METABOLISM OF FLUORINE

Absorption, Retention, Distribution and Elimination of Fluorine, and Its Effect on the Vitamin C Content of Different Tissues, and on the Iodine Content of Thyroids of Rats and Monkeys.

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Summary

(i) Studies have been carried out on the absorption, retention, distribution and elimination of fluorine, and on its effect on vitamin C content of different tissues, and on the iodine content of thyroids of rats and monkeys. (ii) It has been shown that, when 2 mgm. of NaF dissolved in water is given daily for a period of 20 weeks to rats placed on normal diet, (*a*) the absorption F is almost complete; (*b*) its retention is 57·6% of that absorbed as against 43·9% of that absorbed in the rats placed on diet deficient in Ca and P; (*c*) its distribution and deposition is mainly in the calcified structures, soft tissues containing only a very small fraction of F retained in the system; (*d*) among the soft tissues, the skin together with hair contains the highest amounts of F; brain, heart, small intestine, kidney, liver and adrenals come next in the order; (*e*) among the calcified structures, humerus contains the highest amount of F, and vertebræ and epiphyses come next in order; (*f*) after the further administration of F is discontinued, the elimination of F from the system, in a period of 12 weeks, is 33·74% of that deposited, and is mainly through the urinary system; (*g*) in terms of percents, the elimination of F is greater from the soft tissues than from the blood and calcified structures, and from amongst the calcified structures, it is highest from the incisors, and then, in descending order, from epiphyses, scapula, ribs and other bones. (iii) It has been also shown that, as a result of chronic fluorosis, there is a decrease in the vitamin C content of the tissues, and an increase in the I content of thyroids of both rats and monkeys, and that both these changes are more marked in monkeys than in rats.

Introduction.—The results of the earlier workers[1, 2, 3] emphasize the fact that the extent of retention or excretion of F is not invariant but is governed by a number of factors. It is largely determined by the quantity of F ingested,[4, 5] the chemical nature and the state of F compounds at the time of ingestion,[3, 6–11] the age of the animal,[5] the duration of intake[12] and the composition of the diet.[13–15] Influenced as it is by a number of factors, the metabolism of F needs investigation under conditions wherein all or some

of the above factors do not operate, or operate insignificantly. Studies have therefore been carried out, under strictly controlled conditions, on the absorption, retention. distribution and elimination of F, and on its effect on the vitamin C content of different tissues, and on the iodine content of thyroids of rats and monkeys.

General Plan of the Experiment.—To obviate or minimise the influence of the age of the animal, the duration of intake and the composition of the diet on the metabolism of fluorine, the present studies have been carried out for a period of 20 weeks on young growing rats, 5 to 6 weeks old, and placed on normal diet, consisting of cotton seed globulin 9%, gelatin 4%, nitrogen-free starch 65%, salts 4%, choline hydrochloride in sugar (1 : 9) 1%, cystine in sugar (1 : 19) 1%, vegetable fat 16% and the necessary amounts of vitamins A, D and of B complex, and having the following percentage composition: N = 2·20, calcium = 0·384, phosphorus = 0·27, and F = 0·00045. Similarly, to minimise or regularise the effect of the quantity of F ingested and of the chemical nature and the state of F compounds at the time of ingestion on the metabolism of F, 2 mgm. of NaF, dissolved in water, have been daily administered, per os, to each experimental animal 1 hour before the food has been offered. By this procedure, the difference between the toxicity of a definite quantity of F dissolved in water and that of an equal quantity of F mixed with the diet has been eliminated, and besides, the ingestion of the quantity of F administered has been ensured, as the ingestion of F, when admixed with the diet, cannot be controlled within any specified range, as the amount of F ingested will vary with the food intake, which is markedly lowered as a result of F poisoning. The quantity of F to be administered has been fixed as 2 mgm. of NaF daily per rat, as that has been shown to be the approximate quantity of NaF, which, when ingested over a considerable period, will result in the syndrome of chronic fluorosis.[16] The metabolic periods have been divided into 7 days intervals. At the end of each metabolic interval, the F content of urine and fæces has been determined.

In another set of rats, similarly intoxicated with fluorine, studies have been made on the effect of F on the vitamin C content of different tissues and on the iodine content of thyroids. Similar estimations have also been carried out in monkeys. The methods of feeding and producing fluorosis in monkeys have been the same as previously described.[17]

Analytical Methods.—F in the urine, fæces, different tissues and bones has been estimated by the thorium-nitrate titration method.[18] Vitamin C content of the different tissues has been estimated by the method of

Bessey,[19] and I content of the thyroids, by the method of Stimmel and McCullagh.[20]

Absorption and retention of F.—The data on the absorption and retention of F are given in Tables I and II.

