Lillie[1] showed that the activity of sea-urchin sperm is increased upon the addition of egg water. The preparations of limpet and of sea-urchin agglutinins also have this effect, as shown by direct observation and by measurements of the respiratory rate of the spermatozoa. In Arbacia it has been reported[7] recently that echinochrome causes the increased activity of the spermatozoa. The eggs of Strongylocentrotus do not contain echinochrome and attempts[8] to increase the activity of the spermatozoa with this substance have proven unsuccessful.

In both the limpet and the sea-urchin, the agglutinin is obtained in highest titer when the jelly surrounding the eggs is dissolved by means of acid. Other evidence points to the agglutinin being either identical with the jelly or located in it, but not continuously produced by the eggs. The limpet agglutinin is extremely heat-stable, being half-inactivated only after 24 hours boiling at pH 3. The sea-urchin agglutinin is half-inactivated after 5 minutes boiling. Both sea-urchin and limpet agglutinins are stable in isotonic NaCl but not in distilled water. At alkaline pH in sea water the agglutinins are absorbed by the precipitate of calcium and magnesium carbonates and hydroxides that forms. In isotonic NaCl the agglutinins are rapidly inactivated above pH 11 and below pH 2.

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THE CONTROL OF EXPERIMENTAL ALCAPTONURIA BY MEANS OF VITAMIN C[1]

IN a study of the relation of vitamin C to the metabolism of the melanin pigment precursors tyrosine and dihydroxyphenylalanine in the guinea pig[2] it was observed that the animals receiving tyrosine excreted in the urine a substance of melanin-like properties. Upon examination this proved to be not melanin but homogentisic acid. The production of experimental alcaptonuria in the white rat by phenylalanine feeding has been recently reported by Papageorge and Lewis[3] and by Butts, Dunn and Hallman.[4] Our results may be considered as a further example of artificial alcaptonuria although arising from tyrosine feeding and in another species, the guinea pig. In our experiments it appeared that the animals receiving the smaller doses of ascorbic acid excreted the greater amount of homogentisic acid. Experiments were then designed to test this possibility. The guinea pigs were housed in metabolism cages and the urine collected for quantitative analysis. The feeding of 0.5 gm of l-tyrosine with an amount of basal diet containing 0.5 mg of ascorbic acid per day resulted in the excretion of 20–50 per cent. of the theoretical amount of homogentisic acid when determined by the method of Briggs.[5] With the addition of 5 mg of ascorbic acid per day all but a trace of the homogentisic acid disappeared from the urine within one or two days. Subsequent withdrawal of the extra vitamin C resulted in the reappearance of the homogentisic acid to the same extent as before within one to three days. This process could be repeated at will. In order to confirm the results of the quantitative procedure the homogentisic acid was identified by means of the usual qualitative tests, by the characteristic behavior of the lead salt and finally by isolation and identification of the dibenzoyl homogentisamide by the method of Papageorge and Lewis.[3]

The effectiveness of ascorbic acid in controlling this artificial alcaptonuria in the guinea pig led us to perform experiments on two normal human subjects. On a diet practically free of ascorbic acid the daily ingestion of l-tyrosine resulted in the excretion of significant amounts of homogentisic acid which could be completely prevented by the ingestion of reasonably large doses of crystalline ascorbic acid. The lack of alcaptonuric patients in this vicinity has made it impossible to test the effect of extra amounts of the vitamin on an individual who normally excretes homogentisic acid, but we hope that such experiments can be carried out elsewhere in the very near future.

In a further study of this relation of ascorbic acid we have utilized the unnatural d-tyrosine and find that it also causes the excretion of homogentisic acid by the guinea pig although not to the same extent as does the natural isomer. More striking is the fact that we as yet have been unable to prevent the excretion of homogentisic acid arising from d-tyrosine by the administration of the amount of ascorbic acid which completely checks its production from l-tyrosine. This latter point is being further investigated.

The effectiveness of ascorbic acid in influencing the metabolism of tyrosine so as to prevent the excretion of homogentisic acid not only throws new light upon the physiology of this vitamin but furnishes an extremely useful tool in further studies on the intermediary metabolism of the amino acids phenylalanine and tyrosine.

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