THE NATURE AND PHYSICOCHEMICAL PROPERTIES OF HISTONES

By H. J. CRUFT, C. M. MAURITZEN* and E. STEDMAN, F.R.S.

Department of Biochemistry, University of Edinburgh

(*Received* 11 *June* 1956†)

(Plates 5 to 9)

CONTENTS

* Imperial Chemical Industries Research Fellow.

† The Secretaries regret that because of an unfortunate misunderstanding of the authors' intentions the publication of this paper has been delayed for nine months.

The electrophoretic analysis of histones extracted from the cell nuclei isolated from a number of different tissues and species has shown that, while there are some exceptions, these consist in general of three components which have been termed α-, β-, and γ-histones. The α- and γ-components are not, however, necessarily homogeneous proteins, for in those cases where it has been possible to fractionate the total histone either by chemical methods or by sedimentation or by a combination of both these procedures, the former has been found to consist of at least three components and the latter probably of two. The components of the α-fraction have been distinguished by the symbols α_1, α_2, and α_3, respectively. The β-histones correspond essentially to the 'main', and the α- and γ-fractions to the 'subsidiary', histones of the earlier provisional nomenclature. The β-histones possess the typical composition of traditional histones. They are further characterized by their ability to form aggregates of increasing size as the pH's of their solutions are raised, a phenomenon brought to light by measurements of their diffusion coefficients and sedimentation constants. Subsidiary histones differ from β-histones both in amino acid composition and in physical properties. In particular, they do not exhibit the phenomenon of aggregation shown by the latter. Nevertheless, all of them resemble the β-histones in possessing strongly basic properties, a fact which is evident from their mobility-pH curves. The isoelectric points of a number of β-histones have been determined from such curves.

Further evidence supporting the conception that histones are cell-specific is recorded. This has been provided mainly by a study of abnormal cells. Only small differences in isoelectric points and in electrophoretic mobilities have so far been detected between the β-histones from the liver cells and thymocytes of the ox.

Examination of the histones from a number of tumours, both natural and experimental, has shown that β-histones from these sources usually differ from normal β-histones in possessing much smaller solubilities and electrophoretic mobilities and markedly greater capacities to aggregate. No combination occurs between histone and azo dye during the induction of hepatomas in rats by this carcinogen. Some dye is nevertheless bound in the nucleus during the induction period. It is presumably bound by chromosomin. It is concluded that the carcinogenic action of the dye is due to its combination with, and consequent modification or inhibition of, one or more genes.

In conformity with the results of this and former investigations the terms protamin and histone have been defined more precisely than has hitherto been possible.

I. INTRODUCTION

The investigation described below is devoted largely to an examination and characterization from a physicochemical standpoint of the histones isolated from a variety of both normal and malignant tissues. It was commenced immediately after the demonstration in this laboratory (Stedman & Stedman 1950, 1951) that such histones are, in general, of a complex nature. A confirmation and extension of the latter results by electrophoretic analysis is contained in a Thesis by one of us (Cruft 1953). During the further progress of

the work, publications by a number of authors (Gregoire & Limozin 1954; Davison & Butler 1954; Davison, James, Shooter & Butler 1954; Daly & Mirsky 1955; Crampton, Moore & Stein 1955) confirming the heterogeneity of the histone from the thymus gland of the calf, one of the many histones examined by Stedman & Stedman, have appeared in the literature.

As a result of the present work it is apparent that many histones are much more complex than appeared from the chemical fractionation procedure used by Stedman & Stedman. Those from the thymus gland of the calf and the liver of the ox, for example, contain six different components. It has therefore proved necessary to supplement the classification of Stedman & Stedman, who divided these basic proteins into main and subsidiary histones, by one based, in accordance with the procedure adopted for other proteins, on their electrophoretic mobilities. The terms 'main' and 'subsidiary' do not thereby lose their usefulness, for the main histone, originally defined as quantitatively the major component of the unfractionated or total histone, has now been shown to consist substantially of the component designated as β in the new nomenclature. Since, moreover, the physical properties of the β-histones from animal sources differ markedly and in a characteristic manner from the remaining components, it is frequently useful to refer to the latter collectively or individually as subsidiary histones or components. This is particularly the case when they have not been completely characterized according to their electrophoretic mobilities.

The unravelling and characterizing of the individual components of which the complex histones are composed do not constitute the sole purposes of this investigation. Considerable attention has also been paid to the question of the cell specificity of histones, a phenomenon already shown to exist among some of the basic proteins of cell nuclei (Stedman & Stedman 1944, 1947*a*, *b*, 1950, 1951), and to the examination in the light of this phenomenon of the nature of the histones present in malignant cells.

A brief and preliminary account of some of the results recorded have been published elsewhere (Cruft, Mauritzen & Stedman 1954).

II. MATERIAL

The animal tissues used for the preparation of histones were obtained from various sources according to their nature. Normal tissues from domestic animals (ox, pig, sheep, fowl) were collected directly from the slaughter house. When smaller animals (rabbit, rat, mouse) were involved, they were sacrificed in the laboratory and the required organs dissected from the carcasses. Normal human tissues were derived from post-mortem material, which also provided the source of some of the malignant growths examined. Other malignant tissues were obtained from experimental tumours.

When practicable, the tissue was worked up immediately after its removal from the animal. In those cases when this was impossible the material was frozen hard either in solid carbon dioxide or in a deep-freeze and stored in this condition until used. This procedure was particularly useful when only small amounts of the required tissue could be obtained at a given moment. For example, it was thus possible to accumulate a reasonable amount of hepatoma tissue to work up in one operation from rats in which the tumour matured at somewhat different times.

Whichever of the above procedures was adopted, the first stage in the preparation of the histone was the isolation from the tissue of the cell nuclei. This operation was effected essentially as described in former communications from this laboratory (Stedman & Stedman 1951). It has been found, however, that the ease with which 4% acetic acid effects the release of nuclei from their cells varies with the nature of the cells and with the species from which they originate. Provided the tissue is quite fresh, the liberation occurs rapidly with, for example, ox liver. On the other hand, it is a relatively slow process with rat liver. As a result it is necessary when preparing nuclei from the latter tissue, to keep the pulp in 4% acetic acid, with intermittent stirring, for a period of 18 h. This finding makes it possible that the nuclei obtained from rat livers by Mauritzen, Roy & Stedman (1952) were in fact, as was suggested at the time, contaminated with a proportion of whole cells.

Some improvements in general technique have also been made. Whereas earlier preparations of cell nuclei were made at room temperature, which however never exceeded 15° C, the new ones have been effected in a cold room maintained at a temperature not greater than 2° C, the many necessary centrifugations being done in a refrigerated centrifuge at the same temperature. While this change has made no detectable difference in the nature of the product obtained, it has, nevertheless, proved to be a great advantage because it has permitted the process of fractional centrifugation necessary for the purification of the nuclei to be prolonged beyond the period possible under the old conditions. Preparations which would have formerly been rejected because they could not be brought to completion within the limited time available (2 days), have now been completely purified. Low-temperature operation has also been of material assistance in the preparation of the nuclei from fowl erythrocytes, for it has prevented the coalescence of some of the nuclei to a gel during the extraction of the haemoglobin from the lyzed cells, a phenomenon which previously presented some difficulty (Stedman & Stedman 1951).

The method of extraction of the histones from the dry and defatted nuclei by means of dilute mineral acid has remained unmodified. For the precipitation of the histone sulphate from the extracts with sulphuric acid, however, a large excess of acetone has been used in place of the alcohol previously employed. This has produced no evident alteration in the nature of the final product, but the change has been made because acetone is reputed to possess a smaller denaturing action than alcohol.

III. METHODS

(1) *Electrophoresis*

The instrument used for the electrophoresis experiments was supplied by Hilger & Watts, Ltd in 1950. It is based on that designed by Tiselius (1937, 1938) and incorporates the diagonal schlieren optical system developed by Philpot (1938). A medium size U-tube of 11 ml. capacity and a bath temperature of 0·5 to 0·7° C have been employed in all experiments.

For measurements over the pH range 2·5 to 9·6 veronal buffers, made from sodium barbitone, sodium acetate, sodium chloride and hydrochloric acid, according to Michaelis (1931), have been consistently employed. For more alkaline solutions glycine buffers have been prepared from glycine, sodium chloride and sodium hydroxide. These two buffer

systems have been chosen because, when used at the same ionic strength, the transition from one to the other causes no discontinuity in the mobility-pH curve.

The ionic strength of the solvent adopted for all the mobility measurements described is 0·176. This value was chosen after a few preliminary trials with histones at $\frac{1}{2}\Gamma = 0{\cdot}05$, 0·10, 0·15 and 0·20, which showed that, at the higher ionic strengths, there was not only a better separation of the components of the histone, but also a sharpening of the peaks in the electrophoretic pattern. The use of this high ionic strength possesses the additional advantages that the conductivity of 0·3 to 0·5 % protein solutions is virtually identical with that of the buffer with which they have been brought into equilibrium by dialysis; that, with low protein concentrations, the δ-boundary is usually small and difficult to detect after 30 min migration; and that it affords a greater possibility of causing the complete separation of associated proteins.

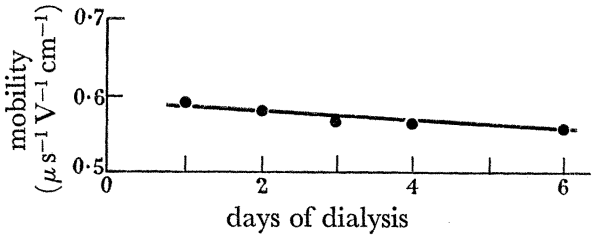


FIGURE 1. Mobility-time of dialysis graph for 0·5 % solution of β-histone from rat spleen nuclei at pH 8·5 and ionic strength 0·176.

The histone solution was prepared for electrophoresis by dissolving, where practicable, 30 to 70 mg of the air-dry histone sulphate or chloride in 12 ml. of buffer or 1 ml. of dilute hydrochloric acid followed by 10 ml. buffer, enclosing the resulting solution in a Cellophane membrane (Visking) and dialyzing it against 250 ml. of the appropriate buffer solution for 50 to 70 h with three or four changes of the latter. Experiments with a 0·5 % solution of histone from rat spleen at pH 8·5 indicated that equilibrium between buffer and protein solution is virtually reached in less than 2 days (figure 1). In later experiments dialysis was carried out in stoppered flasks. This prevented the evaporation of the solvent, with a consequent increase in ionic strength of the buffer, if the period of dialysis was unavoidably prolonged. It was obviously also necessary when using the more alkaline buffers.

The conductivities of the buffer solutions were determined at 4° C with a Mullard conductivity bridge. Measurements of the pH of the buffers were made at 15° C with a pH meter manufactured by Industrial and Scientific Instruments Ltd. This is an a.c. mains, direct-reading instrument using a universal glass electrode and a calomel electrode.

The U-tube of the electrophoresis apparatus was filled by an unusual but rapid and convenient method. The sliding surfaces of the clean tube were coated with a thin layer of the low-temperature lubricant and the two bottom sections pressed together in alinement. The dialyzed protein solution was then pipetted into them until the liquid meniscus rose just above the level of the upper sliding surface. Any visible bubbles inside the tube were removed with a long platinum wire, when the two sections were placed in the U-tube holder. The next section was then slid into position without allowing the bores of the tube to come into alinement, the surplus trace of solution squeezed out of the lower section being absorbed by a piece of filter paper. This section was then filled with the buffer, and the top

section slid on in precisely the same manner, when the remainder of the assemblage was carried out in the customary way. This method of filling the U-tube invariably gave a leak-proof assembly. It possesses the advantage of making it possible to remove any persistent bubbles of air formed in any of the sections during filling.

All electrophoresis experiments were carried out with a uniform current of 30 mA. They were continued for either 2·5 or 3 h, photographs being taken every 30 min.

Electrophoretic mobilities were calculated from the descending boundary only using the equation

$$\mu s^{-1}\ V^{-1}\ cm^{-1} = (d_d q K_s / 36000 A i) \times 10^4,$$

where d_d = the average distance in millimetres moved by the image of the boundary on the plate in 1 h, q = the cross-sectional area of the U-tube, K_s = the specific conductance of the solution, A = the amplification factor of the optical system and i = the current in amperes.

(2) *Diffusion coefficients.*

Solutions of histones required for the determination of diffusion coefficients were prepared in precisely the same manner as those used for electrophoresis. Identical buffer systems and ionic strengths (0·176) were also employed in both cases.

The determinations were made by the process of free diffusion in the electrophoresis apparatus described above using, however, a bath temperature of 20·0° C which was maintained constant within the limits of ±0·003° C. The standard 11 ml. Tiselius cell with an adapted top was used to form the boundaries between solution and solvent, which were photographed at intervals over a period of from 1h to between 2 and 3·5 days after their formation, the actual length of an experiment depending on the rate of diffusion of the histone. In all cases the validity of the Boltzman relation was verified by plotting $\sqrt{t}$ against x (see Geddes 1945). Occasionally it was necessary to use the graph so obtained to correct t, i.e. to give the effective initial time of boundary formation.

As the distribution curves so obtained did not always conform to the ideal Gaussian form, a point which will be discussed later (p. 111) the maximum ordinate and the successive analysis methods (Geddes 1945) were both used for the calculation of D, the former being applied to the last three photographs and the latter to the last two. The seven values for D thus obtained were then averaged. The result was found to be virtually identical with that obtained with the maximum ordinate-area method in the few cases in which this was tested. A correction for the viscosity of the solvent was finally applied to yield D_{20}.

(3) *Sedimentation*

The sedimentation experiments reported were carried out in a Spinco (model E) ultracentrifuge which has been described by Kegeles & Gutter (1951). The analytical rotor A and the preparative rotor A have been used, respectively, for analytical and preparative work. The limiting radii about the axis of rotation of the 12 mm cell used with the former are 5·7 and 7·3 cm, giving an average g value of 259700 at 59780 rev/min. Those for the tubes of the latter are 3·0 and 7·3 cm, giving an average g value of 149670 at 50740 rev/min. The buffers used in both types of work were identical with those previously described for electrophoresis. Unless otherwise specified, the histones were used in 1·33% (w/v) solutions and were dialyzed before use for a constant time of 70 h. During the runs the rotor was kept at a temperature of approximately 20° C by intermittent use of the refrigerator.

(4) *Ultra-violet absorption curves*

These were determined with a Uvispek apparatus supplied by Messrs Hilger & Watts.

(5) *Chemical analyses*

Amino acid estimations were made by the method of Moore & Stein (1954), nitrogen was determined by micro-Kjeldahl and phosphorus by Allen's modification (1940) of King's method.

(6) *Experimental tumours*

Hepatomas were produced in rats by the azo-dye method. For this purpose, male Wistar rats weighing from 150 to 200 g from our own colony were used. They were kept in groups of ten or twelve in screen-bottomed cages and fed *ad libitum* water and the semi-synthetic diet of the following composition: sucrose 77 %, Genatosan (a preparation of casein with a low vitamin content) 12 %, salt mixture (Hawker & Osborne) 4 %, corn oil 6·5 % and cod liver oil 0·5 %. In addition the diet contained the vitamin mixture of Schweigert (1949) except that the riboflavin supplement was reduced from 3 to 1 mg/kg of diet. Sufficient water was added to give the whole a uniform porridge-like consistency on mixing. In order to induce hepatomas in the rats, 0·058 % of 3′-methyl-4-dimethylamino-azobenzene dissolved in corn oil was incorporated in their diet and fed to them for 20 weeks, or until the animals had developed palpable tumours, whichever period was the shorter. They were then maintained on our normal laboratory rat cake (obtained from McGregor & Co., Ltd, Leith) until the tumours were, as judged by palpation, sufficiently large (40 to 60 g) or until the animals became moribund. They were then anaesthetized with ether and their livers perfused with physiological saline *in situ*, or, when this was not possible, the rats were bled to death. Immediately after excision, the tumours were frozen in solid carbon dioxide and stored in a deep freeze. When sufficient material had been accumulated it was used for the preparation of the cell nuclei. The weights of the tumours ranged from 30 to 95 g with an average weight of 55 g.

Normal livers were obtained similarly from rats from the same colony and maintained on the same diet with, of course, the omission of the dye. No difficulty in perfusing the livers was, in this case, experienced.

In view of the observation of Miller & Miller (1947) that a primary step in the induction of the tumour was the binding of the dye to the proteins of the liver during the first few weeks of its administration, 160 male rats were fed the dye for 4 weeks only. They were then immediately sacrificed and the nuclei prepared from their livers.

The *Rd*/3 sarcoma, a tumour originally induced in rats by dibenzanthracene, was propagated by serial subcutaneous transplantation in Wistar rats of our own colony from a specimen kindly obtained for us by Dr Neish from Sheffield University. This tumour tends to become very necrotic in the later stages of its growth. The animals were therefore killed and the tumours excised when the latter had reached a size corresponding to about 10 g in weight. Nuclei were isolated from these in the usual manner.

IV. RESULTS

(A) Normal tissue

(1) *Electrophoretic patterns of unfractionated histones*

The first step in the examination of the physical characteristics of histones has been the electrophoretic analysis of the unfractionated histones from a number of different organs from various species. Corresponding to the earlier chemical results of Stedman & Stedman (1951), this work has shown these are, in general, composite in character. The electrophoretic patterns usually reveal the presence of three components which it is now proposed to term, in descending order of mobility, the α-, β- and γ-components. A closer examination of the α-component indicates, however, that this itself consists, at any rate in some instances, of two or three different molecular species. The α-component will therefore generally be termed the α-fraction and, in those instances in which it is possible to distinguish different components in it, these will be designated by the symbols α_1, α_2, etc.

The presence of the α-fraction and the β- and γ-components are clearly illustrated in the electrophoretic patterns of the unfractionated histone from fowl erythrocytes reproduced in figures 2 and 3, plate 5. The patterns from both the ascending and descending boundaries are, in this case, shown in order to demonstrate that these are symmetrical with one another. With histones from other sources only the latter are, in general, reproduced. Nevertheless, it must be emphasized that in all cases the same symmetry obtains as with the histones from fowl erythrocytes. Other histones from the fowl which present similar pictures are those from the liver (figure 4, plate 5) and spleen (figure 5, plate 5). With both of these, however, the composite character of the α-fraction is evident.

Unfractionated histones from ox thymus (figure 6, plate 5), ox liver (figure 7, plate 5), ox spleen (figure 8, plate 5), pig liver (figure 9, plate 5), pig spleen (figure 10, plate 5), and rabbit liver (figure 11, plate 5) also clearly contain β- and γ-components as well as an α-fraction. The latter is not, it is true, clearly visible in the electrophoretic patterns reproduced in figures 7 and 11, plate 5. Its presence was, however, definitely shown in photographs, not reproduced, taken after shorter periods of electrophoresis.

In a number of unfractionated histones from other sources evidence for the existence of α- and γ-components is less complete. Thus, the electrophoretic pattern of the histone from human thymus (figure 12, plate 5), while showing the presence of a small amount of an α-fraction, seems to be lacking in the γ-component. That for children's livers (figure 13, plate 5) apparently lacks both and would appear to consist of an electrophoretically pure β-histone. It is possible, however, that some loss of the subsidiary histones occurred during the isolation of these preparations for, owing to the nature of the tissue involved, they were both necessarily made on such a small scale that their precipitation from the acid extracts may not have been complete. They were, moreover, prepared before the discovery of subsidiary histones, when the necessity for the use of very large excess of the precipitating solvent to bring about the complete precipitation of the latter was not realized. However this may be, it is certain that subsidiary histones are present in some human cell-nuclei, for a recent preparation of the unfractionated histone from an adult human liver has exhibited an electrophoretic pattern (figure 14, plate 5) in which the presence, albeit in small amount, of both the α- and γ-components is evident.

It is, similarly, sometimes difficult to demonstrate electrophoretically the presence of subsidiary histones in the products from cell nuclei of the rat. That from the liver, for example, gives an electrophoretic pattern (figure 15, plate 5) which appears to be that of a homogeneous substance. Nevertheless, ultracentrifugal analysis (see p. 126) has shown that both types of component are present. In view of such a result it is clear that unfractionated products described as virtually electrophoretically homogeneous may contain subsidiary histones. In contrast to the product from the liver, that from the spleen of this species (figure 16, plate 5) evidently contains relatively large amounts of both α_1- and α_2- histones; a small amount of the γ-component seems also to be present. The histones from the kidney (figure 17, plate 6) present much the same picture, except that no γ-component is visible.

Two preparations of histone from the mouse, one from the spleen (figure 18, plate 6) and the other from the liver (figure 19, plate 6) appear, apart from the presence of the γ-component in the former, to be electrophoretically homogeneous.

Finally, figure 20, plate 6 shows the electrophoretic pattern of the unfractionated histone obtained formerly by Stedman & Stedman (1951) from wheat germ. This must to some extent be placed in a separate category from the other histones on the grounds that it was not prepared from isolated nuclei and that it originated from a plant. Like most of the other histones, however, it contains more than one component. But because the properties of the principal component deviate considerably, as will be shown below, from those of animal β-histones, it is not proposed at present to extend the system of nomenclature used above to these histones.

(2) *Electrophoretic patterns of main histones*

In their original demonstration of the presence of two types of histone in the unfractionated extracts of the basic proteins isolated from various types of cell nuclei Stedman & Stedman (1951) provisionally termed the one present in greater amount the main histone, and the other the subsidiary histone. No general evidence of the number of subsidiary histones present in each nucleus was at that time obtained, although it was demonstrated that at least two were contained in fowl erythrocyte nuclei. In order to correlate these earlier preparations with the components now shown to be present in unfractionated histones, some of the old main histones have been examined electrophoretically. The pattern obtained with that from ox thymocytes, which was one of the actual preparations used in the former work, is reproduced in figure 21, plate 6 and is typical of the picture given by main histones from other sources. It is clear that it consists essentially of the β-component, but is contaminated with a small percentage of the γ-component. The particularly sharp outline of the pattern in this photograph is due to the use of a higher concentration (1 %) of histone than usual.

The electrophoretic patterns of two other main histones, namely, those from fowl thymocytes (figure 22, plate 6) and from cod sperm (figure 23, plate 6) are also illustrated. They have been reproduced because, owing to the great difficulty in obtaining the starting material, it has not as yet been possible to prepare new specimens of the unfractionated histones. They thus serve to show, not only that these main histones are β-histones contaminated with the γ-component, but also that the original unfractionated histones must have conformed to the normal pattern in so far as they contained γ-components.

(3) *Electrophoretic patterns of subsidiary histones*

The method formerly used (Stedman & Stedman 1951) for the removal of the α-fraction from the bulk of the β- and γ-components has been simplified and made much more effective by a procedure illustrated in the following example. It is believed to be of general applicability to unfractionated histones although it has so far only been used with those from ox thymocytes and fowl erythrocytes.

75 g of unfractionated histone sulphate obtained from ox thymocytes were stirred with 2750 ml. of cold distilled water and the suspension left overnight in the refrigerator. A small and negligible amount of insoluble material was then removed by centrifuging the solution at 1500 *g* at 0° C, when the pH of the supernatant was brought to 6·3 by the careful addition of about 300 ml. of 0·4 M sodium bicarbonate. After standing for 7 h in the refrigerator, 1500 ml. of absolute alcohol, cooled to −12° C were added with stirring, precipitation of the histone commencing after the addition of about 150 ml. of the alcohol. This material was stored overnight in the refrigerator when the precipitate was removed and dried in the customary manner with cold alcohol and ether. As will be shown below, the solid so obtained consisted essentially of the β- and γ-components of the histone. The α-fraction was precipitated from the mother liquors by enclosing the latter in a long Cellophane sausage, suspending it in a long glass tube closed at the bottom and dialyzing it successively against 50 and 70% aqueous alcohol and finally against two changes of absolute alcohol. The first treatment with aqueous alcohol was, of course, designed to remove the sodium sulphate formed from the bicarbonate. During the dialysis procedure the α-fraction separated as a precipitate in the cellophane sac. It was collected in the centrifuge and dried in the customary manner.

The electrophoretic patterns of these preparations are shown in figure 24, plate 6 and figure 25, plate 6, respectively. It is evident from the former that the less soluble material consists essentially of a mixture of the β- and γ-components. The latter, which represents the α-fraction, clearly contains three such components; subsequent work (p. 118) has indeed confirmed this for the α-fraction from liver. There is also a possibility that it is contaminated with a small proportion of the γ-component.

The α-fraction obtained from the histone from fowl erythrocytes by a similar procedure appears to contain at least three α-components, as is evident from the electrophoretic pattern in figure 26, plate 6.

The electrophoretic pattern of an old sample of the α-fraction obtained formerly (Stedman & Stedman 1951) from the histone from cod sperm (figure 27, plate 6) is also reproduced. This serves to demonstrate the existence of an α-fraction in the histone from this source, although a specimen of the unfractionated histone is not available.

(4) *Electrophoretic mobilities of histones*

The electrophoretic mobilities of a large number of histones have been calculated over a wide range of pH from the appropriate electrophoretic patterns. The results have been plotted in the form of mobility-pH curves which are reproduced in the accompanying figures. As most of the preparations of histones used in these experiments were unfractionated histones, it has been possible to plot on many of the figures the mobilities of each

of the components present. Values calculated in this way from measurements made on mixtures of proteins may not, of course, represent the absolute mobilities of the various components.

This is particularly the case with the α- and γ-components, for these subsidiary histones are present in smaller amount than the β-component. Their boundaries with the buffer become, moreover, rather diffuse, with the result that there is a considerable diminution in the sharpness of the corresponding peak on the electrophoretic pattern. On the other hand, the mobility of the β-histone is probably not significantly changed by the presence

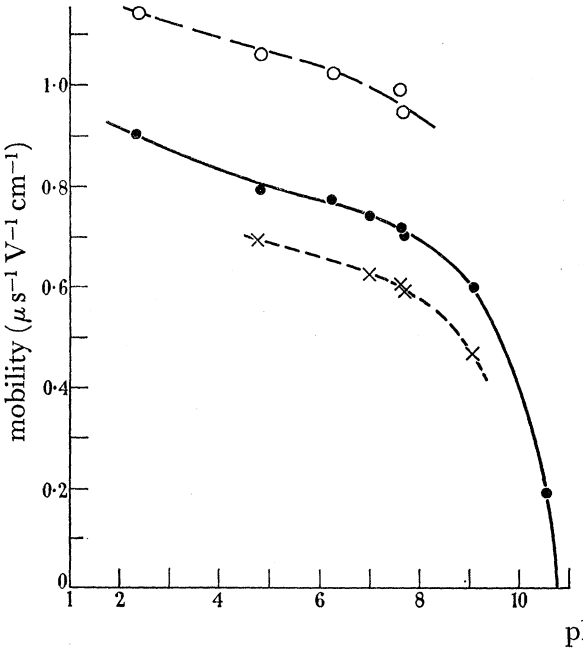


FIGURE 28. *Chicken erythrocytes.* Mobility-pH graph of histones from chicken erythrocyte nuclei. ○, α-fraction; ●, β-component; ×, γ-component.

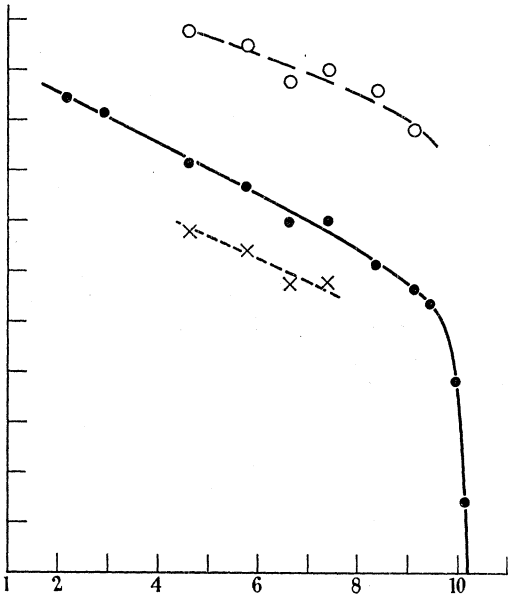


FIGURE 29. *Chicken liver.* Mobility-pH graph of histones from chicken liver nuclei. ○, α-fraction; ●, β-component; ×, γ-component.

of the subsidiary histones, for it does not seem to alter appreciably with its progressive purification. In view of the discovery (p.114) that some electrophoretically pure β-histones still contain appreciable amounts of subsidiary histones it is not certain that this statement will remain valid for β-histones from which the subsidiary components have been completely removed.

