PHYSIOLOGICAL PHENOMENA

Analisis Experimental de los Fenomenos Fisiologicos Fundamentales. By JOSÉ J. IZQUIERDO. xxii + 334 pp. Mexico, 1939.

DR. IZQUIERDO presents in this volume, which has been prepared for serious students of biology, a well-selected series of illuminating experiments accompanied by explanations and by stimulating questions. The procedure is that of letting the experiments lead the student from observations on physical and physico-chemical states and processes to observations on the properties and responses of living structures as affected by these conditioning agencies. Thus, diffusion, osmotic pressure and the characteristics of solutions are first considered, and then these phenomena are applied to an understanding of changes induced in simple biological units. Similarly, hydrogen-ion concentration, colloidal solutions and properties of interfaces, the polarization of membranes, chemical equilibria and the processes of oxidation and reduction are dealt with in such manner as to yield insight into an understanding of biological events. The final sections of the book are devoted to the phenomena of excitability and the contractions of cilia and muscles. Throughout, the student learns not only the important facts of general physiology but the application of the physical and chemical methods which are employed in examining biological responses to experimental tests. An introduction to the use of mathematics in evaluating data obtained by experimentation is a feature of the discipline imposed by the book. Frequent references to the original investigators whose experiments the student repeats bring him in touch with the masters of the subject.

In a preface Dr. Merkel H. Jacobs has characterized Dr. Izquierdo's book as "an admirable introduction to the fundamental principles of physiology." This judgment is well warranted. Spanish-speaking students who follow Dr. Izquierdo's excellent experimental course in the basic aspects of biological reactions should not only be very soundly instructed but should be stimulated to further studies.

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SPECIAL ARTICLES

SPERM AGGLUTINATION IN THE KEYHOLE LIMPET AND THE SEA-URCHIN

THE spermatozoa of the giant keyhole limpet, *Megathura crenulata,* show a striking agglutination reaction upon addition of egg water. The reaction differs from that described by Lillie[1] and Just[2] in the sea-urchin and other animals in that it does not spontaneously reverse. Instead of the clumps breaking up they continue to enlarge by fusion with one another and, in a sufficiently high concentration of the agglutinin, one large agglutinate forms containing most of the sperm. The spermatozoa agglutinate by the tips of their tails as well as by their heads. The agglutinates are spherical in shape with a thin shell of sperm heads at the surface separated from the central mass of sperm by a distance roughly equal to the length of the tail. In small agglutinates, the tips of the tails occupy the center. The reaction resembles that described by Sampson[3] for chiton sperm and by Henle, Henle and Chambers[4] for bull sperm in anti-sera. The head agglutination is, however, not due to a separate head agglutinin present in the egg water but represents an aggregation reaction.

The limpet agglutinin precipitates in nearly saturated ammonium sulfate, and it is retained by a collodion membrane. By these means active concentrates have been prepared. The preparations give definite xanthoproteic, Millon's and biuret tests. The agglutinin is inactivated by solutions of crystalline trypsin or chymotrypsin (supplied by the courtesy of Dr. J. H. Northrop). Complete inactivation is obtained in 6 days with 1 per cent. chymotrypsin at pH 8 and 22° C.; in 7 days with 1 per cent. trypsin. The controls retain practically their full activity during this period. During the first 3 to 4 days of digestion there is no appreciable loss of activity. After complete inactivation of an agglutinin solution of such titer as to give, when mixed with an equal volume of 1 per cent. sperm, a 10 second macroscopic reaction, the formol titration showed 8×10^{-6} equivalents of $-COOH$ per ml. of solution for the trypsin digests and 13×10^{-6} for the chymotrypsin.

In the sea-urchin, *Arbacia punctulata,* Glaser[5] reported obtaining no definite protein tests except for a faint xanthoproteic reaction. In *Strongylocentrotus purpuratus* we obtain, with material prepared by $(NH_4)_2SO_4$ precipitation and dialysis of concentrated egg water (care being taken to avoid injury to the eggs), the same protein tests as with the limpet. Also, the sea-urchin agglutinin is rapidly inactivated by one of the crystallized proteinases, namely, chymotrypsin.

[1] F. R. Lillie, "Problems of Fertilization," The University of Chicago Press. 1919.
[2] E. E. Just, *Protoplasma,* 10: 300, 1930.
[3] M. M. Sampson, *Biol. Bull.,* 50: 301, 1926.
[4] W. Henle, G. Henle and L. A. Chambers, *Jour. Exp. Med.,* 68: 335, 1938.
[5] O. Glaser, *Biol. Bull.,* 26: 367, 1914.

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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[7] M. Hartmann, O. Schartau, R. Kuhn and K. Wallenfels, *Naturwissenschaften*, 25: 433, 1939.; R. Kuhn and K. Wallenfels, *Ber. des deutsch. Chem. Ges.*, 72: 1409, 1939.

[8] A. Tyler, *Proc. Nat. Acad. Sci.*, 25: 523, 1939.

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] R. R. Sealock, B. Ziegler and R. L. Driver, *Jour. Biol. Chem.*, 128: lxxxix, 1939.

[3] E. Papageorge and H. B. Lewis, *Jour. Biol. Chem.*, 123: 211, 1938.

[4] J. S. Butts, M. S. Dunn and L. F. Hallman, *Jour. Biol. Chem.*, 123: 711, 1938.

[5] A. P. Briggs, *Jour. Biol. Chem.*, 51: 453, 1922.