of detonation by means of data on the production ratio based on yields of thermal-neutron fission production from U^{235} (*3*). However, I^{131} and Ba^{140} suffer heavily from fractionation effects in the atmosphere (*4*). A calculated date far removed from any reported test date (26 July 1968) is therefore not too surprising. If one instead assumes a production date of 24 August (date of first French thermonuclear test), the production ratio of I^{131} to Ba^{140} calculated from the experimental data is 0.23 ± 0.08. This is in good agreement with the observed production ratio of I^{131} to Ba^{140} (0.17 ± 0.05) obtained for the Chinese test of 16 October 1964 in which U^{235} was used as the fissioning material (*5*). Thus the time required for the transport of the fission debris from a mid-southern to a mid-northern latitude is of the order of 22 days.

Rainfall activity was continuously checked through 3 December 1968 for the possible arrival of a second "wave" of debris from the second French thermonuclear test of 8 September 1968. No additional fresh debris was observed at this laboratory (Table 1). Therefore, even though it is impossible to definitely assign the observed short-lived activity to the first French thermonuclear test, it can be concluded that debris from the French tests required a maximum of only about 3 weeks for the interhemispheric transport.

B. D. PALMER

Department of Chemistry,
Henderson State College,
Arkadelphia, Arkansas 71923

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Cell Population Kinetics: A Modified Interpretation of the Graph of Labeled Mitoses

Abstract. *Graphs of labeled mitoses, derived from autoradiographs of cell populations with ^{3}H-thymidine, show depressions in the curves at their midpoints. These depressions reflect interruption of DNA synthesis midway through S phase. Such interruptions revealed by the method of labeled mitoses should be considered when determining cell-cycle times.*

The method of labeled mitoses (*1*) has been used extensively in studies of cell population kinetics of tissues to determine duration of the cell reproductive cycle and its components. One such study (*2*), of oral mucosal epithelium of the rat, has given a graph (Fig. 1) which differs from that of Quastler in that the plateau of each curve has a depression at approximately the midpoint. These depressions are not unique to this investigation; similar configurations, though not always described, are evident in other published graphs of cell population kinetics (*1, 3, 4*). These depressions are phenomena revealed by the method of labeled mitoses, and the second curve, despite its two peaks, represents only one population of cells. When the graph is interpreted in this way, cell-cycle time is represented by the horizontal distance between the two curves at the deepest points of their depressions.

Data (Fig. 1) were obtained from autoradiographs of Feulgen-stained squash preparations of the mucosal epithelium on the hard palate of 6-month-old male Wistar albino rats. Rats were injected intraperitoneally, at the same time of day, with ^{3}H-thymidine (specific activity, 3.0 c/m*M*; concentration, 1 mc/ml; dose, 0.2 μc per gram of body weight). Autoradiographs were prepared with Kodak AR.10 fine-grain stripping emulsion and were exposed for 6 weeks. The proportion of labeled mitoses was derived, in each instance, from 100 mitoses.

Heretofore no attempt has been made to explain the depression in the first curve although, in one instance, a low point has been attributed to the possibility of a faulty injection of radioisotope (*4*).

There are two possible explanations for the shape of the second curve if it is assumed to be bimodal and to represent two subpopulations of cells, each with a different cycle time: (i) The two peaks of the second curve may represent two subpopulations of cells differing in the duration of the G_1 phase (*4*). (ii) One peak may represent a subpopulation of cells dividing for the first time after a prolonged G_2 period (*5*).

The first hypothesis is rejected because, whereas two subpopulations with different cycle times should manifest an increasing separation of peaks at each subsequent division, in this study—even when there is a third curve representing a third wave of labeled mitoses—the separation of its peaks differs only slightly from the original (Fig. 2). Furthermore, this hypothesis does not explain the dip in the first curve.

The second hypothesis is also rejected because in a given area in tissue section autoradiographs the number of labeled cells present at 1½ hours had doubled by 16 hours, thus indicating that all of the cells, or almost all of them, had divided by this time. Furthermore, if one of the peaks in the second curve were to represent a subpopulation of cells with a G_2 period exceeding 2 hours, the proportion of labeled mitoses would not have reached 100 percent at 2 hours; nor does this hypothesis account for the dip in the first curve.

