The University of Chicago

ATTEMPTS TO PRODUCE EXPERIMENTAL THYROID HYPERPLASIA

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE

OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

BY

GEORGE EMANUEL BURGET

Private Edition, Distributed By
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE AMERICAN JOURNAL OF PHYSIOLOGY, VOL. 44, NO. 4
NOVEMBER, 1917

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 44, No. 4,
November, 1917

ATTEMPTS TO PRODUCE EXPERIMENTAL THYROID HYPERPLASIA

G. E. BURGET

From the Hull Physiological Laboratory of the University of Chicago

Received for publication August 23, 1917

The following series of experiments were carried out at the suggestion of Doctor Carlson to determine certain factors that have been reported to induce thyroid hyperplasia in man and experimental animals.

I. HIGH PROTEIN DIET

Attention was called to the effect of high protein diet on the thyroid by C. Watson in 1904. At this time he found that prolonged administration of raw meat to healthy fowls induced certain changes in these glands making them different histologically from the glands of fowls on an ordinary diet. He confirmed these results in 1906 by feeding rats. Later ('07) he found that wild rats kept in captivity and fed on a bread and milk diet developed hyperplastic thyroids.

Reid Hunt ('11) reported finding large vascular thyroid glands in mice fed on a diet of oatmeal and liver. The same year Marine and Lenhart in their observations on brook trout found goitre endemic in all hatcheries where the salmonidae were artificially fed. Its severity was related to hygienic conditions, food, water supply and degree of crowding.

An altered condition of the thyroid with an increased cellular element was induced by C. Watson ('12) in rats and mice by feeding a diet of flour alone.

Marine ('14) produced hyperplasia of the thyroid in brook trout by feeding liver and heart. He was able to bring about an involution of this condition by changing the diet to fresh sea fish.

Bensley ('14) noticed that the opossum developed hyperplasia in a relatively short time after being put into captivity. He was able to control the degree of hyperplasia in this animal at will by diet.

Fresh and stale liver was fed young rats by Marine ('15). All his rats gained in weight. Those receiving liver one, two and three days

old showed distinct hypertrophy as judged by the reduction in amount of stainable colloid and the increase in the size of the thyroid cells. Those given fresh liver and liver four, five and six days old had normal or nearly normal glands.

The rats in the following experiments were carefully weighed at the beginning of the experimental feeding period and again before killing at the close of the period. All rats were anesthetized and killed by bleeding. The thyroids were removed as quickly as possible and weighed in moist weighing bottles. They were then fixed in formalin Zenker or acetic osmic bichromate. The cages were cleaned and fresh water given daily. (Table 3 forms an exception of which mention will be made later.)

Bread soaked in milk was used as the standard diet and fresh liver and lean beef (muscle) was given for the high protein diet. The liver or muscle was usually mixed with a little oatmeal or bread crumbs since this enables the rat to live indefinitely. The full amount that the animal would eat each day was given but no more. The rats fed on a standard diet gained rapidly in weight as a rule while those fed on meat grew if young but if mature usually lost weight.

Histologically these glands presented a somewhat uniform appearance. Ordinarily there was a fair amount of colloid, the follicles were round and the cells slightly columnar. The glands of most animals on high protein diet showed a greater or less degree of hyperplasia. Mitosis was rarely seen. A few showed secretion antecedent with Dr. Bensley's new brasilin stain. Since the weight of the thyroid gland has been shown by the above author to be a criterion as to its degree of hyperplasia, I have made use of this method in drawing my conclusions.

I have placed alongside the actual weight of the gland in each case for purposes of comparison the weight as computed by the method given by Hatai based on the body weight of the rat. In each case the body weight at the time the animal was killed was used for computing the thyroid weight.

Table 1 represents the results from six full grown rats. Numbers 1, 2 and 3 received daily fresh liver ground up with a little oatmeal. Numbers 4, 5 and 6 were given bread soaked in milk. Number 6 for some unknown reason lost considerably in body weight but the thyroid weight was below normal showing that loss in body weight does not necessarily indicate a hyperplastic tendency of the thyroid gland.

