with the ox-liver enzyme, and its replacement by a proton does not cause steric hindrance.

Inasmuch as the inhibition of glycosidases by aldono-lactones closely parallels their substrate specificity (Conchie & Levvy, 1957), the present work supports the statement of Wallenfels & Malhotra (1960) that β -galactosidase, in ox-liver preparations at least, hydrolyses β -D-fucosides. On the other hand, to judge by the differential response of the enzyme in different mammalian tissues to galactonolactone and fuconolactone, the relationship of β -D-fucosidase to β -D-galactosidase activity is a complex one, reflecting the differences observed in the properties of β -galactosidase itself. This problem is at present under investigation.

SUMMARY

1. Mammalian β -galactosidase differs in its pH optimum and other properties according to the tissue of origin. The simplest situation was seen in the rat, where there was apparently the same enzyme in different organs, with a sharp optimum at pH 3–4. The most complex situation was seen in the ox: the enzyme in liver differed from that in the genital organs and had a broad optimum, from pH 4 to 7. Attempts to separate the ox-liver enzyme into two fractions failed.

2. Ox liver differed from the other mammalian tissues examined in that the β -galactosidase activity was relatively resistant to inhibition by galactono-1,4-lactone. This was, however, the only

mammalian tissue studied in which β -galactosidase was inhibited by D-fucono-1,4-lactone.

3. Galactono- and fucono-1,5-lactone are much more powerful enzyme inhibitors than the corresponding 1,4-lactones. This can be explained from the effect of the configuration on the structure of these molecules, as compared with the substrate.

The authors are greatly indebted to Robert Lawson and Sons (Dyce) Ltd. for the supply of material from cattle and pigs.

REFERENCES

- Conchie, J. (1954). *Biochem. J.* **58**, 552.
 Conchie, J. & Findlay, J. (1959). *J. Endocrin.* **18**, 132.
 Conchie, J., Findlay, J. & Levvy, G. A. (1959). *Biochem. J.* **71**, 318.
 Conchie, J. & Hay, A. J. (1959). *Biochem. J.* **73**, 327.
 Conchie, J., Hay, A. J. & Levvy, G. A. (1961). *Biochem. J.* **79**, 324.
 Conchie, J. & Levvy, G. A. (1957). *Biochem. J.* **65**, 389.
 Findlay, J., Levvy, G. A. & Marsh, C. A. (1958). *Biochem. J.* **69**, 467.
 Hudson, C. S. (1910). *J. Amer. chem. Soc.* **32**, 338.
 Hudson, C. S. (1911). *J. Amer. chem. Soc.* **33**, 405.
 Levvy, G. A. (1952). *Biochem. J.* **52**, 464.
 Levvy, G. A. & McAllan, A. (1961). *Biochem. J.* **80**, 433.
 Levvy, G. A., McAllan, A. & Hay, A. J. (1961). *J. Endocrin.* **23**, 19.
 McIlvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
 Richtmyer, N. K., Hann, R. M. & Hudson, C. S. (1939). *J. Amer. chem. Soc.* **61**, 340.
 Wallenfels, K. & Malhotra, O. P. (1960). In *The Enzymes*, 2nd ed., vol. 4, p. 409. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press Inc.

Biochem. J. (1962) **82**, 232

An Investigation of Suggested Physiological Functions of Carnitine

By A. E. BENDER AND E. P. ADAMS

Research Department, Bovril Ltd., 148 Old Street, London, E.C. 1

(Received 14 June 1961)

Carnitine (β -hydroxy- γ -butyrottrimethylbetaine) was first isolated by Gulewitsch & Krimberg (1905), and simultaneously, under the name novain, by Kutscher (1905). Its significance in metabolism was investigated sporadically during the next 25 years, but interest subsequently waned until Carter, Bhattacharyya, Weidman & Fraenkel (1952) showed that vitamin B₁₂, essential for the development of the meal worm, *Tenebrio molitor*, was identical with carnitine. A further stimulus to the renewed interest in this substance came from its commercial synthesis (Deschamps, Buu-Hoi, Le Bihan & Binon, 1954).

