SUMMARY

An enzyme has been found in rat tissues, which forms thiosulfate from mercaptopyruvate and sulfite. Liver, kidney and blood are the most active tissues. When sulfite is replaced by sulfinates, thiosulfonates are formed. The formation of the salts of two new compounds, aminoethanethiosulfonic acid (thiotaurine) and alaninethiosulfonic acid has been demonstrated. The significance of these reactions in sulfur metabolism is discussed.

REFERENCES

1 J. H. GAST, K. ARAI AND F. L. ALDRICH, *J. Biol. Chem.*, 196 (1952) 875.
2 CL. FROMAGEOT AND A. ROYER, *Enzymologia*, 11 (1945) 361.
3 B. SÖRBO, *Biochim. Biophys. Acta*, 21 (1956) 393.
4 J. PARROD AND M. RAHIER, *Bull. soc. chim. France*, (1947) 109.
5 T. F. LAVINE, *J. Biol. Chem.*, 113 (1936) 583.
6 E. BRICAS, F. KIEFFER AND CL. FROMAGEOT, *Biochim. Biophys. Acta*, 18 (1955) 358.
7 B. SÖRBO, *Biochim. Biophys. Acta*, 23 (1957) 412.
8 S. BERNTSON, *Anal. Chem.*, 27 (1955) 1659.
9 F. CHATAGNER, B. BERGERET AND CL. FROMAGEOT, *Biochim. Biophys. Acta*, 9 (1952) 340.
10 A. GUTMAN, *Z. anal. Chem.*, 47 (1908) 294.
11 A. MEISTER, P. E. FRASER AND S. V. TICE, *J. Biol. Chem.*, 206 (1954) 561.
12 T. P. SINGER AND E. B. KEARNEY, in W. D. MCELROY AND B. GLASS, *A Symposium on Amino Acid Metabolism*, Johns Hopkins Press, Baltimore, 1955, p. 558.
13 CL. FROMAGEOT AND M. A. ROYANÉ, *Helv. Chim. Acta*, 29 (1946) 1274.
14 J. L. WOOD AND H. FIEDLER, *J. Biol. Chem.*, 205 (1953) 231.
15 B. SÖRBO, *Acta Chem. Scand.*, 8 (1954) 694.
16 H. FIEDLER AND J. L. WOOD, *J. Biol. Chem.*, 222 (1956) 387.
17 CL. FROMAGEOT, E. WOOKEY AND P. CHAIX, *Compt. rend.*, 209 (1939) 1019.
18 F. CHATAGNER AND G. SAURET-IGNAZI, *Bull. soc. chim. biol.*, 38 (1956) 415.
19 E. L. SMITH AND E. F. TULLER, *Arch. Biochem. Biophys.*, 54 (1955) 114.
20 B. H. SÖRBO, *Acta Chem. Scand.*, 7 (1953) 1137.

Received November 26th, 1956

DIALYZABILITY OF HISTONES*

BOHDAN BAKAY, LOUISE B. KIRSCHNER AND GERRIT TOENNIES

Lankenau Hospital Research Institute and Institute for Cancer Research, Philadelphia, Pa. (U.S.A.)

Recent investigations by ultracentrifugation[1–4], electrophoresis[3–8], salting-out[8,9], isoelectric precipitation[8], or chromatography[5,10,11] have shown that histone is a complex which can be resolved into several fractions. The findings differ in regard to the number and ratio of histone components and in regard to their amino acid content. The inconsistencies appear to be due in part to the natural tendency of histones to form aggregates, and in part to the application of non-quantitative fractionation procedures. For example, it has been our experience[2] and also that of DAVISON *et al.*[4] and CRAMPTON[11] that dialysis has marked effects upon histone preparations and seems to

* Aided by research grants from the National Cancer Institute of the U.S. Public Health Service (C-1249) and the American Cancer Society (BCH-55), and by an institutional grant of the American Cancer Society.

References p. 333.

alter the quantitative relationship of the natural components. Therefore, the following investigation was undertaken.

EXPERIMENTAL

Methods

The histones used for dialysis were prepared from a purified calf thymus nucleoprotein preparation (TNP) obtained by a modification of the method of Huiskamp[2]. One histone preparation, TPNH, was obtained by our chloroform-octyl alcohol procedure[2], and the other, TPNH', was prepared by repeated extraction of TNP with 0.1 *N* HCl as described by DAVISON *et al.*[4]. Both histone extracts were neutralized to pH 7.0 and lyophilized.