Number 5 of table 2 showed a very hyperplastic gland in spite of the fact that it was rapidly gaining weight.

The animals represented in table 3 were kept in uncleaned cages. The excreta was allowed to collect in the cage and the drinking water of the animal became highly contaminated with fecal refuse. All the animals with one exception gained in weight during the experimental

TABLE 1

The effect of a high protein diet on the thyroid in adult rats

NUMBER OF RAT	DIET	INITIAL WEIGHT	CHANGE IN WEIGHT	WEIGHT OF THYROID		DURATION OF EXPERIMENTS
				Actual	Hatai computed	
		grams	*grams*			*days*
1	High protein diet	252	−32	0.0540	0.0344	34
2		187	− 9	0.0310	0.0294	26
3		258	−20	0.0300	0.0368	26
4	Standard diet	148	− 3	0.0170	0.0252	25
5		157	21	0.0210	0.0294	25
6		273	−73	0.0240	0 0322	35

TABLE 2

This group received a similar diet to the rats represented in table 1

NUMBER OF RAT	DIET	INITIAL WEIGHT	CHANGE IN WEIGHT	WEIGHT OF THYROID		DURATION OF EXPERIMENTS
				Actual	Hatai computed	
		grams	*grams*			*days*
1	High protein diet	112	5	0.0110	0.0214	32
3		103	37	0.0190	0.0243	32
5		120	48	0.0750	0.0284	32
7		123	23	0.0300	0.0253	32
9		320	−33	0.0350	0.0421	32
11		317	−52	0.0300	0.0401	32
2	Standard diet	113	25	0.0270	0.0243	32
4		150	28	0.0300	0.0295	32
8		158	26	0.0250	0.0294	32
10		355	−69	0.0250	0.0421	32

feeding. The average body weight of the rats on the high protein diet was about the same as that of the rats fed on a normal diet, yet the average thyroid weight was 0.0108 gram greater in the case of the former. The normal fed animals had an average thyroid weight considerably above the Hatai computed weight. This would indicate, as

McCarrison ('14) has reported, that there is some hyperplasia induced in rats fed on a normal diet and kept under unhygienic conditions. All the liver fed animals showed distinctly heavier thyroids with the exception of number 1 and this was a young, growing rat.

In table 4 all the meat fed rats were young and every one grew well on the diet. None showed marked hyperplasia. This would suggest that the young growing rat can thrive on a high protein diet and its thyroid gland is not affected as in the case of the more mature animals.

TABLE 3

The effect of high protein diet and standard diet on the thyroid with the additional factor of unhygienic cages

NUMBER OF RAT	DIET	INITIAL WEIGHT	CHANGE IN WEIGHT	WEIGHT OF THYROID		DURATION OF EXPERIMENTS
				Actual	Hatai computed	
		grams	*grams*			*days*
7	High protein diet	97	78	0.0230	0.0290	31
8		110	77	0.0440	0.0310	40
9		177	18	0.0610	0.0316	37
10		252	−12	0.0550	0.0370	37
11		137	82	0.0550	0.0344	40
12		110	35	0.0280	0.0250	31
1	Standard diet	119	57	0.0250	0.0290	31
2		137	63	0.0280	0.0325	40
3		155	50	0.0270	0.0328	37
4		240	15	0.0520	0.0353	37
5		97	75	0.0350	0.0290	40
6		155	31	0.0350	0.0304	31

Table 5 represents a fairly even litter of rats from Donaldson's laboratory, Wistar Institute. These animals were kept in an iodine-free room in Doctor Bensley's laboratory, thus eliminating any possibility of error which might be introduced by the presence of free iodine. For a high protein diet the rats were given lean beef (muscle). All the rats on this diet lost in weight and most of them showed distinct hyperplasia of the thyroid gland. The bread fed animals all gained in weight and none showed hyperplasia.

