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Introduction

Investigators agree that environmental temperature and the thyroid gland affect fertility; however, the role which both of them play and their interrelationships are not clear. Since both fertility and the output of thyroxine decline in hot weather, it has been suggested that the administration of thyroxine might improve fertility when the weather is hot. Another factor to be considered is whether the high temperature to which the animal is exposed is intermittent or continuous.

The present study was conducted to determine the effect of high environmental temperature with and without added thyroprotein on fertility. Since farm animals are subjected to high temperatures during the day and there is some cooling at night, it seemed appropriate to have one group of rabbits subjected to intermittent heat to compare with the controls and those under continuous heat. Thiouracil was fed to one group, making it possible to differentiate the effect of heat directly upon the testis from the indirect effect in which hypothyroidism is created by the heat.

Two separate experiments were carried out. Mature male rabbits about a year of age were used. Rabbits have the advantage of being small experimental animals, and they, like cattle, are non-sweating animals—a characteristic which makes them react in the same manner as cattle when exposed to high environmental temperature (Brody, 1945).

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Review of Literature

The literature pertaining to the present study will be reviewed in the following five sections: thyroid gland and fertility, environmental temperature and fertility, thyroid gland and environmental temperature, thyroid gland and thyroprotein or thiouracil, and body weight and thyroid gland.

Thyroid gland and fertility

Although it was early reported that the growth of the testes and epididymides was but slightly retarded in thyroidless rats (Hammet, 1923), there is considerable evidence to indicate that thyroxine secretion is essential for the reproductive process in different species (Koger and Turner, 1943; Turner, et al., 1943; Brody, 1945; Richter and Winter, 1947; and Blaxter, et al., 1949).

Many workers claim that at least three organs are associated with reproduction: the testis, the pituitary, and the thyroid gland (Berliner and Warbritton, 1937; Smelser, 1939; Asdell, 1949; and Blaxter, et al., 1949).

In man, thyroid deficiency is associated with loss of libido, sterility, decrease in number of sperm, and an increase in abnormal sperm forms (Lane-Roberts, et al., 1939). Thyroidectomy of the male bovine has been reported by Peterson, et al., (1941) and by Spielman, et al., (1945) to cause complete inhibition of libido but with no effect on spermatogenesis or normality of sperm. However, Bogart and Mayer (1946) found that the thyroid gland of the ram appears to be intimately concerned with spermatogenic activity of the testes but has little or no effect on interstitial tissue or accessory organs.

Results from feeding thyroprotein to rams in an area in which no extremely hot summers occur do not indicate that it was beneficial since thyroprotein-fed groups were not as good as the controls in semen characteristics (Eaton, et. al., 1948). In Missouri, where summers are often hot, "summer infertility" in rams was partly corrected by thyroxine or thyroprotein, but fertility could not be brought up to the level of the normal breeding season (Bogart and Mayer, 1946).

Thiouracil, a drug which prevents thyroxine production by the thyroid gland, reduces fertility in the ram (Bogart and Mayer, 1946). Rations containing 0.2 to 0.5 per cent thiouracil were fed to cocks for 18 weeks. The effect of thiouracil feeding on semen volume was variable. Sperm concentration was not affected. Initial motility was considerably lower than the control, and so also was the sperm survival. Thiouracil in the ration caused significant reduction in actual male fertility as determined by inseminating hens artificially (Shaffner and Andrews, 1948).

An important feature to be considered before interpreting results is the fact that very little of the work on hormonic treatment of sterility is controlled adequately. Where adequate controls have been set up, hormonic treatment has failed to demonstrate efficiency in altering fertility (Asdell, 1949). Thyroxine controls the basal metabolism and thus is essential for the efficient function of every cell of the body. As there is evidence from gynecology that hypothyroidism is often associated with infertility, this hormone must be considered in any account of hormone therapy (Asdell, 1949).

Environmental temperature and fertility

The association of fertility with temperature is a well-known fact. Though treating scrotal testes of rabbits and rams with ice for 10 minutes caused disintegration of many sperm without effect on sex drive or spermatogenesis (Chang, 1943), the application of slightly higher than normal body temperature to the external surface of the scrotum of several species caused degeneration of the generative portion of the testes. Continuous applications are more effective in producing degeneration than intermittent applications (Moore, 1924; and Phillips and McKenzie, 1934).

An increase in scrotal temperature of the bull accompanies increasing air temperature. Environmental temperature of 77° F. is considered as the critical temperature region at which certain physiological factors of heat regulating mechanism become modified. Reproductive ability declines with rising temperature above 80° F. Thermoneutrality is 12° to 18° F. below body temperature (Brody, 1945).

High temperature tends progressively to decrease sperm motility from untreated rams. Data also show that prolonged exposure to high environmental temperature results in progressive decrease in semen volume of rams but has no apparent effect on interstitial cells (Bogart and Mayer, 1946).

Apart from the actual temperature to which the animal is exposed, the length of exposure during the day has a greater effect (Anderson, 1945).

Thyroid gland and environmental temperature

Results indicate that the thyroid gland secretes its active principle, thyroxine, more rapidly in cold than in warm environments (Ring, 1939; Dempsey and Astwood, 1943; Hurst and Turner, 1947 and 1948; and Lee and Phillips, 1948). In chickens, thyroxine output increased during October to nearly double the summer level under Missouri conditions (Reineke and Turner, 1945).

A recent report stated that anatomical studies of cows from various areas showed a definite tendency for the thyroid glands of cows in the southern states to be much smaller than the thyroid glands of cows of the same breed in the northern states (Swett and Mathews, 1949).

Thyroid gland and thyroprotein or thiouracil

The administration of certain sulfonamides or thiourea results in an enlarged, hyperemic, and hyperplastic thyroid gland coincident with the state of hypothyroidism. This thyroid hyperplasia, which is considered to be compensatory to the failure of thyroid hormone synthesis, is abolished by feeding thyroid powder or by hypophysectomy (Astwood, et al., 1943).

Feeding thyroid depresses the production of thyrotropic hormone and therefore depresses thyroxine production causing the thyroid gland to atrophy (Brody, 1945; Leonard, 1947). On the other hand thiouracil interferes with the formation of thyroxine and the resultant low blood-thyroxine level leads to increased thyrotropic hormone by the anterior pituitary (MacKenzie and MacKenzie, 1943; and Leonard, 1947).

Thiouracil administration results in a significant increase in thyroid gland weight and hypertrophy and hyperplasia of the thyroid epithelia in the chicken (Shaffner and Andrews, 1948; and Blakely and Andrews, 1949), and in pigs (Acevedo et al., 1948). On the other hand, atrophy of the thyroid gland follows thyroprotein feeding (Shaffner, 1948).

Body weight and thyroid gland

Since the thyroid gland controls the rate of metabolism, the administration of thyroprotein or thiouracil presumably has an effect on growth and fattening. It is agreed that feeding thyroprotein in large quantities to different species causes a loss in body weight (Shaffner, 1948). In small dosage, however, thyroprotein increases the rate of gain in young animals (Reineke et al., 1948; and Blaxter et al., 1949).

Some investigators report that thiouracil administration has no effect on body weight (Barrick et al., 1949), while others claim that thiouracil feeding retards growth in pigs (Acevedo et al., 1948; Blakely and Andrews, 1949; and Willman et al., 1949), and in chickens (Andrews and Schnetzer, 1946; and Glazener and Jull, 1946), and still others report a beneficial effect on the rate of gain by including thiouracil in the ration (Shaffner and Andrews, 1948).

These discrepancies in results obtained by various workers might be explained by differences in thyroid activity, environmental temperature under which the experiment was run, species studied, age of the animal under investigation, individual response to the treatment, and many other factors. For instance, it was found that the dog has a much greater capacity than man to inactivate exogenous oral thyroid or intravenous thyroxine (Danowski et al., 1946). Also, the metabolic processes of the dog are less dependent on the production of thyroxine than are those of man and most animals (Glock, 1949).

Materials and Methods

A room 1,460 cubic feet was equally divided into two parts, one well insulated (referred to as the hot room), the other not insulated and not heated (referred to as the cool room). The hot room was maintained at a temperature of 90° to 92° F. by means of an electric air heater supplied with a fan and a thermostat. The temperature in the cool room varied with the outside temperature but ranged from 55° to 65° F. The relative humidity was controlled in neither room, but was fairly constant, being about 30 per cent in the hot room and about 60 per cent in the cool room. In both rooms light was supplied only by electricity for 12 hours daily using a 150 watt lamp in each room. A thermohygrograph was alternated between the rooms for recording the temperature and relative humidity.

