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# Studies in Mineral Metabolism XXXVI.

# Fluorine Metabolism in Rats and Bovines.

By P. J. DU TOIT, D. B. SMUTS, and A. I. MALAN, Section of Biochemistry and Nutrition, Onderstepoort.

## INTRODUCTION.

THE supplementary feeding of minerals has become an essential factor in the nutrition of farm animals. Due to its beneficial effects in general on growth and reproduction and its favourable amounts and proportions of the two chief deficient mineral elements calcium and phosphorus, bone meal is the most popular and generally used mineral compound. In recent years, minerals are fed on a more extensive and elaborate scale than formerly, and consequently the economic aspect of such a supplementation comes up for consideration. There is a growing demand for other sources of calcium and phosphorus, which would be less expensive yet efficient in supplying the deficient minerals. As a result attention was directed to the naturally occurring calcium phosphate better known as rock phosphate. This compound contains approximately the same amounts of calcium and phosphate as bone meal. From this it would appear, that we might expect to find in rock phosphate a suitable substitute for bone meal. However, the results obtained by the addition of rock phosphate as a mineral supplement do not at present justify its adoption. The results in this direction are very variable. Some investigators have found abnormal growth and even detrimental effects through the administration of rock phosphate, others have noticed no such ill-effects. The injurious effects of this mineral have invariably been attributed to its fluorine content. It is apparent from the divergency in the results obtained, that the level of feeding or the concentration of fluorine present, is the factor determining the presence or absence of irregularities. At this Institute definite toxic symptoms have been obtained by feeding rock phosphate to bovines. These results partially exclude the possibility of utilizing rock phosphate as a mineral supplement. However, there is no evidence available as to what limit fluorine containing minerals may be fed without affecting the general welfare of the animal. In order to get some information on this point, four groups of bovines consisting of three animals each were given increased concentrations of fluorine. This experiment is still in progress and will be reported on in detail later.

During the course of this experiment, it became evident that an opportunity was offered to study the metabolism of fluorine in bovines. Consequently this study was undertaken which deals chiefly with the retention, excretion and paths of elimination of this element.

## LITERATURE.

That fluorine is a normal constituent of body tissues is now a recognised fact. The work of Sonntag (1916), Gautier and Clausmann (1913), Zdarek (1910) and Sharpless and McCollum (1933) has settled this issue beyond doubt. The latter workers conclude that rats normally contain considerable fluorine in their bones and teeth and that this element within certain limits increases with age. According to Gautier and Clausmann (1913) animal tissues can be arranged in the following order in respect to their fluorine content:—dental enamel 118 to 180 mgm., bone 56 to 87 mgm., epidermis 16·4 mgm., hair 6·1 to 19·7 mgm., Thymus 3·9 to 11 mgm. and blood 2·3 mgm. All these figures are based on 100 grams dry material. It appears that the great majority of studies on the fluorine content of tissues were conducted on bones and teeth. Wrampelmeyer (1893) found the percentage of fluorine in bone to vary from ·05 per cent. to ·32 per cent. Gautier and Clausmann (1913) report 150 mgm. fluorine per 100 grams dry dental enamel and 46 mgm. per 100 grams of bone ash. While bones and teeth contain the highest percentage of fluorine of all tissues, it is interesting that the percentage of this element may vary quite considerably in the different types of bone. Lars Slagsvold (1934) in his extensive observations on fluorine poisoning finds that the humerus of cattle contain ·14 per cent. fluorine, whereas the rib of the same animals only contains ·09 per cent. fluorine. The human skull contains ·18 per cent. fluodine.

Whether fluorine is an indispensable component of a ration is still a problem of the future. If, however, it participates in certain vital physiological functions, then only minute quantities must be normally adequate to fulfil this rôle. Sharpless and McCollum (1933) on a ration exceedingly low in fluorine found, that rats grew normally, gave normal reproduction and exhibited no structural changes in their teeth. It must be mentioned that some slight proliferation of the capillaries in the tooth pulp and surrounding bone was noticed. A similar condition was also noticed in some of the stock rats. It is therefore doubtful whether this abnormality was due to a fluorine deficiency.

