Pyruvate Kinase Isozymes from Rat Intestinal Mucosa. Characterization and the Effect of Fasting and Refeeding†

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ABSTRACT: Five electrophoretically distinct zones of pyruvate kinase activity were observed in extracts of rat jejunal and ileal mucosa. The major form is pyruvate kinase 5 corresponding to the predominant isozyme in rat kidney and the minor isozyme in rat liver. Four of the five bands are neutralized by rabbit anti-rat pyruvate kinase 3 (type M). Pyruvate kinase 1 (type L) was not neutralized. Only four peaks with pyruvate kinase activity could be resolved by DEAE-Sephadex chromatography and the first three peaks had the electrophoretic mobility of pyruvate kinase 5. The fourth peak contained two electrophoretically distinct bands, one with the mobility of pyruvate kinase 1 and the other with a mobility between pyruvate kinase 2 and 3. Rat jejunal pyruvate kinase 5 and 1 both displayed sigmoidal kinetics with respect to phosphoenolpyruvate, pyruvate kinase 5 was slightly activated, while pyruvate kinase 1 was strongly activated by fructose 1,6-diphosphate. Jejunal pyruvate kinase activity decreased about 50% between 24 and 48 hr after withdrawal of food. When the rats were refed, jejunal pyruvate kinase activity increased and reached a peak 72 hr after refeeding that was about 50% greater then control levels. Thereafter, the enzyme levels returned to control values. The changes in pyruvate kinase activity were shown by immunological techniques to be due to changes in enzyme protein. It was demonstrated that all five electrophoretic forms of the enzyme were affected similarly by the fasting and refeeding.

Pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) is one of several glycolytic enzymes that have been shown to exist in multiple molecular forms in various animal tissues (Wilkinson, 1970; Osterman and Fritz, 1973a). The enzyme is a tetramer and the isozymes apparently arise as a result of the combination of two different types of subunit (Steinmetz and Deal, 1966; Cardenas and Dyson, 1973). The enzyme is thought to play an important role in the regulation of glycolysis and gluconeogenesis in view of the response of the major liver isozyme (pyruvate kinase 1)[1] to dietary and hormonal alterations (Krebs and Eggleston, 1965; Tanaka *et al.*, 1965) as well as to changes in liver pyruvate kinase 1 during postnatal development in rats (Middleton and Walker, 1972; Osterman *et al.*, 1973).

It has been reported that pyruvate kinase activity from rat jejunal mucosa resembles liver pyruvate kinase 1 in that the activity decreases during fasting (Stifel *et al.*, 1969a), but that the intestinal enzyme differs from the major liver isozyme in that it is not decreased in diabetes (Tyrrell and Anderson, 1971). The present investigation was undertaken to establish the pyruvate kinase isozyme pattern of rat jejunal mucosa and to determine if dietary changes affect specific isozymes. We found that the rat jejunal mucosa contains five pyruvate kinase isozymes, and that all five isozymes are affected similarly by fasting and refeeding. A preliminary account of parts of this work has been presented previously (Osterman and Fritz, 1973b).

Experimental Section

Substrates and Reagents. Na_2ADP, Na_4Fru-1,6-P_2, NADH, tricyclohexylamine phosphoenolpyruvate, and DEAE-Sephadex were obtained from Sigma. Lactate dehydrogenase from rabbit muscle was obtained from Boehringer as a crystalline suspension in 2.4 M ammonium sulfate. All other reagents were the best quality commercially available.

Animals. Male adult Sprague-Dawley rats (300–400 g) were housed in air conditioned rooms (24 ± 1°) on a schedule of alternating periods of light (7:00 a.m. to 7:00 p.m.) and dark (7:00 p.m. to 7:00 a.m.). The animals had Purina laboratory chow and water *ad libitum*, unless specified otherwise. Rats were always killed between 8:00 a.m. and 9:00 a.m.

Tissue Preparation. Rats were decapitated and exsanguinated. A segment of jejunum located from 15 to 30 cm beyond the pylorus was removed and washed free of contents by flushing it with ice-cold buffer containing 10 mM Tris-HCl, 135 mM KCl, 5 mM $MgSO_4$, and 1 mM EDTA (pH 7.4). The jejunal segment was then slit longitudinally and the mucosa was obtained by scraping the intestine with a glass slide. The mucosa was homogenized in four volumes (1:4, w/v) of the same buffer in a Potter-Elvehjem type homogenizer. The homogenates were centrifuged at 105,000*g* for 60 min, and the clear, fat-free supernatants were used for the assay of enzymatic activity and protein concentration. The jejunal extracts prepared in this way did not show any loss of pyruvate kinase activity within the first 12 hr if kept on ice. However, dilution in the same buffer (tenfold or more), resulted in a 25% reduction of the enzymatic activity by 90 min.