Carnitine is an essential growth factor for at least six organisms, *Tenebrio molitor* (the meal worm), *Tenebrio obscurus*, *Palorus ratzeburgi*, *Gnathocerus cornutus*, *Tribolium confusum* and *Tribolium castaneum*, and is widely distributed in plant and animal tissues (Fraenkel & Friedman, 1957). Although it is present in aqueous extract of animal muscle to the extent of about 3.5% of the dry weight of the protein-free extract (Bender, Wood & Palgrave, 1958) its physiological function has not been ascertained. Various functions have been postulated and some of these have been examined in the present work. It has been reported

that carnitine (*a*) stimulates appetite and weight gain in premature infants (Canlorbe, Deltour, Borniche & Scholler, 1956; Rovinski & Camous, 1957) and poorly developed children (Criscione & Genoese, 1957) and stimulates growth in tissue culture (Liébecq-Hutter, 1960); (*b*) stimulates the secretion of salivary, gastric, pancreatic and biliary juices (Kutscher & Lohman, 1906; Krimberg, 1925; Charlier, 1954, 1955; Chassagne & Jerome, 1958); (*c*) is involved in fat metabolism. It was reported to be a component of lipocaic (Binon & Deltour, 1955), of vagotonin (Binon & Deltour, 1956*a*) and of the phospholipids of blood (Binon & Deltour, 1956*b*); to increase the rate of output of thoracic duct lymph in dogs, to increase the fat in the milk of cows (de Schaepdryver & de Vleeschhouwer, 1957, 1960), to stimulate fatty acid oxidation in isolated liver preparations (Fritz, 1955; Fritz & McEwen, 1959; Fritz & Kaplan, 1960) and it was suggested that acetylcarnitine is an acetyl donor to coenzyme A (Friedman & Fraenkel, 1955).

The suggested effects of carnitine on growth, on stimulation of protein synthesis and on gastric secretion have been examined. The suggested presence of carnitine in fractions from pancreas has been re-examined. We failed to confirm any of these claims.

METHODS

Isolation of lipocaic. The method of Entenman, Chaikoff & Montgomery (1944) was used.

Extraction of pancreas. Fresh ox pancreas, trimmed of large blood vessels and connective tissue, was chopped and minced in acetone in the Waring Blendor, and the acetone solution filtered and evaporated to dryness (fraction *A*). The dry residue was extracted for 10 hr. in a continuous extractor with ether (*B*). A sample of the dehydrated, defatted tissue was extracted continuously for 5 hr. with methanol to extract any free carnitine (*C*).

Samples of the acetone fraction (*A*), the ether extract (*B*), and the insoluble residue were hydrolysed by refluxing with 6*N*-HCl, *A* and *B* for 12 hr., and the insoluble residue for 22 hr. The free fatty acids were removed from the hydrolysates with light petroleum (b.p. 60–80°) and the hydrolysates evaporated to dryness. All six preparations, *A*, *B*, their hydrolysates, a hydrolysate of the residue, and fraction *C*, were examined for the presence of betaines by chromatography and by reineckate precipitation followed by chromatography of the reineckates.

Chromatography. Paper chromatography was done as described by Adams, Ballance & Bender (1960).

Muscle and liver phospholipids. Excess of fat and large blood vessels were removed and the tissue was homogenized in a Waring Blendor. The homogenate was extracted by refluxing with ethanol-ether (3:1, v/v) for 30 min., and the solution cooled, filtered and evaporated to dryness under vacuum. The residue was extracted with chloroform, in which free carnitine is insoluble, and the chloroform solution evaporated to dryness. The residue of crude phospholipids was extracted three times with light petroleum (b.p. 40–60°) and the combined extracts were dried with Na_2SO_4 .

The phospholipids were precipitated by dilution with an equal volume of acetone, followed by further dilution with 12 vol. of ice-cold acetone. The mixture was cooled to 0° for 30 min., then centrifuged, and the supernatant decanted. The precipitated phospholipids were redissolved in light petroleum and reprecipitated with acetone. With the muscle phospholipids extra care was taken to avoid contamination with free carnitine by repeatedly washing the light-petroleum extract with small amounts of water. The phospholipids were hydrolysed by refluxing for 3 hr. with equal volumes of methanol and 6*N*-HCl. Fatty acids were removed by extraction with light petroleum and the aqueous layer was evaporated to dryness and examined for quaternary-ammonium compounds.

