

pletely sterilized at the end of five hours. Albolene after an initial drop at the end of one hour, showed a marked increase in the number of organisms after that time. Cottonseed oil and peach kernel oil showed an increase of organisms at the end of the first hour. Oil of almonds although greatly reducing the number of organisms in five hours showed a marked increase in organisms before that time. The most striking observation in this study is the antiseptic effect of olive oil. This could not be attributed to impurity or acidity of the oil as only the purest product, neutral in reaction, was employed in the tests. The above findings may be of interest to such clinicians as larynologists, rhinologists and others who have occasion to use solutions of antiseptics and other drugs in oil.

ACTION OF OILS AGAINST STAPHYLOCOCCUS PYOGENES AUREUS

Oil.	1 minute.		1 hour.		3 hours.		5 hours.	
	Number of organisms per c.c.	Per cent.	Number of organisms per c.c.	Per cent.	Number of organisms per c.c.	Per cent.	Number of organisms per c.c.	Per cent.
Olive oil .....	110,000	100	16,500	15.	1,350	1.2	0	0.
Cotton seed oil....	136,000	100	145,000	106.6	30,910	22.7	25,450	18.7
Albolene .....	59,000	100	1,500	25.4	67,500	144.4	67,500	144.4
Oil of almonds....	1,400,000	100	5,150,000	367.8	250,000	17.8	16,000	1.1
Peach kernel oil..	530,000	100	1,160,000	218.8	1,885,000	355.6	440,000	83.0

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## On the estimation of organic phosphorus.

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In attempting to use the methods of Bloor and Bell and Doisy for estimating liopoid phosphorus, difficulty was experienced in securing uniform results. A study of these methods was therefore made using pure inorganic phosphate solutions. Complete recovery was rarely possible and the apparent losses were quite variable. After excluding other steps in the processes as possible

causes of error, attention was given to the ashing process, modifications of the Neumann ashing process being used. In this method, which had given satisfaction for the macro estimation of phosphorus for a long time, two possible sources of error were discovered.

Although when heated alone, phosphoric acid does not volatilize until a temperature of about  $260^{\circ}$  C. is reached, when it is heated with sulphuric acid at  $180^{\circ}$ - $200^{\circ}$  C. it volatilizes slowly in the vapors of sulphuric acid—a fact which the writer subsequently found had been demonstrated by Hillebrand and Lundell. This source of loss was found to occur both in the Bloor and Bell and Doisy methods. In addition, when the latter method of ignition was used, some conversion of ortho to pyrophosphoric acid was usually found to occur because of the very small amount of sulphuric acid used.

The fact that volatilization occurs under the conditions present in the Bell and Doisy and Bloor ashing processes was demonstrated as follows:

One c.c. of a phosphate solution containing 1.0 mg. of P was placed in the bottom of a pyrex test tube  $8 \times 1$  and to it were added a few washed quartz pebbles, 8 drops of sulphuric acid and 1 c.c. of nitric acid as suggested by Bell and Doisy, or 1.5 c.c. of a mixture of equal parts of sulphuric and nitric acids as Bloor uses. The upper part of the tube was then drawn out and bent at right angles and the tubes heated as in the ashing processes of these authors. The vapors were collected in a dish of water. The solution containing the vapors was concentrated on the water bath and the acid neutralized with freshly distilled ammonium hydroxide. The amount of phosphorous was then estimated in the distillate and residue. Losses of from 2 to 15 per cent. were found to occur. A typical experiment with the Bloor ignition method is given:

Amount of phosphate used	1.00 mg.
“ “ “ in distillate	.07
“ “ “ “ residue	.94

When the Bell and Doisy ignition method was used the sum of the phosphorus in the distillate and residue was usually less than the amount started with due to conversion of ortho to pyrophosphoric acid.

To avoid these sources of error, several other oxidizing agents were tried in the hope that a lower temperature for oxidation might be used. Redistilled 30 per cent. hydrogen peroxide was finally chosen. (I am indebted to Dr. I. Greenwald for bringing this reagent to my attention). The material is oxidized in a large test tube covered with a watch glass, with 8 drops of sulphuric acid and 0.2 c.c. of the peroxide. Additional hydrogen peroxide is added if necessary—two or three drops at a time.

From this point various methods may be used for the estimation. We have proceeded as follows: The contents of the tube are washed into an evaporating dish and concentrated on a water bath until most of the water is removed. The solution is then transferred to a graduate and the phosphorus estimated according to the colorimetric process of Bell and Doisy.

For estimating lipid phosphorus of blood, the Bloor extraction has been used while other tissues have been dried with plaster of Paris and extracted with ether and alcohol.

No difficulty has ever been experienced in securing complete recovery from pure phosphate solutions by the proposed process. A comparison of results obtained by this method and by that of Bell and Doisy with inorganic phosphate solutions and tissue extracts follows:

	Proposed method.	Bell and Doisy.
1 mg. sodium phosphate.....	1.01 mg.	0.93 mg.
0.2 mg. sodium phosphate.....	0.20 mg.	0.16 mg.
Tissue Extract A.....	0.31 mg.	0.30 mg.
B.....	0.36 mg.	0.34 mg.
C.....	0.28 mg.	0.24 mg.
D.....	0.15 mg.	0.14 mg.
E.....	3.02 mg.	2.62 mg.
F.....	1.50 mg.	1.35 mg.

It should be added that precautions must be observed in redistilling hydrogen peroxide; the distillation is done under diminished pressure and the distillate protected from coming in contact with rough surfaces or organic matter.

All glassware must be very carefully washed with distilled water and all reagents except the carbonate-sulphite solution must be tested to be certain that they are phosphate free.