Table 3. *Weight of lung and nucleic acid phosphorus in the lungs of normal and phosgene-poisoned mice*

Details of mouse		Wet wt. of lung (mg.)	Wt. of lung as % of body wt.	Nucleic acid P of lung	
Exposure	Wt. (g.)			(As % of lung wt.)	(As % of body wt.)
None	19	133	0.705	0.115	0.00081
	23	156	0.678	0.109	0.00073
	21	152	0.722	0.114	0.00082
	19.5	148	0.759	0.108	0.00076
	22	141	0.640	0.107	0.00068
	20	161	0.806	0.108	0.00087
	21.8	146	0.650	0.112	0.00075
	22.5	196	0.871	0.110	0.00096
	18	100	0.556	0.098	0.00055
	19.5	135	0.693	0.108	0.00073
	20.5	143	0.698	0.111	0.00077
	19.5	122	0.626	0.115	0.00072
	20.5	312	1.52	0.062	0.00094
	22	301	1.37	0.069	0.00094
Phosgene <i>Ct</i> =3480 (4½ hr. before estimation)	23	310	1.35	0.064	0.00086
	19.5	314	1.61	0.058	0.00094
	19	296	1.56	0.059	0.00092
	21	320	1.52	0.064	0.00098
	20.5	185	0.903	0.087	0.00078
Phosgene <i>Ct</i> =2240 (22 hr. before)	19	277	1.46	0.051	0.00075
	20	213	1.06	0.081	0.00086
	21	269	1.28	0.069	0.00088
	20	305	1.52	0.051	0.00078
	19	164	0.864	0.087	0.00075
	20.5	185	0.903	0.087	0.00078
	20.5	185	0.903	0.087	0.00078

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The Electrophoretic Analysis of Protein Extracts from Striated Rabbit Muscle

2. DENATURATION IN ACETATE BUFFERS

By J. J. C. JACOB, *Laboratoire de Biologie générale, Université de Liège*

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In a previous paper (Jacob, 1947) some interrelations between the classical muscle proteins (myogen, myoalbumin, myosin, globulin X) and the electrophoretic components of various extracts of striated rabbit muscle were established. In order to complete the characterization of components, the analysis of one of these muscle extracts in acetate buffers of decreasing pH is described here. This is necessary since the isoelectric points of several components lie in the pH range covered by acetate buffers, and in an acid medium, most of the muscle proteins are denatured without flocculating

(Finn, 1932; Weber, 1934; Bate Smith, 1937). These new observations are of interest in the study of denaturation processes.

EXPERIMENTAL

The electrophoretic technique and the procedures for the extraction and preparation of muscle extracts have been described in a previous paper (Jacob, 1947). The extraction solution used here contained 0.048 M- Na_2HPO_4 and 0.006 M- NaH_2PO_4 (pH=7.7, $\mu=0.15$); the dialysis solutions, which also served as supernatant in the electrophoretic experiments, contained 0.1 M-sodium acetate, 0.05 M-NaCl

and a variable amount of acetic acid. Their pH varied from 3.62 to 5.92, with ionic strength constant at $\mu=0.15$.

The extracts were dialyzed for 24–70 hr., and some protein was found to precipitate. When the extract was centrifuged after 3 days' dialysis, the volume of precipitate was greater the lower the pH of dialysis. When the extract was centrifuged several times during dialysis, it was possible to collect a precipitate during the first 36–60 hr., in the pH range 5.0–5.9; whereas, at any lower pH, after the 8–24 hr. precipitates had been removed, no further precipitation occurred.

The total amount of precipitate was larger and its formation more rapid the lower the pH. The dialysis time was sufficient to avoid further precipitation during electrophoresis, though at pH 5.9 failures were frequent. The dialyzed extracts became less turbid as the pH decreased, and below pH 5.0 they were almost limp.

When dialyzed extracts were left at room temperature, a further precipitate appeared; the quantity and rapidity of formation of this increased with the fall of pH, thus illustrating the progressive instability of the extracts. Between pH 3.9 and 3.6, the extracts were extremely viscous and had a tendency to form a thixotropic gel, even when kept at 1°.

