SUMMARY AND CONCLUSIONS.

The p_H studies on Milk of Magnesia made by the standard double decomposition and direct hydration methods have been made with the glass electrode and vacuum tube potentiometer.

The p_H of Milk of Magnesia made by direct hydration and stored in ordinary glass bottles for nine months increased but slightly—about 0.07 p_H.

The p_H of Milk of Magnesia made by the standard double decomposition method and stored in ordinary glass bottles for nine months increased considerably—about 0.6 p_H.

The p_H of Milk of Magnesia made by both methods and containing 0.1% citric acid was 10.10. These milks showed no appreciable change in p_H after standing in ordinary glass bottles for nine months.

The p_H determinations on Milk of Magnesia made by both methods and containing small amounts of citric acid have been made.

The addition of citric acid lowers the viscosity of both milks.

The viscosity of Milk of Magnesia made by direct hydration tends to increase while in storage. Citric acid prevents this.

The viscosity of Milk of Magnesia made by both processes and containing small amounts of citric acid are increasingly lowered by prolonged heating.

ACKNOWLEDGMENT.

We are indebted to E. C. Towle of these laboratories for many helpful suggestions.

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AN IMPROVED COLOR TEST FOR VITAMIN A.*

BY A. E. PACINI AND M. H. TARAS.[1]

The Carr-Price reaction which occurs when Vitamin A is added to a saturated solution of $SbCl_3$ has afforded a basis for computing the vitamin A potency of various fish and animal oils. This reaction is not specific for vitamin A, since carotenoids also show the blue coloration and it has the further disadvantage of changing color within thirty seconds. Various substitute tests have been offered among which are the $AsCl_3$-$CHCl_3$ solution (1), $SbCl_3$ plus *o*-dihydroxybenzene in $CHCl_3$ (2), H_2SO_4 (3), trichloracetic acid (4), pyrogallol (5), anhydrous $FeCl_3$ (6), anhydrous $SnCl_4$ (7), anhydrous H_3PO_4 (8), also colors produced by polyphenols and aromatic and heterocyclic amines. Most of the latter produce vague browns, yellows and intermediate shades which might be due solely to oxidation of the yellow colored cod or other fish liver oil (9).

It has been suggested that the color changes produced, especially in the presence of a phenol compound, are the result of a keto-enol rearrangement of the

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phenol (10). It is impossible to state at this time what mechanism causes the color but it is clearly evident that the presence of certain polyhydroxy phenols assists greatly in augmenting color formation. The presence of chlorine in different combinations is seemingly more effective in the production of colors than the individual metal with which it is combined.

The catechol modification of the Carr-Price reaction suggested by Rosenthal and Erdelyi (11) shows a correspondingly deeper color dependent upon the quantity of *o*-dihydroxybenzene used. The resulting violet-red is formed after warming the vitamin-bearing solution with a mixture of *o*-dihydroxybenzene and $SbCl_3$-$CHCl_3$ solution.

We find that vitamin A develops a bright, almost fluorescent, purple with the addition of 1 cc. of $HClO_4$, and 1 drop of guaiacol in $CHCl_3$ solution. This solution develops maximum intensity in 15 seconds and persists two or three minutes allowing ample time to make comparisons. After three minutes the color slowly changes to brown.

A much more stable color is produced when 1 cc. of $HClO_4$, 1 drop of guaiacol[1] and 2 drops of phenol are added to a 5-cc. solution of cod liver oil (3000 A per Gm.) in $CHCl_3$. The color is a brilliant violet. Within a few minutes the color concentrates in the $CHCl_3$ layer and develops to a bright red.

The following table shows various new color reactions produced with vitamin A bearing oils.

TABLE I.

	Reagent.	Cod Liver Oil.	B-Carotene.
1	H_2MoO_4 + $HClO_4$	Light blue to dark blue	Blue green
2	Diphenylamine + $HClO_4$	Purple—fades at once	Dark brown
3	Hydroxylamine + CH_3COCl	+ Phenol ØOH pink	....
4	$SOCl_2$	Red violet	Yellow
5	$SOCl_2$ + Hydroxylamine	Purple	....
6	$H_3PO_4.12MoO_3$	+ Phenol ØOH deep blue	Dark green
7	$POCl_3$	Purple	....
8	CH_3COCl	Violet	....
9	$POCl_3$ + $ClSO_2H$	Violet	Deep blue to green brown
10	$H_3PO_4.12MoO_3$ + $P_2O_5.12WoO_3.42\ H_2O$	Light green	Medium green
11	$SOCl_2$ + CH_3COCl	Bright red to brown	Yellow color bleached
12	$H_3PO_4.12MoO_3$ + Hydroxylamine	....	Medium green
13	$POCl_3$ + $SOCl_2$	Purple	Color is bleached
14	Phenolsulfonic + Phenoldisulfonic	Purple—fades rapidly	Green brown
15	$KClO_3$	Violet	....
16	P_2O_5	Develops light purple	....
17	P_2O_5 + $POCl_3$	Violet	....
18	CH_3COCl + $ClSO_2H$	Dark brown	Blue green
19	CH_3COCl + Phenol	Pink	....
20	$HClO_4$ + Phenol + *p*-dihydroxybenzene	Bright purple to bright red	Purple to red (weak)

[1] A reagent consisting of 8% guaiacol, 17% phenol and 75% $CHCl_3$ can be prepared to facilitate the new test. This mixture keeps for several days. Add an equal volume to the $CHCl_3$ solution of the oil to be tested (usually 1 cc.) and 1 cc. of $HClO_4$.

The suggested improved test[1] has the advantage of not reacting with inactive carotene and not producing the purple color except in the presence of vitamin A. We have tried the reagent with various vegetable and animal oils including hemp-seed, soy bean, linseed, olive, wheat germ, cocoanut, castor, peanut, herring, lard, hydrogenated shortenings, butter, burbot and cod liver oils. Except in the case of butter, burbot and cod liver oil there was no reaction. Olive oil colored a faint blue but so pale as to be ummistakably negative in A.

SUMMARY.

1. The existing $SbCl_3$-$CHCl_3$ solution has several drawbacks as a vitamin A test since other carotenoids show blue coloration with the reagent. Also the color fades too rapidly to allow for accurate comparison of a series of samples.

2. It is thought that a keto-enol rearrangement of phenol may be a determining factor in producing color changes.

3. The presence of chlorine in certain states seems a decisive factor in producing color reactions.

4. A stable purple color which develops into a bright red is produced when 1 drop of a vitamin A oil is reacted with 1 drop of guaiacol and 2 drops of phenol and 1 cc. of $HClO_4$ in 5 cc. of $CHCl_3$. This color seems specific for vitamin A.

5. Other oils lacking vitamin A do not show this reaction.

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[1] The Rosenthal-Erdelyi modification of the Carr-Price test for Vitamin A is based on the addition of catechol to the antimony trichloride-chloroform solution. The Carr-Price test uses only the $SbCl_3$-$CHCl_3$ solution. The investigation revealed that the latter modification, with the catechol, while producing a more stable color did not produce as quantitative a test as did the simple Carr-Price solution.

The author's modification uses the monomethyl-ether of catechol which seems to develop its color in direct proportion to the vitamin A content.

Carotenoids may be defined as pigments containing carotene in one of its isomeric forms. Since only B-carotene splits to yield vitamin A it is obvious that a colorimetric test *must not* give a positive result with an inactive carotene. Vitamin A is a polymolecular alcohol in the form of a yellow *oil*, not crystalline as is B-carotene.