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SYNERGISTIC ACTION OF IODINE-DEFICIENCY AND FLUORINE-INTOXICATION ON RAT THYROID

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212 Wistar rats were divided randomly into five groups, each of which was fed on one of the following regimes: (1) Normal iodine and fluorine; (2) normal iodine, 10 ppm fluorine; (3) normal iodine, 30 ppm fluorine; (4) flow iodine, normal fluorine; (5) low iodine, 10 ppm fluorine. The experiment lasted seven months. The results showed that severe morphologic and functional damage of the thyroid appeared in the rats drinking water containing 30 ppm fluorine, but only slight abnormal ultrastructural changes of thyroid cells appeared in rats drinking water containing 10 ppm fluorine; rats with iodine deficiency showed proliferative changes of the thyroid; rats on iodine deficient diet and drinking water containing 10 ppm fluorine showed morphologic and functional damage as well as proliferation. The study suggests that there is synergistic action of iodine-deficiency and fluorine-intoxication on the thyroid.

The data of epidemiological and animal experiments show that goiter and hypothyroidism can be induced either by iodine-deficiency or by chronic fluorosis.[1-3] The relationship between iodine and fluorine in thyroid hormone metabolism has attracted more attention recently because both iodine and fluorine belong to the same halogen family and share some common chemical properties.[4] Some investigators report that excessive fluorine may be goiterogenic especially when there is iodine deficiency. However, the relationship between iodine and fluorine in terms of action on the thyroid is not clear.[5,6] In order to study this problem, we performed this study on rats.

MATERIAL AND METHODS

Animals. 212 Wistar rats weighing 120±19 g were divided randomly into five groups. Group 1 (control), 41 rats, female 23 and male 18, were fed normal diet containing 310 ng iodine/g diet and 1.856 ppm fluorine, and drank tap water containing 8.2 ng iodine/ml and 0.4 ppm fluorine. Group 2, 44 rats, female 24 and male 20, were fed the same diet as Group 1, but took water containing 10 ppm fluorine. Group 3, 41 rats, female 23 and male 18, were fed the same diet as Group 1, but given water containing 30 ppm fluorine. Group 4, 41 rats, female 23 and male 18, were fed low-iodine diet containing 20-62.5 ng/g iodine and 1.743 ppm fluorine, but drank deionized water (resistivity 1 000 000 Ohm).[7] Group 5, 45 rats, female 22 and male 23, were fed the same diet as Group 4, but took deionized water to which 10 ppm fluorine was added. The total experimental period was seven months, after which all the animals were sacrificed and weighed.

Biochemical analyses. Iodine and fluorine concentrations in 24-hour urine from six rats in each group were measured by the improved Henty's method and the fluorine-electrode method respectively at the end of four months of the experiment. Thyroid ^{131}I uptake was measured at 2,6 and 24 hours at the end of 5 months of the experiment. Serum T4 and T3 concentrations were measured by radioimmunoassay at the end of the experiment. Thyroid peroxidase (TPO) activity was assayed by the Guaiacol method, which was expressed as Guaiacol Unit (G.U) per 1.00 density by 721-spectrometer. The thyroid gland protein synthetic rate was determined by ^{3}H-leucine uptake. ^{3}H-leucine (20 μCi/100 g B.W.) was injected into the abdominal cavity. The thyroid gland was excised at the 4th hour after injecting ^{3}H-leucine and treated by alkaline-ashing digestion. The ^{3}H-leucine uptake rate, expressed as CPM per 10 mg thyroid tissue, was recorded by YS-A Scintillation counter.

Morphologic studies. The thyroid was weighed. For light microscopy, tissue was embedded in paraffin after fixing in 10% neutral formalin for 24 hours, and then stained with H.E. For transmission electron microscopic examination, fresh thyroid samples were fixed in 2.5% glutaraldehyde, post-fixed in 1% phosphate buffered osmium tetroxide solution, dehydrated in ethanol and embedded in Epon-618. Extra thin sections were double stained with uranyl acetate and lead citrate, and examined by 100 CX-2 electron microscope.