The ration consisted of prepared commercial rabbit pellets. Feed and water were given ad libitum. Each rabbit was kept in a separate wire cage.

The first experiment started in the fall and the second experiment in the spring, so that the outside temperature would be moderate during the course of the experiment. On September 17, 1948, fifteen mature male New Zealand white rabbits that were uniform in size were divided at random into five groups, three rabbits in each group. Group I was maintained in the cool room all the time and served as the control; Group II was in the hot room continuously throughout the period of the experiment (continuous high temperature); Group III was treated the same as Group II except 0.01 per cent of the ration was thyroprotein (Koger and Turner, 1943); Group IV was kept in the hot room for 12 hours daily and then was transferred to the cool room for the rest of the day (intermittent high temperature); and Group V was treated the same as Group IV except again 0.01 per cent of the ration was thyroprotein.

The second experiment was initiated on March 16, 1949. Twenty-four mature male rabbits similar to those used in the first experiment except somewhat smaller were randomly allotted to six groups, four rabbits in each group. The first five groups were treated the same as in the first experiment. A sixth group was kept in the cool room with 0.1 per cent of the ration thiouracil.

Semen was collected by the use of a sheep artificial vagina modified for rabbits by the Missouri Agricultural Experiment Station. Semen collection and the weight of rabbits were taken once a week in the first experiment and once every ten days in the second experiment. Initial weights were taken the first day of each experiment, and semen collection followed. Since no one method of semen evaluation is entirely reliable as a criterion for fertility, several tests were applied: semen volume, initial motility, sperm concentration, percentage of live sperm in both fresh semen and undiluted semen stored for 48 hours at 40° F., and percentage of morphologically normal sperm. Fresh semen is defined as semen immediately after collection. Semen was examined under the low power of the microscope to determine initial motility which was rated as 0 to 5, with 5 being the highest score. The hemocytometer was used to compute sperm concentration. The percentages of live and normal sperm were determined from smears stained with fast green-eosin (developed at the Missouri Agricultural Experiment Station).

The first experiment was conducted for six weeks while the second experiment lasted for seven weeks, after which all rabbits were killed and their testes, epididymides, and thyroid glands were weighed and prepared for histological studies. The tissues were fixed in Bouin's solution, imbedded in paraffin, sectioned at 10 micra and stained with Delafield's haematoxylin and eosin.

The analysis of variance or the "t" test (Snedecor, 1946) was used to determine the significance of the treatment differences in various semen characteristics studied and the weight of testes and thyroid glands. The above differences were considered significant when "F" exceeded 0.05 level.

Results

There were considerable individual variations, but the data show clearly that high temperature affected fertility unfavorably. Rabbits under high temperatures produced semen that progressively decreased in volume, motility, concentration, percentage of live sperm in fresh and stored semen, and percentage of normal sperm. Control rabbits showed no change in quality of semen produced during the experimental period.

In the fourth collection, the rabbits from Group III (continuous high temperature plus thyroprotein) produced practically no normal appearing sperm. Other semen characteristics also changed markedly. For clarity and convenience, the results will be discussed as follows: Semen Volume, Initial Motility, Sperm Concentration, Percentage of Live Sperm in Fresh Semen, Percentage of Live Sperm in Semen Stored 48 Hours, Percentage of Normal Sperm, Body Weight and General Condition, Sex Drive, Mortality, Testes, Epididymis, Thyroid Glands.

Semen volume

FIRST EXPERIMENT: Because the gelatinous fraction of semen was not recorded in this experiment, and since it was observed during the course of the experiment that this fraction was affected by the treatments, figures for semen volume are not of as much value as if the gelatinous fraction were included in semen volume. Nevertheless, data show some decline in the groups subjected to continuous high temperature (Table 1). It is also to be noted that thyroprotein in the ration caused semen to decrease in volume in both Group III (continuous high temperature plus thyroprotein), and Group V (intermittent high temperature plus thyroprotein). Apparently this harmful effect of high temperature and thyroprotein was not pronounced until the third and fourth week of the experiment.

In this experiment the differences in semen volume between groups and collections lacked statistical significance (Table 2).

SECOND EXPERIMENT: Although the same trend in the decline of semen volume was observed in the second as in the first experiment, the differences between groups were more pronounced because the gelatinous fraction was included in semen volume (Table 1). Again the harmful effect of high temperature and thyroprotein on semen volume was manifested more in the third collection and thereafter. High temperature caused a decline in semen volume, the continuous high temperature being more effective than the intermittent one (Table 1). Rabbits fed thyroprotein produced semen less in volume than their mates on the same treatment without adding thyroprotein. Those fed thiouracil produced semen intermediate in volume between the group subjected to continuous high temperature and the group maintained under intermittent heat. It might be of interest to note that on the average Group I (control) produced 8.5 times in volume the semen produced by Group III (continuous high temperature plus thyroprotein), and almost three times that produced by either Group II (continuous high temperature), or Group V (intermittent high temperature plus thyroprotein). In the fifth collection, the control group produced semen amounting in volume to 14.5 times that produced by Group III (continuous high temperature plus thyroprotein) and over two times that produced by Group IV (thiouracil-fed) (Table 1).

Table 1. SEMEN VOLUME (in ml.)

Collection	Groups I	II	III	IV	V	VI	Average
First experiment							
1st	0.8	0.8	0.9	0.9	0.9	....	0.9
2d	0.8	1.0	1.0	0.7	0.6	....	0.8
3d	0.7	0.8	0.6	0.9	0.7	....	0.7
4th	0.8	0.7	0.3	0.8	0.8	....	0.7
5th	0.9	0.4	0.3	0.5	0.6	....	0.5
Average	0.8	0.7	0.6	0.8	0.7	....	0.7
Second experiment							
1st	3.4	1.5	0.8	3.2	1.8	2.1	2.1
2d	5.0	1.6	0.5	3.2	1.5	2.5	2.4
3d	3.1	1.1	0.3	2.4	1.2	1.9	1.7
4th	2.8	0.8	0.2	1.4	0.7	0.7	1.1
5th	2.9	0.8	0.2	1.0	0.7	1.4	1.2
Average	3.4	1.2	0.4	2.2	1.2	1.7	1.7

Table 2. ANALYSIS OF VARIANCE OF SEMEN VOLUME

Source of variation	Degrees of freedom	Sum of squares		Mean square	F
First experiment					
Total	24	1.0			
Collections	4	0.1		0.02	0.4
Groups	4	0.1		0.02	0.4
Remainder	16	0.8		0.05	
Second experiment					
Total	29	38.9			
Collections	4	7.8		1.9	9.5*
Groups	5	27.7		5.5	27.5*
Control vs. five treated groups	1		18.3	18.3	92.0*
Continuous vs. intermittent heat	1		4.3	4.3	21.5*
With vs. without thyroprotein	1		4.4	4.4	22.0*
Interaction of the above two	1		0.1	0.1	0.5
Thiouracil vs. four treated groups	1		0.6	0.6	3.0
Remainder	20	3.4			

* Significant.

In contrast to the first experiment, the differences between groups and collections in semen volume in the second experiment were statistically highly significant (Table 2). The differences in semen volume between the control and the five treated groups, between the groups under continuous high temperature and those under intermittent heat, and between the groups receiving thyroprotein as compared to those getting no thyroprotein, were statistically significant. The differences between the group fed thiouracil and the other four treated groups lacked statistical significance (Table 2), since the group receiving thiouracil was near the average of the other treated groups.

Initial motility

FIRST EXPERIMENT: While motility was about the same during the period of the experiment for semen from Group I (control), motility of semen collected from the other groups showed a continuous reduction as the experiment progressed. Rabbits under continuous high temperature (Group II) showed a marked reduction in their sperm motility, and thyroprotein added to the ration caused a further decline in motility (Group III). Those under intermittent high temperature (Group IV) produced semen intermediate in motility between the control group and the groups under continuous high temperature. Thyroprotein added to the ration of the rabbits under intermittent heat (Group V), brought about some improvement in motility, but this group never reached the control level (Table 3).

Table 3. INITIAL MOTILITY (0 TO 5)

Collection	Groups						Average
	I	II	III	IV	V	VI	
First experiment							
1st	4	3	2	2	3	....	3
2d	3	2	1	1	3	....	2
3d	3	1	1	2	2	....	2
4th	3	1—	0	2	1	....	1
5th	3	0	0	0	1	....	1
Average	3	1	1	1+	2	....	2
Second experiment							
1st	3+	1	2	2	3	1+	2
2d	3+	1	1+	1	2+	2	2
3d	4	1	0	1	3	1+	2
4th	4	0	0	1—	1	1	1
5th	4	0	0	1—	1+	1—	1
Average	4	1—	1—	1	2	1	2

Although the differences in motility between collections lacked significance, those between groups were statistically significant (Table 4). In determining what treatments were effective in giving the significant "between group" variance, it was found that only the differences between the control group and the four treated groups were statistically significant; the other differences lacked statistical significance (Table 4).

