gives first, as shown in the alloxantine series on alkaline salts, a monobasic salt which is colorless; further boiling produces a dibasic neutralization, and the molecule splits into equal parts with trivalent carbon—a free valency—as cause of the absorption in the visible spectrum. Exposure to air in water solution decomposes the split molecules further, giving *o*-carboxyl-mandelic acid, ammonia, carbon dioxide, water and an aldehyde, resp., dioxindone through reduction.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, VANDERBILT MEDICAL COLLEGE.]

THE EFFECT OF THE INGESTION OF ALUMINUM UPON THE GROWTH OF THE YOUNG.

BY J. T. LEARY AND S. H. SHEIB.

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During the investigations carried on by the Board of Scientific Experts of the U. S. Department of Agriculture,[1] to determine the effects of alum baking powder upon man, it was noted by Dr. Taylor that the administration of aluminum caused a distinct decrease of phosphates in the urine and a corresponding increase of phosphates in the stools. The extent of this change was said to be too slight to have any material meaning or effect.

The members of the Referee Board were unable to detect the presence of resorbed aluminum in the blood of four men who had been placed upon a diet of small and known phosphorus content to which aluminum was added. This finding is at variance with the results obtained by Kahn[2] and Steele[3] using dogs. In each of these cases blood was removed from the living animal and aluminum determined quantitatively.

The object of our work was twofold: First, to ascertain the possibility of inhibiting the growth of young animals by diverting the phosphate content of the food to the intestine by means of an aluminum compound, hence limiting the amount of this essential constituent capable of being utilized by the animal; and secondly, to determine whether aluminum was resorbed under these conditions.

The aluminum compound used was aluminum hydroxide, made by the action of sodium bicarbonate upon sodium aluminum sulfate, the resulting product being washed as free as possible from adhering soluble salts.

The experimental animals used were puppies and young white rats. A series of experiments was also run upon full grown white rats. Each animal was fed a diet of low and known phosphorus content. The excreta were analyzed in case of the puppies in order to determine the extent

[1] U. S. Dept. Agr., *Bull.* 103.

[2] Kahn, *Biochem. Bull.*, 1911, I, 235.

[3] Steele, *Am. J. Physiol.*, 28, 94 (1911).

of the deviation of the phosphorus to the intestine. The resulting effect upon the growth of the animals was likewise noted.

At the end of the experiments the animals were killed and an examination of the tissues made with special reference to the presence of aluminum.

The puppies were housed in specially constructed metabolism cages, the urine collected and analyzed daily for ammonia, titratable acidity and total phosphorus. The feces were collected daily and dried. At the end of each period the entire fecal out put was thoroughly mixed and samples analyzed for total phosphorus.

Puppy No. 1 was kept upon a constant meat diet of known phosphorus content throughout the experiment. A preliminary aluminum-free diet was first fed and this was followed for equal periods of time by the same diet to which was added aluminum hydroxide in amounts sufficient to contain daily 100, 200 and 300 mg. of aluminum. After the third and last aluminum periods there was an aluminum-free period similar to the first.

Puppy No. 2 was fed a milk diet and the experiment carried on as above excepting that the last aluminum-free period was omitted.

Experimental.

PUPPY NO. 1.

Preliminary Period.

Black and Tan Female. Weight, 762 g. Diet, 100 g. of meat containing 4.5 mg. P_2O_5 per g.—450 mg.

Urine.

Date.	NH_3 (mg.).	Acidity (cc. 0.1 *N*).	P_2O_5 (mg.).
2/11	0.156	45.3	180
2/12	0.153	43.5	259.5
2/13	0.1897	48.0	262.5
2/14	0.16932	47.1	291.4
2/15	0.22032	53.4	260.25
2/16	0.24684	53.1	298.5
2/17	0.19074	51.6	316.5
2/18	0.1683	57.0	315.0
2/19	0.16626	60.6	322.5
2/20	0.17748	62.4	330.0
2/21	0.18768	60.9	315.0

Amount of P_2O_5 excreted in urine	3,153 mg.
Amount of P_2O_5 excreted in feces	543 mg.
Total excreted	4,257 mg.
Total ingested	4,950 mg.
Retention	1,255 mg.
Weight at end of preliminary period	1,030 g.
Gain in weight	268 g.
Average daily excretion of NH_3	0.183 mg.
Average daily urinary acidity	52.9 cc. 0.1 *N* HCl
Average daily retention of P_2O_5	114 mg.

