

## II. THE TOXICITY OF SODIUM PYROPHOSPHATE ADMINISTERED IN FOOD; WITH A NOTE ON TOXIC COTTON SEED MEAL.

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Our attention was drawn to the comparative physiological activity of ortho- and pyrophosphate of sodium in the course of observations on the physiological effects of some camphene-phosphonates, which appeared to bear to one another the same relation as that which exists between the phosphates in question.

In the course of this work [1911] we compared the activity of the phosphates, in solution, by intravenous injection, and confirmed the findings of earlier workers, viz., that the orthophosphate is relatively inert, whilst the pyrophosphate is much more toxic.

We attributed the activity of the pyrophosphate, in part, to the alkalinity of its solutions, in part to its acid radicle. Its solutions after "neutralisation," with hydrochloric acid, to litmus, to phenolphthalein, and to methyl orange, though less active, were still markedly toxic. Almost simultaneously with the above, Starkenstein [1911] referred the toxicity of pyrophosphate solutions, in part to their anions, in part to their kations. He further stated, in agreement with Gamgee, Priestley, and Larmuth [1877], that pyrophosphate administered by the mouth was not poisonous.

Crawford [1910] on the other hand, administered sodium pyrophosphate to rabbits, by the mouth, with fatal results. The salt, given in doses of 0.5-1.0 g. dissolved in 25-40 cc. of water, was usually lethal within three days, the post-mortem appearances described by him, suggesting that the deaths were due to an irritant poison.

It seemed therefore worth while to test afresh the results of administering the salt by the alimentary canal. This has been done on rabbits, cats, and rats, with the result that large doses, administered for prolonged periods, have failed to produce acute poisoning.

A sheep, on the other hand, died after taking 21 grams of the salt.

In this, as in our earlier paper, the chemical side has been the work of Gardner, whilst the physiological portion is that of Symes.

#### *Observations on Rabbits.*

The first set of observations was made on three pairs of young rabbits, fed with the same food, viz., oats, bran, and chopped cabbage.

The first and second pairs received daily doses, of ortho- and of pyrophosphate of sodium respectively, whilst the third was kept to control the effect of outside factors. In the first two cases (pairs) the salt was powdered, mixed with 15 g. of chopped cabbage, and given to each animal separately. This ensured complete ingestion of the salt by each individual, as the animals devoured the cabbage with relish. Apart from the above the animals were fed, and housed, in pairs. They were weighed, singly, at the commencement of the experiment, and at intervals of seven days during its continuance. Table I shows the result of four weeks of this régime. The doses were doubled each week and, molecule for molecule, that of the orthophosphate was much the greater.

TABLE I.

*Body-weights (weekly) of young rabbits, fed on a mixed diet, with and without addition of phosphate.*

Week	On $\text{Na}_2\text{HPO}_4$ 12 Aq.		Daily dose per head	On $\text{Na}_4\text{P}_2\text{O}_7$ 10 Aq.		Daily dose per head	No added phosphate	
	Rabbit A	Rabbit B		Rabbit C	Rabbit D		Rabbit E	Rabbit F
	Kg.	Kg.	g.	Kg.	Kg.	g.	Kg.	Kg.
0	0.90	0.90	—	0.80	0.60	—	1.10	0.60
1	1.10	1.00	0.32	0.98	0.80	.13	1.40	0.82
2	1.10	1.05	0.64	1.00	0.77	.26	1.40	0.80
3	1.15	1.10	1.3	1.10	0.70	.52	1.45	0.90
4	1.20	1.10	2.6	1.00	0.80	1.0	1.70	1.00
Increase in body-weight	} 33 %			25 %	33 %		55 %	67 %

The above figures suggest that, although neither phosphate proved acutely poisonous, each one had, at this stage of life, an adverse influence on growth. This influence was not maintained in later observations on the same animals.

