C. THE EFFECTS OF THE INJECTION OF CERTAIN SALTS ON THE CALCIUM, MAGNESIUM AND INORGANIC PHOSPHORUS OF THE SERUM OF THE RABBIT.

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BROOKFIELD [1933] showed that in the serum of the rabbit there was an inverse relationship between calcium and magnesium, a direct relationship between magnesium and inorganic phosphorus and an inverse relationship between calcium and inorganic phosphorus, the latter of which had been previously observed by Dupré and Semeonoff [1931] and Bourne and Campbell [1932]. The rises and falls in the concentrations of the three ions in the serum were correlated with their relative amounts in the diet. The relationships seemed worthy of further study under other experimental conditions, in order to determine whether the effects observed with diets varying in their mineral contents were of more general significance, and accordingly another series of experiments has been carried out.

Solutions of salts containing Ca^{++} , Mg^{++} and PO_4 ions have been injected subcutaneously into rabbits and their effects on the calcium, magnesium and inorganic phosphorus of the serum observed.

EXPERIMENTAL.

Experiments were made on fully grown male rabbits maintained on a diet of cabbage and oats, which (with one exception) had fasted for about 18 hours. Calcium gluconate, laevulate and lactate, magnesium sulphate and lactate and disodium hydrogen phosphate were the salts employed, while control experiments were carried out with sodium chloride and sodium sulphate. The salts, dissolved in the minimum volume of water, were injected subcutaneously into the flank. 5 ml. of blood were withdrawn from the lateral vessel of the ear immediately before the injection and on three separate occasions afterwards. The effects were observed over periods varying from $1\frac{1}{2}$ to 6 hours. The methods of estimating calcium, magnesium and inorganic phosphorus were the same as those used in the dietary experiments [Brookfield, 1933].

The influence of two factors called for control before the results of the experiments could be interpreted; firstly, the effects of four small haemorrhages recurring at relatively short intervals and secondly, the effects of the injection of a solution of an indifferent salt.

1. *The effects of bleeding.* (a) *Successive small haemorrhages.* 5 ml. of blood were removed from a rabbit four times at half-hourly intervals; 9 days later the same procedure was repeated, but at hourly intervals and after a further 22 days the animal was bled again, this time at 2-hourly intervals. In the

first experiment the haemoglobin percentage was unaltered at the fourth bleeding; in the other two experiments a fall of 8 % was noted. The serum-calcium, magnesium and inorganic phosphorus values are recorded in Table I.

Table I. *Serum-calcium, magnesium and inorganic phosphorus. Small haemorrhages at short intervals.*

Rabbit 20. Weight 2010 g. 4 bleedings of 5 ml. on each occasion.

	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
$\frac{1}{2}$ -hourly intervals			
	16.55	1.952	3.152
	15.70	1.694	2.626
	15.15	1.597	2.551
	14.95	1.590	2.716
1-hourly intervals			
	13.29	2.886	4.382
	13.29	2.886	4.382
	12.94	2.794	4.762
	12.72	2.749	4.794
2-hourly intervals			
	13.53	3.615	3.750
	13.53	3.542	3.936
	13.90	3.357	4.521
	13.67	3.204	4.654

It will be seen that the magnesium value tends to fall, but at a very slow rate. The calcium figures show variable results, depending on the time interval. The four bleedings at half-hourly intervals have caused a fall of 1.6 mg. (10 %). Hourly bleedings cause a slighter fall (4 %) and 2-hourly bleedings none. A fall in the inorganic phosphorus followed by a rise after one hour results from bleeding at intervals of half an hour, while with hourly or 2-hourly bleedings the inorganic phosphorus rises.

(b) *Progressive changes during a single haemorrhage.* The rapidity with which the fall in the serum-calcium takes place is illustrated by the results recorded in Table II.