As in the preceding paper, pH measurements were made at room temperature. To calculate the pH at 0°, the values were corrected by 0.025 pH unit, according to Harned & Ehlers (1932).

The relative amounts of the different components were obtained from the peak areas after correcting for significant superimposed δ and ϵ effects. Depending on the pH, there were two possibilities: (1) the δ and ϵ effects were superimposed on a protein peak located at the extremity of the pattern (here this peak is h); (2) the δ and ϵ effects were superimposed on one or more peaks located in the centre of the pattern (here these effects shift progressively from l to h as the pH decreases).

The first case is often recorded in the literature, and the values obtained from the descending pattern are usually adopted. By this means, the amount obtained for the extreme peak represents in fact the total of the amounts which can be attributed to that peak and to the ϵ effect, which is generally neglected. In our experiments, when the ϵ effect is dissociated from the protein peaks, it represents on the average 4% of the total surface of the diagram. Consequently, the values obtained from the descending pattern were corrected, by subtracting from the apparent amount of the extreme peak the amount due to the ϵ effect (4% of the total surface), and sharing this amount between the peaks, in proportion to their relative magnitude (this is in conformity with the theoretical developments of Longworth, 1942).

When the δ and ϵ effects are superimposed on one or more median peaks, it is sufficient to consider that each of the patterns, the anode and cathode, contains ascending, descending and median peaks. The first step is to determine, on both diagrams, the relative amounts of the descending peaks. The difference between 100% and the total of these relative amounts represents the percentage to be attributed to the median peak and to the ϵ effect. It is then easy to eliminate the ϵ effect by the same procedure as in the first case.

It would have been a great advantage, if it had been possible, to correct the ascending values, in order to measure asymmetries depending on causes other than δ and ϵ effects. Unfortunately, the precision of the measurements was con-

siderably reduced by the poor separation of one ascending peak at any pH higher than 5.0, and by the excessive sharpness of the gradient of another ascending peak at lower pH values.

Finally, it was impossible to establish whether the relative amounts of the different peaks altered during the course of electrophoresis.

RESULTS

Description of the patterns obtained in acetate buffers

At pH 5.9–5.8, the patterns obtained in acetate buffers differ only slightly from those obtained (Jacob, 1947) at neighbouring pH values in phosphate buffers. The separation into three main groups again occurs (Fig. 1). The first group consists of the peak n , which separates early, and of the two components l and m , which only appear after prolonged experiments at lower ionic strength ($\mu=0.10$). The second group contains its usual components k_1 , k_2 and j . Between groups II and III lies a small quantity of protein which is not easily divided between these two groups. For convenience it was called sp (space). The third group consists of a single peak, unimpaired by any turbidity. Probably the turbid protein i has vanished from it, and this peak represents the component h to which it approximates in amount.

The relative concentrations of the components are not widely different, whether the dialysis buffer is acetate or phosphate (Table 1).

As the pH decreases, the patterns change progressively (Fig. 1). The relative area of peak n increases regularly from 18% (pH 5.9) to 99% (pH 3.64) (Table 1, col. C). This increase in concentration is not so regular when expressed as mg. N/ml. because of non-systematic variations (up to 20% at least) in the total protein-N concentration of the dialyzed extracts. If these errors are taken into account, the change referred to the amount of N is also regular (Fig. 2). The concentration of peak n rises progressively to 1.2, 2.0, 2.4, 3.5 mg. N/ml., values which are much higher than the concentration of the substance n in extracts dialyzed against phosphate buffer (0.72–0.90 mg. N/ml.; Jacob, 1947). This peak thus represents not only the substance n , but also other proteins which behaved as separate entities in phosphate buffer. Consequently another designation C was adopted for this peak.

