Is there "Metabolic" DNA in the Mouse Seminal Vesicle?

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ABSTRACT

This study was designed to answer the question: Is H^3-thymidine uptake by nuclei of the mouse seminal vesicle evidence for DNA synthesis and mitosis, or does it signify some "metabolic" function of DNA unrelated to chromosome duplication?

Mice were given an intraperitoneal injection of H^3-thymidine. Six hours later Feulgen squashes of the seminal vesicle epithelium were made and covered with autoradiographic stripping film. The silver grains above labeled nuclei were counted, and the Feulgen dye contents of these same nuclei were determined photometrically after removal of the grains from the emulsion. Unlabeled nuclei were also measured.

The dye contents of non-radioactive nuclei form a unimodal distribution, indicating that polyploidy is absent from this tissue. The radioactive nuclei fall into two groups. In the first, the average dye content is the same as that of the cold nuclei (2C). In the second, the values range from 2C to 4C.

In the 2C to 4C group the grain count is proportional to the dye content, showing that incorporation is correlated with synthesis.

The radioactive 2C nuclei arose by mitosis during the course of the experiment. This is shown by the following facts: (1) They frequently occur in pairs. (2) They average smaller than unlabeled 2C nuclei. (3) Their average grain count is approximately half that of the 4C nuclei. (4) Labeled division figures are found. (5) A mitotic rate estimated from the number of labeled 2C nuclei accords reasonably well with one based on the number of observed mitoses.

Since the incorporation of thymidine accompanies DNA synthesis and precedes mitosis, there is no reason to postulate a special "metabolic" DNA in this tissue.

The incorporation of labeled precursors into deoxyribose nucleic acid (DNA) occurs at a rapid rate in actively dividing embryonic and adult tissues. On the other hand, uptake is low in tissues that are replaced slowly and is absent in non-dividing cells such as sperm (7, 20, 28, 41). Biochemical analyses at the tissue and organ level have been supplemented by a variety of autoradiographic studies on single cells. Many of these have shown, in accord with the original work of Howard and Pelc (14), that incorporation of precursors takes place only during the interphase (22, 31, 46, 48). It is usually assumed that such uptake is coincident with the duplication of DNA and reflects synthesis of new chromosome material. Of considerable interest, therefore, are several recent reports that precursors may be incorporated into the DNA of nuclei which neither divide nor increase their degree of ploidy (17–19, 24–27, 29). It has been suggested that the incorporation in these cases indicates the participation of DNA in such metabolic processes as protein synthesis. Our concern here is primarily with the studies of Pelc (24) and Pelc and Gahan (27). These authors followed the uptake of C^{14}-adenine and H^3-thymidine into the DNA of mouse seminal vesicle epithelium, and concluded that the incorporation cannot be explained by the mitotic activity of the tissue. We have reinvestigated this case, combining autoradiographic and photometric measurements on the same population of cells. Our results

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suggest that the uptake of H^3-thymidine occurs during the synthesis of DNA preceding mitosis.

Materials and Methods

Ten mice of the C (Bagg albino) strain were used in this study.[1] The strain had been inbred by brother to sister matings for over 8 years. At the time of injection the mice were 3½ months old and had been housed singly during the preceding 2 weeks with constant light from 06:00 to 18:00 alternating with darkness from 18:00 to 06:00. The temperature was kept at 24°C. Purina laboratory chow and tap water were available *ad libitum*. Each animal was given a single intraperitoneal injection of H^3-thymidine, 20 μc. dissolved in 0.25 ml. sterile Ringer-Locke saline (thymidine lot #5812 from Schwarz Laboratories, Mount Vernon, New York, specific activity 0.36 c./mM). Five animals were injected at 12:00 and killed at 18:00, five at 00:00 and killed at 06:00. After injection the mice were returned to individual cages and kept on their usual light or dark regimen until sacrificed. The seminal vesicles were fixed for 6 hours in ethanol-acetic acid (3:1). They were hydrolyzed for 10 minutes in 1 N HCl at 60°C. and stained by the Feulgen procedure following the details given in Swift (44). Pieces of the stained tissue were placed in 45 per cent acetic acid and the epithelium was stripped from underlying connective tissue and muscle. Small bits were placed on conventional microscope slides, covered, and squashed. The coverslip was removed by the dry ice technique (6). Squashes of unstained tissue were made in similar fashion. All material was covered with Kodak AR-10 stripping film and exposed for 14 or 24 days at 4°C. The films were developed for 7 minutes in Kodak D-19 at 18°C.

