suffer most. The book, in common with other texts, does not supply an anatomical organization underlying the elaboration of the acquisitive and actional functions which play such a large part in the biologic activity and achievements of the individual.

There are a few minor errors in the book. On the whole the student as well as the teacher will find it a useful text.

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## GENES AND THE MAN

*Genes and the Man.* By BENTLEY GLASS. 386 pp. The Science in Modern Living Series. Teachers College, Columbia University. 1943. $3.50.

ONE of the most encouraging phenomena of our time is that, as Professor Glass says, "throughout the realm of science the narrow, rigid boundaries of specialized fields of subject-matter are at last breaking down. The boundary between genetics and cytology has already disappeared, and it is now evident that embryology and physiology are beginning to enter the amalgam." This volume is not intended to be a new text-book of genetics. Rather, it "has been prepared to indicate a new outlook," namely, "that we should understand the epic sweep of an individual's growth and development up to maturity and the long years of slow decline thereafter, together with those tenuous bonds that link each generation with all before and after . . . by tracing them from their beginnings in protoplasm and the genes." This is a very ambitious undertaking, and considering its novelty and magnitude Professor Glass has mastered the task quite well.

The presentation begins with a discussion of the possibilities of spontaneous generation, of viruses, cell structures, cell division and of elements of cellular physiology. The concept of genes is introduced without reference to Mendelian heredity. In Chapter II we have a description of sex cells, fertilization, meiosis, mutation, Mendelism, linkage and crossing over. On page 118 a gene is defined as "a single member of the linear series of hereditary factors within each chromosome. Its unitary nature is defined by its separability from its neighbors through crossing over." The reviewer is afraid that such a definition may give comfort to those who doubt the existence of genes. A very good account of the genetic basis of sex is found in Chapter III; Chapter IV combines discussions of gene interactions, gene effects in development, embryonic induction, sex hormones, heterogenic growth and the nature-nurture problem. Chapter V is the longest, as it may well be, since it presents a condensed and yet very readable account of human embryology with excursions into comparative anatomy, physiology and endocrinology. The final chapter is concerned with biological aspects of vital statistics and physiology of ageing.

In a book which sets out "to describe the operation and interaction of those factors which make the physical man" one expects to find a discussion of man's evolution and of genetic evolutionary mechanisms, but these topics are almost completely ignored. However, this complaint may not be a fair one, for even as it stands a tremendous amount of diversified information is condensed between the covers of this medium-sized volume. At times one wishes either that some of the less essential information were removed to give a greater prominence of fundamentals, or else that the book were expanded much beyond its present size. In any case, Professor Glass must be congratulated with having produced a new and interesting type of book on general biology.

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# SPECIAL ARTICLES

## CONTROL OF GRAM-NEGATIVE BACTERIA IN EXPERIMENTAL ANIMALS BY STREPTOMYCIN[1,2]

STREPTOTHRICIN, an agent isolated from a soil Actinomyces, was found[3,4] to be effective against certain gram-positive bacteria, as well as against a variety of typical rod-shaped gram-negative bacteria, not only *in vitro* but also *in vivo*. These results were recently confirmed.[5] However, the action of streptothricin upon other gram-positive bacteria, such as *Bacillus mycoides,* and upon some gram-negative bacteria, such as *Pseudomonas fluorescens, Ps. aeruginosa, Proteus vulgaris* and *Serratia marcescens,* is rather limited. Recently, another antibiotic agent, streptomycin, was isolated[6] and found capable of acting upon these bacteria as well; otherwise, it resembles streptothricin in its chemical behavior and mode of action. This agent has been found to be active against various gram-negative bacteria also in the animal body.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

[2] With partial support from a grant made by the Commonwealth Fund of New York.

[3] H. J. Metzger, S. A. Waksman and L. H. Pugh, *Proc. Soc. Exp. Biol. Med.,* 51: 251, 1942.

