partial reactivation by dialysis. Disc electrophoresis showed one main fraction and one or two subsidiary fractions in most crystalline preparations. Enzymic activities were found only in the main fraction and the ratio of the two activities was the same as in the original enzyme preparations.

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Alberty, R. A. (1961). In *The Enzymes*, vol. 5, p. 531. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press.
Gawron, O. & Fondy, T. P. (1959). *J. Amer. chem. Soc.* **81**, 6333.
Massey, V. (1963). *Biochem. J.* **55**, 172.
Nakamura, S. (1962). *Enzymologia*, **25**, 3.

Liver Enzyme Changes in the Developing Rat

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Enzyme assays were carried out on foetuses removed near term and on rats from 0–100 days old, the animals being weaned at 21 days.

In agreement with Villee (1958) it was found that lipogenesis, as measured *in vitro* by incorporation of [^{14}C]acetate into lipid of liver slices, was similar in foetal and adult tissue. Immediately after birth lipogenic activity fell and was maintained at a low level until 20–25 days. Thereafter it rose rapidly to attain a peak at 30–35 days and then declined steadily reaching adult levels at about 70 days.

ATP: citrate lyase (EC 4.1.3.8) levels followed qualitatively those described for lipogenesis, exhibiting a substantial foetal level, a fall after birth, a rapid rise after 20 days, a maximum at 30–40 days and a fall to adult level at about 80 days. Malic enzyme (EC 1.1.1.40) behaves similarly except that no activity could be detected in the foetus, nor were appreciable quantities of enzyme found until the rise after 20 days. Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) also gave a biphasic response similar to that of lipogenesis but the fall after birth was not as pronounced nor as rapid as in that case.

Pyruvate kinase (EC 2.7.1.40) activity in the foetal liver is about half that in the adult. It is maintained at this low level until about 20 days after which it rises to reach adult level at 40–50 days. In confirmation of the results of Burch (1965) Fructose 1,6-diphosphatase (EC 3.1.3.11) activity was found to be very low in foetal liver and to rise very rapidly after birth to attain a maximum at 10 days, decreasing thereafter to reach adult levels at about 30 days. The results described are for male rats. Differences between male and female rats became apparent after 35 days for lipogenesis, malic enzyme and glucose 6-phosphate dehydrogenase; in each case females gave higher values. It is possible that these differences and the depression of enzyme levels found after 35 days are due to hormonal changes consequent to sexual maturation.

The fluctuations in enzyme levels noted here, particularly striking at birth and weaning, are in marked contrast to the steady increases noted in the respiratory enzymes during development (Lang, 1965) and presumably reflect the control exerted on these enzymes by dietary and hormonal factors over this period.

Burch, H. B. (1965). In *Advances in Enzyme Regulation*, vol. 3, p. 185. Ed. by Weber, G. Oxford: Pergamon Press Ltd.
Lang, C. A. (1965). *Biochem. J.* **95**, 365.
Villee, C. A. & Hagerman, D. D. (1958). *Amer. J. Physiol.*, **194**, 457.

Reversible Blocking of Peptide Amino Groups by Maleic Anhydride

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Maleic anhydride has many advantages as a reversible blocking reagent for amino groups of proteins or peptides. Reaction occurs readily at pH 8·5–9·5 and is completely specific for amino groups. With bovine chymotrypsinogen-A (0·04mM) reaction was 98% complete in 5min. using 30mM reagent at pH 9 and 2°. The resulting maleyl proteins or peptides are very soluble at neutral or alkaline pH since the predominant negative charges maximise electrostatic repulsion and minimize aggregation, e.g. denatured myosin, turnip crinkle virus protein and various denatured dehydrogenases were soluble at pH 8 in the absence of urea or guanidine.

The maleyl amino bond (half-life > 20 weeks at 37°) is very stable above pH 6 but is readily hydrolysed below pH 5 (the half-life of ϵ-maleyl lysine is 11hr. at 37°, pH 3·5) by intramolecular catalysis by the un-ionized carboxyl group which is held in a catalytic configuration by the maleyl double bond (cf. Bender, 1957). These mild conditions minimize any other peptide bond cleavage, so maleylation is a useful alternative to trifluoroacetylation (Goldberger & Anfinsen, 1962) for reversible blocking of amino groups, with the advantage of stability of the product at the blocking pH.