TABLE I. *Absorption and retention of F in the rats placed on normal diet*

Quantity of F as NaF administered in the period of 20 weeks = 126·6 mgm.
Quantity of F ingested from and with the diet = 3·15 mgm.
Total quantity of F ingested = 129·75 mgm.

No. of the week	F excreted in		No. of the week	F excreted in	
	Fæces (mgm.)	Urine (mgm.)		Fæces (mgm.)	Urine (mgm.)
1	0·1064	1·215	11	0·1192	2·925
2	0·1100	1·220	12	0·1200	2·960
3	0·1100	1·320	13	0·1142	3·125
4	0·1152	1·398	14	0·1322	3·200
5	0·1148	2·002	15	0·1252	3·252
6	0·1148	2·108	16	0·1345	3·560
7	0·1152	2·252	17	0·1322	3·452
8	0·1200	2·652	18	0·1372	3·672
9	0·1158	2·890	19	0·1412	3·808
10	0·1212	3·000	20	0·1562	3·980
			TOTAL	2·455	53·96

F excreted in fæces by the control rats varied between 0·090 and 0·1120 mgm. per week, and that excreted in the urine was only in traces.
Quantity of F retained = 129·75 − (2·455 + 53·96) = 73·34 mgm.

TABLE II. *Absorption and retention of F in the rats placed on diet deficient in Ca and P*

Quantity of F as NaF administered in the period of 3 weeks = 19·00 mgm.
Quantity of F ingested from and with the diet = 0·50 mgm.
Total quantity of F ingested = 19·50 mgm.

No. of the week	F excreted in	
	Fæces (mgm.)	Urine (mgm.)
1	0·1020	2·980
2	0·1120	3·520
3	0·1210	4·250
TOTAL	0·3350	10·750

Quantity of F retained = 19·50 − (0·3350 + 10·75) = 8·42 mgm.

From the data presented in the above tables, it can be seen that the absorption of F, when F as NaF is given in solution before the food is offered, is almost complete. As the fæcal excretion of F in both the control and experimental animals is nearly the same, it seems safe to conclude that this fæcal F comes from the F of the diet. As a corollary to this conclusion, it can be said that the absorption of F, given in the above quantity and manner, is complete.

In the case of the experimental rats placed on the normal diet, of the 127·3 mgm. of absorbed F, 53·96 mgm. have been excreted in the urine in a period of 20 weeks, and, in the case of the experimental rats placed on diet deficient in Ca and P, of the 19·17 mgm. of absorbed F, 10·75 mgm. have been excreted in the urine in a period of 3 weeks. The urinary excretion of F in the first case is 42·39% of that absorbed, and in the second, 56·1%. If the urinary excretion of F in the first case is calculated for the first 3 weeks only, it is 19·56% of that absorbed, as against 56·1% in the second case. Thus, it can be said that, though the experimental rats placed on normal diet retain more F than the animals placed on diet deficient in Ca and P, the toxicity of the same concentration of F, as judged by the decline in weight, is more pronounced in the latter than in the former. Similar observation has been made by Mazumdar and Ray in bulls.[15]

Distribution of retained F in the different tissues of the body.—After the daily administration of 2 mgm. of NaF for a period of 20 weeks, 3 rats were sacrificed, and the total F content of each animal was determined, and was found to be, on the average, 68·80 mgm. as against the calculated value of 73·34 mgm. The difference in the observed and calculated values may either be due to the excretion of F through channels other than digestive and urinary, or may be due to the sum-total of errors committed in the 20 estimations of urinary F and 20 estimations of fæcal F carried out over a period of 20 weeks. However, the amount of F retained in each rat has been taken as that found by the analysis of the entire body of the rat, *i.e.*, 68·80 mgm., and not that obtained by deducting urinary and fæcal F from that ingested, *i.e.*, 73·34 mgm. In another set of 3 rats, the F content of different tissues and calcified structures was determined. The results are given in Table III.

