LXV. THE EFFECT OF ASCORBIC ACID ON EXPERIMENTAL GOITRE.

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THE experiments of Marine and his collaborators [Marine *et al.*, 1933] show that the development of simple goitre in rabbits, fed on a diet consisting chiefly of cabbage, can be prevented by the feeding of certain fresh plants and indicate that the plants in question contain an antigoitrogenic substance other than iodine. The authors suggest that the substance may be identical with hexuronic acid isolated by Szent-Györgyi [1928] from cabbage, orange juice and suprarenal cortex. They state however that iodine cannot be entirely excluded as a contributory antigoitrogenic factor in these experiments, and that final proof must await the production of thyroid involution by the use of a pure iodine-free preparation of hexuronic acid. The purpose of this paper is to show that in our experience hexuronic acid (ascorbic acid) has no involuting effect on thyroid hyperplasia produced in rabbits by means of cabbage feeding or on that produced in guinea-pigs by daily intraperitoneal injections of acid extract of anterior pituitary. Further, it will be seen that there is no definite evidence that a deficiency of ascorbic acid over a prolonged period has a goitrogenic action in guinea-pigs.

EXPERIMENTAL.

The effect of ascorbic acid on simple thyroid hyperplasia produced by cabbage feeding. The production of simple thyroid hyperplasia in rabbits fed on a diet consisting chiefly of cabbage was observed by Chesney *et al.* [1928], and confirmed by Marine *et al.* [1929] in the United States, by McCarrison [1931] in India and by Spence *et al.* [1933] in England. In the present experiment considerable thyroid enlargement and marked hyperplasia were produced in a batch of eight rabbits, aged 3 months and weighing 960–1537 g., by feeding them for one month on fresh cabbage in amounts equivalent to 75 Cals/kg. of body weight. The diet was supplemented with 35 g. of rolled oats three times weekly. The condition of the thyroid glands was observed surgically. The animals then continued with their cabbage diet, but two received daily by mouth 5 mg. and two 20 mg. of pure ascorbic acid, freshly dissolved in boiled distilled water. The remaining four served as controls. At the end of 20 days of this treatment the thyroids were again inspected surgically and also microscopically. The glands of two of those which had received ascorbic acid were perhaps slightly less hyperplastic than on the previous occasion, but this might well have been due to some diminution in the potency of the cabbage, as one of the controls had similarly regressed and two had not increased in size (Table I).

By the same means marked thyroid hyperplasia was produced in a second batch of eight rabbits, aged 3 months and weighing about 1500 g. The animals continued with their cabbage diet, but four of them received daily by mouth 50 mg. of ascorbic acid. The remaining four as controls received no medication.

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At the end of 3 weeks there was no diminution in the size of the thyroid glands, and histologically all showed marked hyperplasia with no evidence of involution (Table I).

Table I. *The effect of ascorbic acid on simple thyroid hyperplasia in rabbits.*
(Period: 3 weeks.)

No.	Sex	Age (months)	Initial wt. g.	Previous condition of thyroid	Daily dose of ascorbic acid g.	Final wt. g.	Final condition of thyroid	
							Size or wt. g.	Histology (hyperplasia)
158	M	5	960	++	5	1067	+	Moderate
161	F	5	1295	++	5	1448	++	Marked
179	F	4	1310	+	20	1392	+ -	Moderate
182	F	4	1310	+	20	1325	+	"
134	F	6½	1537	++	Nil	1655	++	Marked
143	M	6	1348	+	"	1580	+ -	Moderate
176	M	4	1117	- +	"	1268	+ -	Marked
177	M	4	1032	- +	"	1193	- +	Moderate
222	M	3	1475	+	50	1982	283	Marked
223	M	3	1875	++	50	2322	580	"
225	F	3	1675	- +	50	1975	224	Moderate
232	F	3	1620	+ -	50	2172	432	Marked
229	F	3	1400	++	Nil	1842	477	"
233	F	3	920	- ?	"	1315	286	Moderate
234	M	3	1410	++	"	1935	650	Marked
235	M	3	1602	- +	"	1800	297	"

++ = More than twice normal size, marked hyperaemia.

+ = About twice normal size, marked hyperaemia.

+ - = About 1½ times normal size, moderate hyperaemia.

- + = Slightly enlarged, slight hyperaemia.

- ? = Possibly slightly enlarged, possibly slight hyperaemia.

(The average weight of the normal thyroid of an adult rabbit is about 150 mg.)