Employing an assembly based on the apparatus of STEGEMANN AND TOENNIES[12] both histones were dialyzed at 25°, with a maximum of agitation, against a continuous stream of 0.01 *M* citric acid, of pH 2.6, distilled H_2O or 0.01 *M* sodium borate buffer of pH 9.0.

As shown in Fig. 1, a cellulose casing (A) ("Nojax" casing 8/32" diameter, Visking Corp., Chicago 38, Ill.) containing 5 ml of histone solution (approximately 15 mg nitrogen) and a magnetic stirring bar (B) covered with polyethylene[12] was placed in a 10 × 140 mm i.d. tube (C) with a fritted coarse filter disk (D) (similar to the fritted disk of Buchner funnels No. 36060, code 418100-2C, Corning Glass Works, Corning, N.Y.) on top and a valve joint (E) (right angle ST 12/30 stopcock from a J-310 absorption tube, Scientific Glass Apparatus Co., Inc., Bloomfield, N.J.) on the bottom. The top of the tube (C) was connected by polyethylene tubing (F) to a 5 cm long capillary (G) of 0.5 mm diameter and a coarse gas dispersion tube (H) (39533 tube, code 404260-12C, Corning Glass Works, Corning N.Y.). This filter was placed in a 250 ml Erlenmeyer flask (I) which contained the dialyzing liquid (J). The level of the liquid was maintained constant by connecting the bottom flask (I) with a 10 × 150 mm o.d. slanted tip glass tube (K) to a 3000 ml bottle (L). The effluent from the nozzle (M) was collected in 40 ml fractions by an automatic fraction collector at a rate of 1 ml per 2 minutes. The solution in the cellulose casing was agitated continuously by a slowly rotating magnet (N) as described by STEGEMANN AND TOENNIES[12]. Determinations of total nitrogen, ninhydrin reaction and absorption of U.V. light at 260 mμ were used to characterize the successive effluent fractions.

Results

Figs. 2 and 3 indicate that in the case of both TNPH and TNPH' the amount of histone material which dialyzes, decreases rapidly with time and with increasing pH. For

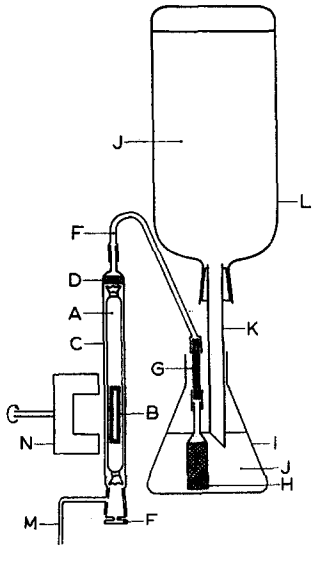

Fig. 1. Diagram of dialyzing assembly.

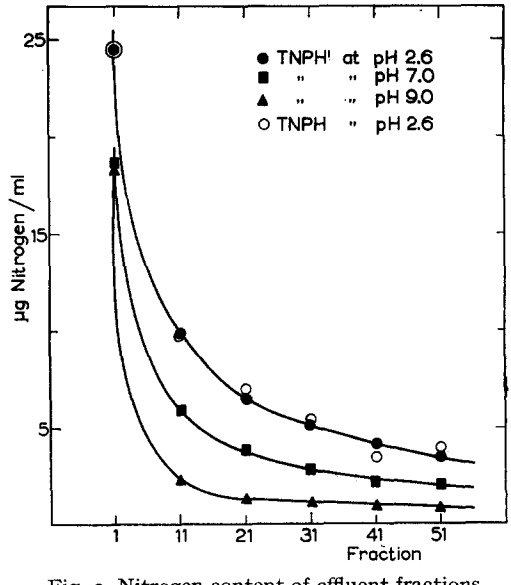

Fig. 2. Nitrogen content of effluent fractions.

References p. 333.

