quite out of the question, thus checking the simple, and often less efficient, calculation on the head-tails basis.

[4] Because of the small and variable number of figures available in the variation of the individual data from their column means and the different weights thereby implied by the different columns, no precision was carried in the arithmetical operations leading to this table which sufficed to determine precisely the unit in the last place of the entries.

VARIATION IN THE AMOUNTS OF DESOXYRIBOSE NUCLEIC ACID (DNA) IN CELLS OF THE SAME TISSUE AND ITS CORRELATION WITH SECRETORY FUNCTION

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Introduction.—It has long been known that the nuclei of the salivary gland of the snail *Helix pomatia* vary greatly in size, and that this variation is in some way correlated with the formation of secretions. Cells with large spherical nuclei show few or no secretory products in the cytoplasm whereas cells with small, irregularly shaped nuclei are characterized by large quantities of such materials. Confirming Barfurth,[1] Hirsch[7] and Krijgsmann[9] have pointed out that changes from one to the other of these types are regular and cyclical. On a cytological basis alone there is thus strong presumptive evidence that the nuclear contents are implicated in the formation of secretions. This was further strengthened by the ultraviolet studies of Caspersson[5] which demonstrated that the decrease in size of the salivary nuclei involves an actual decrease in the amount of the contained nucleic acid.

But the type of nucleic acid, whether Ribose nucleic acid (RNA) or DNA, that is concerned in these processes could not be determined by the ultraviolet method. Its determination has now become a matter of importance, for during the recent years a considerable number of investigators has claimed that the amount of DNA is constant in all the nuclei of an individual or species. It might, therefore, be supposed that in the varying amounts contained in these salivary gland nuclei we are concerned with RNA rather than DNA, but an actual identification of the nucleic acid concerned is obviously necessary. This we have proceeded to do, keeping in mind at the same time that any further information on the nature of the correlation between nuclear contents and cytoplasmic secretion products is of considerable interest.

In the present paper evidence is presented that the DNA of the cells of the *same tissue* varies in the order of magnitude of 30:1 from nucleus to

nucleus, and that such changes in the amount of DNA in individual cells are directly correlated with the secretory function of these cells.

Materials and Methods.—All work was done on the salivary glands of *Helix aspersa* Müller,[23] which were fixed in Carnoy's fluid (alcohol-acetic acid, 3:1), embedded in paraffin and cut at different thicknesses according to the requirements of the investigation. In all features at issue, the salivary glands of this species correspond to those of *Helix pomatia.*[24] The DNA content in individual cells was determined by the microspectrophotometric analysis of the Feulgen reaction as previously described.[10, 18, 22]