Figures 28 and 29 show respectively the mobility-pH curves of the histones from fowl erythrocytes and liver cells. Because no appreciable resolution of the α-fraction occurred under the conditions used in these experiments (compare figure 4, plate 5) it has been necessary to calculate the mobility of this fraction as if it were a single component. Nevertheless, this fraction is, in fact, composite, for, when it is separated from the β- and γ-components it undergoes resolution in the Tiselius apparatus (figure 26, plate 6) into three fractions which have been termed the α_1-, α_2- and α_3-components. The mobilities of these have been determined over a restricted range of pH and are plotted in figure 30. With the unfractionated histones from the spleen (figure 5, plate 5) of the fowl the α-fraction clearly resolves itself into α_1 and α_2 components; their mobilities have therefore been

calculated without the separation of the α-fraction from the β- and γ-components, and are shown in figure 31 together with those for the two latter. Unfortunately, no specimen of the unfractionated histone from the thymocytes of the fowl was available for examination. It has, however, been possible to determine the mobilities of the β- and γ-components by using the main histone previously obtained by chemical fractionation. The results are shown in figure 32.

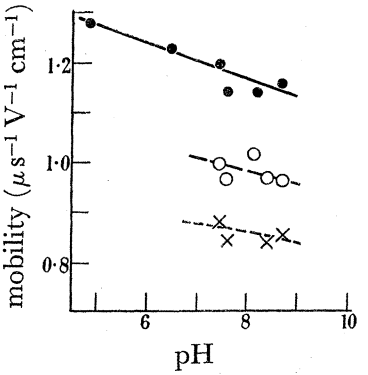


FIGURE 30. *Chicken Erythrocytes.* Mobility-pH graph of the α-fraction of histones from chicken erythrocyte nuclei. ●, α_1-component; ○, α_2-component; ×, α_3-component.

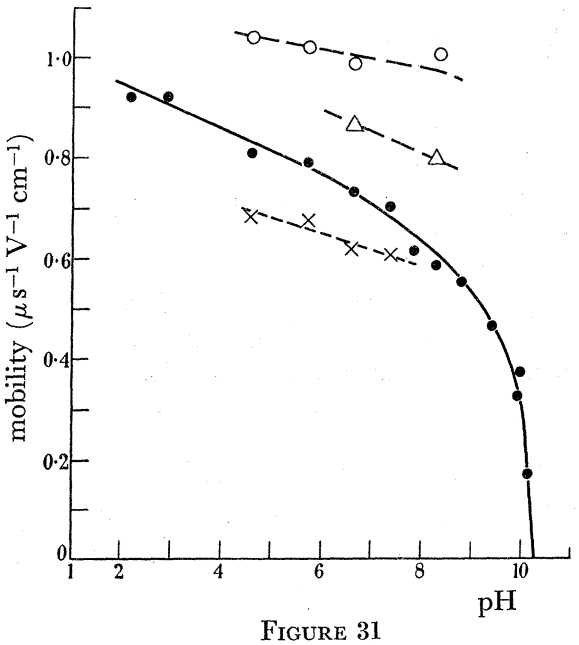


FIGURE 31

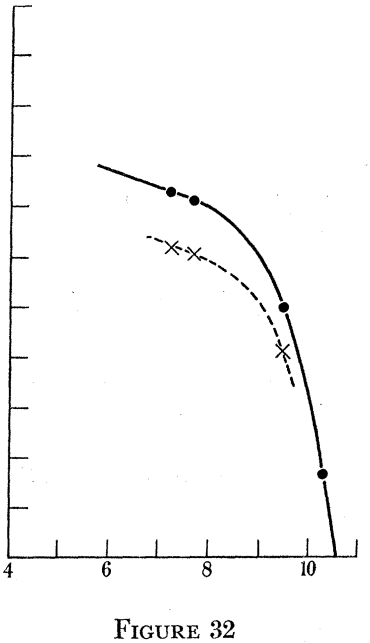


FIGURE 32

FIGURE 31. *Chicken spleen.* Mobility-pH graph of histones from chicken spleen nuclei. ○, α_1-component; △, α_2-component; ●, β-component; ×, γ-component.

FIGURE 32. *Chicken thymus.* Mobility-pH graph of fractionated histone from chicken thymus nuclei. ●, β component; ×, γ-component.

The mobilities of the unfractionated histones from pig liver are given in figure 33. The picture presented resembles that of other histones. That no inflexion is shown in the curve of the γ-component in this, and in some other, examples, is due to the invisibility of this component in the electrophoretic pattern when solutions more alkaline than those shown are used. The probable explanation of this invisibility of the γ-component is that its

mobility in the more alkaline solutions approaches that of the β-component. It may, however, be due to the low concentration of histones which were necessarily employed above pH 8·0. In the histones from the liver of the rabbit the inflexion in the curve of γ-component is evident (figure 34). The α-fraction was, however, only visible after short times of electrophoresis and hence accurate mobilities of this fraction could not be calculated.

As previously pointed out (p. 101) subsidiary histones are not clearly visible in the electrophoretic pattern of the unfractionated histone from rats' livers. Only one mobility curve for this material, which represents the β-component, has therefore been plotted in figure 35. For the histones from the spleen of the same species mobility curves for the α_1- and α_2-, as

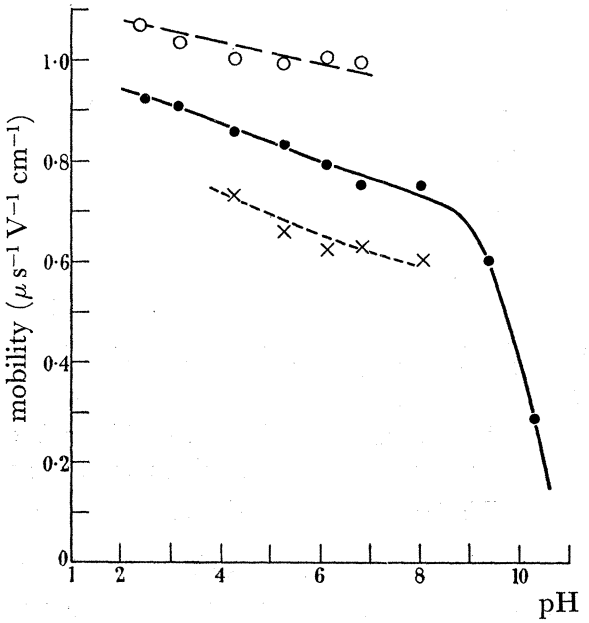


FIGURE 33. *Pig liver.* Mobility-pH graph of histones from pig liver nuclei. ○, α-component; ●, β-component; ×, γ-component.

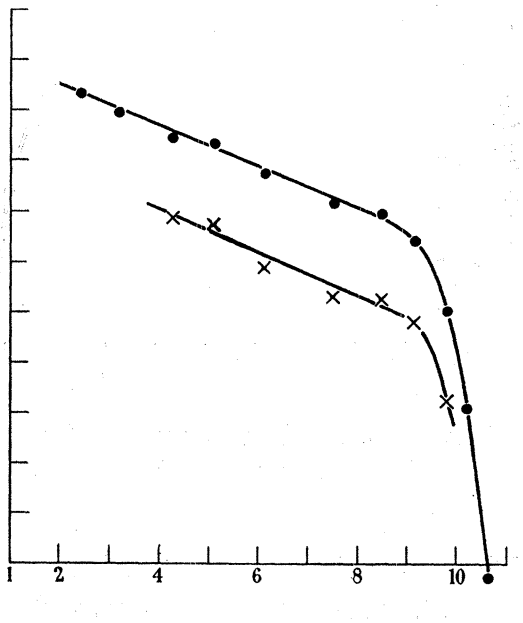


FIGURE 34. *Rabbit liver.* Mobility-pH graph of histones from rabbit liver nuclei. ●, β-component; ×, γ-component.

well as the β-components have been plotted (figure 36). On the other hand, with the product from spleens of mice it has only been possible to calculate and plot the mobilities of the β-component (figure 37). The γ-component is certainly present in this material, but it does not separate from the β-component sufficiently distinctly for its mobility to be calculated.

The mobility-pH curves for the two chief components present in the histone from wheat germ are reproduced in figure 38. The fast or α-component requires no special comment, but it should be pointed out that the curve for the principal component differs markedly from the corresponding curves for animal β-histones in that it exhibits, doubtless because of its content of aspartic and glutamic acids, a pronounced point of inflexion at a pH of between 4 and 5. Why this feature is absent from the curves for animal β-histones, which also contain considerable quantities of acidic amino acids, is not clear. No doubt it is a reflexion of some difference in structure between the histones from the two sources. In this connexion, it may be significant that the histone from wheat germ also differs from animal histones in exhibiting no Tyndall effect in solution, in remaining soluble at and around its isoelectric point and in its inability to aggregate (see p. 113) at pH's from 4 to 5 upwards.

A feature of this histone not apparent from the mobility curve is the separation of a third component at pH's on the alkaline side of the isoelectric point. That this is not an hydrolysis artifact is indicated by the appearance in the sedimentation pattern at pH 7·6 of three components of S values 0·7, 2·5 and 7·5, respectively.

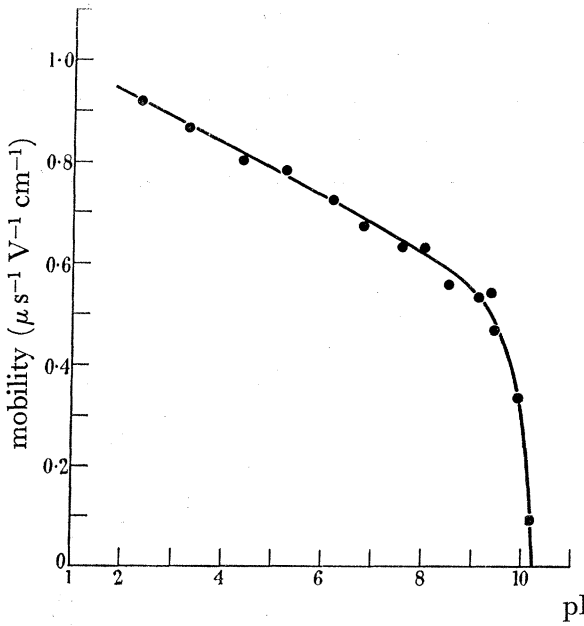


FIGURE 35. *Rat liver.* Mobility-pH graph of β-histone from rat liver nuclei.

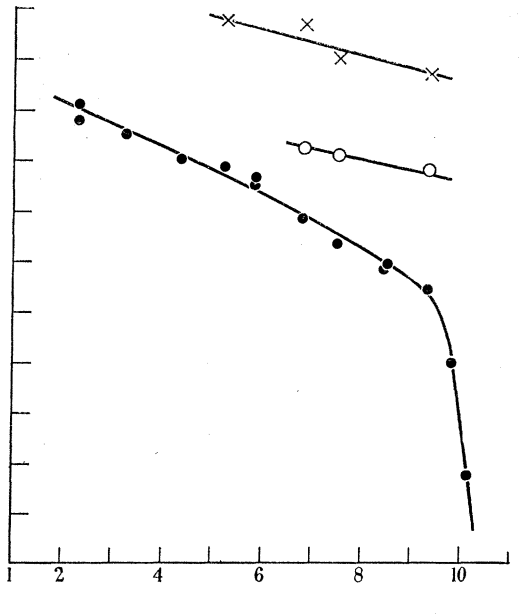


FIGURE 36. *Rat spleen.* Mobility-pH graph of histones from rat spleen nuclei. ×, α_1-component; ○, α_2-component; ●, β-component.

The results of mobility determinations on two different specimens of the unfractionated histones from human livers have yielded apparently abnormal results inasmuch as the mobility curves for the two specimens (figure 39) differ from one another. No real explanation can at the moment be offered for this anomaly. Nevertheless, there is a possibility that it is related to the fact that one specimen was obtained from the livers of three young babies, 5 weeks, 2 months and 5 months old, respectively, whereas the second specimen was prepared from a normal adult liver. The mobility curves for the β-component from human thymus glands (figure 87) and human spleen (figure 88) are shown in a later section.

Particular attention has been directed towards the accurate determination of the mobility-pH curves of the β-histones from the liver cells and thymocytes of the ox because the materials from which they are prepared can readily be obtained in large quantities. Considerable amounts of these histones have therefore been available and it has thus been possible, in determining the curves, to use a large number of entirely different preparations of different degrees of purity and made at widely different times. Thus, the mobility-pH curve for ox thymus histone (figure 40) includes four points measured with the unfractionated histone. The remainder of the estimations have been made with various preparations, some old and others new, from which the α-fraction had been removed. According to their electrophoretic patterns these preparations differed considerably in their content of the γ-component, the amount being, in a few cases, barely detectable electrophoretically. That all the mobility points should, as they do, nevertheless, fall satisfactorily about a

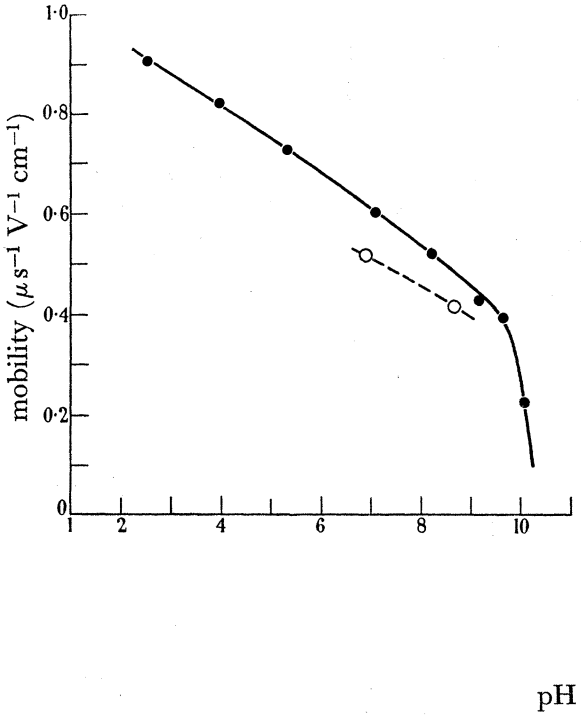


FIGURE 37. *Mouse spleen.* Mobility-pH graph of β-components from the histones of normal and abnormal mouse spleens. ●, normal mouse spleen; ○, leukaemic spleens from line I mice.

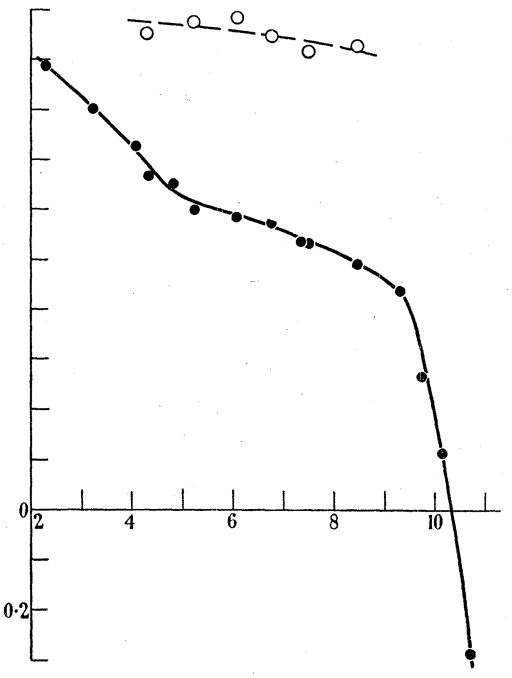


FIGURE 38. *Wheat germ.* Mobility-pH graph of histones from wheat germ. ●, principal component; ○, α-component.

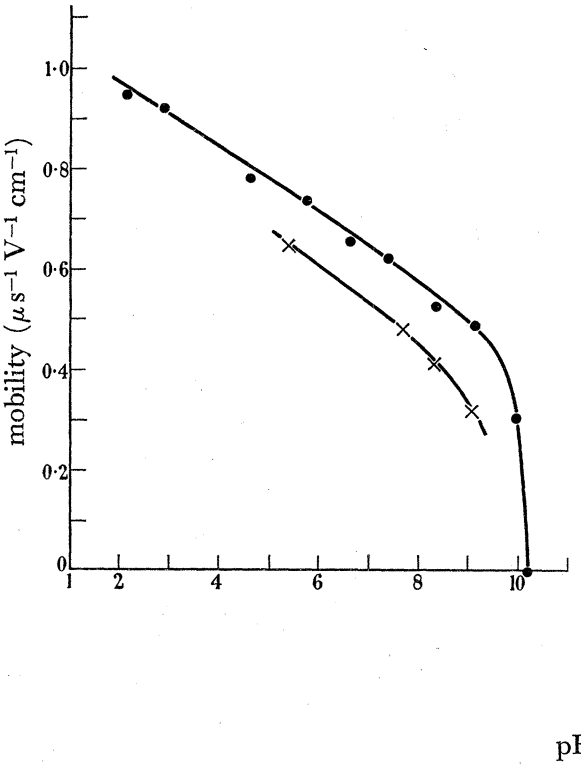


FIGURE 39. *Human livers.* Mobility-pH graph of β-histones from human liver nuclei. ●, adult human liver; ×, child human liver.

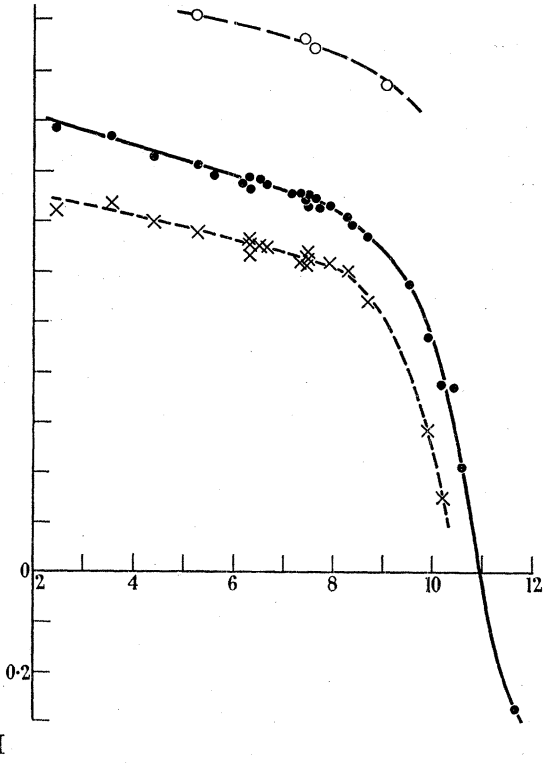


FIGURE 40. *Ox thymus.* Mobility-pH graph of histones from ox thymus nuclei. ○, α-component. ●, β-component. ×, γ-component.

smooth curve gives considerable support to the suggestion made at the commencement of this section that the mobility of the β-component of histones is not appreciably affected by the presence of subsidiary histones in amounts comparable with those found in unfractionated histones from ox thymocytes. In addition to the mobility curve of the β-components those for the α- and γ-components are also shown. The α-fraction has been calculated, as was done for the corresponding fraction in the histones from fowl erythrocytes, as one component although three are known to be present. That such is the case is evident both from the electrophoretic pattern in figure 25, plate 6 and from figure 41, which shows the mobility curves for the α_1- and α_2-components obtained with separated material. Most of the above remarks regarding the material used for the determination of the mobility curves of the histones from ox thymocytes are directly applicable to that employed for curves, shown in figure 42, for the histones from ox liver cells. There is one difference: no unfractionated histones were used with the latter. For this reason, and

---

Description of Plate 5

Figure 2. Electrophoretic pattern at pH 7·62, of a 0·6 % solution of unfractionated histone from chicken erythrocyte nuclei; ascending boundary after 1½ h; knife-edge angle $\theta = 7°$.

Figure 3. Electrophoretic pattern at pH 7·62 of a 0·6 % solution of unfractionated histone from chicken erythrocyte nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 7°$.

Figure 4. Electrophoretic pattern at pH 5·72 of a 0·6 % solution of unfractionated histone from chicken liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 5. Electrophoretic pattern at pH 6·60 of a 0·6 % solution of unfractionated histone from chicken spleen nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 6. Electrophoretic pattern at pH 6·40 of a 0·6 % solution of unfractionated histone from ox thymus nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 7. Electrophoretic pattern at pH 7·60 of a 0·5 % solution of unfractionated histone from ox liver nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 15°$.

Figure 8. Electrophoretic pattern at pH 6·02 of a 0·6 % solution of unfractionated histone from ox spleen nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 14°$.

Figure 9. Electrophoretic pattern at pH 6·10 of a 0·6 % solution of unfractionated histone from pig liver nuclei; descending boundary after 2 h; knife edge-angle $\theta = 7°$.

Figure 10. Electrophoretic pattern at pH 6·62 of a 0·6 % solution of unfractionated histone from pig spleen nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 12°$.

Figure 11. Electrophoretic pattern at pH 5·07 of a 0·6 % solution of unfractionated histone from rabbit liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

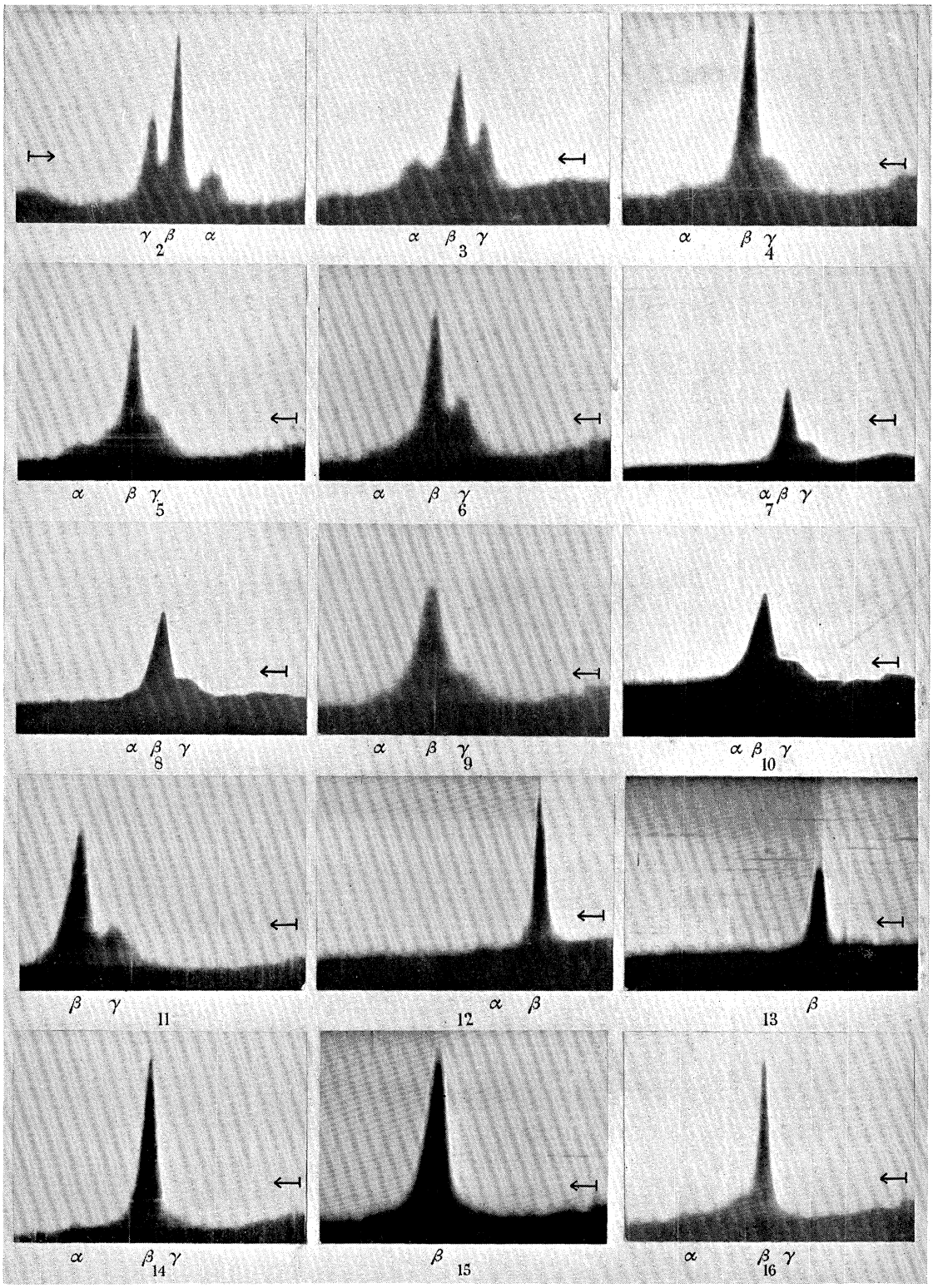
Figure 12. Electrophoretic pattern at pH 7·65 of a 0·5 % solution of unfractionated histone from human thymus nuclei; descending boundary after 1 h; knife-edge angle $\theta = 10°$.

Figure 13. Electrophoretic pattern at pH 7·65 of a 0·3 % solution of unfractionated histone from human child's liver nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 10°$.

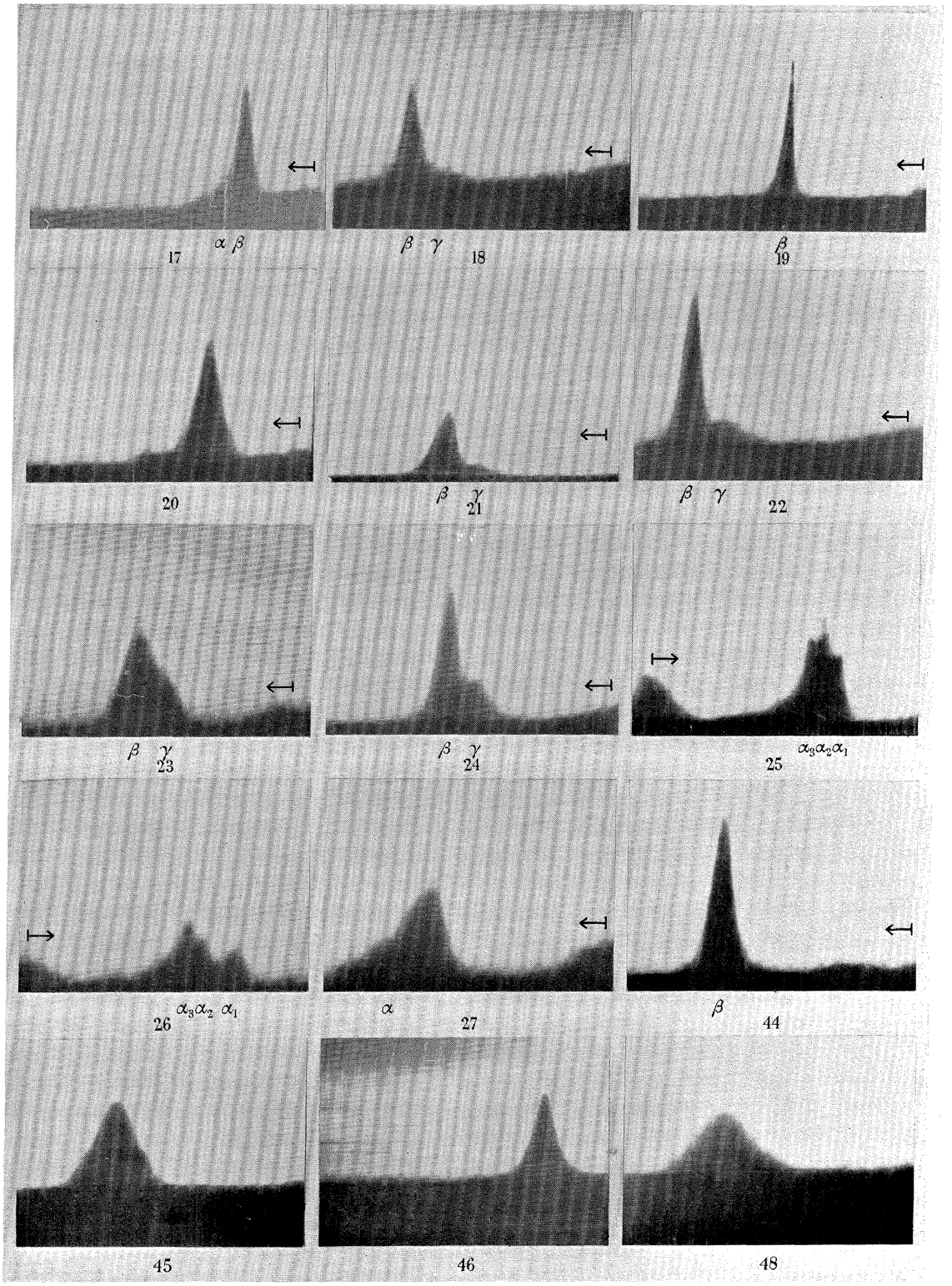
Figure 14. Electrophoretic pattern at pH 6·60 of a 0·6 % solution of unfractionated histone from human adult liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 15. Electrophoretic pattern at pH 5·24 of a 0·6 % solution of unfractionated histone from normal rat liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 16. Electrophoretic pattern at pH 6·78 of a 0·6 % solution of unfractionated histone from rat spleen nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.