Statistical analysis. Groups 2, 3, 4 and 5 were compared with Group 1. Results were analyzed by Student's *t* test.

RESULTS

Body weght, urine fluorine and iodine concentrations. The average rat body weight in Group 3 was significantly decreased. Urinary flourine concentrations were increased in all rats drinking water with excessive fluorine. The urinary iodine concentration declined in all rats fed with low-iodine diet (Table 1).

Table 1. Body weight and fluorine and iodine concentrations in the urine

Group	Body weight (g)		Urine fluorine (ppm)		Urine iodine (μg/24h)	
	n	M±SD	n	M±SD	n	M±SD
1	41	293±57	6	1.23±0.22	6	1.110±0.226
2	44	294±85	6	6.66±0.91*	6	1.215±0.357
3	41	254±68*	6	6.16±0.68*	6	1.150±0.287
4	41	289±72	6	1.23±0.26	6	0.095±0.020*
5	45	308±63	6	6.23±0.[illegible]*	6	0.099±0.017*

Note: n = number of animals; *: $P < 0.01$, compared with Group 1.

¹³¹I thyroid uptake. The 24th hour ¹³¹I thyroid uptake was significantly decreased in Group 3, but the 2nd, 6th and 24th hour ¹³¹I uptake was markedly increased in Groups 4 and 5 (Table 2).

Serum T4 and T3 assay. Serum T4 concentrations were significantly lowered in Groups 3, 4 and 5. Serum T3 levels were also lowered in Group 3, but increased in Group 4 (Table 3).

Thyroid peroxidase. TPO activity was statistically significantly decreased in Groups 3 and 5, but increased in Group 4 (Table 4).

³H-leucine thyroid uptake. H-leucine uptake was decreased in Group 3 but increased in Group 4 (Table 4).

Gross thyroid examination. Thyroid weight and size were decreased in Group 3, but increased in Groups 4 and 5 (Table 5).

Table 2. Thyroid ¹³¹I uptake rate

Group	Animal	¹³¹I uptak rate (% M±SD)		
		2nd hour	6th hour	24th hour
1	15	33.28±2.53	42.31±3.29	47.37±5.66
2	15	33.00±4.03	38.97±4.99	44.74±5.14
3	15	33.90±3.17	38.94±3.75	42.73±4.31*
4	15	44.80±5.65*	57.36±8.16*	58.40±9.54*
5	15	43.98±5.53*	58.28±5.81*	59.05±7.59*

*: $p < 0.01$, compared with Group 1.

Table 3. Serum T4 and T3 concentrations

Group	Animal	Serum T4 (μg/dl) M±SD	Serum T3 (ng/dl) M±SD
1	20	3.64±1.45	70.65±30.29
2	25	3.02±1.48	61.96±26.02
3	7	1.44±0.39*	43.00±11.31*
4	16	0.76±0.70*	95.81±25.18*
5	21	0.65±0.57*	68.05±21.96*

*: P < 0.01, compared with Group 1.

Table 4. Thyroid TPO activity and 3H-leucine uptake

Group	TPO activity (G.U./100g B.W.)		^{3}H-leucine uptake (CPM/10mg)	
	N	M±SD	N	M±SD
1	8	2.04±0.22	12	1808±358
2	8	1.98±0.51	12	1728±790
3	8	1.73±0.24*	10	1258±293*
4	8	2.57±0.44*	13	2252±683*
5	8	1.75±0.21*	13	1804±459

*: P < 0.01, compared with Group 1.

