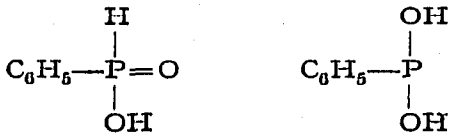
We can assign the so-called phenylphosphonous acid two chemical forms, *viz.* A and B.



(A) (B)

If the phosphonous acid is present in form B, the electrophoretic curve of the phosphonous acid must show two inflection points, considering that the rate of the hydrogen exchange reaction of hydrogen directly bonded to oxygen atom was much higher than that bonded to the phosphorus atom[3] and that two inflection points were found in the curve for phosphonic acid. The actual results that the curve had one point is in agreement with the form A, which must be named phenylphosphinic acid.

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A starch gel electrophoretic demonstration of the effect of pH on the aggregation of arginine-rich histones

The arginine-rich histone f3 comprises about 20 % of the histones of calf thymus and can be prepared by the extraction of deoxyribonucleoprotein with ethanol–1.25 *N* HCl (80:20, v/v) and subsequent precipitation by dialysis against ethanol[1]. The molar ratio of lysine to arginine is about 0.65 when the fraction has been purified by reprecipitation[2], and alanine is the main N-terminal amino acid (40–50 μmoles/g of protein), amounting to over 95 % of all such groups found.

The analytical evidence suggests that this is substantially a single protein but

the patterns obtained by electrophoresis in starch and polyacrylamide gels are complex, often containing a multiplicity of bands[3,4].

An investigation was therefore carried out on the effect of pH on the patterns obtained in starch gel using the arginine-rich histone f3.

Experimental and results

Preparation of histone fraction f3. The arginine-rich histone f3 was prepared as described previously[1] and purified by reprecipitation by dialysis against ethanol[2]. The total and N-terminal amino acid analyses and starch and polyacrylamide electrophoresis patterns have been given previously[5,6].

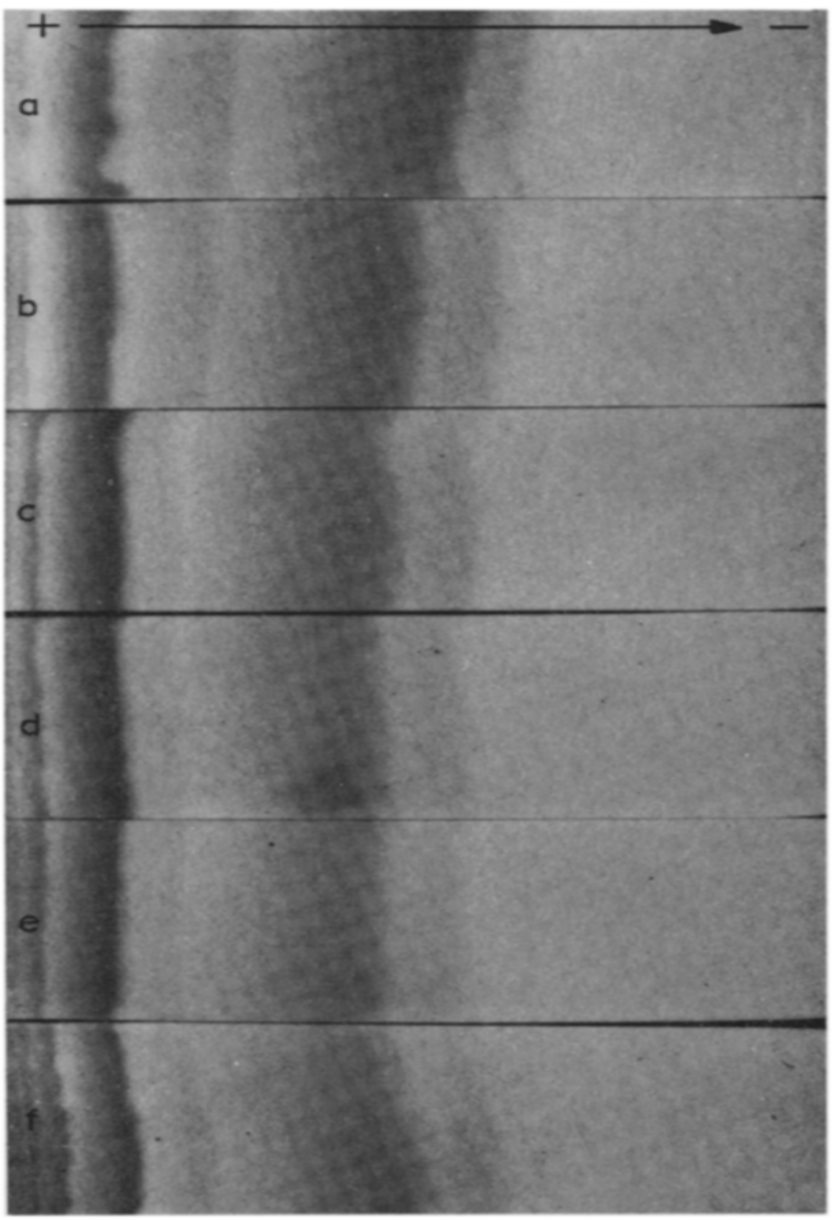

Fig. 1. Starch gel electrophoresis patterns of arginine-rich histones f3 applied in solutions of varying pH. For details see text. (a) pH 2.0; (b) pH 3.0; (c) pH 4.0; (d) pH 5.0; (e) pH 6.0; (f) pH 7.0.

Starch gel electrophoresis. Electrophoresis was carried out at pH 2.3 by a modification of the method of SMITHIES[7] described by JOHNS, PHILLIPS, SIMSON AND BUTLER[8]. In this method the sample is normally applied in 0.01 *N* HCl at approximately pH 2. In these experiments however the conditions were varied as follows. Histone f3 (10 mg) was dissolved in 3 ml of 0.01 *N* HCl, adjusted to pH 2, and a 0.15 ml sample taken. The pH of the solution was then raised slowly by adding 0.1 *N* NaOH and samples (0.15 ml) taken at pH values of 3, 4, 5, 6 and 7. All solutions were allowed to stand at room temperature for 30 min, and then run in starch gel at pH 2.3 in the usual manner[8]. The results are shown in Fig. 1. It can be seen that the bands near the origin are increasing in number and intensity with increasing pH.

Because of this apparent aggregation of histone f3 with increasing pH it was thought necessary to test the other three histone groups in a similar manner. The results for histones f1, f2(a) and f2(b) show that under these conditions there is no aggregation similar to that obtained with histone f3.

Discussion

It can be seen from the results given in Fig. 1 that with increasing pH the arginine-rich histone f3 aggregates giving a multiplicity of bands unable to migrate far into the gel, presumably polymers of a basic unit. This type of aggregation is not easily reversible since the aggregates remain stable on entering the gel which is at pH 2.3.

It is of interest that f3 is the only histone fraction to aggregate in this manner under these conditions. It is unlikely to be due to its high content of arginine (12–13 %) since fraction f2(a)1 has a similar arginine content but does not aggregate in a similar manner. Histone f3 is however the only histone fraction to contain thiol groups[9] which may be connected with the mechanism of aggregation.

It is apparent that a multiplicity of bands on starch gel electrophoresis under the conditions described here is not valid evidence of heterogeneity and that when working with arginine-rich histones the pH should be kept low (say between pH 1 and 3) since the aggregation which takes place at pH 7 is not easily reversed by lowering the pH.

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