Table II. *Serum-calcium and magnesium values during bleeding. Blood taken in two samples of 5 ml. each.*

Rabbit no.	Time of bleeding	Calcium mg./100 ml.	Magnesium mg./100 ml.
19	11.45-12.15	15.28	1.722
	12.15-12.45	14.44	1.694
21	4.30-4.45	15.73	1.895
	4.45-5.0	15.35	1.886
19	10.45-11.0	12.60	1.907
	11.0-11.10	12.25	1.896
20	11.15-11.25	14.35	1.963
	11.25-11.30	14.25	1.951

Each animal was submitted to a single haemorrhage and the blood collected in two successive 5 ml. samples. In one experiment where the time of bleeding was rather prolonged, a fall of 0.8 mg. (6 %) was noted. The effects of haemorrhages on the serum-calcium of rabbits have been referred to by several writers. A decrease of 10 % or more has been reported when observations have been made

at a short interval after the haemorrhage by Stransky [1915], Clark [1920], Stewart and Percival [1927] and Culhane [1927]. Seven hours after bleeding, Moritz [1925] found a slight and inconstant decrease in the serum-calcium value. A fall in the magnesium also was noted by Stransky. These results show that the variations in the concentrations of calcium, magnesium and inorganic phosphorus caused by bleeding are so slight as to have little or no significance in the interpretation of the findings in the injection experiments, which will now be discussed.

2. *The effects of the injection of an indifferent salt.* Sodium chloride was injected in an amount somewhat in excess of those of the other salts employed. The results are recorded in Table III.

Table III. *Injection of sodium chloride. Serum-calcium, magnesium and inorganic phosphorus values.*

Rabbit 24. Weight 2300 g. 5 ml. bleedings. Fasting.

Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
0	13.29	2.786	3.820
0.36 g. NaCl (0.0062 g. equivalent per kg. in 9 ml. water injected subcutaneously).			
$\frac{1}{2}$	13.29	2.806	4.154
1	12.67	2.726	3.434
4	13.06	2.557	3.701

There is a slight fall in all three constituents after one hour with a tendency on the part of the calcium and inorganic phosphorus to return to their initial values after 4 hours. These changes are unimportant in comparison with the results which follow, and they show that probably any effect observed in the injection of salts containing Ca^{++} , Mg^{++} , or $\text{PO}_4^{=}$ was to be attributed to these ions themselves.

3. *The injection of solutions containing the Ca^{++} , Mg^{++} , $\text{PO}_4^{=}$ and $\text{SO}_4^{=}$ ions.* An attempt was made, in each case, to introduce an amount of salt sufficient to cause a marked effect on the several blood-constituents under review without producing untoward symptoms. The success achieved was only partial. No symptoms were noted after the injection of the calcium salts. Slight ataxia of the hind-limbs resulted from the injection of magnesium sulphate and lactate. Definitely toxic effects were observed however with disodium phosphate and sodium sulphate. In both cases there was some degree of collapse after the injection. The animal injected with disodium phosphate showed marked muscular twitching but ultimately recovered. The animal which received sodium sulphate was found dead 2 days later. No urine was passed during the period of any of the experiments except by the rabbit which received sodium sulphate. None of the animals suffered from diarrhoea.

Results.

For purposes of comparison the amounts of the salts have been expressed as g. equivalents per kg. body weight. The results of the injections of the calcium salts are set out in Table IV, of the magnesium salts in Table V, of sodium sulphate in Table VI and of disodium phosphate in Table VII.

1. *The interrelations of calcium and magnesium.* In each of the 4 experiments where calcium salts have been injected approximately equivalent amounts of calcium have been given. A moderate increase in the serum-calcium has been the result amounting on the average to 3.0 mg. or 23 % of the initial value.

The rise to the maximum takes place within one hour and is followed by an almost equally rapid fall. The rise in the serum-calcium is accompanied by a progressive fall in the serum-magnesium. In the case of the injection of calcium gluconate, there may be a slow return towards normal at 6 hours. The average fall amounts to 1.0 mg. or 37 % of the initial value though it should be noted that in 3 of the experiments (Table IV) the fall appears still to be continuing at the time of the final observation.