It is seen that a very considerable portion of the retained F is deposited in the skeletal structures, and that the soft tissues contain only a very small fraction of F retained in the system. Among the tissues, the skin together with hair contains the highest amount of F; brain, heart, small intestine, kidney, liver and adrenals come next in order. The thyroids have been

TABLE III. *F content of the different tissues and calcified structures of rats, containing, on the average, 68·80 mgm. of F*

Tissues	F content (mgm.)	Bones	Weight of the bone (mgm.)	F content of the bone (mgm.)	F content of the dry fat-free bone (%)
Stomach ..	0·042	Ribs ..	..	..	0·910
Small intestine	0·184	Vertebræ ..	..	..	1·528
Duodenum ..	0·029	Fibula ..	10·00	0·120	1·200
Kidney ..	0·174	Radius ..	34·6	0·460	1·330
Liver ..	0·174	Ulna ..	53·8	0·655	1·220
Heart ..	0·184	Humerus ..	99·8	1·604	1·607
Spleen ..	0·072	Scapula ..	57·4	0·770	1·342
Aorta ..	0·012	Femur ..	142·5	1·794	1·259
Lungs ..	0·069	Tibia ..	129·3	1·590	1·227
Brain ..	0·253	Epiphyses ..	..	..	1·447
Skin with hair	1·001	Incisors ..	..	..	1·250
Thyroid ..	0·014				
Pancreas ..	0·098				
Adrenals ..	0·115				
Blood ..	0·016 (per gm.)				

found to contain a very small quantity of F. In this regard may be mentioned the results of Chang *et al.*[21] who observed the thyroid of the cow suffering from chronic fluorosis to contain 160 mgm. of F per 100 gm. of the dry matter. Among the calcified structures, the greatest deposition of F is in humerus; vertebræ, and epiphyses come next in order. The deposition of F in the remaining bones seems to be more or less of the same order. The greater deposition of F in the vertebræ has also been reported by Wolff and Kerr[22] in humans, and by Mazumdar and Ray in bulls.[15]

Elimination of stored F from the system.—The rats, after daily receiving 2 mgm. of NaF for a period of 20 weeks, and after having retained in the system, on the average, about 68·80 mgm. of F, were placed on the same diet, but the further administration of F was discontinued. Metabolic studies of F were then carried out in the manner given above for a period of 12 weeks. At the end of each week, the quantity of F excreted in the urine and fæces was determined. At the end of this 12 weeks period, 3 rats were sacrificed, and the total F content of each rat was determined. In another set of 3 rats, the F content of different tissues and calcified structures was similarly determined. The results are given in Tables IV and V.

TABLE IV. *Elimination of F from the body of the rat containing, on the average, 68·80 mgm. of F during a period of 12 weeks*

Quantity of F ingested from and with the diet during the period of 12 weeks = 2·97 mgm.
Total quantity of F = 71·77 mgm.

No. of the week	F excreted in Fæces (mgm.)	F excreted in Urine (mgm.)	No. of the week	F excreted in Fæces (mgm.)	F excreted in Urine (mgm.)
1	0·1022	3·250	7	0·1120	1·220
2	0·1022	2·500	8	0·0980	1·200
3	0·1100	1·810	9	0·1022	1·250
4	0·0982	1·520	10	0·1122	1·002
5	0·0982	1·250	11	0·0890	1·202
6	0·0950	1·282	12	0·0964	1·110
			TOTAL	1·2156	18·596

Total quantity of F eliminated in 12 weeks = 19·8 mgm. The quantity of F still retained in the system, as obtained by calculation, is 51·97 mgm., and as obtained by analysis of the whole body of the rat, is 47·52 mgm.

TABLE V. *F content of the different tissues and calcified structures of rats containing, on the average, 47·52 mgm. of F*

Tissues	F content (mgm.)	Bones	Weight of the bone (mgm.)	F content of the bone (mgm.)	F content of the dry fat-free bone (%)
Stomach ..	0·010	Ribs ..	..	..	0·61
Duodenum ..	0·009	Vertebræ ..	..	..	1·40
Small intestine	0·054	Fibula ..	10·52	0·100	0·95
Kidney ..	0·110	Radius ..	37·2	0·350	0·94
Liver ..	0·072	Ulna ..	58·2	0·498	0·85
Spleen ..	0·032	Humerus ..	105·2	1·220	1·16
Heart ..	0·058	Scapula ..	60·25	0·400	0·66
Aorta ..	0·007	Femur ..	150·6	1·350	0·89
Lungs ..	0·009	Tibia ..	137·8	1·120	0·81
Brain ..	0·102	Epiphyses ..	..	..	0·72
Skin with hair	0·428	Incisors ..	..	..	0·49
Thyroid ..	0·005				
Pancreas ..	0·003				
Adrenals ..	0.060				
Blood ..	0·009 (per gm.)				