The results obtained in the following experimental periods will be expressed as averages:

Period No. 2.

Weight, 1030 g. Diet contained 100 mg. Al daily.

Average daily excretion of NH_3	0.1695 mg.
Average daily urinary acidity	39.65 cc. 0.1 *N* HCl
Average daily retention of P_2O_5	69.3 mg.
Weight at end of period	1,142 g.
Gain in weight	112 g.

Period No. 3.

Weight, 1142 g. Diet contained 200 mg. Al daily.

Average daily excretion of NH_3	0.1365 mg.
Average daily urinary acidity	20.1 cc. 0.1 *N* HCl
Average daily retention of P_2O_5	6.7 mg.
Weight at end of period	1,177 g.
Gain in weight	35 g.

Period No. 4.

Weight, 1,177 g. Diet contained 300 mg. Al daily.

Average daily excretion of NH_3	0.165 mg.
Average daily urinary acidity	13.7 cc. 0.1 *N* HCl
Average daily loss of P_2O_5	17.9 mg.
Weight at end of period	1,170 g.
Loss in weight	7 g.

Period No. 5.

Weight, 1,170 g. Diet contained no aluminum.

Average daily excretion of NH_3	0.1625 mg.
Average daily urinary acidity	39.77 cc. 0.1 *N* HCl
Average daily retention of P_2O_5	4.3 mg.
Weight at end of period	1,307 g.
Gain in weight	137 g.

PUPPY No. 2.

Preliminary Period (7 Days).

Female bull pup. Weight, 1,410 g. Diet, 350 cc. of milk containing 630 mg. P_2O_5.

Average daily excretion of NH_3	0.0782 mg.
Average daily urinary acidity	18.3 cc. 0.1 *N* HCl
Average daily retention of P_2O_5	403 mg.
Weight at end of period	1,552 g.
Gain in weight	142 g.

Period No. 2.

22-day period.

Weight, 1,552 g. Diet contained 300 mg. Al daily.

Average daily excretion of NH_3	0.090 mg.
Average daily urinary acidity	5.1 cc. 0.1 *N* HCl
Average daily retention of P_2O_5	226 mg.
Weight at end of period	1,520 g.
Loss in weight	32 g.

Period No. 3.
11-day period.
Weight, 1,520 g. Diet contained 500 mg. Al daily.

Average daily excretion of NH_3	0.067 mg.
Acidity of urine disappeared during this period.	
Average daily retention of P_2O_5	108 mg.
Weight at end of period	1,455 g.
Loss in weight	65 g.

Discussion.

The addition of 100 mg. of aluminum daily to the diet of Puppy No. 1 caused a noticeable decrease both in the acidity of the urine and in the weight gained during the period. The decrease in the amount of P_2O_5 excreted in the urine and the increase in the feces is also quite evident.

Upon increasing the daily aluminum ingestion to 200 mg. in Periods No. 3 the decrease of both the urinary acidity and body weight is quite striking, as is also the increased fecal and decreased urinary excretion of P_2O_5.

The increase of the daily aluminum ingestion in Period 4 to 300 mg. did not decrease the urinary excretion of P_2O_5 to a greater degree than in the previous period. The urinary acidity was but slightly lower than in the previous period. The fecal excretion of P_2O_5 was slightly increased with a resulting loss to the body both in P_2O_5 and in weight.

During the aluminum free period there was a great increase in the acidity and P_2O_5 excretion of the urine and in body weight, together with a corresponding large decrease in the fecal output of P_2O_5. The P_2O_5 retention during this period was very slight.