Enzyme Assay. Pyruvate kinase activity was assayed by following the oxidation of NADH at 340 nm using a Gilford Model 240 recording spectrophotometer. The assay mixture contained (final concentrations): 50 mM Tris-HCl buffer (pH 7.5), 75 mM KCl, 8 mM $MgSO_4$, 2 mM tricyclohexylamine

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[1] The IUPAC-IUB Commission on Biochemical Nomenclature recently issued recommendations on the Nomenclature of Multiple Forms of Enzymes (1971), *Biochemistry 10*, 4825. In this paper we follow these recommendations. The pyruvate kinase isozymes from rat tissues are thus designated as pyruvate kinase 1–5 on the basis of their electrophoretic mobility. Pyruvate kinase 1 is the most rapidly migrating anodal band, followed by isozymes 2–5, in order of decreasing anodal mobilities. However, in references to specific work, the nomenclature as used by the authors will be cited to avoid misunderstanding.

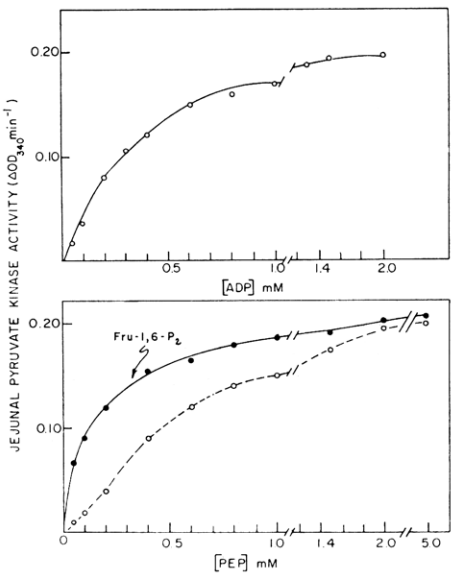

FIGURE 1: ADP and P-enolpyruvate (PEP) saturation curves of unfractioned rat jejunal mucosal pyruvate kinase, and the effect of Fru-1,6-P_2. The concentration of P-enolpyruvate was 2 mM (upper panel). The concentration of ADP and Fru-1,6-P_2 were 2 and 0.2 mM, respectively (lower panel).

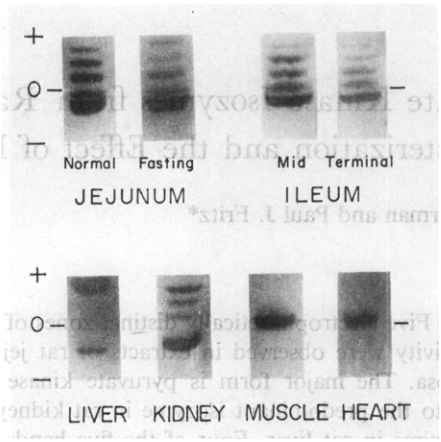

FIGURE 2: Electrophoretic pattern of pyruvate kinase isozymes from several rat tissues. Electrophoresis was performed as described in the Experimental Section.

phosphoenolpyruvate, 2 mM ADP, 0.15 mM NADH, 1 mM EDTA, and 1.0 unit of lactate dehydrogenase in a final volume of 2 ml. In several experiments, as indicated in the text, different concentrations of ADP and P-enolpyruvate were used, and the effect of 0.2 mM Fru-1,6-P_2 was determined. Temperature was strictly maintained at 25°. One unit corresponds to the amount of enzyme that converts 1 μmol of P-enolpyruvate to pyruvate per min at 25°.

Protein Determination. Protein was determined by the biuret method (Gornall *et al.*, 1949) with crystalline bovine serum albumin as standard.

Electrophoresis was performed on cellulose polyacetate strips as previously described (Susor and Rutter, 1971; Osterman *et al.*, 1973) except that it was carried out for 4 hr.

