the phosphorylase activation system is not impaired. Our data are consistent with this view, since the available activator, catecholamine, increased rather than decreased.

The significantly higher concentration of catecholamine content in the normal male of this species is also noteworthy. This is consistent with observations that cardiac output is greater in the rooster than in the hen under a variety of environmental circumstances(8). The predominant catecholamine in the heart of the normal chicken is norepinephrine, epinephrine constituting one-third of the total, or less(9).

Summary. Dystrophy in chickens due to Vit. E and sulfur deficiency is associated with an elevation in myocardial catecholamines. The catecholamine content of the heart of the normal rooster is significantly greater than that of the normal hen.

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Influence of Thyroid State on Positive Inotropic Effect of Ouabain on Isolated Rat Ventricle Strips.* (28417)

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While it is a common belief that heart failure accompanying disorders of thyroid gland function is relatively refractory to the effects of cardiac glycosides(1), there have been few systematic evaluations of this concept. Frye and Braunwald(2) observed an increased requirement for acetylstrophanthidin or digoxin in patients with chronic atrial fibrillation when treated with triiodothyronine, using slowing of the ventricular rate as an index of glycoside action.

Rosén and Moran(3) and Morrow *et al.* (4), have studied the effects of cardiac glycosides in hyper- and hypothyroid dogs. Both groups found ouabain to augment cardiac contractile force in all animals. Rosén and Moran noted a lesser effect of small doses of ouabain in hypothyroid dogs than in controls whereas in hyperthyroid dogs the response to low doses was similar to that of euthyroid dogs but the effect of high doses was depressed. Morrow *et al.*, observed a greater effect of ouabain on contractile force of hypothyroid dogs compared with normal and hyperthyroid animals.

The following experiments were designed in an attempt to evaluate the influence of altered levels of thyroid activity on the positive inotropic effect of ouabain on isolated ventricle strips of rats.

Methods. Male, Sprague-Dawley, albino rats (170-200 g) were divided into 3 groups. Group I (11 rats) were thyroidectomized (with concomitant removal of parathyroid glands). Group II (8 rats) received daily subcutaneous injections of sodium *dl*-thyroxine (20 μg/100 g). Group III consisted of 18 normal rats. Calcium lactate was added to the drinking water (1% w/v) of the thyroidectomized rats to prevent hypocalcemic tetany. Three weeks after thyroidectomy propylthiouracil was added to the diet (0.1% w/w). All animals were given water and food *ad libitum*.

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Oxygen consumption, determined by the closed circuit method of Holtkamp *et al.*(5), and rectal temperatures were measured at approximately weekly intervals and on the day prior to or the day of sacrifice.

Thyroidectomized animals were sacrificed 58 days after operation, the thyroxine-treated animals after 36 days of treatment, and the control animals at intervals of 2 to 4 weeks after the initial determinations. Animals were killed by decapitation, and strips of right ventricle, 2 mm wide and about 15 mm long, were prepared according to the method of Feigen *et al.*(6). They were suspended in 70 ml of a solution of the following composition (7) (as grams of anhydrous material/l of solution): NaCl, 7; KCl, 0.42; $CaCl_2$, 0.24; $MgCl_2$, 0.20; $NaHCO_3$, 2.1; dextrose, 1.8. Oxygen (95%) and carbon dioxide (5%) were dispersed into the solution through a fritted glass disc to give a pH of 7.4. Bath temperature was 37 ± 0.5°C.

Isometric contractile force was measured with a Grass FT-03 force displacement transducer on a Sanborn Poly-Viso oscillograph. A resting tension of 0.75 g was maintained.

The strips were stimulated through bipolar stainless steel electrodes with a Grass Model S4A stimulator at a frequency of 90/min, pulse duration of 10 to 15 msec and voltages approximately twice threshold. After beginning stimulation the strips were allowed to equilibrate for one hour. Ouabain was then added to make a final concentration of 1 μg/ml. After a maximal change in force was attained the strips were removed from the bath, blotted dry and weighed.