The shape of C differs in the anode (descending) and cathode (ascending) patterns. Up to pH 4.0, the characteristics of this asymmetry are classical (Tiselius & Horsfall, 1939; Longworth & MacInnes, 1940). The ascending boundary is sharp and very homogeneous; the descending boundary is wide, and its 'buffer' side is steeper than its 'protein' side; this becomes more typical and almost excessive, as the pH decreases. Below pH 4.0, the descending

Table 1. *Composition of muscle extracts dialyzed in acetate buffers*

No.	pH	Protein-N (mg./ml.)	Relative amounts of the components from corrected descending values (%)					
			<i>C</i>	<i>l+m</i>	<i>k</i> ₁	<i>k</i> ₂ + <i>j</i>	<i>sp</i>	<i>h</i>
1	5.92	1.80*	19.5	54.5	8.0†	9.0†	3.0	6.0
<i>P</i> †	5.79	—	19.5	55.0	18.0	6.0†	2.0	5.5
2	5.78	—	23.5	[55.0]			2.5	5.5
3	5.62	—	33.0	51.0	5.0†	4.5†	2.5	4.0
4	5.61	3.61	35.5	48.0	5.0†	5.5†	2.0	4.0
5	5.45	2.52	34.5	50.0	5.0†	4.0†	2.5†	4.0
6	5.36	3.02	40.5	43.0	6.0	4.5†	2.0†	4.0
7	5.33	3.05	39.5	43.5	6.0	3.5†	1.5†	6.0
8	5.22	3.87	44.5	39.5	6.0	4.0†	2.0†	4.0
9	5.17	2.69	46.0	[40.5]	[4.5]	3.0†	2.0†	4.0
10	5.05	4.32	48.0	40.0	4.0	2.0†	2.0†	4.0
11	5.02	2.86	50.0	32.5	7.0	2.5†	1.5†	6.5
12	4.91	2.71	57.5	34.5	31.0	3.0†	2.0†	5.0
13	4.85	4.00	62.0	31.0		2.0†		5.0†
14	4.80	3.51	66.5	27.0	24.0	2.0†	2.5	4.5†
15	4.69	—	69.5	24.0		2.5		4.0†
16	4.45	—	77.5	18.5	14.5	1.0	—	4.0†
17	4.34	3.66	81.0	14.5				3.5
18	4.17	3.80	[86.5]	[9.5]	—	—	—	4.0
19	3.91	3.92	98.0	—				2.0
20	3.64	—	99.0	—	—	—	—	1.0

* Artificially reduced value, not accounted for in Fig. 2.

† Indicates the peaks to which the ϵ effect was added.

P† Experiment with phosphate buffer.

[] Indicates the peaks whose separation was not satisfactory.

boundary splits in two and then several streaks form, whereas the ascending boundary apparently becomes more homogeneous. This suggests that the progressive spreading of the anode curve from pH 5.0 to 4.0 is a prelude to its cleavage at all lower pH values.

The peak common to *l* and *m* remains homogeneous down to pH 5.30 and its relative amount decreases from 54.5 to 43%. From pH 5.2 to 4.45, this boundary undergoes several successive scissions. The patterns become complex and are characterized by asymmetries which even involve the number of peaks. Simultaneously, the total amount corresponding to these peaks falls as low as 18.5%.

Between pH 5.20 and 5.05, *l+m* divides into two components whose relative amounts differ in the anode and cathode compartments (peak 1 (the faster) and peak 3); there is nothing to facilitate identification of these peaks with the components *l* and *m*.

In the neighbourhood of pH 5, an additional peak (peak 2) appears between 1 and 3; 1 becomes gradually smaller and simultaneously migrates closer to *C* and, mainly on cathodic patterns, its final inclusion in *C* can clearly be followed.

From pH 4.9 downwards, the asymmetry between the two patterns becomes more complex. In the anode pattern a component (*c*) appears whose gradient is relatively very sharp, and no corresponding peak forms in the cathode pattern.

Below pH 4.45, the group is simplified by the fusion with *C* of the residue of the streaks developed from the cleavage of boundary *l+m*.

The peaks of group II disappear progressively. Component *k*₁ separates less from peak 3, mainly on the cathode diagram, and below pH 5.00 it merges into peak 3. Component *k*₂ is sharply defined down to pH 4.9. It decreases in amount from pH 5.30 to 4.9 and at pH 4.8, no trace of it remains. Component *j* is marked by a slight inflexion on the side of *k*₂, which gets less and less distinguishable. Component *h* is, of all components, the one which undergoes the least alteration. Down to pH 4.1, its quantity measured from the descending boundary does not diminish, and it shows no peculiar characteristic. Below pH 4.0, it decreases, but at pH 3.64, it is still evident as a small inflexion. It is the only one among the peaks occurring at pH 5.9 which does not completely disappear, or is not completely absorbed by *C*.