Photometric measurements and grain counts were made on one Feulgen slide from an animal injected at 12:00 (exposure time 14 days). One hundred and eleven hot nuclei were selected in the following fashion. The slide was scanned along predetermined coordinates, and all labeled nuclei were chosen except (*a*) those few elongated ones which obviously belonged to connective tissue or muscle cells (Fig. 1), (*b*) those which overlapped other nuclei, and (*c*) those with fewer than 5 grains. The background averaged less than 1 grain per nucleus, but the occurrence of local grain clusters due to cosmic rays, static, etc., made it advisable to select a lower limit on grain count. Each radioactive nucleus was photographed in green light for determination of size and in red light for grain count. Mechanical stage settings were recorded for each photograph. One hundred and one cold nuclei were chosen arbitrarily from the same slide.

After the photographs had been made, the silver grains were removed from the emulsion by a 15 second dip in 0.75 per cent $K_3Fe(CN)_6$ followed by 3 minutes in 20 per cent $Na_2S_2O_3$. The slide was thoroughly washed and remounted in oil of refractive index 1.580. Darkfield observations showed that the preparation scattered only a small amount of light at this refractive index. Preliminary measurements on another slide showed that the ferricyanide treatment did not affect extinction values significantly.

The previously chosen nuclei were relocated and measured photometrically by the one-wavelength method at 560 mμ (45). The instrument used was modeled after that of Pollister and Ris (34). The diameter of the measured area was approximately half the nuclear diameter. Total dye contents were expressed as extinction × the product of the long and short diameters of the nucleus. Most nuclei were circular or slightly elliptic, and we assumed that they may be considered thin, flattened cylinders. Ten of the hot nuclei (2 metaphases and 4 telophase pairs) were not measured photometrically because of their irregular shape and small size. They are considered in the discussion of mitotic rate but do not appear in the graphs.

TABLE I

Per Cent of Labeled Nuclei in the Seminal Vesicle Epithelium 6 Hours after a Single Intraperitoneal Injection of H^3-Thymidine

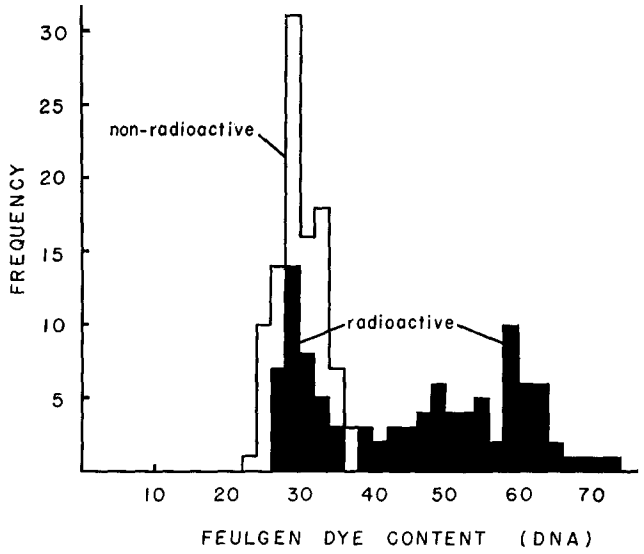
Injected at 12:00		Injected at 0:00	
Animal	Per cent labeled nuclei	Animal	Per cent labeled nuclei
1	0.18 (15/8389)	6	0.30 (25/8221)
2	0.85 (71/8326)	7	0.19 (16/8407)
3	0.81 (81/10034)	8	0.17 (13/7637)
4	0.38 (31/8134)	9	0.39 (31 '7978)
5	1.25 (105/8410)	10	0.84 (68/8073)
Average 0.69 ± 0.19		Average 0.38 ± 0.12	

RESULTS

The frequency of labeled nuclei in the seminal vesicle epithelium was determined on one slide from each of the ten injected mice; the values are given in Table I. Six hours after injection of H^3-thymidine, 0.69 ± 0.19 per cent of the nuclei were radioactive in the day group, 0.38 ± 0.12 per cent in the night group. The difference between the means of the two groups is not significant ($t = 1.4$, $p = 0.2$).

