tration of virus. Virus in these tissues could potentially infect any animal passing through the birth canal. However, the mammary gland and its milk revealed virus in only one of the 5 rabbits tested and would suggest infrequent virus transmission postnatally by this route.

Present reports of severe herpes infections in newborns in man(1,4,5) suggest transplacental passage of virus within the last few days prior to delivery. These experiments demonstrate transplacental transfer can occur in rabbits, though only "traces" of virus crossed the placenta of hamsters(8). It is possible the anatomical differences of the placentas of these three animals may also be a factor in transplacental transmission of herpes virus(12). Previously, it had been thought infection with herpes virus was usually acquired from the maternal cervix or vagina at the time of delivery or from herpes infection of the lips or mouth of the mother or nursery personnel.

Summary. Two different concentrations of herpes simplex virus were given intravenously to pregnant rabbits. Low concentrations of virus failed to cross the placenta and disappeared from the blood rapidly. A higher concentration of virus produced a viremia of 60-120 minutes and caused the death and resorption of 30% of the fetuses. Virus was recovered from both maternal tissues as well as fetuses, fetal membranes, and fetal fluids.

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Influence of Glucose and Ammonium Ions on Endogenous Respiration.* (32198)

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The rate of endogenous respiration has been reported(1) to be repressed, unchanged, or increased during the oxidation of exogenous substrates by washed suspensions of microorganisms. The influence of the exogenous substrate appears to depend upon its nature, the past history of the cells employed in a particular test, and test conditions. Most studies have dealt with washed cells suspended in a buffer solution but recently Gronlund and Campbell(2) compared the influence of growth conditions on the endogenous respiration of *Pseudomonas aeruginosa* with that of cells suspended in a buffer solution or in the buffer to which glucose and/or ammonium ions were added. Marked suppression of endogenous $C^{14}O_2$ production by growth-labeled cells was noted in the growth medium and to a lesser extent in the presence of glucose in a Tris (hydroxymethyl) aminomethane buffer. In the present study all tests were carried out in a buffered salt solution of the same composition as employed for the growth medium and to which glucose and/or ammonium sulfate could be added in the same concentrations as employed in

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the growth medium. The behavior of 3 Gram-negative species was compared under conditions which would permit growth in the complete medium for several generations.

Materials and methods. Pseudomonas aeruginosa U-21a, *Escherichia coli* K-12 or *Serratia narcescens* Z-4 were grown for 16 hours in 50 ml of buffered salt solution(3) containing 1% glucose (plus 20 μc U-C^{14}-glucose) and 0.1% $(NH_4)_2SO_4$. The cells were separated from the culture medium by centrifugation and resuspended in a volume of sterile buffered salt solution equal to that of the culture. Five-tenth ml of this suspension was added per 6.5 ml of the complete medium or of the buffered salt solution either as such or containing 1% glucose or 0.1% $(NH_4)_2SO_4$. Three ml samples of the suspensions were placed in Warburg flasks together with 0.2 ml of 20% KOH in the center well and 0.2 ml of 10% H_2SO_4 in the side arm. The acid was tipped into the suspension of cells at appropriate times to halt respiration and to release bound CO_2. Fifteen minutes after addition of the acid the Warburg flasks were removed from the bath and the KOH was transferred quantitatively to the flasks employed for $C^{14}O_2$ determinations in the Dynacon system as previously described(3,4). The initial C^{14}-content of the cells varied from experiment to experiment but generally was in the range of 40 to 60 mμc per Warburg flask.

Results and discussion. In the absence of glucose the amounts of O_2 consumed were too small to be measured with accuracy by the Warburg technique owing to the low concentration of cells employed in these tests. For the first 1.5 hours the O_2 consumption in the complete medium was very low and was about the same as in the presence of glucose alone. The rate began to increase thereafter in the complete medium as the cultures entered the log phase of growth. At about 3 hours the cells in the growth medium had increased in numbers to a sufficient extent to impart a perceptible turbidity to the cultures. Turbidity of the other suspensions remained too low to be measured in the Klett-Summerson colorimeter (No. 54 filter). The suspensions employed in these tests were very dilute (to allow growth to occur) as compared with those used in most studies with washed cells where 1-10 dilutions frequently give readings greater than 100 in the Klett-Summerson colorimeter.