DEAE-Sephadex Chromatography. The resin was equilibrated in buffer containing 10 mM Tris-HCl, 0.25 M sucrose, 1 mM EDTA, and 1 mM dithiothreitol (pH 7.5). Jejunal mucosa was homogenized in this buffer, centrifuged as above, and 4.0 ml of the supernatant was applied to the column (2.5 × 18 cm). The column was washed with the same buffer until the unadsorbed pyruvate kinase activity was recovered, then the column was eluted by a linear gradient of KCl from 0 to 0.25 M in the above buffer. The whole procedure was carried out in the cold room (0–4°). Fractions (3 ml) were assayed for pyruvate kinase activity and protein concentration (absorbance at 280 nm).

Immunological Procedures. Antibody to purified rat skeletal muscle pyruvate kinase was prepared and immunoprecipitation tests were carried out as previously described (Osterman *et al.*, 1973), except that the samples were analyzed 45 min after addition of antiserum or control serum to jejunal extract. We found that incubation for a longer time resulted in nonspecific losses of jejunal pyruvate kinase activity. In contrast, the skeletal muscle enzyme was very stable.

Results

Substrate Saturation Curves and the Effect of Fru-1,6-P_2. A wide range of values have been reported for the total rat jejunal mucosal pyruvate kinase activity (Stifel *et al.*, 1969a,b, 1970a,b, 1971; Anderson and Zakim, 1970; Rosensweig *et al.*, 1970; Stifel and Herman, 1971; Tyrrell and Anderson, 1971). In all these studies the original assay method of Bücher and Pfleiderer (1955) apparently was used. This method was designed for use with rabbit skeletal muscle pyruvate kinase. Our initial measurements of rat jejunal pyruvate kinase activity were higher than those previously reported, but we used higher concentrations of ADP and P-enolpyruvate (1.3 and 2.0 mM, respectively), the substrate concentrations usually employed for assaying hepatic enzymatic activity. This prompted us to determine ADP and P-enolpyruvate saturation curves for the jejunal enzyme. The results are shown in Figure 1. In the upper panel is the ADP saturation curve using 2.0 mM P-enolpyruvate, and in the lower panel is shown the P-enolpyruvate saturation curve using 2.0 mM ADP. It is obvious that by using substrate concentrations of 0.23 mM ADP, and 0.78 mM P-enolpyruvate as in the original method of Bücher and Pfleiderer (1955), one does not assay jejunal mucosal pyruvate kinase activity under saturating conditions. Fru-1,6-P_2 (0.2 mM) strongly activated jejunal mucosal pyruvate kinase, shifting the kinetics from sigmoidal to hyperbolic, and thus resembling the kinetics of liver pyruvate kinase (Taylor and Bailey, 1967). In all subsequent assays we used 2.0 mM ADP and 2.0 mM P-enolpyruvate unless stated otherwise.

Electrophoretic Patterns of Pyruvate Kinase Isozymes. Figure 2 shows the electrophoretic pattern of pyruvate kinase isozymes from rat jejunal and ileal mucosa. For comparative purposes isozyme patterns from rat liver, kidney, skeletal muscle, and cardiac muscle are also shown. Five zones of pyruvate kinase activity in jejunum and ileum are noted. Pyruvate kinase 5 is the major form in the intestine, corresponding to the predominant isozyme in the kidney and the minor isozyme in the liver. We previously designated the minor liver isozyme (Osterman *et al.*, 1973) as pyruvate kinase 4, but it is now clear that it is pyruvate kinase 5. Pyruvate kinase 1, which is the major liver isozyme, occurs also in the intestine, but as the least intense form. Isozymes 2 and 3 occur both in intestinal mucosa and in the kidney. It was not possible to electrophoretically separate skeletal muscle pyruvate kinase from jejunal pyruvate kinase isozyme 3, when a mixture of jejunal and skeletal muscle extracts was subjected to electrophoresis. Pyruvate kinase 4 was detected only in the intestine. Although fasting resulted in about a 50% decrease in the total jejunal mucosal pyruvate kinase activity (see

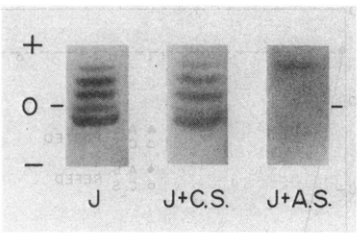

FIGURE 3: Electrophoretic patterns of rat jejunal mucosal pyruvate kinase isozymes before and after reaction with rabbit anti-rat skeletal muscle pyruvate kinase serum. Reactions and electrophoresis were performed as described in the Experimental Section: jejunal enzyme (J); jejunal enzyme + control serum (J + C.S.); jejunal enzyme + antiserum (J + A.S.).

below), it did not appreciably change the relative proportions of isozymes as detected by electrophoresis.

