the plastic intensifies the color. Hematoxylin has been tried, but has not produced a satisfactory stain. The difficulty lies in adjusting the intensity of the stain, which is increased by the plastic.

(3) The specimens are dehydrated in a series of alcohols (70, 83 and 95 per cent and absolute), the time depending upon the size of the specimens.

(4) The next stage of the process—removing the alcohol—presented many difficulties. At first, various compatible solvents were tried, but these proved unsuccessful. The plastic in and around the specimens was diluted, producing internal strains resulting in cleavages and fractures in the blocks. To overcome this, the specimens are transferred from absolute alcohol to anhydrous ether. Here again the time is varied from an hour for early chick embryos to several hours for larger specimens. The anhydrous ether displaces the alcohol and removes any traces of water from the specimen.

(5) The specimens are next transferred into the uncatalyzed plastic and evacuated in a vacuum desiccator to draw off the ether and allow the plastic to impregnate the specimen. This must be done very slowly and carefully until a vacuum is produced (500–700 mm. of Hg.) and left to stand until all the ether has evaporated. Too rapid evacuation causes the ether to boil, destroying the specimens.

(6) A quantity of the monomer is weighed and the catalyst (tertiary butyl hydroperoxide) added in the proportion of 0.1–0.5 per cent, depending upon the thickness of the block. The lesser percentages of the catalyst are used as the thickness of the block increases. The catalyst is stirred completely into the monomer and allowed to stand until all bubbles rise to the surface and break.

(7) A supporting layer of the catalyzed monomer is poured into a Pyrex dish or tray and allowed to gel at room temperature, until it is firm enough to support the specimens. This requires from 1 to 3 hours.

(8) The second layer is prepared in the same manner and poured onto the first.

(9) The specimens are transferred from the uncatalyzed monomer to the trays, care being taken to carry over as little as possible, and are then oriented on the first layer.

(10) The trays are left to stand at room temperature until the second layer has gelled.

(11) After the gelation is complete, the trays are placed in an oven to cure for approximately 2–4 hours. The heat is raised slowly from 100° F. to 250° F. and maintained at 250° F. for ½–1 hour.

(12) After cooling, the blocks are easily removed by merely inverting the trays and allowing the blocks to drop out.

The plastic, being thermosetting, does not require any elaborate or special equipment for cutting or polishing. A fine-toothed band saw is used for trimming and cutting, while polishing is done on a soft-rag wheel using tripoli and rouge.

The process, as outlined above, has worked successfully on chick embryos, pig embryos, jellyfish, flukes, and other similar forms. While no extensive experimental work has been done on forms which may present different problems, we feel confident that in time these other forms can be embedded in this type of resin.

In general, the unsaturated polyester resins are a light-yellowish color, but some of these are available in a purified form; while not as water-white as the methacrylates or styrene, these have the advantage of a much simpler curing schedule. Also, the polyesters are more resistant to abrasion than the methacrylate. Occasionally, the blocks will have to be repolished. This can be done easily on a soft-rag wheel or by hand, using pumice paste, finishing with rouge, and then wiping with a waxed cloth. It will be found that by using a little care in handling, much unnecessary work can be avoided.

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A New Spectrophotometric Method for the Determination of Vitamin A[1]

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The spectrophotometric determination of vitamin A is not generally applicable to materials low in vitamin A and containing appreciable amounts of other nonsaponifiable materials (*2*). In an attempt to develop a method which would be suitable for the determination of vitamin A from tissues of vitamin A-depleted animals, we have examined the possibility of purifying the nonsaponifiable fraction by chromatographic methods before spectrophotometry. We wish to report briefly on the difficulties encountered in chromatography, the use of floridin in vitamin A analyses, and the application of this technic to margarine.

[1] This work was supported by a grant from the Nutrition Foundation.

Adsorption of the nonsaponifiable fraction from petroleum ether solutions has been studied, using MgO according to the method of Wilkie and DeWitt (*4*) and alumina columns. In all instances difficulties were experienced in obtaining satisfactory chromatograms when margarines or vegetable oils containing vitamin A were used. It was found that sufficient sterols were present in the petroleum ether extract of the nonsaponifiable fraction to act as an eluting agent and thus prevent the formation of sharp rings which could be observed in ultraviolet light. Interference due to the sterols could be avoided by treatment of the nonsaponifiable extract with digitonin or by chilling the extract in methanol in a mixture of dry ice and ethyl alcohol and filtering before chromatography. Subsequent chromatograms were sharp, but material subsequently removed from the column by elution or by cutting the column did not give a typical vitamin A curve. The interfering material appeared to consist of tocopherols and their breakdown products, and we have not been able to achieve any satisfactory separation of these compounds from vitamin A on such columns.