Down to pH 4.85, the diagrams are mixed (Jacob, 1947), i.e. both the anode and cathode diagrams include a δ and an ϵ effect. The most obvious of these, the cathode δ effect, shifts progressively from *l* to *sp* between pH 5.9 and 4.9; it then reaches streak *h*, which it lifts, as far as pH 4.4, and then separates completely. The ϵ effect shows very clearly in the anode diagrams below pH 4.4.

Finally, the slight turbidity is no longer associated with groups II or III as in the phosphate experiments, but migrates with group I from pH 5.6 downwards.

In short, with the progressive fall of pH, the proteins tend to migrate as a single boundary (*C*).

The other components, with the exception of h , finally disappear either because they are incorporated in C , or because they precipitate in the course of the preliminary dialysis (which is probably the case for j and k_2). Before disappearing, some components undergo alterations (in the case of $l+m$ and k_2).

Fig. 2. Concentrations (in mg. N/ml., left scale) and relative amounts (in %, right scale) of boundary C as a function of the pH. The relation of the two scales was established with 100% as an average of the total amounts of protein N in the dialyzed extracts (3.4 mg. N/ml.). The streaked area is obtained by joining the points corresponding to the concentrations increased and lowered by 20%. ● Relative amounts of boundary C (acetate buffer); ○ relative amounts of boundary n (phosphate buffer – values previously published (Jacob, 1947)). The corresponding pH values have since been corrected according to Bates & Acree (1945).

Among more than thirty experiments, a single one—executed at pH 5.15—gave aberrant patterns in which the normal components maintained their individuality; the reason for this divergence could not be found.

Complex formation between components

The progressive incorporation of the protein components of the extracts into one electrophoretic peak can have two interpretations: either (a) the mobilities of different components become so similar in some zones of pH that no electrophoretic separation

is possible under the conditions of the experiment; (the resulting peak has no special implication) or (b) a complex is formed between two or more proteins, and migrates with a characteristic mobility, distinct from those of the original proteins.

The first possibility seems likely, since the mobility curves of several components finally coincide with the curve of C (Figs. 3, 4), but this cannot wholly account for the behaviour of C , since before the fusions occur the amount of C increases considerably (from 18% at pH 5.9 to 57.5% at pH 4.90). Further, the contribution of each protein does not account quantitatively for the increase of C (for instance, from pH 4.90 to 4.40, the amount of C rises from 57.5 to 77.5%, whereas the amount of peak 1 is, at the most, 7–8%); finally, the increase of C is not irregular but almost continuous (Fig. 2). Probably C is primarily a complex composed of n and a portion of the other components.

Interactions are likely to occur between complex C and several other components, at all pH values lower than 4.9. Indeed, we notice the existence on the anode pattern of a peak (c), which does not appear on the cathode pattern.

A plausible interpretation is the following (according to Longworth & MacInnes, 1942). On the anode (descending) side, the components 1, 2 and 3 migrate in a medium devoid of C . They have a tendency to re-form the complex C , but in the absence of n which has also left the medium, the aggregation product migrates more slowly than C and is represented by peak c. The mobility of c is not widely different from the average mobilities of 1, 2 and 3 at pH 4.8 and 4.9, and of the average mobilities of 2 and 3 at all lower pH's when 1 has been completely absorbed by C . This does not occur on the ascending side where components 1, 2 and 3 migrate in a medium containing complex C . On the contrary, one would expect complex C to dissociate when it migrates alone; this probably occurs, but the errors involved in the area measurements of the ascending peaks make it difficult to establish.

Further, important asymmetries occur between the ascending and descending mobilities. It is, in general, very difficult to estimate the part to be attributed to the 'classical' asymmetry, and to the 'interaction' asymmetry, but this is not the case for peak h below its isoelectric point. For this component, the 'classical' asymmetry is almost negligible owing to its low concentration (Jacob, 1947). This is true down to pH 4.67, but below this pH, i.e. when h begins to form part of the complex, the asymmetry becomes considerable (Fig. 4). Undoubtedly interactions occur which cannot otherwise be detected, owing to the difficulty of measuring exactly a peak of such small area.