[4] H. J. Robinson, "Some Toxicological, Bacteriological and Pharmacological Properties of Antimicrobial Agents Produced by Soil Microorganisms." Thesis, Rutgers University, 1943.

[5] H. J. Robinson, O. E. Graessle and D. R. Smith, SCIENCE, 99: 540, 1944.

[6] A. Schatz, E. Bugie and S. A. Waksman, *Proc. Soc. Exp. Biol. Med.,* 55: 66, 1944.

The preparation of streptomycin used in these studies was still in a fairly crude state, having about 30,000 dilution units[7] per gram of crude dry material. The toxicity of this preparation was LD0 = 35 mg and LD100 = 135 mg per 20 gm mouse, when injected intraperitoneally. The results of a typical experiment on the effect of streptomycin upon *Salmonella schottmülleri* are presented in Table 1. 6.4 mg of the material, equivalent to a total of 190 units, was sufficient to give complete protection to mice weighing 18–20 gm. The effect of streptomycin upon *Pseudomonas aeruginosa,* an organism known to be highly resistant to the antagonistic action of other bacterial or of antibiotic substances, since it itself produces two active antibacterial agents, is brought out in Table 2.

TABLE 1

ACTIVITY OF STREPTOMYCIN IN MICE INFECTED WITH S. SCHOTTMULLERI

| No. of mice | Treatment | Dilution of bacterial culture used for infection | Survival of mice, in hours | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 18 | 24 | 30 | 42 | 48 | 72 |
| 5 | Control* | $10^{-5}$ | 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | Control | $10^{-6}$ | 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | Control | $10^{-7}$ | 1 | 0 | 0 | 0 | 0 | 0 |
| 3 | Control | $10^{-8}$ | 2 | 0 | 0 | 0 | 0 | 0 |
| 5 | Streptomycin, 6.4 mg† | $10^{-5}$ | 5 | 5 | 5 | 5 | 5 | 5 |
| 5 | Streptomycin, 12.8 mg† | $10^{-5}$ | 5 | 5 | 5 | 5 | 4 | 4 |

* No streptomycin.
† Divided in 4 doses, every 6 hours.

TABLE 2

ACTIVITY OF STREPTOMYCIN IN MICE INFECTED WITH PS. AERUGINOSA

| No. of mice | Treatment | Dilution of bacterial culture | Survival of mice, in hours | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 18 | 24 | 30 | 42 | 72 | 168 |
| 5 | Control | $10^{-4}$ | 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | Control | $10^{-5}$ | 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | Control | $10^{-6}$ | 2 | 3 | 2 | 2 | 0 | 0 |
| 3 | Control | $10^{-7}$ | 3 | 2 | 2 | 2 | 2 | 2 |
| 5 | Streptomycin, 6.4 mg* | $10^{-4}$ | 4 | 3 | 3 | 0 | 0 | 0 |
| 5 | Streptomycin, 12.8 mg | $10^{-4}$ | 5 | 4 | 3 | 1 | 1 | 1 |
| 5 | Streptomycin, 6.4 mg | $10^{-5}$ | 5 | 5 | 5 | 5 | 5 | 4 |
| 5 | Streptomycin, 12.8 mg | $10^{-5}$ | 5 | 5 | 5 | 5 | 5 | 4 |

* Divided into 4 doses, injected every 6 hours.

The addition of 6.4 to 12.8 mg of streptomycin per mouse gave excellent protection against infection by this organism as well.

