CCLXX. STUDIES ON THE EXPERIMENTAL PRODUCTION OF SIMPLE GOITRE¹.

By ALLAN WILLIAM SPENCE,
FREDERICK HERMAN AITKEN WALKER
AND ERIC FRANK SCOWEN.

From the Dunn Laboratories, St Bartholomew's Hospital, London.

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It is generally agreed at the present time that the immediate cause of simple hyperplastic goitre is an iodine deficiency, whereby the thyroid gland becomes hyperplastic in an attempt to manufacture an adequate amount of its iodine-containing hormone. This is amply proved by the work of Marine who has shown that the administration of adequate amounts of iodine will prevent the occurrence of thyroid hyperplasia no matter what the goitrogenic agent. Iodine deficiency may be absolute or relative, or a combination of the two. In absolute iodine deficiency there is a deficient intake; in relative deficiency the intake is normal, but either (1) the absorption of iodine from the alimentary tract is impaired, or (2) the tissues for some reason utilise larger amounts of the thyroid hormone than the gland can normally supply without enlarging. It is this second factor which is of importance in studying the fundamental cause of simple goitre, for it is probably the essential cause of those thyroid enlargements which occur at puberty, during pregnancy and lactation and at the menopause.

The production of goitre by cabbage feeding.

Chesney, Clawson and Webster [1928] discovered that their stock rabbits, maintained on a diet consisting of a daily ration of fresh cabbage, a ration of oats three times a week and one of hay once a week developed large hyperplastic goitres. This was subsequently confirmed by Marine *et al.* [1929], and by McCarrison [1931]. It was further shown by Marine *et al.* [1929] and Webster *et al.* [1931] that the production of the thyroid hyperplasia was not due to an absolute iodine deficiency but to a powerful goitrogenic agent contained in the cabbage. Samples of cabbage grown in different parts of the country had approximately the same iodine content but varied greatly in their goitrogenic potency, and goitrogenic cabbage when dried in a current of air at room temperature lost its power of producing goitre [Marine *et al.*, 1930]. The observation that the goitrogenic power of cabbage is not dependent on an absolute iodine deficiency has recently been confirmed by Hercus and Aitken [1933] in New Zealand. An active sample of cabbage may produce in rabbits a thyroid enlargement two or three times its normal size in three weeks. Steaming the cabbage increases its activity [Marine *et al.*, 1929]. The goitrogenic substance in cabbage is extractable with ether [Baumann *et al.*, 1931]. Various vegetables of the *Brassica* group, such as Brussels sprouts (*B. oleracea*, var. *bullata gemmifera*) and cauliflower (*B. oleracea botrytis*) were found to produce thyroid hyperplasia approximately to the same degree as cabbage (*B. oleracea capitata*) [Marine *et al.*, 1929].

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Realising the importance of being able to produce goitre in rabbits by means of cabbage feeding, we made the attempt in London in 1929, reproducing as nearly as possible the conditions to which Chesney's animals were subjected. The animals used were young Dutch rabbits, their weights at the beginning of the experiment ranging from 1 to 2 kg. They were housed in separate cages and were not exposed to direct sunlight. Wood shavings were used as bedding and were renewed once a week. The cages were cleaned out once a week. Six rabbits received as their diet a daily ration of fresh cabbage obtained from a greengrocer, a ration of whole oats three times a week and hay once a week. They received no water to drink. Six rabbits as controls received a daily ration of hay, bran and greenstuff such as pea-pods. Before the animals were put under experimental conditions, the neck was opened under ether anaesthesia and the thyroid found to be normal in size and appearance. A small portion of the gland removed for section was normal. On February 12th the animals were put under experimental conditions which were maintained until December 3rd, a period of 295 days. Their nutrition and growth were good, but at the end of this time their thyroid glands were normal in size and in histological appearance.

Marine *et al.* [1929], Webster *et al.* [1931], McCarrison [1931] and McCarrison and Madhava [1932-33] demonstrated later the occurrence of seasonal variations in the goitrogenic activity of cabbage, cabbage maturing in the autumn and winter being active and cabbage maturing in the spring and summer being practically inactive. This factor could not account for the negative results in this experiment, as the animals received both varieties, and winter cabbage was fed for the two months preceding the end of the experiment. It was considered at this time that English cabbage was non-goitrogenic.

