if not completely, interfered with the ordinary depressive influence of α-estradiol[3] on the rat prostate. (Table 1, exp. 1.) This finding in a sense confirms, but also extends Hertz' recent observations in the chick (*2*). 2. Furthermore, the folic acid antagonist possessed no androgenic potential itself, as judged by its inability to stimulate prostatic growth in castrate adult, and intact immature rats. (Table 1, exps. 2A and 2B.) 3. Lastly, no interference with androgen[3] stimulation of tissue growth is occasioned by the folic acid antagonist in castrate adult or intact immature rats. (Table 1, exps. 2B and 3.) From this we may reason that folic acid is not a prerequisite for hormonally induced growth in general, but is only essential for the proper utilization of estrogen, either in a stimulant or depressant capacity.

Further investigation of this problem is in progress, using other folic acid antagonists than the one employed here. These studies will be reported subsequently in a paper which will also include complete histological details.

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Metabolism of I^{131} in Severe Anoxic Anoxia

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Clinical reports (*3, 4, 6*) have indicated symptomatic improvement of hyperthyroid patients who sojourn at high altitudes. Rats exposed to severe anoxic conditions demonstrated an oxygen consumption 40–50% of normal (*1, 7*). These results suggest involvement of the thyroid gland in accommodation to anoxia. The present investigation was undertaken to determine whether any significant alterations in metabolism of radioactive iodine could be detected in relatively acute exposures to severe anoxic anoxia.

Eighteen rats were fed a low iodine diet (*5*) for 8 weeks, after which half of the animals served as controls and the other half were exposed to 268 mm Hg air pressure (which stimulated a 27,000-ft altitude) at 15–20° C. The low pressure rats were subjected to this anoxia for 12 hr and were then returned to sea level for injection of I^{131}. Within 3 min they were re-exposed to the low pressure for an additional 24 hr, after which they were sacrificed. The sea level control animals were also kept at 15–20° C and likewise injected with I^{131} 24 hr before they were sacrificed. All injections of radioactive iodine consisted of 10 μc in 0.5 cc of physiological saline (pH = 8.0) administered intraperitoneally. In each rat the total I^{131} was determined in the thyroid gland and in a sample of plasma.

[3] Crystalline α-estradiol and testosterone were obtained through the courtesy of the Schering Corporation. The daily dose of these substances was 0.1 mg in sesame oil; the same injection schedule was used as that for aminopterin.

TABLE 1

FRACTIONATION OF PLASMA RADIOACTIVE IODINE

Experimental conditions	Male rats		Female rats	
	% Plasma I^{131}			
	Filtrate*	Ppt.†	Filtrate*	Ppt.†
Sea level controls	22	78		
	22	78		
	14	86	62	38
	47	53	46	54
	26	75	46	54
avg	26	74	51	49
Anoxic rats	88	12		
	90	10	98	2
	95	5	98	2
	95	5	97	2
avg	91	8	98	2

* Trichloro-acetic acid filtrate.
† Trichrolo-acetic acid precipitate (protein bound fraction).

Plasma I^{131} was fractionated by the addition of 5 volumes of 10% trichloroacetic acid to separate "protein bound" I^{131} (*2*). Urinary excretions from the two groups of male animals were pooled as two samples. The data are summarized in Tables 1 and 2.

The anoxic rats demonstrated a pronounced reduction in protein bound I^{131} in plasma. The average values expressed as percent of plasma I^{131} for male animals were as follows: trichloro-acetic acid filtrate of plasma of control rats 26%, and of the anoxic rats 91%; trichloroacetic acid precipitate of plasma of control rats 74%, and the anoxic rats 8%. In the case of female rats, the anoxic animals demonstrated an even greater depression of the protein bound I^{131} fraction, but the control values were also somewhat different from those of the male animals. It appears inadvisable to account for the ap-

TABLE 2

DISTRIBUTION OF I^{131} IN URINE, PLASMA AND THYROID GLAND

Experimental conditions	Male (10 rats 170–230 g)			Female (6 rats 140–180 g)		
	% of injected dose I^{131}					
	Total urine (pooled*)	Plasma (1 cc)	Thyroid gland	Total urine	Plasma (1 cc)	Thyroid gland
Sea level controls	30	0.78	76	(Not determined)	0.23	50
		0.64	76		0.23	53
		0.78	40		0.38	
		0.78	55			
		0.57				
		0.14				
avg		0.62	62		0.28	52
Anoxic	22	0.57	53	(Not determined)	0.57	31
		0.64	43		0.67	28
		0.16	46		0.57	28
		0.71	66			
avg		0.52	42		0.60	29

* Pooled urine sample, calculated as % of dose per rat.

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.

[1] Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

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