Biochemical work on lysozyme

The object of the work was to crystallize ~~an~~ lysozyme from several ~~another~~ species of birds, ~~not~~ on the chance ~~in the hope~~ that one of them would ~~give~~ be more crystallographically suitable than chick lysozyme. It was hoped that they ~~would~~ could be prepared by using only small variations on the standard method for chick.

~~Dis~~

~~Spontaneous crystallisation~~

~~The standard method for ch~~

~~Estimat~~

Amount of ~~y~~ lysozyme present

Lysozyme was estimated throughout ~~this~~ in the usual way with micrococcus lysodiek.... . It was possible to detect with certainty rather less than 0.2 micrograms in 3 cc. Assuming that the different lysozymes ~~had~~ have the same activity per gram, the results were

Species	~~D~~ wt. of white	wt of "lysozyme"
~~Hen~~ Chick	32 gm	0.10 gm ~~0.1 gm~~
Guinea Fowl	(20 gm)	(0.09)
Turkey	50 gm	0.09 ~~0.11 gm~~
Duck	46 gm	0.08 gm ~~0.09 gm~~
Goose	(70)	(0.04 gm).
Lesser black-back gull	46 gm	trace only

Some human tears ~~tested~~ gave an activity rather higher than chick white.

All the above results are very rough, being done on one or two eggs only, and not always with the best testing technique. They show that 3 species have about the same total amount of lysozyme per egg as chick, that Goose has rather less, and therefore is rather dilute, and that the gull has effectively none.

Armour chick lysozyme was used as the standard of comparison, its N content being kindly determined by Dr. H. Outhwaite by micro-Kjeldhal. A parallel check by U.V. absorption gave fair agreement.

Direct crystallisation

Chick lysozyme is prepared by adjusting the homogenised white to pH 9.5 with NaOH, adding 5 % NaCl, and storing in the cold. It is advisable to seed with a small amount of crystalline lysozyme, (Alderton - - -)

After some days the lysozyme crystals are centrifuged off, dissolved in acetate, etc.

Experiments were first done without seeding. After several weeks no crystals had formed (for any species (Goose produced some salt crystals)). Later the chick spontaneously produced crystals.

The five species were then all seeded with Armour chick

lysozyme. The hen chick soon produced copious crystals, the other species none. (Goose produced some such crystals).

~~From we may conclude that the lysozymes of these different species are do not crystallise with chick lysozyme.~~

This suggests that the different species have different lysozymes. It then seemed necessary to obtain some seed crystal for the other species.

Diffusion experiments

An attempt was made to separate the lysozyme by diffusion through a cellophane membrane, as lysozyme being small is reported to go so through cellophane.

The homogenised egg white was put into dialysis sacs diameter ~ 7mm and about 30 cm long. Each sac was mounted in a glass tube internal diameter about 1 cm containing ~~distilled water~~ M/10 phosphate pH 6.2, and allowed to stand in the cold.

Some tubes were slowly rotated by clockwork in the cold, so that a sterile glass bead in the sac, and an air bubble in the glass tube produced occasional stirring.

After 1 week samples were removed and tested for lysozyme, but not much lysozyme had come through - about 10 % for chick. The stirring appeared not to make much difference. After a few more days the remaining outer liquid was removed & evaporated over P_2O_5. After considerable concentration, with some surface precipitation, the pH was roughly adjusted to about 9 using thymol blue. Next day all the tubes had crystals, probably of protein. Unfortunately ~~activity~~ lysozyme

tests showed these crystals had no lysozyme activity.

~~And further manipulation~~ The supernatant was Evaporated ~~supernatant~~ further + manipulated chemically to try to bring down lysozyme crystals, but without success, as only amorphous ~~crystals were~~ precipitates were obtained ~~and~~ (possibly liquid crystals for Duck). These precipitates were used to seed ~~The Tried seeding~~ the egg white in the standard preparation ~~with these~~, and ~~[illegible]~~ some crystals were formed. However on seeding further egg white (of the same species) with these ~~[illegible]~~ no further crystals were formed. ~~so suspect~~

~~[illegible]~~ The original crystals were probable due to the salt ~~[illegible]~~ added with the first lot of seeds. An attempt was made to separate them by the standard method and check their activity, but the bug test showed ~~showed~~ it was very small, + probably due to contaminating white.

