THE OCCURRENCE OF ANTIGOITROUS SUBSTANCES IN PLANTS

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Plates 4 and 5

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We have previously (1, 2) referred briefly to the possible occurrence of antigoitrous substances, other than iodine, in plant juices and it is the purpose of this paper to record in detail the experiments leading to this conclusion.

Preliminary Experiments Suggesting the Existence of Antigoitrous Substances in Plants

The first experiments, which seemed to point to the existence of one or more antigoitrous substances, were planned to investigate the nature of the goiter-producing principle of cabbage following the observations of Chesney and Webster (3, 4) that cabbage when fed to rabbits as their principal food often causes rapid thyroid hyperplasia.

In these experiments, steamed (35 minutes) northern New York winter cabbage was hashed and press juice, equal to 50 per cent of its weight, was removed in a tincture press. To one group of 4 rabbits (Series 10) 300 cc. of press juice concentrated *in vacuo* to 25 cc. was given with 35 gm. of rolled oats, 20 gm. of alfalfa hay and distilled water daily to each rabbit. To another group of 4 rabbits (Series 21) 200 cc. of unconcentrated press juice was given daily with 35 gm. of oats and 20 gm. of alfalfa hay to each rabbit. Distilled water was omitted. After 20 days the thyroids were examined under ether anesthesia and the results are given in Table I.

As a control for these experiments, 4 rabbits (Series 17) were fed with the cabbage cake, the press juice from which was used in the above experiments. On the 11th day all 4 rabbits had palpable thyroids and on examination under ether anesthesia they were very hyperplastic. The data are given in Table II.

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Several other series of experiments of this nature were carried out but as they showed the same general results, they need not be reported in detail. All these experiments have shown quite clearly

TABLE I

Juice of Steamed Cabbage (Series 10, 21)

Rabbit No. and sex	Age	Amount fed per day		Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
		300 cc. concentrated to	Unconcentrated				
	mos.	*cc.*	*cc.*	*days*	*kg.*	*gm.*	
6-78 M	4½	25		20	1.84	*	Not enlarged, not hyperemic
6-79 M	4	25		20	1.40	*	" " " "
6-80 M	5	25		20	2.15	*	" " " "
6-81 F	5	25		20	1.44	*	" " " "
6-64 F	7		200	24	1.98	*	" " " "
6-93 F	5½		200	24	2.31	*	Slightly enlarged, slight hyperemia
6-94 F	6		200	24	2.15	*	Nearly twice normal size, moderate hyperemia
7-04 F	4		200	24	1.36	0.10	Not enlarged, not hyperemia

* Animals not sacrificed.

TABLE II

Steamed Cabbage Cake (Series 17)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*kg.*	
6-66 F	7½	216	11	2.29	Twice normal size, marked hyperemia
6-67 F	8	220	11	2.40	3 times " " " "
6-68 F	7	198	11	2.11	Twice " " " "
6-69 F	8	228	11	2.32	3 times " " " "

Thyroid weights not given because animals were not sacrificed.

that the press juice from 400 to 600 gm. of cabbage when fed daily for 20 days, whether concentrated or not, had no detectable goiter-producing action, while the cabbage cake from the same cabbage

when fed in amounts equal to 75 calories per kilo per day (in all our work the caloric value of whole cabbage has been figured as 3.1 gm. equaling 1 calory, and cabbage cake as 3.1 gm. equaling 2 calories) produced clinically palpable goiters in 11 days (during the winter of 1928–29 but not during winter of 1929–30).

The next series of experiments was carried out to determine whether washing the cabbage cake (steamed cabbage from which 50 per cent of its weight had been removed as press juice) increased or decreased its goiter-producing power.

In this experiment (Series 50) steamed imported Holland winter cabbage was hashed and 50 per cent of its weight removed as press juice. One portion of this cabbage cake was fed directly to 2 rabbits. Another portion of the same cabbage cake was thoroughly mixed with an equal weight of distilled water, allowed to stand a few minutes and then the water was removed in the press. A part of this was fed to 2 rabbits, while the remainder was again mixed with an equal weight of distilled water, allowed to stand for a few minutes and the water pressed out. This material was fed to the third pair of rabbits of the same age and strain and the results are given in Table III.

While all 6 showed some thyroid hyperplasia, it is clear that those rabbits that received the twice washed cabbage cake had much larger thyroids than those receiving unwashed cake. From these experiments we concluded that the goiter-producing principle of cabbage was relatively insoluble in water, while one or more substances soluble in cabbage juice that inhibited the goiter-producing principle of cabbage were removed or destroyed by washing. It did not appear likely that the amount of iodine that could have been removed in this way could account for the magnitude of the changes observed, especially since in the crude washing some of the goiter-producing principle must also have been removed.

These experiments were repeated in many ways with several dozen rabbits and with various kinds of cabbage. The results were essentially similar and need not all be recorded here. For example, the goiter-producing power of northern New York State red cabbage was compared with that of cabbage cake from the same cabbage.

A supplemental diet of 17 gm. of oats and 20 gm. of alfalfa hay twice weekly was given. After 11 days feeding, the thyroids were exposed under ether anesthesia and the results are given in Table IV.