It should be mentioned that dilution of the extract causes a diminution in the amount of complex C (for instance, the extract no. 9, diluted once with acetate buffer of an identical pH contains only 39% of C instead of 46% and likewise, the extract no. 14, diluted in the same manner contains only 55% instead of 66%). This agrees with some observations of Longworth, Cannan & MacInnes (1940),

Fig. 3. Descending pH-mobility curves of the electrophoretic components of muscle extract in acetate buffers of $\mu=0.15$.

$\square=C$, $\bullet=l+m$, $\times=1$, $\odot=2$, $\blacksquare=3$, $\blacktriangle=c$, $\circ=k_1$, $\triangle=k_2$, $\odot=j$, $+ =h$.

The dashed curves (---) were obtained with phosphate buffers of the same ionic strength (Jacob, 1947; for the pH, see note of Fig. 2 and text, p. 77).

Fig. 4. Ascending pH-mobility curves of the components in acetate buffers of $\mu=0.15$.

$\square=C$, $\bullet=l+m$, $\times=1$, $\odot=2$, $\blacksquare=3$, $\circ=k_1$, $\triangle=k_2$, $+ =h$.

The broken (lower) curve (---) is the descending curve of h .

that aggregation phenomena between proteins are less marked when the protein concentration is low.

Interactions are not likely to be artefacts of the moving boundary method (Perlmann & Kaufman, 1945; Svensson, 1946), for dilution does not result in the disappearance of the asymmetric peak *c*, or a significant reduction of the mobility asymmetries of the various components. It may be concluded that the increase in the area of *C* is due mainly to the formation of a complex. Probably this component interacts with the other components present.

Contrary to facts mentioned by Moyer & Moyer (1940) for rabbit serum proteins, and by Longworth (1941) for ovalbumin, the pH mobility curves of muscle proteins—in phosphate and acetate buffers—do not diverge noticeably in the common pH zone (Fig. 3). It is worth mentioning that this agreement is found only if the pH values are corrected to 0°, which can be done by using the data of Bates & Acree (1945) for phosphate buffers, and Harned & Ehlers (1932) for acetate buffers.

phosphate was prolonged (two days, three renewals of the outer liquid), in order to ensure equilibrium between buffer and protein. Its duration does not exceed the limits which were established empirically for the conservation of extracts dialyzed against phosphate buffer alone (Jacob, 1947). In these experiments the identity of the components remaining in solution can be decided from their mobilities, which are very close to those of the components in extracts which were not dialyzed initially against acetate. Very often, they are slightly higher, which may be accidental; there is no evidence of a definite alkaline shift.

The results of the concentration measurements are given in Table 2. If these results are compared with the values obtained for the same extract, after acetate dialysis alone (Table 1), it appears that the loss of concentration is approximately equal to the amount of the complex *C*; and that the amounts of each of the components remaining in solution are close to the amounts of the corresponding peaks of

Table 2. *Composition of muscle extracts after two dialyses*

No.*	pH of acetate buffer	pH of phosphate buffer	mg. N/ml. after 2nd dialysis	Ppt.†	Relative amounts‡ of protein				
					Soluble part				
					<i>M</i> (+ <i>n</i>)	<i>l</i>	<i>k</i> ₁	<i>k</i> ₂ <i>j</i> + <i>sp</i>	<i>h</i>
§	—	7.10–7.80	4.0±1.0	0	47.5	24	±7	±12	±5
7	5.33	7.63	1.70	44	18.5	25	5	3	4.5
13	4.85	7.67	1.86	53.5	19	19	2	2	4.5
17	4.34	7.56	0.66	82	4	10.5	1.5		2
19	3.91	7.52	0.32	92	4.0		1.0		3

* This number indicates the extract of Table 1, a part of which was subjected to the second dialysis.

† Relative amounts are calculated relative to the N concentration of the extract after the first dialysis. They can thus be compared directly with the amounts of corresponding experiments in Table 1.

‡ Includes precipitation and experimental losses etc. (see text).

§ Average results of experiments made after a single phosphate dialysis without any detectable preliminary denaturation (Jacob, 1947).