All our photometric measurements come from a single animal which was given 20 μc. of H^3-thymidine at 12:00 and was killed at 18:00. In the piece of epithelium studied the frequency of labeled

[1] We wish to thank Prof. Franz Halberg for the mice, the use of the animal quarters, and especially for his helpful comments on diurnal rhythms.



TEXT-FIG. 1. Histograms showing the Feulgen dye content of radioactive and non-radioactive nuclei. The cold nuclei form a unimodal distribution about the 2C mean. The hot ones fall into two groups, of which the first lies within the 2C range; the second includes 4C nuclei and numerous intermediates between 2C and 4C.

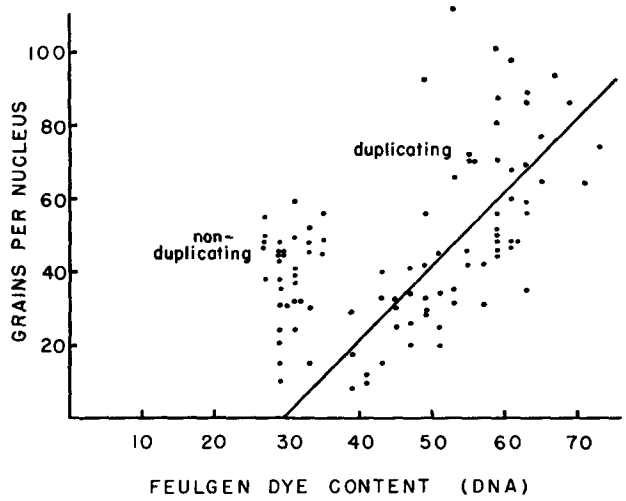
nuclei was 1.25 per cent (105/8410). One hundred and one unlabeled nuclei, chosen arbitrarily, were measured for Feulgen dye content. As shown in Text-fig. 1, the values fall into a unimodal distribution with a mean of 29.9 ± 0.3 (arbitrary units). The spread of values is similar to that usually obtained with the one-wavelength method of measurement; *i.e.*, approximately two-thirds of the values fall within 10 per cent of the mean (45). In accord with the findings of B. M. Richards (39) these data indicate that polyploidy is rare or absent in the mouse seminal vesicle epithelium. A larger sample would be needed to detect a very low percentage of polyploid cells, but the situation in this tissue is certainly different from, say, the liver where 4C and 8C nuclei are common (43). Our sample is not entirely random, since we excluded radioactive nuclei. However, only one or two labeled cells would be expected in a group this size.

When we turn to the Feulgen measurements on the radioactive nuclei, we find an entirely different situation (Text-fig. 1). Here only a little more than one-third of the labeled nuclei (37/101) have Feulgen dye contents that fall within the 2C range. A value of 38 on our arbitrary scale is considered the upper limit of the 2C class, since none of the cold nuclei had a higher dye content. The mean of the labeled 2C nuclei is 30.0 ± 0.2; this does not differ significantly from the mean of the cold nuclei, 29.9 ± 0.3.

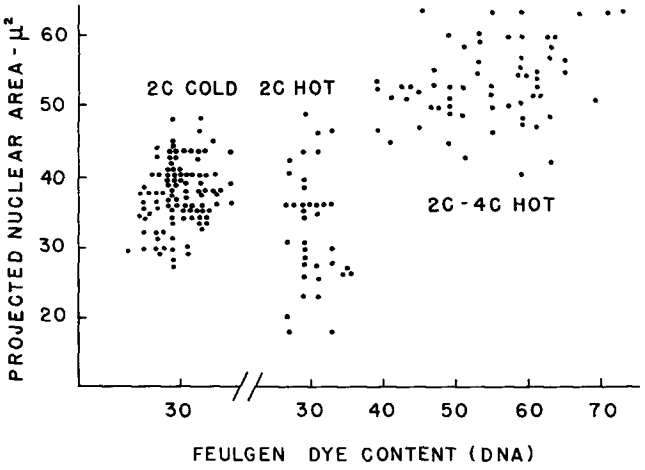
On the other hand, almost two-thirds of the hot nuclei (64/101) have Fuelgen dye contents above the extreme limit of the 2C category. We cannot say with assurance what fraction of these are 4C nuclei and what fraction are still synthesizing DNA, but it appears that roughly half might fall into each group. Most of the nuclei in the peak at 58–60 have probably completed duplication and this would also be true of those with higher values.