For the study of the effect of streptomycin upon fowl typhoid (*Shigella gallinarum*), 11-day-old chick embryos were used. The first four preliminary experiments gave rather striking results; all the controls died from the infection, whereas the greater number of embryos treated with streptomycin were protected. One of the difficulties encountered in these experiments was the fact that the embryos were readily injured by attempts made to give repeated injections, and many of them died from causes other than the infecting organism. The results of such a typical experiment are given in Table 3. A 24-hour broth culture of *Sh. gallinarum,* centrifuged for 12 minutes, at 750 r.p.m., was used. The bacterial count was $88 \times 10^6$ per milliliter. One-tenth ml portions of two dilutions of the bacterial preparation were used for infecting each embryo. The eggs were further incubated for 12 days, and the chicks remaining alive were killed. Cultures were made from the heart and liver of both the dead and the killed embryos and the hatched chicks. The results prove emphatically that streptomycin, if used in sufficient concentration, offers full protection to chick embryos against fowl typhoid, even the dead embryos showing complete freedom from the disease.

TABLE 3

ACTIVITY OF STREPTOMYCIN IN CHICK EMBRYOS INFECTED WITH FOWL TYPHOID

| No. of eggs | Treatment | Dilution of bacterial culture | Survival of embryos in days | | | | Died from fowl typhoid |
|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 7 | 12 | |
| 3 | Control | $10^{-6}$ | 3 | 1 | 0 | 0 | 3 |
| 3 | Control | $10^{-5}$ | 3 | 0 | 0 | 0 | 3 |
| 5 | Streptomycin, 5 mg | $10^{-6}$ | 4 | 3 | 2 | 2 | 1 |
| 5 | Streptomycin, 5 mg | $10^{-5}$ | 5 | 5 | 4 | 3 | 0 |
| 5 | Streptomycin, 10 mg | $10^{-5}$ | 5 | 4 | 3 | 0 | 0 |

Finally the results of an experiment on the effect of streptomycin upon *Brucella abortus* may be reported here. Fifteen-day-old chick embryos were used. A relatively small number of bacterial cells were injected into each embryo. The streptomycin was a crude liquid preparation containing 100 mg of dry, ash-free material per milliliter. Against this pathogen as well (Table 4), excellent protection was afforded by streptomycin.

TABLE 4

EFFECT OF STREPTOMYCIN ON BRUCELLA ABORTUS IN CHICK EMBRYOS*

| Total number of embryos | Treatment | Presence of *Brucella abortus* in embryos† | |
|---|---|---|---|
| | | Membrane | Liver |
| 5 | Control, untreated | 4 | 3 |
| 6 | Streptomycin, 5 mg | 2 | 0 |
| 6 | Streptomycin, 10 mg | 0 | 0 |
| 6 | Streptomycin, 20 mg | 0 | 0 |

* 4,000 cells of *B. abortus* injected into each embryo.
† The tests for the presence of *B. abortus* were made on the death of the embryo or on the 21st day of incubation of the eggs.

Complete protection in experimental animals was also obtained against *Proteus vulgaris.*

The authors are indebted to Dr. F. R. Beaudette, of the Poultry Department, for making the facilities of his laboratories available for the tests on *Shigella,* and to Dr. H. Robinson and Miss D. Smith, of the

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## IN VITRO FERTILIZATION AND CLEAVAGE OF HUMAN OVARIAN EGGS[1,2]

FIRST stages in the cleavage of the fertilized human egg have, as far as we know, never been reported, and while *in vitro* fertilization of tubal eggs of the rabbit has been described,[3] we have found no record of such experiments in higher mammals. A monkey egg fertilized *in vivo* has been cultured *in vitro* from the two- to the eight-cell stage.[4]

Utilizing the surgical material available at the Free Hospital for Women, we have, during the past six years, made numerous attempts to achieve *in vitro* fertilization and cleavage of human eggs obtained from ovarian tissue removed just prior to the expected time of ovulation. Throughout this period of investigation,[5] several factors have been varied; *e.g.*, the conditions of culture of the eggs, both before and after exposure to spermatozoa, the duration of contact of egg and spermatozoa, and the concentration of the sperm suspensions employed.[6] As a result of recent modifications of our method, we believe we have succeeded in three experiments, which constitute the subject-matter of the present report.

