TABLE I.
Effect of Withdrawal Following Chronic Morphine Poisoning on the Respiratory Quotient and Metabolic Rate of the Rat.

	No. of animals	Initial		Final	
		BMR	R.Q.	BMR	R.Q.
Normal	12	51.5 ($t = 55.6$)	.829 ($t = 53.9$)	41.95 ($t = 29.4$)	.812 ($t = 36.2$)
Chronic Morphine	11	53.3 ($t = 29.2$)	.830 ($t = 48.08$)	48.86* ($t = 44.1$)	.764* ($t = 94.8$)

* Values obtained 48 hours after withdrawal of the drug which had been administered in increasing doses (from 20 mg/kg/day to 200 mg/kg/day) over a period of 8 weeks.

was using larger doses and younger rats than were employed in these experiments, that the inhibition of growth was not noted sooner.

It is difficult to interpret the changes in basal metabolic rate of the two groups of animals in the experiments reported here. If the final values were the only ones available to compare, it could be concluded that during the withdrawal period there was an increase in metabolism. However, in view of the fact that the metabolic rate of both groups decreased and since the decrease in each is roughly proportional to the gain in weight, it is possible the final difference is merely due to a difference in weight between the two groups. Nevertheless, the lower respiratory quotient and decreased utilization of food for body growth might indicate an increase in metabolic rate which is not apparent from the values obtained for basal metabolic rates. Further experiments are planned to clarify the questions provoked by these results.

Summary. Chronic administration of morphine in doses increasing from 20 mg/kg/day to 200 mg/kg/day over a period of 8 weeks to young albino rats resulted in a marked suppression of growth. This occurred in spite of a greater food intake per unit of body weight. In addition, the animals receiving morphine demonstrated a significant reduction in their respiratory quotients at the 48 hour withdrawal period. The interpretation of the changes in metabolic rate is not apparent on the basis of the results obtained.

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17072. Value of Calcium Hypophosphite and Other Calcium Compounds as Calcium Supplements in Calcium-Low Diets.

ARTHUR E. MEYER AND JACK GREENBERG.
From The Research Division, Fellows Pharmaceutical Laboratories, New York City.

The value of hypophosphites as therapeutic agents has never been objectively demonstrated and Marriott's criticism of their use[1] has largely been accepted. However, calcium hypophosphite is well worth while being considered as a means of convenient calcium administration, where such therapy is desired, because it is high in this element (23%), does not have the objectionable taste of most calcium salts and, in contrast to gluconate, is adequately soluble. The hypophosphite ion, as a stable acid, balances the alkalinity of the calcium, which effect persists even if there should occur a transformation into phosphoric acid. Therefore, the salt does not shift the acid-base equilibrium to the alkaline side as is the case with salts of carbonate producing acids. The original purpose of the present investigation was a comparison of calcium hypophosphite with dicalcium phosphate and calcium gluconate as a supplement in a cal-

[1] Marriot, McKim, *J. Am. Med. Assn.*, 1916, **76**, 486.

cium-poor diet. According to Marriot's report the hypophosphite anion is largely excreted unchanged; for that reason, the hypophosphite P was regarded as non-available in our first tests. When the early observations suggested that this might not be the case, a study of the influence of readily available P and hypophosphite P at varying Ca/P ratios was included.

Experimental. Groups of 7 to 8 Sprague-Dawley rats were placed on a diet prepared according to Sobel, Rockenmacher and Kramer,[2] which consists of 70 parts yellow corn meal, 16 wheat gluten, 10 brewer's yeast, and 1 NaCl with the addition of cod liver oil to supply 0.1 unit vitamin D per gram. Analysis showed a content of 0.03% Ca and 0.33% P, which is in accord with the figures given by these authors. All animals were kept for 14 days on this diet as a depletion period, and the control group for an additional 25 days. Additions of calcium hypophosphite, gluconate and secondary phosphate were used in the first comparative tests (A to E in table). Nucleic acid was used in some groups to equalize the content in available phosphorus without adding a phosphate. The additions in groups F to O served to vary the Ca/P ratios as widely as the P content in the basic diet would permit, with proper distinction of P, known to be utilized, and hypophosphite P.

