. No further

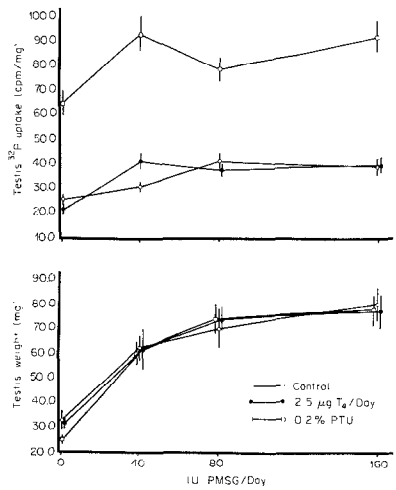

FIG. 4. Testicular responses of 2-week-old hypo- and hyperthyroid chicks to various doses of PMSG. Data are presented as mean ± standard error. N = 8.

significant elevation in absolute testis weights was observed with higher levels of gonadotropin.

and thyroxine-treated animals were practically superimposable. The accelerated uptake of ^{32}P by the testes of hypothyroid animals would thus appear to represent a secondary rather than primary effect of changes in thyroid activity.

DISCUSSION

Accelerated testis ^{32}P uptake has been repeatedly demonstrated in 15-day-old hypothyroid cockerels. Additional data suggest that the effect is observable for at least 3 weeks after hatching and that the physiological adjustments necessary to produce such an effect may be made within the first week of age if treatment has been started when the chicks are 1 day old. The knowledge that 22-day-old cockerels exhibited an increase in isotope uptake following goitrogen administation enabled stronger, more mature animals to be used in thyroidectomy studies. For experiments of 2 weeks duration surgery could be performed when the chicks were 6–7 days old, instead of immediately after hatching

Thyroidectomy in chicks was found to mimic the effect of PTU administration on testis ^{32}P uptake, and would further suggest that the absence of the thyroid gland and its hormones results in an increase in the ^{32}P metabolism of the gonad. This idea is further supported by the fact that thyroxine injections (when given in conjunction with PTU treatment) abolished the testicular effect observed when PTU alone was administered (Lehman, 1970). The results of both studies make the possibility of a direct stimulatory effect of PTU on the testes unlikely.

While hypothyroidism resulted in increased amounts of radiophosphorus taken up by the chick testis, the shapes of the curves shown in Fig. 2 indicate that (1) the time necessary for maximal ^{32}P uptake was the same, regardless of thyroid activity, and that (2) both hypo- and hyperthyroidism had no effect on the turnover of the isotope (i.e., no differential effect on uptake as compared to release) during the 24-hr period following isotope injection. Testis ^{32}P uptake was consistently greater at all time intervals in hypothyroid cockerels, and there was thus no indication that the increased isotope uptake previously noted in PTU-treated birds 18 hr after injection was the result of a delay in reaching the point of maximal accumulation (when extensive release of testicular ^{32}P in hyper- and euthyroid animals might have occurred). Money and Rawson (1955) observed similar uptake curves in their studies, and also found that the gonads of chicks maintained on an iodine-deficient diet for 1 week appeared to concentrate 2–3 times more ^{32}P than did those of animals maintained on standard laboratory ration. These investigators also found that thiouracil administration had little effect on testis ^{32}P uptake, but treatment of the animals had been maintained for only 4 days and goiters had not yet developed. In the present study, the absence of a direct effect of thyroxine on the testis is suggested by the fact that uptake curves for control

The results illustrated in Fig. 3 indicate that the magnitude of this effect was inversely related to the amount of circulating thyroxine. Similarly, increasing levels of isotope uptake in response to PMSG were also observed as the animals became progressively more hypothyroid. However, with the possible exception of the group which had received PTU + 0.3 μg of thyroxine/day, there was no indication that the testes of hypothyroid birds possessed a greater capacity to respond to gonadotropin than did those of control animals. Indeed, since the absolute effect of PMSG was of similar magnitude in all three hypothyroid groups, the results can be interpreted to indicate a simple additive effect of subnormal thyroid activity and PMSG. The response to various degrees of hypothyroidism alone parallels that of hypothyroidism

plus PMSG and thus suggests that the hypothyroid state and PMSG affect testis ^{32}P uptake by the same mechanism.

As with other birds which have been studied (Bageshwar and Thapliyal, 1969; Wieselthier and Van Tienhoven, 1971) equivalent testis weight responses to gonadotropin in hypo- and euthyroid birds also suggests that decreased levels of thyroid activity had no effect on the sensitivity of the testes to PMSG. These results contrast with those of other investigators (Janes, 1954; Nocenti and Leathem, 1956; Leathem, 1958; Adams and Leathem, 1966) who have found that in rats the gonadal weight response to gonadotropin is greater in hypothyroid individuals than in untreated controls. Differences in treatment duration may partially account for these discrepancies, as may species differences. Mice and rats whose thyroid activity has been altered differ in their response to PMSG (Meites and Chandrashaker, 1949). Presumably chicks and rats might also respond differently.

Changes in thyroid function also did not appear to affect the shape of the dose–response curves obtained following gonadotropin administration. This would suggest that the mechanism by which PMSG stimulates the testes is the same regardless of the degree of thyroid activity. The direct physiological cause of accelerated testicular ^{32}P uptake occurring concomitantly with subnormal thyroid activity is still unclear, but alternative suggestions may be made: it is possible that the secretion rates of gonadotropic hormones may be elevated, or that the gonadotropin degradation rates in hypothyroid cockerels may be reduced sufficiently to result in elevated LH and/or FSH titers capable of producing the observed stimulatory changes.

The relationship between thyroid function and the secretion and metabolism of pituitary gonadotropins is an area in need of critical investigation. In recent years the purification of chicken LH and the establishment of a specific radioimmunoassay for this hormone (Follett *et al.*, 1972) has made possible the valid assessment of serum LH levels and turnover rates of LH in this species. The assay has been applied to studies on thyroxine–treated Pekin ducks (Jallageas *et al.*, 1974) and has particular relevance in solving problems of the type described in the present study.

Much of the evidence that thyroidal effects on gonadal function are mediated by pituitary hormones has been supplied by the results of biological assays which have suggested that (1) alterations in pituitary gonadotropic potency occur with changes in thyroid secretion (Contopoulos *et al.*, 1958; Contopoulos and Koneff, 1963) and (2) that thyrotropin itself might have sufficient gonadotropic activity to stimulate to some degree the uptake of ^{32}P by the testes of hypothyroid birds (Lehman, 1970).

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