Immunological Studies. Pyruvate kinase isozyme patterns of rat jejunal mucosa and kidney are similar, except that there is one additional molecular form in the intestine (Figure 2). Our previous study (Osterman *et al.*, 1973) disclosed that antiserum to rat skeletal muscle pyruvate kinase 3 cross-reacted (neutralized) with three out of four kidney isozymes, pyruvate kinase 1 being the only one not cross-reacting. We tested the cross-reactivity of jejunal mucosal pyruvate kinase isozymes with antiserum to skeletal muscle pyruvate kinase. It can be seen (Figure 3) that all isozymes except pyruvate kinase 1 were neutralized.

The immunoprecipitin test was carried out for skeletal muscle and for jejunal pyruvate kinase and the results are shown in Figure 4. Constant amounts of pyruvate kinase activity from skeletal muscle and jejunal mucosa extracts were mixed with increasing volumes of control serum or antiserum. The skeletal muscle enzyme was completely neutralized. More antiserum was required to neutralize corresponding amounts of jejunal pyruvate kinase activity. Finally, 3–4% of the initial jejunal mucosal extract activity, corresponding to isozyme 1, remained unneutralized.

DEAE-Sephadex Chromatography. Four peaks of pyruvate kinase activity were resolved by DEAE-Sephadex column chromatography (Figure 5). The first, major peak was not absorbed onto the resin and had the electrophoretic mobility of pyruvate kinase 5. The second small, and the third larger peak also had the electrophoretic mobility of pyruvate kinase 5. The fourth peak, eluted between 0.10 and 0.15 M KCl, contained two electrophoretically distinct forms, one with the mobility of pyruvate kinase 1, and the other with a mobility between pyruvate kinase 2 and pyruvate kinase 3.

Kinetic properties of the chromatographically separated peaks were determined as shown in Figure 6. The first and the third peak had identical kinetics, and are shown on the upper panel (A). The kinetics of the fourth peak are shown on the lower panel (B). In the absence of Fru-1,6-P_2 both peaks displayed sigmoidal kinetics with respect to P-enolpyruvate. Fru-1,6-P_2 activated peak four more strongly than peak one.

Effect of Fasting and Refeeding on Total Jejunal Mucosal Pyruvate Kinase Activity. Figure 7 shows the response of the total jejunal mucosal pyruvate kinase activity to fasting and refeeding. The decrease in enzymatic activity occurred between 24 and 48 hr of fasting. Refeeding resulted in an increase of the enzymatic activity above the prefasting level and a subsequent return to control levels. In order to determine whether changes during fasting and refeeding were due to changes in the amount of enzyme protein or to inactivation or activation

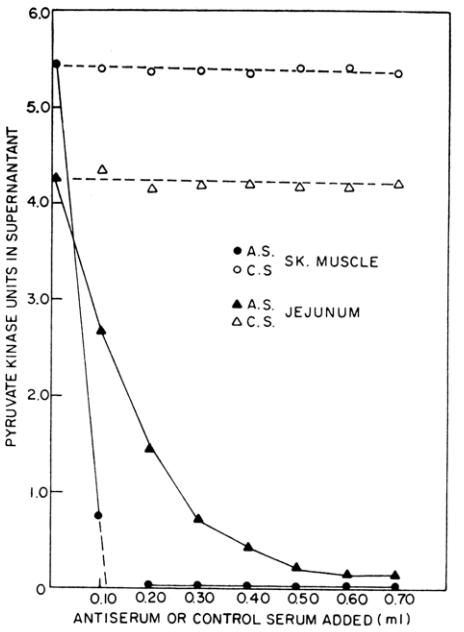

FIGURE 4: Immunoprecipitation of skeletal muscle and jejunal mucosal pyruvate kinase with anti-pyruvate kinase 3 serum. Skeletal muscle (● ○) and jejunal mucosal (▲ △) extracts were prepared and the immunoprecipitation test was carried out as described in the Experimental Section. Constant amounts of enzyme activity were mixed with increasing volumes of antiserum (● ▲) or control serum (○ △).

