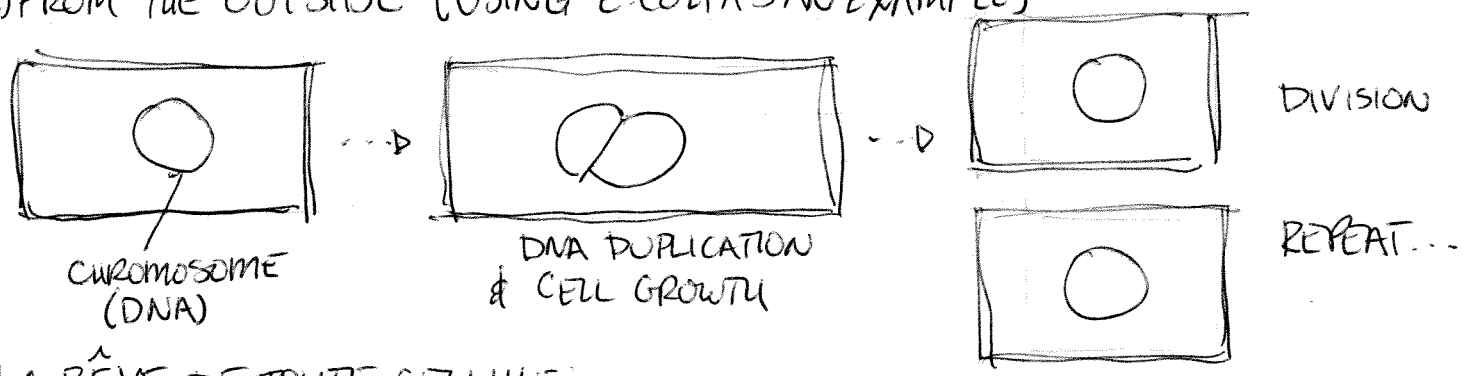
QUANTITATIVE METHODS IN BIOLOGY - BACTERIAL PHYSIOLOGY

QUANTITATIVE METHODS HAVE A LONG HISTORY IN BIOLOGY - PARTICULARLY IN THE STUDY OF BACTERIAL COMPOSITION & GROWTH (CALLED 'BACTERIAL PHYSIOLOGY'). MUCH OF THIS WORK PROCEEDS IN PARALLEL (OR PRECEDES) THE DEVELOPMENT OF OUR MODERN UNDERSTANDING OF BIOLOGY. MATHEMATICS & QUANTITATIVE MODELS ALLOW INFERENCE OF UNKNOWN MECHANISM FROM MACROSCOPIC OBSERVABLES.

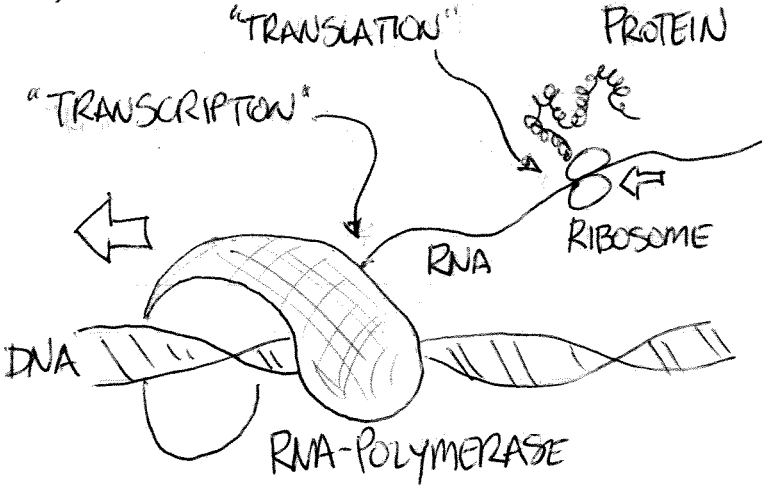
BACTERIAL GROWTH

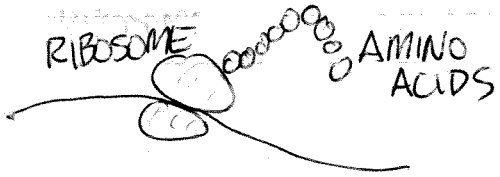
i) FROM THE OUTSIDE (USING E. COLI AS AN EXAMPLE)



"LA RÊVE DE TOUTE CELLULE: DEVENIR DEUX CELLULE" FRANCOIS JACOB
("THE DREAM OF EVERY CELL: TO BECOME TWO CELLS")

ii) ON THE INSIDE





RIBOSOMES POLYMERIZE AMINO ACIDS INTO PROTEINS

[RIBOSOMES ARE THEMSELVES MADE OF RNA (rRNA) & PROTEIN (rPROTEIN).]