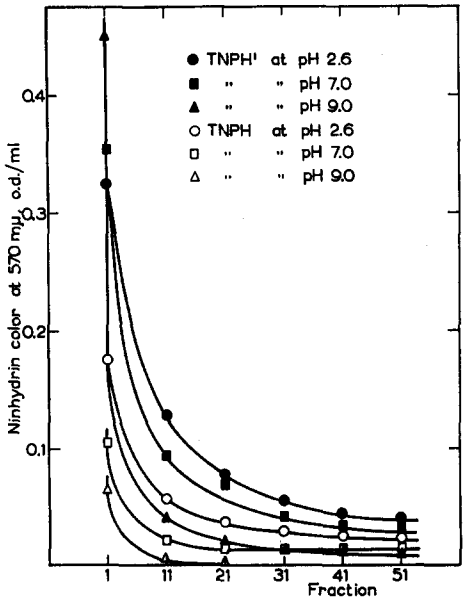

Fig. 3. Ninhydrin color of effluent fractions.

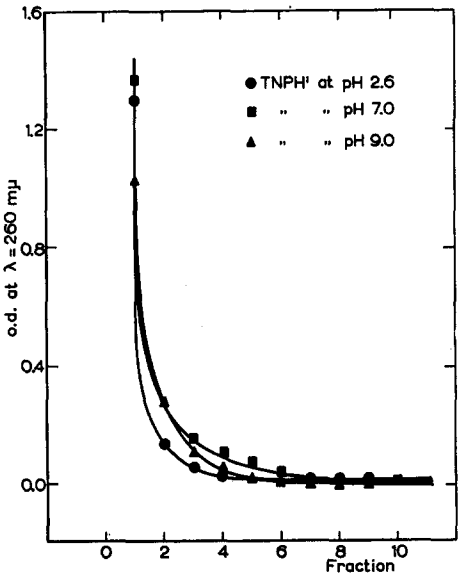

Fig. 4. U.V. absorption of TNPH' effluent fractions.

example, at pH 2.6 TNPH' continued to dialyze after losing 70% of the total nitrogen, but at pH 9.0 dialysis was approaching completion after 28% of the total nitrogen was removed. The table below shows the cumulative yields of N obtained in the permeate after about three days of dialysis.

Dialyzing medium	*% of total N removed by dialysis against 2000 ml medium*	
	TNPH	*TNPH'*
0.01 *M* citric acid pH 2,6	70	69
H_2O	33	58
0.01 *M* sodium borate pH 9.0	12	28

Fig. 3 shows that the yield of ninhydrin color is much greater in the permeate of TNPH' than in that of TNPH.

Fig. 4 shows that the early fractions of the TNPH' permeate contain some nucleic acid degradation products, which add up to approximately 3% nucleic acid when calculated from the U.V. absorption at 260 mμ. Phosphorus determinations[2] and ultraviolet measurements (unpublished) show that the TNPH is practically free of nucleic components.

Fig. 5 shows that in regard to relative ninhydrin color of permeate (ratio of $-NH_2$ to N) the two preparations differ, and also that the fractions obtained at different pH levels differ. Although in terms of nitrogen there is no difference in dialyzability of TNPH and TNPH' at pH 2.6, the ninhydrin color of the latter permeate is approximately twice as high as that of the former.

References p. 333.

In terms of molecular weight considerations it is not surprising that in each of these experiments the more rapidly dialyzing fractions show more reactive $-NH_2$ groups. It is not so readily apparent why in the dialyzates obtained at higher pH values greater relative ninhydrin color is associated with slower dialysis (*cf.* Figs. 2 and 3).

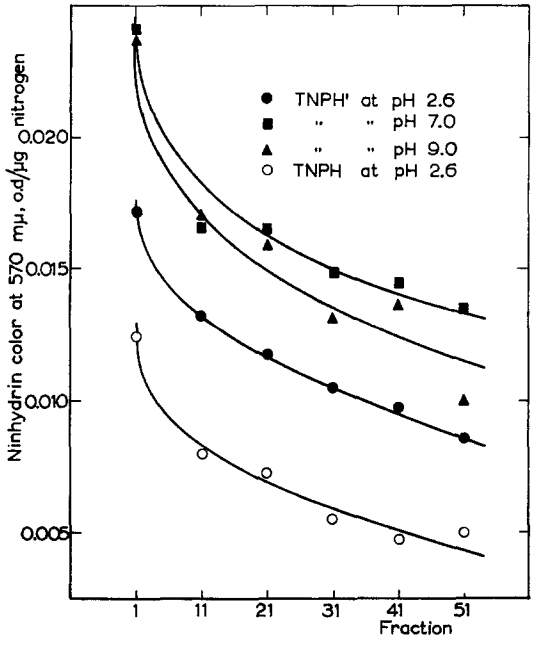

Fig. 5. Ratio of amino nitrogen to total nitrogen in effluent fractions.