Rabbits 8-37 and 8-38 were of the same lot and breed and had not been given iodine or previously used. Rabbits 7-74 and 8-05 had been used in another experiment and each had been given 10 mg. of

TABLE III

Leached Cabbage Cake (Series 50)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*kg.*	*gm.*	
		Whole cake				
7-60 M	3	143	20	1.49	0.27	Twice normal size, marked hyperemia
7-61 F	5	192	20	1.73	*	Slightly enlarged, slight "
		Leached once				
7-62 F	4	166	20	1.59	*	Enlarged, moderate hyperemia
7-63 M	3	144	20	1.34	0.27	Twice normal size, marked hyperemia
		Leached twice				
7-64 F	4	177	20	1.49	*	3 times " " " "
7-65 F	6	208	20	1.78	0.24	2½ " " " " "

* Animals not sacrificed.

TABLE IV

Steamed Red Cabbage (Series 75)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*kg.*	
		Whole			
7-74 M	8	566	11	2.43	Not enlarged, not hyperemic
8-37 M	8	465	11	2.07	Slightly enlarged, moderate hyperemia
		Cake			
8-05 M	5	187	11	1.61	1⅓ times normal size, moderate hyperemia
8-38 M	7	216	11	1.81	Slightly enlarged, " "

Thyroid weights not given because animals were not sacrificed.

KI intraperitoneally. (This is about 20 to 40 times the amount of iodine necessary to involute completely a thyroid weighing 0.25 gm. in a rabbit of about 2 kg. The normal adult rabbit's thyroid weighs between 0.10 and 0.20 gm.) Rabbit 7-74 received KI, 3 months

before the beginning of this experiment, while Rabbit 8-05, which received cabbage cake, was injected only 2 weeks previously and therefore had less chance of losing the excess iodine caught in its tissues. Yet definitely more marked thyroid hyperplasias were obtained by feeding red cabbage cake than with the whole cabbage.

The presence of some antigoitrous substance was shown with a third *Brassica*—kohlrabi (*cavolo rapa*).

Kohlrabi from which the leaves had been cut was steamed and fed to 4 rabbits (Series 54), supplemented by 17 gm. of oats and 20 gm. of alfalfa hay twice weekly for 16 days. The thyroids were then examined under ether anesthesia but were found to be within normal limits. 2 of these rabbits were then fed for 35 days on kohlrabi cake (steamed kohlrabi from which 50 per cent of its weight had been removed as press juice), while the remaining 2 rabbits were fed on the same kohlrabi cake washed once with twice its weight of distilled water. The thyroids were again exposed and the results are summarized in Table V.

The rabbits on the kolhrabi cake developed a slight degree of thyroid hyperplasia while those in the washed kolhrabi cake developed much more marked thyroid enlargement. It appears certain that kohlrabi cake will cause a slight hyperplasia in rabbits, though whole steamed kohlrabi did not in the time and under the conditions obtaining when this experiment was carried out. Further, washed kohlrabi cake from the same lot is still more effective than the unwashed cake.

Here again and with a third *Brassica*, washing removes or destroys one or more substances that allow the small amount of the goitrous substance present in kohlrabi to make itself evident. We have assumed that this effect was due to the removal of antigoitrous substances but from the evidence so far presented these thyroid effects could be due to the removal of iodine as well as to a substance other than iodine.

The Involuting Effects of Various Vegetable Diets on Thyroid Hyperplasia

1. Lawn Grass.—

Two rabbits were fed on fresh cut lawn grass as their principal diet, beginning Apr. 16. They were given, in addition, 17 gm. of oats and 20 gm. of alfalfa hay twice weekly. At the end of 24 days these rabbits were sacrificed, together with

2 controls on our stock diet[1] and the condition of their thyroids noted. The essential data are given in Table VI.

TABLE V

Steamed Kohlrabi (Series 56)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*kg.*	*gm.*	
7-82 F	3	247	16	1.01	*	Normal size, not hyperemic
7-83 F	3	240	16	1.03	*	" " " "
7-84 F	3	245	16	1.01	*	" " " "
7-85 F	3	206	16	0.78	*	" " " "
		Cake				
7-82 F	4	204	35	1.33	0.15	Slightly enlarged, slight hyperemia
7-83 F	4	214	35	1.35	*	" " moderate hyperemia
		Leached once				
7-84 F	4	211	35	1.37	*	Twice normal size, " "
7-85 F	4	172	35	1.15	*	" " " " "

* Animals not sacrificed.

TABLE VI

Lawn Grass (Fresh) (Series 107)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*kg.*	*gm.*	
8-91 F	8	471	23	2.15	0.21	Normal size, not hyperemic
8-77 M	6	496	21	1.27	0.16	" " " "
8-69 M	8	Stock diet	21	2.87	0.44	Twice normal size, marked hyperemia
8-94 F	8	" "	24	2.15	*	1½ times normal size, moderate hyperemia

* Animal not sacrificed.