In table 6 I have made a summary of the results shown in tables 1 to 5. The average in each of the above groups with the difference between the average actual weight and the average computed weight of the thyroid is expressed. The average weight of the meat fed rats

in the five groups was 164 grams; of the bread fed, 179 grams. Yet the average weight of the thyroids of the meat fed rats was 0.0311; and that

TABLE 4

The effect of a high protein diet on the thyroid in growing rats

NUMBER OF RAT	DIET	INITIAL WEIGHT	CHANGE IN WEIGHT	WEIGHT OF THYROID		DURATION OF EXPERIMENTS
				Actual	Hatai computed	
		grams	*grams*			*days*
7		55	57	0.0150	0.0207	31
8		80	25	0.0150	0.0197	31
9	High protein	90	35	0.0190	0.0225	31
10	diet	62	38	0.0200	0.0190	33
11		68	54	0.0270	0.0222	33
12		60	25	0.0130	0.0167	33
1		114	68	0.0210	0.0300	31
2		113	72	0.0240	0.0300	31
3	Standard diet	140	17	0.0280	0.0268	31
4		147	−7	0.0320	0.0243	17died
5		120	70	0.0350	0.0310	31
6		170	7	0.0380	0.0290	31

TABLE 5

The effect of a high protein diet on a litter of rats from Donaldson's laboratory kept in an iodine-free room

NUMBER OF RAT	DIET	INITIAL WEIGHT	CHANGE IN WEIGHT	WEIGHT OF THYROID		DURATION OF EXPERIMENTS
				Actual	Hatai computed	
		grams	*grams*			*days*
8		145	−10	0.0260	0.0235	56
11		130	−40	0.0270	0.0175	56
14	High protein	145	− 5	0.0210	0.0243	56
15	diet	135	−5	0.0140	0.0231	56
16		150	−60	0.0300	0.0173	49
2		117	23	0.0190	0.0243	61
4	Standard diet	142	16	0.0190	0.0268	61
10		132	23	0.0198	0.0268	61

of the bread fed was 0.0280. This shows an increase in weight of the thyroid gland of the animals fed on a high protein diet over that of the animals fed on a standard diet of bread and milk of 10 per cent.

These experiments show that a high protein diet, either fresh liver or muscle, in the course of a few weeks, produces in the adult white rat a distinct hyperplasia of the thyroid gland. The fact brought out by McCarrison ('14) is further emphasized, namely that unclean cages have a tendency to produce goitrous glands in rats.

TABLE 6

Summary of the effects of a high protein diet on the thyroid, detailed in tables 1 to 5

GROUP	DIET	AVERAGE BODY WEIGHT	AVERAGE THYROID WEIGHT IN GRAMS		DIFFERENCE IN THYROID WEIGHT
			Actual	Hatai	
		grams			
I	High protein diet	212	0.0383	0.0335	0.0048
II		187	0.0333	0.0303	0.0028
III*		194	0.0445	0.0313	0.0132
IV		108	0.0182	0.0201	−0.0019
V		117	0.0236	0.0205	0.0031
I	Standard diet	174	0.0234	0.0234	
II		197	0.0268	0.0313	−0.0045
III*		199	0.0337	0.0315	0.0022
IV		172	0.0297	0.0285	0.0012
V		151	0.0193	0.0259	0.0066

* The influence of unclean cages stands out markedly in those rats receiving standard diet as well as in those receiving a high protein diet.

II. INFLUENCE OF FECES FROM GOITRE PATIENTS AND GOITRE DOGS

This series of experiments was undertaken in an attempt to confirm the findings of some recent investigators, namely, that excreta from human or animal subjects is an important factor in the production of goitre.

Marine and Lenhart ('10) found that in regions of endemic goitre the fish may also be affected. This was thought to be direct evidence that goitre was associated with water. Marine ('14) by experiments on brook trout came to the conclusion that goitre was non-infectious and non-contagious.