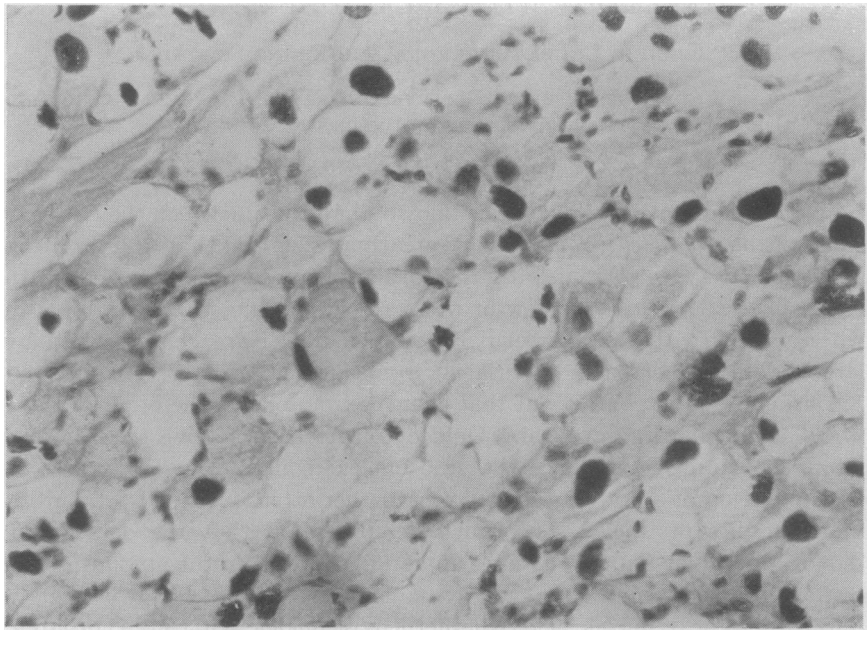

FIGURE 1

Salivary gland of *Helix aspersa* Müller stained by Feulgen-Fast Green. Magnification 330 × approx. Wratten 62.

For the estimation of the polysaccharides the PAS reaction of Hotchkiss[8] was used in conjunction with enzymes as described by Leuchtenberger and Schrader.[12]

Results.—The cytological appearance of the salivary glands of *Helix* is demonstrated in the accompanying photograph. In confirmation of the findings of Krijgsmann,[9] all stages of secretion are present within one gland. A survey, even at a low magnification (Fig. 1), of a Feulgen stained section shows the strikingly varying amounts of DNA in the different sized nuclei. A comparison of the nuclear size and amount of DNA in a non-secreting

cell with those of an actively secreting cell is so impressive in the difference displayed that it seemed hardly necessary to measure photometrically the amounts of DNA. Sections adjacent to those used for the Feulgen preparations, when stained by the PAS reaction for polysaccharides, demonstrate the relationship between nuclear size and amount of DNA on the one hand and the amounts of polysaccharides on the other hand. The larger the nucleus and the greater its content of DNA, the less polysaccharides are encountered; and the smaller the nucleus and the less DNA, the greater is the amount of polysaccharides in the cytoplasm.

In table 1 the results of the photometric measurements of DNA in individual cells are presented. It is evident from this table that the nuclei of the salivary glands of *Helix* contain widely varying amounts of DNA; the highest values are found in the non-secreting cells or in those just beginning secretion, while the lowest values are found in cells with advanced secretion. It is further obvious that there exists a direct correlation between nuclear size and amount of DNA or, in other words, the loss of DNA is accompanied by a decrease in the nuclear diameter.

The inverse relationship mentioned above between the secretion of polysaccharides and the amount of DNA is also apparent in table 1. It thus appears that the DNA is actually utilized in the synthesis of a secretory product which is of polysaccharide nature. Attempts further to identify the chemical nature of this polysaccharide have thus far shown that it is not starch, glycogen or hyaluronic acid. Perhaps we are dealing here with polysaccharides formed from pentose sugars, similar to the so-called pentosans in plants, instead of the usual hexosepolysaccharides. Testing *in situ* for the presence of pentoses in the secretory products of the salivary glands of the snails is now in progress.

It is of interest that the DNA seems to be broken down within the nucleus since in none of the cells studied was Feulgen positive material present either in the cytoplasm or in an extracellular position. These observations are in accordance with the ultra-violet studies of Caspersson,[5] who also noted that in spite of the drop of absorption at 2570 Å in the nucleus during secretion there was never an increase in the cytoplasmic absorption such as would, of course, be expected if the nucleic acid molecule were broken down only *after* leaving the nucleus.