Table IV. *Injection of calcium salts. Serum-calcium, magnesium and inorganic phosphorus values.*

	Salt injected	Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
Rabbit 19. 2520 g.	Gluconate	0	13.17	2.389	3.720
Fasting	(0.6 g. $\equiv$ 0.0028 g. equiv. Ca/kg.)	$\frac{1}{2}$	16.13	1.896	3.861
		2	14.91	1.590	4.106
		6	14.32	1.748	4.152
Rabbit 23. 2000 g.	Gluconate	0	13.29	3.091	4.826
Fasting	(0.75 g. $\equiv$ 0.0035 g. equiv. Ca/kg.)	$\frac{1}{2}$	16.59	2.567	4.700
		1	16.75	2.097	4.609
		$1\frac{1}{2}$	17.77	1.896	4.700
Rabbit 23. 2200 g.	Laevulate	0	12.27	3.044	4.252
Fasting	(0.6 g. $\equiv$ 0.0039 g. equiv. Ca/kg.)	$\frac{1}{2}$	15.19	2.635	3.990
		$1\frac{1}{4}$	14.57	2.136	—
		$2\frac{1}{4}$	13.48	1.860	—
Rabbit 23. 2170 g.	Lactate	0	14.46	2.332	2.977
Fasting	(0.46 g. $\equiv$ 0.003 g. equiv. Ca/kg.)	$\frac{1}{2}$	17.02	1.902	3.106
		1	14.97	1.620	2.927
		2	14.82	1.544	3.247

Though the magnesium salts have been injected in amounts equivalent to those of calcium the effects have been considerably greater. The rise in the serum-magnesium averages 5.6 mg. over the initial value, as compared with a rise of 3.0 mg. in the calcium value caused by injections of corresponding amounts of calcium salts. This rise takes place rapidly but is more sustained than in the case of calcium, and it is clear that either the rate of absorption of magnesium salts is much less rapid than that of calcium salts, or alternatively and more probably

Table V. *Injection of magnesium salts. Serum-calcium, magnesium and inorganic phosphorus values.*

	Salt injected	Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
Rabbit 19. 2680 g.	Sulphate	0	14.69	2.109	2.779
Fed with cabbage and oats	(0.37 g. $\equiv$ 0.003 g. equiv. Mg/kg.)	$\frac{3}{4}$	11.32	8.078	2.080
		$1\frac{1}{4}$	10.74	8.258	1.890
		$4\frac{1}{4}$	13.31	3.634	3.086
Rabbit 25. 2750 g.	Lactate	0	12.62	2.348	3.682
Fasting	(0.37 g. $\equiv$ 0.0031 g. equiv. Mg/kg.)	$\frac{3}{4}$	11.85	6.726	3.262
		$1\frac{1}{4}$	10.54	7.466	3.147
		5	10.61	5.833	3.608

that the removal of excess magnesium from blood is much less readily accomplished. Where magnesium sulphate has been injected (Table V) the serum-magnesium is approaching the normal value after 5 hours, but at the same interval after the injection of the equivalent amount of magnesium lactate (Table V) the serum-magnesium is nearly 3 times greater than the initial value.

These increases in the magnesium concentration have resulted in a marked fall in the calcium value in every experiment. This fall in calcium has been more rapid and greater in extent (4.0 mg. or 27 % of the initial value), following the injection of magnesium sulphate (Table V). The calcium value in this experiment is approaching the initial level after 6 hours. Following the injection of magnesium lactate the serum-magnesium continues at a high level for a considerable time and there is a correspondingly prolonged depression of the serum-calcium, which after 5 hours is still at the low level of 10.6 mg.

The inverse relationship found to exist between calcium and magnesium in the dietary experiments is therefore borne out by the results of these subcutaneous injections of calcium and magnesium salts.