Recovery of native (or 'reversibly' denatured) proteins from the extracts treated with acetate buffers

The native components *h*, *sp*, *k*, *l*, *m* and *n* of the muscle extracts are soluble in a solution containing 0.032M-Na₂HPO₄, 0.04M-NaH₂PO₄ and 0.5M-NaCl, pH 7.60, $\mu = 0.15$ (Jacob, 1947).

If the extracts are first dialyzed against an acetate buffer, centrifuged and then dialyzed a second time against this phosphate solution, a precipitate forms in less than 1 hr. The quantity of protein remaining in solution after removal of the precipitate decreases as the pH of the acetate solution is lowered. The loss is negligible at pH 5.9, but reaches almost 100 % when the pH is 3.64. There is thus a progressive denaturation of the muscle proteins in acetate buffers as the pH falls. This confirms the results of Finn (1932), Weber (1934) and Bate Smith (1937).

The proteins which remain in solution have been examined electrophoretically. Dialysis against

the extract after acetate dialysis alone. This suggests that *C* is the aggregation product of the denatured proteins which precipitate during a second dialysis.

However, the parallelism is not absolute; the loss of concentration represents both the elimination of the precipitate and accidental losses in the course of the second dialysis (osmotic dilution, etc.); it should thus always be greater than the amount of *C*. Yet the opposite is observed in Exps. 13 and 19, and in Exps. 7 and 17 where the loss of concentration is greater than the amount of the complex, this difference being balanced by a fall in the concentration of those peaks which are not yet much involved in the process of progressive aggregation (e.g. *k*, *j*, *sp* and *h* in Exp. 7; *h* in Exp. 7). Presumably the other components remaining in solution are present in a relatively excessive concentration. Consequently, the complex *C* in so far as its real amount is estimated from the descending pattern, is not entirely precipitated. It would thus include, besides the denatured proteins—insoluble in a phosphate buffer—a small proportion of native protein or of denatured proteins soluble in a phosphate buffer.

DISCUSSION

The main object of this study was to characterize the electrophoretic components of extracts from rabbit muscle. In the previous paper (Jacob, 1947), the following isoelectric points were determined: n , 6.75 ± 0.12 ; m , 6.20 ± 0.12 ; l , 6.05 ± 0.12 ; k_1 , 5.50 ± 0.12 (for the correction $+0.12$ see Bates & Acree, 1945). Using acetate buffer, this list is completed with the values: h , 4.65 ; k_2 , 5.25 ; k_1 , 5.70 ; the isoelectric point of j lies between 4.7 and 5.10 .

The isoelectric point of component h is the lowest of all those we have observed. This fraction is not denatured at least down to pH 4.85 (Table 2). This characteristic could not be used to prepare h directly in a purified state; at all pH's lower than 4.90 , the elimination of denatured proteins leaves in solution a substantial proportion of proteins other than h . At pH 3.62 , the undenatured proteins are very dilute, and electrophoresis of the solution at pH 7.6 has for that reason given no reliable information.

This group of characteristics confirms the identification of peak h with the myoalbumin of Bate Smith. The only divergence relates to the position of the isoelectric point: 3.5 according to Bate Smith (1937), 4.65 on our curve. This divergence may be due either to the use of different buffers at different temperatures, or to the fact that Bate Smith's myoalbumin was modified considerably and adsorbed on quartz particles.

The isoelectric point of h is almost identical with that of serum albumin, but h is not identical with serum albumin, however, as has been shown in a preceding paper (Jacob, 1945).

Similarly, the isoelectric points of j and k_2 differ from those given by Green (1945) for the phosphorylases a and b , which seem to constitute, wholly or partly, these two components respectively (compare the curves and diagrams given by Green with our results, obtained in a phosphate buffer). The divergence is probably due to the fact that this author was compelled to extrapolate more extensively than the present data require.

In group I, the water-soluble part of which corresponds with Weber's myogen (Jacob, 1947) component l is not denatured at least down to pH 5.33 . By ultracentrifugation, Gralén (1939) has found that the crystalline myogen A of Baranowski (1939) was stable to a pH of about 5.0 ; l would correspond mainly to this myogen, which is confirmed by unpublished experiments by Distèche in this laboratory.