Table II. *The effect of ascorbic acid on thyroid hyperplasia produced in guinea-pigs by anterior pituitary extract.*

No.	Sex	Initial wt. g.	Daily dose of ascorbic acid (subcutaneous) mg.	Daily dose of anterior pituitary extract (intra- peritoneal) ml.	Final wt. g.	Condition of thyroid		Wt. of supra- renals mg.
						Wt. mg.	Histology (hyper- plasia)	
156	F	230	25 (vi. 28-vii. 9)	1 (vii. 2-9)	250	73	Marked	164
157	F	240	25 (")	1 (")	242	60	"	160
158	M	290	25 (")	1 (")	320	85	"	180
159	M	270	25 (")	1 (")	307	53	Moderate- marked	162
160	M	298	25 (")	1 (")	318	68	"	218
161	M	265	50 (vi. 28-vii. 8)	1 (vii. 2-8)	292	55	Marked	160
162	M	240	50 (")	1 (")	244	37	"	177
163	F	227	50 (")	1 (")	222	68	"	230
164	F	287	50 (")	1 (")	267	73	"	230
165	F	215	50 (")	1 (")	197	35	"	154
166	F	240	Nil	1 (vii. 2-9)	281	55	"	140
167	F	210	"	1 (")	257	78	"	160
168	M	277	"	1 (")	292	54	"	211
169	M	272	"	1 (")	212	124	"	214
170	M	265	"	1 (")	285	80	"	205
171	M	275	"	1 (vii. 2-8)	321	63	"	171
172	M	267	"	1 (")	245	91	"	158
173	F	200	"	1 (")	237	57	"	233
174	F	250	"	1 (")	290	50	"	222
175	F	247	"	1 (")	212	57	"	232

The effect of ascorbic acid on thyroid hyperplasia produced by anterior pituitary extract. Twenty guinea-pigs, ten males and ten females, weighing 200–300 g., were fed on a non-goitrogenic diet containing fresh lettuce three times weekly. They were divided into two groups in which the sexes were equally distributed. Both groups received daily intraperitoneal injections of 1 ml. of acid extract of Organon's anterior pituitary powder, prepared after the method of Loeb and Bassett [Scowen and Spence, 1934]. In one group, however, five received in addition 25 mg. and five 50 mg. of ascorbic acid daily subcutaneously. The administration of ascorbic acid was started 4 days before treatment with anterior pituitary extract was begun. It has been shown by numerous observers, including Aron [1929; 1930], Collip [1933] and Loeb and Friedman [1931], that the initial hyperplasia produced by anterior pituitary extract regresses and involutes to the colloid state in spite of continued administration. In some cases the hyperplasia may reach its maximum in one or two weeks. So that this regression should not be a complicating factor and vitiate the results, the animals were sacrificed after receiving anterior pituitary for 7–8 days. The thyroid glands of both groups, *i.e.* those which received ascorbic acid and those which did not, all showed macroscopically marked hyperaemia and histologically marked hyperplasia. There was no significant difference in the weights of the suprarenals in either group (Table II).

The effect of a diet deficient in ascorbic acid on the thyroid gland of guinea-pigs. Should ascorbic acid be an antigoitrogenic substance, a deficiency of it might

Table III. *The condition of the thyroid gland in acute and chronic scurvy (guinea-pig).*

(Diet: crushed oats, bran and autoclaved milk.)								
No.	Sex	Initial wt. g.	Daily dose of ascorbic acid mg.	Duration of experiment days	Condition of animal	Final wt. g.	Condition of thyroid	
							Size or wt. mg.	Histology
7619	M	267	Nil	22	Acute scurvy	340	Not enlarged	Normal
7620	F	218	"	22	"	340	"	"
7621	M	255	"	22	"	242	"	"
7622	F	265	"	22	"	250	"	"
7623	M	265	"	22	Acute scurvy; pneumonia	225	"	"
7624	F	280	"	22	Acute scurvy	300	"	"
7613	F	255	0.25–0.5	39	Chronic scurvy	245	"	"
7614	M	260	"	44	"	245	"	Marked hyperplasia
7615	M	270	"	39	"	265	"	Slight hyperplasia
7616	F	290	"	44	"	280	"	Moderate hyperplasia
7617	M	255	"	44	"	220	"	Normal
7618	M	260	"	33	"	230	"	"
7761	M	285	"	41	"	362	51	"
7762	F	295	"	41	"	230	50	"
7763	F	275	"	41	"	247	62	Slight hyperplasia
7764	M	295	"	41	"	297	55	"
7765	M	305	"	41	"	315	51	Normal
7809	M	295	"	26	"	330	42	"
7650	F	250	5	44	Normal	370	38	"
7651	F	266	5	44	"	370	41	"
7652	M	275	5	44	"	442	38	"
7653	F	250	5	44	"	372	41	"
7654	F	255	5	44	"	425	40	"
7655	M	285	5	44	"	465	40	"

