THE PLATEAU PHASE OF GROWTH
OF THE L-M STRAIN MOUSE CELL IN A
PROTEIN-FREE MEDIUM

III. THE EFFECT OF ADDED GLUCOSE
ON PROTEIN AND NUCLEIC ACID SYNTHESIS AND
ON CARBOHYDRATE UTILIZATION

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Previous studies employing L-M mouse cells [16] in suspension culture revealed that the onset of the plateau phase is accompanied by a depletion of the original glucose in the medium [23]. The cells become smaller in size and viability drops rapidly [21]. The addition of glucose at this point in the growth cycle preserves cell viability and extends the duration of the plateau phase [21]. Since glucose primarily is an energy source, plateau phase cells normally resort to endogenous metabolism and thereby deplete cell reserves, such as amino acids, glycogen, etc.

The experiments reported here demonstrate that in the presence of an adequate energy source, cells in the plateau phase of growth not only remain viable for prolonged periods but continue to synthesize protein and nucleic acid in an otherwise depleted medium. Moreover, it is speculated that homologous material such as protein and nucleic acid from lysed cells is degraded and possibly reutilized by the viable cells in the population. The potential for such reutilization is shown.

MATERIALS AND METHODS

Populations of L-M strain mouse fibroblasts were propagated in modified medium 199 [11] plus peptone and analyzed for nucleic acid and protein as previously described.

1 This work was supported in part by Grant CA-03720 from the USPHS and by Parke, Davis and Co., Detroit, Mich.
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3 From a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Mich.
Supplemental glucose was prepared by dissolving 50 μc of uniformly labeled D-glucose $^{14}$C (35.9 mg dry sugar) in 10 per cent carrier glucose solution to effect a final concentration of 0.5 μc/ml. The solution was autoclaved at 121°C for 15 min and stored frozen in 5 ml aliquots. At three points during the plateau phase 5 ml of this supplemental glucose solution was added to each of the experimental cultures. Periodically 10 ml samples were withdrawn for biochemical analysis of both cells and medium employing a modified Schmidt-Thannhauser extraction procedure as previously described [11]. Glucose was determined enzymatically by the use of Glucostat® and lactic acid by the Barker and Summersorn method [3]. In the radioactive tracer experiments glucose and lactic acid were not isolated as the pure compounds for radioactive counting purposes. Radioactive counts of lactic acid were performed on the acid soluble fraction after the copper sulfate-calcium hydroxide treatment employed in the Barker-Summerson procedure.

For radioactive counting liquid samples were neutralized by the addition of acid (HCl) or alkali (NaOH) and 0.1 ml aliquots were pipetted to aluminium planchets. One ml of water was added to mix and spread the sample evenly over the surface. Planchets were dried under infrared light and counted in a D-47 gas flow counter having an efficiency of approximately 30 per cent. One tenth ml of a 1:100 dilution of the uniformly labeled glucose solution consistently registered a count of 340–345 disintegrations per minute. On a theoretical basis the 0.0005 μc contained in the 0.1 ml plated should register 1,100 disintegrations per minute if 100 per cent efficiency was achieved. On a 30 per cent basis the count would be 330 disintegrations per minute. All radioactive counting data presented represent the actual count after correction for background count. No correction for efficiency was made. Where problems of self absorption arose, the samples were diluted further in distilled water until this difficulty was overcome. Specific activity, hereinafter mentioned, refers to the radioactive count per minute per microgram of the material analyzed. The “combined specific activity” hereinafter mentioned refers to the total radioactive count of material isolated from the cells and medium in counts per minute per total micrograms of material isolated.

Radioactive CO$_2$ was trapped in 20 ml of 2 N sodium hydroxide as previously described [11]. Ten ml aliquots were transferred to a manometric apparatus as described by Abrahamson [1] and CO$_2$ was liberated in the system by addition of lactic acid. The volume of CO$_2$ released was determined manometrically and transferred to a Bernstein-Ballentine gas counting tube [4] for radioactive count at an efficiency of approximately 70 per cent.

Homologous nucleic acid-protein fractions were prepared as follows: Late in the plateau phase an entire culture, which had been exposed previously to $^{14}$C radioactive glucose was placed at 4°C and the cells were allowed to sediment. The medium was withdrawn and 200 ml of cold distilled water was added to resuspend the cells and induce lysis. After 5 min of agitation in the spinner culture vessel, cold perchloric acid was added to effect a final concentration of 3 per cent. The suspension was agitated for an additional 5 min on a magnetic stirrer and then kept in the cold until the precipitate had sedimented. The acid soluble supernatant fluid was removed.