HOW DID THIS PICTURE EMERGE? AMAZING EXPERIMENTS FROM 1940 - 1970.

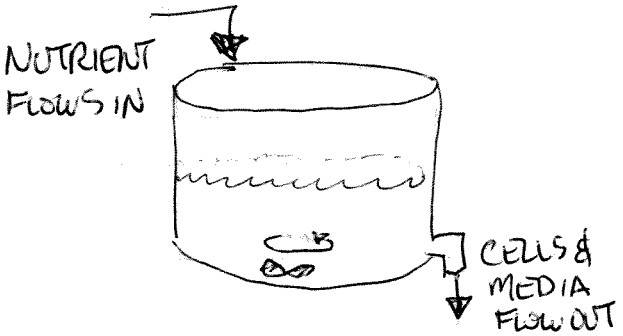
WE'LL EXAMINE SOME HISTORICAL CASE-STUDIES IN DETAIL.

1. MONOD (1949) GROWTH OF BACTERIAL CULTURES: SURVEYS GROWTH OF MANY TYPES OF BACTERIA; IDENTIFIES IMPORTANT FEATURES WORTHY OF OBSERVATION.

TWO CLASSICAL METHODS FOR GROWING BACTERIA.

A) BATCH CULTURE: PUT BACTERIA & GROWTH MEDIA IN A FLASK OR TEST TUBE. SHAKE VIGOUROUSLY (TO AERATE) AT CONSTANT TEMPERATURE (e.g. 37°C FOR ENTERIC E. COLI). IMPORTANT THAT ALL NUTRIENTS ARE IN EXCESS AT THE START OF THE EXPERIMENT. IN THIS MODE, BACTERIA DICTATE THEIR OWN GROWTH RATE.

B) CONTINUOUS CULTURE (CHEMOSTAT OR TURBIDOSTAT)



BY ADJUSTING THE CONCENTRATION OF A GROWTH-LIMITING NUTRIENT IN THE INFLOW, BACTERIA ARE KEPT HUNGRY & GROWTH RATE IS REDUCED RELATIVE TO BATCH CULTURE. IN THIS MODE, GROWTH RATE IS MODULATED BY FLOW/DILUTION RATE.

IN THIS COURSE, WE WILL ONLY CONSIDER BATCH CULTURE.

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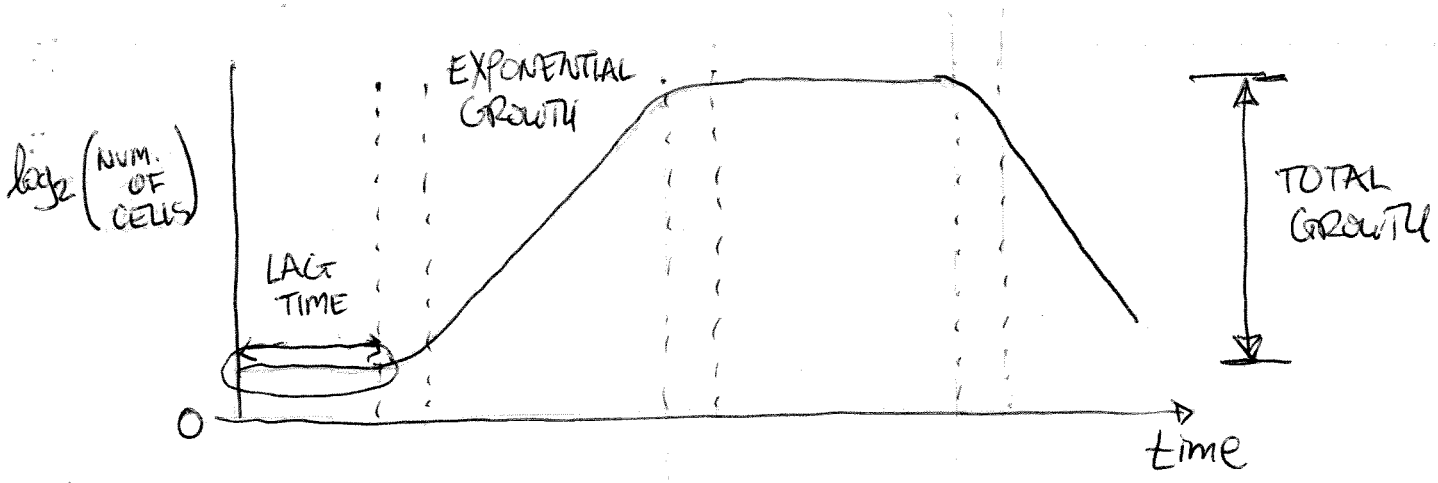
IN BATCH CULTURE, MONOD (AFTER HENRICI/BUCHANAN) IDENTIFIED SEVERAL DISTINCT 'GROWTH PHASES' IN THE BACTERIAL 'LIFE CYCLE' AND FURTHER IDENTIFIED SEVERAL IMPORTANT PHENOMENOLOGICAL MACROSCOPIC OBSERVABLES THAT APPEAR TO OBEY SIMPLE MATHEMATICAL RELATIONSHIPS.