Tryptic digestion of denatured maleyl proteins will yield large fragments with *C*-terminal arginine which can be fractionated on anion-exchange resins without employing urea, unblocked, and then further digested by trypsin at lysyl bonds. Also we

have developed a diagonal electrophoretic procedure (cf. Brown & Hartley, 1966) which selectively purifies lysine and amino terminal peptides from digests of maleyl proteins. Strips are cut from a paper electrophoresis of such digests and exposed to the vapour of pyridine-acetate buffer, pH 3·5 at 60° for 16 hr. After re-electrophoresis the unblocked maleyl peptides emerge decisively from the diagonal position, having sustained a charge change of +2. Applied to a chymotryptic digest of pig β-melanophore-stimulating hormone this method gave the amino terminal peptide and lysine peptides described by Harris & Roos (1959). Since no other peptides were found to be off the diagonal, we believe that the method is specific and represents an alternative to the trifluoroacetyl diagonal method of Jones & Perham (1967) for lysine peptides.

This reagent is being used in this laboratory in the study of several proteins.

Brown, J. R. & Hartley, B. S. (1966). *Biochem. J.* **101**, 214.
Bender, M. L. (1957). *J. Amer. chem. Soc.* **79**, 1258.
Goldberger, R. F. & Anfinsen, C. B. (1962). *Biochemistry*, **1**, 401.
Harris, J. I. & Roos, P. (1959). *Biochem. J.* **71**, 434.
Jones, G. M. T. & Perham, R. N. (1967). *European Journal of Biochemistry*, (in the Press).

3-Methylhistidine in Actin and other Muscle Proteins

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During the course of routine amino acid analysis of highly purified actin a small but persistent shoulder was observed on the trailing edge of the histidine peak. The ninhydrin-staining material responsible for this has been shown to be identical with 3-methylhistidine by ion-exchange chromatography using the Beckman systems for protein hydrolysates and physiological fluids (Spackman, Stein & Moore, 1958) and the standard 20 hr. Technicon Autoanalyzer system. The identity was confirmed by ionophoresis at pH values of 1·8, 3·5, 6·5 and 9·0 and by chromatography in four different solvent systems. The compound was Pauly-negative but gave a positive I_2 reaction for the imidazole nucleus (Smith, 1958). In a preliminary note Asatoor & Armstrong (1967) have also reported the presence of 3-methylhistidine in actin.

3-Methylhistidine has been identified in only one of the 18 peptide fractions obtained by preparative Dowex-1(×2) column chromatography of the complete tryptic digest of actin. The average values for rabbit actin correspond to 1 residue of 3-methylhistidine per 7·8 residues of histidine. This would give a minimum mol. wt. of 49000 and suggests that 3-methylhistidine is present in only one of the peptides obtained on tryptic hydrolysis, although three peptides were present in the 3-methylhistidine-containing fraction obtained by Dowex chromatography of tryptic digests. From the known arginine and lysine contents of actin a unique peptide sequence of 49000 mol. wt. would give rise to approximately twice the number of peptides actually obtained on tryptic hydrolysis by us and other workers (Carsten & Katz, 1964).

Actin from skeletal muscle of the 28-day-old rabbit foetus was also found to contain 3-methylhistidine although significant methylation of carnosine to anserine occurs some time after birth in rabbit skeletal muscle (Kendrick-Jones, 1965). 3-Methylhistidine has also been shown to be present in actin samples from trout and fowl.

3-Methylhistidine could not be detected in purified creatine phosphokinase or tropomyosin B, both isolated from rabbit skeletal muscle, but small amounts were present in purified rabbit myosin and in the large fragment obtained from myosin after succinylation (Oppenheimer, Barany, Hamoir & Fenton (1966). It was also present in heavy meromyosin and subfragment I but not in light meromyosin. The evidence so far available suggests that 3-methylhistidine is present in peptide combination in myosin at approximately 2 residues per 500000 mol. wt.

Asatoor, A. M. & Armstrong, M. D. (1967). *Biochem. biophys. Res. Commun.* **15**, 519.
Carsten, M. E. & Katz, A. M. (1964). *Biochim. biophys. Acta*, **90**, 534.
Kendrick-Jones, J. (1965). Ph.D. Dissertation, University of Birmingham.
Oppenheimer, H., Barany, K., Hamoir, G. & Fenton, J. (1966). *Arch. Biochem. Biophys.* **115**, 233.
Smith, I. (1958). *Chromatographic Techniques*, 1st ed. p. 144. London: W. Heinemann.
Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.