Previous observations of the effects of the injection of calcium and magnesium salts on the calcium and magnesium content of serum are fragmentary and in some cases conflicting. Salvesen *et al.* [1924, 2] found that the magnesium content of the blood of dogs was depressed by the intravenous injection of calcium chloride. Stransky [1915] and Meneghetti [1927] observed a fall in the serum-calcium of rabbits after the injection of magnesium sulphate, the latter worker also showing that the diffusible calcium was increased. A number of observers have shown that the injection of magnesium salts leads to an increase in the calcium excretion and *vice versa*. Thus Mendel and Benedict [1909] found that the injection of calcium chloride into dogs and rabbits led to loss of magnesium, while the injection of magnesium sulphate and chloride increased the excretion of calcium. Greenwald and Gross [1925] noted a temporary increase in the excretion of magnesium in dogs injected with calcium chloride. After introducing metallic magnesium under the skin of rabbits, Reding and Slosse [1923] noted a considerable increase in the output of calcium, and loss of calcium was also observed by Schiff [1920] and by Schiff and Stransky [1920] in infants injected with magnesium sulphate. While a fall in the serum-calcium, accompanied by an increased excretion of calcium, was noted by Pribl [1929] in rabbits after the injection of magnesium sulphate, a rise in the serum-calcium value with calcium retention following the injection of magnesium lactate was reported. Condorelli's [1926] findings are also at variance with those recorded above, in that he observed a considerable elevation of the serum-magnesium following the injection of calcium lactate into rabbits.

2. *The effects of the sulphate ion.* One possible complicating effect in the experiment with magnesium sulphate is the presence of the sulphate ion, for the fall in the calcium value was less when magnesium was introduced as the lactate. Since the injection of phosphate depresses the serum-calcium it was possible that the injection of sulphate might have had a similar effect, and this was controlled in an experiment in which sodium sulphate was injected in amount equivalent to twice that of the magnesium sulphate used. Table VI shows that this has

Table VI. *Injection of sodium sulphate (1.0 g. Na_2SO_4 , $10\text{H}_2\text{O} \equiv 0.006$ g. equiv. Na/kg.). Serum-calcium, magnesium and inorganic phosphorus values.*

Rabbit 23. Weight 2500 g. Fasting.

Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
0	14.34	2.629	4.700
$\frac{1}{2}$	13.52	2.629	3.536
$1\frac{1}{2}$	12.20	2.505	3.106
4	11.20	2.557	3.572

resulted in a considerable fall in the serum-calcium but one different in character from that caused by the injection of magnesium sulphate. The fall is much more gradual, and although still continuing at the time of the last observation, 4 hours after the injection, is less than that produced by half the equivalent amount of magnesium sulphate (Table V). It is of interest that Stransky [1915] found a much increased calcium excretion after the injection of sodium sulphate into a rabbit. Table VI shows that while sodium sulphate has no effect on the serum-magnesium, the serum-inorganic phosphorus is depressed by 1.6 mg. (34 %) to rise again after one and three quarter hours, when it moves in a direction opposite to that of calcium.

3. *The interrelations of calcium and inorganic phosphorus.* The changes in the serum-inorganic phosphorus after the injection of the three calcium salts (Table IV) are so slight that it appears that injected calcium has no direct effect on the inorganic phosphorus. When, however, disodium phosphate is injected there is an immediate fall in the serum-calcium (Table VII). In this experiment the phos-

Table VII. *Injection of disodium hydrogen phosphate (0.57 g. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} \equiv 0.0047$ g. equiv. P/kg.). Serum-calcium, magnesium and inorganic phosphorus values.*

Rabbit 19. Weight 2300 g. 5 ml. bleedings. Fasting.			
Time after injection (hours)	Calcium mg./100 ml.	Magnesium mg./100 ml.	Inorganic phosphorus mg./100 ml.
0	12.97	2.529	4.121
1	11.39	2.308	10.01
2	10.74	2.423	10.51

phate was injected in amount equivalent to 1.5 times the amounts of the salts used for the calcium injections. The rise in the serum-inorganic phosphorus was rapid and relatively prolonged: at the end of one hour it was 5.9 mg. (140 %) above the initial value, and at 2 hours was at a slightly higher level. At the same interval after the injection, the serum-calcium had fallen by 2.2 mg. or 17 %. Samples were unobtainable after 2 hours owing to the collapsed state of the animal.