Animal experiments: net protein utilization. The carcass analysis method of Bender & Miller (1953) and Miller & Bender (1955) was used. Four litters of rats, 30 days old, were arranged in groups of four animals, comprising one from each litter. The groups were arranged so that the combined body weights of each group were the same to within 2 g.; sex differences were ignored. One group was given non-protein diet, the second group 30% casein diet, and the third group 30% casein supplemented with carnitine (120 mg./4 rats/day, added to the top of food). The non-protein diet was composed of 15% margarine fat, 10% uncooked potato starch, 20% glucose, 50% rice starch, 5% mineral salt mixture (Hawk, Oser & Summerson, 1947) and a complete vitamin supplement (Miller & Bender, 1955). For the protein diets casein replaced the rice starch in amounts sufficient to give a 30% protein level. The animals were fed for 10 days, then killed with ether and dried to constant body weight in an oven at 105° (24 hr.). The nitrogen content of the carcasses was calculated from the water content (Bender & Miller, 1953) by using the N:H₂O ratio for 40-day-old rats predetermined on the Bovril Research colony. The net protein utilization of casein fed at 30% was the difference between the carcass nitrogen of the casein and non-protein groups divided by the nitrogen intake (Bender & Doell, 1957*a*). Duplicate assays were carried out 2 weeks later on a different set of litters.

The protein retention efficiency was calculated from the difference in body weight of the casein and non-protein groups, divided by protein intake, and multiplied by 16 (Bender & Doell, 1957*b*).

The protein efficiency ratio is the weight gain of each casein-fed group divided by protein intake.

Gastric secretion. This was determined in intact tubes of isolated gastric mucosa in the Warburg vessel as described by Davies (1948).

Carnitine. The carnitine used was an aqueous 10% solution of the chloride purchased from Labaz Laboratories, Belgium, under the trade name Bicarnesine.

RESULTS

Lipocaic. Only choline was found when examined by reineckate precipitation and chromatography of the reineckate.

Ox pancreas. Carnitine was detected in traces only in the methanol extract and in the tissue residue.

Muscle and liver phospholipids. Only choline was found in these fractions when examined by

reineckate precipitation and chromatography of the reineckate.

Protein synthesis. The results in Table 1 show that net protein utilization of casein, with and without carnitine, was 30. Carnitine could not be shown to have any effect when the results were calculated as protein retention efficiency and as protein efficiency ratio.

Growth. Thirty-two weanling albino rats, obtained from the Mousery, Rayleigh, Essex, were divided into four groups: (a) eight males and (b) nine females, as controls; (c) seven males and (d) eight females, as experimental groups, and the animals were fed *ad lib*. The diet contained 20% casein, the remainder being as described above. The animals were weighed weekly. Carnitine chloride (0.2 ml. of a 10% solution/cage of 4 rats) was added to the surface of the food daily for 6 days of each week; after 2 weeks the dose was doubled. Carnitine had no significant effect on the weight gain of male or female rats over a 5½-week experiment.

Growth of nurslings. Six litters, comprising eight to twelve animals, were selected, and five litters of similar size were used as controls. Carnitine (1 mg./g. body wt.) was fed to the six mother rats from the day of birth of their litters on 5 days each week for 3 weeks. The litters were weighed on the day of birth and at 7, 14, 21 and 28 days. They

were weaned at 21 days. There was no significant difference in the weights of the litters from carnitine-treated and control nursing mothers at 14 days and at 28 days of age.

Effect of injected carnitine. A litter of nine rats, 42 days old, was divided into five treated (3M and 2F) and 4 control animals (2M and 2F). The treated rats received subcutaneous injections of 78.5 mg. (1.0 ml.) of carnitine chloride (as a neutralized solution) four times, on the first, third, eighth and tenth day of experiment and all were weighed at weekly intervals for 4 weeks. A second litter of 7 rats, 30 days old, was similarly divided into 4 treated (2M and 2F) and 3 control (2M and 1F) animals. The treated rats received 100 mg. (1.3 ml.) of neutralized carnitine chloride subcutaneously three times, on the first, fourth and sixth day of the experiment. There was no evidence of any significant effect of injected carnitine on body weight. The mean weight increase of the five treated males was 115% in 4 weeks compared with 111% for the four controls, and for the four treated females was 70% compared with 69% for the three controls.

Gastric secretion. When a closed bag prepared from toad gastric mucosa was shaken in 'bicarbonate saline' (Davies, 1948) with CO₂ + O₂ (1:19, v/v) there was a small steady uptake of O₂ corresponding to acid secretion into the middle of the bag. The rate varied with different mucosae but in each case remained reasonably constant. Addition of histamine (final concentration 0.047 µg./ml.) or meat extract (0.97 mg./ml.) produced a marked stimulation of O₂ uptake but carnitine (up to 34 µg./ml.) had no effect (0.97 mg. of meat extract contains 34 µg. of carnitine). Mucosae that failed to respond to carnitine did respond to the subsequent addition of histamine or meat extract.