The rats at the beginning of the depletion period were of an age specified for vitamin D assay in the U.S.P. It turned out that the range of permissible variations in weight and age, as set for that test, was too wide than was best suited for these experiments. The rats of the last group were apparently a few days older (Nos. A_3 to O), and consequently had larger calcium reserves. For that reason, each group can be compared only with its own control group. The animals were weighed at the beginning and end of the 25 days' test period, then killed and the femora dissected. The bones, dried at 80°, were freed of all adhering tissue, weighed, incinerated and the ash determined.

Results. It is evident that the assimilation of calcium is about the same whether supplied as hypophosphite, gluconate, phosphate or carbonate. While 130 mg Ca per 100 g diet is inadequate for maximal calcification of the bones, 530 mg gave results equal to 730 and 1030 mg as shown in Groups I to O. The final body weight at the end of the test period was within the same range in all groups disregarding the calcium intake, with exception of group A_1. While it is possible that the smaller animals were more sensitive to Ca deficiency, it must be pointed out that group B, with equally low Ca feeding, showed only a slight depression in weight. The weight and ash content of the femora reflect more definitely any calcium deficiency than the body weight.

The experiments on Ca/P ratio were limited by the 330 mg P content in the basic diet, which was found to be high enough to prevent any acute P deficiency. As a consequence the results appear to be exclusively a function of the Ca supply. A ratio of Ca/P of 1:2.54 (Group F) was positively not detrimental to bone formation, and neither was one of 1:4.9, (Group H) assuming that hypophosphite P is utilized. This, however, we were unable to prove because of the impossibility to produce a P deficiency with this diet and of the latitude in tolerated Ca/P ratios.

Shohl[3] has claimed that a low Ca/high P ratio lowers the ash content in the bones of the rats. He used, indeed, a ratio of 1:16, but only by sacrificing the absolute Ca content in the diet. When 0.12% Ca was given with 2% P the ash was 36%, but with 0.12% Ca and 0.5% P the ash was the same. This is in keeping with our conclusion that the quantity of Ca and not the Ca/P ratio is the limiting factor. Since, according to Shohl's statement, excessive quantities of phosphorus are toxic, it is difficult to prove a detrimental effect of a low Ca/P ratio at an adequate Ca intake.

Some of the ulnae and radii of the A_1 to E groups were examined by the Ag line test as used in vitamin D assay, but no differences were detected.

It seems that bone formation is not the most

[2] Sobel, A. E., Rockenmacher, M., and Kramer, B., *J. Biol. Chem.*, 1945, **158**, 475.

[3] Shohl, Alfred, *J. Nutr.*, 1936, **11**, 275.

TABLE I.

	Addition to basic diet	Mg Ca per 100 g	Mg P per 100 g	Ratio, Ca/P	Wt. at beginning of test period	Wt. at end	Femora wt., mg	Ratio femora mg to wt. g	% ash in femora
A_1		30	330	1:11	68.0	91.0	301.5	3.13	31.24
					±7.6	±10.8	±22.5	±0.51	±1.71
B	Nucleic acid	30	330	1:13.6	72.5	110.1	320.5	2.91	31.45
			77		±10.1	±14.5	±16.4	±0.30	±0.68
C	$CaHPO_4$	30	330	1:3.13	60.7	122.8	352.7	2.87	41.73
	$2H_2O$	100	77		±3.2	±8.8	±14.6	±0.13	±1.74
D	$Ca(H_2PO_2)_2$	30	330	1:3.13	64.0	134.9	395.6	2.93	40.76
	Nucleic acid	100	77		±3.0	±4.4	±18.8	±0.15	±1.08
			(155)	(1:4.3)					
E	Ca gluconate	30	330	1:3.13	61.1	115.6	378.7	3.28	41.86
	Nucleic acid	100	77		±3.8	±9.0	±23.0	±0.18	±1.53
A_2		30	330	1:11	83.9	131.7	268.4	2.04	30.70
					±7.8	±7.9	±16.5	±0.08	±0.40
F	$Ca(H_2PO_2)_2$	30	330	1:2.54	83.2	135.1	341.5	2.54	39.22
		100	(155)	(1:3.73)	±9.0	±13.8	±27.5	±0.17	±1.27
G	Ca gluconate	30	330	1:2.54	88.4	135.9	329.3	2.42	39.06
		100			±10.8	±9.8	±32.9	±0.19	±1.01
H	$Ca(H_2PO_2)_2$	30	330	1:2.54	83.5	131.4	324.6	2.48	40.49
	$Na(H_2PO_2)$	100	(155)	(1:4.9)	±3.1	±9.2	±17.2	±0.12	±1.29
	H_2O		(155)						
A_3		30	330	1:11	95.2	129.3	231.2	2.56	36.9
					±7.7	±10.7	±39.7	±0.23	±1.2
I	$Ca(H_2PO_2)_2$	30	330	1:0.32	96.0	121.2	472.6	3.93	52.5
		1000	(1550)	(1:1.8)	±8.4	±10.7	±45.1	±0.12	±1.2
K	Ca gluconate	30	330	1:0.32	97.2	128.8	466.9	3.65	51.6
		1000			±5.0	±19.1	±59.4	±0.29	±2.0
L	Nucleic acid	30	330	1:0.61	96.7	125.2	476.6	3.82	53.0
	$Ca(H_2PO_2)_2$	1000	300		±6.4	±9.3	±24.6	±0.24	±0.8
			(1550)	(1:2.0)					
M	Nucleic acid	30	330	1:0.9	96.6	125.3	458.9	3.66	52.4
	$Ca(H_2PO_2)_2$	700	300		±2.8	±11.3	±35.7	±0.18	±1.1
			(1085)	(1:2.4)					
N	$Ca(H_2PO_2)_2$	30	330	1:0.62	101.4	140.3	504.2	3.60	53.2
		500	(775)	(1:2.09)	±7.6	±13.6	±44.9	±0.24	±1.6
O	$CaCO_3$	30	330	1:0.32	90.8	132.8	462.1	3.48	53.4
		1000			±6.4	±8.2	±61.2	±0.10	±2.2