be expected to bring about hyperplasia of the thyroid gland in a considerable percentage of animals. To test this hypothesis we examined the thyroid glands of guinea-pigs which were maintained on scorbutic diets as suggested by Dr S. S. Zilva of the Lister Institute, where he kindly carried out the experiments for us. Twenty-four guinea-pigs weighing 200–300 g. were fed on a scorbutic diet consisting of a daily ration of 15–20 g. of a mixture of 1 part by volume of crushed oats, 2 parts by volume of bran and about 40 ml. of reconstituted dried milk, autoclaved at 120°. They were divided into three groups. The first group consisting of six animals was fed on this diet for 22 days, and at the end of this period all were in a condition of acute scurvy. The second group consisting of twelve guinea-pigs received the diet for periods varying from 26 to 44 days, and was maintained in a condition of chronic scurvy by the daily administration of ascorbic acid in doses varying from 0.25 to 0.5 mg., the amount being regulated by the animal's weight and daily condition. The third group of six guinea-pigs received the diet for 44 days, but each animal was fully protected against scurvy by a daily dose of 5 mg. of ascorbic acid. At the end of their respective periods the animals were sacrificed. The thyroids of the six guinea-pigs with acute scurvy were normal, both macroscopically and microscopically. The thyroids of the six guinea-pigs which were fully protected against scurvy were also normal. Of the twelve animals suffering from chronic scurvy, one showed marked, one moderate and three slight hyperplasia. There was no definite enlargement of the glands (Table III).

CONCLUSION.

In view of the antigoitrogenic effect of plant juices the consideration of the part played by vitamin C (ascorbic acid) in the aetiology of simple goitre is important. Contradictory results have been obtained in studying the effect on the thyroid gland of vitamin C deficiency. McCarrison [1919–20] found that guinea-pigs fed on a scorbutic diet consisting of crushed oats and autoclaved milk and deficient in essential salts developed thyroid enlargement, mainly as a result of congestion and haemorrhage. He does not state whether hyperplasia was present. In later experiments he found no thyroid enlargement in rats fed on a synthetic diet deficient in vitamin C but complete in other respects [McCarrison, 1930]. Harris and Smith [1928], on the other hand, observed a slight degree of thyroid hyperplasia in guinea-pigs in which experimental scurvy had been produced by means of a diet of alfalfa meal, wheat flour, oats and water.

In the experiment described above it has been seen that in acute scurvy there was no evidence of thyroid hyperplasia, but that in twelve guinea-pigs with chronic scurvy thyroid hyperplasia was present in a fairly marked degree in two and in a slight degree in three animals. We feel that this finding occurred in too small a percentage to have any definite significance. It has also been shown that the daily administration of large doses of ascorbic acid does not prevent the occurrence of thyroid hyperplasia in guinea-pigs receiving injections of anterior pituitary extract containing thyrotropic hormone, a finding which is not in agreement with the conclusions of Marine *et al.* [1934]. Neither has ascorbic acid any involuting effect on the hyperplastic goitres produced in rabbits by means of cabbage, which itself is rich in vitamin C. We are consequently of the opinion that ascorbic acid cannot be regarded as an antigoitrogenic substance.

SUMMARY.

1. The administration of ascorbic acid in daily doses of 5, 20 and 50 mg. for 3 weeks did not cause involution of hyperplastic thyroids produced in rabbits by means of cabbage.

2. The administration of ascorbic acid in daily doses of 25 and 50 mg. for 12 days did not prevent the occurrence or diminish the degree of thyroid hyperplasia in guinea-pigs which received at the same time 8 daily injections of thyrotropic hormone of the anterior pituitary.

3. In six guinea-pigs with acute scurvy, there was no evidence of thyroid hyperplasia; in twelve guinea-pigs with chronic scurvy there were varying degrees of hyperplasia in five animals, but there was no significant thyroid enlargement.

4. It is concluded that ascorbic acid is not an antigoitrogenic substance.

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