lowing tentative conclusions: Irradiation of 2 proteins, casein, and egg-white, in the dry, solid state changes them so that digestion *in vitro* by pepsin and trypsin are slightly inhibited. Metabolism experiments on young male rats, on the other hand, demonstrate a slightly higher body-weight when the protein fraction is irradiated. Since the nitrogen-retention on the low protein diet is slightly less for the irradiated than for the non-irradiated protein, the improvement of the body-weight curve appears to be due to something other than improved utilization of the protein. Vitamin D as an antirachitic agent is ruled out since the diet contains an ample supply for that purpose. Some other factor, stimulating in character is suggested.

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## Further Studies on Etiology of Goiter with Particular Reference to the Action of Cyanides*

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The *immediate* cause of thyroid hyperplasia in all probability is a *relative* or an *absolute* deficiency of iodine.[1] The fundamental or essential cause of goiter is unknown, but the search for the essential cause, as we have often suggested, appears to resolve itself into determining the cause or causes of the iodine deficiency. As most iodine deficiencies are relative rather than absolute, the search further limits itself largely to determining the factors which create the increased needs of the organism for the iodine containing hormone. The simplest way of increasing the need of the thyroid for iodine would be by depressing the utilization of oxygen in the tissues, and the discovery by Chesney and Webster[2] that the prolonged feeding of cabbage caused thyroid hyperplasia in rabbits appeared to offer a practical means of testing this hypothesis.

It has been shown that there are great seasonal and climatic variations in the goitrogenic activity of cabbage,[3] that drying in a current

* Aided by a grant from the Ella Sachs Plotz Foundation.

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[1] Marine, D., *Arch. Int. Med.*, 1923, **32**, 811.

[2] Chesney, A. M., Clawson, T. A., and Webster, B., *Bull. Johns Hopkins Hosp.*, 1928, **43**, 261.

[3] Webster, B., Marine, D., and Cipra, A., *J. Exp. Med.*, 1931, **53**, 81.

of air or *in vacuo* causes a loss of the goitrogenic agent,[4] that prolonged steaming does not impair and under certain conditions may increase its goitrogenic power,[5] that boiling for 30 minutes at pH 3.0 (HCl) does not injure it,[6] that the goitrogenic substance may be extracted from cabbage with ether and other ethereal solvents[7] and that this substance is but slightly extracted by prolonged aqueous leaching.

Since all the *Brassicae* so far tested may produce goiter and since mustard oils (isothiocyanates) are the most characteristic constituents of these plants it was thought that their goitrogenic activity might be connected in some way with these substances or with their cyanide precursors. Several of the mustard oils (allyl, ethyl, phenyl) have been fed to rabbits with negative results.

In view of these observations, together with the isolation of nitriles by Hoffmann[8, 9, 10] from several of the *Cruciferae,* the idea that cyanides were the substances which increase the needs of the organism for the thyroid hormone by partially blocking tissue oxidation strongly suggested itself. Accordingly we first tested the least toxic of the cyanides (acetonitrile). When this substance was injected subcutaneously into 4-months-old rabbits daily in doses of 0.1 to 0.25 cc. for 21 days very striking thyroid hyperplasia was produced in animals maintained on a diet of alfalfa hay and oats. In order to determine whether this thyroid reaction was characteristic of cyanides generally we have tested allyl-, propio-, phenylaceto-, phenylpropio- and benzonitrile. Experiments have also been made with phenylisocyanide, potassium cyanide, cyanamide, and sodium thiocyanate. The data of representative experiments are given in Table I.

It will be seen that thyroid hyperplasia may be produced in rabbits by all the cyanides so far tested but in greatly varying degrees. Acetonitrile produced the greatest reaction and the aromatic nitriles as a group the least. These differences were not altogether due to the doses of cyanide. Cyanamide also produced only a slight reaction and sodium thiocyanate none at all.

[4] Marine, D., Baumann, E. J., Webster, B., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1025.

[5] Marine, D., Baumann, E. J., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 822.

[6] Webster, B., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1026.

[7] Baumann, E. J., Cipra, A., and Marine, D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1017.

[8] Hoffmann, A. W., *Berichte d. chem. Ges.*, 1874, **7**, 1293.

[9] Hoffmann, A. W., *Berichte d. chem. Ges.*, 1874, **7**, 520.

[10] Hoffmann, A. W., *Berichte d. chem. Ges.*, 1874, **7**, 518.

