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

interval was allowed before the cysts were transferred to a suitable culture medium which in this investigation was 10 per cent horse serum in Locke's solution diluted with 50 per cent distilled water. The cysts were counted at the time of their transfer to the culture medium and the reaction of a representative number of the cysts to Donaldson's iodine-eosin was noted. The cultures were kept under observation until all possibility for further excystment had occurred. Tests were made with sixty-four different cultures which had been placed in chlorine-water, the percentages of free chlorine which ranged from .006 to 4.

The following general conclusions may be drawn:

1. Two per cent of free chlorine in water is the strongest solution which will permit excystment and development of cysts of *Hartmanella hyalina* after having been exposed to the solution ten minutes. This is approximately the amount of free chlorine liberated by placing 32 gm. or eight teaspoonsfull of chlorinated lime in one pint of water.
2. Red cysts and plasmolyzed cysts do not undergo excystment and development.
3. Partially plasmolyzed cysts may, after a delayed period, take the eosin stain but the greater the degree of plasmolysis, the less likelihood there is for the penetration of the eosin.
4. All cysts which appear normal at the time of transfer and assume the green color produced by the iodine are not viable, but some may, after a period, ranging from a few hours to 72 hours, stain red by the eosin.
5. This lag period varies according to the concentration of the chlorine water used and probably also varies according to the resistance of the cysts in question and according to the species involved. It should be considered in future experimental work on the resistance of Protozoan cysts where the eosin-criterion is used for determining viability.