because viscosimetric methods usually are considerably more sensitive than other methods. As the substrate is a polyvalent acid, the ionic strength in the reaction mixture must be kept constant (e.g., 0.1), and the concentration of multivalent cations should be as low as possible. Addition of cysteine or other compounds containing sulphhydryl groups causes a steady decrease in the viscosity of carboxymethyl chitin solutions. However, this difficulty can be overcome— in the same way as in the case of carboxymethyl cellulose — by adding a small amount of potassium ferrocyanide.

Sonju and Okimae have described the preparation of hydroxyethylchitin, which is probably a suitable substrate for chitinase determination but this has not yet been proved.

1. Zechmeister, L. and Toth, G. Naturwiss. 27 (1939) 367; Enzymologia 7 (1939) 165.
4. Hultin, E. and Sjögårdh, I. Forthcoming article.

The Zimmermann Method

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This method is the most frequently used in steroid analysis. The sample is dissolved in ethanol, made alkaline with KOH, and stained with m-dinitrobenzene. If the sample contains steroids with a ketogroup in position 17, the extinction curve will show a flat maximum at 5 900 Å. In their absence the curve will only slope towards longer wave-lengths. The reactions causing the staining are not known. None of the many variants of the method is accurate. One source of error is the oxidation of ethanol by the nitro compounds. The aldehyde thus formed polymerises in alkaline solution.

m-Dinitrobenzene is soluble in most organic solvents. As no staining will occur unless pH < 2, the only suitable solvents for the reaction are the simplest alcohols. The tertiary butanol is the only alcohol not oxidised by m-dinitrobenzene. It was used for an investigation of the Zimmermann reaction. The following results were obtained:

1. Alkaline solutions of pure m-dinitrobenzene in tert. butanol have the same extinction curves as those described for stained 17-ketosteroids.
2. The intensity of the colour depends mainly on the concentration of OH-. It explains why the colours disappears after a couple of days.
3. Some carbonyl compounds as acetaldehyde, acetone, cyclohexanone, and 17-ketosteroids increase the extinction, and shift the absorption maximum over to longer wavelengths (5 600 Å). Easily condensed ketones and formaldehyde have no or only slight influence on the colour.


The Incorporation in vivo of Amino Acids into Subfractions of Cytoplasmic Particles

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It has been shown previously that in chick liver the incorporation in vivo of labeled amino acids is particularly high in the submicroscopic particulate components of the cytoplasm. C14-glycine, N18-glycine, or N18-DL-alanine were injected intravenously into chicks or rats. The animals were killed 1—5 minutes after the administration of the isotope. Fractions of large and small submicroscopic particles were prepared from homogenates of the livers by differential centrifugation. (After removal of the mitochondrial fractions, the homogenates were centrifuged for 20 minutes at 20 000 g, and subsequently for 40 minutes at 105 000 g). After several washings, the particles were repeatedly treated at 0°C with 0.2 M NaHCO3, pH 8.4, and with 0.6 % deoxycholate, pH 8.4. The cholate extracts were further fractionated by means of ethanol at low temperatures.

The isotope contents of the proteins were consistently higher in the large microsomes than in the small ones. In both fractions, the carbonate extracts (which contained a major part of the ribonucleic acid of the particles) showed the highest isotope contents. In the deoxycholate extracts, the proteins of the higher ethanol fractions showed a higher isotope level than the proteins of the more readily precipitable fractions. The non-soluble residues obtained after the deoxycholate treatments, had considerably lower isotope contents.

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


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 and into orotic acid by rat liver slices. 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:

\[ \text{NH}_4^+ + \text{CO}_2 + \text{ATP} + \text{aspartylglutamate} \rightarrow \text{Compound X} + \text{ADP} \]

(1)

\[ \text{Compound X} + \text{aspartate} \rightarrow \text{USA} + \text{aspartylglutamate} + P_i \]

(2)

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

\[ \text{Compound X} + \text{ornithine} \rightarrow \text{Citrulline} + \text{aspartylglutamate} + P_i \]

(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_4^+ was used for the investigation. The formation of labeled USA from citrulline-ureido-C^14 could readily be demonstrated in the presence of aspartylglutamate, 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^4-citrulline + acetylglutamate + ATP → C^4-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^4CO_2. Maximal amounts of labeled CO_2 were obtained only in the presence of aspartylglutamate and ATP. Significant enzymatic breakdown of C^4-citrulline also took place without aspartylglutamate, though the presence of this compound increased C^4CO_2 formation up to ten times. When asparagine was substituted for phosphate, 4–6 times more C^4CO_2 was observed. The asparagine reaction was not stimulated by aspartylglutamate or by ATP. It seems likely that this enzyme system contains a "citrullinase" comparable to that previously described in bacteria.


On the Nature of the Salt Inhibition of the Phosphoribomutase Reaction

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The inhibitory effect of salts on the reaction:

\[ \text{Glucose-1,6-diphosphate (GDP) + Ribose-1-phosphate (R-1-P) = Ribose-1,5-diphosphate + Glucose-6-phosphate} \]

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 \( K_m \)

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