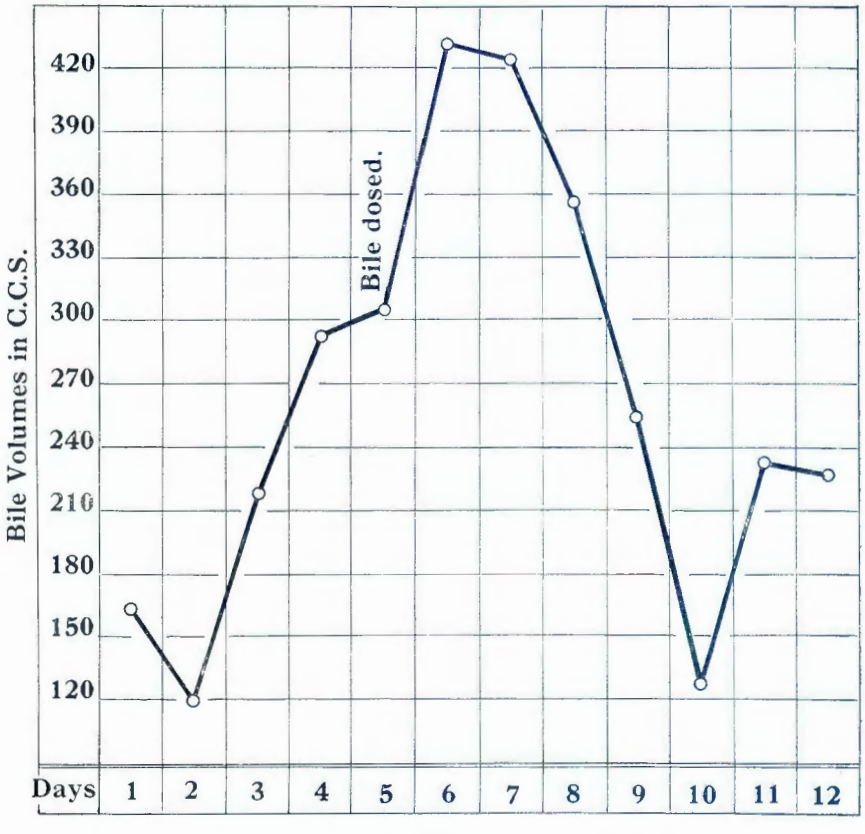
While animals may apparently thrive quite well on a low fluorine ration, they invariably show toxic symptoms when the concentration of fluorine reaches a certain limit. This limit varies with the different fluorine compounds as well as with the different susceptibilities of species. It is an interesting phenomenon, that a toxic substance like fluorine is retained by the tissues of the animal. Slagsvold (1934) found that the ash content of fluorine poisoned sheep contained 0·60 per cent. fluorine, while that of the normal control sheep contained only 0·10 per cent. Christiani and Gautier (1925) are of the opinion, that the tendency of fluorine is to accumulate in the bony tissue and produce a condition resembling

bilirubinaemia and hence the extent to which bile was being eliminated from the liver. The following materials were administered to different sheep suffering from the effects of Lippia poisoning, i.e. as soon as a definite bilirubinaemia had been established and the first sign of clinical jaundice had made its appearance. All animals received 500 grams Lippia as such or its equivalent amount of alcoholic extract dosed through a stomach tube:—

1. Bile dosed repeatedly in large quantities (1,000 c.c.).
2. Sodium taurocholate ·5 gram intravenously.
3. Calomel dosed in amounts of ·5 to 1 gram.
4. Magnesium sulphate per os in amounts of 50 grams to 70 grams.
5. Aloes 1-2 grams per os.
5. Castor oil 60 c.c.
7. Castor oil 60 c.c. + 5 drops croton oil.
8. Olive oil 120 c.c.
9. Hexamine and cholic acid 1 gram each per os.
10. Mercurochrome ·05 gram intravenously.

GRAPH No. 5.

Effect of dosing bile on flow of bile in sheep with fistula.



All the above-mentioned materials are usually considered to exert a purgative action and possibly also some degree of stimulation of the bile flow. In every case however the effect produced on the icterus of sheep suffering from Lippia poisoning was very disappointing. Although purging was eventually caused by the large doses of calomel, and magnesium sulphate, this only followed after repeated dosing. The jaundice however remained unaffected, the bile flow being scanty and the serum charged with large amounts of bile pigments.

In addition to the above, various other forms of treatment were tested out in an endeavour to stimulate bile flow after the onset of icterus, e.g. by dosing sodium salicylate, quinine sulphate, copper sulphate and subcutaneous injections of adrenalin without any of these however producing any beneficial effect.