Although moderately satisfactory results could be obtained by correcting the spectrophotometric curves for the tocopherols estimated from the extinction at 293 mμ, we felt that it would be worth while to explore the possibilities of floridin (floridin XXS, 60–80 mesh),[2] which has been employed to remove vitamin A and sterols from tocopherols (*1*). Although we were able to confirm the observation that vitamin A can be quantitatively adsorbed from floridin with benzene, and the tocopherols quantitatively recovered in the filtrates, we have been unable to recover the vitamin A from the floridin. This is doubtless due to the reaction which takes place on floridin (*3*). Since vitamin A is the only material having an absorption between 300 and 350 mμ which is adsorbed on floridin, this treatment offers a method for differential determination of vitamin A.

The following method has been applied to margarines and vegetable oils. A 5-gram sample is saponified for 30 minutes with 5 ml. of 50 per cent KOH and 20 ml. of 95 per cent ethyl alcohol. The hydrolysate is made to a total volume of 80 ml. with water and alcohol to give a final alcohol concentration of 50 per cent. Extraction is carried out for 3½ hours with 120 ml. of Skellysolve A in a continuous extracter. The petroleum ether extract is washed twice with water, three times with 0.5 per cent sodium hydrosulfite, and four times more with water. The petroleum ether is then removed by evaporation in a stream of nitrogen, and the residue is taken up in 10 ml. of benzene. Five ml. of this solution are diluted to 25 ml. with benzene, and the spectrophotometric measurements are made at 300, 332, and 350 mμ. The remaining 5 ml. of benzene solution are poured onto a layer of purified floridin, 4 mm. thick, in a chromatographic column, 20 mm. in diameter. The floridin is first wet with 5 ml. of benzene and the extract added carefully before the benzene has disappeared from the column. (When considerably thicker layers of floridin were used, it was found that substances other than vitamin A were removed from the extract and the results were inaccurate.) The column is then washed with about 15 ml. of benzene, the combined washings are made up to a volume of 25 ml., and readings made on the spectrophotometer at the wave lengths indicated above. The calculation of vitamin A concentration is made from the difference between the readings for the two solutions at 332 mμ, and the differences between the readings at 300 to 350 mμ serve to demonstrate the completeness of removal of the interfering substances (Fig. 1). In calculating the vitamin A it must be remembered that its absorption curve in benzene dif-

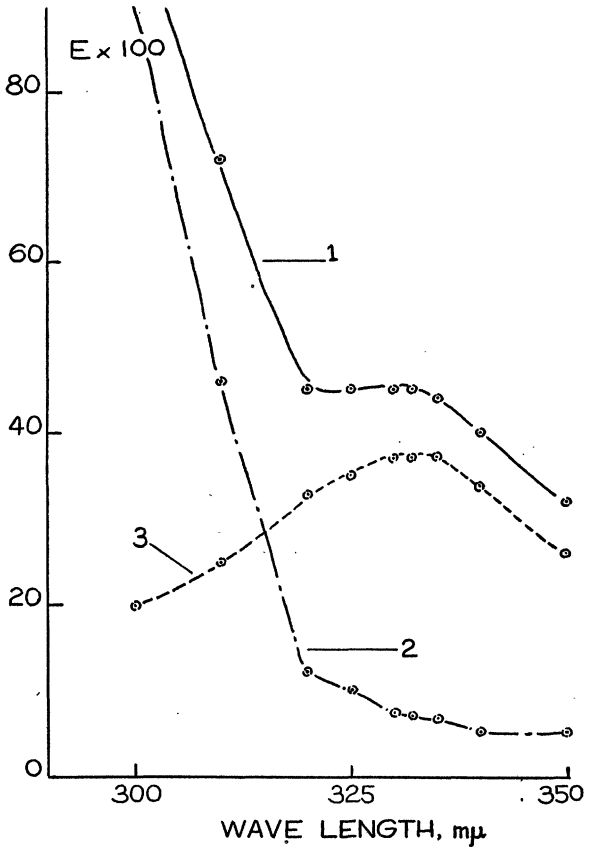

FIG. 1. The extinction (× 100) of the nonsaponifiable extract of margarine in benzene (Curve 1), of same extract after floridin treatment (Curve 2), and of vitamin A obtained by difference between Curves 1 and 2 (Curve 3).