Grain counts were made for all of the radioactive nuclei. They are plotted in Text-fig. 2 against Feulgen dye contents. Once again the labeled nuclei fall into two discrete groups. Most of those in the 2C category have between 30 and 50 grains per nucleus. The remainder, that is, those above the 2C class, show a clear correlation between grain count and extent of DNA synthesis. The regression of grain count on Feulgen dye content has been calculated and is shown as the straight line in Text-fig. 2. This line passes through the point (29.2, 0); that is, the number of grains is proportional to the amount of Feulgen dye above the 2C mean (30.0).

There is a wide spread of grain counts for a given dye content. The major sources of this variation are probably the following. First, the



TEXT-FIG. 2. Plot of silver grains per nucleus against Feulgen dye content. The straight line is the regression of grain count on dye content calculated for the duplicating 2C to 4C nuclei only.



TEXT-FIG. 3. Distribution of nuclear sizes in the seminal vesicle epithelium. Nuclei in the 2C to 4C range are consistently larger than 2C nuclei. Among the latter the cold ones average somewhat larger than the hot.

standard deviation of the grain count due to chance alone will be $\sqrt{n}$, in which n is the observed number of grains. Second, photometric errors introduce uncertainty into the measurements of dye content. Third, the range of the tritium electrons is very short, averaging only 1 or 2 micra in tissue (15, 16), so that irregularities in flattening, overlap of cells, and the like will lead to variability in the number of electrons reaching the emulsion. And finally, grain-counting itself involves a fair amount of subjective decision, as can be seen in Fig. 2. Somewhat better results might have been obtained by using a shorter exposure time, since the more heavily labeled nuclei have too many grains for convenient counting.

Our first examination of the autoradiographs suggested that radioactive nuclei are larger than non-radioactive ones. Measurements of projected nuclear area, taken from the photographic enlargements, confirm this impression (Text-fig. 3). The duplicating nuclei, with a mean area of 53.5 $\pm$ 0.7 μ^2, are larger than all others. The cold 2C nuclei form a fairly homogeneous population with a much lower mean value, 38.1 $\pm$ 0.6 μ^2. The labeled 2C nuclei are the smallest, 32.6 $\pm$ 1.3

μ^2, and their variability is greater than that of the cold nuclei. The difference between the means of the labeled and unlabeled 2C nuclei is significant ($p \sim 10^{-4}$). It is interesting to note that all the duplicating nuclei are large, even those which have just begun DNA synthesis. Enlargement apparently begins before duplication, and DNA synthesis itself is marked by little or no further increase in size. A similar situation has been reported in root tip nuclei of the bean, *Vicia* (36).

DISCUSSION

The basic question which we wish to resolve is the following: Is the observed incorporation of H^3-thymidine in the epithelial nuclei of mouse seminal vesicle a concomitant of DNA synthesis and mitosis? Or must we postulate, with Pelc (24) and Pelc and Gahan (27), that the uptake reflects metabolic activity of the DNA unassociated with nuclear duplication?

The labeled nuclei which fall outside the 2C range show a correlation between grain count and Feulgen dye content. Clearly the uptake of thymidine in these nuclei is associated with DNA synthesis. Since polyploidy is absent the 4C nuclei which are formed must (*a*) go through mitosis, (*b*) become sloughed off, or (*c*) return to the 2C level by some unspecified mechanism. We feel that mitosis is almost certainly the means by which these nuclei halve their DNA content, and that the seminal vesicle presents no unusual features in its DNA metabolism.

As already mentioned, slightly more than one-third of the radioactive nuclei are included within the 2C class. That these are derived from 4C nuclei which divided during the course of our experiment is suggested by the following arguments.

(*a*) Among the labeled nuclei were 2 metaphases and 4 telophase pairs. Therefore, sufficient time elapsed during the experiment to allow duplicated, radioactive nuclei to reach division.

(*b*) Most of the labeled 2C nuclei occur in pairs, as would be expected if they are derived by mitosis (Fig. 3). Among the 37 nuclei there were 15 pairs; the squashing technique could have separated the remaining nuclei from their sisters.

(*c*) The smaller size of these nuclei is further indication that they have recently emerged from the dense telophase packing. Almost half of the labeled 2C nuclei are less than 30 μ^2 in area, whereas only 10 per cent of the unlabeled ones are so small (Text-fig. 3).