SECOND EXPERIMENT: The same trend found in the first experiment was also observed in the second one. The control group in this experiment produced semen of better motility than that produced in the first one. Rabbits getting thiouracil in their ration produced semen with motility a little better than that of rabbits under continuous high temperature (Group II), and quite similar to that

Table 4. ANALYSIS OF VARIANCE OF INITIAL MOTILITY

Source of variation	Degrees of freedom	Sum of squares		Mean squares	F
First experiment					
Total	24	43.6			
Collections	4	11.4		2.8	2.8
Groups	4	16.6		4.2	4.2*
Control vs. four treated groups	1		13.1	13.1	13.3*
Continuous vs. intermittent heat	1		2.1	2.1	2.1
With vs. without thyroprotein	1		0.01	0.01	0.01
Interaction	1		1.4	1.4	1.4
Remainder	16	15.6		1.0	
Second experiment					
Total	29	48.7			
Collections	4	5.9		1.5	5.4*
Groups	5	37.3		7.5	27.6*
Control vs. five treated groups	1		27.1	27.1	90.3*
Continuous vs. intermittent heat	1		4.4	4.4	14.7*
With vs. without thyroprotein	1		2.1	2.1	7.0*
Interaction of the above two	1		1.5	1.5	5.0*
Thiouracil vs. four treated groups	1		2.2	2.2	7.3*
Remainder	20	5.5		0.3	
Both experiments					
Total	49	110.5			
Replications	1	0.1		0.1	0.1
Collections	4	15.5		3.8	2.5
Groups	4	51.2		12.8	8.2*
Interaction	16	6.5		0.4	0.2
Remainder	24	37.4		1.6	

* Significant.

produced by rabbits under intermittent high temperature (Group IV), but not as good as semen produced by Group V (intermittent high temperature plus thyroprotein) (Table 3).

Differences in motility between both collections and groups were found to be statistically significant (Table 4). The differences in initial motility between the control group and the five treated groups, and between the groups under continuous high temperature and those under intermittent heat were significant. Also, the differences between the groups receiving thyroprotein and those getting no thyroprotein, and between the group fed thiouracil and the other four treated groups were statistically significant (Table 4). In pooling such experiments together, it was found that the differences in motility between collections lacked statistical significance, but the differences in motility between groups were statistically significant (Table 4).

Sperm concentration

FIRST EXPERIMENT: Sperm concentration declined markedly when rabbits were subjected to high temperature. Again rabbits under continuous high temperature showed a more marked decline in sperm concentration than those under intermittent high temperature. Thyroprotein added to the ration seemed beneficial in the group under intermittent high temperature, but without effect on rabbits under continuous high temperature. On the average, Group I (control) produced about 2.5 times more sperm than Group III (continuous high temperature plus thyroprotein). After the fourth

Table 5. SPERM CONCENTRATION (x 10,000 in cmm.)

Collections	Groups						Average
	I	II	III	IV	V	VI	
First experiment							
1st	115	91	88	105	86	......	97
2d	110	100	115	90	109	......	105
3d	81	40	15	46	62	......	49
4th	123	7	4	61	55	......	50
5th	106	2	0	47	56	......	42
Average	107	48	44	70	74	......	69
Second experiment							
1st	107	59	53	91	95	59	77
2d	122	47	53	68	77	45	69
3d	92	35	22	50	59	41	49
4th	103	17	4	37	42	24	38
5th	112	9	0	21	30	17	31
Average	107	33	26	53	60	37	53

week, rabbits in Group II (continuous high temperature) and Group III (continuous high temperature plus thyroprotein) produced semen which was practically devoid of sperm (Table 5).

Differences between groups and collections in sperm concentration were statistically significant (Table 6). Also, the differences in sperm concentration between the control and the four treated groups, between the groups under continuous high temperature and those under intermittent heat were significant. On the other hand, the differences between the groups receiving thyroprotein and those getting no thyroprotein lacked statistical significance (Table 6).

Table 6. ANALYSIS OF VARIANCE OF SPERM CONCENTRATION

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
First experiment				
Total	24	38,336.8		
Collections	4	17,759.8	4,439.9	8.8*
Groups	4	12,555.0	3,138.7	6.3*
Control vs. four treated groups	1	9,235.2	9,235.2	18.4*
Continuous vs. intermittent heat	1	3,251.2	3,251.2	6.5*
With vs. without thyroprotein	1	0.05	0.05	0.0
Interaction	1	68.55	68.55	0.1
Remainder	16	8,021.4	501.3	
Second experiment				
Total	29	33,342.0		
Collections	4	9,539.0	2,384.7	22.5*
Groups	5	21,684.6	4,336.9	40.9*
Control vs. five treated groups	1	1,704.2	17,604.2	166.1*
Continuous vs. intermittent heat	1	3,672.0	3,672.0	34.6*
With vs. without thyroprotein	1	0.05	0.05	0.0
Interaction of the above two	1	252.05	252.05	2.4
Thiouracil vs. four treated groups	1	156.3	156.3	1.5
Remainder	20	2,119.4	106.0	
Both experiments				
Total	49	78,882.8		
Replications	1	840.6	840.6	1.3
Collections	4	23,357.1	5,839.3	9.3*
Groups	4	31,138.1	7,784.5	12.4*
Interaction	16	8,489.1	530.5	0.8
Remainder	24	15,057.9	627.6	

* Significant.

SECOND EXPERIMENT: It is of interest to note that results in this experiment were almost a duplicate of those obtained in the first one. The main difference between the two experiments was the fact that while the average sperm concentration in semen from the control group was the same in both experiments, sperm concentration in semen from the treated groups in the second experiment was lower than in the first one. Apparently high temperature affected the rabbits more severely than in the first experiment. With the exception of the above difference, the features of both experiments were the same. Thyroprotein added to the ration was of beneficial effect when rabbits were under intermittent high temperature, but it was of harmful effect when they were kept under continuous high temperature. Rabbits getting thiouracil in their ration (Group VI) produced semen with a sperm count a little better than that from rabbits under continuous high temperature (Group II), but worse than semen from those under intermittent heat (Group IV). While in the fifth collection rabbits in Group III (continuous high temperature plus thyroprotein) had ejaculates devoid completely of sperm, Group I (control) produced semen with sperm counts better than that produced in the first collection (Table 5).

Again collections and groups differed significantly in the sperm count. (Table 6). The differences in sperm concentration between the control and the five treated groups and between the groups under continuous high temperature and those under intermittent heat were statistically significant. On the other hand, the differences between the groups receiving thyroprotein and those getting no thyroprotein and between the thiouracil-fed group and the other four tested groups lacked statistical significance (Table 6).

When both experiments were analyzed together, the differences between groups and collections were statistically significant (Table 6).

Percentage of live sperm in fresh semen

FIRST EXPERIMENT: The percentage of live sperm in fresh semen was decreased when rabbits were subjected to high temperature and this was more marked when high temperature was continuous than when intermittent (Table 7). The addition of thyroprotein, though it might have benefited the rabbits under intermittent heat, was of the reverse effect on those under continuous high temperature. In the fourth week, while Group III (continuous high temperature plus thyroprotein) produced semen with practically all sperm dead, semen from Group I (control) had 91 per cent live sperm. On the average, semen produced by Group III (continuous high temperature plus thyroprotein) contained only about one-third as many live

Table 7. PERCENTAGE OF LIVE SPERM IN FRESH SEMEN

Collection	Groups I	II	III	IV	V	VI	Average
First experiment							
1st	94	88	74	92	83	....	86
2d	84	65	50	42	80	....	65
3d	87	42	40	58	73	....	60
4th	91	12	2	68	71	....	49
5th	84	30	0	84	72	....	54
Average	88	47	33	69	76	....	63
Second experiment							
1st	89	64	53	71	72	69	70
2d	88	47	72	68	75	70	70
3d	82	56	12	64	69	62	58
4th	86	34	3	57	60	45	47
5th	80	11	0	42	47	11	32
Average	85	42	28	60	65	51	55

sperm as in semen produced by Group I (control), and only one-half as many as in semen produced by Group V (intermittent high temperature plus thyroprotein).

Differences in the percentage of live sperm in fresh semen between collections and between groups were statistically significant (Table 8). The differences between the control and the four treated groups and between the group under continuous high temperature and those under intermittent heat were statistically significant. The differences between the groups receiving thyroprotein and those getting no thyroprotein lacked statistical significance (Table 8).