of existing enzyme, constant and equal volumes of similarly prepared extracts from fasting and fasting–refed jejunal mucosa were mixed with increasing volumes of antiserum or control serum. It can be seen (Figure 8) that more antiserum was needed to neutralize the mucosal enzyme activity from refed rats, thus suggesting that the observed changes resulted from changes in the amount of enzyme protein. It should be noted also that the remaining, unneutralized mucosal enzymatic activity, corresponding to isozyme 1, represented about the same percentage (3–4%) of the total enzyme activity of both fasted and fasted–refed rats. This, as well as the previously shown electrophoretic pattern of extracts of fasting jejunal mucosa (Figure 2), suggests that the decrease of total enzyme activity during fasting did not result from a decrease in the activity of a specific isozyme, but rather that all molecular forms were similarly affected.

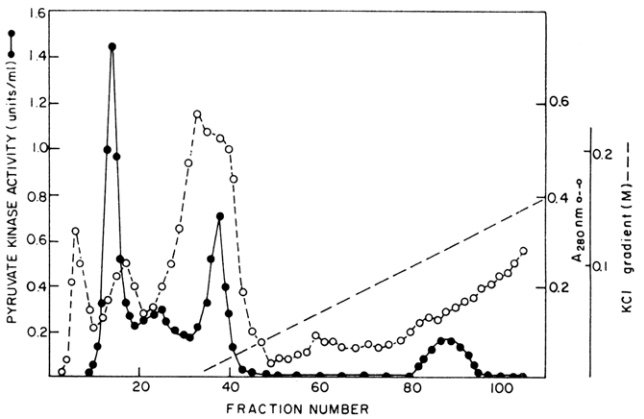

FIGURE 5: DEAE-Sephadex chromatography of rat jejunal mucosal pyruvate kinase. Details are given in the Experimental Section. Recovery of enzyme activity was approximately 65%.

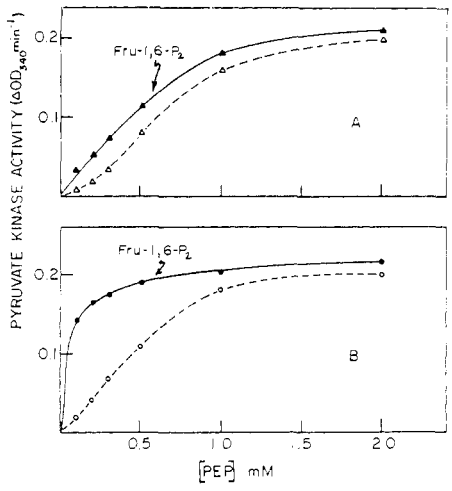

FIGURE 6: Effect of the concentration of P-enolpyruvate (PEP) on the activity of pyruvate kinase isozymes from rat jejunal mucosa in the presence and absence of Fru-1,6-P_2 (0.2 mM): (A) chromatographically separated fraction corresponding to pyruvate kinase 5; (B) chromatographically separated fraction corresponding to pyruvate kinase (isozymes 1 and 2).

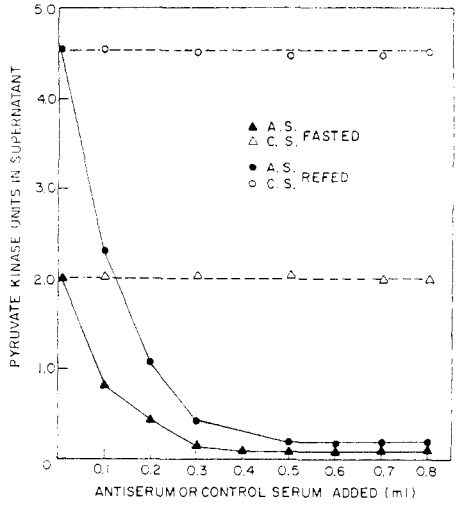

FIGURE 8: Immunoprecipitation of jejunal mucosal pyruvate kinase from fasted and fasted–refed rats with anti-pyruvate kinase 3 serum. One group of three rats fasted for 72 hr. The other group of three rats fasted for 72 hr and then had free access to food for 72 hr. Jejunal mucosa was pooled from respective groups, and tissue extracts were prepared as described in the Experimental Section. Constant and equal volumes (0.2 ml) of similarly prepared extracts of the two groups reacted with increasing volumes of control serum or antiserum.