Table 1. *Progressive fall in net protein utilization of casein fed to the rat at increasing concentrations in the diet*

Four rats were used as a group in a 10-day test and the carcass nitrogen was calculated from the water content. Net protein utilization is (carcass N of test group minus carcass N of a group of litter mates fed a non-protein diet) divided by nitrogen intake from the test protein. Duplicate assays were carried out in separate experiments on different litters. Carnitine was added to the diet at a concentration of 30 mg./rat/day.

Casein in diet (%)	Net protein utilization
8	60, 63
11.6	56, 60
15.6	55, 51
18.2	49, 49
22.3	49, 51
28.2	40, 36
29.9	30, 31

Absence of effect of carnitine on the reduced protein utilization of casein fed to the rat at 30% of the diet

	Casein alone	Casein + carnitine
Net protein utilization	30, 31	29, 30
Protein retention efficiency	34, 35	35, 35
Protein efficiency ratio	1.3, 1.7	1.4, 1.7
Weight gain (g.)	115, 150	137, 152

DISCUSSION

Adams *et al.* (1960) were unable to confirm the claim of Binon & Deltour (1956b) that carnitine constitutes one-third of the nitrogenous base of blood phospholipids. Since this finding was published G. Deltour has stated (unpublished work) that the original claim was based on the separation of reineckates and the growth of *Tenebrio molitor* and that carnitine has not been specifically identified.

A similar explanation can be put forward for the claims that carnitine is a component of vagotonin and lipocaic (Binon & Deltour, 1955, 1956a). In the pancreas examined in the present work only a trace of carnitine was found in the methanol extract and in the tissue residue and this is probably similar in quantity to the carnitine known to be present in various tissues (Fraenkel & Friedman, 1957).

The properties of carnitine as a stimulant for growth in poorly developed children and premature babies have apparently been so well accepted clinically that the substance is commercially available as a pharmaceutical preparation in several countries in Europe. Kieny (1956) showed that carnitine stimulated the growth of articular cartilage in a synthetic medium *in vitro*, and Liébecq-Hutter (1957, 1960) observed a stimulation of growth of rudiments of tibia and fibula of chick embryos. Silakova (1958) linked this growth stimulation with that observed on premature babies and infants by Canlorbe *et al.* (1956), Rovinski & Camous (1957) and Criscione & Genoese (1957).

Canlorbe *et al.* (1956) gave 10 mg./kg. body wt./day for periods of 7 days to 2 months to 28 nurslings aged 1 month to 2 years hospitalized for infection and showing anorexia. Twenty-two cases showed a 'rapid improvement in appetite' and 21 increased in growth rate. Nineteen infants over 2 years of age suffering from retarded growth were given 10–15 mg. of carnitine/kg. for periods of 2–7 months and nine showed increased growth. Of five premature infants four responded with improved appetite and three with increased growth.

Borniche & Canlorbe (1960) gave 200–500 mg. of carnitine/day for periods up to 4 months to 34 infants, aged from 1 month to 6 years, suffering from 'post-infection malnutrition'. There was an 'improvement in weight gain accompanied by improved appetite', a rise in serum albumin and a fall in globulins. Alexander (1956) (also Alexander, Peeters & Vuylsteke, 1959) treated 122 infants, of 3–6 years of age, with 600 mg. of carnitine/day for periods of 2–5 months. All were suffering from some form of primary tuberculosis accompanied by anorexia. He reported weight gains of 8.7, 9.7, 9.2 and 12.2% in groups aged 3, 4, 5 and 6 years, respectively, whereas in 133 controls the gains were 4.3, 4.8, 6.3 and 7.9%, respectively.

Strack, Rotzsch & Lorenz (1960) claimed that carnitine (0.1 mg./g.) fed to nursing rats caused a gain in weight of the nurslings, 'especially of the poorer specimens', but no data were provided. Strack & Rotzsch (1960) claimed that subcutaneous administration of carnitine (0.25–0.5 mg./g.) had a nitrogen-sparing effect on urinary nitrogen, and reversed the negative nitrogen balance induced by thyroxine. Individual values of nitrogen excretion of five rats were given but there was no significant difference between the urinary nitrogen of carnitine-treated and control rats, or between thyroxine-treated and thyroxine-carnitine-treated rats. Alexander *et al.* (1959) found no change in nitrogen balance in four children given carnitine. The results of the present work show no change in growth rate of young rats given carnitine

by mouth or by subcutaneous injection, no effect on nurslings when the mother was given carnitine and no effect on protein synthesis.