SECOND EXPERIMENT: The features observed in the first experiment as to the effect of high temperature and thyroprotein on the percentage of live sperm in fresh semen were about the same in the present one. Again high temperature decreased the percentage of live sperm in fresh semen, the continuous high temperature being more effective than the intermittent one. Thyroprotein, while it helped somewhat in overcoming the harmful effect of high temperature on sperm when rabbits were kept under intermittent high temperature, had the reverse effect when they were under continuous high temperature (Table 7). Rabbits fed thiouracil produced semen that had a percentage of live sperm falling in the midway between that produced by Group II (continuous high temperature) and that produced by Group IV (intermittent high temperature).

On the average, semen ejaculated by Group III (continuous high temperature plus thyroprotein) contained only one-third as many live sperm as that from Group I (control), and less than half as many as that from Group V (intermittent high temperature plus thyroprotein).

Differences between both collections and groups in the percentage of live sperm in fresh semen were statistically significant (Table 8). The differences between the control and the five treated groups and between the groups under continuous high temperature

Table 8. Analysis of Variance of the Percentage of Live Sperm in Fresh Semen

Source of variation	Degrees of freedom	Sum of squares		Mean squares	F
First experiment					
Total	24	19,175.8			
Collections	4	4,153.4		1,038.3	3.1*
Groups	4	9,766.0		2,441.5	7.4*
Control vs. four treated groups	1		4,019.6	4,019.6	12.2*
Continuous vs. intermittent heat	1		5,120.1	5,120.1	15.6*
With vs. without thyroprotein	1		64.8	64.8	0.2
Interaction	1		561.5	561.5	1.7
Remainder	16	5,256.4		328.5	
Second experiment					
Total	29	19,110.3			
Collections	4	6,233.2		1,558.3	9.5*
Groups	5	9,607.5		1,921.5	11.6*
Control vs. five treated groups	1		5,292.5	5,292.5	32.3*
Continuous vs. intermittent heat	1		3,726.4	3,726.4	22.7*
With vs. without thyroprotein	1		130.1	130.1	0.8
Interaction of the above two	1		432.4	432.4	2.6
Thiouracil vs. four treated groups	1		26.1	26.1	0.1
Remainder	20	3,269.6		163.5	
Both experiments					
Total	49	36,291.5			
Replications	1	538.1		538.1	2.4
Collections	4	7,348.1		1,837.0	8.3*
Groups	4	19,177.7		4,794.4	21.6*
Interaction	16	3,913.7		244.6	1.1
Remainder	24	5,313.9		221.4	

* Significant.

and those under intermittent heat were also statistically significant. On the other hand, the differences between the groups fed thyroprotein and those getting no thyroprotein and between the thiouracil-fed group and the other four treated groups lacked statistical significance (Table 8).

Likewise, in analyzing the results of both experiments together, the differences between groups and collections were also found to be statistically significant (Table 8).

Percentage of live sperm in semen stored 48 hours at 40° F.

FIRST EXPERIMENT: The percentage of live sperm in stored semen followed the same pattern as that in semen as collected (Table 9). While in the fifth week semen from Group I (control) had 67 per cent live sperm after 48 hours storage at 40° F., the groups under continuous high temperature produced semen that had 0 to 15 per cent live sperm after storage. The groups under intermittent heat produced semen with about 51 to 59 per cent live sperm after storage. Again thyroprotein caused a decrease in the number of live sperm in stored semen when fed to rabbits kept under continuous high temperature, but it was somewhat beneficial in overcoming the harmful effect of heat when fed to those under intermittent high temperature (Table 9).

Differences in the percentage of live sperm in stored semen between collections lacked significance, but differences between

Table 9. PERCENTAGE OF LIVE SPERM IN SEMEN STORED FOR 48 HOURS AT 40° F.

Collection	Groups						Average
	I	II	III	IV	V	VI	
First experiment							
1st	59	58	60	38	49	....	53
2d	67	47	50	38	71	....	55
3d	64	31	35	40	63	....	47
4th	74	3	0	58	53	....	38
5th	67	15	0	41	49	....	34
Average	66	31	29	43	57	....	45
Second experiment							
1st	66	53	41	60	69	53	57
2d	79	37	62	61	70	62	62
3d	73	43	6	56	66	55	50
4th	79	24	0	47	55	34	46
5th	72	4	0	31	39	6	25
Average	74	32	22	51	60	42	47

groups were statistically significant (Table 10). The differences between the control and the four treated groups and the differences between the groups under intermittent heat were statistically significant. The differences between the groups receiving thyroprotein and those getting no thyroprotein lacked statistical significance (Table 10).

SECOND EXPERIMENT: The same results were obtained in the present experiment as in the first one. Rabbits fed thiouracil (Group VI) produced semen that had a percentage of live sperm intermedi-

Table 10. ANALYSIS OF VARIANCE OF THE PERCENTAGE OF LIVE SPERM IN STORED SEMEN

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
First experiment				
Total	24	11,202.0		
Collections	4	1,612.4	403.1	1.5
Groups	4	5,274.4	1,318.6	4.9*
Control vs. four treated groups	1	2,756.1	2,756.1	10.2*
Continous vs. intermittent heat	1	2,020.1	2,020.1	7.5*
With vs. without thyroprotein	1	186.0	186.0	0.7
Interaction	1	312.0	312.0	1.2
Remainder	16	4,315.2	269.7	
Second experiment				
Total	29	16,977.4		
Collections	4	5,091.5	1,272.9	8.5*
Groups	5	8,884.2	1,776.8	11.8*
Control vs. five treated groups	1	4,384.8	4,384.8	29.2*
Continuous vs. intermittent heat	1	4,032.8	4,032.8	20.2*
With vs. without thyroprotein	1	3.2	3.2	0.02
Interaction of the above two	1	460.8	460.8	3.1
Thiouracil vs. four treated groups	1	2.6	2.6	0.01
Remainder	20	3,001.7	150.1	
Both experiments				
Total	49	26,066.4		
Replications	1	79.4	79.4	0.9
Collections	4	4,836.9	1,209.2	13.4*
Groups	4	13,643.1	3,410.8	37.6*
Interaction	16	5,333.9	333.3	3.7*
Remainder	24	2,173.1	90.5	

* Significant.

ate between that produced by Group II (continuous high temperature) and Group IV (intermittent high temperature). On the average, stored semen from Group III (continuous high temperature plus thyroprotein) contained less than one-third the percentage of live sperm as that found in semen produced by the control group and less than half that in semen from Group V (intermittent high temperature plus thyroprotein) (Table 9).

Differences in the percentage of live sperm in stored semen between both collections and groups were statistically significant (Table 10). While the differences between the control and the five treated groups, and between the groups under continuous high temperature and those under intermittent heat were significant, the differences between the groups receiving thyroprotein and those getting no thyroprotein, and between the thiouracil-fed group and the other four groups lacked statistical significance (Table 10).

In pooling the results of the two experiments together, the above differences between groups and collections were likewise statistically significant (Table 10).

Percentage of normal sperm

FIRST EXPERIMENT: The percentage of normal sperm decreased markedly when rabbits were subjected to high temperature. Rabbits under continuous high temperature were more severely affected than those under intermittent high temperature and thyroprotein apparently accentuated this effect in the former but was of somewhat beneficial effect on the latter group (Table 11). Semen from the control group had more than three times the normal sperm of that produced by Group III (continuous high temperature plus thyroprotein) and more than twice the normal sperm in semen from either Group IV (intermittent high temperature) or Group V (intermittent high temperature plus thyroprotein). While the group fed thyroprotein under continuous high temperature produced semen in the fourth week that contained practically no normal sperm, semen from rabbits in the control group contained 88 per cent normal sperm (Table 11).

Differences in the percentage of normal sperm between both groups and collections were statistically significant (Table 12). The differences between the control and the four treated groups, between the groups under continuous high temperature and those under intermittent heat, and the differences between the groups fed thyroprotein and those receiving no thyroprotein were statistically significant (Table 12).

SECOND EXPERIMENT: Results in this experiment on the percentage of normal sperm are almost identical to those obtained in the first one. Thyroprotein fed to rabbits under intermittent high temperature was more beneficial than in the first experiment. The percentage of normal sperm in semen from rabbits fed thiouracil (Group VI) was lower than that in semen from Group IV (intermittent high temperature), but it was higher than that in semen produced by Group II (continuous high temperature).

It might be of interest to note that the percentage of normal sperm in most groups, especially in the second experiment, was about the same as the percentage of live sperm in stored semen (Tables 9 and 11).