(*d*) The mean grain count for the labeled 2C nuclei is 39; that of the 4C nuclei is 60. According to Taylor (47) sister chromatids as well as daughter nuclei are equally labeled at the first mitosis following isotope uptake. Even if sister chromatids themselves are unequally labeled (33), the distribution of radioactivity between *whole* daughter nuclei would seldom deviate much from equality in an organism like the mouse, which has a high chromosome number (32). Two circumstances may help to account for the fact that the 2C nuclei have somewhat more than ½ the grain count of the 4C. First, it is possible that the earliest nuclei to pass through mitosis may receive thymidine of higher specific activity than later ones. And second, the overlapping of grains above the more heavily labeled nuclei may lead to inaccurate, low counts (*cf.* Fig. 3).

(*e*) If the 37 labeled 2C nuclei arose by division, they represent 37/2 or 18½ mitoses during the 6 hour period of the experiment. The sample must have averaged at least 3 mitoses per hour (mitotic rate of 3/8890 or 0.03 per cent per hour). We say "at least" because some of the nuclei which divided during the first part of the experiment would be unlabeled and hence go undetected.

Our only direct estimate of mitotic rate comes from the 6 mitoses in progress at the end of the experiment (2 metaphases and 4 telophase pairs). To interpret these in terms of a rate we must make some assumption about the duration of mitosis. Lacking direct evidence let us suppose that division takes about 1 hour. The data of Quastler and Sherman (35) suggest that mitosis in mouse intestinal epithelium may be shorter than this, but probably not longer. The six division figures would then correspond to a rate of 6 per hour for this sample (6/8890 or 0.07 per cent per hour). It is clear that the rate estimate is sensitive to the figure chosen for mitotic duration and also to statistical fluctuation due to the small number of observations. The rate might be higher if unlabeled mitoses are present at the end of the experiment. However, the data of Hughes *et al.* (16) and Quastler and Sherman (35) show that almost all mitoses in mouse intestinal epithelium are labeled 6 to 7 hours after administration of H^3-thymidine.

These data are clearly inadequate for a critical evaluation of mitotic rate in this tissue. However, we believe they do reflect a reasonable degree of internal consistency between the number of labeled

2C nuclei and the number of mitoses. We stress this point since Pelc and Gahan (27) base their argument for metabolic DNA on the supposed major discrepancy between the rate of labeling and the rate of mitosis.

Pelc originally studied the uptake of C^{14}-adenine (24), while later with Gahan (27) he followed the utilization of H^3-thymidine. In the adenine study 40 per cent of the seminal vesicle nuclei were said to contain labeled DNA 1 day after administration of the isotope. Since adenine is incorporated into both RNA and DNA, the DNA label was demonstrated by prior digestion of the sections with either RNAse or cold perchloric acid. After treatment the cytoplasm retained some radioactivity and continued to stain lightly with pyronin. Pelc presents evidence that the cytoplasmic label represents incorporation into protein, but the pyronin staining suggests incomplete removal of RNA as a complicating factor. In this case the nuclear uptake may represent largely incorporation into RNA. Moreover, H^3-thymidine, which is a specific precursor of DNA (9, 11, 38), gives a very much lower percentage of labeled nuclei.

Pelc and Gahan base their argument for metabolic involvement of DNA on a comparison between the per cent of mitosis observed 6 hours after injection of colchicine and the per cent of labeled nuclei seen 24 hours after administration of H^3-thymidine. These figures are respectively 0.05 ± 0.02 per cent for colchicine (or 0.04 per cent using the data of Allen (2)), and 4.4 ± 0.9 per cent for thymidine. The ratio between these numbers is 4.4/0.05 or 88. When similar data were collected for mouse esophageal epithelium, the ratio was found to be 1.9. Pelc and Gahan assume that in the esophagus all incorporation leads to mitosis and then conclude that the uptake of H^3-thymidine in the seminal vesicle is "approximately forty times higher than could be expected from the known rates of proliferation in this organ." The comparison of seminal vesicle with esophageal epithelium does demonstrate a marked difference in mitotic pattern between the two tissues, but it does not provide a quantitative basis for the authors' general conclusion. The real issue is whether there must be some specified ratio between the number of labeled nuclei and the number of mitoses in an experiment of this sort.