In another series of experiments, preventive treatment was undertaken against the jaundice. Thus, before and also after dosing Lippia, sheep were kept on an exclusive laxative feed of green barley and green lucerne for two weeks. This however had no influence on the severity of the jaundice. Similarly the regular daily dosing of 250 grams of glucose so as to increase the glycogen content of the liver had no effect on the jaundice. In order to prevent the Lippia from acting on the liver, attempts were then made to inactivate the toxic principle while passing through the digestive tract. Thus two sheep were dosed daily with 5 grams sulphur for 14 days before dosing the Lippia. In both these cases however the jaundice that followed was particularly severe. Three sheep which were dosed with 500 grams Lippia and immediately afterwards with 4 grams potassium permanganate in water remained healthy without any signs of photosensitisation or jaundice developing afterwards. When however in three other sheep dosed with Lippia, the potassium permanganate was given 24 hours later, it had no effect in preventing the onset of jaundice which was of the usual severity. This indicates that potassium permanganate most probably through its oxidative action, can inactivate the Lippia toxic principle before its absorption from the intestinal canal.

### Experiments with Other Liver Poisons.

Of the various substances known to exert a poisonous effect on the liver, chloroform, carbon tetrachloride, phosphorus, and manganese chloride are amongst the most well-known ones. Experiments on Merino sheep were undertaken with each of these materials especially with the view of ascertaining to what extent they might influence the bile flow and thus lead to symptoms of jaundice.

*Chloroform.*—A full-grown sheep dosed by stomach tube with 8 c.c. chloroform in olive oil died within 24 hours. The lesions found were those of acute pulmonary oedema and congestion accompanied by severe fatty changes of the liver and kidneys, without however any signs of clinical jaundice. This indicated that chloroform was highly toxic for sheep thus necessitating the administration of smaller amounts in subsequent experiments. Thus four sheep dosed with amounts from ·5 c.c. to 2 c.c. daily for 5 days showed no untoward effect. Regular examination of the serum however revealed

administration of fluorine below a certain concentration, probably the lethal concentration. These authors also draw attention to the fact that fluorine is accumulated in the hair, nails and epidermis and may be excreted in this way.

## EXPERIMENTAL.

As mentioned before the bovines used in this study actually served in a major experiment in which the toxicity of fluorine at different levels was tested out. Two groups of animals were used. One group received 60·6 mgm. fluorine present in precipitated $CaHPO_4$ and the other 738·4 mgm. fluorine in the form of CaHPO4 and $CaF_2$. These animals received the above quantities of fluorine for several months before the metabolism studies were conducted. Before the actual collection statrted, the animals were put in metabolism stalls for 7 days to accustom them to their new surroundings. The minerals were well mixed with the basal ration and just so much of the ration was given as they would clear up per feed. Consequently there was no food left over and the wastage was reduced to nil. The metabolism stalls were carefully cleaned and washed before the animals were admitted. During the collection period, which lasted 10 days, the faeces and urine were collected daily and a 10 per cent. aliquot taken and stored. With every collection the metabolism stalls were washed with a known amount of distilled water and an aliquot of the washings taken. At the end of the period the aliquots were thoroughly mixed and analysed in duplicate.

Along with the metabolism studies on bovines a subsidiary test on similar lines was conducted with rats. Twelve rats were selected from the same litters and paired in triplicates according to age, sex and weight. One lot was killed at the start, ashed, and total ash, calcium and phosphorus and fluorine of each rat determined. The pairs of the remaining two groups were treated similarly and received the same amount of food except that members of the one group received fluorine while the others received the basal ration without the addition of fluorine. The rats were first put on the basal ration for 7 days and then a collection period of 7 days. This was followed by another period of 7 days on the basal ration plus fluorine and a collection period of the same duration on the same feed. At the end of the experiment members of both groups were killed, ashed and analysed for total ash, calcium, phosphorus and fluorine. The metabolism studies were executed on the same lines as that practised by Smuts (1934).