From these results it is plain that the inverse relationship between the calcium and inorganic phosphorus of serum observed in the dietary experiments is only partially maintained when calcium or phosphate is injected. The results of the injection of phosphate are in accord with the dietary experiments inasmuch as the serum-calcium falls as the inorganic phosphorus rises. Several previous workers have reported a depression of the serum-calcium on injecting phosphate. Binger [1917] produced a fall of the serum-calcium to 6 mg./100 ml. with the development of tetany by injecting dogs intravenously with disodium phosphate. Tisdall [1922] repeated his experiments with a similar effect, while Salvesen *et al.* [1924, 1] obtained the same results when phosphates were administered orally. The calcium thus displaced from the serum is apparently largely excreted since Greenwald and Gross [1925] found a rise in the calcium excretion in dogs after the injection of neutral sodium phosphate, while Boyd *et al.* [1930] observed, in addition to a fall in the serum-calcium, an increased urinary excretion of calcium after injecting sodium glycerophosphate into the same animals.

In the present investigation injection of calcium has produced no definite fall in the serum-inorganic phosphorus, so that in this respect there is no effect analogous to the inverse relationship of the dietary experiments. The results of other workers are somewhat variable. Bomskov [1930] found that a fall in the

inorganic phosphorus followed the injection of calcium gluconate into rabbits. Salvesen *et al.* [1924, 2] and Collip [1926], using dogs, reported a small rise in the inorganic phosphorus value on injecting calcium chloride. Condorelli [1926] found a greater rise in the rabbit injected with calcium lactate. Greville [1931] found the inorganic phosphorus of the serum of two rabbits slightly elevated after the intravenous injection of calcium laevulate, while Apitzsch [1931] observed that the serum-inorganic phosphorus of human subjects was raised after the oral administration of calcium lactate. Thus the balance of evidence suggests that there is no disappearance of phosphorus from the blood, though it is noteworthy that Greenwald and Gross [1925] found an increased excretion of phosphorus in the dog after the injection of calcium chloride.

4. *The interrelations of magnesium and inorganic phosphorus.* Following the injection of magnesium (Table V) there is a fall in the serum-inorganic phosphorus amounting, after the injection of the sulphate to 0.9 mg. (32 %) and after that of the lactate to 0.5 mg. (15 %). The somewhat greater effect of magnesium sulphate is possibly accounted for by the synergic action of the sulphate ion which itself lowers the serum-inorganic phosphorus (Table VI). It will be seen that the injection of phosphate (Table VII) caused no appreciable change in the serum-magnesium, just as the injection of calcium caused no effect on the serum-inorganic phosphorus. These results are not in accord with the direct relationship between magnesium and inorganic phosphorus observed in the dietary experiments.

DISCUSSION.

The injection experiments differ from the earlier dietary experiments in two important respects which may account to some extent for some of the divergences in the results of the two series. Firstly, whereas in the injection experiments salts were introduced subcutaneously and rapidly absorbed into the circulation, in the dietary experiments much smaller amounts of the relevant ion were slowly absorbed from the intestine over a period occupying 2 hours or more. Secondly, while two ions only were concerned in the injection experiments, a number of ions were being absorbed simultaneously in the dietary experiments, as well as other constituents which may themselves exercise important effects.

These differences do not appear to be of sufficient importance materially to affect the relationship existing between the calcium and magnesium of serum, which in both series of experiments is inverse. It is noteworthy that the effects of the injections have not been as drastic as might be anticipated. In the dietary experiments the rises and falls in the serum-calcium corresponded with similar rises and falls in the serum-magnesium, whereas in the injection experiments the greater and more rapid increase in the concentration of the one ion in the blood has produced a fall in the other hardly greater than those seen in strictly physiological conditions. It is evident that there exist mechanisms whereby the organism can resist attempts to lower a blood constituent to an undesirable level.

