The experiments show that the submicroscopic liver cell particles are not homogeneous but are composed of subordinate proteins with different anabolic backgrounds or different rates of rebuilding.

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Synthesis of Ureidosuccinic Acid (USA) from Citrulline with Rat Liver Enzymes

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The isotope from citrulline-ureido-C^{14} is incorporated into position 2 of polynucleotide pyrimidines of the pigeon *in vivo* [1] and into orotic acid by rat liver slices [2]. It has been proposed that this incorporation takes place *via* argininosuccinic and ureidosuccinic acids (citrulline + aspartate → argininosuccinate → USA → orotate → polynucleotide pyrimidines).

With enzyme preparations from rat liver mitochondria evidence has earlier been obtained for the following reactions in the biosynthesis of USA [3]:

$NH_3 + CO_2 + ATP$ + acetylglutamate → Compound X + ADP (1)

Compound X + aspartate → USA + acetylglutamate + P_i (2)

Reaction (1) has been described by Grisolia and Cohen [4] as one step in citrulline formation, the other being:

Compound X + ornithine → Citrulline + acetylglutamate + P_i (3)

The possibility has now been investigated that USA might be formed from citrulline by a reversed reaction (3) followed by reaction (2) rather than by the proposed mechanism *via* argininosuccinate. The rat liver mitochondrial enzyme system which forms USA from aspartate, CO_2 and NH_3 was used for the investigation. The formation of labeled USA from citrulline-ureido-C^{14} could readily be demonstrated in the presence of acetylglutamate, L-aspartate and P_i. Addition of ATP and an ATP regenerating system greatly stimulated the formation of USA. It seems therefore possible that USA is formed by reactions (4) + (2)

C^{14}-citrullin e + acetylglutamate + ATP → C^{14}-compound X + ornithine + ADP (4)

rather than by a reversed reaction (3) + reaction (2).

The formation of compound X from labeled citrulline could be studied directly if aspartate was omitted from the system. Under those circumstances the CO_2 that was fixed in compound X could be released at acid pH and measured as $C^{14}O_2$. Maximal amounts of labeled CO_2 were obtained only in the presence of acetylglutamate and ATP. Significant enzymatic breakdown of C^{14}-citrulline also took place without acetylglutamate, though the presence of this compound increased $C^{14}O_2$ formation up to ten times. When arsenate was substituted for phosphate, 4—6 times more $C^{14}O_2$ was observed. The arsenate reaction was not stimulated by acetylglutamate or by ATP. It seems likely that this enzyme system contains a "citrullinase" comparable to that previously described in bacteria.

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On the Nature of the Salt Inhibition of the Phosphoribomutase Reaction

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The inhibitory effect of salts on the reaction:

Glucose-1,6-diphosphate (GDP) + Ribose-1-phosphate (R-1-P) ⇌ Ribose-1,5-diphosphate + Glucose-6-phosphate

which is catalyzed by phosphoglucomutase preparations from muscle extract has been studied. The reaction was assayed spectrophotometrically in the presence of triphosphopyridine nucleotide and an excess of Zwischenferment. Comparison of the effect of a number of different salts suggest that the inhibition is caused by anions. The influence of the concentration of R-1-P indicates that the salt inhibition can be overcome at infinitely high concentration of R-1-P. The inhibition is, therefore, probably due to competition of anions with R-1-P for the enzyme. The K_m

for R-1-P and the inhibition constants for chloride, phosphate and sulphate have been determined. While GDP inhibits, when it is present at certain high concentrations, no such inhibitory effect has been observed for R-1-P. Since the constants obtained suggest that the binding is dependent primarily on the charge of the anion the experiments indicate that the group of the enzyme which "activates" R-1-P binds this and any other anion by a salt linkage. This linkage might be of the same type which is found in some synthetic anion exchangers. The results also indicate that when GDP serves as a substrate in the reaction it is attached to a group of the enzyme which is different and possibly of different nature from that to which R-1-P is attached.

The Content of Polyglucose of Glycogenic Nature * during the First Hours of Growth in *Escherichia coli* B

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The following experiment is intended to elucidate the normal metabolism of *E. coli* B as measured by the "glycogen" content at different states of growth.

Experimental. Freeze-dried cells of *E. coli* B were inoculated into, and grown in a salt medium (pH 7) and cultivated for 18 hours with Na-lactate as the only source of carbon [1]. The volume of the culture was 5 liter. After centrifuging and washing the bacteria with fresh medium an amount of bacteria was inoculated into two 50-liter bottles, each containing 40 liter of the medium, to give a density of approximately 10^8 bacteria/ml. The praeculture and the 40-liter cultures were constantly shaken at 37° C and aerated through sterile cottonwool filters at a rate of 1 liter per minute and liter of medium. The bacterial suspension was cooled to 6° C within three minutes after harvest of culture fluid. The specimens obtained at different times after inoculation were centrifuged at 0° C for two hours at 2 500 r.p.m. in an International refrigerated centrifuge with four 1-liter cups. The bottom was covered with a net of stainless steel and the cup was divided into four chambers by means of a baffle in order to prevent the bacteria from whirling up when the speed of the centrifuge slowed down. In this way it was possible to spin down the bacteria quantitatively with a loss not exceeding 1.7—2.4 %. The bacteria were washed with 0.9 % NaCl and freeze-dried.

The "glycogen" was isolated by the usual alkali treatment and precipitation with alcohol. The precipitate was hydrolyzed for three hours in 0.6 *N* HCl. The glucose thus obtained was purified by passing the hydrolysate through Dowex 50 in the H+-form and Dowex 2 in the acetate-form. No other sugar than glucose was found on the paper-chromatogram. The "glycogen" could be hydrolyzed by salivary amylase.

The estimations of glucose were performed according to Dische [2].

Results. A very rapid increase of "glycogen"/mg of dry cells was found, reaching its maximum at 30 minutes (Table 1). This indicates that the "glycogen" synthesis precedes the synthesis of the bulk of the cell-material.

Table 1.

Time after inoculation minutes	Number of cells/ml $\times 10^{-6}$	"Glycogen" % of dry weight	Mg "glycogen" per 1 000 ml culture	"Glycogen"/cell mg $\times 10^{-12}$
0	111	1.1	0.3	1.7
15	112	10.9	3.6	17.3
30	111	12.8	4.4	19.5
60	110	11.9	7.1	31.7
90	111	9.8	7.6	34.1
120	175	5.6	7.4	21.0
240	665	1.2	3.5	2.6
360	1 509	1.0	7.4	2.5

"Glycogen"/cell ratio reaches its maximum at 90 minutes after inoculation or immediately before the first cell divisions.

A very rapid decrease in "glycogen"/1 000 mls of culture was found between 90 and 240 minutes, indicating that "glycogen" synthesized during the "lag-phase" of growth was utilized during the first two hours of the logarithmic phase of growth. During this period the number of bacteria/ml increased six times.

Between 240 and 360 minutes after inoculation the amount of "glycogen"/cell remained approximately constant at a level slightly higher than that found in the bacteria at the

* For the sake of brevity called "glycogen".