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 cellulose⁴ — by adding a small amount of potassium ferri-cyanide.

Senju and Okimasu⁵ have described the preparation of hydroxyethylchitin, which is probably a suitable substrate for chitinase determination but this has not yet been proved.

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The Zimmermann Method

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This method¹ 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 5 300 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 $pOH < 2$, 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 extinc-

tion 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 wave-lengths (5 600 Å). Easily enolised ketones and formaldehyde have no or only slight influence on the colour.

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

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It has been shown previously^{1,2} that in chick liver the incorporation *in vivo* of labeled amino acids is particularly high in the sub-microscopic particulate components of the cytoplasm.

C¹⁴-glycine, N¹⁵-glycine, or N¹⁵-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 % desoxycholate, pH 8.4. The cholate extracts were further fractionated by means of ethanol at low temperatures³.

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 desoxycholate 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 desoxycholate treatments, had considerably lower isotope contents.

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 slices². It has been proposed that this incorporation takes place via argininosuccinic and ureidosuccinic acids (citrulline + aspartate $\rightarrow$ argininosuccinate $\rightarrow$ USA $\rightarrow$ orotate $\rightarrow$ polynucleotide pyrimidines).

With enzyme preparations from rat liver mitochondria evidence has earlier been obtained for the following reactions in the biosynthesis of USA³:

Reaction (1) has been described by Grisolia and Cohen⁴ as one step in citrulline formation, the other being:

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)

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) $\rightleftharpoons$ 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