On completion of these observations the animals were all kept on the same mixed diet, without phosphate, for eight weeks. During this period their weights increased as follows:

Rabbit A	from 1.20 kg.	to 1.75 kg.	= 46 %
B	1.10	1.35	= 23
C	1.00	1.60	= 60
D	0.80	0.95	= 19
E	1.70	1.80	= 6
F	1.00	1.35	= 35

The growth of rabbit D during this period is, for its weight, strikingly small. This animal was, from the commencement, weakly, and light in proportion to its apparent bulk, and its subsequent death did not appear to be primarily due to the experiment.

After this interval (eight weeks), the animals were again treated as shown in Table I, with the results given in Table II.

TABLE II.

*Weekly body-weights of rabbits as in Table I.*

Week	On $\text{Na}_2\text{HPO}_4$ , 12 Aq.		Daily dose per head g.	On $\text{Na}_4\text{P}_2\text{O}_7$ , 10 Aq.		Daily dose per head g.	No added phosphate	
	Rabbit A Kg.	Rabbit B Kg.		Rabbit C Kg.	Rabbit D Kg.		Rabbit E Kg.	Rabbit F Kg.
0	1.75	1.35	—	1.60	0.95	—	1.80	1.35
1	1.70	1.70	0.35	1.60	0.80	0.23	1.80	1.30
2	1.80	1.50	0.71	1.80	1.00	0.46	1.90	1.35
3	2.10	1.70	1.4	1.90	1.30	0.93	1.85	1.50
4	2.30	1.70	2.8	1.90	1.45	1.9	2.00	1.70
5	2.30	1.85	„	2.00	1.50	„	2.20	1.80
6	2.40	2.00	„	2.00	1.60	„	2.15	1.90
7	2.45	1.90	„	2.15	1.50	„	2.20	1.95
8	2.50	1.90	„	2.30	1.60	„	2.10	2.00
9	2.70	2.30	„	2.20	1.50	„	2.15	2.00
10	2.65	2.20	„	2.20	1.20	„	2.25	2.20
11	2.60	2.10	1.8	2.15	1.10	1.2	2.30	2.25
12	2.70	2.20	„	2.20	1.10	„	2.25	2.20
Increase in body-wt.	54 %	63 %		38 %	16 %		25 %	63 %

The doses were doubled each week for the first month. That of the orthophosphate was, again, the greater.

Apart from Rabbit D, these figures do not suggest that either phosphate had, with the older rabbits, any adverse effect on growth. This animal which, as above stated, had appeared weakly from the beginning, died about a week after the observations were completed.

The autopsy revealed no gastric or intestinal lesion, nor anything to show that the death resulted from the pyrophosphate.

Towards the end of this series one rabbit of each group was isolated, for one week, for collection of its urine and faeces.

In each case the salt was eaten in full. On estimating the  $P_2O_5$  in the dejecta, by Neumann's method, the whole of the  $P_2O_5$  given, as orthophosphate, was recovered; whilst the  $P_2O_5$  recovered from the urine and faeces of the rabbit taking 2.3 g.  $P_2O_5$  as pyrophosphate only slightly exceeded that from the control animal.

As already mentioned, the whole of the pyrophosphate was eaten. The missing  $P_2O_5$  was not retained in the animal, but deposited as "earthy phosphate" on the floor of the cage, from which it was not dislodged by the water with which the cage was sluiced at the end of each week, but was subsequently, in great part, recovered by washing with hydrochloric acid.

Two further experiments yielded the same result, i.e. all the  $P_2O_5$  given as  $Na_2HPO_4$  was recovered from the dejecta plus water washings; whilst much of that given in  $Na_4P_2O_7$  was found as an almost invisible deposit on the floor of the cage.

On this account, the experiment was repeated on a cat, in the anticipation that the high acidity of its urine would diminish or prevent the deposition of the phosphates.

#### *Observations on Cats.*

The results on two cats were as follows.

The first, a full grown but rather thin cat, was given (and ate) 2 g. of  $Na_4P_2O_7$ , 10 aq., daily, for 15 days, mixed with soaked biscuit and milk. The animal showed no sign of discomfort and gained 100 g. in weight during this period, in spite of slight diarrhoea.