It is more likely that the depressions in the curves are manifestations of interruption of DNA synthesis, for an hour or so, midway through S phase. That such a discontinuity in DNA synthesis, or a change in its rate, can occur has been demonstrated (*6*). For a cell in S phase to escape labeling after the introduction of ^{3}H-thymidine into the system, DNA synthesis must cease, or be reduced in rate, for at least the interval during which the radioactive thymidine is available. The duration of this interval is not known precisely but it has been estimated to be 30 to 120 minutes (*7*). For 2 percent of S-phase cells in an asynchronous population to remain unlabeled, as in this experiment, the interruption of DNA synthesis must be further prolonged by a period equal to 2 percent of the 8-hour synthesis time, or approximately 10 minutes. Consequently, it is estimated that DNA synthesis in this system is interrupted for about 1½ hours. Cells which enter this period of interrupted DNA synthesis shortly after the radioisotope is available, and cells which resume DNA synthesis shortly before the radio-

isotope is eliminated, will incorporate less of it than cells which synthesize DNA throughout the period of radioisotope availability. These cells with interrupted DNA synthesis, along with those that complete S phase shortly after the radioisotope is available and those which enter S phase shortly before the radioisotope is eliminated, form a small population which, by virtue of its reduced incorporation of radioisotope, is distinguished in autoradiographs by a lighter label. Dilution of this label by mitosis makes it hard to recognize the progeny of these lightly labeled cells during a subsequent mitosis, and failure to recognize them accounts for the larger depression in the second curve, as well as the slightly smaller area under it, as compared with the first curve.

Autoradiography, as used in this study, may not be sensitive enough to reveal small quantities of radioisotope incorporated during a diminished rate of DNA synthesis. Hence the occurrence of unlabeled mitoses may not depend on complete cessation of DNA synthesis during a part of S phase. All that is needed is a substantial reduction in rate of synthesis for a period approximately equal to the availability of the radioisotope. Nor is it essential that in each half of each chromosome, for the entire nuclear complement, DNA synthesis begin and end at precisely the same time. Therefore, the hypothesis advanced here is not vitiated by the evidence from autoradiographic studies which have shown variation in the times at which chromosomes complete S phase.

The possibility that the small proportion of unlabeled cells at 4 hours may represent a "false negative" is rejected because: (i) cell squash preparations, unlike paraffin wax sections, contain all of the nuclear radioactive material; (ii) at 4 hours nuclear labeling is usually maximum; (iii) exposure time of the autoradiographs (6 weeks) is relatively long; and (iv) heavily labeled interphase nuclei are also present near each unlabeled mitosis.

The possibility that absence of label might be due to inadequate dosage or to a long G_2 phase in a particular animal was explored by repeating the experiment at the 4-hour interval. The same proportion of unlabeled cells was found in this replication and also in a similar experiment on rats with diabetes mellitus.

It is not clear why DNA synthesis should be interrupted midway through S phase but the interruption could relate to the necessity of duplicating and assembling the protein framework of the chromosome as well as its DNA. Under the circumstances it is possible that the two strands of the DNA molecule are reproduced in sequence rather than in synchrony, their reproduction being separated temporally by the synthesis of the protein for one of the strands or by the conjugation of its protein and DNA. The important constraints are that in each chromosome only one strand of each DNA unit be duplicated at a time and, if the chromosome comprises more than one ribbon of DNA, that in all the units one strand of each be duplicated at approximately the same time. This method of chromosome duplication is compatible with most of the suggested models of chromosome structure.

On the basis of the hypothesis suggested here, Wolfsberg's graph (*4*) could be reinterpreted as a single population of cells with a generation time of about 20 to 24 hours rather than two subpopulations with generation times of about 30 hours and 2 to 4 weeks, respectively.

The existence of a lightly labeled subpopulation of cells, as a result of interrupted DNA synthesis, must be considered in autoradiographic studies that involve a comparison of the number of silver grains appearing over labeled nuclei. Several such studies have in fact revealed proportions of lightly labeled nuclei (*4, 8, 9*). Koburg (*8*) and Wolfsberg (*4*) give various reasons for this population of lightly labeled cells but the hypothesis suggested here offers an alternative explanation.