McCarrison ('11) gave goats water which came through sterilized soil mixed with feces of goitrous individuals. Many of the animals developed diarrhoea and 50 per cent of them showed enlargement of the thyroid. He came to the conclusion that hypertrophy of the thyroid was due to an infecting agent of goitre or some organic impurity

of water. Treatment of simple goitre in individuals by vaccines of coliform bacillus, a spore-bearing bacillus and a staphylococcus with success led him further to believe that the gland undergoes hypertrophy as the results of a stimulus which is commonly a toxic material absorbed from the alimentary tract, the enlargement of the thyroid being due to an effort on the part of the gland to resist some toxic agency. He also proposed intestinal antiseptics as a treatment for goitre. Bircher's work (quoted from McCarrison) on feeding feces with cooked rice and giving subcutaneous injections of feces to rats showed positive results. McCarrison thus concluded that goitre was caused by the toxin of a microörganism that finds its home in the intestine of man. Feeding non-goitrous feces, goitrous feces, aerobic organisms and anaerobic organisms from goitrous feces gave enlargement of the thyroid in rats as did keeping in unhygienic conditions (McCarrison, '14).

Messerli ('14) found that water from a goitrous region produced goitre in rats.

Rosenow ('14) reported the finding of a gram positive diplo-bacillus-like organism in the thyroid in twenty-five out of thirty cases of goitre in man and eight out of twelve cass of goitre in dogs.

Feeding pregnant goats feces, McCarrison ('16) obtained 100 per cent goitrous kids. He concluded that the toxic substance derived from the intestine of the mother produced goitre in the foetus.

Cats were used in the following experiments since these animals are seldom found to have goitre when brought into the laboratory or to develop spontaneous goitre when kept under laboratory conditions. Any goitrous condition occurring among them, therefore, while in the laboratory, might safely be considered to be due to experimental procedure.

All cats in tables 7 and 8 had the left thyroid removed a few weeks before the experimental feeding began. The gland was weighed and fixed in formalin Zenker. The cats in table 7 were given a solution of feces from a goitrous dog. About 100 cc. of this material was injected into the stomach with a stomach tube every second day and was retained. Animal number 5 was kept as a control.

Cats 9, 12, 14 and 17 in table 8 received a solution of feces from an exophthalmic goitre patient every second day. Cats 6 and 13 were given a solution of feces from a normal person. Cat 11 was used as a control.

These animals thrived on a normal diet in spite of the injection of fecal matter. Most of them gained in weight during the experimental

period. The difference in weight of the glands before and after feeding is not enough to be of any significance. The histological changes were negligible. The wide differences in environment and food that must exist among cats procured for laboratory work makes it impossible to describe a normal gland histologically. The two thyroids in the same animal are usually very similar as regards weight, amount and staining

TABLE 7

Cats given feces from a goitrous dog

NUMBER OF CAT	BODY WEIGHT	THYROID WEIGHT		CHANGE IN BODY WEIGHT	DURATION OF EXPERIMENTS
		Left	Right		
	grams	*grams*	*grams*		*days*
1	2060	0.165	0.165	1055	65
2	3780	0.225	0.215	660	65
3	3220	0.080	0.110	295	65
4	2560	0.275	0.150	−820	35
5	2320	0.090	0.105	545	65

TABLE 8

Cats given feces from normal individual and from an exophthalmic goitre patient

NUMBER OF CAT	BODY WEIGHT	THYROID WEIGHT		CHANGE IN BODY WEIGHT	DURATION OF EXPERIMENTS
		Left	Right		
	grams	*grams*	*grams*		*days*
9	3000	0.105	0.108	150	75
12	2900	0.120	0.100	−100	75
14	2720	0.155	0.190	480	90
17*	3200	0.580	0.370	300	90
11	3040	0.210	0.200	260	90
6	4200	0.115	0.120	−260	75
13	3280	0.125	0.145	−340	90

* Had been suckling young.

properties of colloid, size of follicle and dimensions of the epithelial cell. Therefore, the individual comparison of the gland before feeding with the one after feeding was used as a criterion of histological change due to experimentation. No hypertrophy could be said to have been induced in these animals.