1 Nuclear-Chicago, Chicago, Ill.
2 Worthington Biochemicals, Freehold, N.J.
Utilization of glucose by plateau phase cells 149

and the precipitate was extracted twice with 50 ml of neutral 95 per cent ethanol and once with 50 ml of ethanol-ether (1:1) at 35°C. The nucleic acid-protein precipitate was left at 35°C for 3 hr to volatilize any residual ethanol-ether. While still moist it was dissolved in 40 ml of 0.1 N sodium hydroxide and added to cultures in the logarithmic phase of growth.

RESULTS

Figs. 1, 2 and 3 show the results of one experiment in which multiple additions of glucose were administered during the plateau phase to provide an adequate reserve of carbohydrate in addition to an increasing supply of radioactive substrate. The patterns of protein and nucleic acid synthesis were similar to those obtained previously [11], but differed in the rate and time at which cellular RNA and DNA values decreased. Cell number increased slightly from $1.3 \times 10^6$ cells/ml to $1.5 \times 10^6$ cells/ml during the plateau period of 10 days, although cell viability as determined by the dye exclusion technique [25] was only 14 per cent at the end of this period.

When compared to a culture not supplemented with glucose [11], protein

![Graph](image-url)
synthesis (Fig. 1), increased in a similar manner but at a slower rate and cell viability remained relatively high (69 per cent) after 144 hr in plateau. Radioactive glucose was utilized in the synthesis of protein in increasing amounts as the plateau phase progressed, as evidenced by an increase in specific activity. Although the specific activities were very low (0.069 to 0.367 cpm/µg of protein), they represent only 0.36 per cent of the total glucose available to the cells. Theoretically, if all the glucose had been labeled the values obtained would have been increased 280 fold.

The specific activity of the protein found in the medium (0.034 to 0.101 cpm/µg) was less than that of the cellular protein at any point during plateau, but the level of protein in the medium increased 7.5 fold, whereas cellular protein increased only 1.36 fold. The lower specific activity of the former indicated that possibly different kinds of protein were being formed or that dilution of newly synthesized protein occurred in the presence of protein previously synthesized and lost from the cells.

As seen in Fig. 2, the synthesis of RNA followed the same general pattern as in unsupplemented cultures [11] except that cellular RNA was maintained at a constant level during the first 48 hr of plateau. It then decreased but at a much slower rate than in the controls. However, the specific activity continued to increase both in cellular and medium RNA. In every instance the specific activity of the RNA in the medium was considerably higher than that of the RNA in the cells.

Fig. 2.—Effect of multiple additions of glucose on RNA synthesis of L-M cells during plateau phase.

Experimental Cell Research 37
Fig. 3 shows the pattern of DNA synthesis in the culture. The cellular content increased slightly at the onset of plateau and then remained constant up to 120 hr after which it slowly declined. The specific activity of cellular and medium DNA continued to increase as the plateau phase progressed, but there was not a great difference between the two at any one point. This suggested that DNA was being synthesized and replaced at a constant rate, a conclusion which is supported by the incorporation of radioactivity into the DNA.

Table I summarizes the composite picture of DNA, RNA and protein synthesis during the plateau phase in the presence of increasing levels of radioactive carbohydrate. In each instance glucose was utilized in synthesis of nucleic acid and protein in increasing amount as indicated by the rise in specific activity. While cellular RNA decreased to approximately one third the original level after 238 hr, the concentration of RNA in the medium essentially doubled. The total amount of RNA detected in the system decreased gradually during this period, but the combined specific activities indicated an increased utilization of glucose in the synthesis of RNA.

Cellular DNA decreased to two-thirds of its original value by the end of plateau, but total DNA increased gradually and the combined specific activities again indicated increasing utilization of glucose. In most instances
the specific activity of the DNA in the medium was slightly higher than that of the cells. In contrast to the nucleic acids, protein content increased in both the cells and medium, though cellular protein increased only 3.5 per cent whereas the protein in the medium increased 68.2 per cent during this period. The specific activity of the cellular protein was twice that of the medium protein initially and increased to 3.63 fold by the end of the experiment.

Table I. Total protein and nucleic acid synthesis by L-M cells in plateau phase.