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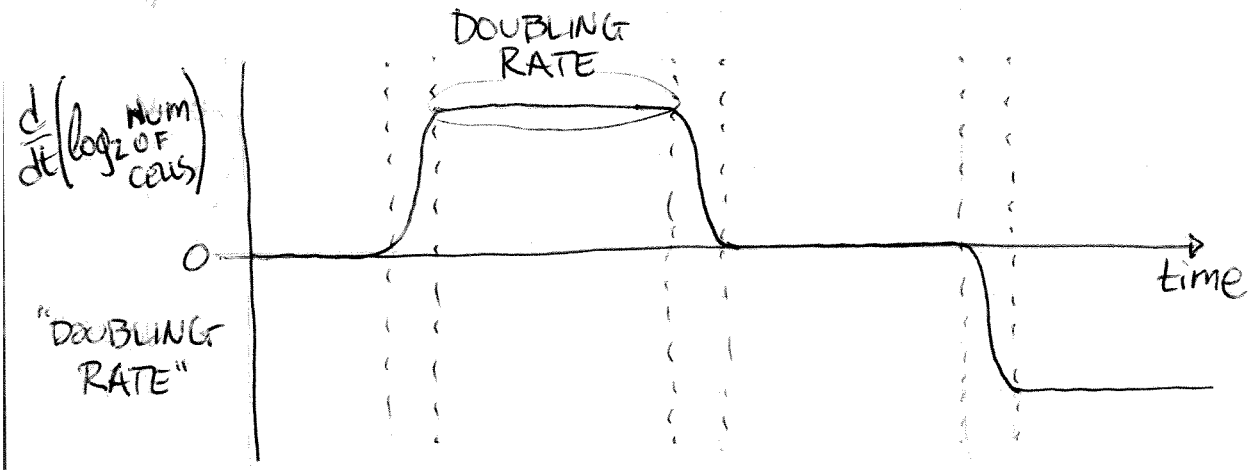
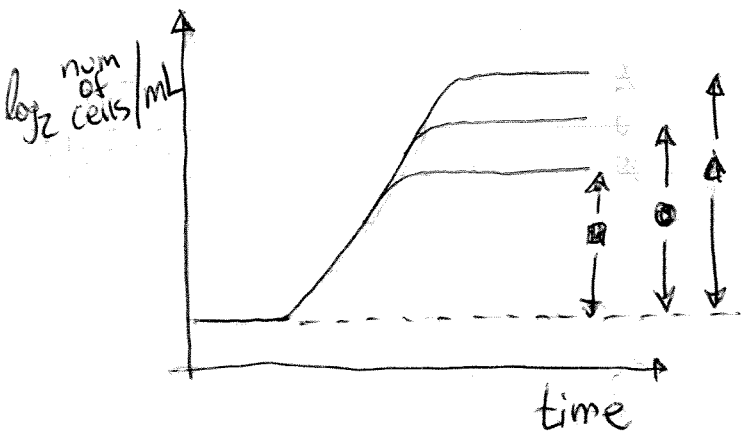
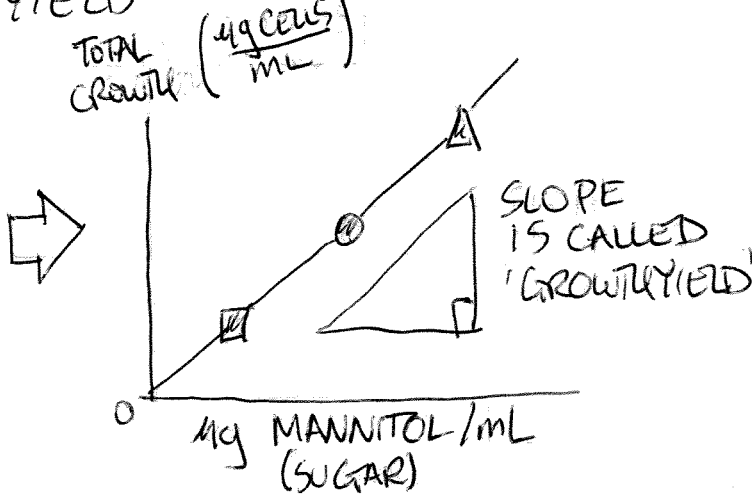