Fuorine was determined by the method of Willard and Winter (1933). Although the method worked well in general, it was found necessary to standardize certain details in the procedure before satisfactory results could be obtained. While no difficulty was encountered in the ashing of faeces a considerable amount of trouble arose when urine and blood were ashed. Usually splattering of the samples took place as soon as they were put in the muffle furnace. Consequently the standardized method fully described by Scott and Henne (1935) was adopted. 40 to 60 c.c. of a saturated solution of lime was added to the aliquots of urine and blood. The amount of

lime added depended on the volumes of aliquots used. They were then carefully evaporated on a steam bath, placed in a hot oven and left there for several days. This procedure was found absolutely essential to prevent splattering and loss of samples. Ashing was completed with the greatest ease afterwards. The addition of lime prevented fusion on ashing as well as a loss of fluorine by volatization. Reynolds and Jacob (1935) as well as Reynolds (1935) draw attention to the fact that the so-called volatization method for the determination of fluorine which involves the distillation of the sample with perchloric acid or concentrated sulphuric acid gave low results in the presence of gelatinous silica and acid decomposable silicates. Willard and Winter (1933) advocate a distillation volume of 100-150 c.c. to overcome the possibility of an adsorption of fluorine in the presence of silica. Reynolds however found that a normal blank as given by clean flasks could only be obtained after the 75 mL distillations, when a flask lightly coated with silica was used. He finds that by boiling out the distillation flask with strong sodium hydroxide no interference with silica was noticed. In applying this method every distillation flask was only used once. After that the entire series of flasks were boiled out with strong sodium hydroxide solution. The distillation volume of every sample was 100 to 120 c.c.

Another possible source of error wrich may arise in the application of the above method is the presence of phosphoric acid in the distillate especially where large samples or samples rich in phosphorus are used. The phosphorus is precipitated as thorium phosphate with the result that the fluorine figure is correspondingly too high. This point was tested out on the magnitude of the different samples used by neutralizing the distillate, evaporating it to a small volume (5-10 c.c.) and re-distilling it with perchloric acid. No significant differences were obtained and it was therefore assumed, that an error due to the presence of phosphorus could be eliminated as all the samples were of the same nature and magnitude as those analysed in this study.

## Experimental Results.

In Tables I and II are given the composition of the rations as used with the bovines and rats. The fluorine content of the basal ration of rats is considered negligible.

Table III gives the retention of fluorine, calcium and phosphorus at different concentrations of fluorine. In the first two instances, where 60·6 mgm. of fluorine was consumed daily, only 12·8 and 18·0 per cent. were retained respectively. Almost five-sixths of the entire intake were excreted in the faeces. However in the two latter cases where the daily intake of fluorine was increased to 738·4 mgm. an altogether different picture presents itself. The faecal fluorine decreased and the urinary fluorine increased in proportion to the difference in intakes of the two groups. The percentage retention increased rapidly with the increased fluorine feeding. Bovine No. 6298 retained 34·0 per cent. and bovine No. 6304,

TABLE I.

*The Fluorine, Calcium and Phosphorus Conten of Rations Fed to Bovines.*

| Ingredients in Ration. | | Daily food Intake. Grams. | Flourine in Ration. Mgms. | Calcium in Ration. Grms. | Phosphorus in Ration. Grms. |
|---|---|---|---|---|---|
| Yellow maize meal | | 1,500 | 3·0 | ·21 | 4·8 |
| Blood meal | | 200 | 1·4 | ·24 | ·26 |
| Lucerne (wet) | | 2,270 | 9·0 | 7·85 | ·69 |
| Teff hay | | 1,589 | 14·3 | 4·08 | 1·74 |
| Minerls (Group II) | | 56·75 | 32·9 | 16·7 | 13·0 |
| Minerals plus (Group III) | | 57·25 | 710·7 | 18·42 | 9·9 |
| TOTAL DAILY INTAKE | Group II. | 5,615·75 | 60·6 | 29·1 | 20·5 |
| | Group III | 5,615·75 | 738·4 | 30·8 | 17·4 |

TABLE II.

*The Composition of Ration for Rats.*

| Ingredients of Ration. | Per cent. |
|---|---|
| Yellow maize meal | 67 |
| Linseed meal | 15·5 |
| Crude casein | 2·5 |
| Lucerne meal | 5·0 |
| Bone meal | 1·0 |
| $CaCO^{''}$ | ·5 |
| NaCl | ·5 |
| Butterfat | 8·0 |

*Calcium, Phosphorus and Fluorine Content of Ration for rats.*

| Ration. | Per cent. Calcium. Grms. | Per cent. Phosphorus. Grms. | Per cent. Flourine. Grms. |
|---|---|---|---|
| Basal ration | ·458 | ·192 | ·0015 (negligible) |
| Basal ration plus ·1 % NaF | ·458 | ·192 | ·0409 |

31·6 per cent. of fluorine. It would appear therefore that as the concentration of fluorine is increased the retention automatically increases. Up to what limit this increase will continue is still problematical.

