by February 27th—in about 20 days—the whole animal was bare to the skin with the exception of the head and neck. Considerably later the wool from this area also was shed.

To find out whether thyroxin would have the same effect on the normal sheep, two were selected and each received ¼ mg. subcutaneously every second day beginning on February 13th. In about a month one of these reacted as the thyroidectomized lamb had done, although the shedding of the wool was not so extensive, but the other showed no effect, although later the dose for this animal was increased to 1 mg. every second day and continued till April 27th.

Following thyroidectomy the fleece becomes coarse and ragged giving the animal a tattered, unkempt appearance but the complete and rapid denudation produced by the subcutaneous injection of thyroxin has not been observed in our flock to follow removal of the thyroids. A more detailed statement will follow.

ABSTRACTS OF COMMUNICATIONS.

Peking Branch.

Eighth meeting.

Peking, China, April 10, 1924.

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Some new observations concerning the effects of dilute acids and alkalies on proteins.

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Although it has been long known that proteins are subject to changes commonly called denaturation whereby their solubility is altered, our knowledge of the nature and the products of these changes is very limited.

The present study of the effects of dilute acids and alkalies on proteins originated from an observation made by one of us[1] some

[1] Wu, H., *J. Biol. Chem.*, 1922, li, 33.

time ago, that serum proteins which had been dissolved in dilute NaOH solution gave more color with the phenol reagent of Folin and Denis than the same proteins which had not been dissolved in NaOH. With the hope of developing a method based on this observation for the differentiation of proteins, a study of the change in chromogenic value of a number of proteins, both in HCl and in NaOH solutions, was undertaken. Changes of chromogenic value were observed in so dilute solutions of acids and alkalies that no question of the decomposition of the protein could arise, and it occurred to us that the phenomenon under observation may be related to the process of denaturation of proteins. Since there has been no investigation on this subject in recent years, it has seemed desirable to study not only the change in chromogenic value but also other effects which dilute acids and alkalies may have on proteins.

We have studied the problem from three points of view:

1. Change of solubility as evidenced by the formation of a precipitate upon neutralizing the solution.

2. Increase in the amount of color obtained when a definite amount of the protein solution is allowed to react with the phenol reagent of Folin and Denis in the presence of sodium carbonate.

3. Liberation of non-protein substances which can be determined after the removal of the protein.

The routine procedure of our experiments was as follows: An approximately 1 per cent protein solution made faintly acid to methyl red was mixed with an equal volume of N/10 HCl or NaOH, giving a solution about 0.5 per cent in protein and 0.05 N in acid or alkali. The same solution was used for the study of all the three effects mentioned above.

For the study of change of solubility, 100 cc. portions of the solution were measured out and neutralized at different intervals with 50 cc. N/10 NaOH or HCl, as the case may be. The precipitate formed was filtered off and the protein which remained in the filtrate was determined by the Kjeldahl method.

For the study of chromogenic value 1 cc. portions were measured into 50 cc. Erlenmeyer flasks, and at different intervals, diluted with 20 cc. H_2O followed by 0.5 cc. of phenol reagent and 3 cc. of 20 per cent Na_2CO_3. The color thus developed was compared with that of a solution prepared at the same time and in a similar manner from 1 cc. of the original protein solution which had been diluted with an equal volume of H_2O.

For the study of non-protein substances, 100 cc. portions of the solution were measured out, and at different intervals, neutralized with 5 cc. N HCl or NaOH as the case may be; 10 cc. each of 10 per cent sodium tungstate and 2/3 N sulfuric acid were then added and the precipitated protein filtered off. Convenient amounts of the filtrate were taken for the determination of ammonia and chromogenic substances. For the determination of the latter substances tyrosine solutions were used as standard.

The results of our investigation are as follows:

1. All the albumins and globulins which we have studied show change of solubility in 0.05 N HCl or NaOH. The list of these proteins includes the albumins of the egg white of hen, pigeon, goose, duck, serum globulins and albumins of sheep and horse, and the globulin of hempseed, edestin. The solubility of gelatin, proteose and peptones were not changed by dilute acids and alkalies.