It will be seen that the rabbits fed with lawn grass had completely involuted, pale, colloid-rich thyroids, while both the controls had

[1] Alfalfa hay (1929 crop) 20 to 30 gm., oats 35 gm. and fresh water daily. Greens twice weekly.

thyroids at least twice normal size, dark red and actively hyperplastic. (See Fig. 1.) It should also be pointed out that 2 of these rabbits, Nos. 8-91 and 8-94, had been fed on cabbage for 29 days and their thyroids were examined just before starting the feeding on lawn grass. Both had hyperplastic thyroids, of about twice normal size and dark red in color. At the end of the experiment, Rabbit 8-91 had a small, pale, completely involuted thyroid, while the thyroid of No. 8-94, kept on stock diet, was unchanged. It is evident that a diet of fresh lawn grass will involute thyroid hyperplasia in 24 days.

2. Fresh Alfalfa (Lucerne) versus Old Alfalfa Hay.—

Four rabbits which had been fed on cabbage from 19 to 32 days in order to produce thyroid hyperplasia were put on a diet of fresh alfalfa, supplemented by 17 gm. of oats twice weekly. This alfalfa was grown in northern Westchester county, cut by the authors at intervals of 7 and 10 days and kept in cold storage until used. As controls, 3 rabbits with hyperplastic thyroids were placed on our stock diet. After 23 days feeding the thyroids were examined under ether anesthesia and notes of their condition, together with other essential data, are given in Table VII.

TABLE VII

Alfalfa (Fresh) (Series 111)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*kg.*	*gm.*	
8-97 M	5	331	22	1.79	0.19	Slightly enlarged, moderate hyperemia
8-99 F	5	325	22	1.60	0.12	Normal size, no hyperemia
9-02 M	5	333	22	1.87	0.25	Slightly enlarged, slight hyperemia
9-03 M	5	332	22	1.80	0.16	" " " "

Again it is evident that the thyroids of those animals getting the relatively fresh alfalfa were nearly completely involuted while their controls were as hyperplastic if not more so than at the beginning of the experiment. The involuting effect of alfalfa was not as striking as was that of lawn grass. This difference might be partially accounted for by the fact that the lawn grass was kept only 2 to 3 days while the alfalfa was kept as long as 9 days, and a loss of antigoitrous substances might have taken place during storage.

**3. Recently Cured versus Old Stored Alfalfa Hay.—*

Six rabbits were given 2.6 mg. KI intraperitoneally on Sept. 30 and Oct. 1. 4 days later 3 were placed on a diet of 35 gm. of the 1930 crop of dry alfalfa hay plus 35 gm. of oats, and 3 others were placed on a diet of 35 gm. of the 1929 crop of dry alfalfa hay that had been in the laboratory for 1 year. After 67 days on this diet, their thyroids were examined under ether anesthesia, and their condition is given in Table VIII.

TABLE VIII

Recently Cured versus Old Alfalfa Hay (Series 127)

Rabbit No. and sex	Age	Amount fed per day	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*gm.*	*days*	*gm.*	*gm.*	
		Old alfalfa				
8-57 F	13	35	67	2460	0.425	Twice normal size, marked hyperemia
9-35 M	6	35	76	2532	0.632	3 times " " " "
9-37 M	9	35	67	2942		1½ " " " moderate hyperemia
		New alfalfa				
8-59 F	12	35	67	2645		Possibly slightly enlarged, slight "
9-36 M	9	35	67	2215		Slightly enlarged, slight hyperemia
9-38 M	9	35	67	2433		1½ times normal size, slight hyperemia

It will be noted that those animals fed on old alfalfa hay had much larger and more vascular thyroids than those fed on the new hay. Both groups received the same lot of oats. Iodine determinations were not made on the two lots of hay. Findings similar to the above have been noted during the past 12 years, in which the hay obtained in the early autumn did not produce thyroid hyperplasia, while after 6 months storage the same hay regularly produced thyroid hyperplasia.

4. Skunk Cabbage (Symplocarpus foetidus) versus Stock Diet.—

In the early spring of 1929, after finding that skunk cabbage had a very high iodine-absorbing value (6 to 8 times that of the *Brassicae*), we fed a series of 4

*This paper was withdrawn in order to recheck certain data, and on resubmission this section (No. 3) was added. No other essential change has been made.

rabbits with steamed leaves as their principal food and found that it markedly involuted their thyroids in 15 days. (See Fig. 2.) This experiment was repeated in the spring of 1930 with the same result and the essential data of both experiments are given in Table IX.