The possibility of residual functioning thyroid remnants in the thyroidectomized rats was evaluated as follows: 1) Two days prior to sacrifice 2.37 μc of NaI^{131} were administered i.p. to the thyroidectomized rats and to each of 10 normal rats. Twenty-four hours later the radioactivity of the anterior neck region and of the medial thigh region of one hind leg was determined separately by suspending the area over a Nuclear Chicago DS-5, 1¾ × 3 inch, NaI (T1), deep well type crystal for one minute. Duplicate determinations were obtained for each region. There was no significant difference between the uptake of I^{131} in neck and thigh regions in thyroidectomized rats compared with a 5-fold greater uptake in the necks of normal rats. 2) Microscopic examination of serial sections of the laryngeal regions of 4 thyroidectomized rats showed no evidence of thyroid tissue.

Statistical evaluations were by Student's *t* test or by the *t* test for paired comparisons(8).

Results. Daily administration of thyroxine for 36 days increased oxygen consumption to a final mean value (± S.E.) of 2.20 ± 0.13 ml/g per hour compared with 1.43 ± 0.01 for normal rats ($p < 0.001$). Thyroidectomy reduced O_2 consumption to a minimum value of 1.22 ± 0.06 ml/g per hour ($p < 0.02$) within 25 days following surgery, but an increase occurred over the subsequent 4 weeks so that the O_2 consumption at time of sacrifice was equivalent to that of normal animals. Body temperature was increased by thyroxine to 39.7 ± 0.18 compared with that of normal rats of 38.56 ± 0.15°C ($p < 0.02$ by paired *t* test). The body temperature of thyroidectomized rats fell to 37.3 ± 0.11°C prior to sacrifice ($p < 0.001$ by paired *t* test). In spite of the secondary increase in O_2 consumption the thyroidectomized animals were considered to be hypothyroid on the basis of the myxedematous appearance, the sustained low body temperatures and inability to demonstrate residual thyroid tissue. Consistent with this conclusion is the report of Taurog *et al.*(9) showing no evidence of extra thyroidal formation of thyroxine in rats 53 days following thyroidectomy.

Contractile force of ventricle strips from thyroxine-treated animals was less than that of strips from euthyroid rats whereas strips from thyroidectomized animals contracted with greater force (Table I). The increase in force produced by ouabain was less in the experimental groups than in the control when expressed in absolute values. In terms of percent increase, the strips from hyperthyroid animals responded similarly to those from euthyroid animals (Table I).

Discussion. Our results confirm the conclusions of Rosén and Moran(3) and Morrow *et al.*(4) that alterations of the thyroid state of animals do not abolish the responsiveness of the heart to cardiac glycosides. The latter 2 studies were performed on anesthetized dogs

with cardiac contractile force changes measured *in situ* with a strain gauge arch, a procedure which does not allow comparison of absolute contractile force among animals. Therefore, our observation of increased isometric cardiac contractile force in hypothyroidism and decreased force in hyperthyroidism has no counterpart in the work of Morrow *et al.*, and Rosén and Moran.

Differences in contractile force in the 3 groups must be considered on the possible basis of differences in oxygenation of the tissues. All strips were cut to approximately the same length and width; thickness was not measured but was estimated to be about 1 mm. Thus, differences in weight may reflect differences in thickness. On this basis the greater contractile force of the hypothyroid heart strips might be explained on the basis of greater diffusion of O_2 to the central fibers of thinner strips. However, the reduced contractility of the hyperthyroid strips is not so easily explained on this basis since these strips did not differ in weight from the control strips and presumably therefore did not differ in thickness. Finally, Hirvonen & Lybeck(10), using isolated rat atria, and Whitehorn *et al.*(11), using small trabeculae carnae of rats, found diminished contractility after thyroid administration. Both of these tissues are thin enough to ensure adequate O_2 diffusion.

