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ON A FERMENTATION CAUSING THE SEPARATION OF CYSTIN. (Preliminary Communication.) By Sheridan Delépine, M.B., B.Sc.

During the months of March and April 1889, I analysed for Dr Lauder Brunton, and under his direction, a number of specimens of urine containing cystin. The estimation of the amount of this substance present in the samples examined was carried out by Löbisch's process, and revealed certain variations which were of interest as connecting the elimination of cystin with the processes of digestion. In carrying out this work, I was struck with the fact that the amount of cystin precipitated from the same specimen was greater under certain circumstances than under others. Thus, (1) when specimens were *strongly acidified with acetic acid*, as recommended by Löbisch, the precipitation took place more slowly than if the specimens were allowed to undergo a *spontaneous acid fermentation* (which never caused the reaction to become very strongly acid). (2) When the fluids were *carefully filtered*, the precipitation of cystin was delayed, often for several days. (3) When a specimen in which cystin had begun to separate was *carefully filtered*, the precipitation was interrupted for several days. (4) When portions of a urine which was proved by collateral experiments to yield cystin were *kept at a temperature of* 60° C., no cystin could be separated afterwards by the usual processes. (5) *Evaporation* did not seem to increase materially the amount of cystin obtainable from a given specimen. (6) The largest amounts of cystin could be obtained by allowing the *specimens to stand at the ordinary temperature for several days*, provided the precipitate was separated whilst the urine was still acid. (7) A similar amount of cystin could be obtained more rapidly by keeping the fluid at a *temperature higher than the normal, but less than* 40° C., for twenty-four to thirty-six hours. (8) When a drop of urine from which cystin was being deposited was added to a portion of the same urine carefully filtered, a deposit of cystin occurred in twenty-four hours, while another

portion of the filtrate protected from organisms deposited no cystin for ninety-six hours. From all these facts I venture to conclude—

(1) That the *simple* addition of an acid in which cystin is not soluble is not sufficient to separate cystin from the urine, and, therefore, that the *theory generally held as to the state of combination of cystin in the urine is probably inaccurate.*

(2) That a compound exists in *certain* urines which under the *influence of a fermentation* yields cystin.

(3) That the fermentation is due to the *growth of an organism*, which can apparently be separated from the urine by ordinary filtration, and must therefore be a *large organism, possibly* (*but not necessarily a torula*).

(4) That the cases recorded in which cystin has been found deposited in the kidneys and liver indicate that the *separation may begin in the system* (whether owing to a fermentation, or to an oxidation process brought in some other way, cannot be decided at present).

APPENDIX.

In order to facilitate the understanding of the above facts, which may at first sight seem to be not quite conclusive, the following explanations may be found useful. (The numbers correspond to those used in the text.)

(1) *a.* Acetic acid is added with the view to displace cystin from a compound in which it is supposed to be fully formed (other acids are not used, because, with the exception of tartaric acid, cystin is soluble in them). If it were owing to the acid properties of acetic acid that cystin is precipitated, acetic acid should cause precipitation much more readily than the feeble acidity due to the ordinary acid fermentation of urine. *The reverse, however, takes place.*

β. Micro-organisms generally grow better in feebly alkaline or feebly acid solution than in strongly acid fluids, therefore, if the precipitation of cystin is due to a fermentation, strong acidification should delay it, *and that is what occurs.*

(2) The delay in precipitation caused by filtration, shows that before cystin is precipitated there is something in the fluid which can be separated by filtration, and in the absence of which cystin is not precipitated readily. The fact that precipitation is only delayed, shows that the body after being separated can form again in the fluid (under ordinary circumstances).

(3) That filtration stops the process of precipitation, even after it has begun, shows that the precipitation is not due to a simple chemical change taking place in the fluid either under the influence of an

enzyme or some other soluble compound; but that a gradual change takes place under the influence of a particulate body large enough to be separated by filtration through Swedish filter-paper.

(4) A temperature of 60° C. is much below that at which cystin is decomposed; it is, however, sufficient, when continuous, to stop the growth of micro-organisms.

(5) The fact that evaporation does not increase the amount of cystin obtainable from a given quantity of fluid, shows that during the fermentation process concentration of fluid has nothing to do with the separation of the crystals.

(7) A temperature of less than 40° C. does not cause the death, and favours the growth of many organisms; the accounts for the difference then is in the results obtained when the fluid is kept at temperatures above or below 40° C. See above (4).

(8) Inoculation is simply a confirmatory test of the conclusions which had been arrived at from the previous observations.

It is therefore evident—

(1*st*) That conditions which favour the growth of micro-organisms in a urine capable of yielding cystin, accelerate and increase the amount of cystin obtainable from that fluid.

(2*nd*) That conditions which hinder the growth or cause the removal or death of micro-organisms, retard or prevent the precipitation of cystin.

(3*rd*) That the direct addition of micro-organisms to the fluid caused a more rapid precipitation than simple acidification.

(4*th*) That some of the methods used in separating organisms from the fluid, showed that the most important of these organisms must be a large one, such as a torula.

Hence the general conclusions arrived at.