This breakdown of the DNA molecule within the nucleus of cells which are synthesizing secretory products differs markedly from the degradation process encountered in pycnosis or necrosis. While a loss of DNA from the nucleus occurs also in such dying cells, the DNA is there found in the cytoplasm and extracellularly, which indicates that the DNA leaves the nucleus and probably even the cytoplasm, still as a complete molecule, though perhaps depolymerized.[10]

Such contrasting behavior between synthesizing and dying cells may

TABLE 1

AMOUNT OF DNA (MICROSPECTROPHOTOMETRY OF FEULGEN REACTION) IN INDIVIDUAL SECRETING AND NON-SECRETING CELLS OF THE SALIVARY GLAND OF HELIX

TYPE OF CELL	NUCLEAR DIAMETER IN MICRA	AMOUNT OF DNA PER NUCLEUS IN ARBITRARY UNITS	PRESENCE OF POLYSACCHARIDES (PAS REACTION)
Non-secretory cell "Ruhekern"	18.0 × 17.9	20.0	−
	18.1 × 17.3	20.0	−
	18.0 × 18.0	20.0	−
	18.7 × 18.5	19.0	−
	17.9 × 17.8	18.1	−
	18.9 × 18.2	17.3	−
	17.1 × 16.6	17.2	−
	20.0 × 18.1	17.3	−
	20.0 × 13.5	16.8	−
	18.9 × 13.8	16.8	−
	18.5 × 14.5	16.7	−
	18.6 × 14.3	16.7	−
	18.0 × 18.0	16.7	−
	18.8 × 14.8	16.2	−
	17.5 × 14.9	16.0	−
Beginning secretion	17.9 × 13.1	15.0	(+)
	20.7 × 13.5	15.4	(+)
	16.5 × 16.2	13.5	(+)
	16.2 × 13.4	13.4	(+)
	16.2 × 16.5	13.4	(+)
Moderate secretion	16.9 × 15.3	12.7	+
	16.2 × 13.5	11.7	+
	13.9 × 12.1	10.0	+
	14.4 × 12.6	9.7	+
	13.7 × 11.9	9.2	+
	15.1 × 11.5	8.6	+
	13.7 × 11.9	8.5	+
	13.2 × 9.6	7.7	+
	15.0 × 11.0	7.0	+
Advanced secretion	11.3 × 11.0	3.8	++++
	9.9 × 9.5	3.7	++++
	10.0 × 9.0	3.2	++++
	9.0 × 8.3	3.2	++++
	7.9 × 7.9	2.9	++++
	9.0 × 7.2	2.8	++++
	10.4 × 7.2	2.6	++++
	10.3 × 7.4	2.6	++++
	9.9 × 8.3	1.9	+++
	10.1 × 8.3	1.9	+++
	10.0 × 8.9	1.7	++++
	8.3 × 8.0	1.6	++++
	8.3 × 7.9	1.6	++++
	8.1 × 8.1	1.4	++++
	8.0 × 7.9	1.4	++++

TABLE 1—*Continued*

Advanced secretion	8.1 × 7.0	1.2	++++
	8.6 × 7.6	1.1	++++
	8.1 × 7.0	1.1	++++
	7.2 × 5.4	1.2	++++
	5.6 × 4.3	0.8	++++
	5.8 × 5.4	0.8	++++
	5.9 × 5.4	0.8	++++
	6.5 × 6.3	0.8	++++
	6.1 × 7.4	0.8	++++
	6.1 × 6.1	0.7	++++
	6.5 × 4.7	0.7	++++
	6.1 × 6.1	0.7	++++
	6.5 × 5.2	0.6	++++
	6.2 × 5.4	0.6	++++
Duct lining cells	9.4 × 4.5	3.2	−
	10.1 × 4.5	1.0	−
	10.8 × 4.5	2.2	−
	11.7 × 4.9	2.3	−
	9.4 × 5.0	1.2	−
	10.2 × 4.8	1.4	−
	10.7 × 4.6	2.4	−
	10.0 × 4.8	2.0	−
	10.0 × 4.0	1.5	−
	10.7 × 4.5	2.4	−

− = negative. (+) = doubtful. + = Small number of PAS positive secretary granules. ++++ = Large number of PAS positive secretory granules.
+++ = Large number of PAS positive secretory granules.

perhaps be regarded as supporting evidence for the view of Krijgsmann that after the maximum amount of secretory material has gathered and is extruded, this same cell then repeats the cycle of building up a nucleus and converting its content once more into secretory products. The absence of mitosis or other type of division, which might indicate that new cells are being formed to replace the spent ones, seems further to support Krijgsmann's idea of a rhythmic repetition of the process in the same cells. Nor are any replacement cells, such as are regularly found, for instance, in the basal layer of the human epidermis, ever encountered here in the salivary gland of the snail. In short, the available evidence seems to favor Krigjsmann's[9] hypothesis, though our own findings are obviously inadequate to give a final answer to this particular question.