~~Thus it is probable, but not certain, that~~

~~[illegible] Later an attempt was me~~

~~Thus it is probable but not certain that~~

Thus it is possible that even if seed crystals ~~were~~ are available the other species may not crystallise out of egg white under the ~~same~~ conditions which chick does.

Crystalline lysozyme was successfully prepared ~~for~~ ~~[illegible]~~ by the standard method from one of the chick eggs.

Bentonite preparation

A start was made on the standard bentonite preparation. Preliminary work on Turkey & Duck showed the yield was lower than on chick. However no crystals were produced even for chick, possibly because the quantities used were too small. The amorphous precipitate of Turkey & Duck were used as seeds in the standard direct crystallisation, but without success.

Conclusion

The eggs of guinea fowl, turkey, duck and to a lesser extent goose have lysozyme activity comparable to that of chick.

The lysozymes of these species are probably not identical with chick lysozyme. The direct crystallisation method evolved for chick appears not to work for the other species, though it is just possible it might do so if some crystals of each species were available as seeds. The yield of the

Appendix on the Seq test

The initial culture of micrococcus lysodeikticus was supplied by Dr. Gale of the Microbiological Unit. It was cultured on agar slants in boiling tubes, the inoculation being done with a platinum loop. Thus the cultures were dry cultures and seemed to be better than those grown by the Low Temperature Station's wet method. The agar was a '1/10 casein in nutrient agar' supplied by

. After innoculation they were incubated for about 2 days at 30°C, and then allowed to stand, if necessary, for a few days at room temperature till required. If not used they were subcultured after a month or so, or sooner as required.

On the morning of the experiment a sterile glass bead was added to each culture, and 1 c.c. of sterile (boiled) phosphate. This was M/10 pH 6.2 phosphate which was used throughout. After shaking, the suspension of bugs was poured off. A further 1 c.c. was added, shaken & poured off. This dense suspension was now diluted with about 12 to 15 times its volume of standard buffer, so that as used in the test (ie diluted by half) its "optical density" at 560 mμ on the Unicam was about 1·2. This was checked.

In the test 3cc of this bug suspension was ~~diluted~~ mixed with 3 cc of the test ~~tube~~ solution, ~~suitably diluted with standard buffer~~ in a small test-tube and incubated for 1 hour at 37°C. The "optical density" at 540 mμ was then measured on the unicam. To obtain uniform results it was important to shake the bug suspension continually when pipetting out the 3 cc and to mix thoroughly by pouring backwards + forwards, as the bugs ~~tend to~~ sediment very easily. The test-tube was also inverted when adding the test solution at the end of the incubation. It was briefly cooled to room temperature before measuring. In spite of all precautions the results were rather variable, ~~parallel determ~~

The test solutions were diluted with the standard buffer to give a final estimated strength of about 0·2 micrograms lysozyme per cc. ~~Dilution~~ A series of ~~accurate~~ Several dilutions, usually in steps of 2, were tested. ~~expected~~ A ~~standard solution of~~ lysozyme solution of known N content was used as a standard, about 6 dilutions (over a 1 to 10 range) being used ~~as to~~. From the results a curve of optical density versus lysozyme concentration was plotted.

The unicam is really too good an instrument to use for this test. It would have been better to have a simpler ~~an~~ instrument which could measure the ~~turbid~~ suspension in the ~~test~~ test tube, as this would

speed up the test. With the unicam ~~the~~ one can only test about 1 tube every 4 minutes, ~~or they~~ and they have to be put into the bath as well as taken out at ~~these~~ this interval.

A complete test of about 6 or 8 samples, dilution, working out and all, takes the best part of a days work, ~~as the dilution thereof~~