The addition of 300 mg. of aluminum to the daily diet of Puppy No. 2 greatly decreased the urinary acidity as well as the average daily excretion of urinary P_2O_5. The daily average of fecal P_2O_5 was proportionately increased. The average daily retention of P_2O_5 was decreased about 45 per cent.

Upon raising the daily ingestion of aluminum to 500 mg. the urine lost its previous acidity and the elimination of P_2O_5 through this channel had practically ceased. The retention of P_2O_5, though greatly decreased, remained about 37% of that of the preliminary aluminum-free period.

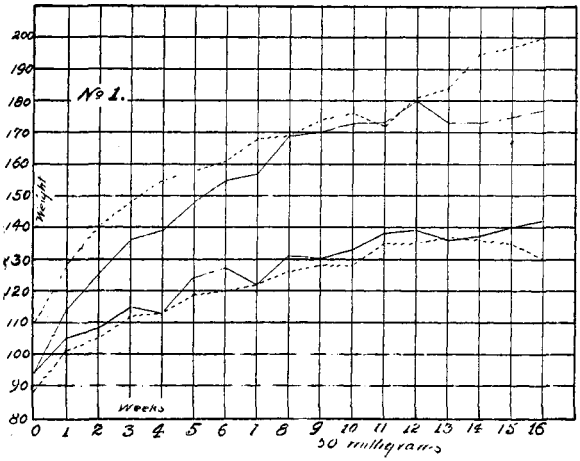
At the end of the experiments the animals were killed with chloroform and their various organs removed and tested for resorbed aluminum.

Macroscopically, no abnormal conditions were observed. Upon chemical analysis, aluminum was found in some of the organs, the deposits in the livers being quite copious. Small deposits were found in the kidneys of the animals.

Experimental Part No. 2.

In order to further study the effects of aluminum hydroxide upon the growth of the young, when consumed in considerable quantities for a

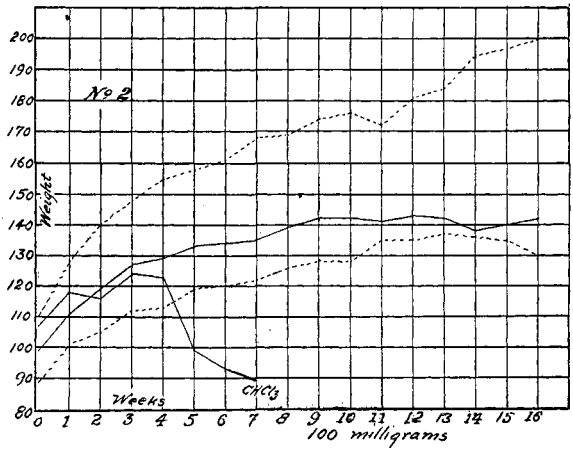
relatively long period of time, young white rats were fed for a period of about four months' duration with puppy biscuit, to which was added varying amounts of aluminum hydroxide. The white rat was selected because of its omnivorous nature and the rapidity of its growth.



A dozen young white rats were employed, whose average weight at the beginning of the experiment was about 100 g. Each rat received a daily ration of 15 g. of puppy biscuit containing 12.25 mg. of P_2O_5 per g. of biscuit. The rats were paired and while one pair served as controls, the remaining pairs had added to their daily allowance of puppy biscuit, sufficient amounts of a suspension of aluminum hydroxide, to furnish to each 50, 100, 150, 200 and 300 mg. of aluminum daily.

Each rat was placed upon an aluminum-free preliminary period of a week's duration before aluminum hydroxide was added to the diet. The rate of growth and the general effect of the aluminum ingestion were noted and the animals weighed weekly for a period of 16 weeks.

At the end of the experiments, the animals were dispatched with chloroform, the viscera examined macroscopically and the various organs incinerated and tested for resorbed aluminum.