γ β α
2
α β γ
3
α β γ
4
α β γ
5
α β γ
6
α β γ
7
α β γ
8
α β γ
9
α β γ
10
β γ
11
α β
12
β
13
α β γ
14
β
15
α β γ
16

(*Facing p.* 108)


17 α β
18 β γ
19 β
20
21 β γ
22 β γ
23 β γ
24 β γ
25 α3α2α1
26 α3α2 α1
27 α
44 β
45
46
48

Description of Plate 6

Figure 17. Electrophoretic pattern at pH 7·50 of a 0·6 % solution of unfractionated histone from rat kidney nuclei; descending boundary after 1 h; knife-edge angle $\theta = 10°$.

Figure 18. Electrophoretic pattern at pH 5·31 of a 0·3 % solution of unfractionated histone from mouse spleen nuclei; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 19. Electrophoretic pattern at pH 6·25 of a 0·4 % solution of unfractionated histone from mouse liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 12°$.

Figure 20. Electrophoretic pattern at pH 6·15 of a 0·6 % solution of unfractionated histone from wheat germ; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 9°$.

Figure 21. Electrophoretic pattern at pH 7·95 of a 1·0 % solution of fractionated histone from ox thymus nuclei; descending boundary after 2 h; knife-edge angle $\theta = 25°$.

Figure 22. Electrophoretic pattern at pH 7·66 of a 0·5 % solution of fractionated histone from chicken thymus nuclei; descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 12°$.

Figure 23. Electrophoretic pattern at pH 7·64 of a 0·6 % solution of fractionated histone from cod sperm; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

Figure 24. Electrophoretic pattern at pH 6·65 of a 0·7 % solution of $\beta+\gamma$-fraction from ox thymus histone; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 25. Electrophoretic pattern at pH 7·43 of a 1·0 % solution of α-fraction from ox thymus histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 9°$.

Figure 26. Electrophoretic pattern at pH 8·41 of a 0·8 % solution of α-fraction from chicken erythrocytes histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

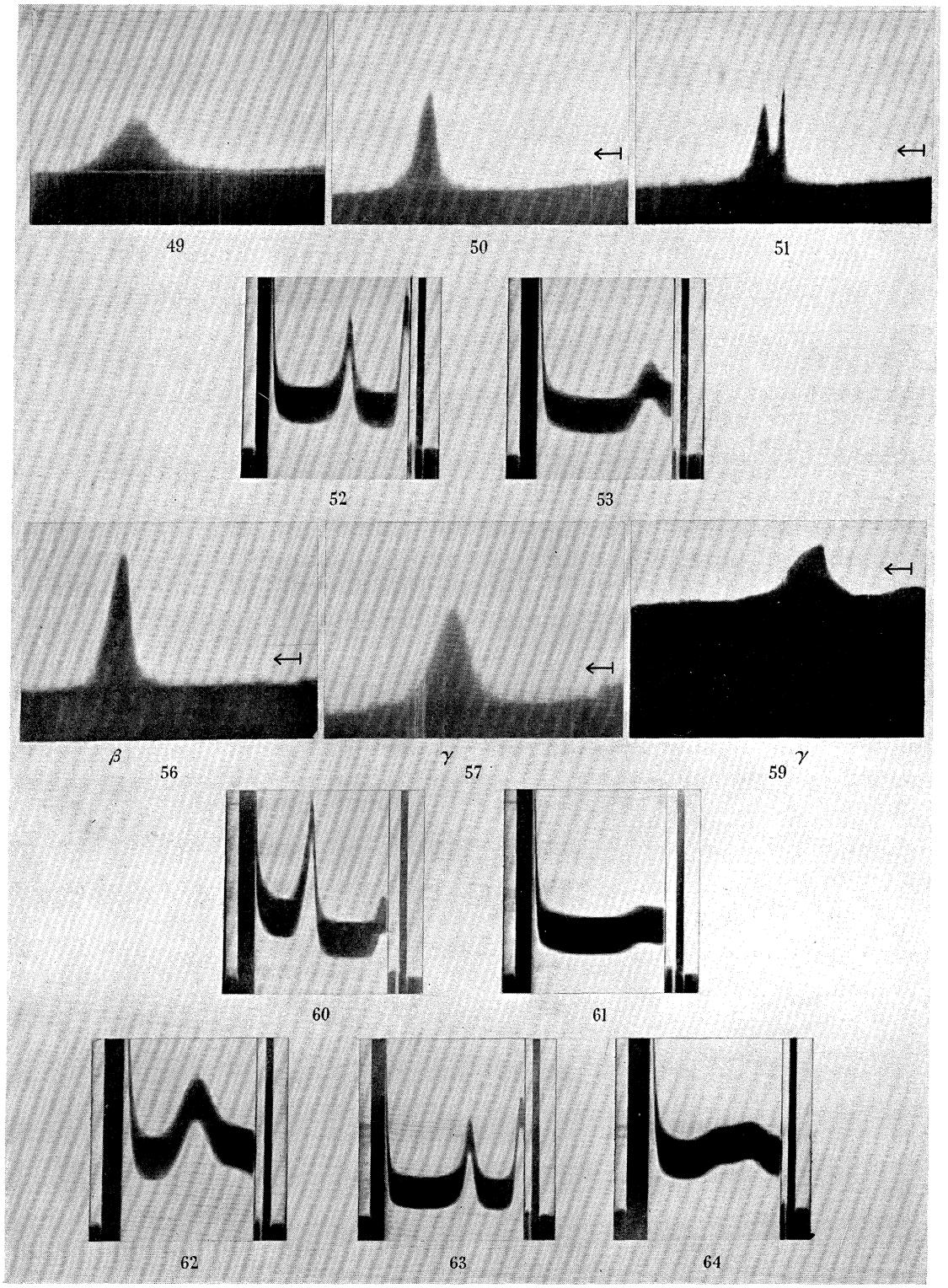
Figure 27. Electrophoretic pattern at pH 7·66 of a 0·55 % solution of α-fraction histone from cod sperm; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

Figure 44. Electrophoretic pattern at pH 7·50 of a 0·7 % solution of the β-fraction (electrophoretically prepared) from ox thymus histone; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 45. Diffusion pattern at pH 1·40 of a 0·5 % solution of ox thymus electrophoretically pure β-histone after 21 h diffusion; knife-edge angle $\theta = 9°$.

Figure 46. Diffusion pattern at pH 7·05 of a 0·5 % solution of ox thymus electrophoretically pure β-histone after 48 h diffusion; knife-edge angle $\theta = 11°$.

Figure 48. Diffusion pattern at pH 3·15 of a 0·5 % solution of α-fraction from ox thymus histone after 24 h diffusion; knife-edge angle $\theta = 7°$.


49
50
51
52
53
β
56
γ
57
59
γ
60
61
62
63
64

Description of Plate 7

Figure 49. Diffusion pattern at pH 6·85 of a 0·5 % solution of α-fraction from ox thymus histone after 18 h diffusion; knife-edge angle $\theta = 8°$.

Figure 50. Electrophoretic pattern at pH 8·65 of an artificial mixture of ox thymus and ox liver electrophoretically pure β-histones 0·25 % each, dialyzed together; descending boundary after 3 h; knife-edge angle $\theta = 10°$.

Figure 51. Electrophoretic pattern at pH 8·65 of an artificial mixture of ox thymus and ox liver electrophoretically pure β-histones, 0·25 % each, dialyzed separately; descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 10°$.

Figure 52. Sedimentation pattern at pH 7·78 of a 1·33 % solution of the $\beta+\gamma$-fraction from ox thymus histone after 12 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 53. Sedimentation pattern of the above solution (figure 52) after 96 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 56. Electrophoretic pattern at pH 6·15 of a 0·6 % solution of the β-component (ultracentrifuge preparation) from ox thymus histone, descending boundary after 2 h; knife-edge angle $\theta = 12°$.

Figure 57. Electrophoretic pattern at pH 6·15 of a 0·6 % solution of the γ-component (ultracentrifuge preparation) from ox thymus histone, descending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 59. Electrophoretic pattern at pH 9·14 of a 0·45 % solution of the γ-component (ultracentrifuge preparation) from ox thymus histone, descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

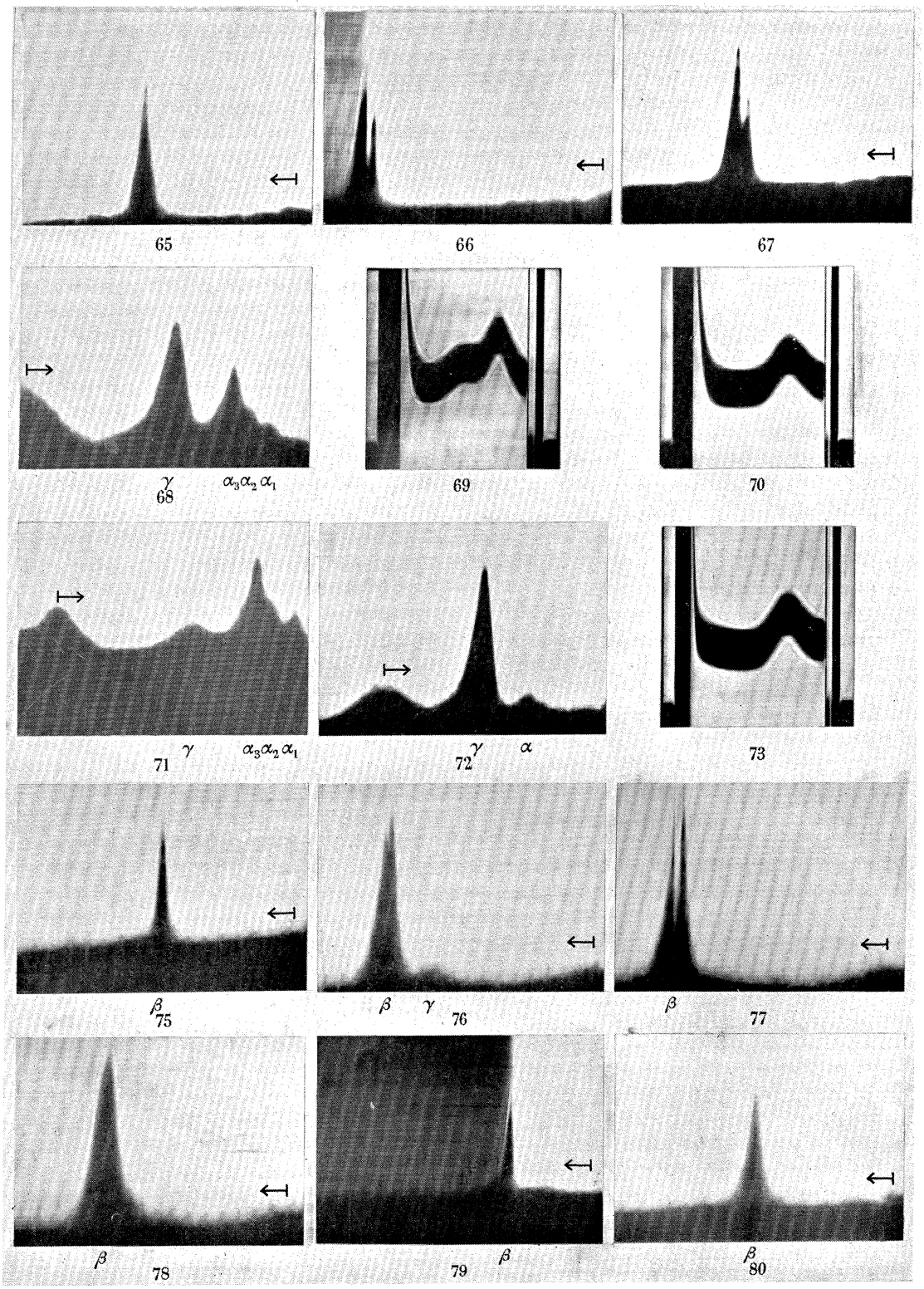
Figure 60. Sedimentation pattern at pH 7·70 of a 1·33 % solution of the β-component (ultracentrifuge preparation) from ox thymus histone, after 16 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 61. Sedimentation pattern of histone solution above (figure 60) after 80 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 62. Sedimentation pattern at pH 7·70 of a 1·33 % solution of the γ-component (ultracentrifuge preparation) from ox thymus histone, after 176 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 63. Sedimentation pattern at pH 8·10 of a 2 % solution of unfractionated histone from ox liver nuclei, after 8 min at 59780 rev/min; bar angle $\theta = 40°$.

Figure 64. Sedimentation pattern of the above solution (figure 63) after 168 min at 59780 rev/min; bar angle $\theta = 45°$.


65
66
67
γ α3α2α1
68
69
70
γ α3α2α1
71
γ α
72
73
β
75
β γ
76
β
77
β
78
β
79
β
80

Description of Plate 8

Figure 65. Electrophoretic pattern of an artificial mixture of 0·25 % each of β-histones (ultracentrifuge preparations) from ox thymus and ox liver dialyzed together at pH 7·54, descending boundary after 2 h; knife-edge angle $\theta = 11°$.

Figure 66. Electrophoretic pattern of mixture as above figure 65, dialyzed separately at pH 7·54; descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 10°$.

Figure 67. Electrophoretic pattern of an artificial mixture 0·3 % with respect to each of β-histone (electrophoretically pure) from ox liver and β-histone (ultracentrifuge preparation) from ox liver dialyzed separately at pH 7·89 descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 68. Electrophoretic pattern at pH 8·47 of a 1·0 % solution of the $\alpha+\gamma$-fraction (ultracentrifuge prepared) from ox liver histone, ascending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 69. Sedimentation pattern at pH 8·50 of a 2 % solution of the $\alpha+\gamma$-fraction (ultracentrifuge prepared) from ox liver histone after 240 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 70. Sedimentation pattern at pH 8·50 of a 2 % solution of the α-fraction (ultracentrifuge prepared) from ox liver histone after 326 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 71. Electrophoretic pattern at pH 8·68 of a 0·8 % solution of the α-fraction (ultracentrifuge prepared) from ox liver histone; ascending boundary after 2 h; knife-edge angle $\theta = 6°$.

Figure 72. Electrophoretic pattern at pH 8·68 of a 0·8 % solution of the γ-fraction (ultracentrifuge prepared) from ox liver histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 9°$.

Figure 73. Sedimentation pattern at pH 8·50 of a 2 % solution of the γ-fraction (ultracentrifuge prepared) from ox liver histone after 144 min at 59780 rev/min; bar angle $\theta = 40°$.

Figure 75. Electrophoretic pattern at pH 5·24 of a 0·25 % solution of fractionated histone from human glands (lymphatic leukaemia); descending boundary after 2 h; knife-edge angle $\theta = 8°$.

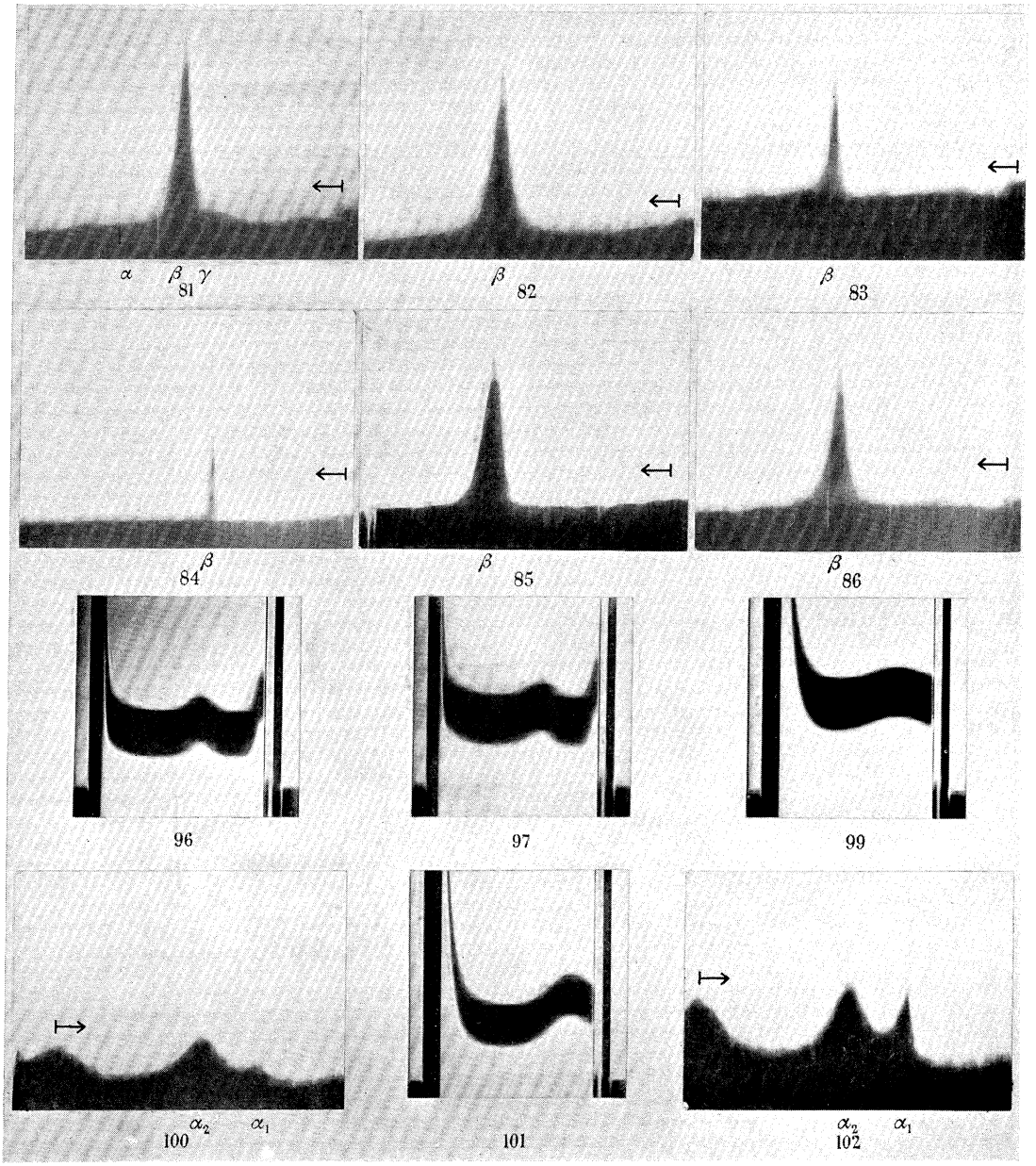
Figure 76. Electrophoretic pattern at pH 7·41 of a 0·6 % solution of fractionated histone from human spleen nuclei (case of lymphatic leukaemia above); descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 77. Electrophoretic pattern at pH 7·69 of a 0·6 % solution of the unfractionated histone from human spleen nuclei (case of lymphatic leukaemia above); descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 6°$.

Figure 78. Electrophoretic pattern at pH 4·33 of a 0·6 % solution of fractionated histone from human bronchogenic carcinoma; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 79. Electrophoretic pattern at pH 7·70 of a 0·4 % solution of unfractionated histone from human leucocytes (case of chronic lymphatic leukaemia); descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 10°$.

Figure 80. Electrophoretic pattern at pH 5·74 of a 0·4 % solution of unfractionated histone from human myelocytes (case of chronic myeloid leukaemia); descending boundary after 2 h; knife-edge angle $\theta = 7°$.


α β γ
81
β
82
β
83
β
84
β
85
β
86
96
97
99
α2 α1
100
101
α2 α1
102

because the α-fraction in ox liver cells is present in too small a proportion for its mobility curve to be determined in the unfractionated histone, no curve is shown for this material.

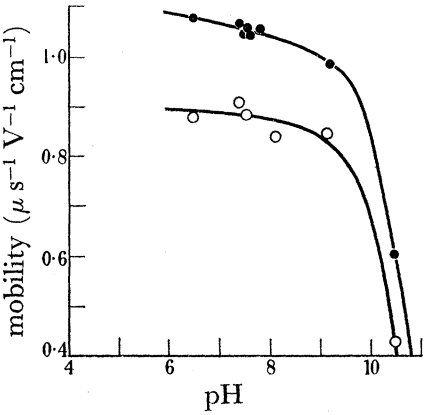


FIGURE 41. *Ox thymus.* Mobility-pH graph of the α-fraction of histones from ox thymus nuclei. ●, α_1-component; ○, α_2-component.

(5) *Isoelectric points of some histones*

Some of the mobility curves described above are sufficiently extensive to enable the isoelectric points to be determined with a reasonable degree of precision. Such isoelectric

---

DESCRIPTION OF PLATE 9

FIGURE 81. Electrophoretic pattern at pH 7·05 of a 0·4 % solution of unfractionated histone from human undifferentiated tumour; descending boundary after 2 h; knife-edge angle $\theta = 7°$.

FIGURE 82. Electrophoretic pattern at pH 5·65 of a 0·4 % solution of unfractionated histone from chicken lymphocytes (case of lymphatic leukaemia); descending boundary after 2 h; knife-edge angle $\theta = 7°$.

FIGURE 83. Electrophoretic pattern at pH 8·70 of a 0·1 % solution of unfractionated histone from leukaemic spleens (mouse line I); descending boundary after 2 h; knife-edge angle $\theta = 5°$.

FIGURE 84. Electrophoretic pattern at pH 8·70 of a 0·08 % solution of unfractionated histone from Walker 256 rat carcinoma; descending boundary after 1½ h; knife-edge angle $\theta = 7°$.

FIGURE 85. Electrophoretic pattern at pH 4·85 of a 0·4 % solution of unfractionated histone from rat hepatoma nuclei; descending boundary after 2 h; knife-edge angle $\theta = 7°$.

FIGURE 86. Electrophoretic pattern at pH 5·07 of a 0·3 % solution of unfractionated histone from rat sarcoma *Rd*/3 nuclei; descending boundary after 2 h; knife-edge angle $\theta = 7°$.

FIGURE 96. Sedimentation pattern at pH 5·29 of a 0·42 % solution of unfractionated histone from normal rat liver nuclei, after 16 min at 59780 rev/min; bar angle $\theta = 40°$.

FIGURE 97. Sedimentation pattern at pH 5·29 of a 0·42 % solution of unfractionated histone from rat hepatoma nuclei, after 16 min at 59780 rev/min; bar angle $\theta = 35°$.

FIGURE 99. Sedimentation pattern at pH 8·47 of a 2 % solution of the α-fraction (ultracentrifuge prepared) from rat hepatoma histone, after 410 min at 59780 rev/min; bar angle $\theta = 35°$.

FIGURE 100. Electrophoretic pattern at pH 8·47 of a 0·8 % solution of the α-fraction (ultracentrifuge prepared) from rat hepatoma histone; ascending boundary after 1½ h; knife-edge angle $\theta = 6°$.

FIGURE 101. Sedimentation pattern at pH 8·47 of a 2 % solution of the α-fraction (ultracentrifuge prepared) from normal rat liver histone, after 216 min at 59780 rev/min; bar angle $\theta = 45°$.

FIGURE 102. Electrophoretic pattern at pH 8·47 of a 0·8 % solution of the α-fraction (ultracentrifuge prepared) from normal rat liver histone, ascending boundary after 1½ h; knife edge-angle $\theta = 6°$.

points have been collected in table 1. Where mobilities have been determined on both sides of the isoelectric points they are considered to be essentially accurate. When the result has been obtained by extrapolation of the curve the approximate nature of the determination has been indicated.

TABLE 1. ISOELECTRIC POINTS OF SOME HISTONES IN GLYCINE BUFFER ($\frac{1}{2}\Gamma = 0{\cdot}176$)

species	organ	component	i.p.
ox	thymus gland	β	11·0
ox	thymus gland	$\gamma(S \simeq 1{\cdot}6)$	10·1
ox	liver	β	10·8
chicken	thymus gland	β	*ca.* 10·5
chicken	liver	β	*ca.* 10·2
chicken	spleen	β	10·3
chicken	erythrocyte	β	*ca.* 10·7
rat	liver	β	*ca.* 10·2
rat	spleen	β	*ca.* 10·4
rat	hepatoma	β	*ca.* 10·2
man	thymus gland	β	*ca.* 10
man	liver	β	10·2
rabbit	liver	β	10·6
mouse	spleen	β	*ca.* 10·4
wheat	germ	principal	10·3

(6) *Comparison of mobility*-pH *curves of β-histones obtained from different cells of the same species*

The extensive series of measurements involved in the determination of the electrophoretic mobilities recorded in the preceding section was carried out primarily with the object of characterizing the various histones examined. Another purpose was, however, kept in view. It was reasonable to suppose that such measurements of physical properties would provide a sensitive method for distinguishing between different histones. If this proved indeed to be the case, it would facilitate comparison of a larger number of histones than has hitherto been possible, and thus assist in ascertaining whether or no the former finding that histones are cell specific could be broadened into a generalization. Such a method would perhaps also remove one obstacle to this generalization which had been previously (Stedman & Stedman 1951) encountered, namely, the failure, with the limited analytical methods then available, to detect any chemical difference between the β-histones from the thymus gland and liver of the ox. For these and other reasons mentioned in the preceding section, considerable attention has been directed towards the accurate determination of the mobilities of these two histones over as wide a range of pH as possible. When the results are plotted on the same diagram, as has been done in figure 43, it is evident that the mobility curves of the two histones differ over the whole pH range examined. The difference is certainly small. Nevertheless, it is outside the limits of experimental error involved in the actual measurements. That it is a true difference is, moreover, supported by the fact that, despite the large number of measurements involved, there is not a single point which does not clearly belong to its own curve rather than to that of the other histone. While the results thus seem to establish a physical difference between the β-histones present in the thymocytes and liver cells of the ox, the magnitude of this difference is so small as not, perhaps, to carry complete conviction. Confirmation of the conclusion has therefore been sought by other methods, which will be described in following sections.

