exudate had left the blood during the first hour of inflammation. The figures for the 2nd, 3rd and 4th hours of inflammation were, respectively, 32%, 25% and 9%, making a total of 102%. If significant numbers of unlabeled granulocytes from the tissues had entered the exudate, the summation of these percentages should have been substantially less than 100%. Consequently, these studies provide no evidence for the existence of a tissue pool of granulocytes.

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Distribution of Mutarotase in Subfractions of Rat Intestinal Mucosa and Rat Kidney.* (29041)

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An enzyme with the ability to accelerate mutarotation of glucose was first demonstrated in extracts of the mold *P. notatum* by Keilin and Hartree(1). Presence of the enzyme was first noted by the fact that action of notatin (glucose oxidase) from the same mold upon glucose was accelerated by the presence of an enzymatic impurity. It was subsequently shown that glucose oxidase was specific for the β-form of glucose, and that mutarotase accelerated the conversion of α-glucose to the form utilized by notatin. This property has been used to assay for mutarotase(2).

Keston(3) detected large amounts of a similar enzyme in extracts of rat kidney. It was proposed that the enzyme functioned in tubular resorption and membrane transport of glucose by accelerating conversion of glucose to a preferentially diffused form. The theory fell into disrepute when it was shown that 1-deoxyglucose(4) and α-methylglucoside(5), compounds containing no mutarotable hydroxyl group, were, however, actively absorbed.

It has been suggested recently, however(6), that mutarotase activity is only a coincidental function of the enzyme protein. In support of this proposal it was shown that both 1-deoxyglucose and α-methylglucoside interact with the mutarotase active center. Both compounds were efficient competitive inhibitors of the enzyme acting on α-glucose.

It has been shown(7) that during intestinal absorption of sugars the concentrative gradient for actively transported sugars is established at the epithelial cells lining the villi. Crane(8) has also demonstrated recently a

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technique for separation of a "brush border" fraction from intestinal epithelium which retains most of the activity of certain intestinal hydrolytic enzymes. It was of interest, therefore, to examine the distribution of mutarotase in intestinal mucosa. This paper describes the assay of the enzyme in rat kidney and rat intestine and the distribution of the enzyme in certain subfractions of these tissues.

Materials and methods. Male white rats (Wistar strain) of 150-200 g were maintained on water and commercial pellets fed *ad libitum.* Purified α-glucose (Fisher AR grade) was recrystallized from ethanol/water, dried over calcium chloride *in vacuo,* and ground in a mortar to a fine powder. The powder was sieved through 200 mesh gauze and stored in preweighed amounts in a vacuum desiccator until used. For measurement of spontaneous mutarotation of glucose, 48 mg of the powder was dissolved in 12 ml of .005 M EDTA buffer, pH 7.4 at 25°C. The solution was well mixed by stirring for 60 seconds and then transferred to a 1 dm. polarimeter tube. Readings of optical rotation were made at 1 minute intervals, using the Keston attachment to the Beckman model DU spectrophotometer at wavelength of 589 mu. The instrument was precalibrated using freshly dissolved sucrose at different concentrations. Readings of optical density were translated to degrees rotation, using the linear calibration curve. Optical rotation, α, was plotted against time and extrapolation of the curve gave a value for the rotation at 0 time (α_0). A final rotation was measured after standing overnight and gave a value for equilibrium rotation (α_E). The first order rate constant for the spontaneous mutarotation (K_{spont}) was then measured from the slope of the plot $\log \frac{\alpha_0 - \alpha_E}{\alpha_T - \alpha_E}$ against time (Fig. 1). When the mutarotational rate in the presence of enzyme was being measured, the procedure was the same except that the enzyme solution diluted to 12 ml with .005 M EDTA buffer, pH 7.4 was added to the preweighed α-glucose powder. The first order rate constant for the catalyzed reaction (K_{cat}) was then measured as described above.

One unit of mutarotase activity was defined as that which produced a 10% increase in the first order rate constant of mutarotation of α-glucose. Total enzyme units in a solution were then given by the expression:

$$\text{Mutarotase units} = \frac{K_{cat} - K_{spont}}{K_{spont}} \times 10$$

The validity of the unit was shown by plotting first order rate constants for glucose in the presence of increasing amounts of a kidney mutarotase extract. A linear relationship between K_{cat}–K_{spont} and enzyme concentration was obtained (Fig. 1). Invertase was assayed in intestinal homogenates by the method of Borgstrom and Dahlqvist(9).