Discussion

The pyruvate kinase isozymes have presented a special challenge to biochemists in that the activity and amounts of these enzymes are affected by such a large number of parameters and in such a way that it appears certain that they are involved in the regulation of glycolysis and gluconeogenesis (Krebs and Eggleston, 1965; Tanaka *et al.*, 1965, 1967; Taylor and Bailey, 1967; Weber *et al.*, 1967; Bailey *et al.*, 1968; Sillero *et al.*, 1969; Llorente *et al.*, 1970; Jiménez De Asúa *et al.*, 1970). Numerous reports have appeared dealing with the metabolic effects of activation and inhibition of the pyruvate kinase isozymes as well as changes in the levels of the enzymes due to dietary or hormonal effects. Many of the reports have been conflicting, leading to a certain amount of confusion as to the true role of these enzymes in regulating metabolic pathways. The present paper reveals one source of this confusion for, as is well known by enzymologists, it is essential to use saturating concentrations of substrates if one expects to measure the true amount of an enzyme in an *in vitro* assay. Unfortunately, a number of investigators seem to have used the unmodified assay conditions of the original

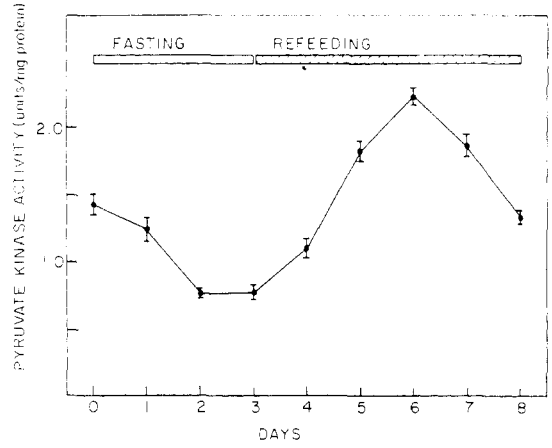

FIGURE 7: Effect of fasting and refeeding on total jejunal mucosal pyruvate kinase activity. The results are means ± S.E. of 5–11 animals.

Bücher and Pfleiderer (1955) method. This method was designed for use with the rabbit skeletal muscle enzyme and used 0.23 mM ADP and 0.78 mM P-enolpyruvate. According to Tanaka *et al.* (1967), the ADP concentration necessary for half-maximal velocity of rat muscle pyruvate kinase is 0.27 mM and for rat liver pyruvate kinase it is 0.10 mM. The corresponding values for P-enolpyruvate are 0.075 mM and 0.84 mM for rat muscle and liver pyruvate kinases, respectively. Our estimates for the enzyme from rat jejunal mucosa (Figure 1) are 0.28 mM for ADP and 0.45 mM or 0.11 mM for P-enolpyruvate in the absence and presence of fructose 1,6-diphosphate, respectively. Thus, it is clear that the concentrations of ADP and P-enolpyruvate used in the original Bücher and Pfleiderer method are unsuitable for any of these pyruvate kinases. Krebs and Eggleston (1965) may have been aware of this problem, because in measuring rat liver pyruvate kinase, they modified the Bücher and Pfleiderer method by increasing the ADP concentration from 0.23 to 1.3 mM. However, they did not change the P-enolpyruvate concentration. Szepesi and Freedland (1969) also used the Bücher and Pfleiderer method to measure rat liver pyruvate kinase, but increased the P-enolpyruvate concentration to 3 mM. Of particular concern to us are the numerous reports on pyruvate kinase from rat jejunal mucosa (Stifel *et al.*, 1969a,b, 1970a,b, 1971; Anderson and Zakim, 1970; Rosensweig *et al.*, 1970; Stifel and Herman, 1971; Tyrrell and Anderson, 1971). These authors apparently used the Bücher and Pfleiderer method with no modification of ADP or P-enolpyruvate concentration and thus were assaying the enzyme under less than saturating substrate concentrations. This no doubt accounts for the wide variation in pyruvate kinase levels they have reported.

The electrophoretic patterns of rat jejunal and ileal pyruvate kinase (Figure 2) are similar to those reported for rat intestine by Imamura and Tanaka (1972). These authors did not indicate what part of the intestine they were working with, but it is clear from our studies that there are quantitative if not qualitative differences in the pyruvate kinase patterns of various parts of the rat small intestine.