The differences of the magnitude of effect of ouabain among the 3 groups of rat ventricle strips are similar to the differences in effect of small doses of ouabain found by Rosén and Moran. That is, hypothyroid heart muscle responds less than euthyroid myocardium, both in absolute force and percentage change, and hyperthyroid myocardium responds to approximately the same degree as euthyroid heart muscle. The reduced response of hypothyroid myocardium may be spurious in view of the high control contractile force of the strips; that is the contractile force may be near a maximum limiting value.

In contrast, Morrow *et al.*, found nearly comparable increases in cardiac contractile force in response to ouabain in hyper- and euthyroid animals but an increased effect in hypothyroid dogs. Explanations for these different results from those of Rosén and Moran are not immediately obvious. Rather, the one

TABLE I. Comparison of Contractile Force and Response to Ouabain of Right Ventricle Strips from Normal, Athyroid and Thyroxine-Treated Rats.

	No. exp	O_2 consumption, ml/g hr	Body temp, °C	Strip wt* (wet), mg	Contractile force			
					Before ouabain	After ouabain	Change in force	
					mg/mg of strip wt		mg	%
Normal	18	1.43 ± .01	38.6 ± .15	47.4 ± 4.9	15.2 ± .89	22.8 ± 1.39	7.75 ± .61	51.75 ± 3.08
Thyroxine	8	2.20 ± .13†	39.7 ± .18†	50.5 ± 4.0	7.3 ± 1.28‡	10.2 ± 1.36‡	2.90 ± .27‡	44.8 ± 6.07
Athyroid	11							
after 25 days		1.22 ± .06†	37.3 ± .11†					
after 58 days		1.5 ± .06	37.3 ± .17†	38.9 ± 1.9‡	21.8 ± 1.45‡	25.0 ± 1.81	3.34 ± .39‡	15.05 ± 1.12

All values are means ± standard errors.

Thyroxine, 20 μg/100 g s.c., was given for 36 days.

* Each strip was cut to be approximately 15 × 2 mm. Mean lengths were 15.75, 15.13 and 15.00 for normal, thyroxine-treated and athyroid rats, respectively.

† Compared with control by paired *t* test $p < 0.01$. Others not significant.

‡ Compared with control by Student's *t* test. Others not significant.

common factor in all of these studies is the unquestionable ability of ouabain to augment myocardial contractile force regardless of the level of thyroid activity of the animals.

The altered responsiveness of ventricle strips from thyroidectomized rats may not be due entirely to the athyroid state. These animals were treated with propylthiouracil and calcium and it is possible that the changed myocardial response to ouabain was due to one or both of these agents. Assuming an elevation of serum calcium one would expect increased contractile force, which was observed, and increased response to ouabain on the basis of the additive effect of glycoside and calcium on the heart(12), which was not observed. Furthermore, we have no evidence that propylthiouracil influences myocardial contractility. Also Rosén and Moran observed reduction of the positive inotropic effect of a small dose of ouabain in athyroid dogs. However, until these factors are definitely eliminated one can only tentatively conclude that the changes in myocardial responsiveness to ouabain are a result of the absence of circulating thyroid hormone.

The nature of these changes in the heart is obscure. One consistent change observed in heart muscle which may be correlated with the changes in contractility is in the oxygen consumption. Several investigators(13,14,15, 16) have demonstrated that heart tissue removed from rats treated with thyroid powder or injected with thyroxine consume oxygen at a faster rate than hearts from normal rats. Also, Goh and Dallam(15) observed a reduced respiration of heart muscle removed from thyroidectomized rats. Thus, the force of contraction appears to be inversely related to the oxygen consumptoin. Benforado(17) has suggested that the decreased contractile force of heart muscle from hyperthyroid animals and the increased contractile force of heart muscle from hypothyroid animals is related to the uncoupling of oxidative phosphorylation produced by thyroid hormone. However, Olsen and Piatnek(18), on the basis of analysis of partition of acid soluble phosphorus in the heart of dogs treated with thyroid powder, concluded that since hyperthyroidism does not alter the storage of high-energy phosphates in myocardium, it does not affect the coupling of oxidation and phosphorylation. Limited knowledge of the action of thyroxine on tissue metabolism and the many divergent actions in *in vivo* and *in vitro* systems preclude attempts to relate specific biochemical effects to changes in contractile activity.