Focal infections such as may occur in tonsils, teeth, sinuses of the head and joints of the body, appear to bear some relation to goitre

(Billings, '14; Brown, '14; Buford, '15). Evans, Middleton and Smith ('16) have reported findings in a number of cases of goitre in endemic goitre regions in Wisconsin which lead them to believe tonsillar infection may be one means by which goitre may be induced. With this in mind an attempt was made to infect the tonsils of three cats by injecting virulent streptococci directly into the tonsil with a hypodermic syringe.

A bouillon suspension of virulent streptococci was injected into the tonsils of the cats while under an anesthetic. Several injections were made with an interval of seven days between each. In no case were we able to produce a tonsillar infection or thyroid hyperplasia by this means.

III. UNION OF THE PHRENIC AND CERVICAL SYMPATHETIC NERVES

This was an attempt to reproduce some work done by Cannon, Binger and Fitz ('16). These authors fused in the cat the anterior root of the right phrenic nerve with the right cervical sympathetic cord. Thus, after regeneration had taken place, the impulses over the phrenic would be sent to the superior cervical ganglion. Out of the six cats operated on four showed, after four months, according to these authors, the following symptoms: marked tachycardia, loose movements of the bowels, falling hair, increased excitability and increased metabolism. One cat also showed exophthalmos and respiratory hippus on the operated side and on autopsy the adrenals were found to be three times their normal size. A. Troell performed the same operation on cats and dogs. His animals survived from twenty to one hundred and seventy-five days. None showed the evidences of hyperthyroidism noted by Cannon and his coworkers.

Langley ('04) in testing the union of different kinds of nerve fibers united the peripheral end of the cervical sympathetic to the root of the phrenic on the left side. The lower half of the cervical sympathetic was excised on the opposite side. The paralytic effects were equal on the two sides for about two months after which they gradually diminished on the left side. This animal lived for six months without showing any change in the nictitating membrane, eyelids or pupil in correspondence with respiration, or any changes similar to those reported by Cannon and his coworkers.

I made an end-to-end suture of the central end of the phrenic and anterior end of the cervical sympathetic cord in ten cats and three

rabbits. These animals survived from one to twelve months. In no case was I able to find any of the changes indicative of excessive thyroid secretion. The animals recovered from the operation readily, ate heartily and were apparently normal in every respect. This would seem to indicate that a physiological anastomosis was not formed or, if formed, the stimuli thus carried were not efficient in increasing the output of thyroid secretion to the point of producing the pathological changes accompanying toxic thyroid hyperplasia in man.

IV. EFFECT OF REMOVAL OF A SECTION OF THE CERVICAL SYMPATHETIC NERVE ON THE THYROID

Missiroli ('08) cut the cervical sympathetic nerve in rabbits and examined the thyroid histologically. He reported an excess of secretion immediately after with a gradual reversion and final atrophy. Wiener and Floresco (quoted from Wilson and Durante, '16) made similar findings.

TABLE 9

Results after removal of a section of the cervical sympathetic nerve on the right side in cats

NUMBER OF CAT	WEIGHT OF THYROIDS		DURATION OF EXPERIMENTS
	Left	Right	
	grams	*grams*	*days*
1	0.070	0.085	45
2	0.130	0.130	14
3	0.180	0.150	33
4	0.125	0 143	49
5	0.050	0.050	65
6	0.150	0.155	51
7	0.100	0.105	111
8	0.080	0.080	96
9	0.090	0.075	169
Average	0.108	0.108	

Manley and Marine ('15) removed a part of the thyroid gland and transplanted a part. They found that the transplant underwent hyperplasia exactly as did the stump of the gland in place. It also reacted in the same manner to iodine. These authors conclude that at least secretory nerves are not essential to the thyroid.