It is obvious from these experiments that steamed skunk cabbage causes a more rapid involution of thyroid hyperplasia than anything yet tried except iodine. On the possibility that this effect with skunk

TABLE IX

Steamed Skunk Cabbage (Series 42, 108, 109)

Rabbit No. and sex	Age	Amount fed per day	Total amount eaten	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*gm.*	*gm.*	*days*	*kg.*	*gm.*	
		1929					
7-12 M	6	325	4875	15	1.41	*	Normal size, not hyperemic
7-14 F	5	298	4470	15	1.46	*	Slightly enlarged, not hyperemic
7-16 F	5	363	5445	15	1.86	0.21	" " " "
7-22 F	5	255	2040	8	1.22	0.12	Normal size, not hyperemic
		1930					
8-95 F	7	423	3684	26	1.38	0.11	" " " "
8-96 F	8	442	6438	25	1.82	0.16	" " " "
8-80 M	7	514	5637	26	1.95	0.15	" " " "
8-84 M	7	512	3465	26	1.86	0.16	Slightly enlarged not hyperemic
9-06 F	5	414	7387	34	1.72	0.15	Normal size, not hyperemic
8-66 F	8	542	5727	31	2.03	0.16	" " " "
2-31 M	5	Control		23	1.91	0.25	1½ times normal size, moderate hyperemia
2-32 M	5	"		23	1.36	0.22	Twice normal size, marked hyperemia
2-33 M	5	"		17	1.83	0.22	1½ times normal size, moderate hyperemia
9-05 F	5	"		17	1.95	*	1½ " ". " " "

* Animals not sacrificed.

cabbage could have been due to iodine, specimens were examined for iodine in this laboratory and also by Dr. Roe E. Remington of the South Carolina Food Research Laboratory. The determinations made here on different samples gave 2000, 1860, 2130, 1520 and 1820 parts per billion; that is, an average of 1.86 mg. iodine per kilo dried material. The determinations made at the South Carolina Food Research Laboratory showed 916, 1238, 1224 and 1448 parts per billion; that is, an average of 1.206 mg. iodine per kilo of dried material.

According to these figures the iodine content of skunk cabbage is among the highest found in land plants. Those rabbits which were fed for 15 days would therefore have received a minimum of 0.767 mg. of iodine and it is possible that this quantity administered as it was in the most favorable dosage for producing thyroid involution could have brought about this result. Therefore proof of the existence of an antigoitrous substance, other than iodine, was still lacking.

5. Effect of Skunk Cabbage Juice Containing Different Amounts of Iodine-Absorbing Material.—It had become evident that the involuting effect of the various foodstuffs was somewhat proportional to the capacity of the food to absorb iodine. It was also found that the iodine-absorbing value of a given plant (cabbage, skunk cabbage, alfalfa, etc.) was highest during the early, rapidly growing stages and fell off sharply at maturity (9). This observation made it possible to compare the effect of skunk cabbage juice containing a large amount of iodine-absorbing material with a juice containing small amounts.

The experiments were carried out as follows:

Fresh skunk cabbage was brought to the laboratory every 2nd or 3rd day and placed in cold storage. A batch of this material was steamed each day, the juice pressed out and concentrated *in vacuo* to 1/10 or to 1/15 of its original volume. This concentrate was fed to rabbits by pipette in approximately 10 to 20 cc. quantities daily. All rabbits were kept on our stock diet. The principal data are given in Table X.

Four series of experiments with concentrated skunk cabbage press juice are included in Table X. The data have been arranged to show the daily and total amounts of iodine-absorbing material fed, the equivalent of this in press juice, and the number of days during which the material was fed. It will be seen that those rabbits sacrificed on the 5th and 6th days, even though receiving large doses of iodine-absorbing material, showed slight thyroid involution, while those sacrificed after 11 days showed advanced or complete involution. (See Fig. 3.) Rabbits 9-15, 9-16, 7-73 and 7-78 were fed a moderately goitrous cabbage as their principal food during the experiment in order to make the test for the involuting effect of the concentrate more severe. In spite of the cabbage diet, thyroid involution was complete when these were examined on the 20th and 27th days. All

the other rabbits were fed our regular stock diet which, as already pointed out and as the controls of this series show, is slightly goitrous in the late winter and spring months. (See Figs. 4 and 5.)

TABLE X

Steamed Skunk Cabbage Juice Concentrate (Series 110, 113, 116, 120)

Rabbit No. and sex	Age	Amount press juice fed per day		Total units	Duration of experiment	Body weight	Thyroid weight	Condition of thyroid found at operation
	mos.	*cc.*	*units†*		*days*	*kg.*	*gm.*	
8-04 F	10	92	100	1663	16	2.08	0.20	Normal size, not hyperemic
8-23 F	12	102	110	1211	11	2.14	0.24	" " " "
8-76 F	13	79	85	3432	40	2.60	0.17	" " " "
9-07 F	5	139	150	878	6	1.75	0.09	" " " "
8-73 M	8	162	175	1890	11	1.98	0.19	" " " "
9-08 F	5	149	160	818	5	2.08	0.38	Twice normal size, moderate hyperemia
9-10 M	4	84	90	805	9	1.47	0.22	Normal size, not hyperemic
9-11 M	4	65	70	1312	19	1.92	0.12	" " slight hyperemia
9-12 M	4	65	70	1299	19	2.15	0.24	1½ times normal size, moderate hyperemia
9-13 M	4	72	80	1479	19	1.93	0.15	Normal size, slight hyperemia
9-00 M	5	56	60	993	16	1.98	0.26	" " " "
9-15 M	4	79	85	2316	27	1.83	0.19	Slightly enlarged, nor hyperemic
9-16 M	4	79	85	2316	27	1.64	0.20	Not enlarged, not hyperemic
7-73 M	11	79	85	2316	27	2.18	0.20	" " " "
7-88 M	12	84	90	1798	20	2.01	0.29	1½ times normal size, not hyperemic
8-62 M	9	125	2.5	53	21	1.98	0.26	Slightly enlarged, slight hyperemia
8-83 M	9	125	2.5	53	21	2.40	0.30	Twice normal size, marked hyperemia
9-20 M	5	125	2.5	53	21	1.78	0.19	Normal size, slight hyperemia
9-21 M	6	125	2.5	53	21	1.83	0.28	Twice normal size, moderate hyperemia
2-30 M	6	Control				2.34	0.39	" " " " "
7-20 M	17	"				2.04	0.50	" " " " "
8-64 M	9	"				1.99	*	Slightly enlarged " "
9-05 F	6½	"				1.95	*	Twice normal size, " "
9-17 M	4	"				1.91	0.23	" " " marked "
9-18 M	4	"				2.00	0.27	Slightly enlarged, not hyperemic
7-98 M	13	"				1.93	0.28	Twice normal size, moderate hyperemia
8-41 M	6½	"				2.30	0.42	3 times normal size, marked "