The second, a younger cat, was given (and ate) the same dose for 13 days. This animal vomited once, but gained 100 g. in weight during the observation. The same cat, two months later, took 2 g. of  $Na_2HPO_4$ , 12 aq., daily, for 9 days, also without any symptom of acute poisoning. Both phosphates produced slight diuresis, and some tendency to diarrhoea. In consequence of the latter, the faeces were not collected. The urine, collected during a week of each régime, in comparison with that of a week on the same diet without addition of phosphate yielded  $P_2O_5$  as shown in the following table.

TABLE III.

*Urine of a cat on a mixed diet, and on the same diet plus phosphate.*

Régime	Volume (one week)	Sp. Gr.	P <sub>2</sub> O <sub>5</sub> recovered	P <sub>2</sub> O <sub>5</sub> given as salt	Difference between P <sub>2</sub> O <sub>5</sub> recovered, and P <sub>2</sub> O <sub>5</sub> given as salt
Control	748 cc.	1026	2.0 g.	0.0 g.	2.0 g.
Orthophosphate	819 cc.	1040	4.7 g.	2.4 g.	2.3 g.
Pyrophosphate	776 cc.	1038	5.5 g.	3.8 g.	1.7 g.

It is obvious that both phosphates were absorbed, and that loss by deposit was not great.

*Observations on Rats.*

A series of observations was made on three pairs of rats. The animals were fed on bread moistened with water and on oats. The first pair was given, daily, with the soaked bread, 0.5 g. of Na<sub>2</sub>HPO<sub>4</sub>, 12 aq.; the second pair an equal weight of Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, 10 aq.; whilst the third pair had no phosphate added to its ration. The animals were fed in pairs, and weighed (also in pairs) once a week. Table IV shows the body-weights under nine weeks of this régime.

TABLE IV.

*Body-weights (weekly) of pairs of white rats, fed on a mixed diet, with and without addition of phosphate.*

Week	On Na <sub>2</sub> HPO <sub>4</sub> 12 Aq. 0.5 g. daily	On Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> 10 Aq. 0.5 g. daily	No added phosphate
0	217 g.	175 g.	229 g.
1	229 „	193 „	233 „
2	232 „	197 „	248 „
3	235 „	192 „	254 „
4	238 „	206 „	267 „
5	244 „	213 „	263 „
6	259 „	224 „	263 „
7	256 „	220 „	273 „
8	256 „	222 „	277 „
9	245 „	219 „	285 „
	Increase in } body-weight } 13 %	25 %	25 %

The pyrophosphate pair, starting 25 % below the controls, grew at the same nett rate as these, whilst the orthophosphate pair starting at the same level (as the controls) grew less rapidly. Both of the phosphate pairs lost weight after the sixth week.

It was noted that the pyrophosphate ration was eaten with less relish than the others, and that on 21 days (out of the 63) some of it was left, thereby entailing loss of some of the phosphate. But since on the remaining 42 days the dose was fully ingested, the table confirms the indications of the earlier series, that neither phosphate was acutely poisonous.

*Observations on a Sheep.*

On account of the supposed presence, and toxicity, of pyrophosphate in some samples of cotton seed meal, it was thought well to test the effect of pyrophosphate on a sheep, and we are indebted to the Director of the Lister Institute for affording us facilities for this portion of the work.

A full grown wether was given, daily, 1.0 g. of  $\text{Na}_4\text{P}_2\text{O}_7$ , 10 aq., in 50 g. of dry oatmeal, in addition to hay, for a week. The meal was eaten with relish and produced no noticeable effect. After a week's interval, the daily dose of pyrophosphate was recommenced and doubled. This was still taken with relish, but produced some thirst and restlessness, and the animal was found dead on the morning following the seventh dose, i.e. after a total intake of 21 g. of pyrophosphate.