The answer to this question depends to a large extent, but not exclusively, on the degree of mitotic synchronization. In the extreme case, exhibited by meiosis in some plants, all divisions take place at once. Here one can obtain complete labeling with no mitosis, or the converse, depending upon the timing of the experiment. In the mouse, diurnal rhythms are well known. Barnum *et al.* (3, 4) have reported a 24 hour cycle in the rate of P^{32} uptake into DNA of both immature and regenerating mouse liver. In the same tissue there is a marked diurnal periodicity in mitotic activity: around noon, the high point of the cycle, the mitotic count is 30 to 40 times greater than at midnight. The peak of P^{32} incorporation precedes the peak of mitosis by about 8 hours. Similarly mitoses in the skin epidermis and parenchyma and stroma cells of the adrenal cortex have a diurnal rhythm (13), although here the ratio of maximum to minimum is not so great as in the liver.

We injected one group of our mice at noon, the other at midnight on the chance that we might hit high and low points of a daily cycle. As already mentioned the means of the two groups differed by a factor of two, but the difference is not statistically significant. A large scale experiment with a shorter labeling period and many more animals would be needed for an adequate periodicity analysis. Until such data are available we cannot assess the meaning of the ratio reported by Pelc and Gahan.

Finally, to make an interpretation of the ratio between labeling and mitosis, we need to know the various time parameters of the mitotic cycle (*e.g.*, 35), as well as the duration of isotope availability. There is also a possibility that colchicine may influence the ratio by slowing down cells that have begun mitosis or by lowering the rate of entry into mitosis (12). In view of our general ignorance of these factors, we feel that the data of Pelc and Gahan provide a hazardous basis for conclusions about the metabolic activity of DNA.

Our experiment affords some information about the availability of injected thymidine. As shown in Text-fig. 2, nuclei in the 2C to 4C category show a proportionality between grain count and Feulgen dye content. This fact suggests that radioactive thymidine or its derivatives is still being incorporated at the end of the 6 hour period. There may have been some drop in specific activity, since the grain count of the labeled 2C nuclei averages somewhat more than half that of the 4C, but the isotope has certainly not been exhausted. Hughes *et al.* (16) studied the uptake of H^3-thymidine by mouse tissue following intraperito-

neal or intravenous injection. They found that thymidine leaves the blood stream within a few minutes and passes into the tissues, possibly in the form of thymidylic acid. Subsequent loss of thymidylic activity from the tissue is likewise rapid. They suggest that all labeling of nuclei may occur during the first hour after injection. They are careful to point out that this conclusion follows only if the cells synthesizing DNA exhaust thymidine at the same rate as the whole tissue. Our data raise the interesting possibility that cells about to synthesize DNA may sequester a pool of precursors which is not freely exchangeable. This pool could remain available for synthesis during the latter part of the experiment, when the thymidylic activity of the tissue as a whole is low.

We will comment briefly on three other cases of "metabolic" DNA mentioned in the recent literature. Koenig (17, 18) has studied incorporation of C^{14}-adenine and C^{14}-orotic acid in the central nervous system of the cat. Beginning the 2nd day after injection, ribonuclease-resistant label appears in nuclei of oligodendrocytes and Schwann cells. At first, only a small fraction of cells is labeled, but in long term experiments lasting up to 51 days, as many as 30 to 40 per cent of the oligodendrocyte nuclei may be involved. The label is removed by DNAse. The case is of interest here, since mitosis is generally supposed to be absent from the central nervous system of adult mammals. However, a low rate of division, of the order of 1 percent per day, would explain Koenig's findings; and there is some evidence for cell division in the nervous system. Allen (1) long ago reported some mitosis in the cerebrum of adult albino rats. Messier, Leblond, and Smart (21) have demonstrated H^3-thymidine incorporation in dividing cells of the mantle layer in the brain of young adult mice. Penfield (30) discusses the well known fact that oligodendrocytes may increase in number under certain pathological conditions, although he ascribes the increase to amitosis. Spectrophotometric data on the labeled oligodendrocytes would help resolve the question of synthesis versus metabolic lability.

