parently different results obtained from the two sexes (see Tables 1 and 2).

Table 2 indicates that the influences of anoxia upon I^{131} in unfractionated plasma, in thyroid, or in urine were not as striking as the effect upon protein bound I^{131} of plasma (Table 1).

The data in Table 1 may result from an almost complete suppression of a portion of the thyroid activity under these experimental conditions. More detailed experiments are necessary to substantiate such a conclusion.

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The Use of Detergents for Quantitative Fat Determinations
1. Determination of Fat in Milk[1]

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The determination of fat content has long been an important aid in clinical investigation and in the dairy industry, involving in most instances the use of long, technically difficult procedures and extensive laboratory equipment. Babcock's method and modifications thereof are widely used in the dairy industry because of their comparative simplicity, but even these have certain disadvantages. Sulphuric acid, used in the tests, often causes charring and consequent inaccurate results; caution must be observed in handling the acid and glassware to prevent burns; and as many as three different centrifugations must be carried out for each test. The method outlined in this paper was created to obviate these difficulties.

In this investigation a comparatively new principle is used—formation of a protein-detergent complex to break up emulsions and liberate the fat contained therein. The dispersion principle of detergents was originally used clinically to increase the bactericidal effect of antiseptics (*2*) and is now also used in the laboratory to dissolve sputa for the purpose of isolating and concentrating tubercle bacilli (*3*). The combined solvent action of the two detergents described here has resulted in a very satisfactory method for the determination of fat in milk and shows promise as a universal procedure for the measurement of fat.

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Materials. (A) A supersaturated solution of a fat dye is prepared by mixing 500 mg of oil red O in 100 ml of isopropyl alcohol. Two and one-half ml of the clear solution is added to a mixture of 100 g of a standardized nonionic detergent, polyoxyethylene sorbitan monolaurate, and 65 ml of 95% ethyl alcohol, and the new mixture is shaken. (B) The other reagent is a standardized anionic detergent, dioctyl sodium phosphate.

Procedure. (1) A well-mixed sample (17.6 ml) of milk is placed in a Babcock milk bottle. (2) Seven ml of solution (A) is placed in the vessel, which is then shaken immediately to mix the contents intimately. (3) Thirteen ml of reagent (B) is added without further shaking so that this last addition forms a layer at the bottom of the container. (4) The container is immersed in a water bath at 180° F. The water level in the bath should be approximately equal to that in the bottle. (5) After 5 min in the bath, water at 180° F is added to the Babcock flask until the fluid level reaches the top of the graduated portion. (6) The flask is then set aside at room temperature for 10 min, after which the percentage of fat (as in the Babcock test) is read by subtracting the lower meniscus reading from that of the upper meniscus.

One hundred duplicate samples of milk were tested for quantitative fat content. The readings obtained by this method were the same as those resulting from the Babcock test. Detailed comparison of readings obtained with various types of milk, and comparison of methods for cream and ice cream will be presented soon.

The test is based primarily upon three factors: (1) a proper order in mixing of reagents and specimen to obtain maximum effect of the reagents individually and combined; (2) correct proportioning of the reagent emulsion complex by adjusting the quantity of the nonionic detergent to the type of emulsion (milk, ice cream, etc.) from which the fat is to be extracted; and (3) a proper temperature at which the reaction takes place optimally. In addition to these factors, intervals between steps should not be prolonged; the shaking, when prescribed, should be sufficiently thorough to distribute the contents evenly; and the time of reaction should be exact.

Milk fat exists in milk in the form of minute globules constituting a true emulsion of the oil-in-water type, the fat globules being in the dispersed phase. Each globule of fat is surrounded by a very thin film of protein in the serum of the milk, concentrated on the surface and held in place by surface attractions or adsorption. The protein layer may contain some lecithin to form a protein-lecithin layer on the surface of the fat molecule. This concentration of the milk proteins around the fat globule is one factor which assists in maintaining the stability of the fat emulsion in milk.

Dioctyl sodium phosphate is an anionic compound in which the hydrophilic portion is negatively charged. It has a long chain of carbon atoms with a strong polar group located near the center of the carbon chain. With a given chain length of a detergent, the position of the hydrophilic groups is an important variable in the determination of the surface active properties of the resultant

molecule. Dreger and his co-workers (*1*) showed that the activity of detergents increased when the hydrophilic groups were attached near the center of the carbon chain.