Table 5. Thyroid weight and morphometric indices

Group	Weight (mg/g)		Height (M±SD)			N to P (M±SD)	Follicle diagram (μ) (M±SD)
	N	$\bar{X}$±SD	N	Cell (μ)	Nucleus (μ)		
1	41	9.97±3.52	8	5.53±0.41	3.32±0.18	1:1.67±0.12	36.83±2.90
2	44	9.58±2.40	10	5.38±0.03	3.22±0.18	1:1.63±0.06	36.65±1.76
3	41	7.90±2.37*	8	5.40±0.20	3.26±0.13	1:1.65±0.06	36.36±1.70
4	41	19.91±11.23*	8	7.83±0.50*	4.35±0.20*	1:1.81±0.06*	21.17±1.61*
5	45	20.13±22.10*	10	7.91±0.27*	4.29±0.28*	1:1.85±0.07*	23.10±1.47*

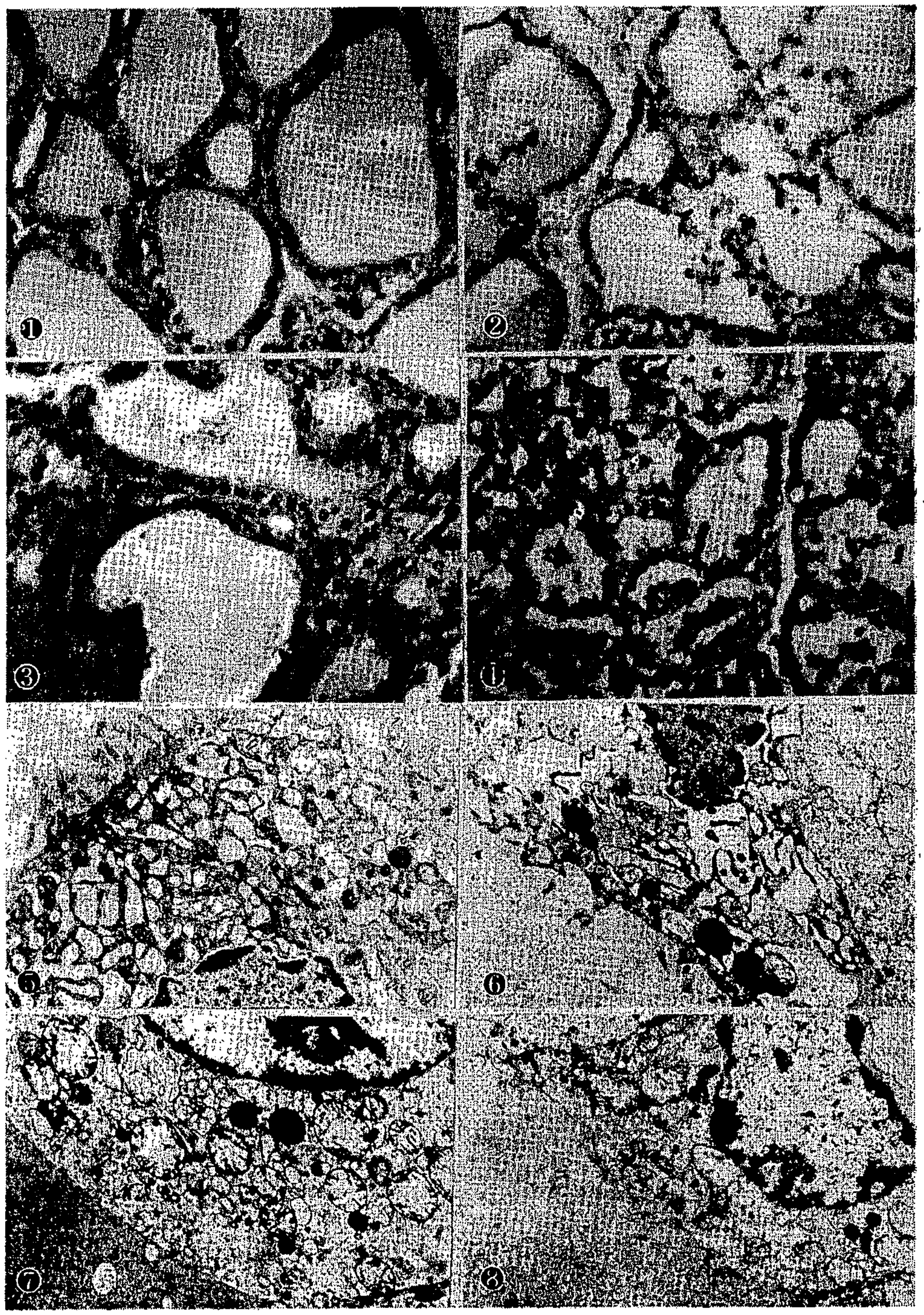
*: P < 0.01, compared with Group 1.

