original nucleoside triphosphate. Identical results were obtained with synthetic UTP, obtained by courtesy of Professor A. R. Todd, Cambridge.

Inosine triphosphate (ITP) — previously found to be inactive [2] without the addition of a magnesium salt [3] — gave a volume change of actomyosin gel comparable in magnitude to that given by the other nucleoside triphosphates, but somewhat slower. No addition of magnesium salt was required, although the concentration of magnesium in the reaction mixture was below 4×10^{-7} *M*.

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Deamination of Adenosine Diphosphate by Adenylate Deaminase Preparations

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The deamination of adenine nucleotides by muscle preparations is generally assumed to proceed via adenosine 5′-monophosphate (AMP) through the action of a specific adenylate deaminase associated with myosin. In recent reports, evidence was presented for an additional route of deamination: direct deamination of adenosine diphosphate (ADP) by actomyosin gel [1] and washed myofibrils [2]. Deamination of ADP can likewise be effected by preparations of adenylate deaminase obtained from water extracts of skeletal muscle by the method of Kalckar [3]. Whereas only AMP is deaminated under the usual conditions [3] of the adenylate deaminase test (1—5 μg protein/ml and a reaction time of up to 60 min.), higher protein concentrations and longer reaction times lead to the deamination of ADP as well. 80—90 % deamination was observed in reaction mixtures containing 5 mg protein and 7 μmoles ADP per ml in 0.1 *M* succinate buffer, pH 6.0 at 20° C and using a reaction time of 4 hours. Ion exchange [4] and two-dimensional paper chromatography [5] were used for analysis of the deproteinized reaction mixtures. Inosine diphosphate (IDP) was found to be the main product of the deamination of ADP and was isolated as the barium salt from pooled fractions of the effluent from ion exchange columns and identified by chemical analysis (hypoxanthine : ribose : acid labile P : total P). Beside IDP, minor amounts of inosine 5′-monophosphate (IMP) were detected and a further inosine nucleotide fraction, which precedes IDP in the ion exchange effluent and contains hypoxanthine, pentose, acid labile and acid stable phosphate in the proportions 2 : 2 : 1 : 2, corresponding thus in its composition to an equimolar mixture of IMP and IDP, or to a diinosine triphosphate.

Experiments on the pH-dependence of ADP deamination by adenylate deaminase preparations point to a pH optimum of near pH 6, in harmony with previous findings on actomyosin gel [1].

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On the Deaminase Component of Actomyosin

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It has been shown previously [1] that dephosphorylation of adenosine triphosphate (ATP) by actomyosin is followed by deamination of the diphosphate initially formed, yielding inosine diphosphate (IDP) — or an IDP compound converted to IDP during isolation [2] — as the final reaction product. At 20° C and pH 6.8—7.1 (without addition of buffer), using 5—25 mg (dry weight) actomyosin gel and 5 μmoles ATP per ml, dephosphorylation is complete within 30 minutes, whereas deamina-

tion needs 4 hours or more for completion. In experiments where the reaction mixture was divided into insoluble protein sediment and supernatant fluid by centrifugation after 30 minutes reaction, complete deamination was obtained nevertheless, both in the sediment and the supernatant fluid, after several hours standing. Only the sediment is able to dephosphorylate added ATP; thus, the deaminase component of actomyosin has been partly released into the supernatant fluid in the reaction with ATP, free from ATPase, in agreement with recent observations [3] on the partial release of ATPase free adenylate deaminase from washed myofibrils by treatment with ATP. Addition of adenosine 5′-monophosphate (AMP) or adenosine diphosphate (ADP) to the supernatant fluid from actomyosin — ATP mixtures results in deamination, whereas no deamination follows the addition of ATP. The specificity range of ATPase-free deaminase thus includes only AMP and ADP but not the triphosphate. Deamination of ADP proceeds more slowly in the sediment-free supernatant than in the complete system, presumably because of the incomplete extraction of deaminase. Repeated treatment of the protein sediment with ATP releases further amounts of deaminase, free from ATPase. In different experiments 2—5 % of the total actomyosin protein was recovered in the supernatant after reaction with ATP for ½—24 hours. According to Engelhardt [4], the deaminase component of myosin represents 6—10 % of the total protein.

24 hours dialysis against distilled water at 0° C results in partial precipitation of the deaminase protein from the ATPase-free supernatant. On chromatography, both protein and nucleotide material are retained by Dowex-1 chloride (200—400 mesh). On elution with 0.1 *M* potassium chloride the deaminase protein appears in the first fractions of the effluent, practically free from nucleotide material.

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On the Dissolving Power of the Bile for Cholesterol

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The fractional extraction procedure on lyophilized bile described by Isaksson [1] gives the following separation of the main components: cholesterol is found in the ethyl ether extract, lecithin and part of the bile salts in the subsequent chloroform extract and the rest of the bile salts in the following ethanol extract. The constituents of the chloroform appear in rather constant proportions, 1 mole lecithin per 3 moles bile salts. This system is soluble in water and has the power to dissolve cholesterol. Thus, it seemed to be of value to interpret the importance of lecithin for the dispersion of the bile cholesterol. Since the works of Andrews *et al.*[2] the bile salts have been known as the only components of bile of interest in that respect.

Experiments on human bile: Normal bladder biles were lyophilized and extracted as described earlier [1]. Part of an ether extract was mixed with an equivalent part of either the chloroform or the ethanol extract, the solvents evaporated *in vacuo* and the residue taken up in a phosphate buffer (pH 6.0, 7.0 or 8.0) at 37° C. A quite clear solution was obtained with the chloroform but not with the ethanol residue. Addition of bile salts to the ethanol extract in amount equal to that in the chloroform extract was without influence. This must mean that the magnitude of the bile salts in normal bile is not sufficient to keep the actual amount of cholesterol in solution, in contrast to the reports of Andrews [2]. Moreover, as lecithin and bile salts are the only components found in the chloroform (except bile pigments) the two seem to be obligatory components in the solving system. The minimal relation of lecithin-bile salts system to cholesterol for complete solution was titrated out and found to be 11/1—12/1, it means a molar relation lecithin: bile salts: cholesterol = 2:6:1.

Experiments on artificial systems: Pure lecithin and bile salts were mixed in different proportions and tested for their power to dissolve added cholesterol with pH and temperature conditions as above. The best weight relation was found to be about 35/65 (*i.e.* 1 mole lecithin per 3 moles bile salts). The desoxycholic salts were slightly superior to the cholic salts, but no significant difference between tauro and glyco conjugates was found.