The fact that antibody to rat skeletal muscle pyruvate kinase (pyruvate kinase 3) will neutralize four out of the five jejunal pyruvate kinase isozymes (Figure 3) is good evidence that these isozymes have subunits in common and that the fifth isozyme (pyruvate kinase 1), which does not cross-react with anti-pyruvate kinase 3, does not have subunits in common with the other isozymes. These observations are consistent with the report of Cardenas and Dyson (1973) who isolated pyruvate kinase from bovine liver and skeletal muscle and by treating these enzymes with guanidine hydrochloride were able to produce five electrophoretically distinct bands with pyruvate kinase activity, three of which were apparently hybrid enzymes designated by Cardenas and Dyson as M_3L, M_2L_2, and ML_3. We prefer to designate the subunits A and B since the M-L nomenclature leads to confusion when dealing with, for example, enzymes from the intestinal mucosa. It is interesting to note from the work of Cardenas and Dyson that the major form of pyruvate kinase in bovine muscle is apparently the homopolymer A_4, whereas in rat skeletal and cardiac muscle the major form is probably A_2B_2.

The situation in rat tissues may not be as clear cut as it apparently is in bovine tissue for, as originally reported by Tanaka *et al.* (1967), the major isozymes isolated from rat skeletal muscle and liver have molecular weights of 250,000 and 208,300, respectively. That the situation in rats is more complicated is supported by our observations on the chromatographic behavior of rat jejunal pyruvate kinase isozymes (Figure 5). Judging from our previous experience with lactate dehydrogenase isozymes (Fritz *et al.*, 1970) we would have expected that enzymes as electrophoretically distinct as the pyruvate kinase isozymes could have been cleanly separated by DEAE-Sephadex column chromatography. Instead, we found (Figure 5) that an electrophoretic band with the mobility of pyruvate kinase 5 was eluted in three places from the column and that the fourth peak with pyruvate kinase activity contained two electrophoretically distinct bands. What we are calling rat jejunal mucosal pyruvate kinase 5 on the basis of its electrophoretic and chromatographic behavior apparently has different kinetic properties than the rat skeletal muscle pyruvate kinase described by Tanaka *et al.* (1967). As seen in Figure 6A, chromatographically separated rat jejunal pyruvate kinase 5 displays sigmoidal kinetics with respect to P-enolpyruvate and is slightly activated by fructose 1,6-diphosphate. By contrast, rat skeletal muscle pyruvate kinase 3 (type M) displays hyperbolic kinetics with P-enolpyruvate as substrate and is not activated by fructose 1,6-diphosphate (Tanaka *et al.*, 1967). Our findings may be related to the recently reported observations of Van Berkel *et al.* (1973) that reduced and oxidized forms of rat liver pyruvate kinase have altered kinetic and allosteric properties. Also, Pogson (1968) has reported that a bivalent cation may be involved in the interconversion of two forms of rat adipose tissue pyruvate kinase. In addition, Ibsen and Trippet (1972) have reported that the pyruvate kinase isozymes from rat kidney cortex may exist in several conformations. Such observations serve to emphasize the risks involved in using kinetic criteria to determine levels of different forms of pyruvate kinase during development (Middleton and Walker, 1972) or at other times when the enzyme levels might be changing.

Several laboratories have reported changes in rat liver pyruvate kinase levels during fasting and upon switching to a low or high carbohydrate diet (Krebs and Eggleston, 1965; Tanaka *et al.*, 1965; Bailey *et al.*, 1968; Szepesi and Freedland, 1969). The effect of diet on rat intestinal mucosal pyruvate kinase has also been investigated (Stifel *et al.*, 1969a; Anderson and Zakim, 1970) and although these authors reported decreases in enzyme activity after a 72-hr fast as we also noted (Figure 7), the actual values that they reported were extremely low. The difficulty apparently lies in the conditions they are using to assay the enzyme as discussed before.

Tanaka *et al.* (1965) found that in rats fed a low carbohydrate diet there was a decrease in the major liver isozyme, pyruvate kinase 1 (type L in Tanaka's nomenclature), as well as in the minor isozyme (pyruvate kinase 5 or type M) but that the greatest loss was in type L, since the L/M ratio changed from 2.35 to 0.88. We have not quantitated the changes in pyruvate kinase isozymes of rat jejunal mucosa during fasting but it is apparent from the electrophoretic patterns (Figure 2) that all five isozymes are decreased.