Having seen in Marine's laboratory the considerable thyroid enlargement which may be produced in 3 weeks by cabbage feeding, and taking into consideration the fact that the goitrogenic power of cabbage shows considerable variations in different years [Webster *et al.*, 1931], we repeated these experiments in the spring and summer of 1933. The cabbage used was summer cabbage and was obtained from Cornwall. Negative results were obtained in a batch of four rabbits aged 9 weeks which were fed with steamed whole cabbage in amounts equivalent to 75 calories per kg. per day (the food value of cabbage being calculated as 3.1 g. equivalent to 1 calorie), with the addition of 17 g. of whole oats and 20 g. of hay twice weekly.

Although we did not expect positive results with summer cabbage, from the negative results which we had been obtaining with other known goitrogenic agents we began to suspect that our previous failure to produce thyroid hyperplasia by means of cabbage feeding might have been due to an antigoitrogenic property of the hay and oats which supplemented the animals' diet. Consequently we fed a batch of eight rabbits, aged 2 to 4 months, on fresh cabbage (from Buckinghamshire) daily; four received in addition 35 g. of whole oats daily, and four 35 g. of rolled oats daily. They received no hay and no water. At the end of 21 days those on cabbage and rolled oats had marked thyroid hyperplasia and enlargement; those on cabbage and whole oats had hyperplasia and enlargement, but less marked (Table I). Three animals with marked thyroid hyperplasia then continued with their diet of cabbage and oats, with the addition however of hay, for a further 21 days. At the end of this time their thyroids had involuted. This was not due to the inactivity of this sample of cabbage, since in a concurrent experiment it produced thyroid hyperplasia.

These experiments show fairly definitely that the hay and whole oats obtained in this country have an antigoitrogenic effect, and may explain our

Table I. *The effect of hay, whole oats and rolled oats on the production of goitre in rabbits by feeding with equivalent amounts of cabbage daily for 21 days.*

Hay and whole oats					Whole oats. No hay					Rolled oats. No hay				
No.	Sex	Age weeks	Weight in g.	Condi- tion of thyroid	No.	Sex	Age months	Weight in g.	Condi- tion of thyroid	No.	Sex	Age months	Weight in g.	Condi- tion of thyroid
71	F	9	1095	—	75	F	3½	1155	— +	69	M	4	1420	+
72	M	9	1017	—	77	F	3	1252	+	80	M	3	1217	+
75	F	9	844	—	91	M	2	807	— +	107	M	2½	1300	+
76	F	9	972	—	92	M	2	947	— +	106	M	2	892	— +

+ = about twice normal size, marked hyperplasia. — + = slightly enlarged, moderate hyperplasia. — = normal.

previous failure to produce thyroid hyperplasia by cabbage feeding. It is of interest that the batch of cabbage used in this experiment matured in the early summer and yet in the absence of hay was markedly goitrogenic. A subsequent batch of cabbage, however, obtained from the same district in the late summer during and just after a period of considerable drought showed little if any goitrogenic power. This was of value in demonstrating that the rolled oats had no goitrogenic effect. It is interesting to note in this connection that McCarrison and Madhava [1932–33] found that the goitrogenic potency of cabbage attained its maximum after the heavy autumn rains.

The production of goitre by methyl cyanide.

It was suggested by Marine *et al.* [1929] that the goitrogenic agent in cabbage acts by depressing some oxidation system, an effect which the thyroid attempts to overcome by producing a larger amount of its iodine-containing hormone with consequent hyperplasia. In studying the chemistry of the Cruciferae, to which the cabbage family belongs, it was found that the presence of a mustard oil (*isothiocyanate*) was a specific characteristic of this group of plants [Gilde-meister and Hoffmann, 1916]. Weith [1873] had observed that when a mustard oil is distilled under suitable conditions it is converted into its corresponding cyanide (nitrile), and about the same time Hoffmann [1874] had succeeded in isolating organic cyanides from several of the Cruciferae. These observations, and the fact that cyanide is a powerful depressor of tissue oxidation led Marine *et al.* [1932] to determine the effect of the daily subcutaneous injection of small doses of various organic cyanides on the thyroid gland of young rabbits maintained on a diet of alfalfa hay and whole oats. Striking thyroid hyperplasia and enlargement were obtained after 21 days in about 70 % of the animals by the daily subcutaneous injection of 0.1 to 0.15 cc. doses of methyl cyanide, the least toxic of the nitriles. This has been confirmed by McCarrison [1933]. Several other organic cyanides produced thyroid reactions to a less degree. The production of thyroid hyperplasia by means of organic cyanides is dependent entirely on the gradual liberation of hydrocyanic acid in the organism, and it was found that organic cyanides which are relatively stable, such as phenyl cyanide (C_6H_5CN), produced no thyroid reaction. Further, animals such as chickens, in which hydrocyanic acid is liberated only to a very small degree even from such a nitrile as methyl cyanide, failed to develop thyroid hyperplasia [Spence, 1933]. The production of thyroid hyperplasia by means of cyanides could be prevented by the administration of iodine [Marine *et al.*, 1932], and is an illustration of the importance of a relative iodine deficiency in the aetiology of goitre. The particular nitrile which may be present in cabbage has not been isolated; in McCarrison's

laboratory acid hydrolysis of cabbage yielded 6.3 mg. of cyanogen per kg., which did not necessarily represent the total amount present [Ranganathan, 1933].