Discussion.—The preceding analysis shows that in the great variability of nuclear size in the *Helix* salivary gland we are actually dealing with changes in the quantity of nuclear DNA. Our results, therefore, run counter to the prevailing view that DNA is constant in amount in all the nuclei of an individual or species. This view, first advanced by Boivin, Vendrely and Vendrely[2] in 1948 has since received the support of a whole series of investigations.[6, 11, 14, 22]

The present report is, however, not the first to question the universality of the constancy rule for exceptions have also been noted in certain cells of *Tradescantia*,[4, 19] as well as for various cells in embryonic tissues.[15, 16] Indeed, even in the liver of rodents which has offered some favorable evidence in support of the constancy rule, Leuchtenberger, Vendrely and Vendrely[11] have pointed out that though the *mean* amount of DNA may be the same as for all diploid nuclei of the species, measurements of *individual* nuclei may show a variation reaching 50%. It is, of course, probable that smaller departures from the constancy rule are attributable to errors inherent in the Feulgen-photometric method,[10] but the recent report of Leuchtenberger, Leuchtenberger, Vendrely and Vendrely[13] shows that the different method of ultra-violet spectrophotometry discloses a very similar range in the DNA content of individual nuclei. In short, although it is not to be contested that in the cells of some tissues, as for instance the male germ cells, there obtains a remarkable constancy of DNA, there are various conditions and types of cells where the generalization is not applicable.

Our analysis further indicates that the decrease of DNA in salivary gland nuclei is the result of its utilization in the formation of secretory products out in the cytoplasm. This secretory product is of polysaccharide nature, which raises the question how the breakdown products of DNA may be utilized for the synthesis of the polysaccharides. While in animals polysaccharides, as, for instance, glycogen, are usually formed from hexose sugar, the possibility that in the salivary gland of the snail the *pentose* sugar of DNA is utilized for the synthesis of pentose polysaccharides is worth consideration. The presence of an enzyme digesting pentose-polysaccharides within the digestive glands of *Helix*[21] seems to favor such a view. It would be intriguing indeed if the synthetic pathway of the formation of pentosans in plants, which is at present still obscure,[3] could be explained in a similar manner.

The utilization of the breakdown products of the DNA for the formation of polysaccharides might appear to be a new role for DNA, but a very similar process has also been encountered in the ovary of certain insects, such as *Acanthocephala*, by Schrader and Leuchtenberger,[20] where the products of the breakdown of DNA contribute to the nutritive materials which are transferred to the growing egg.

The prevalent view that the amount of DNA per nucleus is constant throughout the individual and species is thus evidently subject to modification and this, in turn, must affect questions concerning the deeper significance of DNA. Our investigations on *Helix* and *Acanthocephala* show that DNA may be used in the elaboration of other cellular products, which incorporates the suggestion that its role in metabolism and differentiation may be a much more direct one than is usually assumed.

Summary.—1. Evidence is presented that the different sizes of nuclei present in the salivary gland cells of *Helix* correspond to different amounts of DNA carried by them. These amounts may vary in the order of magnitude of 30:1.

2. As the size of a nucleus and its DNA content decrease, the amount of secretion in the cytoplasm increases. It appears that the DNA is actually utilized in the manufacture of such cytoplasmic secretions.

3. Since these secretory products contain polysaccharide, the possibility of formation of polysaccharides from degradation products of DNA is discussed.

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