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Separation of Anthranilate Synthetase Components I and II of *Escherichia coli*, *Salmonella typhimurium*, and *Serratia marcescens* and Determination of Their Amino-Terminal Sequences by Automatic Edman Degradation†

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ABSTRACT: Amino-terminal sequences are presented for polypeptide components I and II of the anthranilate synthetase of *Escherichia coli*, *Salmonella typhimurium*, and *Serratia marcescens*. The respective amino-terminal sequences are homologous in all three species despite the fact that component II of *E. coli* and *S. typhimurium* is three times as large as component II of *S. marcescens*, *ca.* 60,000–65,000 *vs. ca.* 21,000 and that component II of the former species catalyzes the second or anthranilate phosphoribosyltransferase reaction of tryptophan biosynthesis while component II of *S. marcescens* does not.

Anthranilate synthetase (ASase)[1] catalyzes the initial reaction unique to tryptophan biosynthesis: chorismate + L-glutamine → anthranilate + L-glutamate + pyruvate (Zalkin, 1973). In all bacterial species studied to date ASase exists as an enzyme complex containing two nonidentical polypeptide chains (Zalkin, 1973) designated components I and II (Ito and Yanofsky, 1966). Two general types of ASase complex have been observed in bacteria. One type also catalyzes the second or anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase (PRTase) reaction of tryptophan biosynthesis: anthranilate + 5-phosphoribosyl-1-pyrophosphate → 5-phosphoribosylanthranilate + pyrophosphate. Such complexes, present in *Escherichia coli* (Ito and Yanofsky, 1966, 1969; Ito *et al.*, 1969), *Salmonella typhimurium* (Bauerle and Margolin, 1966; Henderson *et al.*, 1970a,b; Nagano and Zalkin, 1970; Hwang and Zalkin, 1971; Henderson and Zalkin, 1971), and *Aerobacter aerogenes* (Egan and Gibson, 1966), are composed of polypeptides of similar size. The second type of ASase complex, present in *Serratia marcescens* (Robb and Belser, 1972; Zalkin and Hwang, 1971; Robb *et al.*, 1971), *Bacillus subtilis* (Kane and Jensen, 1970; Kane *et al.*, 1972), *Acinetobacter calcoaceticus* (Sawula and Crawford, 1972, 1973), *Chromobacterium violaceum* (Wegman and Crawford, 1968), and various *Pseudomonas* species (Queener and Gunsalus, 1970; Queener *et al.*, 1973) does not have PRTase activity and consists of two polypeptides of very different sizes. Component I of both types of complex can convert chorismate to anthranilate in the absence of component II when L-glutamine is replaced by ammonia as amino donor, *i.e.*, component II provides the glutamine amidotransferase function which allows glutamine to serve as the amino source in anthranilate formation. In *E. coli* and organisms with a similar ASase complex, component II also provides the PRTase activity of the complex. Genetic and biochemical studies have shown that the amino-terminal third of component II of *E. coli* (Yanofsky *et al.*, 1971) and *S. typhimurium* (Hwang and Zalkin, 1971; Grieshaber and Baurele, 1972) provides the glutamine amidotransferase function while the carboxyl-terminal two-thirds of the polypeptide is sufficient to perform the PRTase reaction (Jackson and Yanofsky, 1974). Component II of these organisms has a mol wt of 60,000–65,000 (Ito *et al.*, 1969; Henderson and Zalkin, 1971). By contrast component II's of *S. marcescens* and organisms with the second type of ASase have molecular weights of 14,000–21,000 (Kane and Jensen, 1970; Queener and Gunsalus, 1970; Zalkin and Hwang, 1971; Robb and Belser, 1972; Queener *et al.*, 1973; Sawula and Crawford, 1973) and lack PRTase activity. In view of the differences between the ASase complexes of the bacterial species mentioned, it was of interest to examine the primary structures of the respective components for evidence of homology. In this paper we describe the isolation and separation of ASase components I and II of *E. coli*, *S. typhimurium*, and *S. marcescens* and amino-terminal sequence determinations by automatic Edman degradation. A preliminary report of the

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[1] Abbreviations used are: ASase, anthranilate synthetase; PRTase, anthranilate 5-phosphoribosylpyrophosphate phosphoribosyltransferase; TSase, tryptophan synthetase; Quadrol, *N,N,N',N'*-tetrakis(2-hydroxypropyl)ethylenediamine; PhNCS, phenylthiohydantoin.