2. The velocity of denaturation measured by the change of solubility varies with different proteins. In solutions containing about 0.5 per cent protein in 0.05 N HCl or NaOH the time required for half denaturation of some of the proteins is as follows:

	HCl	NaOH
Hen egg albumin	9 hours	2 hours
Goose egg albumin	3 days	1 minute
Duck egg albumin	2(?) days	2.7 minutes
Pigeon egg albumin	3 hours	——
Horse serum euglobulin	6 minutes	4(?) seconds
Horse serum albumin	——	15 minutes
Edestin	very rapid	very rapid

3. The hydrogen ion concentration decreases in acid protein solution and the hydroxyl ion concentration decreases in alkaline protein solution. This, we believe, is due to an increase in the acid and base binding power of the protein.

4. The velocity of denaturation increases with increasing hydrogen ion concentration or hydroxyl ion concentration. An exception is found in horse albumin which remains unchanged in 0.05 N HCl although it can be denatured in 0.02 N HCl.

5. The velocity constant calculated on the assumption that denaturation is a monomolecular reaction, falls off rapidly with increase in time. Three explanations are offered: (1) the decrease in hydrogen ion concentration or hydroxyl ion concen-

tration already mentioned; (2) only a part of the original protein molecule forms insoluble product, while the remainder is soluble; (3) the insoluble protein undergoes further change into soluble products.

6. All the proteins which show change of solubility also show increase in chromogenic value in 0.05 N NaOH or HCl solution. Gelatin, proteose and peptone show no increase of chromogenic value.

7. The form of the chromogenic value-time curve resembles closely that of the denaturation-time curve.

8. The increase in chromogenic value is not due to the formation of new reactive groups towards the phenol reagent, but rather to an increase in the reactivity of protein molecule so that more groups take part in the reaction. This increase in reactivity can be explained by the protein molecule becoming smaller.

9. The precipitability of the protein by complex acids (*e. g.*, phenol reagent) decreases as the chromogenic value increases.

10. The chromogenic value of the protein denatured by alkali is different from that of the same protein denatured by acid.

11. Ammonia and non-protein chromogenic substances which are probably tyrosine and tytrophane are liberated from the proteins in 0.05 N NaOH at a measurable rate, but only very slowly in 0.05 N HCl solution. The course of liberation of these substances does not run parallel with those of change of solubility and of chromogenic value. The curve for the former change is nearly linear when those for the latter have come practically to a standstill.

12. Hydrogen sulfide is split off from the proteins in alkaline solution but not in acid solution. H_2S forms an inappreciable portion of the non-protein chromogenic substances.

From the above mentioned facts the following conclusions may be drawn:

1. The change in solubility and the increase in chromogenic value of proteins are the results of the same underlying reaction which is commonly called denaturation.

2. The velocity of denaturation, being different for different proteins, should prove of great value for the differentiation of proteins.

3. The underlying reaction of the denaturation of proteins by dilute acids and alkalies is a hydrolysis, probably of some especially labile bonds.

4. The liberation of non-protein substances, NH_3, H_2S and other chromogenic substances is not an essential feature of the denaturation of the protein.

5. The products of denaturation by acids and by alkalies are different.

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The application of the eosin-criterion for the viability of protozoan cysts to cysts of Hartmanella hyalina treated with chlorine-water.

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Owing to the fact that no satisfactory method has been developed to induce the excystment and growth of the amebæ parasitic in the human intestinal tract, no absolute criterion has been established to distinguish the viable from the non-viable cysts. The penetration of the cysts by analine dyes is regarded as an indication that the cysts are dead and the use of eosin as an indicator has been generally accepted. The ultimate fate of the cysts which fail to take the eosin stain has not been finally determined. It has been shown that cysts which undergo a plasmolysis may not stain red by the eosin, though they are considered to be incapable of development.

The present investigation was undertaken in order to determine the percentage of free chlorine in water necessary to retard the development of cysts of *Hartmanella hyalina* and at the same time to determine the fate of the cysts which presented an apparently normal appearance. It is hoped that by comparing the resistance of the cysts of the human intestinal Protozoa with the resistance of the coprozoic ameba under consideration that information may be procured regarding the relative value of methods employed in disinfecting fruits and vegetables for table use and regarding the general resistance of Protozoan cysts.

Cysts of *Hartmanella hyalina* were placed in water containing different percentages of free chlorine in solution. A ten minute