Similar slight differences in electrophoretic mobility have also been found in less extensive experiments with the β-histones obtained from different cells of the fowl. This will be evident if the mobility curves for the β-histones from the erythrocytes, liver and spleen of this species shown in figures 28, 29 and 31 are plotted on one diagram.

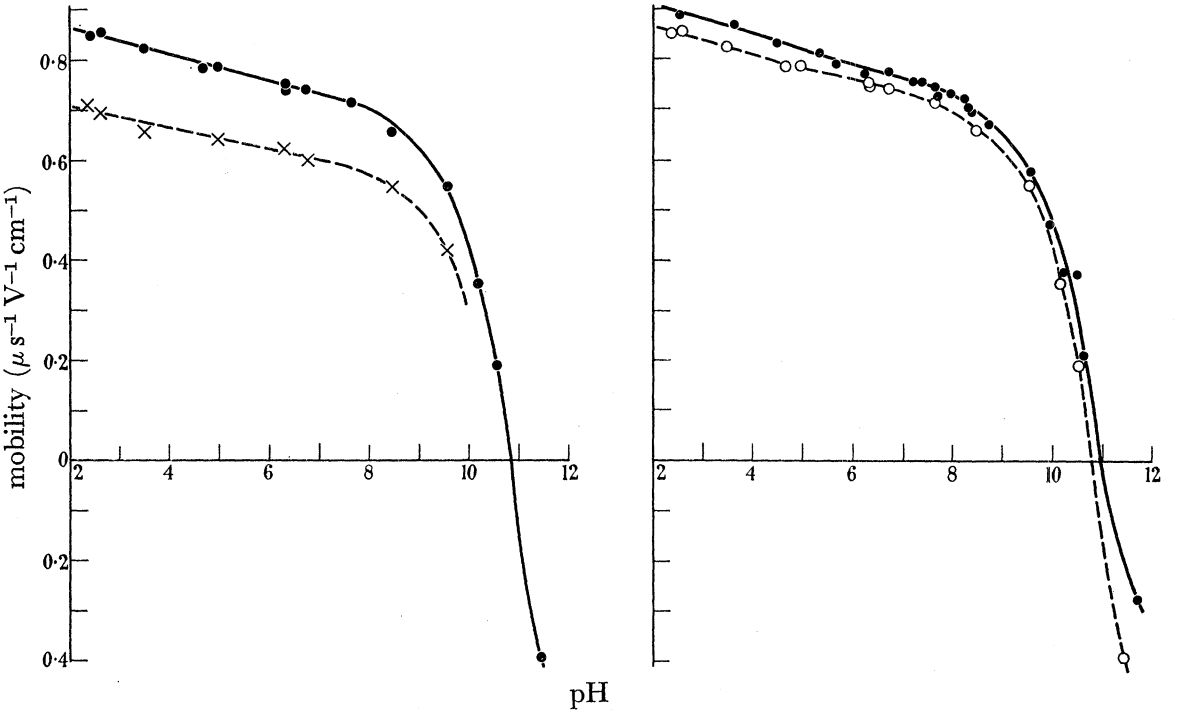


FIGURE 42. *Ox liver.* Mobility-pH graph of fractionated histone from ox liver nuclei. ●, β-component; ×, γ-component.

FIGURE 43. Mobility-pH graph of the β-components from ox thymus and ox liver plotted together. ●, ox thymus β-histone; ○, ox liver β-histone.

(7) *Diffusion phenomena*

Measurements of the diffusion coefficients of the β-histones from the livers and thymus glands of the ox were made partly for other purposes and partly with the object of characterizing, and perhaps distinguishing between, these two histones by means of another physical property. The preparations of the β-histones used were virtually electrophoretically pure, as is evident from figure 44, plate 6, which is the electrophoretic pattern of the thymus histone used in these experiments. The diffusion measurements were made over a pH range of from 1·4 to 8, using initially ox thymus β-histone. At pH values up to about 4 the diffusion patterns closely approached true Gaussian curves (figure 45, plate 6, but as the acidity of the solution was diminished they deviated in progressively increasing amounts (figure 46, plate 6) from this form, the ordinates for the lower portions of the curve being greater than required by theory. This suggests the presence of one or more components of small particle size, a suggestion which is supported by the sedimentation behaviour of β-histones described on p. 114. The patterns were nevertheless used for the calculation, made in the manner previously described (p. 98), of diffusion coefficients. The results obtained are plotted against pH in figure 47. Two points subsequently determined with ox liver β-histone are also included in this figure. It is clear that the latter fall

accurately on the curve for the thymus histone. It was therefore concluded that the two histones diffuse at identical rates under the same conditions and are subject to the same influence of pH.

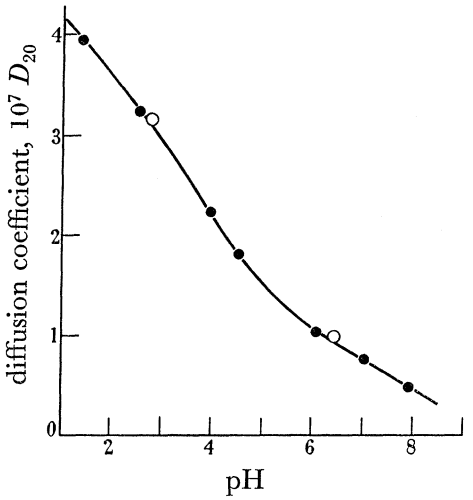


FIGURE 47. *Ox thymus diffusion.* Graph of diffusion coefficient against pH for β-histones from ox thymus and ox liver nuclei at 20° C, ionic strength 0·18 and concentration 0·5 %. ●, ox thymus histone; ○, ox liver histone.

While the measurements thus failed to distinguish between the two β-histones, the results nevertheless seemed of interest from another point of view. If the decrease in the numerical value of the diffusion coefficient with increase in pH is interpreted as indicating an increase of the particle weight in the solutions of the histones, it is clear from figure 47 that both the β-histones used are unusual amongst proteins in possessing a very great capacity to aggregate. Thus, the value of $10^7 D_{20}$ falls from 4·2 at pH 1·4 to 0·5 at pH 8·0. The significance of this can be better appreciated from the corresponding values of the particle weights. Using standard methods (Edsall 1953), ignoring the non-Gaussian form of the diffusion patterns, and assuming an axial ratio, calculated from viscosity measurements, for the protein particles of 22:1, the particle weights have been calculated from the actual diffusion coefficients and viscosities of the solutions to be $7{\cdot}6 \times 10^4$ and 7×10^6 at pH 1·4 and 7·0, respectively. There is thus nearly a hundred-fold increase in particle weight over this range. Confirmation of the great capacity to aggregate which β-histones from animal sources possess has been obtained in ultracentrifugal studies described in a later section. This capacity does not, however, extend to subsidiary histones so far as they have at present been examined. Thus the α-histones from ox thymocytes admittedly probably a mixture of the α_1-, α_2- and α_3-components and possibly also including a small proportion of γ-histone, gave diffusion patterns which were identical at pH 3·15 and 6·85 and indicated a D_{20} of $7{\cdot}44$ and $8{\cdot}09 \times 10^{-7}$. Both patterns were, moreover, closely Gaussian in shape (figure 48, plate 6, figure 49, plate 7). In addition to demonstrating that α-histones do not share with β-histones a capacity to aggregate, these results suggest that the various α-components, and possibly also the γ-component, of the histone present in ox thymocytes do not differ appreciably in particle weight. Diffusion experiments with the unfractionated histone from wheat germ also indicate that both components of this histone resemble subsidiary rather

than β-histones. The failure of wheat germ histone to show signs of aggregation on raising the pH of the solution constitutes a second difference between this and histones of animal origin (cf. p. 105).

As a first interpretation of the diminution of the diffusion coefficient of the two β-histones with increase in pH it was postulated that these histones exist in acid solution either in a monomeric form or possibly as small aggregates, and that aggregation proceeds progressively as the hydrogen ion concentration of the solution is reduced. On the basis of this postulate, and assuming the process to be a reversible one, each increase in the degree of aggregation, would be associated with the formation of a greater range of particle sizes than previously existed. But subsequent experiments with the ultracentrifuge have shown that material such as that used in the diffusion experiments contains a subsidiary histone which does not aggregate with increases in pH. This clearly offers a more satisfactory explanation of the non-Gaussian form of the diffusion patterns.

(8) *Electrophoretic separation of mixtures of β-histones from different cells*

The differences between the electrophoretic mobilities of the β-histones from the liver cells and thymocytes of the ox (figure 44) although small, are of a sufficient magnitude to warrant the belief that, if the two were admixed, it would be possible to demonstrate their separation from the mixture by electrophoresis. Success in such an experiment would, moreover, offer unequivocal confirmation of the difference between the two histones. Several such experiments have accordingly been carried out, but without success. The peak in the electrophoretic pattern given by the mixture did, it is true, appear to be broader than those of the unmixed histones, but no sign of a separation into two peaks could be detected.

A possible explanation of this failure of the two histones to separate is afforded by the aggregation phenomena described above. These histones resemble one another closely in chemical composition and physico-chemical properties. According to the interpretation given to the diffusion data they also possess identical capacities to form aggregates, capacities which depend in exactly the same way upon the pH of their environment. It is not, therefore, unreasonable to suppose that the two histones are also capable of forming aggregates with one another. The methods used in the preparation of the solutions for electrophoresis, namely, dialysis of an acid solution of the histones for several days against a buffer of the chosen pH, are, moreover, ideally suited to the formation of such mixed aggregates, for aggregation is at a minimum in the acid solution of the histones first prepared for dialysis. There would thus be every opportunity for the formation of mixed aggregates to occur as the pH was slowly raised to that of the buffer, and, if it did actually take place, it is evident that it would prevent the separation of the two histones by electrophoresis.

Assuming that the aggregation of histones is a relatively slow process, as there is every reason for believing it is in neutral or alkaline but not in acid, solution, it was thought that this difficulty could be overcome by dialyzing the two histones separately against the same buffer and then mixing the solutions immediately prior to their introduction into the electrophoresis apparatus. If the two histones were different substances, they should then separate under these conditions. Accordingly, experiments were carried out with the

β-histones from the liver and thymus glands of the ox in which the histones from the two sources were dialysed separately against a buffer of pH 8·6. At the same time a solution of a mixture of the two histones in roughly equal amounts was prepared and used as a control. In order to obviate the production of differences in pH or ionic strength among the different solutions, they were all dialyzed together in the same flask. When the control solution was examined in the Tiselius apparatus, the mixture behaved as an electrophoretically pure substance (figure 50, plate 7). On the other hand, the solution prepared by mixing together the separately dialyzed solutions of the two histones immediately before filling the U-tube gave an electrophoretic pattern (figure 51, plate 7) which left no doubt as to the presence of two substances. In order to exclude the possibility that this result was due to an artifact caused by the changed dialysis procedure, two different preparations of main histones from ox thymus glands were treated in exactly the same way. No separation occurred in either case.

At the time these experiments were carried out it was concluded that the results demonstrated unequivocably that the β-histones from ox livers and thymus glands were different substances. Since that time, however, an ultracentrifuge has become available to us and its application to this problem has given results, recorded in the next section, which have caused us to doubt the unequivocal nature of this conclusion so far as pure, as distinct from electrophoretically pure, β-histones are concerned.

(9) *Behaviour of histones on sedimentation in the ultracentrifuge*

The examination of the behaviour of histones during sedimentation was originally planned as a separate study and was not commenced until the remainder of the work reported in this communication was finished and being prepared for publication. The early experiments with the ultracentrifuge, while giving general confirmation of the aggregation phenomena exhibited by β-histones and described above, nevertheless, indicated that electrophoretically pure β-histones were probably contaminated to a smaller or greater extent, according to the particular preparation, with γ-histone. As this finding might invalidate the interpretation which we had placed on our electrophoretic experiments with regard to the non-identity of the β-histones from ox thymocytes and liver cells, it was decided to delay the publication of our results until some data bearing on this problem had been obtained. The work with the ultracentrifuge here recorded must therefore be regarded as of a preliminary nature in the sense that it represents only the first experiments of a much more extensive investigation which it is proposed to make in this sphere. For most of the experiments on the β-histone from ox thymocytes, a sample of the histone from which the α-components had been removed, but which contained both the β- and γ-components was used. The preparation of this sample and its electrophoretic behaviour are described on page 102. When examined in the ultracentrifuge it was at once evident that, corresponding with the results obtained by electrophoresis, two components were present (figures 52, 53, plate 7). These differed markedly, however, in their response towards change in pH. With increase in pH the sedimentation constant (S value) of the fast component, while showing little change between pH 1·4 and 5·0, rose rapidly and continuously with further increases. On the other hand, the S value of the slow component remained approximately constant over the pH range of 1·4 to 9·4 (figure 54). This result suggests that the fast and

slow components consist essentially of the β- and γ-histones, respectively. It also confirms the aggregation phenomena revealed with β-histones in the diffusion experiments described above and gives an explanation of the departure of the diffusion patterns from a true Gaussian curve, for at no pH could one expect particles of uniform size to be present. It is, moreover, evident that γ- differ fundamentally from β-histones in lacking the ability to aggregate with increase in pH.

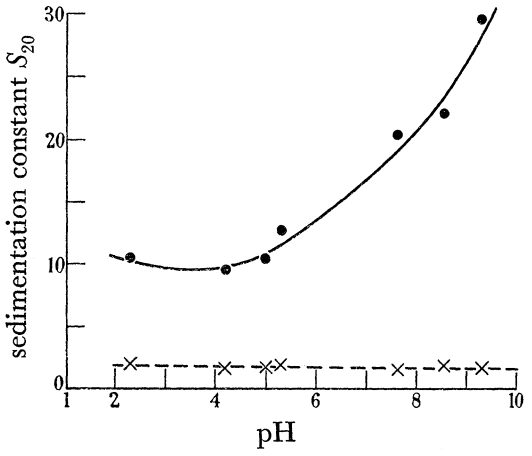


FIGURE 54

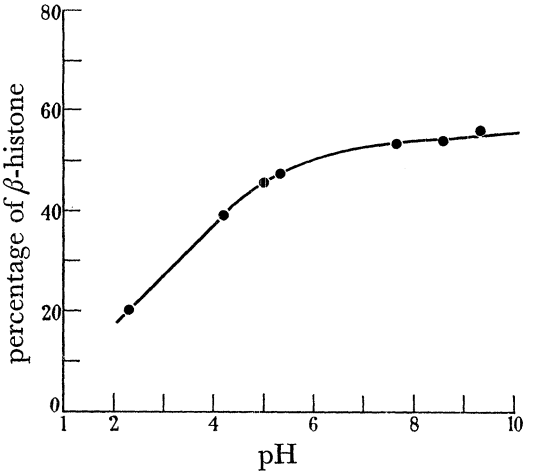


FIGURE 55

FIGURE 54. *Ox thymus sedimentation.* Sedimentation constants in svedbergs of β- and γ-histones from ox thymus nuclei plotted against pH, after 72 h dialysis; solutions of 1·33 % concentration and of ionic strength 0·18. ●, β-component. ×, γ-component.

FIGURE 55. The percentage of β-histone ($S > 10$) obtained during the sedimentation runs on ox thymus $\beta+\gamma$-histone shown in figure 54 are plotted against pH.

Measurements of the relative amounts of slow and fast components present at different pH's were calculated by determining the areas of the corresponding peaks in the schlieren patterns. On plotting the results (figure 55) it appeared that these varied in a regular manner with the pH. At low pH's the slowly sedimenting or γ-fraction contained material which was transferred to the fast or β-fraction as the pH was raised. It is evident that the acid solution contains material which will give rise to the aggregated β-component on increasing the pH. This may be due to the presence at low pH's in the slow fraction of unaggregated β-histone, either in equilibrium with its aggregated form or in loose combination with the γ-component. An alternative and more probable explanation is that the γ-histone combines with the β-component and is incorporated into the aggregates of the latter in increasing amounts as the pH rises.

Measurements of the relative amounts of the β- and γ-components made from an electrophoretic pattern of this preparation at pH 6·65 gives values which differ from those obtained at the same pH in the above ultracentrifuge experiments. The proportion of the β-component found electrophoretically is greater than that calculated from the sedimentation data for the same pH even though the proportion has here reached its highest value. The values are: electrophoresis, β, 70 %; γ, 30 %; sedimentation, β, 52 %; γ, 48 %.

Whatever the final explanation of the above phenomenon may prove to be, it immediately became evident that a large-scale separation of the β- and γ-components could be

effected in the preparative rotor of the ultracentrifuge, particularly if this were done at pH's well above 5·0. This was accordingly carried out with a 2% solution of the above-mentioned sample of histone containing the β- and γ-components. The solution was first dialyzed for 4 days at room temperature at pH 8·5 against a veronal buffer of the usual ionic strength and then centrifuged at 50740 rev/min for 6·5 h. The clear, colourless supernatant solution containing the bulk of the slow component was poured without difficulty from the centrifuge tubes leaving a clear gel with a pale yellowish colour. The latter was dissolved in 0·1 N sulphuric acid and the two solutions dialyzed separately against dilute sulphuric acid of the same concentration to remove salts. The histones were

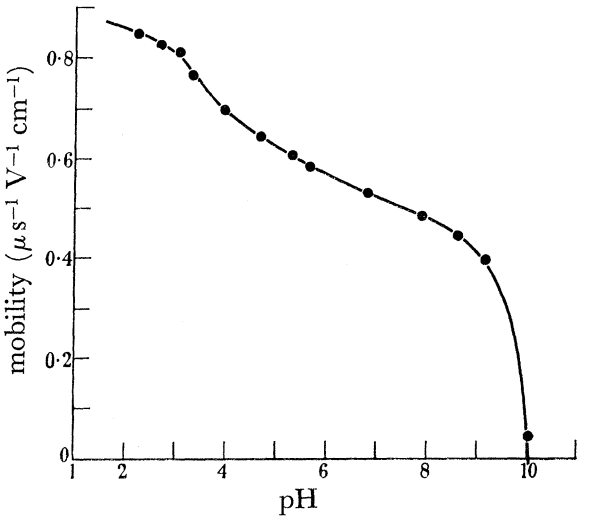


FIGURE 58. Mobility-pH graph of the γ-histone prepared in the ultracentrifuge from ox thymus histones.

finally precipitated with acetone and dried in the usual way. Each fraction when analyzed electrophoretically at ordinary pH's was found to consist of one component (figures 56, 57, plate 7). Moreover, the mobility of the fast or aggregated fraction fell on the curve (figure 40) for the β-component, while that for the slow or unaggregated component gave a mobility curve (figure 58) which is close to that for the γ-component (figure 40). It should be noted that in the region of the isoelectric point of the γ-component, which is at pH 10·1, a small amount of another component becomes visible (figure 59, plate 7). This is presumably the second γ-component to be referred to later. Another feature of this curve which distinguishes it from animal β-histones is the presence of a point of inflexion between pH 2·5 and 3·5. This explains the absence of points on the mobility curves of γ-components determined with unfractionated histones in acid solution.

But despite the fact that these two fractions had been separated from one another by sedimentation, neither fraction behaved as a monodisperse substance when examined analytically in the ultracentrifuge. At pH 7·7, the fast fraction, presumed to consist essentially of the β-histone, exhibited, as was to be expected, the presence of a small amount of the slow component (figures 60, 61, plate 7). The slow or presumed γ-histone, on the other hand, contained no material of S value greater than 1·6. Nevertheless, the schlieren pattern for this component was not symmetrical. After long centrifuging material appeared with an S value of about 0·8, which is approximately that of α-histones (figure 62, plate 7).

It is possible indeed, that it does represent an α-component, for the preparation from which this slow fraction was obtained might well have contained traces of α-histones. Nevertheless, it is by no means excluded that it represents a γ-histone.

With the object of increasing the purity of the β-component, the fast fraction obtained above was run for a second time in the preparative ultracentrifuge. The pH selected was 8·5 and the duration of run was curtailed in order to diminish the amount of the slow fraction deposited with the fast.

A similar sample of the β-component from ox liver histone was also prepared, although the experimental details were somewhat different. The unfractionated histone which constituted the starting material for this preparation exhibited initially in the analytical ultracentrifuge two peaks (figure 63, plate 7) of which the slower moving one ultimately resolved itself (figure 64, plate 7) into two components with S values of 1·6 and 0·75, respectively. In this case, the unfractionated histone was separated directly in the ultracentrifuge into fast and slow fractions, the former consisting essentially of the β-component and the latter of the subsidiary histones ($\alpha+\gamma$). A 2·5 % solution of the histone dialyzed for 4 days at pH 8·5 and with $\frac{1}{2}\Gamma = 0{\cdot}176$ was used. The solution obtained by dissolving the gel of the fast fraction in 0·1 N hydrochloric acid was directly dialyzed against the appropriate buffer, run again in the preparative ultracentrifuge, and the whole process repeated once more. The gel of the β-histone was then dissolved in 0·1 N sulphuric acid and precipitated and dried in the usual way. The supernatant solution from the first preparative run was also dialyzed against 0·1 N sulphuric acid and similarly precipitated and dried; the examination of these subsidiary histones is described later.

By the above procedure two preparations of β-histones, one from the liver and one from the thymus gland, were obtained which could reasonably be expected to be pure, as indeed they were electrophoretically. When examined, however, in the ultracentrifuge a slow component was found to be present in each of them. The nature of this material is unknown. It was present in such small amount that it was not possible to calculate its S value nor could an accurate estimate of its concentration be made. It is, nevertheless, possible to say that it represented less than 5 % of the total protein.

It is conceivable that this consisted of unaggregated β-histone, although the possibility that it was a subsidiary histone cannot be excluded. A decision between these alternatives might have been made by amino acid analysis, but when the slow moving material was isolated from the supernatant liquid in a subsequent preparative run in the ultracentrifuge, the amount obtained was too small to permit this.

A comparison in the ultracentrifuge of these preparations with earlier electrophoretically pure samples of β-histones demonstrated that they represented the purest specimens of these histones which we have hitherto succeeded in obtaining. It therefore seemed desirable, particularly as the earlier electrophoretically pure preparations have now been shown to be polydisperse, to repeat with these new preparations the former experiments (p. 114) which seemed to show conclusively that ox liver and thymus β-histones are different substances. The results completely confirmed the earlier ones. When dialyzed together the two histones behaved electrophoretically as a single component (figure 65, plate 8), but separated from one another (figure 66, plate 8) when dialyzed separately and mixed immediately before their introduction into the U-tube. It was judged advisable, however,

to compare one of these histones with earlier and less pure samples from the same source. For this purpose a sample of ox liver β-histone, prepared by preparative electrophoresis and found to be electrophoretically pure in the Tiselius apparatus, was selected. When this specimen was dialyzed at pH 7·9 and then mixed immediately before its examination in the Tiselius apparatus with a similarly treated sample of the liver histone prepared in the ultracentrifuge a separation of two components occurred (figure 67, plate 8). This result has been confirmed in a duplicate experiment as well as in other experiments using either two different preparations of liver β-histones or of ox thymus β-histones. No adequate explanation of this phenomenon can yet be given. Some facts, however, point to the possibility that a subsidiary histone, presumably that designated as γ, enters into reversible combination with the β-histone. When just sufficient γ-histone is present to saturate the β-component, the histone becomes electrophoretically homogeneous. It will not then, as has been shown on page 114, separate from another preparation of the same histone which contains a greater proportion of the γ-component. When, however, the proportion of γ-histone is reduced below that necessary to saturate the β-component, two different preparations of the same β-histone may still contain different proportions of γ-histone. They then behave as different substances when in the aggregated state.

When examined in the Tiselius apparatus, the subsidiary histones from unfractionated ox liver histone, prepared in the ultracentrifuge as described above, were found to consist of four components, namely α_1, α_2, α_3 and γ (figure 68, plate 8). The combined α-components represented 45 % and the γ-fraction 55 % of the total histone present. Analysis in the ultracentrifuge indicated, on the other hand, the presence of only two components with S values of 0·8 and 2·0 (figure 69, plate 8) in the proportion of 70 and 30 %, respectively. Assuming that the particle weights of the α-components are all of the same order of magnitude and correspond with an S value of about 0·8, an assumption which is justified by other evidence, it is clear that the material (70 %) sedimenting slowly in the ultracentrifuge consists of all the α-components (45 %) shown in the electrophoretic pattern augmented by material which has arisen from the 55 % of γ-component also calculated from the same pattern to be present. The γ-component apparent in electrophoresis is thus obviously not homogeneous. While its exact nature has not yet been ascertained a partial solution of the problem which it offers has been afforded by separating the above mixture of subsidiary histones from ox liver into two fractions by controlled treatment with alcohol.

For this purpose the following procedure was, after some trials, adopted. A solution of the mixture at pH 8·5 was stirred with one-fifth of its volume of alcohol. After standing for 5 h the solution, which had become opalescent, was centrifuged for one hour at 50740 rev/min. This yielded a water-clear supernatant solution and a slightly opaque but colourless jelly. The former was decanted, dialyzed against 0·1 N sulphuric acid, precipitated with acetone and dried in the usual way; the latter, after being dissolved in 0·1 N sulphuric acid was treated in the same manner. There were thus obtained two fractions of the subsidiary histones from ox liver which were provisionally regarded as α- and γ-fractions, respectively.

The presumed α-fraction when examined in the ultracentrifuge at pH 8·68 was found to contain no material with an S value greater than 0·8 (figure 70, plate 8). The peak with this value is slightly asymmetric indicating the presence of a component with an S value

of less than 0·8. This asymmetry is not, however, surprising for the fraction was expected to contain several α-components, as, indeed, electrophoretic analysis at pH 8·68 showed that it did. But the electrophoretic pattern (figure 71, plate 8) not only indicates the presence of the α_1-, α_2- and α_3-histones; a fourth peak with a mobility identical with that of the γ-component can also be seen. Evidently a γ-histone with an S value of approximately 0·8 is contained in the subsidiary histones from ox liver. This finding to some extent explains the above apparent discrepancy between the quantitative results obtained by ultracentrifugal and electrophoretic methods when applied to the total subsidiary histones, for it is clear that a comparison was made between the proportion of α-histones determined electrophoretically and that of the material, now shown to contain, in addition to the α-components, a γ-histone with an S value of about 0·8. But it does not explain the nature of the material with an S value of 2·0.

Some light on this problem has, however, been obtained by the examination of the presumed γ-fraction obtained from the total subsidiary histones. The electrophoretic pattern of this material (figure 72, plate 8) shows, as was to be expected from its method of preparation, the presence of small amounts of α-histones. But the greater part of it migrates with a mobility which is hardly distinguishable from, but nevertheless slightly greater than, that of γ-histone. That this material is not, despite its mobility, identical with the γ-component with $S=0{\cdot}8$ follows from the results of its examination in the ultracentrifuge, for apart from a small amount (7 %) of a substance which sediments rapidly and was evidently β-histone, the pattern (figure 73, plate 8) shows one main peak giving $S \doteq 1{\cdot}6$. These results could be explained by assuming the existence of two γ-components with almost identical mobilities but different molecular weights. Another possibility, to some extent supported by the slight asymmetry of the peak in the electrophoretic pattern and by the change in the S value from 2·0 to 1·6 on fractionation, is that the material in question is a complex formed between γ- or other subsidiary histones and unaggregated β-histone.

(10) *Factors influencing aggregation of β-histones*

In view of the possibility that external factors other than pH may influence the formation of aggregates by β-histones the experiments described in this communication have been made under strictly standard conditions of salt concentration, time of dialysis and, except where other conditions rendered it impossible, protein concentration. A few preliminary experiments have, nevertheless, been carried out to ascertain if any of the above-mentioned factors do, in fact, influence the process. Time of dialysis appears to be a most potent factor, for, when a preparation of ox thymus histone containing the β- and γ-components was dialyzed at pH 7·8 and in 1·33 % concentration and the S value of the β-component determined on aliquots of the solution after increasing intervals of time the results plotted in figure 74 were obtained. It is clear that, although the rate of aggregation decreases with time of dialysis, the process is incomplete even after 160 h. In this connexion it should be recalled that the electrophoretic mobility is not susceptible to change by comparable long periods of dialysis (p. 97).