In the columns of Ca and P values, the first figure represents the quantities contained in the basic diet, the following figures are added Ca and P. The parentheses signify hypophosphite P, or in the Ca/P ratio, the inclusion of hypophosphite P in the total P value. The second figures in the data of results are standard deviations $S = \pm \sqrt{\frac{\Sigma(d^2)}{n-1}}$.

sensitive indicator of a deficiency or unbalance of Ca and P. Most of the control rats developed a considerable degree of baldness. This was still more accentuated in Group B, which points to a further damage caused by the change in the Ca/P ratio from 1:11 to 1:13.6. It also appeared markedly in Group K, receiving calcium gluconate without additional P, but was absent in E, where the gluconate was balanced with P. None of the other groups showed loss of hair. By comparing the Ca/P ratio in Group K (1:0.32) with those of the other groups showing baldness (Ca/P, 1:11;1:13.6) it may be concluded that the disproportion in Ca/P ratio to either extreme has a similar effect. The non-appearance of baldness in Group I may point to some assimilation of hypophosphite P, since otherwise this group would have the same Ca/P ratio as K. More data will be required to make a positive statement on this point.

Summary. Calcium hypophosphite is well suited to serve as a supplement for dietary calcium. To what extent the hypophosphite phosphorus is utilized by the organism remains uncertain. The ratio Ca/P in the diet may be varied within a wide range without affecting the calcification of the bones, provided the absolute quantities of each constituent are adequate. Extreme disproportion of Ca and P in either direction seems to cause loss of hair, as was observed in rats receiving either very low or very high relative levels of calcium.

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17073. Extra-Renal Removal of Hemoglobin from Circulation in the Rat. 1. Effect of Parenteral Bovine Albumin.

RICHARD W. LIPPMAN.*

From the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.

Hemoglobin in the circulation, whether resulting from hemolysis of erythrocytes *in vivo*, or introduced by parenteral injection, is removed at least in part by excretion through the kidney. Hemoglobin filtered through the glomerulus may appear in the urine, although part is reabsorbed by the proximal tubule cells.[1-3] That hemoglobin is removed by extra-renal routes, as well, may be inferred from the accumulation of hemosiderin in reticuloendothelial cells, when erythrocytes are destroyed at an abnormally rapid rate.

Parenteral injections of albumin might be anticipated to alter the rate at which hemoglobin is removed from the circulation by extra-renal routes. Since injected albumin is itself removed rapidly from the circulation, a nonspecific increase in protein removal might occur. On the other hand, the mechanism for protein homeostasis might be so saturated that the rate of hemoglobin removal might diminish. Overloading the reabsorption mechanism with bovine albumin is known[4] to affect the renal tubule cells so as to diminish reabsorption of hemoglobin from tubular urine.

Our interest in the extra-renal removal of hemoglobin from the circulation arose during an investigation into the mechanism of proteinuria. Using hemoglobin as an indicator,

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