[2] Obtained from the Floridin Corporation, Warren, Pennsylvania. This was purified as described by Emmerie and Engel (*1*).

fers from the curves in petroleum ether or alcohol, and we have used the relation

$$\text{Vitamin A in I.U./gram} = \frac{(\text{extinction difference at } 332\ m\mu)\,(50)}{0.0442\ (\text{weight of sample})}.$$

solved; the factor 0.0442 is the value taken for the extinction of a solution containing 1.0 I.U./ml. This factor is based on the usual conversion figure of 2,000, corrected for the difference between the absorption in benzene at 332 mμ and petroleum ether at 328 mμ.

TABLE 1

THE VITAMIN A CONTENT (I.U.) OF MARGARINE AS DETERMINED IN SUCCESSIVE DETERMINATIONS WITH THE FLORIDIN TECHNIC BASED ON THE $E^{1\%}_{1\ cm.}$ AT 332 mμ

Sample No.	Vitamin A in 5 grams	Vitamin A added	Extra vitamin A recovered		Margarine vitamin A/pound	
			Total	%	Uncorrected	Corrected average
1, 1	151,169	191*	159,171	83.3, 89.6	13,700, 15,300	16,800
1, 2	161,165	245	200,214	81.6, 87.3	14,600, 15,000	17,600
2, 1	156,159	254	209,219	82.3, 86.3	14,200, 14,400	17,000
2, 2	154,161	257	180,212	70.1,† 82.5	14,000, 14,600	17,300
2, 3	166,170	255	217,231	85.2, 90.5	15,100, 15,400	17,300
Average				85.4	14,630	17,200

* Fish-liver oil added in this test; in other experiments, pure vitamin A alcohol was used.
† Not considered in average or in calculation of corrected average of margarine vitamin A.

The extinction difference is presumed to represent the extinction due to the vitamin A removed by the

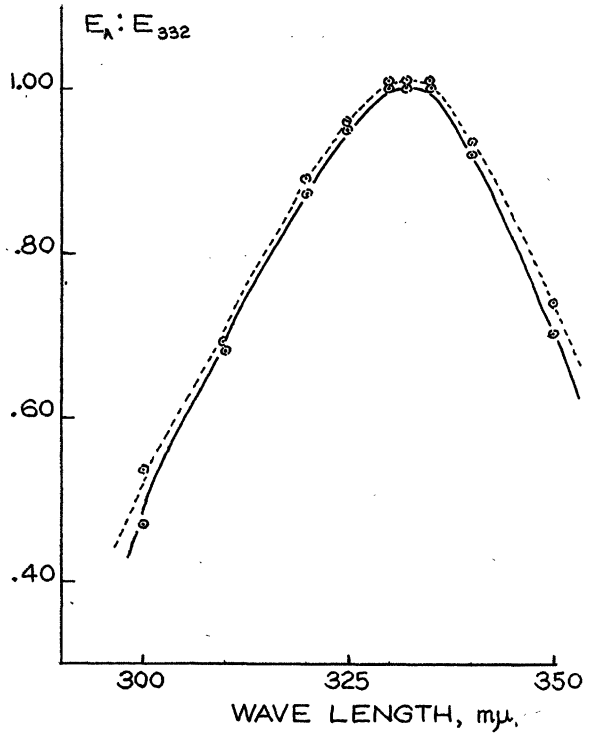

FIG. 2. The ratio of E_λ to E_{332} for vitamin A alcohol (Distillation Products) in benzene (solid line) and for margarine "vitamin A" obtained by difference between absorption of untreated nonsaponifiable fraction and fraction treated with floridin (dotted line).

floridin; the factor 50 corresponds to the final total volume in which the nonsaponifiable fraction is dis-

The validity of the method may be judged by the identity of the difference curves for the "vitamin A" of margarine determined by this procedure and for vitamin A alcohol (Fig. 2).

That the method is satisfactory is also indicated by the uniformity of the uncorrected values for vitamin A in the different samples of margarine. These unselected results obtained on consecutive days indicate that the results are reproducible. Although the corrected figures for vitamin A are based on a recovery factor of only 85 per cent, it is believed that by better extraction procedures this can be considerably improved. However, with the present method using a standardized procedure, a determination with a probable error of ±2 per cent is available after correction for recovery obtained with added vitamin A.

Addendum. With more efficient extractors which we are now employing, we have been able to obtain consistent recoveries of better than 95 per cent of added vitamin A. Uncorrected values for vitamin A in margarine with the new equipment were found to average 18,200 I.U./pound.

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