The evolution of component n is strange; from pH 5.8 it is wholly included in the denaturation complex. All the evidence seems to indicate that the protein n is a centre of formation for the complex C . It is not impossible that, in a phosphate

buffer, n is already a denatured protein (or a protein aggregate) which has undergone an alkaline shift (cf. Neurath, Greenstein, Putman & Erickson, 1944). This hardly seems probable, for in a phosphate buffer, the amount of n does not change much with the pH (Fig. 2). On the other hand, when an extract is denatured in a phosphate buffer by ageing, there is no increase in n , but the turbid matter collects at the level of group II, a group which migrates with a mobility approximately equal to the average mobility of all muscle components (cf. an analogous phenomenon for serum, Davis, Hollaender & Greenstein, 1942). The evolution of n gives rise to many hypotheses which it will be possible to examine only when purified muscle proteins are isolated and studied.

The denaturation of a mixture of muscle proteins in acetate buffers is mainly characterized by the formation of a complex. A similar phenomenon occurs in heated serum (Van der Scheer, Wyckoff & Clarke, 1941; Kleczkowski, 1941), in serum irradiated with ultra-violet rays (Davis *et al.* 1942) and when a mixture of casein and antibody is heated (Krejci, Jennings & Smith, 1942). It is generally admitted that this aggregation is a phenomenon distinct from denaturation proper; it would be made possible by the emergence of new reactive groups in a denatured protein molecule.

The points which characterize the formation of the complex C are: (1) its continuous increase with decrease of pH, (2) the probable existence of interactions between the complex and certain other proteins of the mixture.

If the interpretation given of these interactions is really accurate, complex formation would be an equilibrium reaction, the constant of which would be determined essentially by the pH (or by the concentration of acetic acid). The condition of equilibrium would also be influenced by the concentrations of the proteins involved. This phenomenon can be interpreted either (a) as an equilibrium between the original and the denatured forms of a protein (the latter form being the only one liable to enter into the formation of the complex); or (b) as a probability of aggregation of molecules which would all be denatured, but to varying degrees, according to the pH (for instance, at a given pH, all molecules are unfolded more than at the immediately higher pH, but less than at the immediately lower pH). This leads us to the hypothesis of Neurath, Cooper & Erickson (1942). The facts presented have, besides, a certain analogy with the experiments on 'reversible' denaturation by urea and guanidine, described by the authors. Indeed, both in Neurath's experiments and in ours the elimination of the denaturing agent brings about the partial precipitation of proteins. This process differentiates the proteins into irreversibly denatured proteins and others which remain soluble, of which in our case we cannot be certain whether they are identical with or slightly different from the original proteins.

We cannot develop any further this idea of equilibrium, and the very numerous problems which it suggests and could solve. The mixture analyzed here is too complex and too poorly defined.

Lastly, the aggregation complex is altered at low pH; this is shown on patterns such as Fig. 1, pH 3.9, which resemble diagrams given by Longsworth (1941) for the acid denaturation of ovalbumin. The interpretation of such asymmetrical diagrams is still unelucidated, but the similarity stresses the analogy of the action of acetic acid, in high concentration, on such widely different proteins as crystalline ovalbumin and a complex of modified muscle proteins.

SUMMARY

1. Protein extracts of rabbit muscles have been submitted to electrophoresis, in buffers containing 0.1M-Na acetate, 0.05M-NaCl and various concentrations of acetic acid (total $\mu=0.15$, pH varying between 3.6 and 5.9). As the pH decreases, the patterns diverge more and more from the type of pattern obtained with the same extracts in a phosphate buffer.

2. The analysis of the changes reveals the formation of an aggregation complex and the prob-

ability of interactions between this complex and other components of the mixture.

3. If the extracts dialyzed against acetate buffer are then subjected to a second dialysis against a phosphate solution at pH 7.6, a precipitate appears. The latter increases the lower the pH of the acetate buffer, and its amount corresponds approximately to the amount of the aggregation complex formed at that pH.

4. The relation between the electrophoretic components of our extracts and the 'classical' muscle proteins are discussed.

5. The meaning of the aggregation complex (denaturation complex) and its evolution in the course of electrophoresis are interpreted, as far as the great complexity of the observed mixture allows.

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