Changes in salt concentration have little effect on the aggregation of β-histones provided the ionic strength is kept between 0·1 and 0·2. Between ionic strengths of 0·3 and 0·5 and presumably at higher ones there is, however, an enormous increase. Thus, the β-component

in a mixture of the β- and γ-components of ox thymus histone has S values of 100 and 300, respectively, at these ionic strengths.

Variations in protein concentration have a slight but definite influence on the rate of aggregation of β-histones. This is shown by the increase in S value from 15 to 18 by raising the concentrations of thymus $\beta+\gamma$-histone from 0·25 to 2·0 % at pH 7·8 and 65 h dialysis.

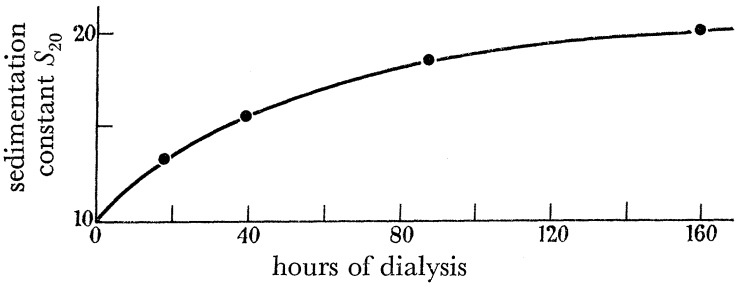


FIGURE 74. Sedimentation constants in svedbergs of the β-histone from ox thymus nuclei plotted against time of dialysis for a 1·33 % solution at pH 7·8 and at room temperature.

(B) TUMOUR TISSUE

(1) *General*

Concurrently with the above work on normal histones the examination of the histones from a number of tumours has from time to time been made as suitable material became available. One of the objects of such work was to compare the physical properties of histones from the two sources, for, if the view that all histones are cell specific is true, those from malignant tissues might possibly exhibit differences which are detectable and characteristic. The first material used was obtained from human malignant tissue. Two types were examined: the metastases from a case of bronchogenic carcinoma and the glands from one of lymphatic leukaemia. The histones from these tissues had been previously examined chemically (Stedman & Stedman 1951) and shown by paper chromatography and amino acid analysis to possess an amino acid composition similar to that of normal β-histones. In particular they gave the characteristic high arginine values. They were also extracted from the cell nuclei by the normal procedure and precipitated as sulphate, the formation of which salt is a general property of histones. In these respects, therefore, they completely resembled normal histones.

But when they were dissolved and dialyzed with a view to the examination of their electrophoretic behaviour a marked difference in solubility became apparent. Whereas most normal histones in 0·5 % solutions showed no tendency to precipitate when dialyzed against the usual buffers, except when in the immediate vicinity of their isoelectric points (pH 10 to 12), the tumour histones separated almost completely in a few hours at all pH's above about 5·0 and 6·0, respectively, for the bronchogenic and leukaemic material. In view of these results other histones accumulated from time to time from both human and experimental tumours were also examined. The former comprised the metastases from a breast sarcoma, the leucocytes from cases of chronic myeloid and chronic lymphatic leukaemia and an 'undifferentiated' tumour which was obtained from, and may have originated in the pancreas. The lymphocytes from a fowl which succumbed to lymphatic leukaemia was another example of a natural tumour. The experimental tumours examined

were: the Walker rat carcinoma 256, the rat sarcoma *Rd*/3, the azo-dye-induced rat hepatoma, and leukaemic spleens from the mouse (line I). A few comments should perhaps be made about some of this material. The histone from the breast sarcoma was available only in very small amount owing to the minute yield of histone sulphate, which amounted to only 4 % of the dry weight of the nuclei. The malignant lymphocytes from the fowl were isolated from the whole liver, which consisted of little else than malignant lymphatic tissue. To make certain that this material was not significantly contaminated with liver cells a large nodule of lymphatic tissue was dissected from the metastases and worked up separately. The results given by the two batches of material so obtained were indistinguishable. In preparing the nuclei from the human leucocytes, the whole blood was centrifuged and the white cells, which were present in very large amount (500000 per ml.), pipetted off, washed with saline and lyzed with 4 % acetic acid according to our normal procedure.

When 0·5 % solutions of the histones from the following tumours were dialyzed against veronal buffers the bulk of the protein precipitated at about the pH's indicated after about 3 days: human bronchogenic carcinoma, pH 5·0; human leukaemic glands, pH 6·0; human chronic myeloid leukaemia, pH 6·0; human chronic lymphatic leukaemia, pH 6 to 7; human breast sarcoma, pH 6·0; fowl lymphatic leukaemia, pH 7 to 8; Walker rat carcinoma, pH 6 to 7; rat sarcoma *Rd*/3, pH 6 to 7; rat hepatoma, pH 6 to 7; mouse spleen (line I) pH 7.

The histones from the human 'undifferentiated' tumour could not, however, be distinguished from a normal histone with respect to its solubility, nor could that isolated from the spleen from the case of lymphatic leukaemia the glands of which yielded the very insoluble histone mentioned above. So far no anomalous properties have been detected in the histone from the undifferentiated tumour, but that from the spleen certainly exhibits, as will be shown below, an abnormally high electrophoretic mobility.

One abnormality was found amongst histones from 'normal' tissue. The preparation from the livers of the mouse (strong A) precipitated from solution under the above standard conditions at pH 8·0 if the concentration was greater than 0·1 %. Unfortunately, no data obtained from liver histones from normal stock mice is available for comparison with this figure.

(2) *Electrophoretic patterns of histones from tumours*

(i) *Spontaneous tumours*

The electrophoretic behaviour of the histones isolated from the tumours mentioned above has been examined with the object of comparing their patterns with those given by histones from normal tissue. For this purpose, it has been necessary in most cases, because of their small solubility in neutral or alkaline solution, to restrict the study to acid solutions. When alkaline solutions have been employed it has been necessary to use a very low concentration. The resulting pattern has then sometimes been obscured owing to the high Tyndall effect (figure 79, plate 8). Some of the histones were, moreover, only available in the form of 'main histones', i.e. histones which had been fractionated to remove subsidiary ones. This was the case with the preparation from the human glands (lymphatic leukaemia), which gave an electrophoretic pattern (figure 75, plate 8) showing only one component. α- and γ-histones were, however, present in the material separated during fractionation.

The histone from the spleen of the same case of lymphatic leukaemia gave anomalous patterns both in the fractionated (figure 76, plate 8) and unfractionated (figure 77, plate 8) form. In each case the main peak corresponding with the β-histone separated above pH 7·0 into two components in nearly equal amounts. Both patterns also contained the γ-component, while an α-fraction was evident in the unfractionated material. The separation of the β-histone into two components is distinctly abnormal, for it has never been observed with the histones from any other source.

The histone from the lung tumour (bronchogenic carcinoma), examined in the fractionated form, gave a pattern (figure 78, plate 8) corresponding with an electrophoretically pure substance.

Both the histones from the leukaemic bloods examined gave patterns (figures 79, 80, plate 8) indicating that they were electrophoretically homogeneous even in the unfractionated form. That from the undifferentiated tumour (figure 81, plate 9) appeared, as it did with respect to solubility, to be quite normal in containing both a γ-component and an α-fraction as well as the usual β-histone.

Like the histones from the above two human leukaemias, that from the fowl was electrophoretically homogeneous (figure 82, plate 9).

(ii) *Experimental tumours*

The electrophoretic patterns of the histones from the experimental tumours are shown in figures 83, 84, 85 and 86, plate 9. All indicate the presence of only a single component.

(3) *Electrophoretic mobilities of histones from tumours*

(i) *Spontaneous tumours*

Where possible the mobility curves of the β-components of the spontaneous tumours have been grouped in the illustrations into related cell types for purpose of comparison. The curve of a normal β-histone has also usually been included in each diagram. Figure 87 shows the curves obtained with the β-components from human glands (lymphatic leukaemia), leukaemic blood (lymphatic leukaemia) and normal thymocytes (child). It is at once evident that the mobility curves of the two histones of malignant origin differ widely, not only from that of the thymus but also from any normal histone which has hitherto been examined. That from the human glands suggests that the histone possesses an isoelectric point in the region of pH 6·5 to 7·0. It must be pointed out, however, that, although this would not materially alter the shape of the curve, the last point may be subject to appreciable error owing to the impossibility of using more than a minute concentration of protein for its determination. Moreover, the high Tyndall effect indicated that the histone was on the verge of precipitation. Its insolubility at higher pH's precluded further investigation of the curve. The β-histone from the leukaemic blood exhibits the same phenomenon but to a lesser degree.

Owing both to its insolubility and to the lack of material it was not possible to determine the mobility curve of the β-histone from the bronchogenic carcinoma. The mobility at pH 4·3 was, however, measured. The value obtained was $0{\cdot}77\ \mu\,s^{-1}\,V^{-1}\,cm^{-1}$, the point thus falling on the curve for that from the leukaemic glands.

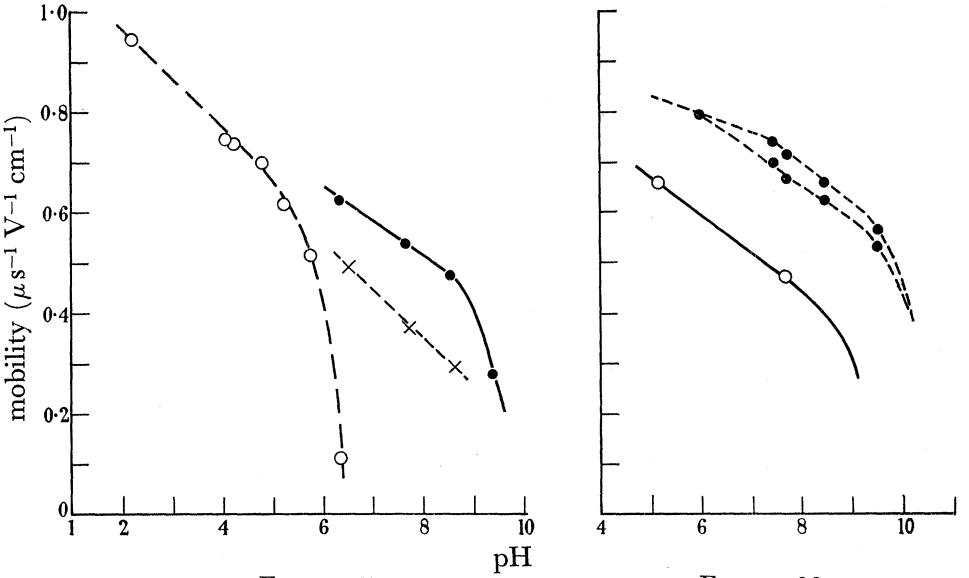


FIGURE 87 FIGURE 88

FIGURE 87. *Human histones.* Mobility-pH graph for β-histones from: ●, normal human thymocytes (child); ×, human leukaemic blood (lymphatic leukaemia); ○, human glands (lymphatic leukaemia).

FIGURE 88. *Human histones.* Mobility-pH graph for β-histones from: ○, normal human spleen; ●, spleen from the case of lymphatic leukaemia.

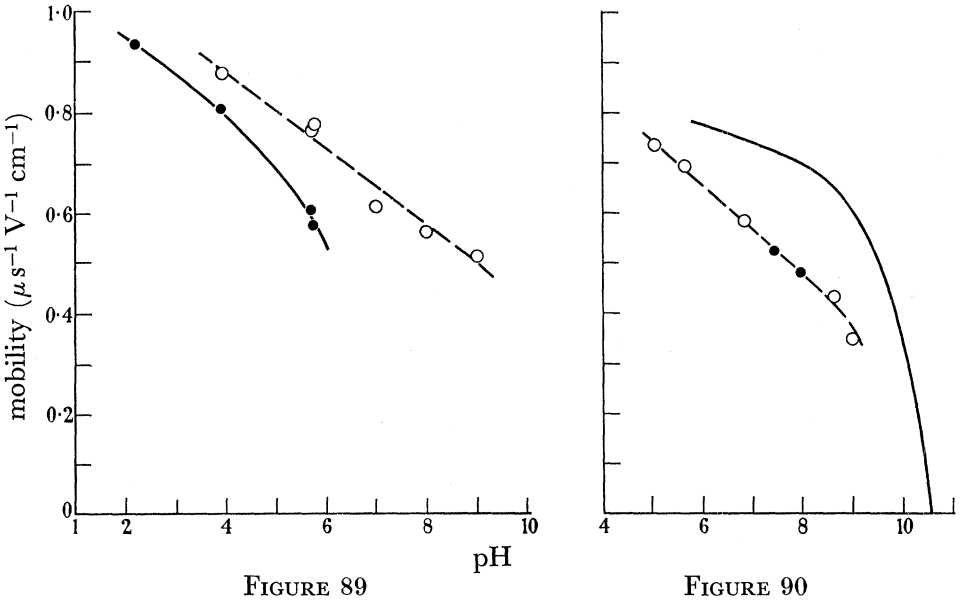


FIGURE 89 FIGURE 90

FIGURE 89. *Human histones.* Mobility-pH graph of the β-histones from: ○, human undifferentiated tumour; ●, human leukaemic myelocytes.

FIGURE 90. *Chicken lymphatic leukaemia.* Mobility-pH graph of the β-histone from the malignant lymphocytes in the liver of a fowl with lymphatic leukaemia. ●, from a single large nodule of lymphatic tissue; ○, from remaining lymphatic tissue in the liver; ———, β-histone from normal thymocytes.

The β-histone from the lymphatic spleen already referred to differed from both normal and the above abnormal histones in possessing a relatively high mobility. This is shown in figure 88, where its mobility curve is plotted together with that from a normal human spleen. It also differed, as already mentioned, from any β-histone hitherto encountered in separating into two components over a large pH range.

On figure 89 the mobilities of the β-histones from the leukaemic myelocytes and of the undifferentiated tumour are plotted. No curve for suitable normal material could be included in this diagram. It is, nevertheless, evident that corresponding with its low solubility, the former histone, possesses a low mobility, whereas the latter is not distinguishable from a normal β-histone.

The last spontaneous tumour cells to be considered in this section are the malignant lymphocytes from fowl liver. The mobility curve of its β-histone has been compared in figure 90 with that of normal chicken thymocytes. The former again exhibits the low mobility characteristic of so many of the histones of malignant origin.

(ii) *Experimental tumours*

Owing to the small amounts of histones from experimental tumours available for this work, it has not been possible for us to determine the mobility curves of an extensive series of such preparations. The two chief examples of this type of tumour examined have been derived from the rat. In addition to these, however, two mobilities at different pH's have been measured with the histone prepared from the leukaemic cells present in the spleens of mice of the strain known as line I. The nuclei from which the histone used in these measurements was prepared were isolated in 1947 during a visit by one of us to Cold Spring Harbour, the spleens of the leukaemic mice being kindly prepared for us and put at our disposal by Miss M. J. Taylor. For purposes of comparison these two points have been plotted in figure 37 together with the mobility curve for the histone from the spleens of normal laboratory mice. It is at once apparent, despite the lack of sufficient determinations to enable a complete mobility curve to be plotted for the leukaemic histone, that the mobility of the latter is much smaller than that of the histone from normal lymphocytes.

The histone from the rat sarcoma *Rd*/3 gave a mobility curve which was lower practically throughout its whole length than either normal liver or spleen histone. It has been plotted in figure 91 which also includes, for comparison, that from normal spleen.

It was fortunately possible to compare the mobility of the histone from the rat hepatoma directly with that from the corresponding normal liver cells. The two curves deviated considerably from one another except at extremes of pH. This is shown in figure 92, where the low mobility of that from the hepatoma is evident. A second preparation of this histone from a second batch of rats gave a mobility curve, shown in figure 93, which clearly differs little, if at all, from the first one.

On the same diagram is plotted the mobility curve for the histone obtained from rat livers after the animals had been maintained on the azo-dye diet for 1 month. This material was examined for the following reason. It had been shown by Miller & Miller (1953) who have examined azo-dye carcinogenesis in great detail, that during its administration the dye becomes bound to proteins present in all cell fractions of the liver (Price, Miller & Miller 1948). This binding reaches a maximum after 4 weeks administration and thereafter

despite continued feeding of the dye progressively diminishes, reaching a half maximal value in the emergent tumour (Miller, Miller, Sapp & Weber 1949). It was, therefore, of interest to ascertain if the dye was at this period bound to the histone or, alternatively, if the histone itself was in any way different from the one present in the normal tissue. While the isolated cell nuclei contained appreciable amounts of bound dye, the histone extracted from them was completely free from it, the dye remaining bound to the nuclear residues.

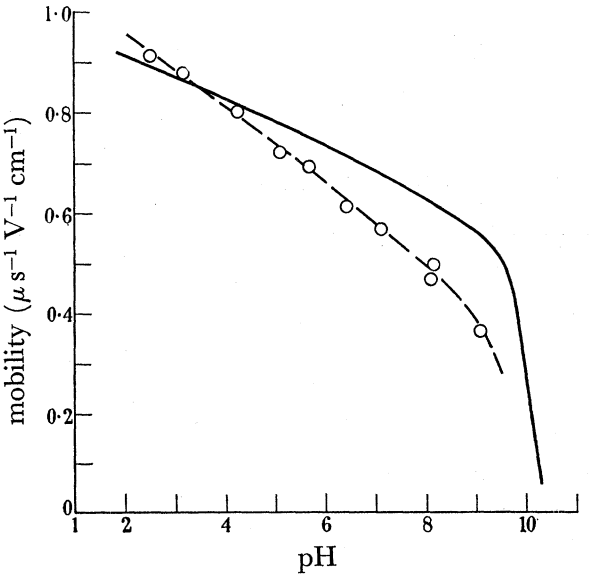


FIGURE 91. *Rat histones.* Mobility-pH graph for β-histone prepared from the rat sarcoma *Rd*/3. ○, tumour histone; ——, β-component of normal rat spleen for comparison.

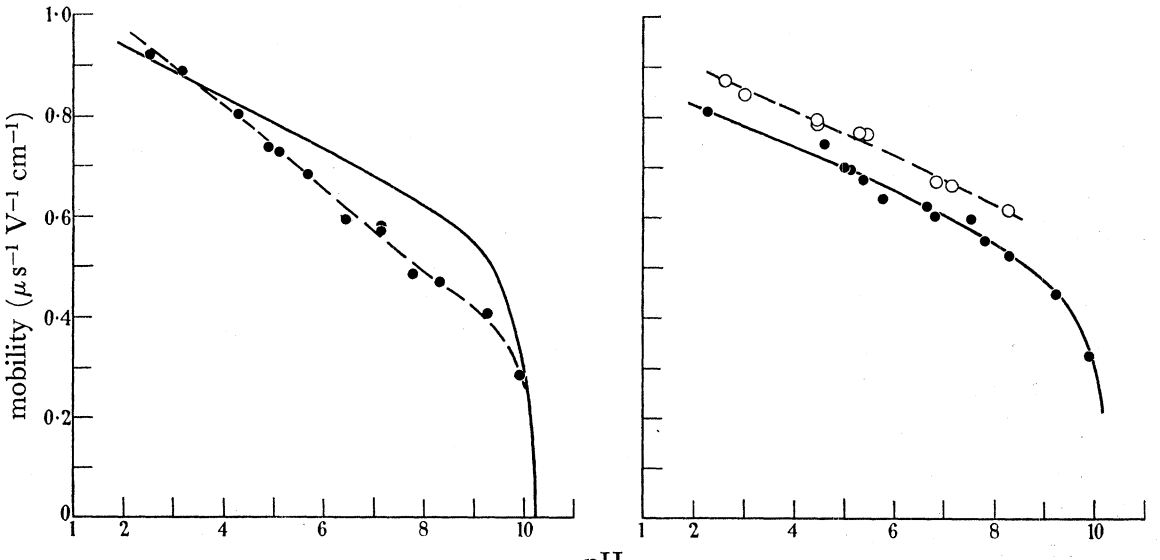


FIGURE 92

FIGURE 93

FIGURE 92. *Rat histones.* Mobility-pH graph for β-histone from rat hepatoma nuclei, first preparation. ●, hepatoma histone; ———, β-component from normal rat livers.

FIGURE 93. *Rat histones.* Mobility-pH graph for β-histone from rat hepatoma nuclei, second preparation. ●, hepatoma histone; ○, β-histone from the livers of rats which had been maintained on the azo-dye diet for 1 month; this is indistinguishable from β-histone of normal rat livers.

Moreover, as the above mobility curve indicates, it was indistinguishable electrophoretically from that from normal rat liver.

Although the differences between the mobility curves of the histones from hepatoma and liver of rats seemed to show conclusively that they are qualitatively distinct, it appeared desirable to test the possibility of separating them from artificial mixtures of the two. As with the ox thymus and liver histones (p. 114) this only occurred when the two proteins were dialyzed separately and mixed immediately prior to electrophoresis. Some doubt has since arisen as to the significance of this result because of the findings mentioned on p. 118 and because of the subsequent discovery, discussed in the following section, that, although electrophoretically homogeneous, these histones are, in fact, composite.

(4) *Diffusion and sedimentation phenomena*

During the preceding work it was observed that the β-histones from many tumours not only differed from normal β-histones in solubility, but also in exhibiting a much stronger Tyndall effect under comparable conditions. This suggested that they might be capable of aggregating to an even greater degree than normal ones. Confirmation of this was obtained by measurements of the rate of diffusion of the fractionated histone from the leukaemic glands. The results are given in figure 94 in which the diffusion coefficients are plotted against pH. The corresponding curve for ox thymus histone is included for comparison. There is no doubt of the greater capacity of the tumour histone to aggregate with increasing pH. It should also be noted that the sharp fall in the value of the diffusion coefficient which it shows at a pH of about 5 corresponds with the pH at which the onset of marked insolubility occurs. As was the case with the normal histones already discussed the diffusion patterns deviated from the true Gaussian form. No doubt the same cause is operating here; for ultracentrifugal studies have shown that, at pH 2·0, two components are present in this material, a fast component, obviously consisting of the β-histone and representing the bulk of the protein and a slowly sedimenting subsidiary histone.

Corresponding to the difference between their mobility curves, the histone from rat hepatoma and normal rat liver yielded different diffusion coefficients and sedimentation constants. The former are plotted against pH in figure 95. Below pH 5·3 the diffusion coefficients do not differ very appreciably from one another, while above this figure they diverge progressively ultimately showing widely different values. At pH 7·7 the diffusion coefficient for the histone from the hepatoma approached zero during the first 46 h of the experiment; thereafter it slowly precipitated from the solution.

Sedimentation experiments were performed on aliquots of the solutions used in the above diffusion experiments and were carried out within a few hours of the commencement of the latter. The behaviour of these two histones in the ultracentrifuge was similar to, although not identical with, that of those from ox thymus and liver. Both gave patterns (figures 96, 97, plate 9) indicating the presence of two components throughout the pH range (2·2 to 7·7) studied, although they were, as has been shown above, electrophoretically homogeneous. In each case one of these components, which in the light of the experiments with ox thymus and liver histones, must be designated the β-histone, sedimented rapidly and aggregated progressively with increase in pH from 5·0 upwards. The other sedimented slowly and gave an S value of 0·8 which did not alter with change in pH. One feature

which distinguished these histones from ox thymus and liver histones was the absence of a component with an S value of 1·6. Because of the use in these experiments of very dilute solutions (0·6 %) it was not possible to make accurate calculations of the relative proportions of the two components revealed by sedimentation. There did, however, appear to be an increase in the amount of the fast component at the expense of the slow with the histone from the hepatoma, but not from that from the liver, as the pH was raised from 2 to 5.

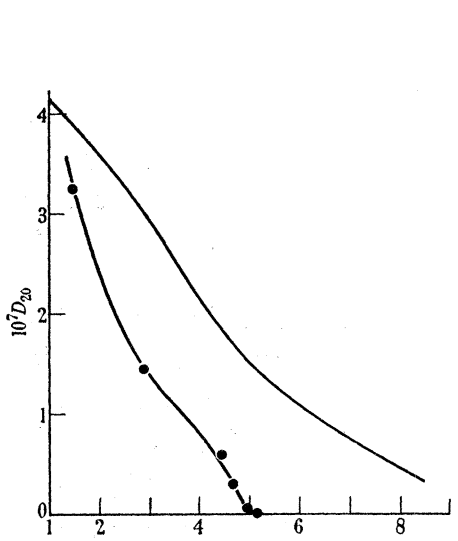


FIGURE 94

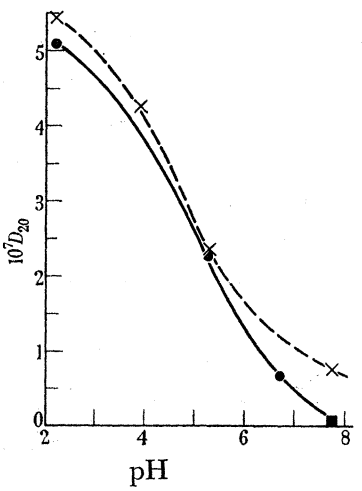


FIGURE 95

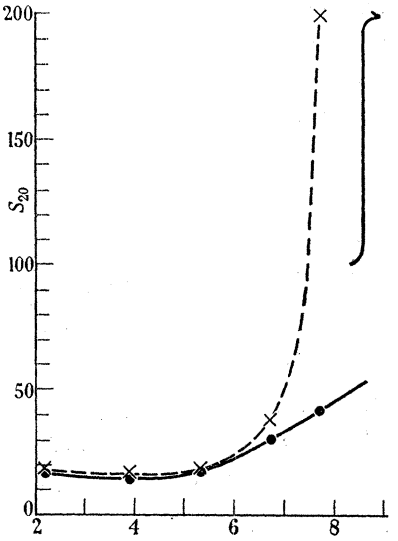


FIGURE 98

FIGURE 94. Diffusion coefficient-pH graph for the β-histone from human glands (lymphatic leukaemia) at 20° C and 0·3 % concentration. ●, tumour histone. ———; β-histone from ox thymus nuclei for comparison.

FIGURE 95. Diffusion coefficient-pH graph for the β-histones from rat hepatoma and normal rat livers at 20° C. Concentrations were 0·5 % for points up to pH 6 and less than this for higher pHs. ●, rat hepatoma histone; ×, normal rat liver histone.

FIGURE 98. Sedimentation constants in svedbergs of the β-histones from rat hepatoma and normal rat livers are plotted against pH; the solutions are the same as those used for the points in figure 95. ●, normal rat liver histone; ×, rat hepatoma histone.

As is evident from figure 98, the S values of the β-components of the two histones from the rat differ little from one another in acid solution although they are both ($S=15$ to 18) higher than that from ox thymus histone ($S=10$). But at pH's above 6·5 a striking difference is apparent, the aggregates formed by the β-histone from the hepatoma possessing at pH 7·7 S values ranging from 100 to 300, while that from the liver has one of only 45.