Preparation of brush border from rat intestinal mucosa. A modification of the method of Crane(8) developed by Gallo and Treadwell(10) was used. The small intestine of a non-fasted rat was removed, rinsed through with ice-cold saline and everted over a brass rod. The mucosa was removed by scraping with a glass microscope slide and weighed. The weighed mucosa (1 g) was dispersed in 100 volumes of ice-cold .005 M EDTA buffer, pH 7.4. The dispersion was homogenized for 30 seconds in a Potter-Elvehjem homogenizer with a loose fitting Teflon pestle allowing only 2 up and down passes of the homogenizing tube over the pestle at 1200 rpm. The homogenate was split into 2 portions. One portion (30 volumes) was retained for further homogenization and measurement of enzyme in "whole homogenate." The second portion (70 volumes) was strained through silk to remove gross particles and the brush border fraction was sedimented at 3500 rpm (1475 × *g*) in a Servall RC-2 refrigerated centrifuge fitted with a SS-34 rotor. The supernatant was assayed for mutarotase and invertase. Brush border sediment was resuspended in 70 volumes of .005 M EDTA buffer, further homogenized and split into 2 portions. One portion was used for invertase assay and a second was sonicated for 3 minutes (model MSE 20 kc at 200 watts with ice bath cooling). The solution was clarified by centrifugation at 17,000 rpm (34,800 ×