Investigations in which unlabeled mitoses were found at the midpoint of the first curve in the graph of labeled mitoses, as well as those revealing variations in the rate of incorporation of ^{3}H-thymidine into DNA, provide evidence to support the findings of the present study. The explanation of these findings, namely, that a marked reduction in the rate of DNA synthesis occurs about midway through S phase, is confirmed by the results of Kasten and Strasser (*6*). This phenomenon is

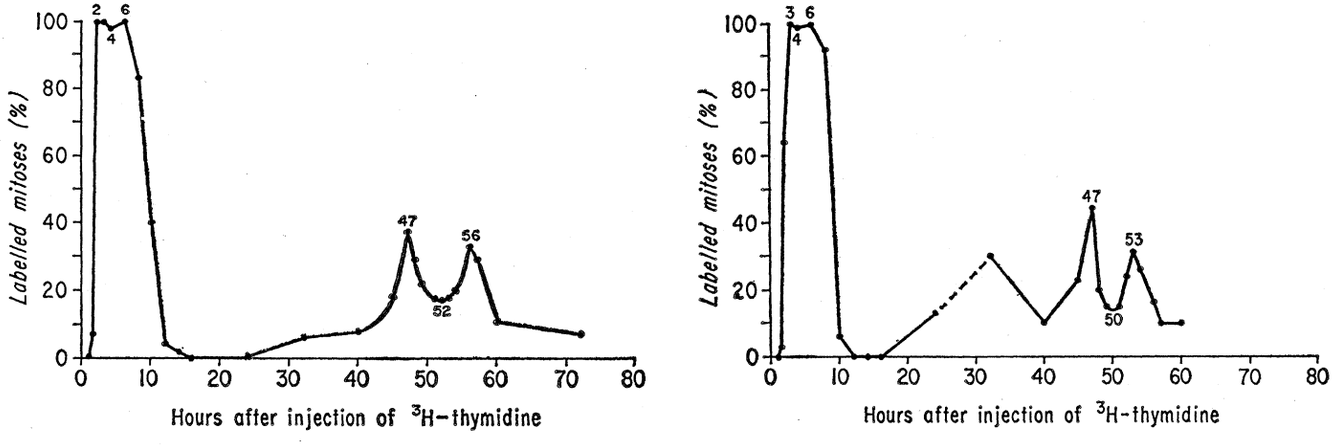

Fig. 1 (left). Functional relation between proportion of labeled mitoses in epithelium of hard palate and time after injection of ^{3}H-thymidine. Each point on the curve, except those at 4 and 57 hours, represents the value obtained from one experimental animal; at 4 hours the values from two animals were identical, and at 57 hours the values from two animals differed by 1 percent. Phase duration in hours: G_1, 38; S, 8; G_2, 1; M, 1; and C, 48. Fig. 2 (right). Functional relation between proportion of labeled mitoses in epithelium of soft palate and time after injection of ^{3}H-thymidine. Each point on the curve represents the value obtained from one experimental animal. The curves for the first and third waves of labeled mitoses are complete; the broken line indicates the missing part of the curve for the second wave of labeled mitoses. Phase duration in hours: G_1, 14; S, 7; G_2, 1; M, 1; and C, 23.

revealed in the graph of labeled mitoses by depressions in the plateaus of the curves at approximately their midpoints. Hence the curves, though comprising two peaks, represent only one population of cells whose cell-cycle time is expressed by the horizontal distance between two consecutive curves at the deepest points of their depressions.

A. IAN HAMILTON
Department of Operative Dentistry and
Department of Biological Structure,
University of Washington,
Seattle 98105

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Immunochemistry of Newly Found Substituents of Polysaccharides of Rhizobium Species

Abstract. *Acetyl and pyruvic acid groups were detected and estimated in the extracellular polysaccharides of* Rhizobium trifolii *and* R. meliloti. *Pyruvic acid was found also in the specific polysaccharide of pneumococcal type 27, and is believed to explain the cross-reactivity between antiserum to pneumococcal type 27 and these and other pyruvate-containing bacterial polysaccharides. Removal of pyruvate from a* Rhizobium *polysaccharide rendered it inactive with its homologous antiserum and with antiserum to pneumococcal type 27, but it then reacted with several antiserums to other pneumococcal types with which the intact material was unreactive.*

