4. The stability to water of the various types of halogenated esters varies as follows: the aromatic acid chloride-aliphatic aldehyde addition compounds are the most stable; the aromatic acid halide-aromatic aldehyde next; and the aliphatic acid halide-aliphatic aldehyde compounds the least stable.

5. The aliphatic acid halide-aliphatic aldehydes form condensation products in 50 to 70% yields, the aromatic acid halide-aliphatic aldehydes form products in 40 to 60% yields.

6. The action of ammonia, certain amines, pyridine, quinoline, potassium hydroxide and water, on chloromethyl acetate and benzoate was studied. The reactions of the halogenated esters from aliphatic acid halide-aliphatic aldehydes and aromatic acid halide-aliphatic aldehydes resemble those of the aromatic acid halide-aromatic aldehyde compounds described in a previous paper.

URBANA, ILLINOIS.

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A METHOD OF PURIFYING CERTAIN KINDS OF PROTEINS.[1] (Preliminary Paper.)

BY ADA M. FIELD.

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The importance of the iso-electric point in connection with the behavior of proteins has been emphasized by a number of investigators since it was first recognized and defined by Michaelis.[2] Jacques Loeb[2] recently reported a series of experiments on gelatin showing that on one side of the iso-electric point gelatin behaves as an acid, combining with positive ions only, while on the other side it behaves as a base and combines with negative ions only. A compound such as calcium gelatinate must therefore give up its calcium ions when brought into equilibrium with acid of greater hydrogen-ion concentration than the iso-electric point of gelatin, and gelatin chloride likewise give up its chloride ions if the hydrogen-ion concentration of the solution falls below that of its iso-electric point. These considerations suggested a simple method for purifying proteins having iso-electric points differing considerably from the reaction of pure water. Any protein with iso-electric point at a hydrogen-ion concentration greater than that of water will lose any combined basic elements when dialyzed for a sufficient length of time against dilute acid, and the resulting acid-protein compound can then be completely hydrolyzed and the acid removed by dialysis against pure water. Likewise a protein with iso-electric point at a concentration of hydrogen ion below that of

[1] Michaelis, *Biochem. Z.*, **24**, 79–91 (1910).

[2] Loeb, *J. Gen. Physiol.*, **1**, (1918–1919).

water might be purified by treating first with dilute alkali and then with water.

Since powdered gelatin is easily handled the method was first applied to this substance. The iso-electric point of gelatin, according to Michaelis,[1] is at $C_{H^+} = 2.5 \times 10^{-5}$. Accordingly it was treated first with acid as follows.

Thirty g. of powdered "Silver Label" gelatin was placed on a piece of washed linen lawn about 30 cm. square, the corners of the lawn brought together at the center, and the edges sewed firmly together. This bag was then immersed in 600 cc. of 0.002 *M* hydrochloric acid in a Pyrex beaker and allowed to stand at a temperature below 20° for an hour or more, when it was lifted to a Büchner funnel and drained with moderate suction. This process was repeated with successive fresh portions of the acid. According to Loeb, 4 washings of essentially this character will bring gelatin to its iso-electric point, and our results agree with this statement. After 6 washings the concentration of acid was changed to 0.001 *M* and 4 more washings given. By this time the wash water was practically free from ash, and distilled water was substituted for the acid. The temperature was kept at about 5° and the wash water changed once or twice daily until there was practically no change in the hydrogen-ion concentration of water by standing for 24 or more hours in contact with the gelatin. After the first few washings with acid the solubility of gelatin in cold water is very slight and consequently there was surprisingly little loss.

The chloride-free gelatin, drained under pressure, contained about 90% water. It was made into an approximately 2% solution, centrifuged and filtered through cotton wool in order to remove fibers and dust particles.

The warm solution is opalescent and freely mobile, but it readily solidifies into an opaque white jelly. The addition of either acid or base to this white jelly causes it to become transparent like ordinary gelatin. An amount of the solution containing 1.58 g. of gelatin yielded no ash.

The properties of the purified gelatin are under active investigation, as is also the applicability of the method to the purification of egg albumin.

NEW YORK, N. Y.

[1] Michaelis, *Biochem. Z.*, 41, 373 (1912).