TABLE I. Suppression of Endogenous $C^{14}O_2$ Production by Uniformly Labeled Cells in a Glucose-Basal Salts Medium.*

Hours	1.5	3.0	4.5	6.0
Organism	% Suppression of $C^{14}O_2$ production below that of control suspensions			
P. aeruginosa	74	78	80	82
E. coli	87	—	88	84
S. marcescens	80	79	74	78

* Basal salt solution containing 1.0% glucose and 0.1% $(NH_4)_2SO_4$.

In the first experiments endogenous $C^{14}O_2$ production by cells uniformly labeled during growth on glucose was determined (Table I) in the growth medium and in the basal salts solution at intervals of 1.5 hours over a 6-hr period. The original intent was to compare behaviors noted during the lag and log phases of growth. However, the amounts of endogenous $C^{14}O_2$ produced by cells suspended in the growth medium were very low and the errors of measurement of the small amounts (frequently 0.1 to 0.5 mμc per 1.5 hr) probably are responsible for any differences noted with time in the extent of suppression of endogenous respiration by glucose for a particular organism. The radioactivity of the cells was high but the concentration of cells was very low as indicated above. In some experiments there was a much greater decrease in endogenous $C^{14}O_2$ production in the salts solution after 1.5 hours than in other experiments. This probably reflects differences in the amounts of storage products in the cells, endogenous respiration continuing at a higher level for a longer period of time in those experiments in which the cells were rich in glycogen or other storage products. The above results indicate that there was marked suppression (74 to 88%) of the endogenous respiration of the 3 test organisms under conditions suitable for growth.

In other experiments, reported in Table II, the influence of addition of glucose and/or $(NH_4)_2SO_4$ to the basal salt solution on

TABLE II. Influence of Glucose* and/or Ammonium Ions* on Endogenous $C^{14}O_2$ Production by Uniformly Labeled Cells in Basal Salt Solution Suspensions.

Organism	Hours	% Suppression below endogenous control			Endogenous $C^{14}O_2$, mμc
		Glucose + NH_4^+	Glucose	NH_4^+	
P. aeruginosa	3	80	76	—	3.4
	5	93†	75	9	3.9
E. coli	3	82	69	13	4.5
	5	81	72	33†	5.8
S. marcescens	3	85	82	−9	3.4
	5	85	77	0	3.9

* Glucose in 1.0%, $(NH_4)_2SO_4$ in 0.1% concentration.
† Values are high owing to technical difficulties.

endogenous $C^{14}O_2$ production is reported for 3- and 5-hour periods. Endogenous $C^{14}O_2$ production was suppressed to the greatest extent in the complete medium and to almost as great an extent when glucose alone was added to the basal solution. Ammonium sulfate appeared to have little influence on endogenous $C^{14}O_2$ production, a slight suppression being noted in most experiments. This behavior is in agreement with that reported for *P. aeruginosa* by Gronlund and Campbell(2) who used Tris buffer for suspending the nonproliferating cells rather than the basal salt solution of their growth medium. Some of the results in Table II are suggestive of an additive effect of glucose plus NH_4^+ on the suppression of endogenous respiration. This needs to be studied in more detail. If true it might suggest that glucose suppresses a different type of endogenous respiration than repressed by NH_4^+ ions.

The marked suppression by glucose of endogenous $C^{14}O_2$ production in suspensions of *E. coli* was not in agreement with the behavior reported earlier by Clifton(5) who concluded that the rate of endogenous respiration was increased slightly in the presence of glucose. In this earlier study the cells were not uniformly labeled and the tests were carried out in a Dynacon air recirculation system(6). Also the endogenous value was determined before addition of the substrate rather than simultaneously with the exogenous system. The suspensions were adjusted to readings of around 150 in the Klett-Summerson colorimeter (1-10 dilution, 54 filter) as compared with readings of less than 1 for the suspensions (undiluted) used in the present tests.