† A unit is the amount of reducing substance that absorbs 1 cc. N/100 I.
* Animals not sacrificed.

In the first three series skunk cabbage press juice containing about 1 unit per cc. was used while in the fourth series (Rabbits 8-62, 8-83,

9-20 and 9-21) each cubic centimeter of the press juice contained about 0.02 units. These rabbits, even though fed the concentrate from 125 cc. of press juice daily for 21 days, showed only slight thyroid involution. Thus it would appear that the involuting effect of skunk cabbage juice varies with the amount of iodine-absorbing material.

The iodine content of the press juice has been found to be high (around 0.3 mg. per liter) and this amount of iodine might produce thyroid involution but it does not account for the fact that thyroid involution varies with the amount of iodine-absorbing material, rather than with the quantity of press juice fed, as should be the case if the involuting effect were due entirely to the iodine present.

The Effect of the Injection of Plant Juice Extracts on the Thyroid

In order to further eliminate iodine as the involuting agent and to obtain additional information as to whether the reducing substance was responsible for the goiter-inhibiting effect of plant juice, we have tried to effect some purification. To this end we have prepared several crude extracts and have administered them parenterally.

1. Alcoholic Extract of Cabbage (Brassica oleracea).—

Steamed hashed cabbage was extracted with 95 per cent ethyl alcohol saturated with hydrogen sulfide. After extracting 24 hours at about 5°C. in a stoppered bottle the alcohol extract was drained and concentrated *in vacuo* to a thin syrup. Fatty substances were removed from the syrup by two extractions with absolute ethyl acetate, and the acetate removed in a vacuum desiccator. The syrup was diluted with sterile saline and injected intraperitoneally into Rabbits 7-34 and 7-35. Each received 25 injections in a period of 29 days, containing in all, iodine-absorbing material equivalent to 745 cc. of N/100 I. 2 rabbits, Nos. 7-35 and 7-36, of the same age, weight and stock were placed on our stock diet. The thyroids of all 4 were then examined.

While the 2 animals receiving the cabbage extract had thyroids that were normal, or below, those of the controls were both hyperemic and enlarged. Essentially similar results were obtained with another group of 4 animals treated in the same way (Series 38).

In the method of preparation of this extract a considerable proportion of the iodine present in the cabbage would probably have been carried along. It should be noted, however, that the amount of iodine

in northern New York State cabbage is low (100 parts per billion, Remington).

2. Decomposed Lead Precipitate of Cabbage Juice.—It was thought that this goiter-preventing agent might be identical with the hexuronic acid Szent-Györgyi (5) showed to be present in certain plant and animal tissues. To test this, the first stages of purification described by Szent-Györgyi in his isolation of the substance from cabbage were used.

The first lead precipitate was decomposed with sulfuric acid, the lead sulfate removed and the solution brought to a pH of 6.2–6.4 concentrated *in vacuo* to a small volume, 4 volumes of methyl alcohol added and the precipitated gums and salts removed. Methyl alcohol was removed by vacuum distillation, the material dissolved in sterile water and injected intraperitoneally. It caused considerable peritoneal irritation.

Rabbits 7-09 and 7-21 (Series 40) on our stock diet received, in 29 days, 24 injections of this material containing iodine-absorbing substances equivalent to 906 and 850 cc. N/100 I respectively. On examination both thyroids were small, pale and contained dense colloid with flattened epithelium. (See Fig. 6.) 2 other rabbits, Nos. 7-19 and 7-20, on stock diet served as controls. They had thyroids that showed slight and moderate degrees of hyperplasia respectively. (See Fig. 7.) Similar results were obtained with another group of rabbits (Series 34).

In these experiments practically all of the iodine present in the cabbage juice would have been removed in the preparation of these extracts. This shows that some substance present in cabbage juice precipitable by lead at a pH of 6.8–7.0 is responsible for the involution and that it could be the substance separated by Szent-Györgyi or something closely associated with it. We have evidence that the peritoneal irritation caused by the extracts was not a factor in bringing about these results.