When the relations of calcium and magnesium to inorganic phosphorus are studied, differences in the results of the two series of experiments are at once apparent. Although the injection of phosphate causes a fall in the serum-calcium the converse is not observed, since when a calcium salt is injected, the serum-inorganic phosphorus is virtually unaffected. The inverse relationship between the serum-calcium and inorganic phosphorus as observed in the dietary experiments therefore appears to operate in one direction only. The direct relationship between magnesium and inorganic phosphorus observed in the dietary experiments receives no support from the results of the injections, since while the

injection of phosphate leaves the serum-magnesium unaffected, the injection of magnesium salts depresses the inorganic phosphorus so that the two constituents are actually in inverse relationship.

The lack of reciprocity between calcium and inorganic phosphorus on the one hand and between inorganic phosphorus and magnesium on the other which is indicated by the results of the injection experiments appears to find an explanation when the relative concentrations of these constituents in the blood are considered. Normally the calcium of serum is more than equivalent to the inorganic phosphorus, so that it might be anticipated that, when more phosphate was introduced into the blood-stream, combination with calcium would occur with the deposition in some suitable site of calcium phosphate and a consequent fall in the serum-calcium value. Since calcium is present in excess of inorganic phosphorus, it is hardly surprising that the introduction of further calcium produces no fall in the inorganic phosphorus. Similarly, the inorganic phosphorus of serum is more than equivalent to the magnesium, so that, when more magnesium is introduced, it seems reasonable to expect that some of the magnesium will combine with inorganic phosphorus, the disappearance of which from the blood will lead to a fall in the serum-inorganic phosphorus. On the other hand, the addition of further phosphate when inorganic phosphorus is already in excess need hardly be expected to cause a fall in the serum-magnesium.

The injection experiments suggest that fluctuation of the serum-inorganic phosphorus is the chief controlling factor in the calcium-inorganic phosphorus relationship observed in the dietary experiments. This is in harmony with the explanation suggested by Fraser [1932] who regards the relationship as dependent on the periodical liberation of calcium phosphate from the bones. The calcium plays a passive rôle, in that calcium deficiency causes calcium phosphate to leave the depots with consequent rise in the serum-inorganic phosphorus value, whereas the presence of a sufficiency of calcium in the blood leads to a discontinuance of the process, so that the serum-inorganic phosphorus tends to fall. It is obvious that the depots must normally be replenished from time to time and it may fairly be assumed that a rise in the serum-inorganic phosphorus is instrumental in this effect leading to a deposition of calcium phosphate in the bones. Regarded from this aspect, the mechanism of the reciprocal relationship might be stated thus: A rise in the serum-inorganic phosphorus leads to a fall in the serum-calcium by deposition of calcium phosphate in bone, while a fall in the phosphorus value, as by excess of excretion over intake, allows liberation of calcium phosphate from the bones to proceed with consequent rise in the serum-calcium value.

SUMMARY.

1. With a view to confirming earlier observations of the effects of the inorganic constituents of diet on the relationships between the concentrations of calcium, magnesium and inorganic phosphorus in the serum of the rabbit, solutions containing the appropriate ions have been injected subcutaneously and their effects on the three constituents compared with those noted previously.

2. The inverse relationship existing between the concentrations of calcium and magnesium in the serum, first observed when rabbits are kept on certain diets, has been confirmed by the subcutaneous injection of calcium and magnesium salts.

3. The inverse relationship between the serum-calcium and inorganic phosphorus and the direct relationship between the serum-magnesium and inorganic phosphorus is only supported in so far as the injection of disodium phosphate

causes a fall in the serum-calcium. The injection of the gluconate, laevulate and lactate of calcium has no constant effect on the serum-inorganic phosphorus, nor does the injection of phosphate affect the serum-magnesium. The injection of the sulphate and lactate of magnesium depresses the serum-inorganic phosphorus, and the relationship is therefore the converse of that observed in the dietary experiments.

4. The above findings are discussed in relation to the concentration of calcium, magnesium and inorganic phosphorus, normally existing in the blood.

5. The injection of sodium sulphate depresses the serum-calcium and inorganic phosphorus but leaves the serum-magnesium unchanged.

6. The injection of sodium chloride has no significant effect on the serum-calcium, magnesium or inorganic phosphorus.

7. Small haemorrhages cause a slight temporary depression of the serum-calcium.

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