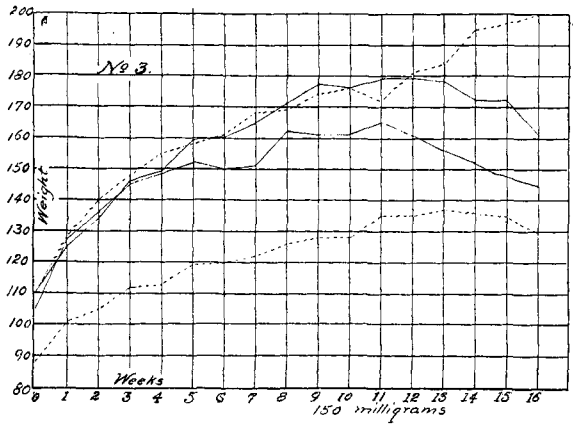


As a check upon the nutritional adequacy of a daily ration of 15 g. of puppy biscuit, five full grown rats of the same species were fed the same daily allotment and in no case was there a decrease in weight.

The weekly weights of the various rats were plotted, the curves of the rats receiving varying amounts of aluminum being compared in each case

with the curves of the control rats, which received no aluminum in their diet.

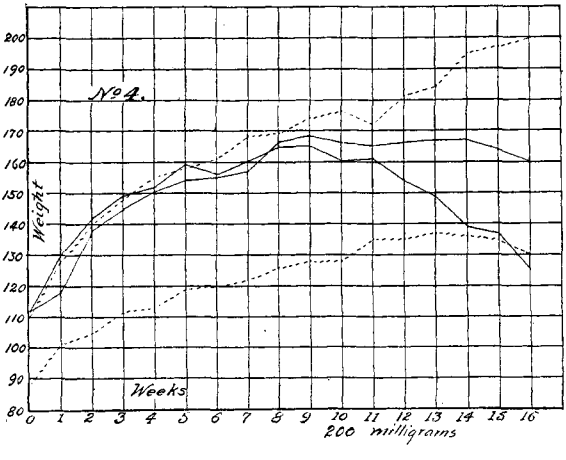
In the curves here presented broken lines represent the control rats; full lines the rats receiving aluminum.



Rat No. 2, receiving 100 mg. of aluminum daily, soon evidenced symptoms of illness and as a precautionary measure was chloroformed. An autopsy, a culture made from the lungs, showed a pneumonic infection.

Rat No. 1, of those receiving 300 mg. of aluminum daily, after a continuous, gradual diminishing in weight, expired at the end of the fifteenth week. Cultures taken from various organs were negative.

When the feeding experiments had been concluded, one rat of each pair was chloroformed, examined macroscopically and the various organs incinerated and tested for resorbed aluminum. No macroscopic lesions were evident. Aluminum was found in the livers of all the rats examined, although but very small amounts were found in those receiving 50 and 100 mg. of aluminum daily.



The rats in each case gained rapidly in bodily dimensions and in most cases in weight. The weights of those receiving the larger amounts of aluminum tended to decrease during the latter part of the experimental feeding.

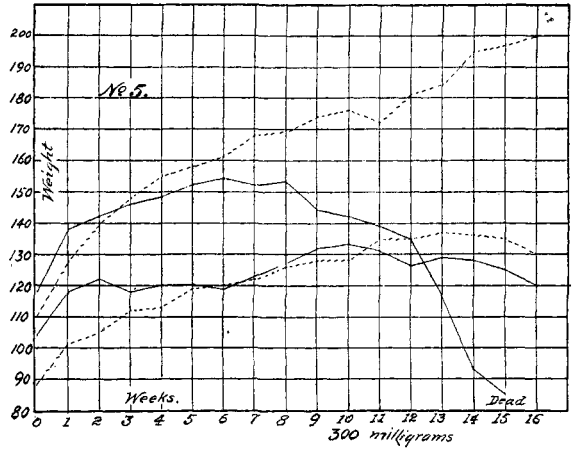
Except in the two cases, above mentioned, no abnormal condition of the rats could be detected during the experiment, the rats retaining their appetites and general good spirits.

Discussion and Results.