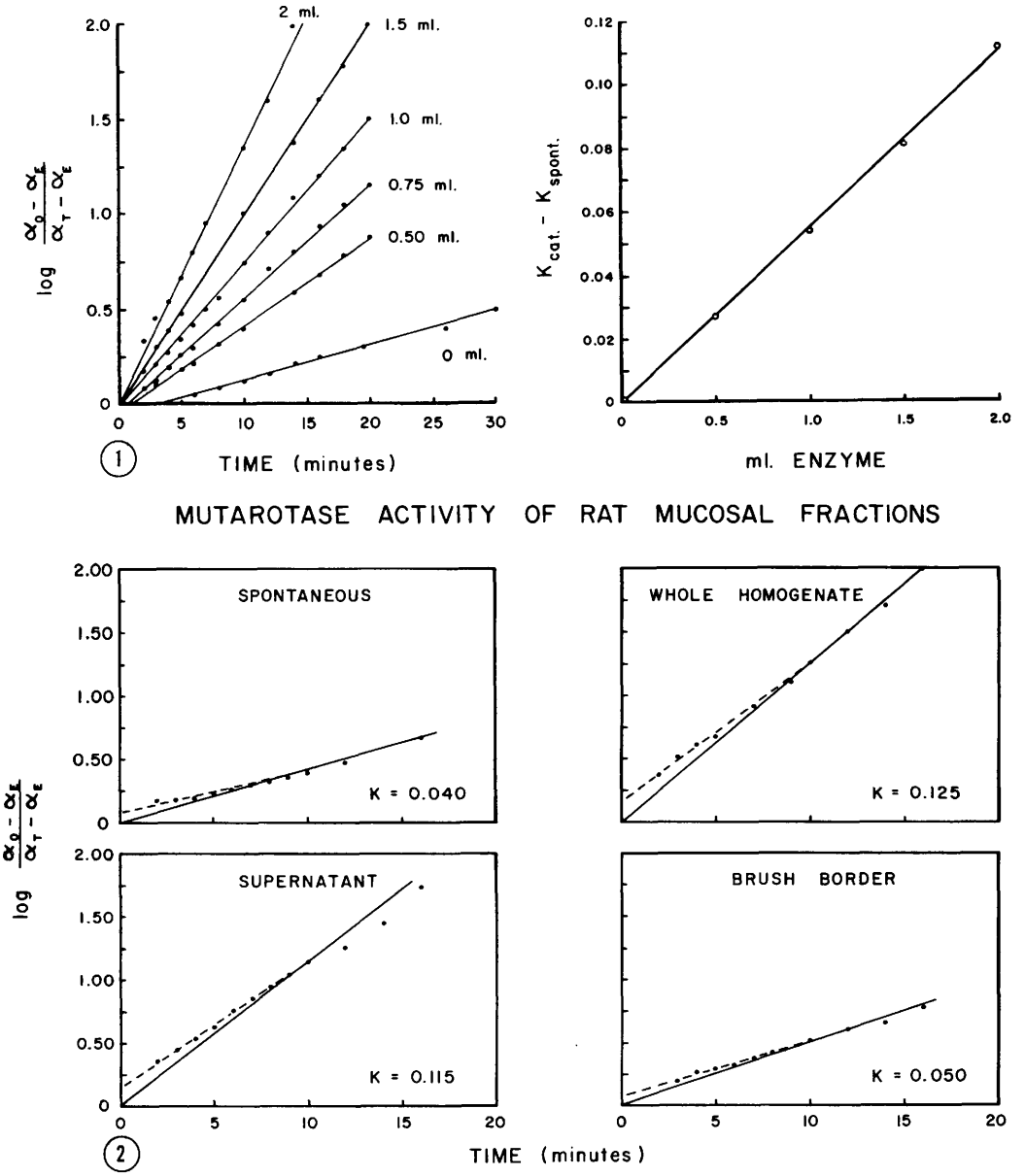

FIG. 1. Linearity of enzyme assay for mutarotase. α-glucose (48 mg) was incubated in a total volume of 12 ml .005 M EDTA buffer, pH 7.4 with 0, 0.5, 1, 1.5 and 2 ml portions of a rat kidney extract. Readings of optical rotation against time were made using the Keston attachment to the Beckman model DU spectrophotometer. First order rate curves were derived from the readings, as described in text. Linearity between $K_{catalyzed} - K_{spontaneous}$ and ml enzyme added was used to establish the definition and validity of the enzyme unit used for assay of mutarotase.

FIG. 2. First order rate curves for mutarotation of α-glucose catalyzed by different fractions of intestinal mucosa. Each curve represents the mutarotation of 48 mg of α-glucose in 12 ml .005 M, pH 7.4 EDTA buffer. Appropriate fractions of intestinal mucosa prepared as described in *Methods*, were added such that each addition was derived from 0.1 g of unfractionated mucosa. Failure of initial points over first few minutes to be on curve passing through origin is due to a combination of mixing artifacts: 1. Due to finite time taken for glucose crystals to dissolve (only partially overcome by using 200 mesh glucose powder); 2. Convection gradients in light path following introduction of solutions to polarimeter tube causing decrease in transmitted light; and 3. Faint opalescence in enzyme preparation causing differential light scattering (only partially overcome by centrifuging enzyme at 105,000 × *g*).

g) in the Servall RC-2 refrigerated centrifuge, and the clear supernatant assayed for mutarotase activity. It was shown in control experiments that sonication for up to 10 minutes followed by centrifugation did not lead to any loss in activity of mutarotase; also the enzyme was not sedimentable from the sonicates when centrifuged at 105,000 $\times$ g in the Spinco model L preparative ultracentrifuge.

Determination of distribution of mutarotase between kidney cortex and medulla. The kidneys were removed from a freshly killed rat and fat was dissected away. Using a razor blade, the outer surface of the kidney was gently scraped and these outer layers of tissue were placed into ice-cold EDTA. Scraping was continued until about 25% of the kidney had been removed. The cortex and residual tissue were homogenized in 100 volumes ice-cold EDTA buffer, centrifuged for 1 hour at 30,000 rpm and the clear supernatants were assayed for mutarotase.

Results and discussion. Mutarotase activity was detected both in rat kidney and rat intestine. The enzyme is in much higher content in kidney (885 units/g) than in whole intestine (150.4 units/g) or intestinal mucosa (184 units/g). In the intestine over 75% of the total enzyme was found in the mucosa, but there was still considerable activity remaining in the serosa after the mucosa had been scraped off. The specific activity of the serosal enzyme (98 units/g) was only about half that of mucosa.

Distribution of the enzyme in rat kidney appears to be consistent with Keston's previous conclusion for hog kidney that the enzyme is concentrated in the tubules(11). The specific activity of the cortex (1720 units/g) was about 3 times that of the tissue remaining (572 units/g) after the outer 25% of the kidney has been removed. This remaining tissue undoubtedly also contains much residual tissue of tubular origin.

