CXX. CHEMICAL CHANGE IN GELATIN RESULTING FROM THE METHOD OF STORAGE.

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THE occurrence of intramolecular change in proteins as the result of physical treatment has been exemplified in earlier researches on gelatin, wherein it was shown, *inter alia*, that the dissolving of gelatin in water at 37° leads to a continuous irreversible change into the soluble form [Schryver and Thimann, 1927]. That the mere keeping of gelatin in a moist state also appears to produce chemical change is the subject of this note.

Previous investigations [Knaggs and Schryver, 1924] have shown that gelatin, after having been freed from ash, may be freed from colloidal impurities by flocculation in an electric field. In using this method it was found that by flocculation of a 1.5 % hydrochloric acid solution of gelatin at p_H 3, the crude gelatin may be freed simultaneously from ash and from colloidal impurity. Only simple apparatus is required, the platinum poles used being enclosed in vessels separated from the gelatin solution by semipermeable collodion membranes (Necolloidine) and washed constantly with a stream of water. As soon as the bulk of the inorganic matter and acid has been removed by the current, the gelatin is precipitated as a gel, free of ash and colloidal impurities but not of soluble gelatin (ash content, 0.001 %). The soluble gelatin can, if desired, be afterwards separated by a similar flocculation from neutral solution, when the insoluble gelatin only is precipitated. It is a point of interest that the gel is periodic in structure, consisting of alternate layers of clear gel and opaque flocculum.

The gel obtained as above consists therefore almost entirely of true insoluble gelatin and soluble gelatin. It can be either stored as gel, under toluene, or dried down in a current of cold air, and these two different methods of storage apparently lead to different products.

It was pointed out in a previous communication [Thimann, 1930] that the amount of nitrogen precipitated from a gelatin hydrolysate by phosphotungstic acid offered a sensitive indication of chemical differences, if, and only if, it was used over a considerable range of nitrogen concentration. A curve similar to that described therein, *i.e.* nitrogen-concentration plotted against

the percentage nitrogen precipitated by phosphotungstic acid, was therefore determined for gelatin prepared as described above, and stored as gel (curve A). In another exactly similar preparation, starting from the same crude material, the gelatin was dried down immediately on completion of the experiment, and the curve again determined (curve B). Though the difference between these two products is only one of the method of storage, it will be seen that the second yields considerably more diamino-acid precipitate.

Fig. 1. Effect of storage of gelatin on diamino-nitrogen fraction.

× Stored as gel. □ Stored dry.
 ○ Stored as gel and afterwards dried down.

In the hope of discovering whether the change is reversible, some of the gelatin stored as gel was afterwards dried down in a current of cold air. Recovery, apparently, did not take place, for the curve obtained (curve C) lies close to that for the gelatin preserved in the moist condition. Nevertheless, there is a curious irregularity, which may indicate some degree of reversibility in the change produced.

It appears, therefore, that moist storage produces a gelatin chemically different from the normal dry-stored product. Another explanation of the differences may lie in the fact that the dried material may be more slowly hydrolysed than the moist gel. If this were so, the curve for the dried material would correspond to that for gelatin exposed for a short time to acid treatment before hydrolysis, that is, it would lie, in general, above the normal curve, as indeed it does. If true this would indicate that for rigid comparison,

proteins must be in the same state of moisture or dryness before hydrolysis. Since, however, the hydrolysis was in all cases carried out by dropping the solid into the boiling acid, this suggestion does not seem probable.

Whichever of these explanations is the true one, the phenomenon further confirms, if confirmation be necessary, the extreme sensitiveness of the phosphotungstic acid precipitation over a range of concentration as an indicator of intramolecular change.

The variations in the curves are unfortunately too slight to allow of their being ascribed to any particular amino-acid.

A possible analogous difference between gelatin dried down as sol and as gel has been pointed out by Gortner and Hoffmann [1927] in regard to the degree of imbibition of water. This, however, although it may have a chemical cause, is a more purely physical phenomenon than the diamino-acid content, and may be explicable on a purely mechanical basis.

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