things not already necessarily implied in the past. "The outstanding fact about organic evolution is the increasing dominance of Mind." "Unless we have quite misunderstood evolution it implies an emergence of novelties. It is like original thinking." In it there is something like the joyous play of the organism at self expression. "It may be well for us, on our own behalf and for our children to ask whether we are making what we might of the well-springs of joy in the world; and whether we have begun to know what we ought to know regarding the Biology or the Psycho-biology of Joy."

Perhaps the most remarkable single matter in these lectures is the suggestion of a sort of cell-intelligence, particularly in the germ-cells. "Just as an intact organism from the Amoeba to the Elephant tries experiments, so the germ-cell, which is no ordinary cell, but an implicit organism, a condensed individuality, may make experiments in self-expression, which we call variations or mutations. Such, at least, is our present view of a great mystery." "The position we are suggesting is that the larger mutations, the big novelties, are expressions of the whole organism in its germ-cell phase of being, comparable to experiments in practical life, solutions of problems in intellectual life, or creations in artistic life." "The germ-cell is the blind artist whose many inventions are expressed, embodied, and exercised in the developed organism, the seeing artist who, beholding the work of the germ-cell, either pronounces it . . . to be good or . . . curses it effectively by sinking with it into extinction."

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SPECIAL ARTICLES

MORE LINKED GENES IN RABBITS

In Science for August 13, 1920, I presented evidence indicating the existence of linkage between the genes for English spotting and dilute pigmentation in rabbits. The evidence consisted of a group of 83 young produced in matings of a male heterozygous for both characters, mated with doubly recessive females. Such matings are expected to produce equal numbers of individuals of four color classes, if no linkage exists. Consistently, in his successive litters of offspring, this male sired more young in the non-cross-over classes than in the cross-over classes, which result indicated linkage of strength 23 on a scale of 100, the cross-over percentage being 38.5.

A second heterozygous male has since been tested, in similar matings with doubly recessive females, for the occurrence of linkage between the same pair of characters as seemed to be linked in the gametes of the first male, but shows *no linkage* with as much consistency as the first male showed linkage. The totals for the first male were 32 cross-over; 51 non-cross-over gametes; for the second male they are 75 : 76, as near equality as possible. The question now arises, Were the results given by the first male statistically significant? The cross-over percentage calculated as 38.5 has a probable error of 3.6 per cent. Hence the departure from 50 per cent. cross-overs (which would indicate *no* linkage) slightly exceeds three times the probable error, a result which would ordinarily be considered significant. Unfortunately no further experimental tests of this animal can now be made as he is no longer living. There can be no doubt about the negative result given by the second male. We are now confronted by this dilemma. Either the result given by the first male was *not* significant, or we may have in the same strain of rabbits two individuals, in one of which two characters show linkage, while in the other they do *not* show linkage. This latter alternative seems improbable, yet it can not be regarded as impossible on the chromosome hypothesis. Gates and Rees [1] in discussing the pollen development of *Lactuca sativa* state that the number of chromosome pairs in the species is nine but that

> Occasionally in diakinesis only eight chromosome bivalents were present, and frequently there were only seven or eight bodies present on the heterotypic spindle. This was found to be due to a tem-

[1] *Annals of Botany*, 35, 1921, p. 394.

porary end to end fusion of certain bivalents, usually the shorter ones, but occasionally the longest being involved. This phenomenon is also likely to disturb Mendelian ratios, causing partial linkage.

This last statement points out clearly the possibility of just such apparently irreconcilable results as we have obtained in the case of these two rabbits. If English spotting and dilution have their genes located in different chromosomes, the two characters will not ordinarily show linkage. If, however, these two chromosomes should form a temporary union with each other in the spermatogenesis of a male rabbit, linkage would result. Such linkage, however, would not be of the same nature as that found in *Drosophila*. Its strength would not be due to the distance apart of genes in a chromosome, but to the persistency of the temporary attachment between chromosomes ordinarily distinct.

The cytology of the rabbit is said to be difficult. Even the number of the chromosomes has not been definitely determined. According to the summary of Miss Harvey,[2] recent observers give the number as 11 or 12 pairs, but in older investigations the number is put at 14–18 pairs. One source of uncertainty as to the number may be the formation of temporary attachments between chromosomes such as Gates and Rees describe for *Lactuca*. While we await the outcome of the study of other cases, it seems reasonable to assume that the two characters, English spotting and dilution, have their genes located in distinct chromosomes, even though these may occasionally be united to such an extent as to produce partial linkage in the gametes of certain individuals.

This case shows the desirability of expressing linkage strength in terms of something less problematical than map-distances, since linkage may occur which varies quite independently of map-distance, as for example linkage between genes lying in different chromosomes. A method of expressing linkage strength on a scale of 100 has been suggested elsewhere.[3] By this method the linkage strength indicated among the gametes of the first rabbit was 23.0 ± 7.26, that for the second rabbit is 0.6 ± 2.7.