Although the number of animals utilized is small, there exist, however, definite indications that fluorine retention disturbs the normal metabolism of both calcium and phosphorus. The average retention of calcium in the low fluorine groups is 40·0 per cent., while in the high fluorine group it rises to 54·2 per cent. Whether this increase is only a chance variation is difficult to say. The consistency and the magnitude of the differences is nevertheless indicative of a higher retention of calcium with the higher level of fluorine feeding.

The phosphorus retention is much higher in the fluorine low group and averages 45·8 per cent. in comparison with 31·3 per cent. of the high fluorine group. This difference seems fairly definite, and in conjunction with the rat experiment on similar lines can only be considered as due to the influence of fluorine.

The fluorine content of the blood varies according to the fluorine concentration of the ration. The average percentage of fluorine in Group I is ·17 mgm. per 100 c.c. blood, while that for Group III is distinctly higher and reaches an average level of ·60 mgm. per 100 c.c. blood. There are no indications that either the blood calcium or phosphorus concentration is impaired by fluorine feeding. The phosphatase figures are variable and no conclusions can be drawn from a limited set of data as variable as these.

In the subsidiary rat tests conducted in conjunction with that of bovines, confirmatory evidence of a definite retention of fluorine is advanced as is shown in Table V. The major part of fluorine is excreted through the faeces, while the urinary excretion of this element is approximately half that of the faecal excretion. The average retention is 38·3 per cent. This figure very closely approximates that of the high fluorine group of bovines, which showed an average retention of 32·8 per cent.

That fluorine has a definite depressing effect on the calcium and phosphorus retention is evident from the retention figures in Table V. The average retention of calcium in the fluorine low period is 34·6 per cent., and in fluorine period 22·4 per cent. This difference is not a chance occurrence and is decidedly the outcome of a fluorine effect. If the variations between the groups are compared with the remainder or error variance by Fisher's t test it is found, that $t = 2 \cdot 846 - 0 \cdot 559 = 2 \cdot 287$ and these degrees of freedom are given by $n_1 = 1$ and $n_2 = 3$. For these degrees of freedom the least value for significance at 1 per cent. probability is given by Fisher as 1·765. This value is greatly in excess of the required minimum and therefore highly significant. According to Students, method Z becomes 5·3, which gives a probability of approximately 1 in 750 that the outcome is not due to chance (for $n_1 = 1$ Fisher's t test is identical with Student's Z test).

With reference to the phosphorus retention the evidence is even more striking and leaves no doubt that the retention of this element is seriously influenced by the presence of fluorine. The average

TABLE III.

*Metabolism of Fluorine, Calcium and Phosphorus of Bovines Receiving Different Concentrations of Fluorine.*

| Animal No. | Live Weight. lb. | Daily Flourine Intake. Mgm. | Daily F in Faeces. Mgm. | Daily F in Urine. Mgm. | Daily F retention. Mgm. | Daily Calcium Intake. Grms. | Daily Ca in Faeces. Grms. | Daily Ca in Urine. Grms. | Ca Balance. Grms. | Daily Phosphorine Intake. Grms. | Daily P in Faeces. Grms. | Daily P in Urine. Grms. | P Balance. Grms. | Ca Retention. Per cent. | P Retention. Per cent. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6328.. | 635 | 60·6 | 49·3 | 3·50 | 7·80 | 29·1 | 16·34 | ·17 | 12·6 | 20·5 | 11·18 | ·14 | 9·2 | 43·3 | 44·9 |
| 6350.. | 555 | 60·6 | 45·0 | 4·64 | 10·96 | 29·1 | 18·21 | ·20 | 10·7 | 20·5 | 9·18 | 1·76 | 9·6 | 26·8 | 46·8 |
| 6298.. | 700 | 738·4 | 230·7 | 196·22 | 251·48 | 30·8 | 14·57 | ·15 | 16·1 | 17·4 | 9·48 | 2·86 | 5·1 | 52·3 | 29·3 |
| 6304.. | 600 | 738·4 | 314·4 | 190·85 | 233·05 | 30·8 | 14·36 | ·04 | 16·4 | 17·4 | 7·81 | 3·80 | 5·8 | 53·2 | 33·3 |



TABLE IV.