In analyzing the results of the second experiment and both experiments together, it was found, as in the first experiment, that the differences in the percentage of normal sperm between groups and collections were statistically significant (Table 12). While the differences between the control and the five treated groups, and between the groups under continuous high temperature and those under intermittent heat were significant, the differences between the groups fed thyroprotein and those getting no thyroprotein and between the thiouracil-fed group and the other four groups lacked statistical significance (Table 12).

It is of interest to note that in 8 out of 11 calculations of different semen tests the differences between the groups fed thyroprotein and those receiving no thyroprotein lacked statistical significance (Tables 2, 4, 6, 8, 10, and 12). This might be due to the fact that the addition of thyroprotein was beneficial to fertility when

Table 11. PERCENTAGE OF NORMAL SPERM IN FRESH SEMEN

Collection	Groups						Average
	I	II	III	IV	V	VI	
First experiment							
1st	78	75	58	57	63	....	66
2d	77	50	41	58	55	....	54
3d	76	47	20	50	50	....	47
4th	78	34	1	44	46	....	41
5th	81	38	0	52	55	....	45
Average	78	47	24	52	54	....	51
Second experiment							
1st	85	59	38	66	70	51	62
2d	78	59	51	62	63	53	61
3d	76	52	19	55	62	54	53
4th	78	16	2	30	48	39	36
5th	75	6	0	22	41	15	26
Average	78	38	22	47	57	42	47

rabbits were kept under intermittent heat but had a harmful effect when rabbits were under continuous high temperature.

In all cases of semen evaluation the differences between the control and the treated groups were statistically significant.

The differences between the groups kept under continuous high temperature and those under intermittent heat were statistically significant in all but one time when the differences lacked statistical significance.

Table 12. ANALYSIS OF VARIANCE OF THE PERCENTAGE OF NORMAL SPERM

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F
First experiment				
Total	24	11,439.0		
Collections	4	2,096.2	524.0	4.4*
Groups	4	7,424.6	1,856.1	15.5*
Control vs. four treated groups	1	4,569.8	4,569.8	38.1*
Continuous vs. intermittent heat	1	1,548.8	1,548.8	12.9*
With vs. without thyroprotein	1	561.8	561.8	4.6*
Interaction	1	744.2	744.2	6.2*
Remainder	16	1,918.2	119.9	
Second experiment				
Total	29	16,853.5		
Collections	4	5,961.0	1,490.2	15.8*
Groups	5	9,003.1	1,800.6	19.1*
Control vs. five treated groups	1	5,728.9	5,728.9	60.6*
Continuous vs. intermittent heat	1	2,354.4	2,354.4	24.9*
With vs. without thyroprotein	1	54.5	54.5	0.6
Interaction of the above two	1	858.0	858.0	9.1*
Thiouracil vs. four treated groups	1	7.3	7.3	0.1
Remainder	20	1,889.4	94.5	
Both experiments				
Total	49	27,127.6		
Replications	1	74.4	74.4	0.9
Collections	4	6,304.1	1,576.0	19.0*
Groups	4	16,079.1	4,019.8	48.4*
Interaction	16	2,658.0	166.1	2.0
Remainder	24	2,012.0	83.0	

* Significant.

In contrast to the previous statement, the differences between the thiouracil-fed group and the other four treated groups lacked statistical significance in all cases but one (motility in the second experiment, Table 4).

Body weight and general condition

Both high environmental temperature and thyroprotein increased the rate of respiration. Rabbits from Group I (control) appeared to be in comfort all the time and they gained in weight during the experimental period. The group under continuous high temperature showed signs of discomfort and breathed at a fast rate. The addition of thyroprotein to the feed of rabbits subjected to continuous high temperature (Group III) caused great distress, marked rate of respiration, and great loss in body weight.

Rabbits under intermittent high temperature (Group IV) showed increased respiration rate and discomfort while in the hot room, but became normal in a short time after being moved to the cool room. The addition of thyroprotein to the ration of animals under intermittent high temperature (Group V) increased their response to the heat. These animals apparently were normal in the cool room, but they were more excitable.

Rabbits from Group VI (thiouracil) were lazy and breathed at a normal rate. Group I (control), Group IV (intermittent high temperature), and Group VI (thiouracil) were heavier at the close of the experiment than their initial weight. On the other hand, Group II (continuous high temperature), Group III (continuous

Table 13. BODY WEIGHT (in pounds)

Intervals	Groups					
	I	II	III	IV	V	VI
First experiment						
1st	9.1	8.2	8.5	9.3	8.6	....
2d	9.2	7.8	7.4	9.1	8.2	....
3d	9.3	7.7	7.2	9.2	7.8	....
4th	9.7	7.6	7.1	9.6	7.8	....
5th	9.9	7.6	6.8	9.7	8.0	....
6th	9.9	7.6	6.7	9.6	8.2	....
Second experiment						
1st	7.4	7.5	7.6	7.8	7.4	7.6
2d	7.8	7.5	7.2	8.1	7.0	7.4
3d	7.8	7.4	6.9	7.8	7.0	7.9
4th	7.9	7.4	6.6	7.8	6.9	7.7
5th	7.8	7.3	6.5	7.7	6.9	7.7
6th	7.9	7.4	6.4	7.9	6.9	7.9

high temperature plus thyroprotein), and Group V (intermittent high temperature plus thyroprotein) weighed less at the end of the experiment than their initial weight (Tables 13 and 14).

In both experiments, rabbits in the control group gained the most of all groups in body weight, followed by Group VI (thiouracil), and Group IV (intermittent high temperature). Group II (continuous high temperature) lost more in the first experiment, but in the second experiment the loss was less than in Group V (intermittent high temperature plus thyroprotein). In both experiments, Group III (continuous high temperature plus thyroprotein) lost the most in body weight when compared with the other groups (Tables 13 and 14).

Feed consumption was reduced by the effect of high temperature, especially the continuous one, and it was further reduced to a great extent by the addition of thyroprotein to the ration. Thiouracil was not of appreciable effect on feed consumption.

Table 14. PERCENTAGE OF GAIN OR LOSS IN BODY WEIGHT AT THE END OF THE EXPERIMENT

	Groups					
	I	II	III	IV	V	VI
First experiment	8.96	—7.63	—20.59	3.35	—5.07	......
Second experiment	5.88	—1.66	—16.39	0.80	—7.56	3.29

Sex drive

Sex drive was normal in Group I (control), almost the same in Group V (intermittent high temperature plus thyroprotein), little impaired in Group IV (intermittent high temperature) and in Group VI (thiouracil), but it was greatly reduced in Group II (continuous high temperature) and especially in Group III (continuous high temperature plus thyroprotein). While semen collection was quite easy from Group I (control), varying degrees of difficulties were encountered in collecting from the other groups.

Mortality

FIRST EXPERIMENT: A rabbit from Group III (continuous high temperature plus thyroprotein) failed to ejaculate. After two days from the start of the experiment it was paralyzed. It died October 14, 1948 after losing 32.2 per cent of its initial weight in 28 days.

SECOND EXPERIMENT: A rabbit from Group V (intermittent high temperature plus thyroprotein) was apparently in good condition, and two ejaculates were collected from it. On April 9, 1949, it died after being paralyzed for two days. It lost 9.0 per cent of its initial weight in 24 days.

Testes

FIRST EXPERIMENT: Testes weight was less in all groups under high temperature than the control. Continuous high temperature caused more reduction in testes weight than the intermittent heat. Thyroprotein added to the ration was apparently without effect when rabbits were under intermittent heat, but caused further reduction in testes weight for those under continuous high temperature. It is interesting to note that one testis from the control group weighed more than both testes from Group III (continuous high temperature plus thyroprotein) (Table 15).

The mean testes weight in the five groups was 4.38 grams with a standard error of 0.57 gram, with only Group I (control) having significantly heavier testes than the other groups.

Table 15. THE WEIGHT OF BOTH TESTES (in grams)

	Groups					
	I	II	III	IV	V	VI
First experiment	6.45	3.52	3.15	4.44	4.35	......
Second experiment	6.62	5.11	3.07	5.61	5.64	6.53

SECOND EXPERIMENT: Although the effect of high temperature and thyroprotein on testes weight was about the same in this experiment as in the first one, apparently high temperature did not have as severe an effect in the second as on rabbits in the first experiment. On the other hand, thyroprotein added to the ration of rabbits maintained under continuous high temperature (Group III) caused a marked decrease in testes weight (Table 15). The same fact repeated itself in this experiment as in the previous one with a testis from the control group weighing more than two testes from Group III (continuous high temperature plus thyroprotein). Rabbits fed thiouracil had testes only excelled in weight by those from the control group. A main difference observed was that the testes from the thiouracil group were flabby, and did not have the same consistency as the testes from the control group.

