CELL INCONSTANCY IN HYDATINA SENTA[1]

A. FRANKLIN SHULL

Department of Zoology, University of Michigan

The highly determinate development of certain invertebrates has long been known. The number as well as the fate of the early embryonic cells has been established in numerous cases, and is exceedingly regular. The number of cells in the embryo may be the same in all individuals, even though the number in the adults of the same species is variable. It is only in comparatively recent years, however, that this constancy of number of cells has been discovered in adult animals of certain species in which the cells are numerous. Martini ('09 a, '09 b, '09 c, '12) has claimed such constancy in nematodes, rotifers, and tunicates and Van Cleave ('14) in Eorhynchus. The latter author cites a number of other cases.

The extension of constancy of cell number to adult animals seems to me to make new demands upon the explanations offered to account for this constancy. Embryonic stages are passed through quickly, and one might suppose that whatever agencies could interfere with the orderly and definite production of cells in the embryo would have little time to do so. Before the adult condition is reached, however, such disturbing factors might have time to operate and the number of cells be thereby altered. The fact of cell constancy in the adult, then, makes greater demand upon its explanation than does the fact of cell const ncy in the embryo. I do not mean to assert that the cause of constancy in the adult is different from the causes of constancy in the cleavage stages; but an explanation which would satisfy the demands of the latter, might be inadequate for the former.

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How rigidly the factors producing cell constancy must operate, therefore, is a matter of some importance. If the number of cells is absolutely invariable in the adult, as well as in the embryo, the explanation of constancy must be of one kind; if the adult number is occasionally aberrant, while the embryonic number is invariable, the explanation may be of a somewhat different order of exactitude. Regarding the invariability of the adult number one is left somewhat in doubt by the statements of Van Cleave and of Martini, chiefly because it is not usually stated just how many individuals have been studied. Van Cleave (op. cit., p. 259) states that, in his work, in no case was the number of individuals examined as small as two, and that in many cases it was as large as two hundred. He reports no variations in the number of cells in any organ, except possibly one in which the number of cells was so large that it was difficult to count them accurately. The one possible exception was presented by two individuals, studied by the reconstruction method from sections, which revealed, respectively, one hundred and eight and one hundred and nine cells in the brain, with the possibility that one of these counts was an error. Van Cleave himself raises the question whether moderate variability would invalidate conclusions drawn from cell constancy, but since he concludes that no such moderate variability was demonstrated, nor even rendered probable, in Eorhynchus, the question was not very pertinent to his own work.

Martini ('12) expresses the conviction that the number of cells in the rotifer Hydatina senta is highly invariable. He states the number of cells (or nuclei, in the case of syncitia) in each organ, adds them together, and gives the total number of cells for the whole organism as nine hundred and fifty-nine. In most of the organs, especially the smaller ones, the number of cells was never found to vary. In others there was some uncertainty. The stomach-intestine, for example, usually contains thirty cells, but in a few instances an aberrant number was found. Martini strongly emphasizes that the aberrant number were always less than thirty, and he was at first inclined to ascribe the reduced number to the probability either that the individual was a young one which had not yet undergone all its cell divisions, or that distortions concealed one or more of them. However, he finally concluded from the positions of the remaining cells, and from supposed protoplasmic remnants, that certain cells had been lost, and suggested that their disappearance was due to the diatomaceous food upon which the animals were reared. So convinced was he that the number of cells in each and every organ is constant, that in the summary of the paper cited, the possibility of exceptions is practically ignored.

Such regularity in this rotifer is surprising to one who has noted its extreme irregularity in other respects. The metabolic processes[2] which result in cell division, and determine the number of cell divisions, must needs occur with clock-like precision; while the metabolic processes that determine the rate of growth and the type of reproduction are subject to great and unaccountable fluctuations. The remarkable contrast of fixity in one set of processes, and apparent lawlessness in another, in the same animal led me to examine a very limited portion of the structure of this rotifer to ascertain whether the number of cells is as nearly constant as Martini supposed.

For the proposed test, two small organs whose cells could be easily counted were selected. These were the yolk gland and the gastric glands. The yolk gland is a large syncitial mass, in the form of a baseball catcher's mitt, closely applied to the stomach-intestine and to the ovary; it was found by Martini to have invariably eight nuclei. The gastric glands are two rounded masses, situated near the upper end of the stomach-

[2] By ascribing the initiation of cell division and the determination of the number of cell divisions to metabolic processes I do not attempt to locate the ultimate cause. I have on one occasion verbally expressed the view that in some way the number of cells is dependent upon some rhythm of the protoplasm. Here again, 'rhythm' is not supposed to name the final agent. Even if cell constancy were attributed to an accurate time relation between the rhythm of cell division and the rhythm of growth and differentiation, such that a given number of cell divisions (no more and no less) had time to occur before cell differentiation made further division impossible, the assumed rhythms would not be the ultimate cause. Doubtless something inherent (hereditary) in the organism determines these rhythms, as is almost certainly true of the rhythm in the life cycle; but this inherent something must work through metabolic processes to attain its end.

intestine, with which they are connected by short canals. They are likewise syncitia, which Martini finds always contain six nuclei.

