tribution could be discovered, when the differences in sensitivity of the assay methods for vitamin H and for biotin and coenzyme R were considered. The solubilities of these three factors in various organic solvents, their heat stability, low molecular weight, adsorption phenomena, stability toward acid and alkali and the effects of reagents such as nitrous acid, benzoyl chloride, acetic anhydride and lead acetate, all pointed to the probable identity of the factors. The strikingly parallel distribution of biotin, coenzyme R and vitamin H activities in the electrodialysate fractions from our vitamin H liver concentrates added particularly convincing evidence for the identity of the three factors. Additional work on biotin and vitamin H which we have since carried out brought still further confirmation.

Conclusive proof of the identity of these principles, as we previously noted, had to await the testing of the pure substances for the mutual activities. This has now been facilitated through the kindness of Professor F. Kögl, who has placed at our disposition for vitamin H assay a solution of 150 γ of crystalline biotin methyl ester in ethyl alcohol. We wish to take this opportunity to express our sincere appreciation for this generosity and cooperation.

In order to obtain some indication of the level at which the biotin methyl ester should be tested for vitamin H activity by the rat assay method, the yeast-growth activity of this solution was compared with that of a solution of known vitamin H activity. A modification of the method of Snell, Eakin and Williams[7] was employed, using *Saccharomyces cerevisiae* Strain 139 as the test organism. Yeast growth was determined by turbidity measurements in the Klett-Summerson photoelectric colorimeter. The curve obtained by plotting turbidity readings against the logarithm of the concentration of the substance being assayed was used to determine the concentration at which half the maximum increase in growth occurred. The half-maximum growth increase was found to be more accurate than either maximum or minimum growth concentrations for comparison of various samples in the calculation of activity. It was found that biotin methyl ester produced a half-maximum growth increase at a concentration of 1 part in 4×10^9. A vitamin H preparation containing 34 units of vitamin H activity per mg produced the same yeast-growth effect at a concentration of 1 part in 1.36×10^7. It could be predicted, therefore, that the biotin methyl ester should show an activity of approximately 10,000 units of vitamin H per mg by the rat assay method if biotin and vitamin H were identical.

Twenty-six rats showing definite vitamin H deficiency symptoms[2,8] were used for assay of the solution of crystalline biotin methyl ester at various levels above and below the amount indicated by the yeast assay. Subcutaneous administration of the biotin ester brought about complete cure of the skin manifestations and resumption of growth in these animals. The minimum effective dose, within the limits of assay error, was found to be 0.1 γ per rat per day for 30 days. This corresponds to an activity of 10,000 units of vitamin H per mg for the methyl ester of biotin. The most potent vitamin H preparation hitherto reported[1] possessed an activity of 215 units per mg.

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MOBILIZATION OF VITAMIN A BY ALCOHOL

THE important observation of Clausen *et al.*[1] that, in dogs, vitamin A is mobilized from tissue stores by ethyl alcohol, as shown by actual blood analyses, probably applies also to humans. The Pett visual test for vitamin A deficiency,[2] has been correlated with blood analyses.[3] Using this instrument, routine daily tests have been carried out on many people during the past two years. On several occasions unaccountably short recovery times (indicating higher blood vitamin A levels) have been observed the day following the taking of alcohol. Table I shows a few such incidental observations.

TABLE I

Subject	Recovery Times			
	Previous average	1st day after alcohol	2nd day after alcohol	7th day after alcohol
	seconds	seconds	seconds	seconds
A	9	5	6	8
B	10	7	8	9
C	15	8	..	12
D	19	9	9	16

* Smaller values equal higher blood vitamin A.

The observations of Clausen and his colleagues would now appear to explain these findings.

L. B. PETT
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