Table 9 represents the results obtained by removing, under aseptic conditions, about 2 cm. of the right cervical sympathetic cord in cats.

The thyroids were removed, weighed and fixed in the usual manner. The time elapsing between the sectioning and the removal of the glands varied from fourteen to one hundred and sixty-nine days. The difference in weight of the two glands was not significant in any one case. On the other hand, the average weight of the glands to which the nerve was cut was almost exactly the same as that of the glands where the nerve was left intact. Histologically there was no difference in the two glands. They showed a similar amount of colloid taking the same degree of staining.

It would seem therefore that in the cat secretory fibers, if there are such fibers present, play no part in the normal mechanism of thyroid secretion.

CONCLUSIONS

Adult rats kept under hygienic conditions and fed a high protein diet develop hyperplasia of the thyroid gland.

Rats kept under unhygienic conditions develop hyperplasia of the thyroid if given a standard diet of bread and milk.

Unhygienic conditions plus a high protein diet bring about a higher degree of hyperplasia in the adult rat than either factor taken alone.

Young, growing rats kept under hygienic conditions do not develop hyperplasia of the thyroid when given a high protein diet which will induce this condition in adult rats.

Feces either from goitre patients or from goitrous dogs fail to induce any changes in the thyroid of the cat when given by stomach.

The central end of the phrenic nerve when sutured to the peripheral end of the cervical sympathetic either does not form a physiological anastomosis or, if formed, the stimuli thus carried are not efficient in increasing the output of thyroid secretion to the point of producing the pathological changes accompanying Graves' disease in man.

Removal of a section of the cervical sympathetic cord produces no change in the thyroid gland in the cat indicating that secretory fibers, if there are such fibers present, play no part in the normal mechanism of thyroid secretion.

BIBLIOGRAPHY

BENSLEY, R. R.: '16. Amer. Journ. Anat., xix, 37, 57.
'14. Anat. Rec., viii, 431.
BILLINGS, F.: '14. Journ. Amer. Med. Assoc., lxiii, 899.
BROWN, J. R.: '14. Northwest Med., vi, no. 12.
BUFORD, C. G.: '15. Surg., Gynec. and Obst., xx, 35.
CANNON, W. B., C. A. L. BINGER AND R. FITZ: '14. This Journal, xxxvi, 363.
EVANS, J. S., W. S. MIDDLETON AND A. J. SMITH: '16. Amer. Journ. Med. Sci., new series, cli, 210.
HATAI, S.: '13. Amer. Journ. Anat., xv, no. 1.
HUNT, R.: '11. Journ. Amer. Med. Assoc., lvii, 1032.
LANGLEY, J. N.: '04. Journ. Physiol., xxxi, 365.
McCARRISON, R.: '11. Proc. Royal Soc., lxxxiv, 155.
'12. Lancet, i, 357.
'13. Lancet, i, 365.
'14. Ind. Journ. Med. Res., ii, 214.
'16. Ind. Journ. Med. Res., iv, no. 1.
MARINE, D. AND C. H. LENHART: '10. Bull. Johns Hopkins Hosp., xxi, 229.
'11. Journ. Exper. Med., xiii, 455.
MARINE, D.: '14. Journ. Exper. Med., xix, 376.
'15. Journ. Exper. Med., xxi, no. 5.
MANLEY, O. T. AND D. MARINE: '15. Proc. Soc. Exper. Biol. and Med., xii, 202.
MESSERLI, F.: '14. Centralbl. f. Bact. u. Parasit. Orig., lxxv.
MISSIROLI, A.: '09. Arch. Fisiol., vi, 582.
TROELL, A.: '16. Allm. Svenska Läkaretid, 137.
WATSON, C.: '04. Proc. Physiol. Soc.
'06. Journ. Physiol., xxxiv, 111.
'07. Proc. Physiol. Soc.
'12. Quart. Journ. Exper. Physiol., v, 229.
WILSON, L. B. AND L. DURANTE: '16. Journ. Med. Res., xxxiv, 273.