In two of these cases (D. D. and R. P.), the egg, after being subjected to certain procedures (to be described later), was found to be in the two-cell stage. In the third case (J. D.), two eggs divided. One of these, when first seen in cleavage, consisted of one large blastomere and two smaller ones, each of the three containing a round, vesicular nucleus. The second egg from this same patient was in a similar stage, but part of the cytoplasm appeared fragmented, and soon proceeded to undergo rapid degenerative changes. In this first report, we will confine our discussion, therefore, to the two eggs in the two-cell stage and the more normal of the two eggs in the three-cell stage.

### THE TWO-CELL STAGE OF THE HUMAN EGG

The first specimen was obtained from Mrs. D. D. (No. 20,768), a 38-year old Para IV, who underwent laparotomy on the 10th day of her menstrual cycle, at a time when the endometrium was in the early proliferative stage. When first observed in the fluid drained from a 2.3-cm bluish follicle, the egg was enclosed within a moderate investment of granulosa cells. It was washed in Locke's solution and incubated for 27 hours in the serum of the same patient. Then it was exposed for one hour at room temperature to a washed sperm suspension in Locke's solution. The watch-glass containing the ovum and spermatozoa was left on the stage of the dissecting microscope and the egg was kept in constant view (at a magnification of $\times 35$). The spermatozoa showed great activity throughout the period of observation; they were clearly seen to travel through the interstices of the loose cellular formation surrounding the egg, and many were noted in active motion just outside the ovular boundary. At the end of one hour, the ovum was transferred to fresh serum from a post-menopausal patient. As the egg was pipetted into the culture flask, the cellular investment suddenly dropped off and it appeared as a single round cell with a fuzzy border. When it was again observed after 40½ hours' culture, it was found to consist of two blastomeres, each measuring 86 μ in diameter, and was enclosed within a zona pellucida of uniform width, measuring 14 μ. A sketch of the specimen was made and it was fixed in Bouin's solution, but in the lengthy process of dehydration, it was, unfortunately, lost.

A stained section of the follicle from which this egg was obtained showed a typical preovulatory phase.

Of the second egg in the two-cell stage we have a complete series of stained sections. Essentially the same procedure, as described above, was carried out on an ovum, washed out of a follicle of Mrs. R. P. (No. 14,518), a 31-year old Para VI, Gravida VIII, who was operated upon on the 11th day of her cycle. The endometrium at this time was in the early to mid-proliferative phase of its development.

Thirteen eggs in all were recovered from the follicles

[7] A unit of streptomycin is that quantity of the antibiotic agent which inhibits the growth of a given strain of *Escherichia coli* in 1 ml of nutrient broth or agar.

[1] From the Department of Gynecology, Harvard Medical School, Boston, and the Fertility Clinic Laboratory, Free Hospital for Women, Brookline, Mass.

[2] Aided by grants from the William F. Milton Fund of Harvard University, the Committee for Research in Problems of Sex, National Research Council, and the Carnegie Corporation of New York.

[3] G. Pincus and E. V. Enzmann, *Proc. Nat. Acad. Sci.*, 20: 121, 1934.

[4] W. H. Lewis and C. G. Hartman, *Carnegie Inst. Wash. Pub. 443, Contrib. to Embryol.*, 24: 187, 1933.

[5] Nearly 800 human follicular eggs have been isolated and studied during the course of this investigation; of these, 138 have been observed after exposure to spermatozoa.

[6] We very gratefully acknowledge the invaluable advice and encouragement generously given us by Dr. Gregory Pincus, as well as the helpful assistance furnished at various stages by Dr. Nicholas T. Werthessen, Miss Lotte Lee Sichel, Miss Eleanor C. Adams, James M. Snodgrass and Dr. Harold Brown. We are also deeply indebted to Dr. Austin M. Brues for his help in the early part of this investigation, and to Dr. Arthur T. Hertig for his constant encouragement, advice and material aid through his grant from the Carnegie Corporation of New York.