Mixing this detergent with milk and heating the combination effects a dispersion of the protein layer around the fat globule, liberating the fat so that it can combine with other fat molecules. However, the separation is not complete. When a quantity of the strongly hydrophilic, nonionic detergent is added to the mixture, a clear solution and complete separation results.

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On the Food Selectivity of Oysters

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The question whether oysters and other closely related mollusks can, in selecting their food, discriminate between the different types of microorganisms has been debated since the end of the last century, but no general agreement has been reached as yet. Among others, Lotsy (*5*) and Grave (*2*) thought that, in their feeding, oysters show a definite selection of particles having food value. Another group, represented by Kellogg (*3*) and Yonge (*7*), maintains that in lamellibranchs the selection of particles is purely quantitative. Yonge (*7*) thinks the main objective of this selection is "the reduction of the quantity of matter passed to the mouth, large particles or many small particles embedded in mucus being rejected and smaller particles or mucus masses passed on to the mouth quite irrespective of their food value."

My observations and experiments make me agree with Lotsy and Grave that oysters (*O. virginica*) do show some selectivity in feeding. In several of our feeding experiments in which yeast cells in very small numbers were added to running sea water many oysters rejected most of the yeast in pseudofeces, while the true feces were composed largely of plankton forms and detritus normally present in our waters (Loosanoff and Engle, *4*). It is significant that the yeast cells were rejected even if their size (about 5 μ diam) was equal or even smaller than that of the many forms ingested by oysters. This clearly indicated that the discrimination against yeast cells was not based upon their size.

Recently I had the opportunity to observe even more striking cases of selectivity shown by oysters in their feeding. In the summer and fall of 1948, during periods when sea water contained relatively little food material, we were adding at a constant rate small quantities of plankton culture to the water flowing into the trays containing experimental oysters. This culture, which was grown outdoors in a 3000-gal wooden tank, contained a variety of different algae, flagellates, and bacteria. The color of the culture was usually light brown or a purple-brown. In feeding this culture to the oysters I noticed that in many cases the pseudofeces formed were purple or pink, while the color of the material that was swallowed by the oysters and passed through the digestive system was greenish-brown.

Microscopic examination showed that the purple pseudofeces consisted principally of a round-shaped form measuring 2 to 3 μ diam. The true feces, on the other hand, consisted of plankton normally present in our water and of relatively small numbers of the purple form. On the basis of morphological examination, this form has been tentatively identified by S. F. Snieszko of our service as being a species of the genus *Chromatium perty*, which contains purple sulfur bacteria.

On several occasions I was able to grow, in flasks, cultures consisting predominantly of chromatia. The cultures developed best if placed near a southern window in strong light. The color of the good cultures was almost purple. When these cultures were added to the greenish-brown cultures fed to the oysters the latter soon formed purple pseudofeces composed largely of chromatia, while the feces remained a normal, greenish-brown color.

I have noticed that the most energetic rejection of the purple form by oysters took place usually during the first few days after it was added to the water. Later on, some of the oysters evidently developed tolerance to this form and ingested it without apparent discrimination. As usual, the oysters showed considerable individual variations in their feeding behavior, i.e., while some of them ingested *Chromatium* within a few hours after it was first added to the water, the others continued to reject it even after several weeks of contact.

Because *Chromatium* is smaller than many other forms ingested by oysters, we cannot ascribe its rejection to its size. It is more probable that as Cobb (*1*) has shown for *Anodonta*, the palps of which responded to a variety of stimuli, including those of a chemical nature, the palps of oysters may possess specialized cells which act as chemoreceptors, and may be sensitive not only to the physical characters of plankton forms, such as their size and shape, but also to their chemical properties. Nelson (*6*) says that feeding of oysters also is a complex process involving the interaction of the muscular, ciliary, secretory, and nervous tissues. Thus, I think, the selection of food may be based in part on the nature of the secretions of different species of microorganisms reaching the palps, and therefore, as the observations on the rejection of chromatia indicate, at least in some instances, oysters can select their food not only quantitatively but also qualitatively.

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