I have recently discovered in rabbits a case of linkage which is not doubtful, since it is found in the gametes of all rabbits so far studied, and in a strength which is beyond question statistically significant. This involves the same dominant character, English spotting, as was involved in the other case. It is strongly coupled with angora coat, a recessive character. The average linkage strength is over 80 on a scale of 100. Table I. summarizes the evidence for this case. In the production of the doubly heterozygous parents used in these test-matings, English and angora were derived one from the father, the other from the mother. Consequently the linkage here takes the form of "repulsion." The English young are regularly short-haired, the non-English young are regularly long-haired (angora), except in about one case in ten, when a crossover occurs.

TABLE I

Classes of Young Produced by Rabbits Doubly Heterozygous for English Spotting and Angora Coat, when Mated with Non-English Rabbits either Homozygous or Heterozygous for Angora Coat

Heterozygous English Parent	English Short	Non-English Angora	English Angora	Non-English Short	Per cent. Cross-overs
♂ 4595 (× hom. ♀ ♀)	25	25	2	1	5.6
" (× het. ♀ ♀)	18	14	3	1	11.1
♂ 4388 (× hom. ♀ ♀)	9	8	3	0	15.0
" (× het. ♀ ♀)	4	5	3	0	25.0
Het. ♀ ♀ (× hom. ♂ ♂)	16	17	0	1	2.9
Totals	72	69	11	3	9.0 ± 1.5
	Non-cross-overs		Cross-overs		

[2] *Jour. Morphol.*, 34, 1920.

[3] *Am. Nat.*, 54, May, 1920.

In order to increase the number of test matings, the males, 4,595 and 4,388, were mated with females which were merely *heterozygous* for angora coat, animals which were themselves short-haired but which had one parent an angora. Therefore only half the gametes of these females, viz., those which bore angora, would be useful in the test matings. Accordingly half the total young from such matings have been deducted before entering the totals in Table I., and of course the deductions have been made from the *short-haired* classes, equal numbers being deducted from the English and the non-English groups. Apparently male 4,595 gives a lower percentage of cross-overs than male 4,388, and the female double heterozygotes give a lower percentage than either male, but the totals are not large enough to give much weight to these ideas. The average result for all test matings is a cross-over percentage of 9.0 ± 1.5, which means linkage of strength 82 ± 3, on a scale of 100. This certainly is a significant result, which indicates that the characters English and angora have their genes in the same chromosome.

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THE HYDROGEN-ION CONCENTRATION OF CULTURES OF CONNECTIVE TISSUE FROM CHICK EMBRYOS

In view of the fact that tissue cultures in Locke-Lewis solution were to be used in observing the behavior of living cells when exposed to bacteria and other foreign substances, it became necessary to determine the optimum and the final hydrogen-ion concentration of the cultures themselves. For the purpose several hundred cultures of connective tissue of chick embryos were prepared, in Locke-Lewis solution with varying hydrogen-ion concentrations and containing different amounts of dextrose.

The normal solution was composed of 85 c.c. of Locke's solution (NaCl 0.9 per cent. plus KCl 0.042 per cent. plus $CaCl_2$ 0.025 per cent. plus $NaHCO_3$ 0.02 per cent.), together with 15 c.c. of chicken bouillon and 0.5 per cent. dextrose. This solution has a hydrogen-ion concentration between 6.6 and 7, depending upon that of each lot of bouillon. For the experiments the hydrogen-ion concentration was varied from pH 4 to pH 9.2 with an increment of 0.2, and the amount of dextrose was varied from 5 per cent. to none at all.

The hydrogen-ion concentration of the cultures explanted into these solutions was determined at different stages of their growth, namely, when they failed to grow, when they exhibited extensive and healthy growth, and when they had degenerated after vigorous growth. This determination was made by a colorimetric method devised by Felton (1921) by means of which it is possible to test the small hanging drop of a culture.

Early in the investigation it was discovered that not all kinds of coverglasses were suitable for the experiments because of the change in hydrogen-ion concentration exhibited by control drops (without explant) when incubated upon this glass. It became necessary, therefore, to select coverglasses on which the control drop remained constant when incubated for a period of three weeks.

When cultures of embryonic chick tissue were prepared on reliable coverslips, those explanted into a medium with a hydrogen-ion concentration of 4 to 5.5 seldom showed any growth; those in a medium pH 5.5 exhibited growth in a few instances; while those in media having a hydrogen-ion concentration from pH 6 to pH 9 usually showed abundant growth. Approximately one hundred cultures were explanted into solutions pH 6, 7, 8, and 9. The percentage of growth which occurred in these cultures was respectively 71, 93, 89 and 81, while that of the normal cultures (pH 6.6–7) was 90 per cent. The optimum hydrogen-ion concentration seemed to be about pH 7.

When the hydrogen-ion concentration of these cultures was tested at different stages of their growth, it was noted that while it differed markedly, this was dependent much more upon the state of the culture at the time the test was made, and also upon the amount of dextrose in the medium, than upon the initial hydrogen-ion concentration of the medium.