With the object of examining the electrophoretic behaviour of the two components revealed by the sedimentation experiments, their separation by preparative ultracentrifugation was attempted, parallel experiments being carried out with the histones from the hepatoma and liver. Using 3·2 and 2·5 g, respectively, of the histone sulphate from the hepatoma and the liver, the material was, in each case, dissolved in 100 ml. of 0·1 N hydrochloric acid and the solution centrifuged at 1500 g to remove a trace of insoluble material. The volume was then brought to 130 ml. by the addition of water and the solution dialyzed in Cellophane sacs for 90 h against six changes of veronal+acetate buffer

of pH 8·5. At the end of this treatment the solutions exhibited intense Tyndall effects and had become so viscous as almost to constitute gels. By vigorously and repeatedly squeezing the dialysis sacs, however, a considerable diminution in the viscosity occurred thus enabling the solutions to be filled into the analytical cells and preparative tubes of the ultracentrifuge. In the analytical runs the β-histones, as judged by the movement of the Tyndall effect, sedimented rapidly at between 20000 and 25000 rev/min. Owing to the light scattering caused by this phenomenon no normal schlieren patterns from which the proportions of components could be calculated were obtained. In the light of these results the preparative runs were made in rotor *A* at 50740 rev/min for 2 h. The supernatant fluids, which were water clear, were dialyzed against 0·1 N sulphuric acid, treated with six volumes of acetone and the precipitate dried in the usual way. After dissolving in 0·1 N sulphuric acid the pale yellow gel of β-histone was similarly dialyzed and dried. The yields of the air-dry products were hepatoma, 1·47 g of β-fraction and 1·18 g soluble fraction; liver, 1·24 g β-fraction, 1·02 g soluble fraction.

The soluble fractions from these two preparations were analyzed in both the ultracentrifuge and the Tiselius apparatus. That from the hepatoma gave a sedimentation diagram (figure 99, plate 9) which showed only one component with $S=0{\cdot}75$. Electrophoresis at pH 8·5 resolved the material into two components (figure 100, plate 9) with mobilities of 1·05 and 0·74 $\mu s^{-1}\ V^{-1}\ cm^{-1}$, respectively. The corresponding results with the soluble material from the liver were: sedimentation (figure 101, plate 9) one component with $S=0{\cdot}70$; electrophoresis (figure 102, plate 9), two components with mobilities of 1·05 and 0·71 $\mu s^{-1}\ V^{-1}\ cm^{-1}$. From their mobilities and *S* values it is clear that the soluble components from the two sources are indistinguishable and that they consist of α-histones. They are therefore designated as α_1- and α_2-fractions, respectively.

The two β-histones, when analyzed in the ultracentrifuge at pH 2·5, were found to be contaminated with approximately 10 % of material of low *S* value. Because of this they have not yet been examined in detail. Nevertheless, their electrophoretic mobilities have been provisionally determined at pH 5·0 and 5·7 and have given values which are close to, although slightly smaller than, the values given by the curves for the unfractionated histones.

(C) Chemical analyses

(1) *Amino acid composition of histones*

Amino acid analyses have been made of a large number of preparations of histones, but as the majority of them were carried out with the object of following the course of the fractionation of the various products they obviously do not represent the true composition of any single, pure component. It is, therefore, not proposed to publish these figures as a whole. There are, nevertheless, a few of the analyses which illustrate important points germane to this investigation. These are recorded below.

(i) *β-Histone from ox thymus glands*

Since the first isolation of a histone from the thymus glands of the calf (Lilienfeld 1894), much work has been carried out by many authors on its amino acid composition, particular attention being directed towards the basic amino acids. On the tacit assumption that the

material investigated was a pure substance, it has been generally recognized that this histone is characterized by a high content of arginine which, according to the older workers, accounts for about 25 % of its nitrogen content (cf. Felix & Rauch 1931). With the discovery (Stedman & Stedman 1950, 1951) that this and other histones, when extracted directly from isolated cell nuclei, are composite in character and contain basic proteins with a high content of lysine but a low one of arginine, the significance of the earlier analyses becomes uncertain. Stedman & Stedman (1951) found for their main histone from the thymus gland of the calf an arginine nitrogen content of about 30 % of the total nitrogen. But, as they emphasized at the time, the method of analysis which they used, while giving closely reproducible values, might give high ones because of the possible inclusion of a small percentage of nitrogen from sources other than arginine. Since that time, this method for the determination of arginine has been examined in detail in this laboratory by Purves (1953), who has found that it does, as was suspected, give high values. When the sources of error were eliminated, Purves found that the arginine nitrogen content of the actual main thymus histones used by Stedman & Stedman, as well as a new preparation which was electrophoretically homogeneous, fell to almost exactly 26 %. Since that time, the present investigation has shown that the main histones obtained by Stedman & Stedman, although in some cases electrophoretically homogeneous, consist of a mixture of β- and γ- histones. Of these, the β-component is, as shown by the sedimentation experiments already described, undoubtedly present in preponderating amount. It also represents the only component in ox thymus histone possessing a high arginine content, and must therefore have constituted the greater proportion of the material used for the analyses published in the older literature. Unfortunately, it has not so far proved possible to prepare a sample of this histone which forms, as judged by its behaviour on sedimentation, a monodisperse solution. All preparations, including those made in the preparative ultracentrifuge, exhibit the presence of a small amount of material which is either γ-histone or simulates the behaviour of this substance. In view of this difficulty, and pending its further elucidation, the amino acid analyses of an electrophoretically pure sample of thymus β-histone containing only a small amount of the presumed γ-component are reproduced in table 2, where they are compared with other products described below.

(ii) *γ-Histone from ox thymus glands*

Evidence has been presented on p. 116 which suggests that the γ-fraction from ox thymus histone contains two components with identical electrophoretic mobilities over the greater part of the pH range investigated. The values of their sedimentation constants differ, however, widely from one another, their S values being 1·6 and 0·8, respectively. Considerable support for these conclusions has been obtained by amino acid analyses of appropriate fractions.

The first of these analyses was made on the preparation of γ-histone separated by sedimentation from the $\beta+\gamma$-product (p. 116). In the analytical ultracentrifuge this resolved itself into two components, the major peak in the Schlieren pattern corresponding with $S=1{\cdot}6$ and the minor one, which represented only 18 % of the material, with $S=$ about 0·8. Thus, while this preparation probably contained the second γ- as well as some α-components, the bulk of it apparently consisted of the γ-histone with $S=1{\cdot}6$. Its amino acid

composition is shown in table 2. Unfortunately, its total nitrogen content is not known. The amino acid contents are therefore necessarily only expressed as percentages by weight.

A sample of the γ-histone with $S=0{\cdot}8$ was prepared by a different method. The original preparation of $\beta+\gamma$-histone used above was submitted to preparative electrophoresis at pH 7·4 and the extreme anode fraction collected separately. This procedure was designed to ensure the absence from the latter of any traces of α-components which remained in the $\beta+\gamma$ preparation. The anode fraction was then dialyzed and brought to about pH 8 by the careful addition of dilute sodium hydroxide. It had been hoped that, after allowing this solution to stand for several days to enable the β-component to undergo extensive aggregation, it would be possible to sediment the aggregated material in the ordinary laboratory

TABLE 2. AMINO ACID CONTENTS OF SOME HISTONES FROM THE THYMUS GLAND OF THE OX

	β-component (nitrogen = 17·0 %)		γ-component ($S=1{\cdot}6$)	γ-component ($S=0{\cdot}8$)
	% by weight	N as % of total N	% by weight	% by weight
histidine	2·7	4·4	2·6	1·8
lysine	10·7	12·1	15·0	17·9
arginine	14·9	28·2	8·8	7·3
aspartic acid	5·3	3·3	5·1	4·0
threonine	4·4	3·0	4·0	4·3
serine	3·4	2·7	4·4	4·3
glutamic acid	10·6	5·9	8·7	7·7
glycine	4·8	5·3	5·2	4·6
alanine	7·5	6·9	8·1	13·3
valine	5·1	3·6	5·1	4·4
methionine	1·6	0·9	0·9	0·4
isoleucine	4·9	3·1	3·6	2·8
leucine	9·9	6·2	7·9	7·2
tyrosine	4·5	2·1	3·9	2·5
phenylalanine	2·5	1·3	1·2	1·0

Note: Proline and amide nitrogen, although present, were not determined. Neither cysteine nor cystine was detected.

centrifuge, but this proved to be impossible. A small volume (2 %) of alcohol was therefore added, causing the precipitation of much of the histone. After removal of the precipitate, the addition of excess of alcohol to the mother liquors produced an intense opalescence which slowly transformed itself into a precipitate. This was collected and again submitted to the foregoing procedure, which resulted in the removal of a further small quantity of the presumed β-histone. When the product was examined in the analytical ultracentrifuge, 27 % of it sedimented rapidly and evidently consisted of aggregated material. The bulk of it, however, gave a symmetrical peak with $S=0{\cdot}75$, which was presumed to represent the pure γ-component. Amino acid analysis gave the results recorded in table 2.

The analyses of the above histone fractions, while they obviously do not give figures which represent accurately the amino acid composition of the actual components constituting the bulk of each fraction, nevertheless, do show the direction in which these components differ from one another in composition. The γ-histone with $S=0{\cdot}75$ possesses many of the features, e.g. high lysine and alanine and low arginine contents, which characterized the subsidiary histone obtained by Stedman & Stedman (1950, 1951) from ox

thymus histone during their original demonstration of the composite nature of histones in general. The γ-histone with $S=1{\cdot}6$ differs from β-histone in a similar manner although to a much less marked degree. It clearly resembles the latter more closely in composition than does the other γ-component.

(iii) *Total histones from rat liver and hepatoma*

As previously demonstrated, the histones extracted from the nuclei of both normal rat livers and rat hepatomas behave electrophoretically as pure substances. Analyses were therefore made of their basic amino acid contents with the results shown in table 3. It is clear that, in the unfractionated condition, these two histones show marked differences in their lysine and arginine contents, a result which was originally regarded as confirming the conclusion reached from a study of their physical properties, that they are different substances.

TABLE 3. BASIC AMINO ACID CONTENTS OF UNFRACTIONATED HISTONES FROM NORMAL RAT LIVERS AND RAT HEPATOMAS

	normal livers (N content of histone 15·1%)		hepatomas (N content of histone = 15·7%)	
	% by weight	N as % of total N	% by weight	N as % of total N
histidine	2·1, 2·1	3·8, 3·8	2·5, 2·3	4·3, 4·0
lysine	13·4, 13·1	17·1, 16·6	12·0, 11·9	14·7, 14·6
arginine	11·3, 11·5	24·2, 24·5	12·4, 12·2	25·4, 25·1

(iv) *β-Histones from rat liver and hepatoma*

The subsequent revelation by sedimentation experiments that, despite their electrophoretic homogeneity, both proteins contained considerable proportions of subsidiary histones necessitated a re-examination of their amino acid compositions. So far, it has been possible to do this only with the corresponding β-histones, which were prepared from the total histones by preparative ultracentrifugation. That from normal rat liver was obtained by one such treatment at pH 8·5. In the preparation of the β-histone from the hepatoma, two preparative runs were carried out at pH 8·5 and 7·5, respectively. The products from the two sources consist preponderatingly of β-histones, but it must be mentioned that both still contain a small proportion (less than 10%) of material sedimenting slowly in the ultracentrifuge. The amino acid compositions of these two preparations are recorded in table 4. It is clear from the results that the differences in amino acid composition present in the unfractionated histones have vanished from the purified material. The amino acid compositions of the two β-histones are, within the limits of experimental error, indistinguishable. The physical differences between them have, however, been preserved.

(v) *Ultra-violet absorption curves of histones*

During the course of this work measurements have been made of the ultra-violet absorption curves of many of the histones described above. In general, these are almost complete replicas of that for pure tyrosine. The chief deviation from the tyrosine curve which has been observed has been a greater apparent absorption at the shorter wavelengths

which increases progressively as the wavelength diminishes and varies in extent from preparation to preparation. Since this effect is correlated with the intensity of the Tyndall phenomenon, it is clearly caused by light scattering at the lower wavelengths. Typical results are shown in figures 103 and 104 which represent respectively the curves for main (i.e. principally β-) histones from ox thymus glands and the human leukaemic glands

TABLE 4. AMINO ACID CONTENTS OF β-HISTONES FROM NORMAL RAT LIVERS AND RAT HEPATOMAS

	normal (N content = 16·0 %)		hepatoma (N content = 15·7 %)	
	% by weight	N as % of total N	% by weight	N as % of total N
histidine	2·6	4·4	2·7	4·6
lysine	9·5	11·4	9·0	11·0
arginine	12·9	25·9	12·3	25·2
aspartic acid	5·9	3·9	6·2	4·2
threonine	4·9	3·5	4·8	3·5
serine	3·1	2·6	3·1	2·7
glutamic acid	10·1	6·0	10·5	6·3
glycine	5·4	6·3	5·4	6·4
alanine	7·2	7·1	6·8	6·8
valine	5·5	4·1	5·5	4·2
methionine	1·6	0·9	1·5	0·9
isoleucine	5·1	3·4	5·1	3·5
leucine	9·9	6·6	9·6	6·5
tyrosine	3·8	1·8	4·1	2·0
phenylalanine	2·7	1·4	2·6	1·4

Note: Proline and amide nitrogen, although present, were not determined. Neither cysteine nor cystine was detected.

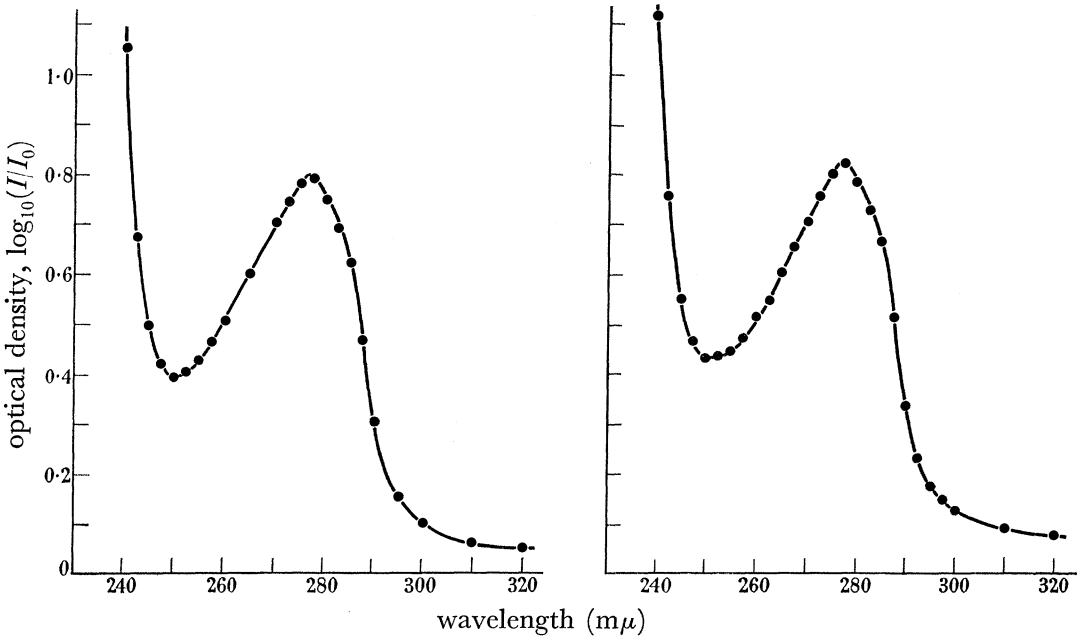


FIGURE 103. Ultra-violet absorption curve of a 0·30 % solution of the β-histone from ox thymus nuclei in 0·1 N sulphuric acid.

FIGURE 104. Ultra-violet absorption curve of a 0·30 % solution of the β-histone from human glands (case of lymphatic leukaemia) in 0·1 N sulphuric acid.

referred to earlier. These curves serve to illustrate those for β-histones in general. They also constitute another example of the manner in which the β-histone from the leukaemic glands resembles normal β-histones despite its marked difference from them in physical properties. In addition they demonstrate the absence of nucleic acid from our preparations of histones, which absence has also been shown by the zero values obtained in phosphorus determinations.

TABLE 5. HISTONE AND *DNA* CONTENT OF CELL NUCLEI

organ or tumour*	species	nucleic acid content (%)	yield of histone sulphate (%)
liver (12)	ox	28·5	21·5
liver	pig	26·5	19·4
liver	man (adult)	28·2	19·1
liver	man (child)	27·5	17·6
liver	rabbit	28·7	22·4
liver (7)	rat	29·4	22·0
liver	mouse	28·9	21·8
liver	dog	28·3	21·1
liver	chicken	26·9	26·0
liver	salmon	32·7	19·2
spleen	ox	34·3	26·1
spleen	pig	35·2	24·6
spleen (normal)	man	36·8	—
spleen (lymphatic leukaemia)	man	29·8	28·2
spleen	rat	33·1	23·5
spleen (normal)	mouse	35·8	28·2
spleen (line I)	mouse	28·7	—
spleen	chicken	31·2	26·0
thymus (25)	ox	35·4	25·0
thymus	man	37·0	27·0
erythrocyte	chicken	33·9	26·0
erythrocyte	salmon	39·6	18·5
sperm	cod	—	23·7
hepatoma (5)	rat	25·5	16·8
Rd/3 sarcoma	rat	24·5	20·5
lymphatic leukaemia	chicken	30·4	12·1
blood (lymphatic leukaemia)	man	36·0	25·0
blood (myeloid leukaemia)	man	32·1	19·4
breast tumour	man	19·6	4·0
bronchogenic carcinoma	man	30·7	20·4
glands (lymphatic leukaemia)	man	30·0	20·1
Undifferentiated tumour	man	24·5	20·0

* Figures in parenthesis represent the number of preparations included in those cases where average figures are given.

(2) *Histone and* DNA *content of cell nuclei*

The foregoing work has necessitated the preparation of cell nuclei from a number of different types of tissue. Many of the preparations have been analyzed for phosphorus, while the yield of histone sulphate obtained by exhaustively extracting them with dilute sulphuric acid has also been determined. In recording these results in table 5, the contents of phosphorus have not been shown as such, but have been converted into nucleic acid contents on the assumption that the nucleic acid contains 10·0% of phosphorus. These results necessarily give the sum of deoxyribonucleic and ribonucleic acid contents. As shown, however, by Mauritzen *et al.* (1952), the content of the latter is almost negligible so that the values quoted refer essentially to deoxyribonucleic acid. In several instances in which a number of preparations of nuclei have been made from the same tissue and species,

average results have been recorded in place of individual ones. None of the latter differed, however, from the average value by more than $\pm 2{\cdot}5\,\%$, which indicates that the method for the preparation of nuclei used in this laboratory yields a uniform product for a particular tissue. Taken in conjunction with the demonstration (Stedman & Stedman 1951) that little is lost from such nuclei during the process of isolation, the values in table 5 must also represent essentially the composition of the nuclei as present in the tissue despite the views held in some quarters (see, for example, Dounce 1951, 1955) that the nucleic content is considerably smaller.

In addition to demonstrating the constancy in composition of nuclei isolated from a particular tissue, these new analyses confirm the finding, first made by Stedman & Stedman (1947*b*), that the nuclei from corresponding cells of different species of mammals contain, within the limits of experimental error, identical nucleic acid and histone contents. This is evident when the results obtained with the various preparations of liver or lymphocyte nuclei are compared with one another. Whether or no this identity extends to species of different classes is not certain. The nuclei from the liver of the salmon appear to contain a higher nucleic acid and a lower histone content, but these nuclei may be somewhat abnormal for they were obtained from fish which had returned to the river to spawn. It is possible that during their sojourn in the river, when they are reputed to fast despite the active protein synthesis necessitated by the formation of germ cells, there is a fall in the histone content of the nuclei with a corresponding rise in that of the nucleic acid.

V. DISCUSSION

The application in the foregoing investigation of physicochemical methods to the analysis of histones isolated from a variety of cell nuclei has shown that these basic proteins are, in general, much more complex than appeared from the chemical fractionation procedures used in the earlier work carried out in this laboratory. Simple electrophoretic analysis of these unfractionated histones usually suffices to indicate their heterogeneity, although some exceptions to this general rule have been encountered. When analyzed in this way, many of the histones examined have yielded a schlieren pattern which indicates the presence in the unfractionated material of at least three components. It was on the basis of this observation that the system of nomenclature in use with other proteins, in which the various components are named with the letters of the Greek alphabet in descending order of their electrophoretic mobilities, was applied to histones (Cruft *et al.* 1954). The three components indicated by the above-mentioned schlieren patterns have accordingly been named α-, β- and γ-histones or components, the α-component possessing the greatest and the γ- the smallest electrophoretic mobility in the pH range around neutrality.

Before we discuss the extension of this system of nomenclature which has since become necessary, some justification for classifying as histones the subsidiary or α- and γ-components should, perhaps, be given, for these might otherwise be considered to be fortuitous contaminants of the true histones. Unfortunately there is no agreed definition of a histone. The term was originally applied (Kossel 1884) to the basic protein extracted from the nuclear material obtained from goose erythrocytes, with the object of distinguishing it from the simple basic proteins, known as protamins, present in the sperm heads of the salmon and herring. This histone differed from the two protamins in possessing a more complex

amino acid composition. When, later, similar basic proteins were shown to be present in the thymus gland of the calf, in the sperm heads of certain fishes and in the nuclei from nucleated erythrocytes of different origin, the term histone was also applied to them. It seems clear that any basic protein present in cell nuclei which was more complex than a protamin was regarded by the early workers on the subject as a histone. But two *ad hoc* committees (1907, 1908), appointed in 1907 to formulate a classification of the proteins as a whole gave, on the information then available to them, a definition of histones which has induced subsequent writers to include globins among this group of proteins, a procedure which is frequently followed in modern textbooks. Parenthetically, it might be mentioned that this practice has not been extended to more-recently discovered basic proteins such as ribonuclease and lysozyme. There are now, however, valid objections to this course, for globins are known to bear little relation to histones chemically and none physiologically. It is true that they do resemble histones in being basic proteins, but even here there is a wide difference between the two, for whereas globins are practically neutral with isoelectric points at a pH of about 7·5, histones are strongly basic. The β-histone from the thymus gland of the ox, for example, has an isoelectric point at pH 11·0, while those from other sources give figures grouped round this value (table 1). Such basic properties as globins possess depend, moreover, largely on a high content of histidine, whereas those of histones are due mainly to the large amounts of arginine and lysine which they contain, their content of histidine being small. Again, histones are characterized by the complete absence of tryptophan from their amino acid composition (cf. Stedman & Stedman 1947*b*), while globin contains this amino acid as an integral part of its molecule. The chemical differences between the two types of proteins are thus so wide that their classification together in the light of present knowledge seems to be quite unwarranted. A comparison of their physiological functions leads *a fortiori* to the same conclusion. Globin occurs as a co-ordination compound with haem and functions in this form as a respiratory pigment. Histones, on the other hand, are found in salt-like combination with nucleic acid in the cell nucleus, where they presumably participate in some way in the genetic control of the cell.

In view of this lack of precision in the definition of a histone, it is proposed that this term should be reserved exclusively for basic proteins, other than protamins, which occur in the cell nucleus. It is, perhaps, unfortunate that protamins should be excepted from this definition for, as pointed out by Stedman & Stedman (1947*b*), protamins and histones are homologous proteins. Moreover, there is at present no clear line of demarcation between them. But the name protamin was given to the first basic protein (salmine) to be isolated from a cell nucleus (Miescher 1897) and should on that account be preserved, especially as this particular protamin differs from histones chemically in containing no aromatic amino acids. Some other protamins, e.g. clupeine from the sperm heads of the herring, share this property with salmine; others do not, but this may be due to the impure nature of the specimens hitherto examined. It would, therefore, be advantageous and give precision to the term if protamins were defined as the basic proteins of cell nuclei which differed from histones in containing no aromatic amino acids. This would make clear the chemical differences between the two types of protein without materially changing existing nomenclature, and without suggesting that the difference between them was more than an extreme example of the species specificity which occurs among homologous proteins. The

few basic proteins which have been termed protamins, but which do not appear to conform to the above definition of a protamin require further investigation, the results of which will doubtless ultimately decide whether they are to be classed as protamins or histones.

These considerations tacitly formed the basis of the decision which Stedman & Stedman (1950, 1951) made to extend the term histone to all the components of the basic proteins extracted from isolated cell nuclei, a decision which was expressed in the words: 'All the components are basic proteins, and we therefore propose, at any rate provisionally, to describe them all as histones, the component present in preponderating amount being termed the main, and the others the subsidiary histones'. It was, nevertheless, realized that some of the latter differed from the traditional histone of the cell nucleus in one important respect; their arginine contents were no greater than those of non-basic proteins, the basic properties which they exhibited being due to a high content of lysine. That this was the case was directly demonstrated for a subsidiary histone obtained from ox thymocytes, a result which has been confirmed by the authors quoted in the introduction, and indirect evidence was also provided that the same held for the subsidiary histones present in many other cells. But this difference does not constitute a valid reason for excluding these proteins from the group of histones for, as Stedman & Stedman demonstrated in their original work, there is present in the nuclei from the erythrocytes of the fowl a subsidiary histone which, while resembling that from the thymus gland of the ox in physical properties, nevertheless, contains a greater proportion of arginine than the main histone from which it was separated. The latter, it should be added, possesses the physical and chemical properties of a traditional histone. The designation of the subsidiary basic proteins of cell nuclei as histones is thus justified on the grounds of their pronounced basic properties, which has been confirmed in the preceding pages by the determination of the mobility curves of many of them; of their occurrence in cell nuclei; and especially of their presence in cell nuclei of many different types and from many different species.

The study, made as part of the present investigation, of the physical properties of some of these subsidiary histones suggests that the solutions which their salts form in water may approach the border line between true and colloidal solutions. However this may be, they do not appear to dialyze through cellophane membranes, although some evidence has been obtained that one or more of them will pass very slowly through exceedingly permeable collodion membranes.

An extension of the system of nomenclature used to designate the various components of the histones extracted from cell nuclei was rendered necessary by the results obtained in the examination of the electrophoretic behaviour of a wider range of histones, and in the more detailed study of the various fractions obtained from the histones from the thymus glands and livers of the ox by either chemical methods or ultracentrifugation or a combination of both. When the histones from many species and types of cell were examined electrophoretically it was found that in some cases the α-component resolved itself after a certain length of time into two components. The α-fraction, prepared by sedimentation from ox liver histone, similarly resolved itself into four components, one of which however, was identified by its mobility as a γ-component. It was evident from results of this kind that the original α-component was, in fact, itself composite and should be termed an α-fraction. The individual components of this fraction have accordingly, again following convention,

been distinguished by the use of suffixes, the three α-components in ox thymus histone, for example, being described in descending order of their mobilities, as the α_1- α_2- and α_3-components. The adoption of this expedient rather than using the alternative method of re-lettering the components in the order of their electrophoretic mobilities has been based on the demonstration that the α-components form a group of similar proteins, all the members of which are sharply distinguished both in their physical properties and chemical composition from the β-components. It thus ensures that the designation of a component as a β-histone implies that it possesses the characteristic properties of such histones the most marked of which is the ability which they possess to undergo progressive aggregation as the pH of their solutions is raised. This property is totally absent from subsidiary histones. There is considerable evidence that the γ-component in ox thymus histone is also composite, consisting of two components with identical electrophoretic mobilities over a wide range of pH, but differing in their sedimentation constants. No separate symbols have, however, been introduced for these two γ-components pending a final solution of the problem which they offer.

The schlieren patterns obtained by the electrophoresis of unfractionated histones do not present an absolutely uniform picture, but vary to some extent according to the species from which they originated. The majority show the presence of an α-fraction together with the β- and γ-components. This happens with the histones from the erythrocytes, liver and spleen of the fowl; the thymocytes, liver and spleen of the ox; the liver and spleen of the pig; the liver of the rabbit; and the liver of man. There is also indirect evidence that the same components are present in the histones from chicken thymocytes and cod sperm. The peaks of the α-fraction in the schlieren patterns of the unfractionated histones rarely, if ever, exhibit the sharpness which would be expected for a pure component. This was at first attributed to a combination of the effects of the low concentration in which it was present, and of its small molecular weight and consequent greater diffusibility. It is now known that it is caused by its composite nature. Apart from other evidence of this, the patterns obtained with the unfractionated histones from fowl liver and spleen and rat spleen and kidney do, in fact, show the presence of two α-components. Two such components are also visible in the soluble fraction from the histone from cod sperm. Sometimes, however, even when two α-components appear quite distinctly, as in the cases of the total histones from rat spleens and kidneys, no γ-fraction is also discernable with certainty. A few histones, namely, those from human thymocytes, children's livers, rat livers and mouse spleens and livers, have, moreover, been encountered which virtually behave as electrophoretically pure substances. There may be, as has already been suggested, some special explanation of this in the cases of the two human histones. None can be offered in that of rat livers, for an α-fraction containing at least two components can readily be separated from this histone by sedimentation in the ultracentrifuge.