In endeavouring to repeat the production of thyroid hyperplasia by methyl cyanide in London we were again impressed with the importance of diet in the prevention of goitre, a fact which McCarrison has repeatedly stressed. Four rabbits, aged $2\frac{1}{2}$ to 3 months and weighing from 600 to 2550 g. received daily subcutaneous injections of methyl cyanide in doses ranging from 0.06 to 0.16 cc. They were maintained on a diet of hay, whole oats and tap water. At the end of 24 days their thyroid glands were normal. Because of these negative results it was considered that the diet which they were receiving might be markedly antigoitrogenic. To test this hypothesis a batch of 24 rabbits, aged 2 to $3\frac{1}{2}$ months, were given 0.1 cc. of methyl cyanide daily, but were divided into four groups, which received various modifications in their diet. All were kept on about 20 g. of hay daily, but group I received daily in addition 35 g. of whole oats and tap water, group II, 35 g. of rolled oats and distilled water, group III, 35 g. of rolled oats and tap water, and group IV, 35 g. of whole oats and distilled water (Table II). Those on whole oats and tap water again had normal thyroid glands at the end of 21 days; of those on rolled oats and distilled water, three had marked thyroid

Table II. *The effect of methyl cyanide on the thyroid gland of rabbits fed on various diets. (Period, 21 days.)*

Hay, whole oats, tap water						Hay, rolled oats, distilled water					
No.	Sex	Age months	Weight in g.	Daily dose of methyl cyanide (subcut.) cc.	Condition of thyroid	No.	Sex	Age months	Weight in g.	Daily dose of methyl cyanide (subcut.) cc.	Condition of thyroid
55	F	$3\frac{1}{2}$	1325	0.1	—	59	M	$3\frac{1}{2}$	1004	0.1	(—)*
65	F	3	1165	"	—	64	F	3	1167	"	+
72	M	$2\frac{1}{2}$	1132	"	—	70	F	$2\frac{1}{2}$	1075	"	++
71	F	$2\frac{1}{2}$	1370	"	—?	73	M	$2\frac{1}{2}$	1069	"	+±
78	M	2	992	"	—+	77	F	2	1102	"	—
79	F	2	1330	"	—	80	M	2	1082	"	—
Controls receiving no cyanide						74	F	$2\frac{1}{2}$	1115	Nil	—
						75	F	$2\frac{1}{2}$	953	"	—
						81	M	2	1175	"	—?
						82	M	2	1140	"	—?
Hay, rolled oats, tap water						Hay, whole oats, distilled water					
No.	Sex	Age months	Weight in g.	Daily dose of methyl cyanide (subcut.) cc.	Condition of thyroid	No.	Sex	Age months	Weight in g.	Daily dose of methyl cyanide (subcut.) cc.	Condition of thyroid
72	M	$3\frac{1}{2}$	1132	0.1	—+	71	F	$3\frac{1}{2}$	1537	0.1	—
68	M	3	1217	"	—	66	F	3	1175	"	—?
69	M	3	1175	"	—	67	F	3	1079	"	—+
100	M	$2\frac{1}{2}$	1157	"	—	97	F	$2\frac{1}{2}$	1157	"	—?
101	F	$2\frac{1}{2}$	1130	"	—?	99	M	$2\frac{1}{2}$	1187	"	—+
86	M	2	992	0.075	—	98	M	2	852	0.075	—
Controls receiving no cyanide						76	F	$2\frac{1}{2}$	1125	Nil	—?
						60	F	$3\frac{1}{2}$	937	Nil	—

++ = more than three times normal size, marked hyperplasia.

+± = more than twice normal size, marked hyperplasia.

+ = about twice normal size, marked hyperplasia.

—+ = slightly enlarged, moderate hyperplasia.

—? = possibly slightly enlarged, possibly slight hyperplasia.

— = normal.