Light-microscopic examination. Group 1. The follicles were regular in shape and their lumina were filled with colloid. The follicular cells were flat or cuboid in shape (Fig 1). Group 2. There were no differences in thyroid histologic structure as compared with Group 1. Group 3. Some of the follicles were disintegrated, and degenerate and necrotic epithelial cells were seen. Stromal edema was found (Fig 2). Group 4. The follicles were irregular in shape and varied in size. The lumina contained only a little colloid. The follicular cells were tall, columnar and proliferated, forming papillae protruding into the lumen. Solid cell masses were found scattered among the follicles (Fig 3). Group 5. The thyroid showed the same pattern as in Group 4, obvious degenerative changes were observed in some areas (Figs 4, 5). Morphometric indices showed that in Groups 4 and 5, the height of the follicular cells and their nuclei, and the ratio of nuclei to plasma (N to P) were increased, but follicular diameter was decreased. No differences in these indices were found compared with Group 1 in the other groups (Table 5).

Electronmicrosocpic examination. Group 2. As compered with Group 1, the microvilli on the apical surface of the follicular cells were slightly decreased and the mitochondria in some of the follicular cells were swollen (Fig 5, 6). Group 3. The microvilli were markedly decreased. Mitochondrial swelling and endoplasmic reticuli dilation were marked. Organele disintegration and disappearance were also observed in some follicular cells (Fig 7). Group 4. The microvilli were thickened and mitochondria and ribosomes were increased in number. Group 5. The microvilli were thickened but scanty. Mitochondrial swelling and endoplasmic reticuli dilation were also present (Fig 8).

DISCUSSION

We successfully produced an animal model of iodine deficiency hyperplastic goiter and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chronic fluorosis by giving an iodine deficient diet and excessive fluorination in the water over a long period in this experiment.

Fluorine is one of the body's essential trace elements but the beneficial range of the amount taken is so narrow that health may be influenced adversely if excessive fluorine is taken. It is indicated that the thyroid fluorine content is frequently higher than in other non-skeletal organs, perhaps due to the thyroid's higher affinity to fluorine. Slight thyroid damage can be detected only by electron microscope in rats drinking water containing 10 ppm fluorine. But in rats drinking water containing 30 ppm fluorine, severe damage occurs, both morphologically and metabolically. We have shown that the thyroid gland became decreased both in weight and size; degeneration and necrosis of follicular cells were noticed; serum T4 and T3 contents, ^{131}I uptake, TPO activity and ^{3}H-leucine uptake were all decreased. The results suggest that iodine-trapping function, protein synthesis, and synthesis or transformation of the thyroid hormones were suppressed, and that the degree of thyroid impairment correlated with the fluorine dose.

Some epidemiologic information indicates a high incidence or goiter in endemic fluorosis areas.[5] Goiter in experimental animals due to excessive fluorine intake has been reported elsewhere.[1] But the incidence of goiter in endemic fluorosis areas in Guizhou Province was not higher than that in non-fluorosis areas.[8] Our experiment confirms further that excessive flourine intake alone does not cause goiter.

In our experiment, thyroid compensatory changes such as goiter formation; enhancement of iodine trapping, protein synthesis and TPO activity were displayed in rats with iodine deficiency. Although serum T4 level declined, the transformation of T4 to T3 was increased so that serum T3 content was raised. Damage to thyriod structure and function besides goiter, was observed in rats with iodine deficiency and excessive fluorine intake. These changes were much more severe than that observed in rats on normal iodine diet and water with 10 ppm fluorine. Abnormal changes in thyroid structure were evident by both light and electron microscopy. TPO activity and serum T4 level declined although ^{131}I uptake was not decreased. No compensatory increase in serum T3 content was noticed. This may show that the transformation of T4 to T3 is suppressed.