SUMMARY

1. A number of claims for suggested physiological functions of carnitine (β -hydroxy- γ -butyrotimethylbetaine) have been examined.
2. The presence of carnitine in lipocic and muscle and liver phospholipids was not confirmed.
3. Carnitine had no effect on protein synthesis as determined by the net protein utilization of casein fed at a level beyond the rat's normal ability to synthesize tissue protein.
4. The growth rate of weanling rats was not affected when carnitine was fed daily for 5½ weeks.
5. The growth rate of young rats was not affected when carnitine was administered by subcutaneous injection.
6. The growth of nursing rats was not affected when carnitine was fed to the mothers.
7. Carnitine did not stimulate acid secretion in the isolated gastric mucosa of the toad.

REFERENCES

- Adams, E. P., Ballance, P. E. & Bender, A. E. (1960). *Nature, Lond.*, **185**, 612.
- Alexander, F. (1956). *Acta paediat. belg.* **10**, 170.
- Alexander, F., Peeters, H. & Vuylsteke, J. (1959). *Proc. 6th Colloq. Protides of biol. Fluids, Bruges*, p. 306. Amsterdam: Elsevier.
- Bender, A. E. & Doell, B. H. (1957a). *Brit. J. Nutr.* **11**, 138.
- Bender, A. E. & Doell, B. H. (1957b). *Brit. J. Nutr.* **11**, 140.
- Bender, A. E. & Miller, D. S. (1953). *Biochem. J.* **53**, vii.
- Bender, A. E., Wood, T. & Palgrave, J. A. (1958). *J. Sci. Fd Agric.* **9**, 812.
- Binon, F. & Deltour, G. (1955). *C.R. Soc. Biol., Paris*, **149**, 932.
- Binon, F. & Deltour, G. (1956a). *Experientia*, **12**, 357.
- Binon, F. & Deltour, G. (1956b). *Rev. Ferment.* **11**, 14.
- Borniche, P. & Canlorbe, P. (1960). *Clin. chim. Acta*, **5**, 171.
- Canlorbe, P., Deltour, G., Borniche, P. & Scholler, R. (1956). *Ann. Pédiat.* **32**, 276.
- Carter, H. E., Bhattacharyya, P. K., Weidman, K. R. & Fraenkel, G. (1952). *Arch. Biochem. Biophys.* **38**, 405.
- Charlier, R. (1954). *Arch. int. Pharmacodyn.* **98**, 251.
- Charlier, R. (1955). *C.R. Soc. Biol., Paris*, **149**, 934.
- Chassagne, P. & Jerome, H. (1958). *Thérapie*, **13**, 400.
- Criscione, C. & Genoese, V. (1957). *Aggiorn. pediatri.* **8**, 425.
- Davies, R. E. (1948). *Biochem. J.* **42**, 609.
- de Schaepdryver, A. F. & de Vleeschhouwer, G. R. (1957). *Arch. intern. pharmacodyn.* **113**, 15.
- de Schaepdryver, A. F. & de Vleeschhouwer, G. R. (1960). *Proc. 7th Colloq. Protides of biol. Fluids, Bruges*, p. 268. Amsterdam: Elsevier.
- Deschamps, G., Buu-Hoi, N. P., Le Bihan, H. & Binon, F. (1954). *C.R. Acad. Sci., Paris*, **238**, 826.
- Entenman, C., Chaikoff, I. L. & Montgomery, M. L. (1944). *J. biol. Chem.* **155**, 573.