*The Fluorine, Calcium, Phosphorus and Phosphatase of the Blood of Bovines.*

| Animal No. | Group No. | F per 100 cc. Blood. Mgm. | Ca per 100 cc. Blood. | P. per 100 cc. Blood. | Phosphatase in 100 cc. Serum. |
|---|---|---|---|---|---|
| 6328..... | I | ·16 | 12·5 | 6·4 | 3·8 |
| 6350..... | I | ·18 | 11·6 | 5·8 | 7·1 |
| 6298..... | III | ·59 | 12·7 | 6·5 | 3·8 |
| 6304..... | III | ·62 | 10·8 | 6·4 | 5·0 |

retention on the fluorine low ration is 49·1 per cent. and on the fluorine ration 15· per cent. A statistical treatment of these results gives according to Fisher a value for $t = 3{\cdot}870 - 0{\cdot}206 = 3{\cdot}6\ 4$, with degrees of freedom $n_1 = 1$ and $n_2 = 3$. This value for t greatly exceeds the required minimum value of t for significance at the 1 per cent. probability level, which according to Fisher for the above degrees of freedom is 1·765.

In Table VI the total ash, fluorine, calcium and phosphorus of the total rats are given. In young rats on a normal ration varying in weight from 38 to 44 grms. the percentage fluorine in the ash varies from ·045 to ·050 per cent. Litter mates of the same age at the start of the experiment show no significant variation in the percentage fluorine of the ash after reaching weights varying from 109 to 139 grams. Normally therefore the fluorine concentration is not increased in the tissues. If, however, fluorine is administered there is a definite inclination for the system to retain and deposit this element in the tissue. This is strikingly demonstrated in the last column of Table VI. Litter mates receiving ·1 per cent NaF in their ration deposited approximately 20 times as much fluorine as the controls. The average percentage fluorine in the ash of the fluorine fed rats is ·845 in comparison with ·044 of the controls. No significant differences appear in the percentage calcium and phosphorus of the ash of the rats under these conditions. The ash per 100 grams empty weight increases as the rats grow older. For rats killed at the start of the experiment and weighing on the average 42 grams, the ash per 100 grams empty weight reaches an average figure of 3·063 grms. This figure is significantly less than the average of 3·708 grms. per 100 grms. empty weight attained by litter mates of an average weight of 126 grms. This difference is normally expected since these rats were growing and continually depositing salts. However any difference which may arise in the ash content between litter mates receiving the same treatment except for the fluorine addition must be ascribed to the action of fluorine.

The mean average of ash per 100 grams for rats on the basal ration is 3·708 and that for rats on fluorine ration 3·888. Analysing the results by Fisher's t (or Student Z) method, where $t = \frac{\text{diff. of means}}{\text{S}\Sigma \text{ of diff.}}$ it is found that the standard deviation (S$\Sigma$) of the differences is ·0608 and $t$ equals 2·961. According to Fisher's probability table, the probabolity P that this difference is due to chance lies between 0·05 and 0·02, which is equivalent to 2 to 5 events out of every 100 trials. This difference is clearly significant and it can safely be concluded that such a difference is due to the operation of fluorine.

The calcium per 100 grams empty weight definitely increases with increase in weight and advancement in age. This finding agrees with the results of Sherman and McCleod (1925) who found that the percentage calcium showed marked successive increases up to the age of 1 year. If fluorine should affect the calcium content of the body it should show up in an analysis of the results between rats of column 2 and those of column 3. A statistical treatment

TABLE V.

*Calcium and Phosphorus Metabolism of Rats on a Fluorine Low and High Ration.*

| Rat No. | Daily F Intake. Mgm. | Daily F in Faeces. Mgm. | Daily F in Urine. Mgm. | Daily F retention. Mgm. | Daily Ca Intake. Mgm. | Ca in Faeces. Mgm. | Ca in Urine. Mgm. | Ca Balance. Mgm. | Daily P Intake. Mgm. | P in Faeces. Mgm. | P in Urine. Mgm. | P Balance. Mgm. | Ca Retained. Percent. | P Retained. Percent. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1..... | — | — | — | — | 43·97 | 25·33 | 3·30 | 15·34 | 18·43 | 8·57 | ·71 | 9·15 | 34·9 | 49·6 |
| 3..... | — | — | — | — | 43·97 | 25·99 | 3·27 | 14·71 | 18·43 | 8·61 | 1·10 | 8·72 | 33·5 | 47·3 |
| 5..... | — | — | — | — | 43·97 | 25·65 | 2·94 | 15·38 | 18·43 | 8·52 | ·71 | 9·20 | 35·0 | 49·9 |
| 7..... | — | — | — | — | 43·97 | 25·40 | 3·20 | 15·37 | 18·43 | 8·73 | ·55 | 9·15 | 35·0 | 49·6 |
| 1..... | 3·15 | 1·16 | ·76 | 1·23 | 37·12 | 25·61 | 4·01 | 7·50 | 14·80 | 11·97 | ·68 | 2·15 | 20·2 | 14·5 |
| 3..... | 3·27 | 1·19 | ·57 | 1·51 | 36·64 | 23·42 | 4·22 | 9·00 | 15·30 | 12·19 | ·80 | 2·31 | 24·6 | 15·1 |
| 5..... | 3·35 | 1·40 | ·76 | 1·19 | 37·55 | 24·20 | 4·63 | 8·72 | 15·74 | 12·23 | ·76 | 2·72 | 23·2 | 17·3 |
| 7..... | 3·43 | 1·50 | ·80 | 1·13 | 38·53 | 25·53 | 4·62 | 8·38 | 16·15 | 13·11 | ·80 | 2·24 | 21·7 | 13·9 |