TABLE I.

| Group | Sex | Age | Substance | How Given | Dose | Condition of Thyroid | | |
|---|---|---|---|---|---|---|---|---|
| | | (mo.) | | | | | | |
| I | M | 4 | Methyl cyanide | With rolled oats | 0.5 cc. | | | + |
| | F | 9 | " " | " " " | " | | +— | |
| | F | 4 | " " | " " " | 0.25 | —+ | | |
| | F | 9 | " " | " " " | " | —? | | |
| II | M | 4 | " " | Subcutaneously | 0.10 | | | ++ |
| | M | 4 | " " | " | " | | | + |
| | F | 4 | " " | " | " | — | | |
| III | M | 3 | Allyl cyanide | " | 0.0125 | | | +? |
| | F | 5 | " " | " | " | | +— | |
| IV | M | 4 | Benzyl cyanide | " | 0.025 | | | +? |
| | M | 4 | " " | " | 0.019 | | +— | |
| | F | 4 | " " | " | 0.0125 | —+ | | |
| V | M | 5 | Phenyl cyanide | " | 0.04 | —? | | |
| | M | 5 | " " | " | 0.02 | —+? | | |
| | M | 5 | " " | " | 0.01 | —? | | |
| VI | M | 4 | Cyanamide | " | 0.20 | —+ | | |
| | F | 4 | " | " | 0.10 | — | | |
| | M | 4 | " | " | 0.05 | — | | |
| VII | F | 5 | Sod. thiocyanate | Intraperitoneally | 0.20 | — | | |
| | F | 6 | " " | " | 0.10 | — | | |
| | M | 5 | " " | " | 0.05 | — | | |
| | F | 5 | " " | " | 0.025 | — | | |

++ = More than twice normal size, very hyperemic.
+ = Twice normal size, very hyperemic.
+— = One and one-half normal size, moderately hyperemic.
—+ = Slightly enlarged, slightly hyperemic.
— = Not enlarged, not hyperemic.

The thyroid response was more marked in young rabbits. So far our experiments to prevent these cyanides from causing thyroid hyperplasia by the administration of sodium thiosulphate intraperitoneally have been negative.

Our experiments so far have demonstrated that substances which depress oxygen consumption may increase thyroid activity and that cyanides are among the most potent of these goitrogenic agents. While a cyanide has not yet been definitely isolated from cabbage, enough evidence is available to show that its goitrogenic activity is due to one. These observations have given us our first definite information concerning the essential cause of thyroid hyperplasia. We have frequently pointed out that a deficiency of iodine, while certainly the immediate cause of thyroid hyperplasia, is in most cases only relative, and is due to the increased demands for iodine caused by a goitrogenic agent.

Iodine administration will prevent hyperplasia of the thyroid caused by cyanides just as easily as the hyperplasia due to meat diets (liver), cabbage feeding, administration of anterior pituitary

extracts, or following the partial removal of the gland. These observations throw some light on why a low iodine intake may not lead to thyroid hyperplasia if the production of cyanide is below the effective concentration or if the mechanism for detoxicating cyanides is sufficient, and why a high iodine intake may not protect against thyroid hyperplasia if there is an excessive cyanide intake or formation within the body.

If cyanides prove to be an essential factor in the causation of goiter, both the exogenous and the endogenous sources of cyanide must be investigated, since it is obvious that in most cases the cyanide must be of endogenous origin. This must exist in the organism in an effective concentration either because of insufficient detoxification or through some modification of metabolism whereby cyanide is formed in excess of its physiological needs or of the organism's capacity to handle it.

The fact that the thyroids of a small percentage of rabbits of the same weight, age and breed did not undergo hyperplasia following injection of cyanides is of biological interest, since it suggests, among other things, that some rabbits have a more efficient physiological mechanism for detoxicating cyanides.

The effect on the thyroid of feeding plants known to contain large amounts of HCN *(Sorghum vulgare, Sorghum sudanense)* has not been studied.

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## Cortical Response to Stimulation of the Optic Nerve.

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In the cortex of the rabbit a small area can occasionally be found that is supplied by one artery and one vein. Such a region can thus be isolated by incisions except for the tongue of tissue where the vessels enter, without serious interference with the blood supply. A metal plate slipped under this tissue and connected to ground serves as an indifferent electrode, and the end of a fine wire resting on the cortical surface serves as a test electrode. When the region so isolated is not active no record is picked up from activity in the rest

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