Determination of the number of nuclei in these glands was made from serial sections. The sections had been prepared for another use in which it was important to have complete series. All imperfections in the series were therefore recorded, and for the counting of nuclei only complete series were used. Moreover, in the yolk gland, the nuclei are so large that five or six successive sections passed through each nucleus, so that any breaks in the series large enough to omit one nucleus would be easily detectible not only from the yolk gland itself, but from the surrounding tissues. In the case of the gastric gland, the nuclei are smaller, and, though imperfections in the series of sections could probably have been detected from the sections themselves, reliance was placed entirely upon the records made when the slides were prepared. In any case, incompleteness in the series of sections could never lead to an erroneous increase in the number of nuclei counted. All aberrant numbers were determined independently by two persons trained in the interpretation of serial sections, and in every case there was agreement.

YOLK GLAND

This organ was examined in two hundred and forty-five satisfactory individuals. In two hundred and thirty-five, the number of nuclei was eight. In the remaining ten, a trifle over 4 per cent of the total, aberrant numbers were found, as indicated in table 1.

TABLE 1

Distribution of the numbers of nuclei in the yolk glands of 245 specimens of Hydatina senta

NUMBER OF NUCLEI	NUMBER OF INDIVIDUALS
5	1
6	0
7	5
8	235
9	3
10	1

The commonest aberrant numbers are seven and nine, while the extremes show a reduction of 37.5 per cent below and an elevation of 25.0 per cent above the usual number (eight).

To discover whether anything in the environment caused these differences in the number of nuclei, or whether any relation between the deviation and other phenomena could be established, all recorded facts were carefully examined. Information was found concerning the following points: 1) Some of the two hundred and forty-five rotifers examined belonged to periods of many male-producers, others to periods of few male-producers; 2) some were young, some middle-aged, some old females; 3) some were reared in spring water, others in manure solution; and 4) some were male-producers, others female-producers. In each of these groups some aberrant yolk glands were found. How frequently the unusual numbers occurred under the several circumstances named, is shown in tables 2, 3, 4 and 5, respectively.

The numbers of specimens aberrant with respect to the number of nuclei in the yolk gland are, of course, too small in any of

TABLE 2

Distribution of individuals of Hydatina senta having aberrant numbers of nuclei in the yolk gland, with reference to periods of many male-producers and periods of few male-producers

PERIOD OF MANY MALE-PRODUCERS			PERIOD OF FEW MALE-PRODUCERS		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
93	5	7, 7, 7, 9, 10	49	3	7, 7, 9

TABLE 3

Distribution of individuals of Hydatina senta having aberrant numbers of nuclei in the yolk gland, with reference to the age of the individuals

YOUNG			MIDDLE-AGED			OLD		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
31	1	9	24	1	7	15	1	7

TABLE 4

Distribution of aberrant numbers of nuclei in the yolk glands of individuals of Hydatina senta reared in spring water and in manure solution, respectively

Spring water			Manure solution		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
47	2	7, 7	56	3	7, 9, 10

TABLE 5

Frequency of occurrence of aberrant numbers of nuclei in the yolk glands of male-producing and female-producing Hydatina senta

Male-producers			Female-producers		
Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with eight nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
47	2	5, 9	188	8	7, 7, 7, 7, 7, 9, 9, 10

these tables to justify the computation of percentages with the expectation of making valid comparisons. There are, however, no striking differences in the numbers of aberrant glands under different circumstances. Yolk glands with other than eight nuclei occur at times of many male-producers, as well as at times of few; in young, middle-aged, and old females; in specimens reared in spring water, and in those reared in manure solution; in male-producers as well as in female-producers. And they occur with what might well be equal frequency, if the numbers examined were large enough, in each of these cases.

It seems unlikely, therefore, that any of the conditions above enumerated have anything to do with the alteration of the number of nuclei. It is also worthy of note that the deviations from the usual number eight are not all diminutions, as Martini (see ante) found in the case of the stomach-intestine. It may also be remarked that the only deviation in a young adult female was a plus deviation (table 3), which cannot therefore be explained by supposing that not all the usual cell divisions had yet occurred.

GASTRIC GLANDS

Degeneration of the gastric glands. It is not uncommon in this rotifer to find the gastric glands more or less atrophied. In some cases the change referred to is evidenced merely by the more homogeneous appearance of the organ, and by its failure to take the stain properly. In more marked cases the glands are plainly reduced in size, the characteristic structure is lost, and there is no longer any connection with the stomach-intestine. In extreme cases one of the glands is entirely missing, and in one specimen I was unable to find either of the glands, though the sections were apparently not damaged in the region proper to these organs. One gland was sometimes atrophied while the other was normal.

In none of these cases does the degeneration take the form of a destruction of some of the cells (here nuclei, since the gastric glands are syncitia) through high physiological activity. The whole organ degenerates simultaneously, and in the early stages the six nuclei are still recognizable.

Number of nuclei in the gastric glands. In determining the number of nuclei in the gastric glands only normal specimens were used. The degeneration described above is easily recognized, and specimens with such glands were rejected.

