As to the second possibility, a destruction of the histamine by the high oxygen tension, it might be expected, if this were the explanation of our findings, that there would be a diminution or failure of the histamine reaction to develop when histamine is introduced intradermally into an arm in oxygen. On the contrary, the reaction is obtained even in very high dilutions. Indeed, under these conditions one observes a well-defined flare, a fact also incompatible with Lewis' belief that this part of the "triple reaction" of histamine is exclusively a reflex arteriolar dilatation.

6099

Effect of Salt Concentration on the Colorimetric Phosphorus Determination.

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In studies involving the fractionation of blood phosphate compounds by means of hydrolysis with N HCl certain difficulties were encountered in the determination of the resulting orthophosphate by Kuttner's colorimetric procedure.[1] The results were usually too low, and analyses of known quantities of P under similar conditions likewise gave values below those expected. Although inclined to attribute our difficulties to the losses of HCl during hydrolysis, we had the same trouble even when we prevented such alterations in concentration. We had no more trouble when we substituted N H_2SO_4 for the N HCl. Our analyses on known quantities of P were also entirely satisfactory when H_2SO_4 instead of HCl was used for the hydrolysis. This led us to investigate the probable influence of different salts in high concentration on the orthophosphate values determined by the Kuttner method. We discovered subsequently that Rimington[2] had already pointed out the effect which the concentration of various salts used as anticoagulants may exert upon the quantitative determination of P in blood by Brigg's procedure. We, nevertheless, present these results to call attention once more to this important matter, and thus save other investigators the time and trouble it has cost us to find out this simple

[1] Kuttner, Th., and Cohen, H. R., *J. Biol. Chem.*, 1927, **75**, 517.

[2] Rimington, C., *Biochem. J.*, 1924, **18**, 1297.

fact. The warning is perhaps especially needed now, inasmuch as hydrolysis by N HCl is generally employed in the study of various phosphate fractions in blood or muscle.

On neutralizing this extra acid before carrying out the colorimetric test a rather high salt concentration is produced, which in our experiments, on account of the final dilution used, amounted to 0.2 M. Not all salts interfere with the colorimetric reaction, as will be shown presently, but the chlorides and nitrates do interfere seriously. We determined in a series of special analyses the various concentrations of sulfates and chlorides at which the colorimetric determination of P is no longer accurate. These results are here summarized:

Salt	Interfering Concentrations
NaCl	0.1 M
NH_4Cl	0.2 M
$(NH_4)_2SO_4$	0.5 M
Na_2SO_4	About 3.0 M

Rimington found the NaF in a concentration of 0.01 M interfered with the phosphate determination. We also find that NaCl interferes seriously, 1.0 M concentration causing a loss of 50% in the P recovered colorimetrically. A 0.1 M $NaNO_3$ gives results that are 20% too low. The fact that the sulfate salts, especially the Na_2SO_4, interfered very much less than the chlorides, accounts for our success in using N H_2SO_4 for hydrolysis. The maximum Na_2SO_4 concentration upon neutralization of the acid and upon dilution of the solution is only 0.1 M, whereas our analyses show that this salt does not interfere even in very large concentrations and at 3 M it actually causes an increase in color by about 7%.

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Plasma Protein and Blood Volume.

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Plasma protein deficiency results in a decrease of the osmotic pressure of the blood and has been held to be part of the explanation of edema in nephrosis and certain cases of undernutrition. If this postulation be correct the capillary pressure would be in excess of the osmotic pressure of the blood and as a result of the increased