DISCUSSION

That different histone fractions differ in ninhydrin color has been shown before: DALY AND MIRSKY[8] as well as DAVISON AND SHOOTER[5] report that lysine-rich histone gives twice as much ninhydrin color as arginine-rich histone and that the non-fractionated histone gives less color than either of these sub-fractions. Correlation of these observations with the results of the present study must await amino acid analyses of dialysis fractions which are now pending. The occurrence of reversible equilibria or of enzymic processes are other questions which remain to be examined.

The present data indicate that the presence or absence of dialysis, as well as its conditions and extent, in the course of preparation, will affect the composition of the histones obtained from calf thymus nucleoprotein.

SUMMARY

A study of the effect of dialysis on histone preparations obtained from calf thymus nucleoprotein by different methods showed that amount and characteristics of dialyzable material vary with the mode of preparation and the pH of the dialyzing medium. A modified assembly for fractional dialysis is described.

References p. 333.

REFERENCES

[1] L. AHLSTRÖM, *Arkiv Kemi, Mineral., Geol.*, 24A, No. 31, (1947).
[2] B. BAKAY, J. J. KOLB AND G. TOENNIES, *Arch. Biochem. Biophys.*, 58 (1955) 144.
[3] J. A. V. BUTLER, P. F. DAVISON, D. W. F. JAMES AND K. V. SHOOTER, *Biochim. Biophys. Acta*, 13 (1954) 224.
[4] P. F. DAVISON, D. W. F. JAMES, K. V. SHOOTER AND J. A. V. BUTLER, *Biochim. Biophys. Acta*, 15 (1954) 415.
[5] P. F. DAVISON AND K. V. SHOOTER, *Bull. soc. chim. Belges*, 65 (1956) 85.
[6] H. J. CRUFT, C. M. MAURITZEN AND E. STEDMAN, *Nature*, 174 (1954) 580.
[7] J. GRÉGOIRE, J. GRÉGOIRE AND J. REYNAUD, *Compt. rend.*, 236 (1953) 192.
[8] M. M. DALY AND A. E. MIRSKY, *J. Gen. Physiol.*, 38 (1955) 405.
[9] P. F. DAVISON AND J. A. V. BUTLER, *Biochim. Biophys. Acta*, 15 (1954) 439.
[10] C. F. CRAMPTON, S. MOORE AND W. H. STEIN, *J. Biol. Chem.*, 215 (1955) 787.
[11] C. F. CRAMPTON, *Federation Proc.*, 15 (1956) 237.
[12] H. STEGEMANN AND G. TOENNIES, *Nature*, 177 (1956) 440.

Received October 17th, 1956

THE TRANSFER OF SODIUM IONS BETWEEN MAMMALIAN MUSCLE AND THE SURROUNDING MEDIUM

H. McLENNAN

Department of Physiology, Dalhousie University, Halifax (Canada)

Previous investigations[1,2] have dealt with the movement of potassium ions across the cell membrane separating the intracellular fluid of mammalian muscle from the extracellular space. These studies showed that the uptake of labelled K by muscle cells when the tissue is immersed in a saline medium containing a physiological concentration of K is a process strongly dependent on the supply of metabolic energy, and that any agency which interferes with the metabolism of the tissue inhibits the uptake of K. In contrast, the loss of K from the cells is by this criterion a passive process. It was further shown that when a muscle is incubated *in vitro* in a solution containing 5 mmoles/l of K, the exchange of tissue K proceeds until some 50% of the analytically determined K has turned over, after which no further exchange takes place. Increasing the concentration of K in the incubation medium brings about an increase in the extent of tissue K exchange, but does not affect the time constant governing the process. Complete exchange is obtained with an external K concentration of 20 mmoles/l.

These studies have now been extended to include the transfer of sodium ions in muscle. No evidence has been found for the presence of difficultly exchangeable Na in the muscle analogous to the inexchangeable K, such as has been reported by CONWAY AND CAREY[3] and by HARRIS AND STEINBACH[4] in frog muscle. The efflux of Na from the muscle and influx of K have been shown to be mutually dependent, inasmuch as changes in the external concentrations of either ionic species affect both fluxes similarly in most cases.

References p. 339.