In the experiments upon Puppies No. 1 and No. 2 the addition of aluminum to the diet increased the fecal excretion of P_2O_5 and decreased the amount excreted in the urine in general in proportion to the amount of aluminum ingested. Accompanying this decreased urinary excretion was a decrease in the titratable acidity. In the case of Puppy No. 1, the amount of P_2O_5 excreted in the urine varied but little when the aluminum ingestion was increased from 200 to 300 mg.

The increase of the aluminum added to the diet of Puppy No. 2 caused a progressive decrease in both these factors, the excretion of P_2O_5 through the urinary channel being practically eliminated and the urine losing completely its former acidity.

The failure of the urinary acidity of Puppy No. 1 to decrease greatly when the aluminum ingestion was increased from 200 to 300 mg. daily was probably due to the fact that this acidity represented the acidity of the organic acids present which presumption is borne out by the rather varied amounts of P_2O_5 accompanying the same degree of urinary acidity. The rather fixed urinary excretion of P_2O_5 during the 200 and 300 mg. periods of aluminum ingestion may have been due to the increase endogenous excretion, or possibly to the fact that the food ingested, meat, contained only so much of a form of phosphorus that could be diverted from the urinary to the intestinal channel by the presence of aluminum, regardless of the amount ingested. The decrease of the nutritional value of the diet when aluminum was added to it can readily be seen from the comparative weights of the animals at the expiration of the various experimental periods.



In the case of Puppy No. 2, the divergence of the mode of P_2O_5 excretion and the change of urinary excretion is very evident. The phosphorus retention was greatly decreased but could not be eliminated even upon increasing the aluminum ingestion to 500 mg. daily. The ill effects noted, due to the addition of aluminum to the diet, were the poorer utilization of the food as is evidenced by the loss of body weight, and a slight diarrhea in the case of Puppy No. 2 during the 500 mg. period due, no doubt, to a

mechanical irritation of the intestine caused by the large amount of aluminum ingested.

The addition of aluminum in varying amounts to the diet of the young rats appeared to have no great effect during the period of our observation. The rats in most cases progressed equally well as was shown by their comparative increase in bodily dimensions and weekly weights. In but one instance, that of the death of a rat receiving 300 mg. of aluminum, could any great ill effect that could be observed during the period of our experiments be attributed to aluminum.

Using amounts of aluminum up to 300 mg. daily for a period of about four months we were unable to perceptibly influence the growth of young rats receiving a diet containing a fixed amount of phosphorus.

Absorption of aluminum takes place both in dogs and rats when fed in the amounts administered by us over a considerable length of time, the liver being the site of greatest deposition.

It will be, of course, recognized that these experiments have no special bearing on the question of the behavior of alum compounds as used in the diet of man, largely because the weights of aluminum employed in our experiments are relatively high in proportion to the body weights of the animals on which the trials were made. Even a very excessive use of alum compounds could scarcely furnish an amount of the metal in excess of 5 or 6 mg. daily per kilo of body weights. With the rats the ingestion of aluminum amounted to 500 to 3,000 mg. daily per kilo of body weight, while in our experiments the aluminum minimum daily was about 100 mg. per kilo of body weight.

Our investigations confirm the statements of members of the Referee Board with reference to the deflection of phosphorus, but we have carried the dosage to this extreme to be able to show just what would happen under these conditions. But it must be kept in mind that many other substances when ingested in greatly excessive amounts, would probably show equally bad effects.

NASHVILLE, TENN.

NOTES.

Note on the Formation of Esters.—In an interesting communication[1] entitled "Studies on Catalysis: The Addition Compounds of Esters with Organic Acids," Kendall and Booge deduce experimental evidence for an intermediate compound theory, and give examples of many compounds formed by organic acids and ethereal salts.

Work of this character is very suggestive to me, since I have endeavored to attack the same problems with Professor Evans, of McGill University. The outbreak of war stopped researches of this kind, and as it is unlikely

[1] THIS JOURNAL, 38, 1712 (1916).