DISCUSSION

The experiments we have reported indicate the presence in certain plant tissues of an antigoitrous substance other than iodine. This substance in plant tissue can be roughly measured by its capacity to absorb iodine, and as we have shown may be identical with the hexu-

ronic acid isolated by Szent-Györgyi. Iodine, however, has not been excluded as a contributing antigoitrous factor in the experiments reported and final proof must await the production of thyroid involution by the use of an iodine-free preparation of this reducing substance or substances.

Many facts support the view that there are antigoitrous substances other than iodine. Thus the seasonal variations observed in the capacity of cabbage to produce goiter can be partially explained by the fact that summer cabbage which contains large amounts of iodine-absorbing material is only slightly, if at all, goiter-producing, while winter cabbage which is low in iodine-absorbing material has a higher goiter-producing power, although both samples of cabbage may have approximately the same iodine content.

We have, also, some experiments which indicate that keeping cabbage cake exposed to air for several days may increase its goitrous power by destroying the reducing substances present. It would be difficult to explain this observation as due to a change in iodine content. We have also observed a similar thyroid phenomenon in our rabbit colony during the past 12 years. Each spring we have observed the occurrence of definite thyroid hypertrophy in our stock, whereas in the autumn this is absent. It has been our custom to purchase a full supply of hay and oats in the early autumn sufficient to last us through the winter, in order to have a constant stock diet. This annually recurring thyroid hyperplasia could not be explained on the basis of any change in the iodine content of the foodstuffs during storage. The gradual loss of the antigoitrous substance associated with prolonged storage, however, would account for the progressive increase in thyroid hyperplasia.

The seasonal variations in the iodine content of the thyroid and the seasonal variations in the incidence of goiter which have been proven to occur in both the northern and southern hemispheres (6, 7) during corresponding months, likewise cannot be explained on the basis of variations in the iodine content. (This does not affect the proven relation between endemic goiter and the low iodine content of soil, water and foods in endemic goiter districts.) It is difficult to imagine that sheep or cattle get more iodine by grazing than by eating hay harvested from the same fields. This seasonal variation can be more

rationally explained by assuming that there is a greatly increased intake of antigoitrous substances (iodine-absorbing material) from eating fresh growing plants which in some way lessen the need for thyroid activity or exert a thyroid-sparing action.

Likewise it has long been known that men and animals living in the tropics and subsisting on tropical vegetation rarely have goiter. This fact also suggests the occurrence of antigoitrous agents other than iodine in fresh fruits and vegetables.

We have for many years insisted that while thyroid hyperplasia was due immediately to a deficiency of iodine, this deficiency could be relative as well as absolute (8). That is, the iodine intake may be high, but owing to increased demands it is utilized so rapidly that it cannot be held in the thyroid in sufficient concentration to prevent the gland from enlarging. The goiters produced by feeding cabbage or liver are illustrations of relative iodine deficiencies apparently produced by positive goitrous agents which inhibit tissue oxidation. In the case of cabbage it probably is a nitrile, while in the case of liver it could be a sulfhydryl compound. Another type of relative iodine deficiency would now appear to be dependent upon a deficiency of some reducing substance normally present in growing plants. Evidence has been presented to show that both the goiter-producing and goiter-inhibiting substances may exist in the same plant (cabbage) in variable amounts.

Our experiments indicate that the iodine-absorbing material that inhibits thyroid hyperplasia may be identical with the hexuronic acid isolated from plants and from suprarenal cortex by Szent-Györgyi. We have found that a similar reducing substance is also present in high concentration in the corpus luteum of the pig. If these reducing substances other than glutathione in the suprarenal cortex and sex glands are antigoitrous, as all the evidence now available indicates, then we have our first knowledge of why and how sex, puberty, pregnancy and the menopause influence the incidence of goiter—a problem that has interested medical men in all ages.

The possibility that glutathione also present in the suprarenal cortex and corpora lutea in high concentration was one of the reducing substances responsible for thyroid involution was considered, and three sets of experiments have been made where 200 mg. of crystalline glu-

tathione was injected intraperitoneally 3 times weekly for a month without any involuting effect on the thyroid. Indeed the opposite effect—a moderate goitrogenic effect—was observed. In looking back over the old experiments of the production of goiter in brook trout (*Salmo fontinalis*) with pig's liver it seems probable that this was in part due to glutathione.

The mode of actions of one of these reducing substances (probably hexuronic acid) in bringing about thyroid involution and of another (glutathione) in preventing involution is unknown. We have suggested that they may provide another means of promoting and regulating oxidations in the body and in this way lessen the need for thyroxine. This is in contrast with the accepted explanation of the antigoitrous action of iodine; *viz.*, that an ample supply of iodine makes it possible for the thyroid to meet any demands for thyroxine without enlarging.