- Fraenkel, G. & Friedman, S. (1957). *Vitam. & Horm.* **15**, 73.
- Friedman, S. & Fraenkel, G. (1955). *Arch. Biochem. Biophys.* **59**, 491.
- Fritz, I. B. (1955). *Acta physiol. scand.* **34**, 367.
- Fritz, I. B. & Kaplan, E. (1960). *Proc. 7th Colloq. Protides of biol. Fluids, Bruges*, p. 252. Amsterdam: Elsevier.
- Fritz, I. B. & McEwen, B. (1959). *Science*, **129**, 334.
- Gulewitsch, W. & Krimberg, R. (1905). *Hoppe-Seyl. Z.* **45**, 326.
- Hawk, P. B., Oser, B. L. & Summerson, W. V. (1947). *Practical Physiological Chemistry*, 12th ed., p. 1273. London: J. and A. Churchill Ltd.
- Kieny, M. (1956). *C.R. Acad. Sci., Paris*, **243**, 320.
- Krimberg, R. (1925). *Biochim. Z.* **157**, 186.
- Kutscher, F. (1905). *Z. Untersuch. Nahr.- u. Genussm.* **10**, 528.
- Kutscher, F. & Lohman, A. (1906). *Pflüg. Arch. ges. Physiol.* **114**, 553.
- Liébecq-Hutter, S. (1957). *Arch. Biol.* **68**, 201.
- Liébecq-Hutter, S. (1960). *Proc. 7th Colloq. Protides of biol. Fluids, Bruges*, p. 245. Amsterdam: Elsevier.
- Miller, D. S. & Bender, A. E. (1955). *Brit. J. Nutr.* **9**, 382.
- Rovinski, J. & Camous, J. P. (1957). *Sem. Hôp.* **33**, 538.
- Silakova, A. J. (1958). *Biokhem. Zh.* **30**, (4), 604.
- Strack E. & Rotzsch, W. (1960). *Proc. 7th Colloq. Protides biol. Fluids, Bruges*, p. 263. Amsterdam: Elsevier.
- Strack, E., Rotzsch, W. & Lorenz, I. (1960). *Proc. 7th Colloq. Protides of biol. Fluids, Bruges*, p. 235. Amsterdam: Elsevier.

Biochem. J. (1962) **82**, 236

Identification of Some Peptides from an Arginine-Rich Histone and their Bearing on the Structure of Deoxyribonucleohistone

By D. M. P. PHILLIPS AND PAMELA SIMSON

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, S.W. 3

(Received 18 August 1961)

It is generally accepted that the histones are attached to the DNA of cell nuclei by salt linkages. Some estimates of the relative amounts of the histones and the DNA indicate that the basic groups in these proteins could, if such is their role, neutralize from 85 to 100 % of the DNA phosphate groups (Davison & Butler, 1956; Crampton, Stein & Moore, 1957; Vendrely, Knobloch-Mazen & Vendrely, 1960). The deficiency of basic groups as compared with phosphate in some preparations may be due to the loss of histone during preparation of the deoxyribonucleoprotein. A model for the structure of deoxyribonucleohistone should therefore show the close equivalence of basic and phosphate groups in salt linkages.

Since the phosphate groups in DNA are regularly spaced along the polynucleotide chains it might perhaps be expected that the neutralizing basic groups in the histones (due mainly to lysyl and arginyl residues) would be evenly spaced along the polypeptide chains. This arrangement would require three non-basic residues between each pair of basic amino acids, since in most of the histones approximately one-quarter of the amino acid residues are basic amino acids. Degradation of such a structure by trypsin (which has a specificity dependent on the arginyl and lysyl residues of the substrate) would then yield a mixture of tetrapeptides which, except perhaps for the C-terminal peptide, would each bear a C-terminal lysyl or arginyl residue.

This work describes some experiments with a tryptic digest of a calf-thymus histone fraction undertaken to establish whether the basic amino acid distribution follows this regular pattern or not. A preliminary account has already been published (Phillips & Simson, 1961).

EXPERIMENTAL

The histone used for most of this work was an arginine-rich fraction (lysine:arginine molar ratio, 0.8:1) of calf-thymus histones prepared by an acid-ethanol-extraction procedure (Johns, Phillips, Simson & Butler, 1960). The amino acid composition and the N-terminal group analyses are given in Table 1. Preparations designated F3/78 and F3/83 were both used for the experiments on the precipitates from the digests, whereas only the F3/83 preparation was used for the study of the small soluble peptides.

Tryptic digestion. Salt-free crystalline trypsin (1 mg.) in 1 ml. of water was added to 105 mg. of the histone dissolved in 9 ml. of 3.3 mm-phosphate buffer, pH 7.7. The digest was kept (with a drop of chloroform) at 40° and within 2 min. a precipitate began to form. After 24 hr. the digest was centrifuged, and the supernatant decanted and reduced in volume to 0.75 ml., so that it then contained about 0.8 mg. of soluble peptides in 0.01 ml. The precipitate from the digest was washed with the same phosphate buffer and suspended in dilute HCl at about pH 3 for storage.

Electrophoresis and chromatography. Electrophoresis of the soluble peptides was carried out on Whatman no. 3MM filter paper which had been previously washed with butan-1-ol-acetic acid-water (2:1:1, by vol.) by downward irrigation for 36 hr. After the paper had been dipped in a