The earlier type of experiment was repeated under the same test conditions as in the present study, except for cell concentrations, with *E. coli* grown in the presence of U-C^{14}-glucose on the basal medium or on nutrient glucose agar. The experiments with the heavy cell suspensions had to be terminated at 30 to 40 minutes since the exogenous glucose appeared to be utilized by that time. Under these test conditions the endogenous $C^{14}O_2$ production was suppressed by 25 to 30% as contrasted with the greater than 80% suppression noted with the light suspensions in this study. Gronlund and Campbell(2) noted a similar influence of concentration of exogenous substrate on the extent of endogenous respiration although they varied the substrate concentration rather than that of the cells. Ribbons and Dawes (7) reported complete suppression by exogenous glucose of endogenous respiration of *E. coli*. Their results, however, were indirect since their calculations were based on intracellular glycogen determinations. It would appear that the amount of substrate (or O_2) available per cell is an important factor in controlling the extent of endogenous respiration in the presence of an exogenous substrate. The nutritional status of the cells also must be considered as evidenced by the studies of Clifton and Cherry(3) with *Bacillus subtilis*. Addition of an exogenous substrate to a suspension of *B. subtilis* prepared and tested as rapidly as possible resulted in

a slight enhancement of the rate of endogenous respiration. Once the endogenous rate of respiration of these cells (which was quite high compared with that for *E. coli*) began to decrease with time, the addition of an exogenous substrate suppressed the rate of endogenous respiration. The results of the various studies lead to the conclusion that addition of an exogenous substrate commonly results in a suppression of endogenous respiration but that the response does vary with the organism and with test conditions.

Summary. *Pseudomonas aeruginosa, Escherichia coli* or *Serratia marcescens* were uniformly labeled during growth on glucose in a buffered basal salt solution. The endogenous $C^{14}O_2$ production of these organisms in dilute suspensions of washed cells was suppressed by glucose under either growth or nongrowth conditions, to a somewhat greater extent under the former conditions. Increasing the concentration of bacteria tended to decrease the extent to which endogenous respiration was suppressed in the presence of glucose. These results suggest that the amount of substrate (or O_2) available per cell is an important factor in controlling the extent of endogenous respiration in the presence of an exogenous substrate.

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Activation of Trypsinogen by Plasmin.* (32199)

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The activation of pancreatic trypsinogen by trypsin is known to involve the hydrolysis of the peptide bond between lysine and isoleucine(1). Plasmin, the fibrinolytic enzyme of blood, is known to hydrolyze esters of the amino acids lysine and arginine as well as fibrin, fibrinogen, casein and other naturally occurring proteins(2,3,4). The experiments to be described demonstrate that plasmin can activate trypsinogen.

Materials. Trypsinogen and trypsin were obtained from the Worthington Biochemical Corp., Freehold, N. J. Trypsinogen was a once-crystallized powder that was 90% protein. According to the supplier, its intrinsic trypsin activity was 0.09% of that reached when it was activated. Lyophilized trypsin was once-crystallized and salt-free.

The lyophilized plasminogen was prepared from Fraction III by the method of Kline (5) and contained 4.35 Remmert & Cohen caseinolytic units per mg of powder. Plasmin was prepared from this plasminogen by activation with minimal amounts of streptokinase and was lyophilized from a solution containing 0.1 M lysine, added to stabilize plasmin. There was 0.70 Remmert & Cohen unit per mg of powder, 10% of which was protein.

Urokinase, prepared by Abbott Laboratories, contained 2100 units per vial.

p-Tosyl-L-arginine methylester, (TAMe), was obtained from Mann Research Laboratories.

Tris (hydroxymethyl) aminomethane

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