SUMMARY

The development of simple goiter in rabbits, which results when they are fed on a diet consisting chiefly of cabbage (or other *Brassica*) can be prevented by the feeding of certain fresh plants. Lawn grass, fresh alfalfa, skunk cabbage and steamed cabbage press juice have been found effective. Washing *Brassicae* with water improves their goiter-producing action, probably by the removal of goiter-preventing substances.

The antigoitrous effect of plant juices was found to vary with the amount of reducing substance other than glutathione present. Skunk cabbage press juice, obtained from growing plants during the spring months, contained a large amount of iodine-absorbing material, and was very effective in involuting hyperplastic glands while juice obtained from mature plants in July when the iodine-absorbing material had nearly all disappeared, was quite ineffective.

Certain extracts from cabbage administered parenterally, containing the reducing material, also had a marked involuting action on the thyroid. These extracts were practically iodine-free.

The substance or substances believed to be responsible are easily soluble in water, ethyl alcohol, aqueous ethyl alcohol and are precipitable by lead acetate at a pH of 0.68–0.70. They are partially

destroyed by exposure to air and by steam at 100°. Hexuronic acid isolated by Szent-Györgyi from cabbage, orange juice and suprarenal glands could be the antigoitrous agent. It cannot be glutathione.

BIBLIOGRAPHY

1. Marine, D., Baumann, E. J., and Cipra, A., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 822.
2. Marine, D., Baumann, E. J., and Webster, B., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 1029.
3. Chesney, A. M., Clawson, T. A., and Webster, B., *Bull. Johns Hopkins Hosp.*, 1928, **43**, 261.
4. Webster, B., and Chesney, A. M., *Am. J. Path.*, 1930, **6**, 275.
5. Szent-Györgyi, A., *Biochem. J.*, 1928, **22**, 1387.
6. Seidell, A., and Fenger, F., *J. Biol. Chem.*, 1913, **13**, 517.
7. Hercus, C. E., Benson, W. M., and Carter, C. L., *J. Hyg.*, Cambridge, Eng., 1925, **24**, 321.
8. Marine, D., *Medicine*, 1924, **3**, 453.
9. Marine, D., Baumann, E. J., and Webster, B., *J. Biol. Chem.*, 1930, **89**, 213.

EXPLANATION OF PLATES

All photomicrographs were made from formalin-fixed thyroids stained with hematoxylin and eosin and magnified approximately 130 diameters.

PLATE 4

FIG. 1. (R1-8-77) complete involution. Fed with lawn grass for 24 days.

FIG. 2. (R1-7-16) complete involution. Fed with steamed skunk cabbage for 15 days (1929).

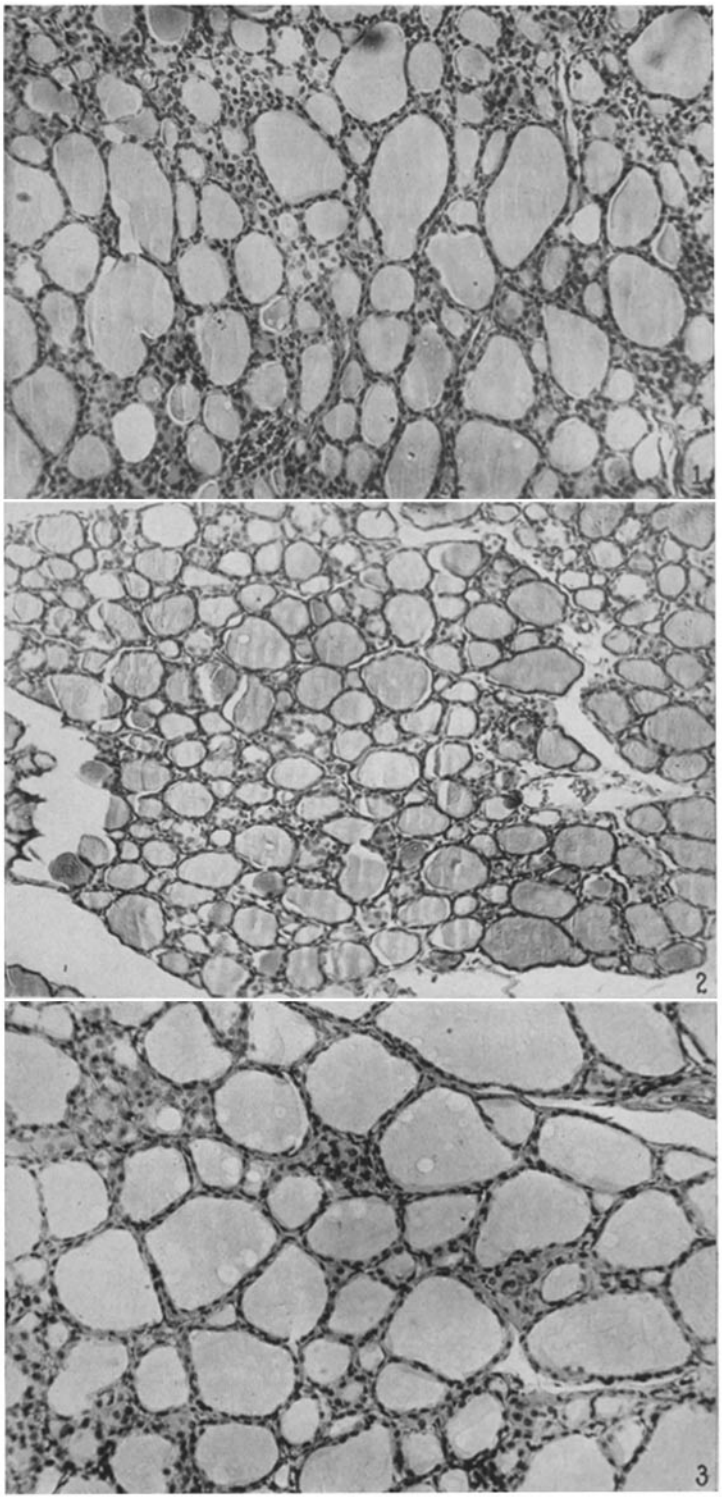
FIG. 3. (R1-8-73) complete involution. Given the concentrate from 160 cc. skunk cabbage juice daily by mouth for 11 days. Stock diet.

