The high results in ether (4 samples out of 5) and the even more markedly low results in chloroform certainly differ from those in alcohol by amounts well outside the limits of experimental error. Chloroform also caused a shift in the position of the maximum to approximately 333 mμ; the other solvents caused no shift detectable with certainty, except that with cyclohexane the maximum appeared to be nearer 330 mμ than 328 mμ. Morton[1] found that chloroform gave low results, but attributed the phenomenon to instability of vitamin A in this solvent. Although vitamin A certainly is unstable in chloroform, this is not sufficient to explain our findings. A chloroform solution of halibut-liver oil gave a value $E^{1\%}_{1cm.}$ 328 mμ = 26·4; the chloroform was evaporated from this solution under reduced pressure and replaced by an equal volume of ether; this ether solution gave $E^{1\%}_{1cm.}$ 328 mμ = 31·7; a direct solution in ether gave $E^{1\%}_{1cm.}$ 328 mμ = 31·8.

The materials listed in the table fall into two groups, comprising on one hand the first two oils, and on the other the third oil and the two concentrates. Relative in each instance to the absorption in alcohol, the first group shows greater enhancement of the absorption in ether and less depression in chloroform than does the second group. These irregularities are probably due to differences in the proportion of *cis-trans* isomers of vitamin A. These isomers may not only have different absorption coefficients in any one solvent, but also the ratio of these coefficients may vary from solvent to solvent. Possible differences between the biological activities of the isomers may help to explain the various factors reported relating $E^{1\%}_{1cm.}$ 328 mμ to biological units.

We have further observed that irradiation of solutions of vitamin A sometimes increased and sometimes decreased (by so much as 40 per cent) the absorption at 328 mμ, but that the absorption almost returned to the original value after the irradiated solution had stood in the dark. Variations in the proportion of *cis-trans* isomers might explain these results and also some of the spontaneous increases as well as decreases in absorption of liver oils and concentrates during periods of storage which have been observed by ourselves and others (*cf.* Heilbron, Gillam and Morton[2]; *cf.* also the experiences of Holmes and Corbet[3] with crystalline vitamin A and of Zechmeister and Tuzson[4] with lycopene).

It is intended to publish full details elsewhere of the work summarized here and additional work now in hand.

Note added to proof. Since submitting this letter for publication we have learned that Dr. A. E. Gillam and Dr. M. S. El Ridi have—independently of ourselves—studied the effect of solvents on the vitamin A absorption spectrum.

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[1] Morton, private communication.
[2] Heilbron, Gillam and Morton, *Biochem. J.*, **25**, 1352 (1931).
[3] Holmes and Corbet, *J. Amer. Chem. Soc.*, **59**, 2042 (1937).
[4] Zechmeister and Tuzson, NATURE, **141**, 249 (1938).

THE above authors have been good enough to show me their letter. The 1934 Conference was concerned with a substitute for the bio-assay with its experimental error of perhaps ± 25 per cent for individual assays. The fact that variations of a few per cent for spectrophometric assays in different solvents like alcohol and cyclohexane are now being noted is an indication of the progress made. Such variations are usual for polar molecules and do not invalidate the spectrophotometric method.

The possibility of variable proportions of geometrical isomerides in different vitamin A containing materials has been obvious since the polyene formula was established. So many isomerides are theoretically possible with five ethenoid linkages (four in a straight chain) that the problem of their occurrence has long defied experimental attack, and it has been reasonable to assume a balancing of effects.

With one double bond as in *cis* and *trans* stilbene and cinnamic acid[1], differences in ε max. are of the order 2 : 1, an effect much larger than that observed except in the valuable irradiation experiment.

I have approached the problem from another angle. Hitherto the ultra-violet absorption (328 mμ) and the $SbCl_3$ colour test maximum (620 mμ) have shown a nearly constant ratio $E^{1\%}_{1cm.}$ 328 mμ/620 mμ of 0·32 for rich preparations. A halibut intestinal oil prepared by Dr. Lovern has been subjected to molecular distillation by the courtesy of Dr. F. H. Carr of Messrs. British Drug Houses, Ltd. In the different fractions the ratio varies from 0·21 to 0·38 and for a range of very rich ester fractions it is *c.* 0·28. A partial separation of *cis-trans* isomerides is a plausible explanation.

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[1] Smakula and Wassermann, *Z. phys. Chem.*, A, **155**, 353 (1931).

Specificity of Disaccharide-splitting Enzymes

DISAGREEMENT exists concerning the substrates which maltases of different sources can hydrolyze. According to the view advanced by Leibowitz[1], there occur in Nature two types of maltase, a gluco-maltase and a glucosido-maltase respectively. The gluco-maltase which occurs in malt and in moulds splits maltose but is inert towards alpha-glucosides and sucrose; the glucosido-maltase which occurs in yeast splits alpha-glucosides as well as maltose. Different investigators[2] have applied the two maltase classification over a wide range of maltases from various sources. Weidenhagen[3], however, has denied the validity of this view, and claims that in sufficient enzyme concentrations, even malt and taka-diastase hydrolyze alpha-methyl-glucoside. All maltases in Nature are said therefore to be glucosido-maltases; they are considered to be identical with the gluco-sucrase among the sucrases, and to split alpha-glucosides and sucrose as well as maltose.

In the course of a series of tests on the heat stability of different lots of taka-diastase, taka-maltase preparations were found which were peculiarly resistant to inactivation by heat. This property has enabled us to make additional observations on the question of the specificity of taka-maltase. If a solution of the enzyme is heated to boiling point, these resistant preparations lose their ability to split sucrose and also what slight activity they formerly possessed to