The mean testes weight in the six groups was 5.43 grams with a standard error of 0.53 grams. Only Group III (continuous high temperature plus thyroprotein) had testes significantly smaller than the other groups.

The testes showed about the same histological structure in both experiments. Histological observations showed a remarkable agreement with various characteristics studied for semen evaluation. Normal spermatogenic activity was clear in the sections of testes from the control group (Figure 1)*, whereas the testes from those under continuous high temperature showed marked derangement (Figure 2). Testes from rabbits under intermittent high temperature showed some derangement (Figure 3), but were not so severely deranged as testes from those under continuous high temperature either with or without added thyroprotein. Thyroprotein added to the ration of rabbits under continuous high temperature produced a more severe effect than when no thyroprotein was given (Figures 4 and 5). Some cells had pycnotic nuclei. However, the addition of thyroprotein to the ration of rabbits under intermittent heat prevented the high temperature from affecting the testes as severely as otherwise, but it did not maintain this group with normal testicular function (Figures 6 and 7). Testes from rabbits fed thiouracil showed some derangement (Figures 8 and 9), a condition that exhibited less derangement than that shown in Group II (continuous high temperature).

Epididymides

The epididymides from rabbits in both experiments exhibited mainly the same histological appearance. Complete agreement was observed in epididymides and testes histology and with various tests studied for semen evaluation. While the epididymides from the control group were packed with sperm (Figure 10), epididymides from the other groups contained few sperm or none. For instance, only debris and complete absence of sperm were found in the epididymis of a rabbit from Group III (continuous high temperature plus thyroprotein) (Figure 11). More sperm were found in the epididymides of rabbits from the group under intermittent heat than in those from rabbits under continuous high temperature. There were more sperm in the epididymides of rabbits receiving thyroprotein under intermittent heat, but the reverse was true when they were fed thyroprotein under continuous high temperature.

Thyroid glands

FIRST EXPERIMENT: Although intermittent heat had no effect on the weight of thyroid glands, continuous high temperature may have caused a reduction in thyroid weight. Thyroprotein added to the ration of rabbits under either continuous or intermittent high

* All photomicrographs of testes are 130 X.

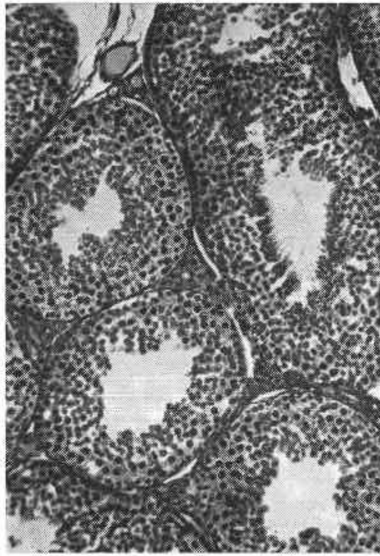

Figure 1. Group I. Control. Note normal spermatogenic activity.

Figure 2. Group II. Continuous high temperature. Note almost complete testicular derangement and lack of spermatogenic activity.

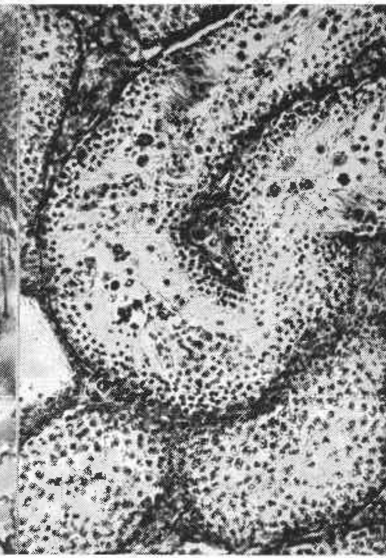

Figure 3. Group IV. Intermittent high temperature. Note testicular derangement in some portions and spermatogenic activity in only a few tubules.

ADDITION OF THYROPROTEIN TO RABBITS KEPT UNDER CONTINUOUS HIGH TEMPERATURE DOES NOT IMPROVE ABNORMAL TESTIS CONDITION CAUSED BY THE HEAT

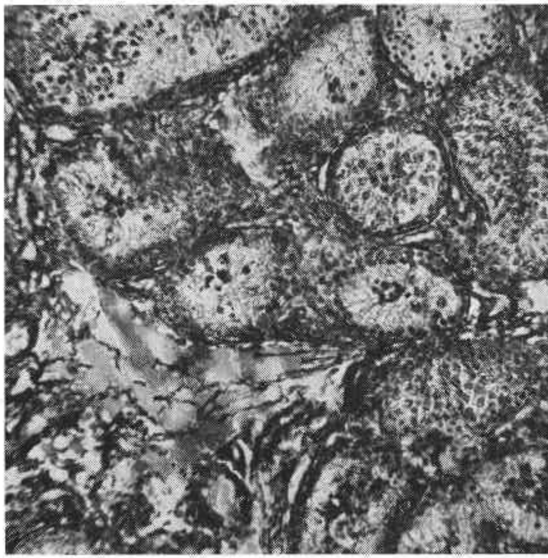

Figure 4. Group II. Continuous high temperature. Note testicular derangement and lack of spermatogenic activity. Compare with Figure 5.

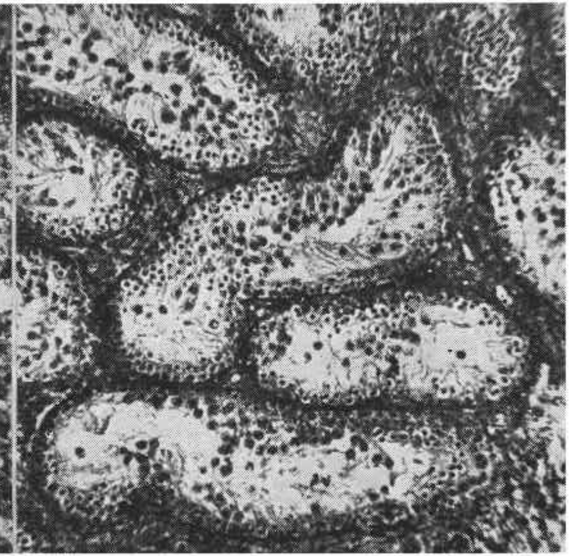

Figure 5. Group III. Continuous high temperature plus thyroprotein. Note severe testicular derangement.

Addition of Thyroprotein to Rabbits Kept Under Intermittent Heat Alleviates Some of the Harmful Effects of Heat on the Testis

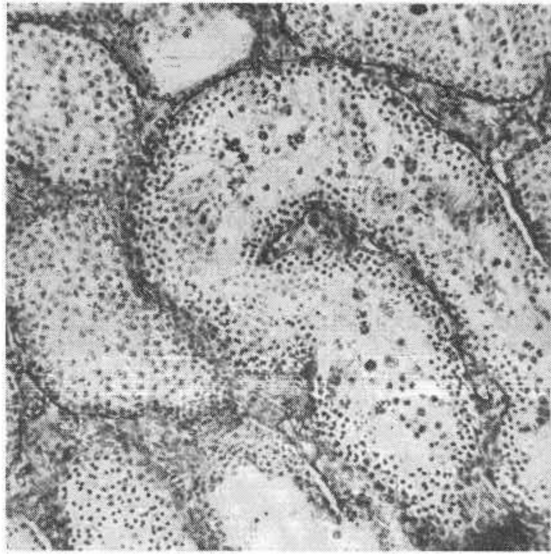

Figure 6. Group IV. Intermittent high temperature. Note some testicular derangement and spermatogenic activity in only a few tubules.

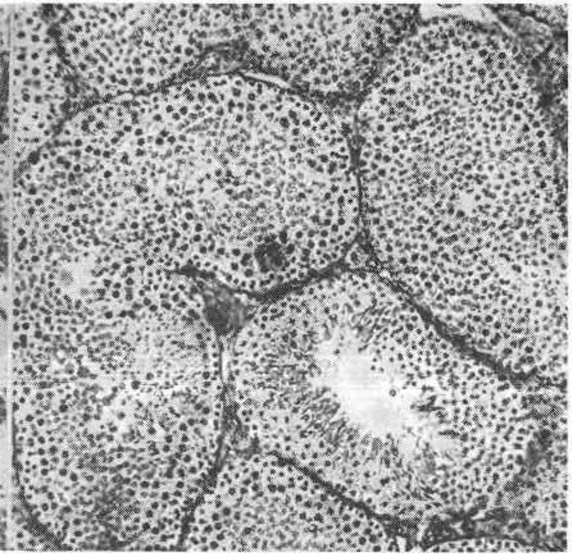

Figure 7. Group V. Intermittent high temperature plus thyroprotein. Note improvement over that shown in Figure 6. However, spermatogenic activity is not equal to control (Figure 1).