Intestinal mucosa was fractionated into brush border and a supernatant fraction according to the modified technique of Crane (8,10), in which the intact nature of the brush border membrane is related to the retention of invertase in the fraction. Using this technique and according to this criterion, preparations which were 95% intact were obtained (Table I). In 3 separate experiments of this type (Fig. 2), however, the mutarotase activity was found in the supernatant fraction and was not associated with the brush border cell membrane (Table I).

TABLE I. Distribution of Mutarotase and Invertase in Subfractions of Rat Intestinal Mucosa.

	Total enzyme units*					
	Invertase			Mutarotase		
Exp No.	Homog	Brush border	Super	Homog	Brush border	Super
1	26.7	23.3	0	212	25	187
2	23.3	19.7	1.7	241	13.2	197
3	43.3	28.3	10	190	0	190
Mean	31.1	23.7	3.9	214	12.7	192
%	100	76	12.5	100	6	90

* Calculated on basis of 1 g mucosa.

Invertase units expressed as μM reducing sugar formed/hr at 37°C. Mutarotase units expressed as $(K_{cat}\text{-}K_{spont}/K_{spont}) \times 10$ with 0.4% α-glucose at pH 7.4 (see text for details). Freshly prepared rat intestinal mucosa was separated into a "brush border" and a supernatant fraction, as described in *Methods* section. Recovery of invertase and mutarotase in both fractions was measured and compared to total enzyme present in the original homogenate. For further details, see text.

As a result of a somewhat striking correlation between ability of the mutarotase protein to interact with certain sugars, and the observed active transport of these sugars(6), a role for the protein in membrane transport of the sugars seemed possible. In kidney the results obtained here confirm those of Keston (11) and are not inconsistent with a concentration of the enzyme in kidney tubules. The *cellular* distribution within the tubule, however, still remains to be determined. The results reported here show fairly conclusively that intestinal mutarotase is not associated with the brush border cell membrane. Any postulated mechanism which seeks to relate this enzyme to sugar transport therefore will have to account for this fact.

Summary. 1. A method for assaying the enzyme mutarotase in rat kidney and rat intestine was developed. 2. Kidney contains considerably more enzyme than intestine and the activity appears to be concentrated in the tubules. 3. Intestinal mucosa was fractionated into brush border and supernatant

fractions by the method of Crane. Brush border membrane contained most of the mucosal invertase but little of the mutarotase. Ninety per cent of the mutarotase remained in a supernatant fraction after removal of brush border. 4. The possible implications of these findings in connection with mechanism of sugar transport are discussed.

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Effects of Hormones and Drugs on Activity of L-Gulono-γ-Lactone Hydrolase.* (29042)

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It has been established that the synthesis of ascorbic acid by rats is diminished following hypophysectomy without concomitant decrease in formation of D-glucuronic acid or activities of L-gulonate NADP oxidoreductase, D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase(1,2,3). The concentration of $NADPH_2$ actually increased(1). Therefore, it has been suggested that the lowered rate of synthesis of ascorbic acid may be ascribed to the depressed activity of L-gulono-γ-lactone hydrolase(2,3). In agreement with this view was the finding that replacement therapy with somatotropin led to marked increases in the activity of L-gulono-γ-lactone hydrolase and the synthetic ability (3). But the production of D-glucuronic acid, and the activity of enzymes other than L-gulono-γ-lactone hydrolase which are responsible for its conversion to ascorbic acid, remained unaffected.

The sequence in which changes in activity of L-gulono-γ-lactone hydrolase and concentrations of ascorbic acid in serum and urine occur following daily administration of somatotropin to hypophysectomized rats has now been studied. The evidence corroborates the view that somatotropin influences the synthesis of ascorbic acid through control of the activity of L-gulono-γ-lactone hydrolase. Although the mechanism whereby somatotropin affects this enzymic activity is not understood, it may be assumed that the hormone stimulates the anabolism of proteins. However, it is doubtful that insulin is essential for the effect of somatotropin on hepatic L-gulono-γ-lactone hydrolase, since the activity of this enzyme remains at normal level in rats made severely diabetic with alloxan. A joint requirement for these two hormones in striated muscle has been observed(4). It is of interest in this connection that DL-ethionine produces changes in the system under investigation which closely mimic those observed after hypophysectomy of long standing.

It is also found that, of 2 possible alternate pathways, the route which circumvents the step catalyzed by D-glucurono-δ-lactone hydrolase must be quantitatively far more important in the synthesis of ascorbic acid. The reported inhibitory effect of Chloretone

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