TABLE VI.

*Total Ash, Fluorine, Calcium and Phosphorus of Litter Mates Killed at the Start of the Experiment.*

| Animal No. | Live Weight. Grms. | Empty Weight. Grms. | Total Ash. Grms. | Fourine in Ash. Per cent. | Ca. in Ash. Per cent. | P. in Ash. Per cent. | Ash per 100 grms. Empty Weight. Grms. | Ca. per 100 grms. Empty Weight. Grms. | P. per 100 grms. Empty Weight. Grms. |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 44·0 | 37·5 | 1·142 | ·050 | 32·5 | 15·5 | 3·04 | ·99 | ·47 |
| 21 | 44·0 | 37·0 | 1·142 | ·050 | 29·4 | 15·0 | 3·09 | ·91 | ·46 |
| 22 | 38·0 | 36·5 | 1·132 | ·045 | 27·2 | 15·3 | 3·10 | ·81 | ·47 |
| 23 | 42·0 | 33·5 | 1·010 | ·048 | 29·9 | 18·2 | 3·02 | ·90 | ·55 |
| *Total Ash, Fluorine, Calcium ond Phosphorus of non-Fluorine Litter Mates Killed at the Termination of the Experiment.* | | | | | | | | | |
| A | 109 | 97 | 3·68 | ·046 | 31·6 | 15·4 | 3·79 | 1·20 | ·48 |
| B | 110 | 96 | 3·69 | ·048 | 31·6 | 14·3 | 3·84 | 1·20 | ·55 |
| C | 139 | 124 | 4·42 | ·044 | 30·8 | 14·6 | 3·56 | 1·10 | ·52 |
| D | 129 | 114 | 4·15 | ·039 | 31·2 | 14·3 | 3·64 | 1·13 | ·52 |
| *Total Ash, Fluorine, Calcium and Phosphorus of Fluorine Fed Litter Mates Killed at Termination of Experiment.* | | | | | | | | | |
| 1 | 101 | 85 | 3·42 | ·802 | 28·8 | 15·4 | 4·02 | 1·16 | ·62 |
| 3 | 106 | 88 | 3·51 | ·891 | 31·6 | 14·4 | 3·99 | 1·26 | ·57 |
| 5 | 102 | 84 | 3·21 | ·886 | 3·04 | 14·6 | 3·82 | 1·16 | ·56 |
| 7 | 126 | 109 | 4·05 | ·791 | 30·2 | 15·4 | 3·72 | 1·12 | ·56 |

of the results gives $t=0{\cdot}497$, which according to Fisher is equivalent to a probability P beween ·7 and ·6. This means that the chances are 6 out of 10 that the difference is merely a chance effect. Consequently any effect which fluorine might have on the Ca content of the ash is quite insignificant. There is slight increase in the phosphorus content with increase in weight and age. This corresponds with the data of Shereman and Quinn (1926) who find an increase of 49 per cent. from the weight of 21 grms. to 71 per cent. at a weight of 300 grms. Fluorine may be considered to have influenced the phosphorus content of the ash. The mean difference in this case is $0{\cdot}06$ and $t$ is equal to $2{\cdot}178$ giving a probability P, according to Fisher, which lies between $0{\cdot}1$ and $0{\cdot}05$. This is more of a border line case, and although the 5 per cent. probability level is not definitely reached, it closely approximates it.

