into fat molecules may have been much faster than the possible passive diffusion of acetate through the extravascular fluid. Second, the rate of acetate utilization for biosynthesis and catabolism in cells adjacent to the milk cistern might be so great that there is a steep diffusion gradient down towards that area, so that the number of acetate molecules moving in the opposite direction is small.

Summary. 1. 2-C^{14} labeled acetate was injected into the milk cistern of the right front quarter of a lactating cow. This quarter was subsequently milked separately from the other 3 quarters and radioactivities of milk fat constituents from each were determined. 2. The data confirm that there is a delay of several hours between synthesis and secretion of milk fat. 3. A surprisingly small amount of the injected acetate diffused into the other 3 quarters or into the rest of the body. This can be accounted for by its rapid utilization for milk synthesis near the site of injection. 4. Fatty acids of all chain lengths and glycerol seem to be synthesized from acetate in the mammary gland itself. The glycerol synthesis, however, is on a smaller scale.

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Further Studies of Prothrombin Derivatives.* (23060)

RICARDO H. LANDABURU AND WALTER H. SEEGERS

Department of Physiology and Pharmacology, Wayne State University College of Medicine, Detroit, Mich.

Activation of purified prothrombin to thrombin can be achieved with the use of several combinations of materials separated from natural sources. All these "activator" factors are dispensable for even in 25% sodium citrate solution thrombin activity develops by autocatalytic mechanisms(1) thus indicating that the site on the prothrombin molecule which gives rise to thrombin activity is a structure arising only from changes in the prothrombin molecule itself. In addition to thrombin other derivatives can be obtained (2,3,4). Certain of these have a function in clotting of blood and it is likely that factor VII and plasma thromboplastin component (PTC) are derivatives of prothrombin(2,3). One derivative can again be reconverted to prothrombin by means of liver mitochondria preparations(5,6).

In the presence of thrombin, fibrinogen activates and subsequently polymerizes; and synthetic substrates, such as tosylarginine methyl ester (TAMe), are hydrolyzed(7). Thrombin can thus be regarded as an esterase, and in accordance with that view we have recently found that it hydrolyzes tributyrin quite effectively. Equally interesting is the discovery, reported here, that clotting activity of thrombin may decrease while the esterase activity is retained. Thus, we have

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TABLE I. Changes in Activity of Thrombin Preparation at 28°C.

Time, hr	Units thrombin activity*		
0	520 (520)	270 (270)	103 (103)
24	380 (515)	160 (280)	75 (100)
50	275 (500)	130 (280)	40 (100)
120	180 (510)	47 (270)	20 (80)
180	105 (510)	25 (150)	0 (40)

* Activity expressed in units/ml. Clotting activity is given first and esterase activity in parenthesis.

found another prothrombin derivative of interest in blood clotting and enzymology.

Methods. Purified biothrombin was prepared from purified bovine prothrombin as described by Seegers and Alkjaersig(8). The preparations were of high quality as indicated by several criteria. For example, the specific activity was equivalent to the highest previously obtained in this laboratory and in the ultracentrifuge virtually a single peak was observed. Clotting activity was measured by the method of Seegers and Smith(9), and esterase activity as described by Sherry, Troll and Wachman(7). For purposes of comparison a unit of clotting activity was regarded as equivalent to a unit of esterase activity.

Results. The biothrombin was placed in 0.9% NaCl solution and adjusted to pH 7.25. At room temperature clotting activity or capacity to activate fibrinogen was gradually lost (Table I). The esterase activity was more stable. Actually 3 different concentrations of the same thrombin preparation were studied. In the lowest concentration all clotting activity was lost while about 40% of the esterase activity remained. In the highest concentration studied, 80% of clotting activity was lost while all esterase properties remained stable. Experiments of this kind have been performed repeatedly and also with citrate thrombin. We find that it is possible to have all of the clotting properties disappear; however, the stability of the esterase is as yet not predictable. It is only possible to say that it is the more stable of the two activities.