One hundred and twenty glands were studied. Among this number, nine had other numbers of nuclei than six. Four was the smallest number observed, seven the largest. The frequency of the various number is given in table 6.

In the case of the gastric glands, as with the yolk gland, it was possible to determine some of the conditions of age, environment,

TABLE 6

The frequency of occurrence of various numbers of nuclei in 120 gastric glands of Hydatina senta

NUMBER OF NUCLEI	NUMBER OF INDIVIDUALS
4	1
5	7
6	111
7	1

TABLE 7

Frequency of occurrence of aberrant numbers of nuclei in the gastric glands of Hydatina senta in periods of many male-producers and in periods of few male-producers

PERIOD OF MANY MALE-PRODUCERS			PERIOD OF FEW MALE-PRODUCERS		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
22	2	5, 5	44	5	4, 5, 5, 5, 5

TABLE 8

Frequency with which aberrant numbers of nuclei occur in the gastric glands of Hydatina senta, in young and middle-aged adults, respectively (no old adults were examined)

YOUNG			MIDDLE-AGED		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
17	2	5, 7	9	2	5, 5

TABLE 9

Proportion of gastric glands having aberrant numbers of nuclei in individuals of Hydatina senta reared, respectively, in spring water and in manure solution

SPRING WATER			MANURE SOLUTION		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
18	1	5	31	4	4, 5, 5, 5

TABLE 10

Proportion of gastric glands, in male-producing and female-producing individuals of Hydatina senta, having aberrant numbers of nuclei

MALE-PRODUCERS			FEMALE-PRODUCERS		
Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers	Number with six nuclei	Number with aberrant numbers of nuclei	Aberrant numbers
36	0	None	75	9	4, 5, 5, 5, 5, 5, 5, 5, 7

and phase of life cycle of the individuals whose glands were studied. The facts, as far as recorded, are given in tables 7, 8, 9 and 10.

With the exception of male-producers, as contrasted with female-producers (table 10), none of the various conditions mentioned in tables 7 to 10 seem to be associated with deviations in the number of nuclei in the gastric glands. The number of nuclei deviates from the usual number six in periods of few or many male-producers, in adults of different ages, and in those reared in spring water or in manure solution. And in view of the small number of individuals studied, it is not impossible that the absence of aberrant glands in male-producers is insignificant. The minimum aberrant number is 33.3 per cent below the normal, the maximum 16.6 above the normal. It is also worthy of note that in only one specimen studied were both gastric glands aberrant, one of them having seven nuclei, the other five.

DISCUSSION

In two of the smaller organs of the rotifer Hydatina senta, an organism said to be highly constant in the number of its cells, it is now found that the number of nuclei varies. In the case of one organ, 4 per cent of those examined contained other numbers of nuclei than those claimed for it; in the other organ, 7.5 per cent were aberrant. In both organs, the highest number found is approximately double the lowest number. In one organ the nuclei are of moderate size, in the other enormous, so that they are readily counted.

If those who have found the numbers of cells in the organs of this rotifer highly constant have been misled in the case of small organs whose nuclei are easily counted, there is no reason to assume that their counts are any more accurate in the case of larger organs where the counting is difficult. If other organs are as variable as the yolk gland and the gastric glands, and if the factors which change the cell number from the 'normal' can operate in the same direction in all organs, then by rare chance the supposed nine hundred and fifty-nine cells in the whole body might be either six hundred and forty or eleven hundred and eighteen. More likely the number would be between these, since there is nothing as yet to indicate that deviation in one organ is associated with deviation in other organs. However, the possibilities are numerous, not single. If each organ contains unusual numbers of cells in 5 per cent of the individuals, and there is no association between the deviation in various organs, the total number of nine hundred and fifty-nine would probably be realized less often than not.

It has not seemed worth while to pursue the re-examination of cell constancy in Hydatina further than the two organs named. A measure of its exactitude has been obtained, and that is all that was sought. The observations described in this paper do not destroy the problem of cell constancy; they merely make its solution easier. Even if every organ of every animal in which constancy of cell numbers has been claimed, should prove to be as variable as the yolk gland and gastric gland of this rotifer, the problem of cell constancy would remain. Although the total number of cells in an organism is the same in only a small percentage of cases, if the number in any one organ is the same in ninety per cent of the individuals, there is a problem of cell constancy which calls for solution. But there is no need of complicating this solution by assuming a degree of constancy that does not exist.

BIBLIOGRAPHY

Martini, E. 1909 a Studien über die Konstanz histologischer Elemente. I. Oikopleura longicauda. Zeit. f. wiss. Zool., Bd., 92.

1909 b Studien, u.s.w. II. Fritillaria pellucida. Zeit. f. wiss. Zool., Bd. 94.

1909 c Über Subcuticula und Seitenfelder einiger Nematoden. IV. Vergleichend-histologischer Teil, Zeit. f. wiss. Zool., Bd. 93.

1912 Studien, u.s.w. III. Hydatina senta. Zeit. f. wiss. Zool., Bd. 102.

Van Cleave, H. J. 1914 Studies on cell constancy in the genus Eorhynchus. Jour. Morph., vol. 25.