LaCour and Pelc (19) and Pelc and LaCour (29) have recently studied the incorporation of H^3-thymidine in roots of the bean, *Vicia faba*. They claim to find rapid uptake in cells which are not synthesizing DNA, specifically in the 4C nuclei of the elongation zone. These nuclei, moreover, are said to become more radioactive than the actively dividing meristematic nuclei at the very tip of the root. Pelc and LaCour say that "nearly all nuclei at a distance of 1.9 mm. from the tip of the root contain 4C (prophase) values of DNA." The data of Deeley, Davies, and Chayen (8), however, do not bear out this statement. As shown in the histograms of Fig. 2 in the paper of Deeley *et al.*, many nuclei in the region between 2 and 4 mm. from the tip have Feulgen dye contents between the 2C and 4C values, both in the cortex and central cylinder of the root. In the zone from 4 to 8 mm. there are numerous intermediate values in the central cylinder, and a few in the cortex.[2] More recently the problem has been reinvestigated by Rasch, Woodard, and Swift (37). They find excellent agreement between the number of interclass nuclei and the number of nuclei labeled in short term isotope experiments. Furthermore, no label occurs in the differentiation zone where interclass values are absent. The heavier uptake in the zone of elongation, as compared to the meristem, is possibly due to the presence there of nuclei intermediate between 4C and 8C.

The third case, also reported by Pelc (25), concerns the loss of DNA from epithelial nuclei of mouse esophagus during the process of keratinization. Pelc finds H^3-thymidine incorporation in the basal layer of cells prior to mitosis. As these cells are displaced upward, some of their radioactivity, which was at first confined to the nucleus, begins to appear in the cytoplasm. Pelc calculates that there is also an over-all loss of radioactivity from the epithelium, which cannot be accounted for by sloughing of cells from the surface. He postulates that the DNA may be used in the synthesis of keratin. This case differs from those already considered in that the proposed metabolic activity concerns the breakdown products of degenerating nuclei. The incorporation of thymidine presumably occurs only at the time of DNA duplication.

The studies discussed in this paper raise several questions about the interpretation of autoradiographs, particularly when DNA precursors are involved. The most important of these is whether incorporation always implies synthesis of new molecules. Probably the majority of biologists who use labeled thymidine assume that uptake by DNA is *prima facie* evidence for duplication. It is useful

[2] See also the recent comments by E. Tschermak-Woess, *Chromosoma*, 1960, **11**, 25.

to have this assumption brought under scrutiny from time to time, since it is possible that exchange of nucleotides may occur without net synthesis. In most cases photometric determinations of DNA content should help to clarify the situation, particularly if grain counts and DNA measurements are made on the same group of nuclei. To the best of our knowledge there is no clear-cut example of nucleotide incorporation unaccompanied by DNA synthesis. We have given detailed reasons for discounting the claims made for the mouse seminal vesicle and bean root tip. In the case of the cat oligodendrocytes the requisite photometric data are not presently available.

After synthesis DNA is usually distributed by mitosis to two daughter nuclei with a consequent return to the 2C condition. Obviously this leads to the production of labeled 2C nuclei, as in the mouse seminal vesicle. On the other hand, mitosis may not ensue; labeled 4C and 8C nuclei can then accumulate, as in the bean root tip. Still a third possibility is that the newly synthesized DNA may be used up in some metabolic process. This apparently happens in keratinizing epithelium, when the nuclei as a whole degenerate.

Much of the recent interest in the metabolic activity of DNA has centered in studies on the giant polytene chromosomes of Dipteran larvae (5, 10, 40, 42). Certain bands on these chromosomes show a disproportionate local increase in DNA content at the time of "puffing" or Balbiani ring formation. Heavy incorporation of thymidine occurs simultaneously. The situation in the polytene chromosomes is of the utmost interest, since puffing may be a cytological expression of gene activity. If a similar phenomenon occurs in other organisms, we may expect to find DNA synthesis as a regular nuclear feature independent of mitosis or polyploidization.

It should be noted, however, that the disproportionate increase involves a very small fraction of the total DNA content of the nucleus, probably less than 1 per cent (40). Such an increase would be unmeasureable by conventional photometric techniques for whole nuclei (23), and would require extremely favorable cytological conditions to be detected in autoradiographs of smaller chromosomes. We feel that mitosis or polyploidization is an adequate explanation for cases like the seminal vesicle and bean root tip, in which the whole nucleus becomes moderately or heavily labeled by H^3-thymidine, and the DNA content doubles.