* Died after four days.

enlargement, two had normal glands and the sixth died during the experiment. The thyroid responses in the other two groups on rolled oats and tap water, and on whole oats and distilled water, were either slight or negative. Four control animals of the same age and breed, which received no cyanide, but a diet of hay, rolled oats and distilled water, had normal thyroid glands. The three rabbits in group II which had marked thyroid hyperplasia were then given whole oats and tap water, instead of rolled oats and distilled water, and continued with their cyanide injections for a further 27 days. At the end of this time their thyroid glands had involuted.

There is thus no doubt that whole oats and tap water are definitely antagonistic to the goitrogenic action of methyl cyanide. From our cabbage experiments there is also evidence that better thyroid reactions could be obtained with cyanide if the hay were eliminated. Experiments are proceeding on these lines, for in order ultimately to test the antigoitrogenic power of various substances it is essential to have a reliable method of producing thyroid hyperplasia, and with this in view it is consequently of importance to use a diet which is not antigoitrogenic. On the other hand, it is preferable to use only one goitrogenic agent, and therefore in producing thyroid hyperplasia by means of methyl cyanide or any other goitrogenic substance, care should be taken to determine that the diet is not goitrogenic. The diet used should be one which McCarrison terms neutral, and this will vary in different localities. Cabbage feeding does not form a reliable method of producing thyroid hyperplasia, because as mentioned previously the goitrogenic activity of cabbage varies so considerably.

With regard to the nature of the antigoitrogenic substance, or substances, in hay, whole oats and tap water, we have not made determinations of the iodine content, as it does not lie within the scope of our present research. The iodine content of London tap water is approximately 4γ (0.004 mg.) per litre [Houston, 1929], which is insufficient by itself to prevent thyroid hyperplasia under the above experimental conditions. In considering the possibility of any difference in the iodine contents of whole oats and of rolled oats, it is unlikely that iodine is lost during the process of producing rolled oats. This process consists essentially in removing the husk by crushing and then passing the oats between hot rollers. Marine *et al.* [1933] have recently drawn attention to the occurrence in plants of antigoitrogenous substances other than iodine. Such substances have to be taken into account in considering the antigoitrogenic action of various foodstuffs.

The relation between the production of thyroid hyperplasia by methyl cyanide (acetonitrile) and the Hunt acetonitrile reaction.

Objections to the hypothesis concerning the mode of production of thyroid hyperplasia by means of methyl cyanide have been raised [Thomson and Collip, 1933] on the grounds that it does not appear to run parallel with the work of Hunt [1923], who showed that the administration of thyroid substance and thyroxine diminished the resistance to acetonitrile of all animals tested, except the mouse. Hunt explained the diminished resistance to acetonitrile following the ingestion of thyroid substance by the increased metabolism, whereby hydrocyanic acid was liberated more rapidly from the molecule of acetonitrile with consequently greater toxic effects. There is no evidence to refute this hypothesis, and we are in complete agreement with it. Superficially it appears that if, according to Marine's theory with which we also concur, thyroid hormone is antagonistic to the action of cyanide, then one would expect the administration of thyroid hormone to increase the resistance to acetonitrile. This, however, does not necessarily follow, for it would hold only if the

antagonism between thyroid hormone and cyanide were a direct one. The antagonism between the two substances is more complicated.

The experiments of Hunt on the diminished resistance to acetonitrile in hyperthyroid animals do not contradict the hypothesis of Marine and his collaborators on the mode of production of thyroid hyperplasia by means of acetonitrile, because the two series of experiments are not comparable, in that they involve two entirely different mechanisms. If thyroid hormone be given before the acetonitrile, so that the organism has an increased metabolism, then one would expect the resistance to acetonitrile to be diminished, as Hunt has explained. If, on the other hand, acetonitrile be given without the previous administration of thyroid, then there is a depression of tissue metabolism which the thyroid endeavours to overcome.

SUMMARY.

1. Thyroid hyperplasia has been produced in rabbits by feeding cabbage which was grown in England, although this cabbage matured in the early summer. Cabbage which matured in the later summer during a period of drought was practically inactive.

2. Previous failures to produce thyroid hyperplasia by means of cabbage were due not necessarily to the inactivity of the cabbage, but to the presence of antigoitrogenic substances in the hay and oats with which the animals' diet was supplemented.

3. The production of thyroid hyperplasia in rabbits by means of the daily administration of small doses of methyl cyanide (acetonitrile) has been confirmed.

4. In attempting to produce thyroid hyperplasia by means of goitrogenic agents, it is essential that the animals' diet be not antigoitrogenic. It is important that their diet should be what McCarrison terms neutral.

5. The relation between the production of thyroid hyperplasia by acetonitrile and the Hunt acetonitrile reaction is discussed.

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