From the data presented in Tables IV and V, it can be seen that, in a period of 12 weeks, out of 71·77 mgm. of F stored in the system, only 24·25 mgm. of F have been eliminated, and that 66·22% of F originally stored in the system is still present in it, indicating that the elimination of F deposited in the system is very slow. The elimination of F from the system is essentially through the urinary system. Save for a very small quantity, probably derived from the diet, F has not been found to be excreted through the intestinal tract. Similar observation has been made by Brun *et al.*[3] in the case of cryolite workers, and a contradictory one, by Lawrenz *et al.*[1] in the growing rats, and by Pandit and Rao[13] in monkeys. On the discontinuation of the administration of F, the elimination of F, in terms of per cent. of that deposited, is greater from the soft tissues than from blood and calcified structures. Among the calcified structures, the elimination of F is the highest from the incisors. Epiphyses, scapula and ribs come next in the order. The higher rate of elimination of F from the incisors of the rats can be due to the fact that the incisors of the rat are continually growing.

Effect of F on the vitamin C content of the tissues of rats and monkeys.— Though some points of similarity between scurvy and F poisoning have been indicated,[23] the data on the vitamin C content of the tissues in fluorine poisoning are not well established.[24–26] Determinations have therefore been carried out of the vitamin C content of the different tissues of rats and monkeys suffering from chronic fluorosis produced in the manner given above. The results are given in Table VI.

TABLE VI. *Vitamin C content of the different tissues of rats and monkeys suffering from chronic fluorosis*

The results are expressed in mgm. per gm. of the tissue

Name of the tissue	Vitamin C content in the case of rat		Vitamin C content in the case of monkey	
	Control	Experimental	Control	Experimental
1. Duodenum ..	0·10	0·086	0·09	0·03
2. Kidney ..	0·15	0·100	0·17	0·05
3. Liver ..	0·28	0·19	0·30	0·16
4. Spleen ..	0·21	0·17	0·28	0·13
5. Heart ..	0·11	0·07	0·10	0·09
6. Brain ..	0·34	0·21	0·29	0·10
7. Thyroid ..	0·22	0·16	0·23	0·09
8. Adrenals ..	2·42	1·82	1·98	0·52
9. Pancreas ..	0·15	0·11	0·25	0·17

It can be seen that, in chronic fluorosis, there is a lowering of the vitamin C content of the tissues of rats and monkeys, and that the lowering is much more marked in the tissues of the monkeys than in those of the rats. The earlier workers (*loc. cit.*), though obtained contradictory results about the vitamin C content of some tissues, have reported an increase in the vitamin C content of certain actively metabolising organs like the anterior lobe of the hypophysis and the suprarenals. Such a difference in these results, without further studies on this point, can, at the present, only be ascribed to the likelihood that, in the earlier studies, the estimation of vitamin C in the tissues was made when the animals probably had not developed chronic fluorosis, whereas, in this case, the studies have been carried out in animals suffering from chronic fluorosis.

Effect of F on the iodine content of thyroids of rats and monkeys.—It has been observed that the ingestion of F lowers the metabolic rate of the body,[27] and causes a hypertrophy of the thyroid.[28, 29] It has been shown[17] that, in monkeys suffering from chronic fluorosis, the thyroids present the condition which resembles colloid goiter, wherein the epithelium is degenerated, and the acini are filled with abundant colloid which stains deeply. With a view to studying further the effect of F on the thyroids, determinations have been carried out of the iodine content of thyroids of rats and monkeys suffering from chronic fluorosis. The results are given in Table VII.

TABLE VII. *Iodine content of the thyroids of rats and monkeys suffering from chronic fluorosis*

The results are expressed in per cents. on the basis of dry thyroid

No.	Percentage I in the thyroids of rats		Percentage I in the thyroids of monkeys	
	Control	Experimental	Control	Experimental
1	0·22	0·23	0·19	0·26
2	0·24	0·24	0·17	0·28
3	0·19	0·23	0·17	0·23
4	0·22	0·24	0·21	0·29
5	0·24	0·26		
6	0·17	0·24		
7	0·21	0·25		
Average ..	0·21	0·24	0·185	0·265

Thus, from the data given above, it can be seen that, in F toxicosis, there is an increase in the iodine content of thyroids, which is more marked in the thyroids of monkeys than in those of the rats. Stormont *et al.*[30] made a similar observation in rabbits, intravenously receiving 3 mgm. of NaF per kg. of body weight for 2 months, but they did not consider the increase as significant, as the probable error due to individual variation in the I content exceeded the differences noted in the I content of the thyroids.

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