In the physico-chemical analysis of histones, the Tiselius apparatus and the ultracentrifuge have, indeed, proved to be essential complementary instruments. Unfortunately, the latter has only been available to us during the later stages of this investigation; its use has therefore been restricted to the examination of a few only of the histones investigated electrophoretically. The histone from rat livers mentioned in the preceding paragraph serves to illustrate its use. When analyzed in the ultracentrifuge this histone,

although electrophoretically homogeneous, gave a schlieren pattern which indicated the presence of two components with widely different S values, the slowly sedimenting one consisting of an α-fraction, the other being a β-component. When the former was analyzed electrophoretically it resolved itself into two components which were classified as α_1- and α_2-components on the basis of their mobilities. The number and kind of components contained in the histone from normal rat liver thus fall into line with those shown to be present in the products from the spleens and kidneys by direct electrophoretic analysis. It is noteworthy that the histones from this particular species differ from those of other species in giving no indication in the electrophoretic pattern of the presence of a γ-component. The electrophoretic evidence, so far as it goes, suggests that the histones from mice share this characteristic, but these have not so far been investigated in sufficient detail to be certain of this.

But it is perhaps in the unravelling of the complex nature of the histones from the thymocytes and liver cells of the ox that the ultracentrifuge has so far given most service. In combination with electrophoresis and chemical methods of fractionation it has been possible with its aid to demonstrate the presence of at least five components in these histones. Of these, three are α-histones which, corresponding with their electrophoretic mobilities can be described as α_1-, α_2- and α_3-components; these all possess S values of about 0·8. The fourth is a γ-component, also with an S value of about 0·8, while the fifth represents the β-component. In addition there is a component with the same electrophoretic mobility as the γ-component (with $S \simeq 0{\cdot}8$) mentioned above but with an S value of about 1·7. All the evidence so far obtained and presented in the experimental section suggests that this is a second γ-component, but a final decision on this point must be reserved because of the remote possibility that it may be an artifact.

From analyses made by Stedman & Stedman (1950, 1951) of the arginine contents of a number of main histones, now known to consist essentially of β-histones, and from further analyses reported in this communication, it follows that the β-histones are distinguished from the other components of the unfractionated histones by their high content of arginine and, in this respect, resemble the traditional histones. This distinction does not, however, hold universally, for, as pointed out above, there is present in the histone from fowl erythrocytes a subsidiary histone which contains a greater proportion of arginine than does the β-histone from the same source. This is probably an exceptional case. Nevertheless, in view of its existence, as well as for other reasons, it is useful to note that the β-histones are sharply distinguished from the subsidiary histones in certain of their physico-chemical properties. The first indication of this distinction arose from measurements of the diffusion coefficients. Using preparations of ox thymus and ox liver β-histones which were electrophoretically pure but nevertheless contained, as was subsequently demonstrated, a certain proportion of γ-component, the diffusion coefficients were found to diminish progressively with increase in the pH of the solution in which the measurements were being made, a phenomenon which obviously indicated that the molecules of the β-histones underwent aggregation to an increasing extent as the pH of their environment was raised. On the other hand, and α-fraction of ox thymus histone containing the α_1-, α_2- and α_3-components, and probably a small amount of the 0·8S γ-component gave diffusion coefficients which were identical at pH's 3·1 and 6·8. The latter diffusion patterns were, moreover, closely

Gaussian in shape. It is legitimate to conclude from these results that the components of the α-fraction all consist of particles of about the same weight and that none of them possesses any tendency to aggregate. These results have been completely confirmed, as reference to the experimental part of this paper will show, by sedimentation studies. This ability to aggregate progressively as the alkalinity of the solution is increased in the direction of the isoelectric point is thus a characteristic property of β-histones and one which differentiates them sharply from subsidiary histones. Provisional experiments, not recorded in this paper, also show that the axial ratios of β-histones, determined from the viscosity increment, are much greater than those of subsidiary ones.

The various electrophoretic mobility curves which have been determined in this work serve, not only to characterize the different proteins to which they refer, but also to demonstrate that all of the components present in the acid extracts of isolated cell nuclei are strongly basic proteins, and can therefore be legitimately called histones in accordance with the nomenclature which has been proposed above. The curves for normal β-histones do not differ extensively from one another although relatively small differences in their position, sometimes made more precise by a difference in the position of the isoelectric point, can be observed in the curves from those obtained from different species or cells. Perhaps the greatest species difference is that exhibited by the β-histones from the livers of the ox and rat, respectively.

All the curves, however, agree in showing no point of inflexion between the pH's of 3·5 and 5·0. Such an inflexion would be expected on the grounds that β-histones contain, despite their strongly basic character, rather large amounts of aspartic and glutamic acids, which undergo ionization between the above pH values. It must therefore be concluded that these two amino acids are largely present in the β-histones, not as the free acids, but as asparagine and glutamine. Such a conclusion is supported by the high values for 'amide' nitrogen given by the main histones analyzed by Stedman & Stedman (1951).

The principal electrophoretic component of wheat germ histone does, however, show a point of inflexion indicating the presence of free carboxyl groups. In this respect it differs from animal β-histones as it also does in its inability to aggregate when the pH is increased. It more nearly resembles the γ ($S \simeq 1{\cdot}6$) component which has been isolated from ox thymus histone and which also shows a point of inflexion between pH 3·5 and 4·5. Wheat germ histones are thus sharply distinguished from the animal β-histones.

During the course of the work discussed above, some attention has been devoted to the question of the cell specificity of histones. This problem is of considerable importance for, if it were established that such specificity is a general phenomenon, it would go far to show that these basic proteins play some part in the differentiation of cells and in the maintenance of their characters. That some histones are cell-specific seems to be quite certain, particularly if protamins, although differing from histones in some of their chemical and physical properties, are assumed for this purpose to be equivalent physiologically to histones. That this assumption is justified follows from the fact, pointed out by Stedman & Stedman (1947*b*), that the protamins of the sperm heads of the salmon and herring take the place of the histone in cod sperm. The two substances are thus evidently homologous proteins in much the same way as are the respiratory pigments haemoglobin and chlorocruorin, despite the fact that this relation is obscured because the members of each pair of

homologues have been differently named in order to emphasize the differences in their chemical and physical properties. If this argument is accepted, it becomes clear that the first and most striking example of cell specificity among the basic proteins of cell nuclei is that which exists between those present in the sperm head and the somatic cells of the salmon for, while the sperm head of this species contains a protamin, the nuclei of the somatic cells, in particular the erythrocytes and liver cells, contain histones in place of the protamin of the sperm head (Stedman & Stedman 1944). There is also evidence that the sperm mother cells, either spermatogonia or spermatocytes or more probably both, also contain histones. In addition to this example of cell specificity, the same material provided evidence that the histones present in the erythrocytes and liver cells were also cell-specific, for their arginine contents differed widely from one another. It is true that this work was carried out before the discovery of subsidiary histones, which might well have been present in different proportions in the unfractionated histones which were examined. Subsequent work (Stedman & Stedman 1951) has, indeed, shown that the histone from the liver cells of the salmon does contain one or more subsidiary histones with a low content of arginine. But in view of the wide difference between the arginine contents of the two unfractionated histones it is improbable that the findings can be explained in this way. By comparison of the erythrocytes and thymocytes of the fowl, further examples of the cell specificity of histones have been found, for the main (i.e. β-) histones from these two types of cells differ appreciably in their arginine contents. Moreover, there is present in the nuclei of the erythrocytes, but not of the thymocytes, a subsidiary histone with an arginine content considerably higher than that of the β-histone. This subsidiary histone is clearly specific to the nuclei of the erythrocytes. A more recent confirmation of cell specificity is the discovery by Daly & Mirsky (1951) of a protamin (gallin) in the sperm heads of the fowl. Despite these examples, it has not hitherto been possible to demonstrate any difference between the β-histones from ox thymocytes and liver cells. In the absence of such a demonstration it is clear that the phenomenon in question cannot be asserted to be a general one, a consequence which may seem to diminish its importance. Considerable efforts have accordingly been made during the past 5 years to examine these two β-histones from a physical rather than a chemical standpoint in the hope that this would lead to a solution of the problem. The first approach of this kind was to determine the electrophoretic mobilities and subsequently the mobility-pH curves of these histones. This work led to the curves reproduced in figure 43, from which it is evident that, despite the large number of experiments which were made and despite the use of a number of entirely different preparations for the determinations, the two histones consistently show a difference in their mobilities throughout the extensive pH range examined. This might, and in fact to us did, seem to be conclusive, for the difference between the mobilities of the two histones, while small, is quite outside the range of experimental error. Nevertheless, it seemed desirable to obtain confirmation of the results, particularly as they themselves suggested an elegant method for doing so, namely by analyzing a mixture of approximately equal amounts of the two β-histones electrophoretically. According to the mobility curves mentioned above, the difference between the mobilities of the two proteins are sufficiently great for two peaks to appear in the schlieren pattern of such a mixture after a few hours electrophoresis. This experiment was performed several times, but on no occasion did two peaks appear in the

pattern. Apart from an apparent broadening of the peak as compared with that given by the single histone, the pattern simulated that of a homogeneous protein. With many types of proteins such a result could have been regarded as conclusively demonstrating the identity of the two components of the mixture. It does not do so, however, with the histones involved, for in view of their ability, mentioned above during the discussion of the diffusion experiments, to undergo extensive aggregation it is conceivable, indeed probable, that two such closely similar histones would form mixed aggregates in solution which would migrate as a single component in the electric field. By preventing, or minimizing, the formation of such aggregates, an end which was effected by dialyzing the separate solutions of the two histones against the same buffer and mixing the dialyzed solutions immediately before their introduction into the Tiselius apparatus, a pattern was obtained which indicated the separation of the two histones. This result appeared to be conclusive, particularly as a control experiment in which two different preparations of ox thymus histone were dialyzed together and separately under the above conditions failed in both cases to give evidence of the presence of more than one component when examined electrophoretically.

The preparations of histones used in these experiments, although electrophoretically homogeneous, have since given evidence of heterogeneity in the ultracentrifuge. Great pains have therefore been taken to prepare pure samples of the two β-histones by preparative sedimentation in the ultracentrifuge. Unfortunately, assessment of the purity of such preparations is at present impossible owing to the uncertainty of the effects produced by the aggregation phenomena exhibited by these histones. All of the preparations are electrophoretically homogeneous, but even the best shows in the analytical ultracentrifuge a small amount of material of relatively low molecular weight. It is difficult to decide what this represents. It may consist merely of unaggregated β-histone; on the other hand, it is not excluded that it represents a γ-histone. Similarly, it is possible that the aggregates formed by β-histones may include a certain quantity of a subsidiary histone. However this may be, the best preparations of ox thymus and liver β-histones obtained with the preparative ultracentrifuge behave during analytical electrophoresis like the earlier less pure preparations in so far as they yield patterns exhibiting two peaks when the formation of mixed aggregates has been prevented. This result would again appear to be conclusive, were it not that some control experiments, judged to be unnecessary when they were performed but carried out as a matter of routine, have given results which suggest the intervention of some factor additional to, but possibly associated with, aggregation. In these control experiments the highly purified β-histone was mixed before and after dialysis, as in the earlier experiments, with a sample of the same material which has been prepared by a combination of chemical fractionation and preparative electrophoresis. The latter was electrophoretically pure but, as judged by analysis in the ultracentrifuge, contained a greater amount of the fraction sedimenting slowly than did the first sample. When a mixture of these two samples of β-histone, made after they had been dialyzed separately against the same buffer, was submitted to electrophoretic analysis two peaks appeared in the schlieren pattern. The result has been confirmed, not only with the same material, but also with both liver and thymus histones. It is our opinion that the conclusion drawn from the experiments with the highly purified β-histones, particularly as it is supported by the mobility curves of these two histones made with less pure material, is still valid, although

this opinion cannot be held so firmly as would have been the case if the control experiments had given the anticipated result. The unexpected outcome of the control experiments is probably associated with the great ability of the β-histones to aggregate. As an explanation of the effect, it is postulated that these aggregates may include subsidiary histones with the consequence that two preparations of the same β-histone containing different proportions of a subsidiary histone will form aggregates of different composition which will migrate at different rates in the electric field. The subsidiary histone involved is almost certainly the γ-component with $S \simeq 1{\cdot}6$ for this is exceedingly difficult to separate completely from a β-histone by any means.

The problem of the cell specificity of histones possesses an intrinsic interest of its own. An added stimulus to its investigation is provided by the knowledge that its establishment as a general phenomenon would carry with it the corollary that malignant cells differ from the cells of their origin in the nature of the histone contained in their nuclei. Such a result would not necessarily imply that the malignancy of a cell is caused by a change in the histone which it contained, although it would suggest, in terms of the hypothesis which forms the basis of this aspect of our work, that the two changes are related to one another and perhaps proceed *pari passu*.

The examination of histones from normal tissues, which has been discussed above, has accordingly been accompanied by the investigation of those from a number of tumour tissues. This has not resulted in the discovery of any special feature in the amino acid composition of the histones from malignant tissue. As far as they have been examined they resemble normal histones in this respect. Nor is it apparent that they differ from the latter in the components which they contain. It is true that the histones from experimental tumours as well as some of those from natural ones appear to be electrophoretically homogeneous β-histones, but there are others in which the usual α- and γ-components are present. Moreover, as has been found with normal histones, the presence of subsidiary histones is not always revealed by simple electrophoretic analysis. This has been demonstrated for the histone from rat livers; a similar result has been obtained with that from rat hepatomas. It may thus well be that, despite their electrophoretic homogeneity, these apparently pure β-histones do contain subsidiary histones the presence of which will only be detected when it is possible to submit them to analysis in the ultracentrifuge.

Despite their general resemblance to normal β-histones, those from tumours have usually been found to be characterized by an abnormally low solubility, a feature which is associated with an anomalous mobility-pH curve, and a greatly intensified capacity to form aggregates as the pH of the medium is raised, a property which manifests itself in both diffusion and sedimentation experiments. The insolubility of the β-histone was sometimes so great that only a few points on the mobility curve could be measured, and even to obtain these it was necessary to use concentrations which were much smaller than those employed with normal β-histones. Because of its insolubility, only one measurement at pH 4·3 could be made with the histone from a human bronchogenic carcinoma. That from the human glands (lymphatic leukaemia) was slightly more soluble with the result that, using low concentrations, it was possible to determine the greater part of the mobility curve on the acid side of neutrality. The majority of the remaining β-histones obtained from tumours, whether natural or experimental, behaved similarly although to a slightly less marked

degree. There were, however, two exceptions among the human tumours. The histone isolated from a human tumour, termed an undifferentiated carcinoma because the tissue of its origin could not be ascertained, showed none of the above abnormalities. Nor did the histone isolated from the spleen derived from the above case of lymphatic leukaemia. This was surprising, for the spleen contained large numbers of small lymphocytes which were indistinguishable histologically from those in the glands. Nevertheless, despite the absence of these abnormalities, others were present. Over a wide range of pH the β-histone possessed abnormally high mobilities. Moreover, on the alkaline, but not on the acid, side of neutrality the peak of this component in the electrophoretic pattern consistently separated into two peaks. This phenomenon has never been observed with any other β-histone which we have examined. Nor would it be expected to occur, for when two β-histones such as those from ox thymocytes and liver cells, or from rat livers and hepatoma tissue are mixed and dialyzed together they form mixed aggregates and then behave as a single component. It is thus evident that, while the β-histone from this spleen does not possess the identical abnormalities found in other histones derived from malignant cells, it nevertheless does behave as an abnormal histone in other respects.

No less striking than the differences in solubility exhibited by the β-histones from normal and malignant tissue is the contrast between their mobility curves. Those for normal histones, while showing minor variations according to the species or cell from which they originate, form a group of closely similar curves. On the other hand, with the two exceptions mentioned above, the mobility curves of the histones isolated from malignant cells not only differ considerably from one another but also diverge widely in general shape from the normal curves. The differences are due to the much greater diminution in the mobilities of the abnormal histones as the pH is increased, a difference which appears to be associated with both their greater capacity for forming aggregates and their smaller solubility. It is, in fact, at the pH at which the concentration has to be reduced below the 0·5 % normally used for electrophoresis that this abnormally low mobility becomes very apparent. Because of the sparing solubility of the β-histone from the bronchogenic carcinoma, no mobilities could be measured for this protein at pH's above 4·3; it mobility curve could not therefore be determined. With that from human glands (lymphatic leukaemia), however, measurements were made up to pH 6. As is evident from figure 87 there is a sharp drop in mobility from pH 5·0 to 6·3 the curve taking a course which suggests that it would cross the abscissa at pH 7·0. With the other histones isolated from tumours the drop in the curve is less marked although quite pronounced.

Special comment should perhaps here be made about the rat hepatoma, for this is the single example in which it has been possible to compare the histone isolated from malignant tissue directly with that from the corresponding normal tissue. Both histones, without fractionation of any kind, were electrophoretically pure. Their basic amino acid contents were therefore determined; these proved (table 3) to differ significantly from one another. Subsequent examination of the histones in the ultracentrifuge indicated, however, the presence of subsidiary histones in both products. These consisted, in each case, of α_1- and α_2-components. The two histones thus resembled one another and differed from histones from many other species in containing no γ-components. After removal of the subsidiary histones in the preparative ultracentrifuge, the β-histones were again analyzed, this time

for practically all of their amino acids. The results, given in table 4, show that the former difference had disappeared with fractionation, for the two sets of analyses were, within the limits of experimental error, indistinguishable. Nevertheless, the two β-histones still differed in their physical properties. Their respective electrophoretic mobilities at pH's 5·03 and 5·70 were: liver 0·75, 0·71; hepatoma 0·69, 0·66 $\mu s^{-1} V^{-1} cm^{-1}$, thus retaining a difference of the same magnitude as that exhibited in the unfractionated material. Both had diminished in solubility, but, as far as could be judged by their qualitative behaviour, their original relative solubilities had been preserved. Although both gave identical S values of about 15 at pH's below 4·0, a value which is higher than that given by the β-histones from the ox, the values of 35·0 and 200, respectively, were obtained for the liver and hepatoma histones at pH 7·72.

These results indicate that the β-histones from tumours differ, usually in a characteristic manner, from those from normal tissue by possessing widely different physical properties. The cause of this difference has not yet been ascertained. It does not seem to be due to any marked variations in amino acid composition, although the possibility that some small variations exist is not entirely excluded. It must therefore be presumed that is is due mainly to a change in the internal structure of the histones in the tumours. Whatever the cause, however, the results conform to the view that β-histones are cell-specific.

A discussion of the bearing of these findings on the mechanism of the genesis of tumours must necessarily be somewhat speculative. Nevertheless, there is one result which suggests that the change in the nature of the histone is, as would be expected from its postulated physiological function, a secondary feature of the process. When hepatomas are induced in rats by feeding these animals with an azo dye the histone of the liver cells neither combines with the dye nor undergoes any other detectable change during the period of its administration, although some dye does combine with another component of the nucleus. With the cessation of its ingestion the dye disappears from the nuclei and the liver cells are slowly replaced by hepatoma cells in which the histone differs from that of the normal liver cells. The sequence of these events clearly corresponds with the suggestion which has long been current, that carcinogenesis is the result of a 'somatic mutation'. The dye presumably combines with one or more genes thereby inhibiting or modifying their properties. As a consequence the character of the cell is changed, proliferation takes place and the histone synthesized in the resulting cells conforms to and maintains their new genetic constitution.

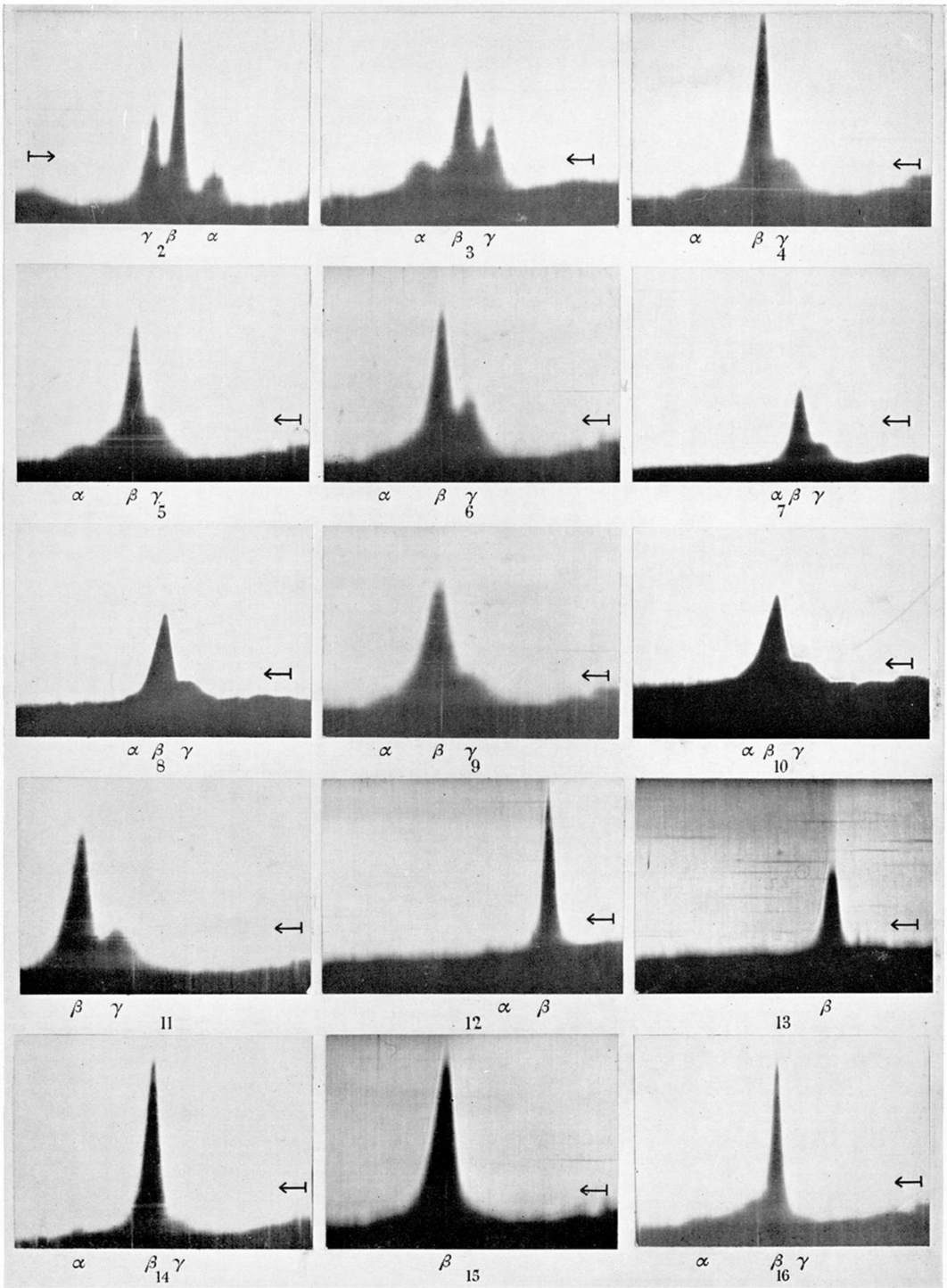
We gratefully acknowledge financial assistance from the Scottish Hospital Endowments Research Trust, the Melville Trust and the Court of Edinburgh University, without which this investigation could not have been undertaken.

We are indebted to the following for supplies of human and animal neoplasms: Dr Helen Russell of the Christie Hospital and Holt Radium Institute; Professor A. Haddow of the Chester Beatty Cancer Research Institute; Dr J. G. Campbell of the Poultry Research Centre, Edinburgh; Professor Sir Stanley Davidson of the Department of Medicine, Edinburgh University; and the members of the Pathology Department of Edinburgh Royal Infirmary.

We also thank Dr J. W. Minnis for micro-weighings and Miss Margaret Rollo, Miss Wilma Sommerville and Mr D. Herkes for skilled technical assistance.

VI. REFERENCES

Allen, R. J. L. 1940 *Biochem. J.* **34**, 858.
Committee on Proteid Nomenclature. 1907 *J. Physiol.* **35**, xvii.
Committee on Proteid Nomenclature. 1908 *Amer. J. Physiol.* **21**, xxvii.
Crampton, C. F., Moore, S. & Stein, W. H. 1955 *J. Biol. Chem.* **215**, 787.
Cruft, H. J. 1953 Ph.D. Thesis, University of Edinburgh.
Cruft, H. J., Mauritzen, C. M. & Stedman, E. 1954 *Nature, Lond.* **174**, 580.
Daly, M. M., Mirsky, A. E. & Ris, H. 1951 *J. Gen. Physiol.* **34**, 439.
Daly, M. M. & Mirsky, A. E. 1955 *J. Gen. Physiol.* **38**, 405.
Davison, P. F. & Butler, J. A. V. 1954 *Biochim. biophys. Acta*, **15**, 439.
Davison, P. F., James, D. W. J., Shooter, K. V. & Butler, J. A. V. 1954 *Biochim. biophys. Acta*, **15**, 415.
Dounce, A. L. 1951 In Summer & Myrback, *The enzymes* **1**, 222. New York: Academic Press.
Dounce, A. L. 1955 In Chargaff & Davidson *The nucleic acids*, **2**, 93. New York: Academic Press.
Edsall, J. T. 1953 In Neurath & Bailey, *The proteins* **1**, *B*, 646. New York: Academic Press.
Felix, K. & Rauch, H. 1931 *Hoppe-Seyl. Z.* **200**, 27.
Geddes, A. L. 1945 In Weissberger, *Technique of organic chemistry, physical methods* **1**, 300. New York: Interscience.
Gregoire, J. & Limozin, M. 1954 *Bull. Soc. Chim. Biol. Paris*, **36**, 15.
Kegeles, G. & Gutter, F. J. 1951 *J. Amer. Chem. Soc.* **73**, 3770.
Kossel, A. 1884 *Hoppe-Seyl. Z.* **8**, 511.
Lilienfeld, L. 1894 *Hoppe Seyl. Z.* **18**, 473.
Mauritzen, C. M., Roy, A. B. & Stedman, E. 1952 *Proc. Roy. Soc.* B, **140**, 18.
Michaelis, L. 1931 *Biochim. Z.* **234**, 139.
Miescher, F. 1897 *Die histochemischen und physiologischen Arbeiten* **1**, **2**. Leipzig: Vogel.
Miller, E. C. & Miller, J. A. 1947 *Cancer Res.* **7**, 468.
Miller, J. A. & Miller, E. C. 1953 *Advances in cancer research* **1**, 339. New York: Academic Press.
Miller, E. C., Miller, J. A., Sapp, R. W. & Weber, G. M. 1949 *Cancer Res.* **9**, 336.
Moore, S. & Stein, W. H. 1954 *J. Biol. Chem.* **211**, 893.
Philpot, J. St. L. 1938 *Nature, Lond.* **141**, 283.
Price, J. M., Miller, E. C. & Miller, J. A. 1948 *J. Biol. Chem.* **173**, 345.
Purves, D. 1953 Ph.D. Thesis, University of Edinburgh.
Schweigert, B. S. 1949 *Vitamins and hormones*, **6**, 56. New York: Academic Press.
Stedman, E. & Stedman, E. 1944 *Biochem. J.* **38**, xxvi.
Stedman, E. & Stedman, E. 1947*a* *Symp. Soc. Exp. Biol.* **1**, 232.
Stedman, E. & Stedman, E. 1947*b* *Cold Spr. Harb. Symp. Quant. Biol.* **12**, 224.
Stedman, E. & Stedman, E. 1950 *Nature, Lond.* **166**, 780.
Stedman, E. & Stedman, E. 1951 *Phil. Trans.* B, **235**, 565.
Tiselius, A. 1937 *Trans. Faraday Soc.* **33**, 524.
Tiselius, A. 1938 *Kolloidzschr.* **85**, 129.