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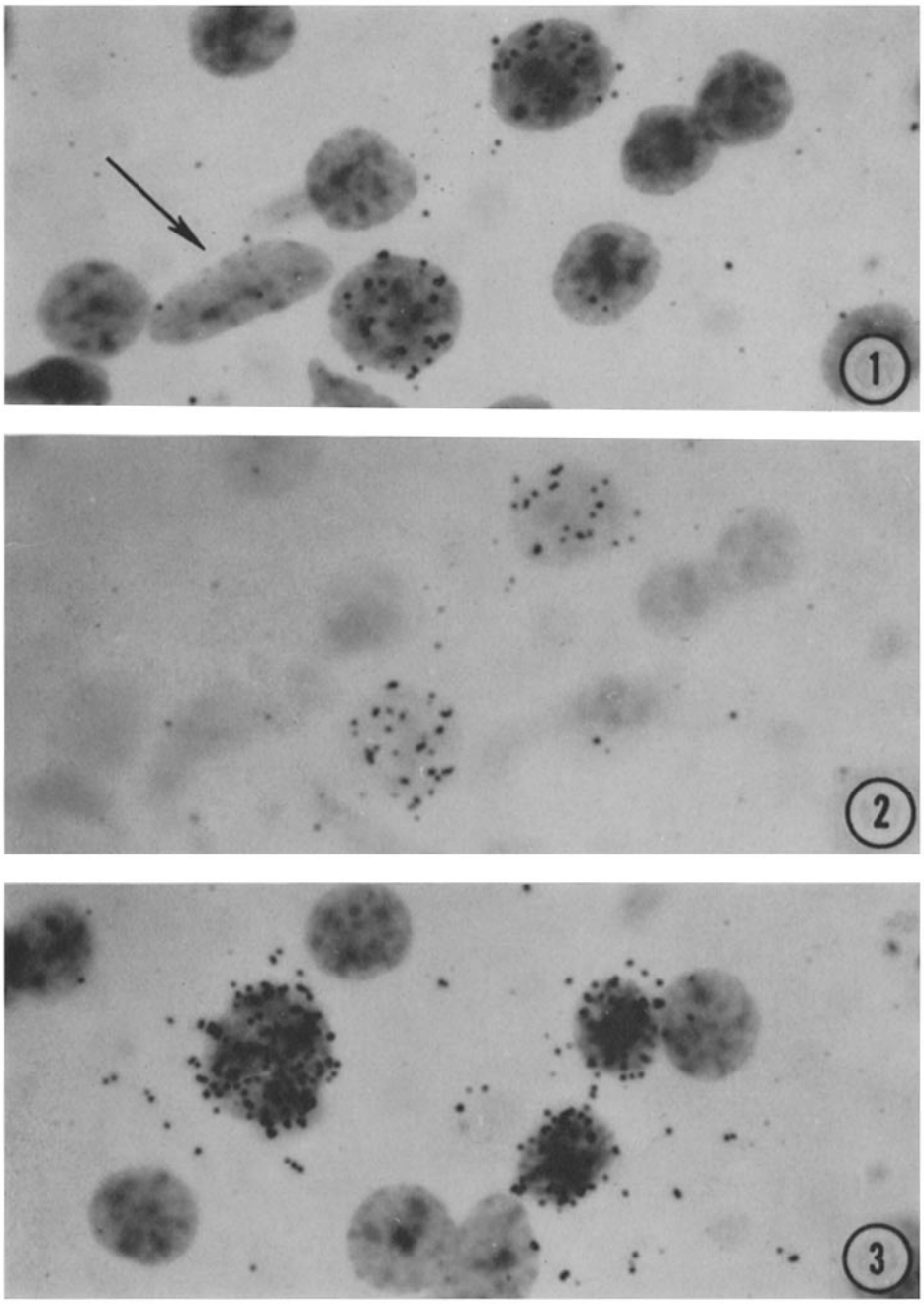
EXPLANATION OF PLATE 326

FIG. 1. Seminal vesicle epithelium of the mouse. Autoradiograph of Feulgen-stained squash preparation, photographed in green light to emphasize the nuclei. The two labeled nuclei are larger than the unlabeled ones, and their DNA content is greater than 2C. In such nuclei the number of silver grains is correlated with the extent of DNA duplication. The arrow indicates a connective tissue nucleus. × 1950.

FIG. 2. The same area, photographed in red light to emphasize the silver grains in the emulsion. × 1950.

FIG. 3. Another area to show a large, heavily labeled 4C nucleus (left) and two labeled 2C nuclei (right). Most hot 2C nuclei occur in pairs and are smaller than cold ones. Both features suggest that they have arisen by mitosis during the experiment. Their grain count averages somewhat higher than one-half that of the 4C nuclei. × 1950.



(Gall and Johnson: "Metabolic" DNA in mouse seminal vesicle)