Ehrlich's reagent according to the technique of Wallace and Diamond. Urine samples which gave a positive reaction before extraction gave the same test after petroleum ether had been used. Petroleum ether, therefore, would apparently serve as a satisfactory method for separating indol from urobilinogen if the former compound occurred in urine. The studies show, however, that need for such a separation occurs seldom, if ever.

In this series containing a fairly large number of patients with intestinal stasis and with jaundice due to various causes, indol could not be demonstrated by a very sensitive qualitative test. While a study of a larger number of extreme conditions might yield some positive results, it is hardly possible that the excretion of indol can serve as a satisfactory method of demonstrating impairment of liver function.

6006

Composition of Bone. XIII. Direct Gravimetric Determination of Ca, Mg, and PO_4.

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The literature contains few analyses of bone accurate enough to serve for the determination of the molecular constitution of the calcium phosphate present. The deviations from the "theoretical" composition obtained by previous investigators have usually been ascribed either to shortcomings of the analytical methods employed or to experimental error. Shear and Kramer[1] pointed out the possibility that these discrepancies may have been due to actual variations in the composition of bone; this possibility has not as yet been ruled out.

It is therefore desirable to obtain data by methods capable of differentiating between these possibilities. The standard macro analytical procedures are not suitable for this purpose.

An extended study was made of the determination of Ca, Mg, and PO_4. Each constituent was studied both in pure solutions and in mixtures. It was found that calcium was completely precipitated in solutions just yellow to thymol blue (pH 3), and that it was completely separated from Mg at this pH. Although both PO_4 and Mg

[1] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1928, **79**, 125.

were present, the Ca precipitate was not contaminated by magnesium phosphate. The Ca was weighed as $CaC_2O_4.H_2O$.

PO_4 and Mg were both weighed as $MgNH_4PO_4.6H_2O$ after reprecipitation.

The effect of varying a number of conditions was studied: volume, temperature, pH, amount of precipitate, excess of reagents, the effect of the presence of citrate, proteins, etc.

Several hundred analyses were performed. With the technique finally adopted, results were obtained for each constituent, in pure solution, that were reproducible to 0.3% and that had an accuracy of 0.3%; equally satisfactory results were obtained in mixtures of all 3 constituents. For solutions containing Ca, Mg, and PO_4 in the proportions in which they occur in bone the procedure may be summarized as follows:

Calcium—Add HCl until the solution is red to thymol blue. Add sufficient oxalic acid to give an excess of between 40 and 140%. To the hot solution add dilute NH_4OH until the full yellow color is obtained. Digest for 3 hr. Filter on a weighed Jena crucible. Wash with dilute NH_4OH. Dry at 105°C. for 1 hr. Cool and weigh as $CaC_2O_4.H_2O$.

Magnesium—Evaporate the Ca filtrate to about 75 cc. Add HCl until a clear red solution is obtained. Add enough H_3PO_4 to give from 10 to 50 times the equivalent of the magnesium present. Add NH_4OH slowly to the blue color; then add NH_4OH in excess. Let stand overnight. Decant and redissolve in HCl. Add enough H_3PO_4 to make the phosphate from 5 to 25 times the Mg equivalent. Reprecipitate as before by adding NH_4OH very slowly. Let stand overnight. Filter on a weighed Jena crucible. Wash with cold, dilute NH_4OH. Then wash with alcohol (redistilled from alkali) and finally with ether (redistilled from alkali). Dry at 37°C. for 1 hr., cool and weigh as $MgNH_4PO_4.6H_2O$.

Phosphate—Evaporate the Ca filtrate to about 100 cc. Add HCl to a red color. Add enough $MgCl_2$ to give from 2 to 4 times the equivalent of the phosphate present. Precipitate by slow addition of NH_4OH as in the Mg determination. Let stand overnight. Decant and redissolve in HCl. Add enough $MgCl_2$ to make the Mg about twice the phosphate equivalent. Add NH_4OH very slowly to precipitate, as before. Let stand overnight. Filter, wash and weigh as in the Mg determination.

Results obtained with known solutions by means of these procedures are illustrated in the table. When large amounts of Mg were taken for analysis, an accuracy of 0.3% and a reproducibility

of 0.3% were obtained, as in the Ca and PO_4 analyses. When one-tenth this amount of Mg was taken for analysis, as in the case illustrated in the table, the same absolute accuracy in milligrams was obtained; the per cent error was of course 10 times as great for a precipitate of 0.03 gm. as for one of 0.3 gm.

TABLE I.
Analysis of Known Solutions.
pH of Ca pptn. = 3.0.

Constituent	calc.	Pure Solution wt. of ppt.	dev.	Mixture of Ca, Mg, PO_4 wt. of ppt.	dev.
	gm.	gm.	gm.	gm.	gm.
Ca	0.3924	0.3915	—0.0003	0.3927	—0.0010
		0.3907	—0.0011	0.3923	—0.0004
		0.3931	+0.0013	0.3946	+0.0009
				0.3939	+0.0002
		0.3918	±0.0009	0.3936	—0.0001
				0.3941	+0.0004
				0.3937	±0.0005
Reproducibility =		0.2%		0.1%	
Accuracy =		0.2%		0.3%	
PO_4	0.3723	0.3721	+0.0009	0.3711	0.0000
		0.3704	—0.0008	0.3716	+0.0005
		0.3710	—0.0002	0.3705	—0.0006
		0.3712	±0.0006	0.3711	±0.0004
Reproducibility =		0.2%		0.1%	
Accuracy =		0.3%		0.3%	
Mg	0.0299	0.0296	0.0000	0.0304	+0.0002
		0.0296	0.0000	0.0300	—0.0002
		0.0295	—0.0001		
		0.0296	±0.0000	0.0302	±0.0002
Reproducibility =		0.0%		0.7%	
Accuracy =		1.0%		1.0%	

When proteins were present in the solution, even in small amount, results of this degree of accuracy were not obtainable.

6007

Increased Growth of a Population of Yeast Obtained with Inosite.

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Following Eastcott's[1] identification of Bios I as inactive inosite it seemed desirable to determine whether or not the medium used in

[1] Eastcott, E. V., *J. Phys. Chem.*, 1928, **32**, 1094.