Description of Plate 5

Figure 2. Electrophoretic pattern at pH 7·62, of a 0·6 % solution of unfractionated histone from chicken erythrocyte nuclei; ascending boundary after 1½ h; knife-edge angle $\theta = 7°$.

Figure 3. Electrophoretic pattern at pH 7·62 of a 0·6 % solution of unfractionated histone from chicken erythrocyte nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 7°$.

Figure 4. Electrophoretic pattern at pH 5·72 of a 0·6 % solution of unfractionated histone from chicken liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 5. Electrophoretic pattern at pH 6·60 of a 0·6 % solution of unfractionated histone from chicken spleen nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 6. Electrophoretic pattern at pH 6·40 of a 0·6 % solution of unfractionated histone from ox thymus nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 7. Electrophoretic pattern at pH 7·60 of a 0·5 % solution of unfractionated histone from ox liver nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 15°$.

Figure 8. Electrophoretic pattern at pH 6·02 of a 0·6 % solution of unfractionated histone from ox spleen nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 14°$.

Figure 9. Electrophoretic pattern at pH 6·10 of a 0·6 % solution of unfractionated histone from pig liver nuclei; descending boundary after 2 h; knife edge-angle $\theta = 7°$.

Figure 10. Electrophoretic pattern at pH 6·62 of a 0·6 % solution of unfractionated histone from pig spleen nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 12°$.

Figure 11. Electrophoretic pattern at pH 5·07 of a 0·6 % solution of unfractionated histone from rabbit liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

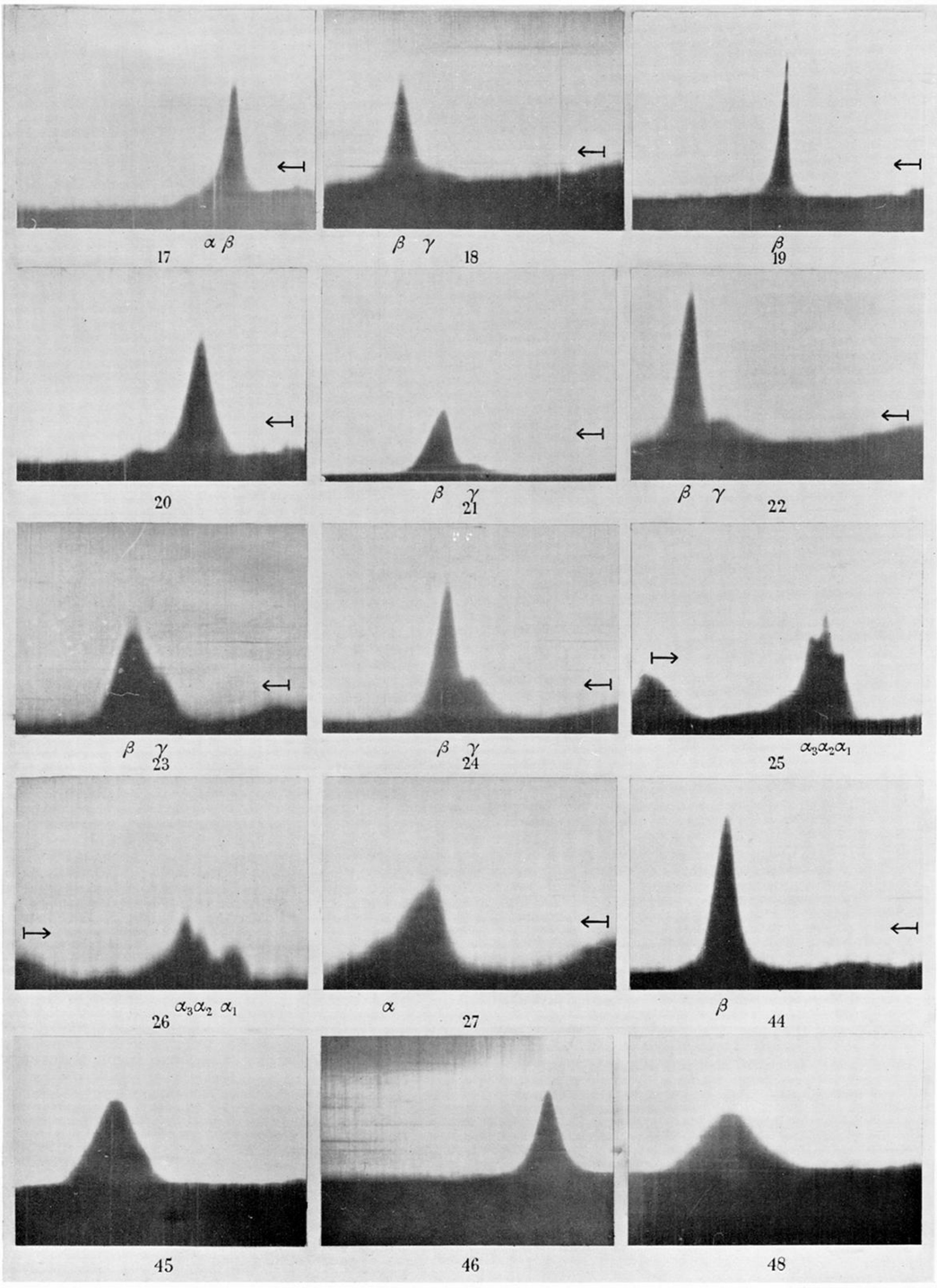
Figure 12. Electrophoretic pattern at pH 7·65 of a 0·5 % solution of unfractionated histone from human thymus nuclei; descending boundary after 1 h; knife-edge angle $\theta = 10°$.

Figure 13. Electrophoretic pattern at pH 7·65 of a 0·3 % solution of unfractionated histone from human child's liver nuclei; descending boundary after 1½ h; knife-edge angle $\theta = 10°$.

Figure 14. Electrophoretic pattern at pH 6·60 of a 0·6 % solution of unfractionated histone from human adult liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 15. Electrophoretic pattern at pH 5·24 of a 0·6 % solution of unfractionated histone from normal rat liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 16. Electrophoretic pattern at pH 6·78 of a 0·6 % solution of unfractionated histone from rat spleen nuclei; descending boundary after 2 h; knife-edge angle $\theta = 8°$.



Description of Plate 6

Figure 17. Electrophoretic pattern at pH 7·50 of a 0·6 % solution of unfractionated histone from rat kidney nuclei; descending boundary after 1 h; knife-edge angle $\theta = 10°$.

Figure 18. Electrophoretic pattern at pH 5·31 of a 0·3 % solution of unfractionated histone from mouse spleen nuclei; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 19. Electrophoretic pattern at pH 6·25 of a 0·4 % solution of unfractionated histone from mouse liver nuclei; descending boundary after 2 h; knife-edge angle $\theta = 12°$.

Figure 20. Electrophoretic pattern at pH 6·15 of a 0·6 % solution of unfractionated histone from wheat germ; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 9°$.

Figure 21. Electrophoretic pattern at pH 7·95 of a 1·0 % solution of fractionated histone from ox thymus nuclei; descending boundary after 2 h; knife-edge angle $\theta = 25°$.

Figure 22. Electrophoretic pattern at pH 7·66 of a 0·5 % solution of fractionated histone from chicken thymus nuclei; descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 12°$.

Figure 23. Electrophoretic pattern at pH 7·64 of a 0·6 % solution of fractionated histone from cod sperm; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

Figure 24. Electrophoretic pattern at pH 6·65 of a 0·7 % solution of $\beta+\gamma$-fraction from ox thymus histone; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 25. Electrophoretic pattern at pH 7·43 of a 1·0 % solution of α-fraction from ox thymus histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 9°$.

Figure 26. Electrophoretic pattern at pH 8·41 of a 0·8 % solution of α-fraction from chicken erythrocytes histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

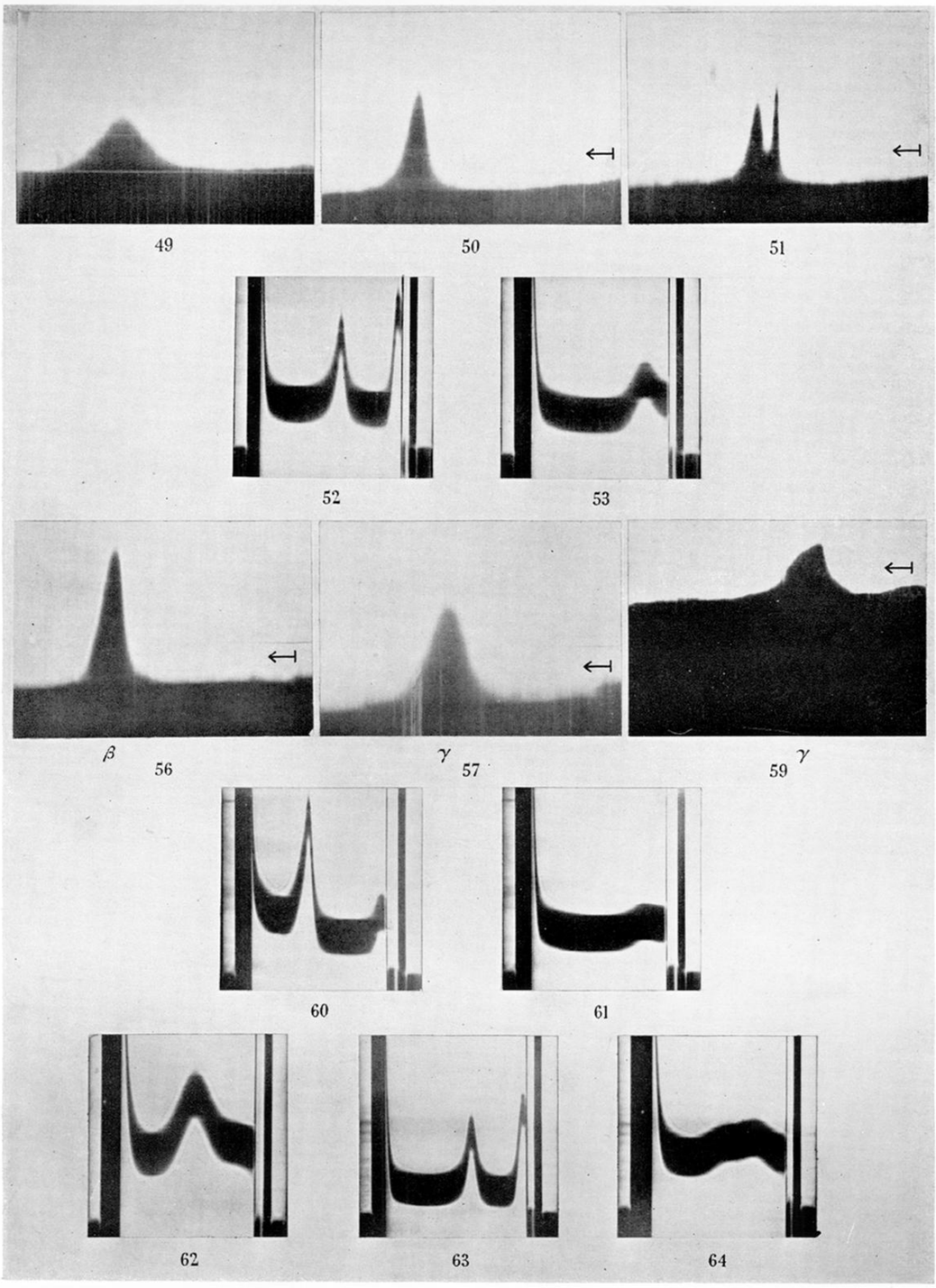
Figure 27. Electrophoretic pattern at pH 7·66 of a 0·55 % solution of α-fraction histone from cod sperm; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

Figure 44. Electrophoretic pattern at pH 7·50 of a 0·7 % solution of the β-fraction (electrophoretically prepared) from ox thymus histone; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 45. Diffusion pattern at pH 1·40 of a 0·5 % solution of ox thymus electrophoretically pure β-histone after 21 h diffusion; knife-edge angle $\theta = 9°$.

Figure 46. Diffusion pattern at pH 7·05 of a 0·5 % solution of ox thymus electrophoretically pure β-histone after 48 h diffusion; knife-edge angle $\theta = 11°$.

Figure 48. Diffusion pattern at pH 3·15 of a 0·5 % solution of α-fraction from ox thymus histone after 24 h diffusion; knife-edge angle $\theta = 7°$.



Description of Plate 7

Figure 49. Diffusion pattern at pH 6·85 of a 0·5 % solution of α-fraction from ox thymus histone after 18 h diffusion; knife-edge angle $\theta = 8°$.

Figure 50. Electrophoretic pattern at pH 8·65 of an artificial mixture of ox thymus and ox liver electrophoretically pure β-histones 0·25 % each, dialyzed together; descending boundary after 3 h; knife-edge angle $\theta = 10°$.

Figure 51. Electrophoretic pattern at pH 8·65 of an artificial mixture of ox thymus and ox liver electrophoretically pure β-histones, 0·25 % each, dialyzed separately; descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 10°$.

Figure 52. Sedimentation pattern at pH 7·78 of a 1·33 % solution of the β+γ-fraction from ox thymus histone after 12 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 53. Sedimentation pattern of the above solution (figure 52) after 96 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 56. Electrophoretic pattern at pH 6·15 of a 0·6 % solution of the β-component (ultracentrifuge preparation) from ox thymus histone, descending boundary after 2 h; knife-edge angle $\theta = 12°$.

Figure 57. Electrophoretic pattern at pH 6·15 of a 0·6 % solution of the γ-component (ultracentrifuge preparation) from ox thymus histone, descending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 59. Electrophoretic pattern at pH 9·14 of a 0·45 % solution of the γ-component (ultracentrifuge preparation) from ox thymus histone, descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 8°$.

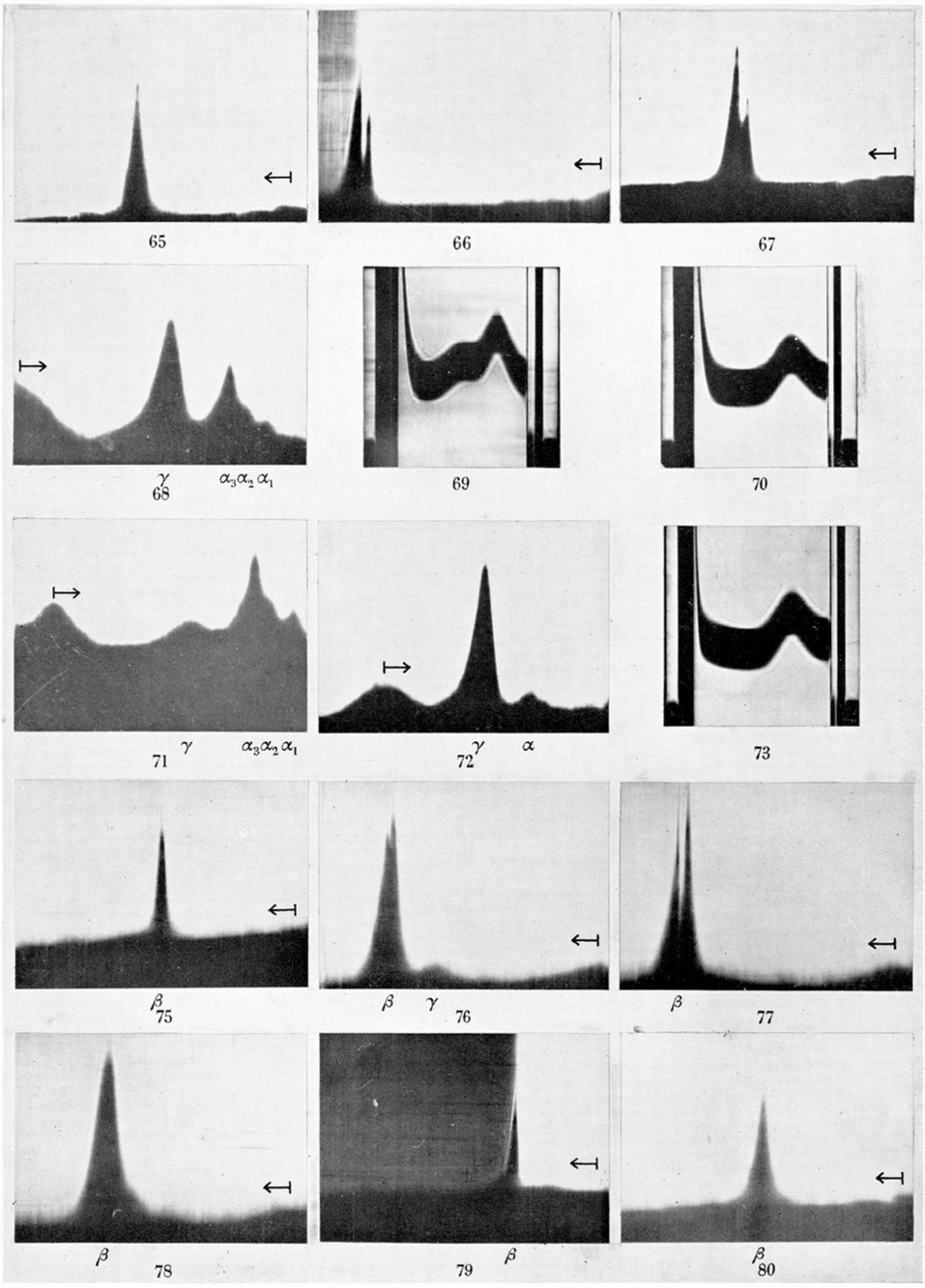
Figure 60. Sedimentation pattern at pH 7·70 of a 1·33 % solution of the β-component (ultracentrifuge preparation) from ox thymus histone, after 16 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 61. Sedimentation pattern of histone solution above (figure 60) after 80 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 62. Sedimentation pattern at pH 7·70 of a 1·33 % solution of the γ-component (ultracentrifuge preparation) from ox thymus histone, after 176 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 63. Sedimentation pattern at pH 8·10 of a 2 % solution of unfractionated histone from ox liver nuclei, after 8 min at 59780 rev/min; bar angle $\theta = 40°$.

Figure 64. Sedimentation pattern of the above solution (figure 63) after 168 min at 59780 rev/min; bar angle $\theta = 45°$.



Description of Plate 8

Figure 65. Electrophoretic pattern of an artificial mixture of 0·25 % each of β-histones (ultracentrifuge preparations) from ox thymus and ox liver dialyzed together at pH 7·54, descending boundary after 2 h; knife-edge angle $\theta = 11°$.

Figure 66. Electrophoretic pattern of mixture as above figure 65, dialyzed separately at pH 7·54; descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 10°$.

Figure 67. Electrophoretic pattern of an artificial mixture 0·3 % with respect to each of β-histone (electrophoretically pure) from ox liver and β-histone (ultracentrifuge preparation) from ox liver dialyzed separately at pH 7·89 descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 68. Electrophoretic pattern at pH 8·47 of a 1·0 % solution of the $\alpha+\gamma$-fraction (ultracentrifuge prepared) from ox liver histone, ascending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 69. Sedimentation pattern at pH 8·50 of a 2 % solution of the $\alpha+\gamma$-fraction (ultracentrifuge prepared) from ox liver histone after 240 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 70. Sedimentation pattern at pH 8·50 of a 2 % solution of the α-fraction (ultracentrifuge prepared) from ox liver histone after 326 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 71. Electrophoretic pattern at pH 8·68 of a 0·8 % solution of the α-fraction (ultracentrifuge prepared) from ox liver histone; ascending boundary after 2 h; knife-edge angle $\theta = 6°$.

Figure 72. Electrophoretic pattern at pH 8·68 of a 0·8 % solution of the γ-fraction (ultracentrifuge prepared) from ox liver histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 9°$.

Figure 73. Sedimentation pattern at pH 8·50 of a 2 % solution of the γ-fraction (ultracentrifuge prepared) from ox liver histone after 144 min at 59780 rev/min; bar angle $\theta = 40°$.

Figure 75. Electrophoretic pattern at pH 5·24 of a 0·25 % solution of fractionated histone from human glands (lymphatic leukaemia); descending boundary after 2 h; knife-edge angle $\theta = 8°$.

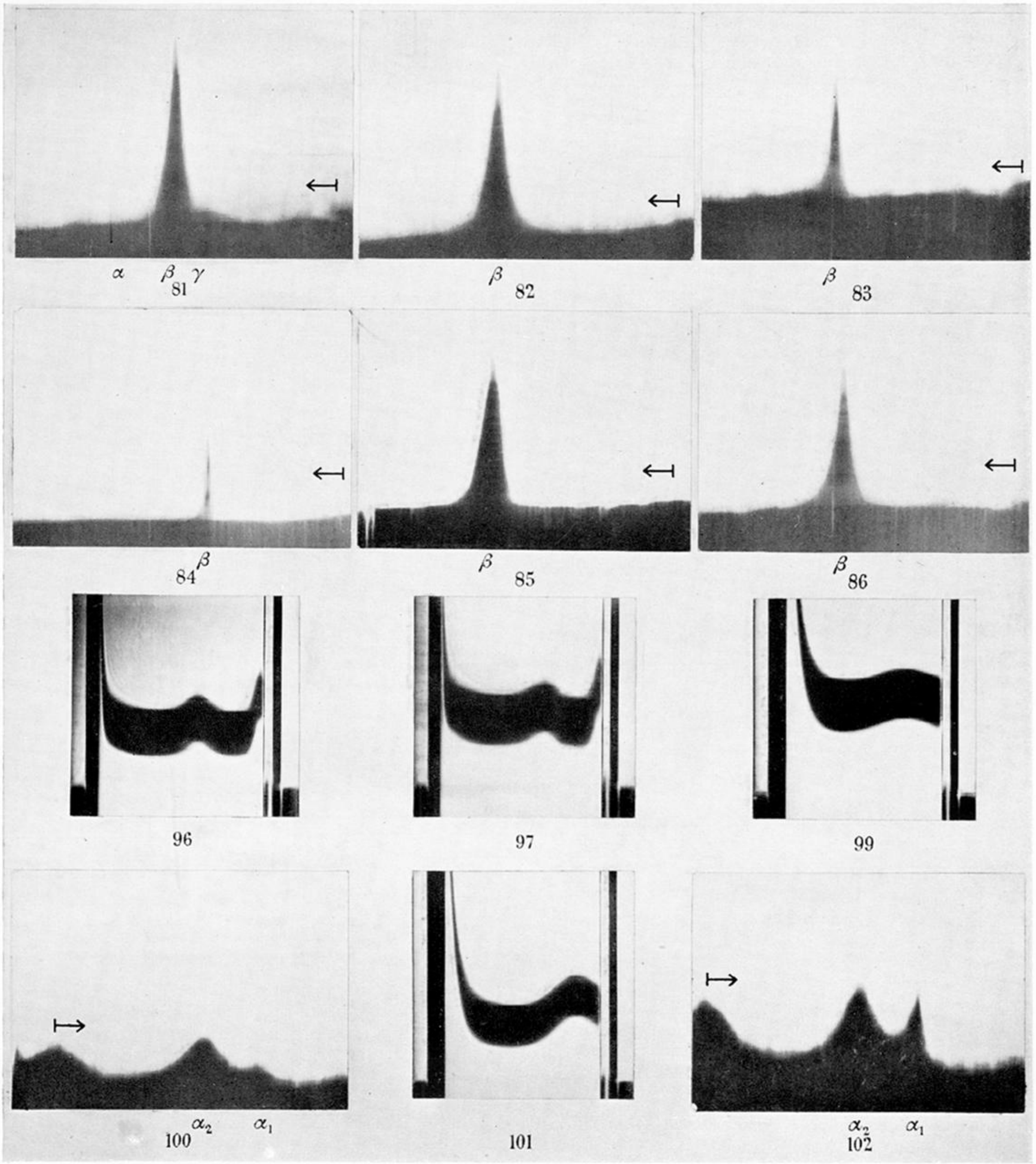
Figure 76. Electrophoretic pattern at pH 7·41 of a 0·6 % solution of fractionated histone from human spleen nuclei (case of lymphatic leukaemia above); descending boundary after 2 h; knife-edge angle $\theta = 8°$.

Figure 77. Electrophoretic pattern at pH 7·69 of a 0·6 % solution of the unfractionated histone from human spleen nuclei (case of lymphatic leukaemia above); descending boundary after $2\frac{1}{2}$ h; knife-edge angle $\theta = 6°$.

Figure 78. Electrophoretic pattern at pH 4·33 of a 0·6 % solution of fractionated histone from human bronchogenic carcinoma; descending boundary after 2 h; knife-edge angle $\theta = 10°$.

Figure 79. Electrophoretic pattern at pH 7·70 of a 0·4 % solution of unfractionated histone from human leucocytes (case of chronic lymphatic leukaemia); descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 10°$.

Figure 80. Electrophoretic pattern at pH 5·74 of a 0·4 % solution of unfractionated histone from human myelocytes (case of chronic myeloid leukaemia); descending boundary after 2 h; knife-edge angle $\theta = 7°$.



Description of Plate 9

Figure 81. Electrophoretic pattern at pH 7·05 of a 0·4 % solution of unfractionated histone from human undifferentiated tumour; descending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 82. Electrophoretic pattern at pH 5·65 of a 0·4 % solution of unfractionated histone from chicken lymphocytes (case of lymphatic leukaemia); descending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 83. Electrophoretic pattern at pH 8·70 of a 0·1 % solution of unfractionated histone from leukaemic spleens (mouse line I); descending boundary after 2 h; knife-edge angle $\theta = 5°$.

Figure 84. Electrophoretic pattern at pH 8·70 of a 0·08 % solution of unfractionated histone from Walker 256 rat carcinoma; descending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 7°$.

Figure 85. Electrophoretic pattern at pH 4·85 of a 0·4 % solution of unfractionated histone from rat hepatoma nuclei; descending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 86. Electrophoretic pattern at pH 5·07 of a 0·3 % solution of unfractionated histone from rat sarcoma *Rd*/3 nuclei; descending boundary after 2 h; knife-edge angle $\theta = 7°$.

Figure 96. Sedimentation pattern at pH 5·29 of a 0·42 % solution of unfractionated histone from normal rat liver nuclei, after 16 min at 59780 rev/min; bar angle $\theta = 40°$.

Figure 97. Sedimentation pattern at pH 5·29 of a 0·42 % solution of unfractionated histone from rat hepatoma nuclei, after 16 min at 59780 rev/min; bar angle $\theta = 35°$.

Figure 99. Sedimentation pattern at pH 8·47 of a 2 % solution of the α-fraction (ultracentrifuge prepared) from rat hepatoma histone, after 410 min at 59780 rev/min; bar angle $\theta = 35°$.

Figure 100. Electrophoretic pattern at pH 8·47 of a 0·8 % solution of the α-fraction (ultracentrifuge prepared) from rat hepatoma histone; ascending boundary after $1\frac{1}{2}$ h; knife-edge angle $\theta = 6°$.

Figure 101. Sedimentation pattern at pH 8·47 of a 2 % solution of the α-fraction (ultracentrifuge prepared) from normal rat liver histone, after 216 min at 59780 rev/min; bar angle $\theta = 45°$.

Figure 102. Electrophoretic pattern at pH 8·47 of a 0·8 % solution of the α-fraction (ultracentrifuge prepared) from normal rat liver histone, ascending boundary after $1\frac{1}{2}$ h; knife edge-angle $\theta = 6°$.