<table>
<thead>
<tr>
<th>Hr in Plateau</th>
<th>μg/1 × 10⁶ cells</th>
<th>Specific activity CPM/μg</th>
<th>% max. radioactivity</th>
<th>μg/ml of culture</th>
<th>Specific activity CPM/μg</th>
<th>% max. radioactivity</th>
<th>μg/ml of culture (Total)</th>
<th>Combined specific activity CPM/μg</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell RNA</td>
<td>RNA in the medium</td>
<td>RNA</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>33.8</td>
<td>0.114</td>
<td>0.125</td>
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<td>76.0</td>
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<td>0.159</td>
<td>0.175</td>
<td>37.0</td>
<td>2.00</td>
<td>1.850</td>
<td>80.0</td>
<td>1.000</td>
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<td>54</td>
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<td>0.159</td>
<td>0.175</td>
<td>40.0</td>
<td>2.35</td>
<td>2.35</td>
<td>83.0</td>
<td>1.200</td>
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<td>27.2</td>
<td>0.216</td>
<td>0.100</td>
<td>45.0</td>
<td>2.60</td>
<td>1.46</td>
<td>82.0</td>
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<td>0.562</td>
<td>0.225</td>
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<td>3.30</td>
<td>1.94</td>
<td>79.0</td>
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<tr>
<td>144</td>
<td>20.0</td>
<td>0.357</td>
<td>0.125</td>
<td>49.0</td>
<td>3.63</td>
<td>2.22</td>
<td>76.0</td>
<td>2.44</td>
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<td>238</td>
<td>10.0</td>
<td>0.333</td>
<td>0.036</td>
<td>58.0</td>
<td>4.27</td>
<td>1.77</td>
<td>72.0</td>
<td>3.46</td>
</tr>
<tr>
<td>Cell DNA</td>
<td>DNA in the medium</td>
<td>DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>14.6</td>
<td>0.236</td>
<td>0.125</td>
<td>10.5</td>
<td>0.383</td>
<td>0.100</td>
<td>29.5</td>
<td>0.305</td>
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<tr>
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<td>0.300</td>
<td>0.150</td>
<td>11.5</td>
<td>0.435</td>
<td>0.125</td>
<td>31.5</td>
<td>0.348</td>
</tr>
<tr>
<td>54</td>
<td>14.7</td>
<td>0.350</td>
<td>0.175</td>
<td>12.5</td>
<td>0.480</td>
<td>0.150</td>
<td>32.5</td>
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<td>11.4</td>
<td>0.444</td>
<td>0.137</td>
<td>18.0</td>
<td>0.444</td>
<td>0.100</td>
<td>34.0</td>
<td>0.559</td>
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<tr>
<td>238</td>
<td>10.0</td>
<td>0.800</td>
<td>0.085</td>
<td>22.5</td>
<td>0.933</td>
<td>0.150</td>
<td>37.0</td>
<td>0.880</td>
</tr>
<tr>
<td>Cell protein</td>
<td>Protein in the medium</td>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>124.6</td>
<td>0.069</td>
<td>0.250</td>
<td>58.0</td>
<td>0.034</td>
<td>0.050</td>
<td>220.0</td>
<td>0.090</td>
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<tr>
<td>30</td>
<td>129.3</td>
<td>0.123</td>
<td>0.500</td>
<td>64.0</td>
<td>0.033</td>
<td>0.100</td>
<td>235.0</td>
<td>0.102</td>
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<tr>
<td>54</td>
<td>132.4</td>
<td>0.133</td>
<td>0.600</td>
<td>68.0</td>
<td>0.103</td>
<td>0.175</td>
<td>245.0</td>
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<tr>
<td>102</td>
<td>135.3</td>
<td>0.282</td>
<td>0.650</td>
<td>94.0</td>
<td>0.106</td>
<td>0.125</td>
<td>276.0</td>
<td>0.222</td>
</tr>
<tr>
<td>122</td>
<td>137.2</td>
<td>0.308</td>
<td>0.725</td>
<td>110.0</td>
<td>0.109</td>
<td>0.146</td>
<td>290.0</td>
<td>0.234</td>
</tr>
<tr>
<td>144</td>
<td>137.1</td>
<td>0.312</td>
<td>0.750</td>
<td>122.0</td>
<td>0.123</td>
<td>0.176</td>
<td>310.0</td>
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<tr>
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<td>0.367</td>
<td>0.586</td>
<td>178.0</td>
<td>0.101</td>
<td>0.122</td>
<td>370.0</td>
<td>0.238</td>
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</table>
Fig. 4 shows that response of the culture in terms of glucose utilization and lactic acid production. A burst of lactic acid production occurred after each addition of glucose. This result was in accord with the experiments of Broda et al. [5] and Danes and Paul [8] who demonstrated that an increased supply of glucose enhances glycolysis and lactic acid production. Moreover, in this experiment the radioactive count of the lactate increased significantly with each addition of glucose and decreased as the lactate was utilized. Glucose in the acid soluble fraction of the cells continued to increase as plateau progressed. The radioactive count also increased, but this count represents the entire acid soluble fraction and may have been due to the accumulation of label in other compounds such as glycolytic and Krebs cycle intermediates.