The autopsy showed marked softening of the mucous membrane of the rumen and a small patch of inflammation, in its wall, opposite the spleen, to which it was firmly adherent.

The reticulum and omasum appeared normal, whilst the abomasum and small intestine were inflamed throughout. No ulcer was seen in any portion of stomach or intestine. This result agrees with those of Crawford, on rabbits, in which the conditions of experiment were substantially those in the case of the sheep. Crawford administered  $\text{Na}_4\text{P}_2\text{O}_7$  in (approximately 2 %) solution, i.e. he introduced a strongly alkaline solution into the stomachs of his animals.

Introduction of dry  $\text{Na}_4\text{P}_2\text{O}_7$  into the already alkaline contents of the sheep's rumen yields, likewise, a strongly alkaline solution. This alkaline solution produced the same effect as Crawford's more concentrated alkaline solutions, though without visible ulceration and less rapidly.

To our rabbits, the pyrophosphate was given as powder in chopped cabbage; to the rats and cats, as powder in moistened bread and biscuit respectively. In these cases, the salt was presumably, in great part, (if not wholly) neutralised as rapidly as it reached the stomach wall. It certainly produced no acute poisoning.

Equally certainly it was largely absorbed, as shown by the analyses of

the urines, and urinary deposits. It would appear, therefore, either that the pyrophosphoric acid, liberated by the gastric juice, was hydrolysed to the less active orthophosphoric acid (as suggested by Starkenstein), or that (as supposed by Gamgee) the absorption of pyrophosphate was balanced by its prompt excretion.

#### NOTE ON TOXIC COTTON SEED MEAL.

In relation to cotton seed poisoning, mentioned above, we may add that a sample of toxic cotton seed meal which has come into our hands, has proved fatal to rats. Four of these animals, fed exclusively (and *ad libitum*) on the meal, died on the 5th, 7th, 13th and 14th days respectively, showing gastrointestinal inflammation without visible ulceration or noticeable softening of the mucous membrane. The appearances were, in short, those common to irritant poisons, not distinctive of any particular one.

As detailed above, our rats consumed, daily, 0.5 g. per head of crystalline sodium pyrophosphate (= 0.3 g.  $\text{Na}_4\text{P}_2\text{O}_7$ ), for nine weeks, without visible harm. On the other hand, four rats fed on oats, and bread, plus 5 g. of cotton seed meal per head, died on the 10th, 15th, 24th, and 25th days respectively. Four other rats fed on oats, bran, and 5 g. per head of the same cotton seed meal, after previous extraction with alcohol and with ether, lived 26, 31, 40, and 50 days respectively; whilst four (control) rats, fed on oats and bread only, maintained their health and gained 77 g. in weight, in the 50 days of the experiment. The  $\text{P}_2\text{O}_5$  content of the (air dry) cotton seed meal was 0.43 %, and amounted to 0.022 g. in the daily ration. This corresponds to 0.07 g. of  $\text{Na}_4\text{P}_2\text{O}_7$ , 10 aq., i.e. less than one-seventh of the quantity tolerated, daily, for nine weeks. It is clear from the reduction of toxicity by alcohol and ether, and from the low phosphate content of the seeds that sodium pyrophosphate cannot be the chief poison in the meal in question.

It was hoped to identify the poisonous principles and to extend the observations to ruminants, but the non-renewal of a small grant from the Board of Agriculture has rendered this, for the present, impracticable.

#### SUMMARY AND CONCLUSIONS.

Sodium pyrophosphate administered, with food, to rabbits, cats, and rats, is devoid of the toxic action that it shows when intravenously injected. This confirms the verdict of Gamgee and his pupils, and of Starkenstein.

Administered to a sheep, in the same way, it has proved lethal, producing effects similar to those described, by Crawford, as following its administration, in aqueous solution, to rabbits.

Such toxic action as sodium pyrophosphate exerts, when administered by the mouth, differs from that of the same compound, intravenously injected, in that it is wholly due to the alkalinity of the salt and not to the acid radicle.

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