Extracellular polysaccharides of strains of *Rhizobium trifolii* exhibited anomalous behavior. The polysaccharide of strain TA1 (hereinafter referred to as TA1), after exhaustive methylation by usual standard techniques showed a methoxyl content of only 31 percent, yet no free hydroxyl groups were detectable in the infrared spectra (*1*). There was also a large discrepancy in the content of uronic acid found in TA1 by decarboxylation (23 percent), by the carbazole reaction (23 percent), and by titration with dilute alkali (42 percent) (*1*). Although the polysaccharides of five strains of *R. trifolii* contained glucose, galactose, and glucuronic acid in the same relative proportions, as judged by chromatographic comparisons, they displayed little serological cross-reactivity, but reacted well with their homologous antiserums by double diffusion in agar. Moreover, when tested with antiserums to pneumococcal types 1 to 29, they failed to cross-react significantly except in antiserum to pneumococcal type 27. This was also one of the antipneumococcal serums that reacted with polysaccharides of *R. meliloti*, which are known to contain very little uronic acid (*2*). Partial hydrolysis of TA1 with 0.01*N* HCl for 20 hours at 100°C eliminated some oligosaccharides, but 65 percent of the polysaccharide was recovered with apparently unchanged composition with respect to the sugars. This degraded TA1 no longer precipitated antiserum to TA1 or to pneumococcal type 27 but now showed precipitation with antiserums to types 6, 7, 8, 9, 10, and 14.

Table 1. Acetyl and pyruvic acid substituents in polysaccharides of *Rhizobium*. Results shown are for air-dried samples.

Polysaccharide	Acetyl (%)	Pyruvic acid (%)
R. meliloti strain B	4.5	4.7
strain F	4.1	5.0
R. trifolii strain TA1	4.1	9.0
strain UNZ29	3.7	10.4
strain WA67	3.3	9.1
strain CC10	2.1	8.8
strain 2480a	4.0	9.2
R. radicicolum	2.2	7.8
Pneumococcal type 27	7.0*	8.7
R. trifolii strain TA1		
Acid-degraded	4.4	0.3
NaOH-treated	0	6.9

* From R. Brown (*14*).

All these results suggested the presence of hitherto undetected "masking" groups or side chains. Accordingly, the polysaccharides were analyzed for phosphate, *O*-acetyl (*3*), and pyruvic acid (*4*). Both acetyl and pyruvate were found in all of the *Rhizobium* polysaccharides examined (Table 1); they were free of phosphate. The acetyl of TA1 and the pyruvate of all the *Rhizobium* polysaccharides were identified chromatographically as the hydroxamate and the 2,4-dinitrophenylhydrazones, respectively, by comparison with authentic standards. The hydroxamates were separated in an ethyl acetate, acetic acid, formic acid, water system (18:3:1:4, by volume) and in an ethyl acetate, pyridine, water system (5:2:5, by volume) and detected with alcoholic acidic ferric chloride; chromatograms of the 2,4-dinitrophenylhydrazones were developed in a butanol, ethanol, water system (5:1:4, by volume) (*5*). These substituents, which the amounts found in TA1 indicate to be present on approximately one-third of the sugar residues, provide an explanation for the low methoxyl and high titratable acid values obtained with this polysaccharide.

Mild treatment of TA1 with acid, as above, resulted in the selective removal of the pyruvate while 0.2*N* NaOH at room temperature for 22 hours displaced the acetyl groups (Table 1). Loss of pyruvic acid abolished serological activity with antiserums to TA1 and to pneumococcal type 27 while removal of acetyl led to loss of reactivity only with antiserum to TAl.

Because pyruvate groups could be implicated in the specificity of pneumococcal type 27, we analyzed the specific polysaccharide of this pneumococcal type for pyruvic acid, and it was found to contain 8.7 percent. Antiserum to type 27 had already been shown to react strongly with the polysaccharides of *Rhizobium radicicolum* and *Xanthomonas campestris* (*6*). The former, which contains glucose and glucuronic acid (*7*), was analyzed and found to contain pyruvate and acetyl (Table 1); the presence of both groups (approximately 10 percent acetyl, 3.0 to 3.5