The results of the experiments are representative of four similar experiments in which radioactive glucose was administered at the onset and during the plateau phase. Two additional experiments in which glucose was
added late in plateau (during a period of rapid decrease in cell viability) revealed that it was ineffective in rejuvenating cell activity and the cultures declined rapidly.

Radioactive carbon dioxide was produced from radioactive glucose during logarithmic and plateau phase as seen in Table II. Theoretically, 1 millimole of glucose, if oxidized completely, would yield 6 millimoles of carbon dioxide. A total of 5.55 mM of radioactive glucose was added to a culture in two increments, one in log growth and one shortly after the onset of plateau phase. If completely converted to CO₂ the stoichiometric yield should have been 33.3 mM of CO₂. A total of 8.07 mM of CO₂ was trapped during the growth cycle, which represented about 25 per cent recovery of the glucose as carbon dioxide. The specific activity of the glucose added was 612,612 counts/min/millimole at a counting efficiency of approximately 30 per cent. The specific activity of the carbon dioxide recovered was measured in a Bernstein-Ballentine counting tube [4] at an efficiency of approximately 70 per cent. At 70 per cent efficiency the calculated specific activity of the glucose added was 1,429,428 counts/min/mM. The activities recovered at any point in the experiment were approximately $\frac{1}{5}$ of this theoretical value. The drop in specific activity of CO₂ as the plateau phase progressed indicated dilution by residual glucose in the culture or utilization of other substrates for energy (e.g. amino acids, glycogen, etc.).

The accumulation of nucleic acid and protein in the medium observed in the plateau phase of growth and reported earlier [11]_plus the cyclic increase in size of a small percentage of the cells during plateau [21], suggested that the viable population may have been re-utilizing cellular fractions released into the medium. To test this hypothesis a crude preparation of nucleic

<table>
<thead>
<tr>
<th>Elapsed time hr</th>
<th>Cell No. $\times 10^6$</th>
<th>% of viable cells</th>
<th>mM CO₂ produced</th>
<th>Specific activity CPM/mM</th>
<th>Specific activity CPM/µg</th>
<th>Time interval hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 0</td>
<td>0.90</td>
<td>88</td>
<td>1.64</td>
<td>50,810</td>
<td>1.15</td>
<td>0</td>
</tr>
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<td>15</td>
<td>1.20</td>
<td>88</td>
<td>2.20</td>
<td>53,434</td>
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<td>40</td>
<td>1.70</td>
<td>88</td>
<td>1.40</td>
<td>50,479</td>
<td>1.15</td>
<td>25</td>
</tr>
<tr>
<td>G 134</td>
<td>1.63</td>
<td>67</td>
<td>1.40</td>
<td>50,479</td>
<td>1.15</td>
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<td>65</td>
<td>1.22</td>
<td>41,566</td>
<td>0.95</td>
<td>55</td>
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</table>

*Experimental Cell Research 37*
Utilization of glucose by plateau phase cells

acid and protein was obtained from intact cells previously exposed to uniformly labelled $^{14}$C glucose and administered to L-M cells during logarithmic growth when cell viability and metabolic activity is optimal. Fig. 5 shows the effect of this material on the generation time of L-M cells in suspension culture. In the first experiment 20 ml of this crude material (containing the nucleic acid-protein complex from approximately $37 \times 10^7$ cells) was added to a spinner culture in early logarithmic growth and the pH was adjusted to 7.4 with 0.3 N hydrochloric acid. Another aliquot was introduced late in logarithmic growth which increased the concentration of this cellular material by 2.5 fold.