Discussion. Many experiments have been performed to activate purified prothrombin so that another derivative besides thrombin is obtained(2,3,4). These derivatives evidently have physical-chemical properties slightly different from prothrombin itself(8) and all have the quality of being refractory to becoming thrombin in the presence of solutions wherein prothrombin ordinarily becomes thrombin. The view that some of these derivatives of prothrombin possess activities formerly attributed to "new" clotting factors, having no relationship to prothrombin, ascribes unusual qualities to a protein molecule. In furtherance of these ideas we suppose that the esterase and clotting activity are a property of a single molecule derived from prothrombin. This is biothrombin. By further modification of the molecule the clotting activity is lost, and only esterase activity remains. This we call *esterase thrombin.* We do not believe that these 2 kinds of activity are possessed by separate molecules derived from 2 kinds of precursors. It is interesting that we have not been able to have clotting activity without the esterase activity. We imagine that esterase thrombin can inactivate antithrombin and lyse fibrin clots in the same way as biothrombin can(10,11); and accordingly, are conducting experiments to see whether it does. Furthermore, an attempt is being made to activate prothrombin directly to esterase thrombin, for in the above experiments only the following sequence was observed: Prothrombin→Biothrombin→Esterase thrombin.

Summary. Purified bovine prothrombin preparations may lose the property of clotting fibrinogen but retain esterase activity. It is likely that a single molecule possesses the 2 activities. When biothrombin has lost clotting activity and only esterase activity remains, it is called *esterase thrombin.*

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Effect of Environmental Oxygen Tension on Intestinal Iron Absorption.* (23061)

DONN L. SMITH, MAX E. BIERWAGEN AND THOMAS K. EARLY

Department of Pharmacology, University of Colorado School of Medicine.

Recent reports by Reismann *et al.*(1) indicate that dissociable iron salts are readily absorbed through anatomically intact intestinal mucosa. Although the actual amount absorbed is small, toxic and lethal effects may result. Numerous studies have shown that intestinal absorption of iron is influenced by various types of anemia and fever(2). Granick(2) has postulated that oxygen content of the intestinal mucosal cells may influence absorption of iron by modification of the redox potential within the cell.

The purpose of this investigation was to study the relative amounts of ferrous iron absorbed at widely divergent oxygen tensions in the environmental air. It was desired to determine the effects of oxygen tension without accompanying anemia, infection, or modification of the diet.

Methods. Male and female albino rats, ranging in size from 150 g to 250 g were employed as test animals. All animals were starved for 24 hours prior to experimentation. Iron was administered in dose of 100 mg/kg of elemental iron as ferrous sulfate by stomach tube. Control serum iron levels were determined for each animal, following which the animals were fed the iron and immediately exposed to environmental oxygen tensions of 3 widely varying amounts. One group was exposed to oxygen tension of 54 mm Hg obtained by placing the animals at a simulated altitude of 26,000 feet. A second group remained at ambient oxygen pressure of 125 mm Hg (Denver altitude, 5,280 feet). A third group was exposed to pure oxygen at total pressure of 1390 mm Hg. The oxygen tensions and values employed are approximate, varying slightly with day to day barometric pressure and temperature. After 2 hours exposure to various oxygen tensions, the total serum iron was again determined. All serum iron values were obtained by modification of spectrophotometric method of Barkan and Walker(3) in which o-phenanthroline is used. Blood samples of 1.5 cc were drawn under light ether anesthesia from the right external jugular vein for serum iron determinations. A large number of control serum iron levels were made. One control group was exposed to 54 mm Hg oxygen and a second control group was placed in 1390 mm Hg oxygen for 2 hours without administration of iron. There was no visible necrosis or significant inflammation noted in the intestinal tract of animals autopsied at end of experimental periods.

Results. The results are tabulated in Table I. There was a significant increase in total serum iron levels in all 3 iron-fed groups. In addition there was a significant difference between serum iron values for each group of animals at each oxygen tension level. There was a small difference from control levels in animals exposed to high or low oxygen tensions, but not administered iron. A large group of control serum iron values were determined to establish the normal levels for the rat colony, including sex differences.

Discussion. From the observed results it

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