Stolo et al[9] reported that fluroine can inhibit the synthesis of T4 and T3 in rats fed with low-iodine diet, but exhibit no influence in rats on a normal level iodine diet. Fluorine shares similar chemical properties with iodine, though the former is more active. There may be competitive antagonism between fluorine and iodine in their thyroid action. Therefore, the action of excessive fluorine and iodine deficiency may accumulate in the thyroid, thus causing more severe damage there.

This study reveals that the degree of impairment of thyroid morphology and function is related with the amount of flourine taken by rats. Goiter occurs in rats with iodine deficiency. Damage to the thyroid is observed in rats on iodine deficient diet and highly fluorinated water. These changes are much more severe than in

Fig 1. Group 1. Normal thyroid follicles and follicular cells. HE × 140

Fig 2. Group 3. Follicles disintegrated. Degenerative and necrotic epithelial cells and stroma edema shown. HE × 140

Fig 3. Group 4. Lumina contain little colloid. Follicular cells proliferated to form papillae. Solid cell masses are scattered between the follicles. HE× 140

Fig 4. Group 5. Similar appearance to Group 4 with obvious degenerative changes. HE × 140

Fig 5. Group 1. Normal thyroid follicular cells.× 10 000

Fig 6. Group 2. Microvilli slightly decreased and mitochondrial swelling in the follicular cells. × 10 000

Fg 7. Group 3. Microvilli markedly decreased. Mitochondrial swelling and dilation of the endoplasmic reticuli are obvious. × 10 000

Fig 8. Group 5. Microvilli thickened but scanty. Mitochondrial swelling and dilation of the endoplasmic reticuli. × 10 000 rats on a normal level iodine diet and highly fluorinated water. This seems to suggest that competitive antagonistic action exists between fluorine and iodine in the thyroid gland.

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ARMY HOSPITAL DEVELOPS UNIQUE SKILLS IN TREATING INFANTILE PARALYSIS

No. 208 Hospital of the People's Liberation Army has been successful in treating and operating on patients with infantile paralysis.

The Hospital, which is the center for the treatment of the affliction, has treated 60 000 sufferers from China and from 13 foreign countries including France, Thailand and Japan. It has carried out 14 000 orthopaedic operations since 1968.

After the treatment, 93.6 percent of patients undergo improved conditions and totally successful operations have helped patients to live normally, according to Ye Mou-sheng, Director of the Hospital.

Su Lin-nan, a 18-year-old girl from Zhejiang Province, could only get around on her hands for 15 years. After her hips and knees were operated on, she was able to stand and walk.

Ye said the Hospital has developed 23 different operations to treat the sequelae of infantile paralysis such as contracture of the knee joint and deformity of the hip joint.

It has also invented a variety of orthopaedic props and shoes.

TRADITIONAL CHINESE DRUG DRYS OUT DRUNKS

It's a sobering thought, but a small package of traditional Chinese medicine called Zuixing has been declared capable of clearing even the head of a dead-drunk tippler within a couple of hours.

In fact, the amazing medicine which may completely eliminate hangovers was recently cited as a major world-class science and technology achievement by China's prestigious State Science and Technology Commission.

Developed by the Zili Traditional Chinese Medicine Factory in Xi'an, capital of Shaanxi Province, the medicine is made in accordance with ancient imperial recipes and the theory of traditional Chinese medicine. The drug should be taken after mixing with water.

Seriously speaking, however, the medicine's most important role is to prevent alcohol from entering the liver, as well as reducing alcohol stimulation to the nervous system, thus aleviating harm to human health.

The beneficial effect of the medicine has been convincingly proven by tests on animals and in clinical use, and no side effects have been observed.