Thiouracil, Which Prevents Thyroxine Production by the Thyroid Gland, Causes Some Damage to the Testis

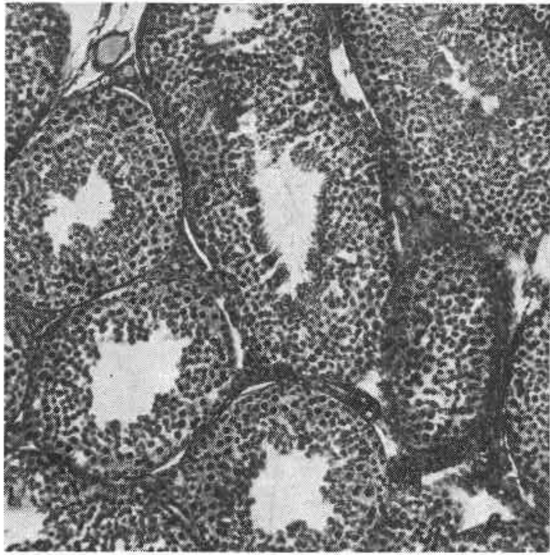

Figure 8. Group I. Control. Note normal spermatogenic activity.

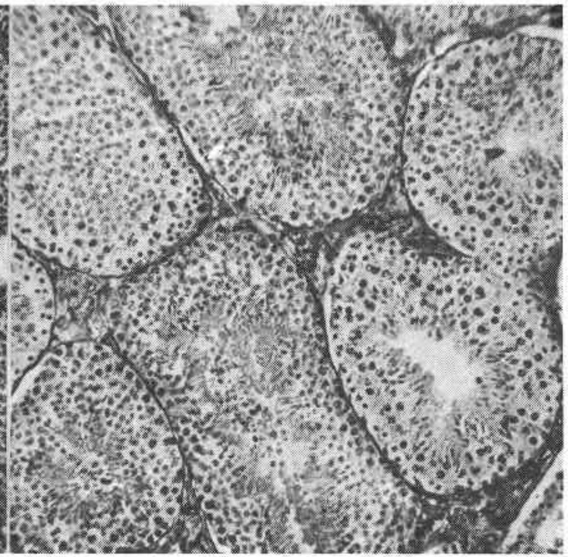

Figure 9. Group VI. Thiouracil. Note some derangement. Compare with Figure 8.

Table 16. THE WEIGHT OF THE THYROID GLANDS (in grams)

	Groups					
	I	II	III	IV	V	VI
First experiment	0.53	0.44	0.40	0.52	0.40	......
Second experiment	0.41	0.26	0.21	0.27	0.24	0.70

temperature may have decreased the weight of the thyroid glands (Table 16).

The mean weight of the thyroid glands of the five groups was 0.458 gram with a standard error of 0.028 gram. The differences in the weight of the thyroid glands between groups were not significant.

SECOND EXPERIMENT: Differing a little from the first experiment, both continuous and intermittent high temperature appeared to have caused a reduction in the weight of the thyroid glands in this present experiment and thyroprotein added to the ration resulted in further reduction in the weight of thyroid glands. Thiouracil fed to rabbits caused a marked increase in the weight of the thyroid glands (Table 16). Control rabbits had thyroid glands weighing almost twice as much as those from rabbits in Group III (continuous high temperature plus thyroprotein). On the other hand, thyroid glands from the thiouracil-fed group weighed over 1.7 times as much as those from the control group.

The mean weight of the thyroid glands of the six groups was 0.348 gram with a standard error of 0.077 gram. Only Group VI (thiouracil) had thyroid glands which were significantly heavier than those of the other groups. On the average, thyroid glands of the individual rabbits in the second experiment (excluding the thiouracil group) weighed less than those from rabbits in the first experiment (Table 16).

Histological studies of the thyroid glands of the control group showed a normal structure and signs of normal activity, whereas those from the groups subjected to high temperature showed varying degrees of atrophy. High temperature, and especially the continuous one, caused marked inactivity in the cells and dissolving of the colloid. The follicles in the thyroid glands of the control group (Figure 12) were closely packed, fairly uniform in size, and separated with very thin connective tissue. The epithelium was low and cuboidal. The colloid was plentiful in amount, stained deeply and but slightly vacuolated. In Group III (continuous high temperature plus thyroprotein) there was an increased vacuolization of the colloid with its diminution in amount and depth of staining. The follicles were dis-

Epididymis of Rabbits Presents the Same Evidence as Semen Evaluation and Testis Morphology in Showing Harmful Effects of High Environmental Temperature on Reproduction

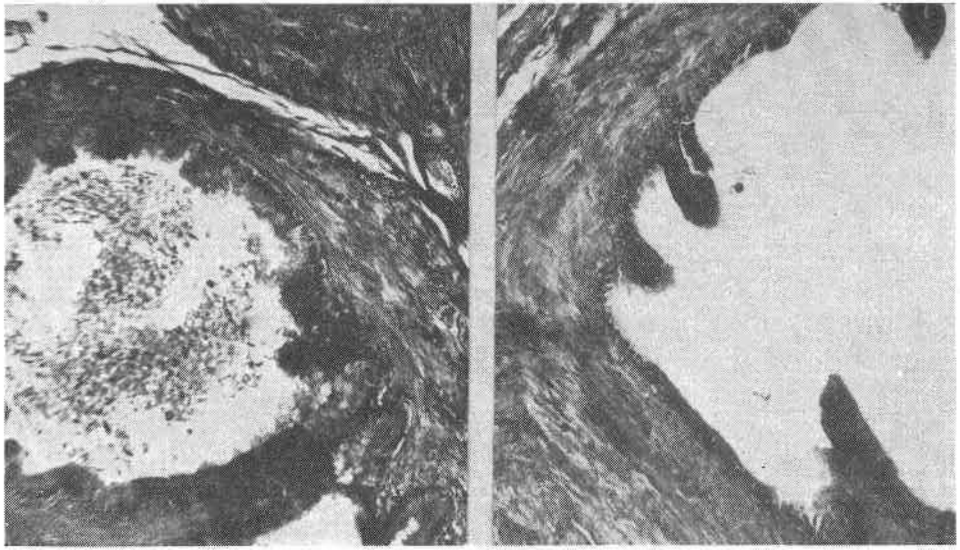

Figure 10. Group I. Control. Note sperm in lumen of epididymis.

Figure 11. Group III. Continuous high temperature plus thyroprotein. Note lack of sperm in lumen of epididymis.

Effect of Thiouracil on the Thyroid Gland of the Rabbit

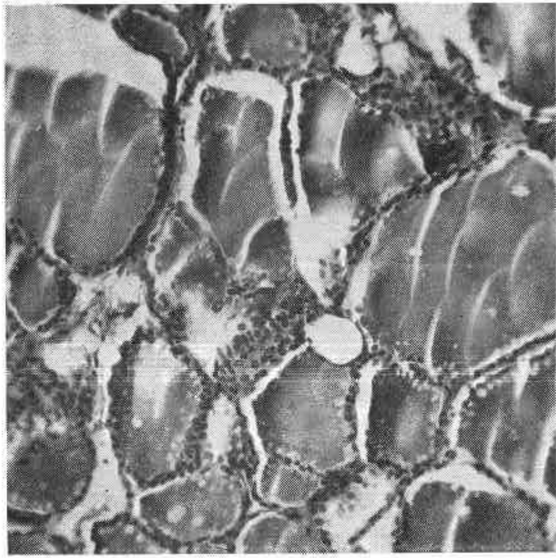

Figure 12. Group I. Control. Note abundance of colloid and low cellular height.