A second experiment was initiated in the same manner, using a nucleic acid-protein preparation from another culture. It contained nucleic acid and protein from approximately one-half the number of cells used to initiate Experiment No. 1, but had a radioactive count of 500 counts per ml. No further addition was given to this culture. The solid black line in Fig. 5 represents the normal growth curve of L-M cells in modified 199 peptone as calculated from cumulative data over a period of two years. The vertical lines crossing the curve indicate the range of variation encountered. The average doubling time falls in the range of 45–55 hr. Despite this wide range, the addition of a homologous nucleic acid-protein complex increased the rate of cell proliferation significantly beyond the normal rate. In both experiments the doubling time was decreased by at least 50 per cent. This effect was observed in three separate experiments of this type.

Fig. 5.—Effect of homologous protein and nucleic acid on generation time of L-M strain cells. G, glucose addition (5 ml of 10% glucose containing 2.5 $\mu$C of $^{14}$C-glucose.)
These cultures were harvested in late plateau phase and fractionated according to the aforementioned procedure. Of the total radioactivity introduced 13.3 per cent was recovered from the cellular nucleic acid-protein fraction in Experiment No. I and 12 per cent in Experiment No. II. Although these figures are very close, it must be pointed out that the total material added to Experiment No. I was approximately 5 times that added to Experiment No. II, whereas the specific activity was 70 per cent less. These results imply that incorporation of the labelled material into the nucleic acid-protein fraction of the cells might have occurred and that re-utilization of large molecules released into the medium was possible.

In the first experiment the amount of homologous material introduced was approximately 2.2 times the quantity present in the total population at the time of initiation. This level was obviously nontoxic for the system and cell viability ranged from 98-100 per cent as compared to the range of 90-95 per cent usually observed.

**DISCUSSION**

The result of the foregoing experiments indicate that glucose, if available, can serve both as an energy source and as a precursor for nucleic acid and protein synthesis during the plateau phase of the cell growth cycle. Relatively small, but significant, amounts of $^{14}$C from glucose were incorporated into proteins and nucleic acids though the bulk of the radioactivity added remained in the acid soluble fractions of the cells and medium. No attempt was made to purify and identify the labelled components in these fractions. It is probable, as Chang [7] has reported, that a sizeable amount of radioactive carbon dioxide was utilized in the production of purine and pyrimidine bases prior to incorporation into the nucleic acids. This together with the recovery of 25 per cent of the added glucose as free CO$_2$ suggests a significant use of oxidative metabolic pathways. It must be pointed out also that glucose was present in excess and that perhaps the cells were operating at maximum efficiency in the conversion of glucose to CO$_2$. The progressive incorporation of $^{14}$C into nucleic acid and protein is explainable on the basis of the increased demand of the cells for replenishment of intra-cellular purines, pyrimidines, and amino acids in a depleted medium. Moreover, the carbohydrate tracer studies of Fischer [12] and Eagle et al. [10] have confirmed that radioactive glucose is utilized by cells in culture for the synthesis of nucleic acid and protein precursors. The carbon dioxide fixation reactions

*Experimental Cell Research 37*
Utilization of glucose by plateau phase cells

reported by Chang et al. [7] and McCoy et al. [20] offer another plausible explanation for the recovery of $^{14}C$ in the nucleic acid fractions.

In view of the progressive incorporation of $^{14}C$ into the various fractions isolated, it appears that cell viability is sustained over a longer period of time if, in the presence of an abundant energy source, the population can utilize this same substrate for the synthesis and replenishment of other constituents. Moreover, the observation that glucose does not extend the duration of the logarithmic or plateau phase indefinitely may be related to the observation of Eagle et al. [10] that radioactive glucose was incorporated only into the non-essential amino acids. The availability of the essential amino acids [9] either free, or as constituents of protein in the medium, probably is the major limiting factor. In light of the progressive accumulation of protein in both the cells and medium, a situation arises in which both the essential and non-essential amino acids or their precursors may be offset by the ability of the cells to synthesize these acids from glucose and thereby to maintain viability over a longer interval of time.