PLATE 5

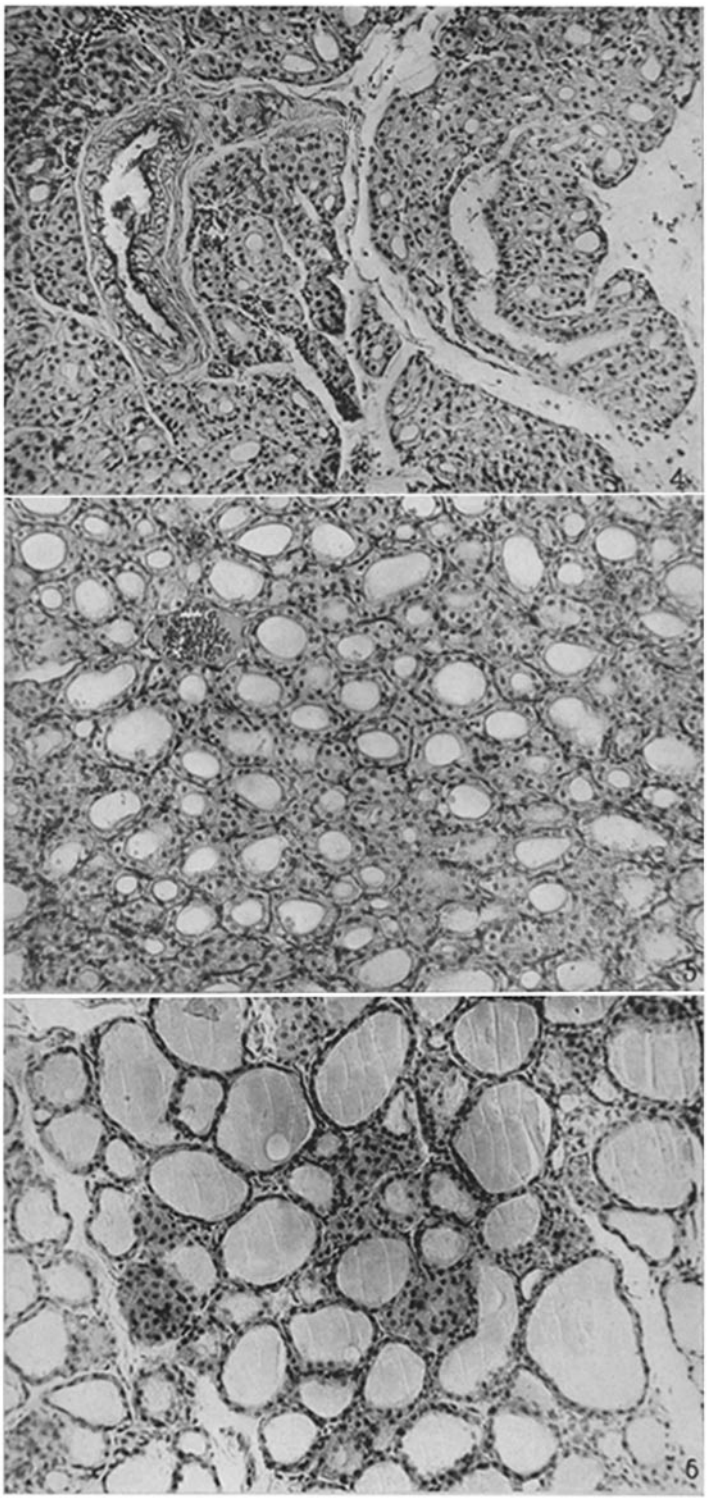
FIG. 4. (S-2-30) marked hyperplasia. Control for R1-8-73 (Fig. 3). Stock diet.

FIG. 5. (R1-7-20) moderate hyperplasia. Control for R1-7-21 (Fig. 6). Stock diet.

FIG. 6. (R1-7-21) complete involution. Daily intraperitoneal injections decomposed lead precipitate.



(Marine *et al.*: Antigoitrous substances in plants)



(Marine *et al.*: Antigoitrous substances in plants)