It is not possible to conclude from available data that basic changes in the myocardium occur in the course of thyroid disorders in human beings which render the heart insensitive to the actions of cardiac glycosides. Certainly our data and those of Rosén and Moran and Morrow *et al.*, suggest that the heart will respond to cardiac glycosides in both hypothyroidism and hyperthyroidism.

Summary. The influence of thyroxine administration and thyroidectomy on the contractility of the myocardium and its responsiveness to ouabain was evaluated in rats. Electrically driven strips of right ventricle, isolated from rats treated for 36 days with thyroxine, contracted with less than 50% of the force of strips (under comparable conditions) removed from normal animals. Ventricle strips removed from rats 58 days after thyroidectomy contracted with greater force than those of normal hearts. Ouabain, 1 μg/ml, increased contractile force in all groups, but the total increase was less in both the thyroxine-treated group and thyroidectomized group than in the control. No explanation can be offered for the altered contractility and responsiveness of myocardium produced by excess thyroxine and by ablation of the thyroid gland.

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Impaired Antibody Production in Chicks Fed Diets Low in Vitamin A, Pantothenic Acid or Riboflavin.* (28418)

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The deleterious effects of specific dietary deficiencies upon antibody production to a variety of antigenic stimuli have been noted (1-3). In a recent investigation(4), diets deficient in pyridoxine and pantothenic acid markedly impaired antibody production to influenza virus in rats, whereas thiamine deficiency failed to exert an effect. Axelrod *et al.*(5) showed that pyridoxine deficiency in guinea pigs depressed circulating antibody formation to diphtheria toxoid in both the primary and secondary phases. Stoerk and Eisen(7) and Stoerk *et al.*(8) found that male albino rats immunized in a state of pyridoxine deficiency developed serum antibody levels far below those of inanition controls (paired weight) and full fed controls.

Most of these studies have been conducted with animals severely deficient in a particular nutrient. There is need for study with animals only partially deprived of a specific nutrient, since the deficiency state in such studies would bear a closer relationship to the degree of nutritional deficiency usually encountered in man.

This study was designed to determine the effect of a partial, single deficiency of Vit. A, pantothenic acid and riboflavin on antibody production to *Salmonella pullorum* antigen in chicks. The authors are unaware of similar work in the chick.

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Methods. Two hundred day-old White Rock male chicks were randomly distributed into 5 groups of 40 each, weighed, wing-banded, and maintained to 4 weeks in thermostatically controlled battery brooders with wire floors. They were allowed feed and water *ad libitum*. Chicks were individually weighed at weekly intervals. Feed consumption was recorded.

The chicks in 3 of these groups were fed a diet suboptimal in riboflavin, pantothenic acid, or Vit. A, respectively, while those in the other 2 groups received complete diets, containing different levels of pyridoxine. A basal diet suboptimal in riboflavin, pantothenic acid, and Vit. A was prepared and these vitamins were added to give the levels in control A diet except for the vitamin studied. The basal diet shown in Table I was calculated to contain per kilogram, 975 I. U. Vit. A, 4.6 mg pantothenic acid, 2.1 mg riboflavin and 5 mg pyridoxine. These levels are 45, 50 and 72% of the chick's requirements listed by the National Research Council(6) for Vit. A, pantothenic acid and riboflavin, respectively. The complete diets (control A and B) supplied 9960 I. U. Vit. A, 24.6 mg pantothenic acid and 9.7 mg riboflavin per kilogram. All diets contain 13 mg of pyridoxine per kilogram, except for control B diet which contains 5 mg per kilogram. This latter level is 175% of N. R. C. requirement. The vitamin levels used in con-