The presence of RNA in the medium with a specific activity higher than that found in the cells suggests that in the presence of both viable and damaged cells, which tend to leak nucleic acid and protein into the medium, sufficient templates and enzymes may be present to permit extracellular synthesis of RNA [6, 14]. It must be borne in mind that this is not an acellular system, but one in which whole cells and materials comparable to those in cell extracts are present in undenatured form. The evidence presented here is not sufficient to warrant the conclusion that extracellular synthesis occurred, but only that it was possible in the presence of DNA and protein in the medium. The progressive decrease of the total amount of RNA in the culture system may be due, in part, to the action of ribonuclease released by the cells [2].

As noted previously by Thomson et al. [28] cellular DNA appears to be more stable than RNA. This is evidenced by the sustained level of cellular DNA during the plateau phase and is supported by the fact that the specific activities of the DNA in the medium (0.263 to 0.800) and the cells (0.383 to 0.933) fall within the same range. Moreover, the total amount of DNA synthesized tends to remain constant or to increase slightly as the plateau phase progresses.

The rapid accumulation in the medium of protein of lower specific activity (0.034 to 0.101) as compared to that (0.069 to 0.367) contained in the cells suggests that: (1) more than one type of protein is being formed; (2) turnover and replacement of unlabelled amino acid components of
protein is occurring at a rapid rate; (3) dilution of newly synthesized protein derived in part from a radioactive substrate by cellular protein produced during logarithmic growth occurs. The production of "collagen-like" material by L strain cells as demonstrated by Merchant and Kahn [22] and fibril formation in stationary cultures of mouse lung cells as observed by Pumper [26] lend support to the first hypothesis that a specialized type of protein is being formed. Furthermore, Paul [24] has observed that fibroblastic cells are generally leakier than epithelial cells and that fibrous protein formation is apparent only in slowly growing cultures. The state of the culture during the plateau phase fulfills these conditions permitting production of extracellular material [17, 22, 24, 26].

The rate of protein turnover in L strain cells during logarithmic growth as reported by Jordan and Schmidt [18] favors the second possibility. However, if only one type or general class of protein is being synthesized, it would be expected that the specific activities of the cellular and medium protein would be the same or approach identity as the plateau phase progresses. It must be pointed out that initially the specific activity of the protein in the cells is twice that of the protein contained in the medium and by the end of the plateau phase it is approximately three and one half times greater. There is a divergence of specific activity and a convergence of the total amount of protein contained in the cells and medium.

Dilution of newly synthesized protein lost to the medium no doubt occurs, but the rate, level and specific activities of material accumulating in the medium suggest an interplay between turnover [18], specialized synthesis [22], and leakage of protein from the cells [24].

The possibility of re-utilization of cellular nucleic acids and proteins accumulating in the medium is indicated by the marked decrease in generation time and the recovery of radioactivity in these same fractions when cultures are exposed to radioactive homologous nucleic acids and protein. Whether prior degradation or incorporation of intact material occurs cannot be determined from the data obtained, but the immediate response of a growing population to preformed homologous material suggests that direct incorporation may be operative. The phenomenon of pinocytosis [15] must not be overlooked. It is possible that preformed nucleic acids and protein are taken up in polymerized form and lie dormant, or are degraded and fractions thereof utilized in the synthesis of protein and nucleic acid de novo.

The observations of Gartler [13], Simbonis [28] and Kutsky [19] lend support to direct utilization of preformed nucleic acid and protein.

As suggested earlier the maintenance of animal cells, for prolonged
periods in a plateau or stationary growth phase, offers many opportunities for study of specialized cell function, factors limiting growth, etc. [21]. The studies reported here have shown a pronounced effect of supplementation of a single component, viz. glucose. Supplementation by other compounds undoubtedly would widen the usefulness of the system.

**SUMMARY**

The addition of radioactive glucose to suspension cultures of L-M strain mouse cells during the plateau phase has been shown to extend the duration of this period from the normal 48–72 hr to 144–240 hr. Supplemental carbohydrate serves both as an energy source, as detected by the production of radioactive lactic acid and carbon dioxide, and as a precursor for nucleic acid and protein synthesis during this period. On the basis of the specific activities of the nucleic acids and proteins recovered from both the cells and the medium, possibilities of synthesis of multiple proteins and of extracellular RNA are discussed. The addition of radioactive homologous nucleic acid-protein fractions to culture in logarithmic growth is shown to reduce the mean generation time by 50 per cent. The recovery of 12 to 13 per cent of the radioactivity in these same fractions isolated from the cells at the end of logarithmic growth suggests that cells have the potential for re-utilization of cellular material release into the medium from other cells in the population.

**REFERENCES**