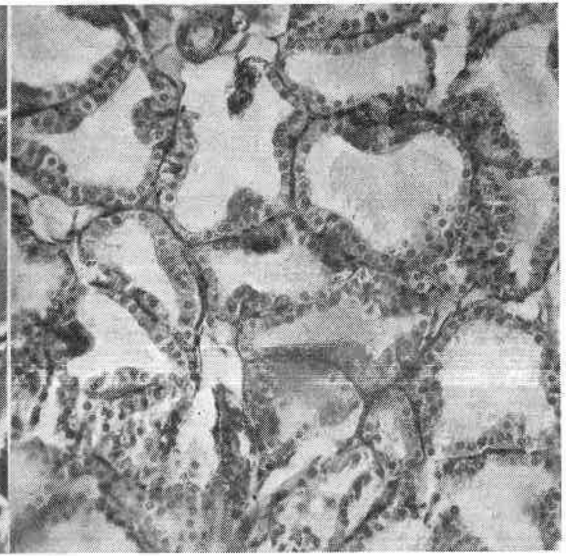

Figure 13. Group VI. Thiouracil. Note absence of colloid and marked increase in cellular height.

tended and filled with non-vacuolated colloid and composed of extremely low epithelium, indicating a high degree of regression. A milder degree of the above picture is appropriate in describing the sections of the thyroid glands from Group II (continuous high temperature), Group IV (intermittent high temperature), and Group V (intermittent high temperature plus thyroprotein). On the other hand, thyroid glands from the thiouracil-fed group were greatly enlarged as a result of hyperplasia and hypertrophy (Figure 13). The thyroid glands in this group showed papilliferous projections of the heightened epithelium into the colloid. The epithelium was double and sometimes several layers in thickness. The colloid sometimes was the same in amount as in the control but generally much less.

Thyroid glands from Group IV (intermittent high temperature) were not as badly damaged as those from Group II (continuous high temperature). Thyroprotein caused a further inactivation of the thyroid glands in rabbits under both continuous and intermittent high temperature.

Discussion

It is evident from the present study and other investigations that environmental temperature is involved in fertility of the male. Since livestock in some regions is exposed during the summer months to high temperature which remains fairly constant day and night, while in other regions the difference in temperature between day and night is great, the effect of continuous and intermittent heat on fertility was investigated. High environmental temperature reduced fertility in the male rabbit with continuous high temperature being of a more detrimental effect than the intermittent heat.

Dempsey and Astwood (1943) have shown that high temperature reduces the output of thyroxine by the thyroid gland, and there is ample evidence that thyroxine is necessary for proper reproduction in the male. Thiouracil was used in this investigation to inactivate the thyroid gland. The hypothyroidism created by the use of this drug can be compared with hypothyroidism produced by high temperature plus the direct effect of heat, and with the direct effect of heat when thyroxine is added to make sure that hypothyroidism does not exist.

When thiouracil was administered, fertility declined to a level somewhat lower than when animals were subjected to intermittent heat. This indicates that intermittent high temperature did not completely inactivate the thyroid gland. Fertility in rabbits receiving thiouracil was not as low as that in the rabbits under continuous high temperature. This indicates that continuous high temperature is acting both by inactivating the thyroid gland (indirectly) and by affecting the testes (directly).

When thyroprotein was added to the ration of rabbits under intermittent heat, fertility was maintained at a higher level than when no thyroprotein was given, though the level of fertility was below that of the controls. This suggests that a part of the reduction in fertility from intermittent heat resulted from the direct effect of the increased temperature on the testes. Apparently thyroxine is not effective in correcting the lowered fertility which results from the direct effect of high temperature on the testes.

The addition of thyroprotein to rabbits under continuous heat caused an increased rate of metabolism at a time when animals would normally reduce their own thyroxine output to decrease the rate of metabolism. This made the rabbits uncomfortable, increased their respiration rate, caused them to lose weight, and reduced their fertility even lower than those under continuous high temperature with no added thyroprotein.

It appears that high temperature is affecting fertility in two ways: (1) directly on the testes and (2) indirectly through the thyroid gland. The normal mechanism for the animal to adjust itself to high temperature is for the thyroid gland to slow down its thyroxine output and this results in a reduced metabolic rate to offset the increased metabolism created by high temperature.

From the previous discussion, the evidence points to the conclusion that both environmental temperature and the thyroid gland affect fertility in the male rabbit, thus agreeing with other workers in a number of various species. But since the fertility of rabbits was not completely restored by feeding thyroprotein, one cannot escape the conclusion that the direct effect of high environmental temperature has a more far reaching influence on fertility than the indirect action by causing the thyroid gland to cut down its thyroxine output.

Discrepancy in the results of hormone treatment reported in the literature may be partly explained by differences in individual response, species studied, environmental temperature at which the experiment is conducted, dosage of hormone given, the quality of feed, and many other factors. However, as was mentioned earlier, thyroxine controls the basal metabolism, and thus is essential for the efficient functioning of every cell of the body. For this reason, thyroxine must be considered in any account of hormone therapy (Asdell, 1949).

This work suggests that thyroxine might be beneficial under practical use only when there is cooling at night. Also the indications are that thyroxine will not completely overcome the harmful effects of high temperature even if there is cooling at night. Perhaps the direct effect of high temperature on the testes during the day, which is independent of its action on the thyroid gland, causes an effect which cannot be alleviated by thyroxine.

Summary and Conclusions

An experiment was conducted in the fall of 1948 and another one in the spring of 1949 using mature male rabbits to study the effects of environmental temperature and the thyroid gland on fertility. Fifteen male rabbits were divided equally into five groups in the first experiment and twenty-four male rabbits were divided equally into six groups in the second experiment. Group I served as control and was kept continuously under room temperature of 55° to 65° F.; Group II was maintained under 90° to 92° F. 24 hours daily; Group III was treated as Group II except 0.01 per cent of the ration was thyroprotein; Group IV was kept under 90° to 92° F. 12 hours daily and 55° to 65° F. the remainder of the day; Group V was treated the same as Group IV except 0.01 per cent of the ration was thyroprotein; and Group VI was kept under 55° to 65° F. all the time but 0.1 per cent of the ration was thiouracil. Light was supplied to all groups by electricity for 12 hours daily.

Semen collections and weights of rabbits were taken every seven days in the first and every ten days in the second experiment. The semen was examined for volume, motility, concentration, percentage of live sperm in fresh and stored semen, and percentage of normal sperm.

The first experiment covered a period of six weeks, while the second one was continued one week more than the first. All rabbits were killed at the end of the experimental period and their testes, epididymides, and thyroid glands were weighed and prepared for histological studies.

High temperature resulted in a decrease in semen volume, motility, sperm concentration, the percentage of live sperm in fresh and stored semen, and the percentage of normal sperm. Continuous heat had a more pronounced effect than intermittent heat. Thyroprotein caused a decline in semen volume but its effect on the other semen characteristics depended on whether or not rabbits were under continuous or intermittent heat. Under continuous heat, thyroprotein caused a further reduction in motility, concentration, the percentage of live sperm in fresh and stored semen, and the percentage of normal sperm. On the other hand, thyroprotein fed to rabbits under intermittent heat resulted in an improvement in motility, concentration, the percentage of live sperm in fresh and stored semen, and of normal sperm when compared with semen from rabbits under the same treatment without feeding thyroprotein. Thiouracil caused a decrease in semen volume, motility, sperm concentration, the percentage of live sperm in fresh and stored semen and in normal sperm.

Sections of the testes showed that high temperature caused marked derangement and reduced spermatogenic activity. Continuous high temperature had a more pronounced effect than intermittent heat. Testes from rabbits fed thyroprotein and maintained under continuous high temperature showed lack of spermatogenic activity and a high degree of derangement. On the other hand, thyroprotein fed to rabbits under intermittent heat was apparently of considerable benefit to the spermatogenic activity. Testes from rabbits fed thiouracil exhibited about the same derangement as those from rabbits under continuous heat.

Both high temperature and thyroprotein increased the rate of respiration, decreased food consumption, and caused a loss in body weight. Rabbits fed thiouracil breathed at a normal rate, were somewhat lazier than normal, and gained in body weight.

Rabbits in the five treated groups were slower in their sex drive than those in the control group.

Thiouracil was without effect on testes weight, whereas both temperature and thyroprotein caused a decrease in testes weight.

High temperature and thyroprotein resulted in a decrease in thyroid gland weight, while thiouracil caused a marked increase in the weight of thyroid glands.

From the above discussion it is evident that high environmental temperature reduced fertility in the male rabbit, continuous high temperature being more detrimental to fertility than intermittent heat. The addition of thyroprotein to the ration was harmful when rabbits were under continuous high temperature, whereas its administration to those under intermittent heat alleviated some of the ill effects of high temperature, but did not give results equal to those with rabbits kept under cool temperature. The addition of thiouracil to the ration lowered fertility.

In conclusion, it might be suggested that environmental temperature affects fertility directly by acting on the testis, and indirectly by affecting the thyroid gland. The addition of thyroprotein may alleviate the indirect action of high temperature which results from a hypothyroid condition, but apparently it has no action in preventing the direct effect of high temperature on the testes at dosage levels here used.

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