## DISCUSSION.

From the results obtained in this study together with the data of Sharpless and McCollum, it appears that although fluorine is a normal constituent of the body, animals do not require fluorine in measurable amounts to complete any of the normal functions, such as growth, reproduction and lactation. In fact, rats varying in weight from 44 to 139 grams contain the same percentage of fluorine. This seems to indicate that unless fluorine is purposely or accidentally administered to animals, the tissues will maintain a fairly constant amount of this element, even under conditions where the fluorine content of the ration is negligible. Whether the fluorine normally present is indispensable to life is still a problem to be solved. Under conditions of fluorine feeding the system can definitely retain appreciable amounts. In bovines the retention is as high as ·2 gms. per day. It seems possible that at a certain limit the tissues must almost become saturated wth this element, and unable to cope with a continual influx. The chances are that at this limit, the natural functioning of the tissues is so seriously upset that death occurs due to fluorine toxicity. A single dose of too high a concentration may on the same principle destroy the activity of the tissues and cause death.

Gautier and Clausmann (1913) have shown fluorine to be present in a number of tissues. Whether the retained fluorine accumulates proportionally in all the different tissues is not known, but it appears from available data that it concentrates in the bone and teeth. These two tissues contain normally chiefly calcium and phosphorus and if any fluorine is deposited there it must be at the expense of either or both of them.

From this study on rats it appears that fluorine interferes with the absorption of both these salts as is witnessed by the proportionately higher faecal excretion of these elemens. On the other hand it may be equally possible that absorption proceeds along the usual lines and that fluorine prevents the normal deposition of calcium and phosphorus from the circulating fluids in the tissues, with the result that these salts are excreted by way of the bowels in significantly larger quantities than under normal conditions. Agren (1935) believes that calcium is excreted in the pancreatic juice

where it exists in higher concentration than in the serum. After injection of calcium chloride the serum of cats in which the pancreas was stimulated by secretin contains less calcium than that of the controls. He suggest that this is the mechanism underlying the excretion of calcium by way of the bowels. It is known, that fluorine has a definite effect on certain enzymatic reactions and it is quite possible that fluorine may enhance the supply of secretin, stimulate or retard their enzymatic reactions and so increase the excretion of calcium and possibly phosphorus by way of the bowels. Whatever the *modus operandi* of fluorine in respect to calcium and phosphorus metabolism may be, the fact remains, that it has a definite depressing effect on the amounts of these elements retained. It appears, however, that the antagonistic action of fluorine is more severe in respect to phosphorus metabolism, since the retention of this element is diminished by approximately 33 per cent., whereas the calcium retention under the same conditions is only diminished by approximately 12 per cent.

With bovines the effect of fluorine on calcium and phosphorus metabolism is somewhat different from that of rats in that the calcium retention is increased, while the phosphorus retention as in the case of rats remains decreased. However the ratio of the percentage calcium to phosphorus retained in the normal and fluorine cases is very nearly alike in both species. In normal bovines the average percentage ratio of calcium to phosphorus retained is 1:1·4 and in normal rats 1:1·4. With fluorine administration these ratios become 1:0·58 for bovines and 1:0·60 for rats. This seems to indicate that although there appears to be an apparent difference in the retention of the salts by the two species, the results nevertheless agree in the fact that phosphorus is eliminated in preference to and in larger amounts than calcium in the presence of fluorine. Consequently it is plausible to assume that in the presence of fluorine certain disturbing conditions in the normal scheme of calcium and phosphorus metabolism are created, which cause an extensive elimination of phosphorus. The probability further exists that by the continual presence of fluorine a condition of phosphorus shortage may ultimately result. Such a condition would then in general be analogous to osteomalacia. The evidence obtained through the retention studies together with the histiological examination of bone sections from bovines and rats at this Institute strongly suggest such a possibility. Slagsvold (1934) on the basis of extensive histiological studies is very emphatic that fluorine poisoning causes osteomalacia. If this is actually the case, it would seem that the fluorine problem is of special practical importance, in that mineral compounds containing a fair amount of fluorine when substituted for bone meal and fed to livestck may actually help to produce osteomalacia instead of preventing it.

## CONCLUSIONS.

1. Appreciable amounts of fluorine is retained by bovines and rats on rations to which fluorine has been added. Bovines retain approximately ·2 grm. and rats 1 mgm. of F per day under the conditions of the experiment.

2. The percentage F occurring normally in ash of rats varies from 0·039 to 0·05.

3. The ash per 100 grams empty weight in rats is increased by fluorine feeding.

4. Fuorine causes a decreased retention of both calcium and phosphorus in rats, a slight increase in calcium retention and a pronounced decrease in the phosphorus retention of bovines.

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