DOUBLING RATE IN THE 'EXPONENTIAL PHASE' IS ADJUSTED BY THE QUALITY OF THE GROWTH MEDIUM; TOTAL GROWTH IS ADJUSTED BY THE QUANTITY.

PLOTTING THE TOTAL GROWTH AS A FUNCTION OF THE INITIAL CONCENTRATION OF THE GROWTH-LIMITING NUTRIENT PROVIDES A MEASURE OF THE 'GROWTH YIELD'





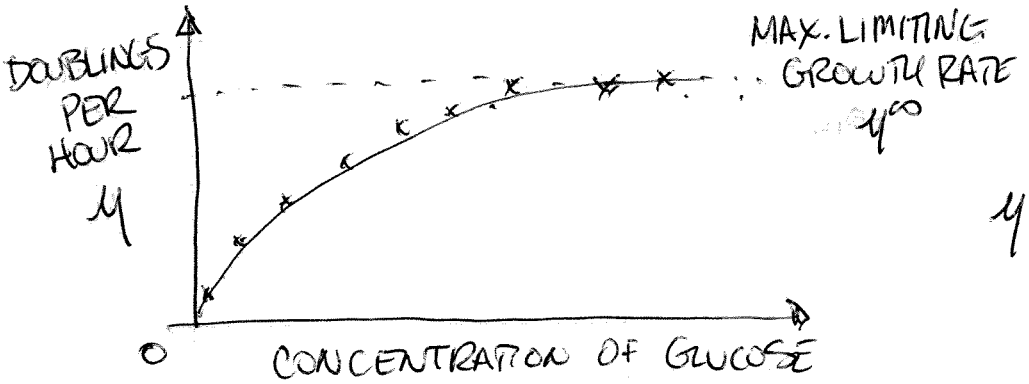
GROWTH YIELD TELLS YOU HOW EFFICIENT THE ORGANISM IS AT TURNING NUTRIENT → BIOMASS.

ie.

$$\text{GROWTH YIELD} = \frac{\text{mg BACTERIA}}{\text{mg NUTRIENT}}.$$

ANALOGY: GROWTH-RATE IS 'TOP SPEED' & GROWTH YIELD IS 'KILOMETERS-PER-LITER).

MONOD'S MOST LASTING CONTRIBUTION FROM THIS PERIOD IS HIS OBSERVATION OF 'MONOD KINETICS': IN BATCH CULTURE, AS THE NUTRIENT CONCENTRATION DROPS, THE EXPONENTIAL GROWTH RATE IS A HYPERBOLIC FUNCTION OF THE NUTRIENT CONCENTRATION:



$$\mu = \mu^{\infty} \frac{[\text{NUTRIENT}]}{[\text{NUTRIENT}] + K_D}$$

K_D: CONSTANT. (FOR A GIVEN STRAIN & NUTRIENT)

TAKE HOME MESSAGE: DESPITE IMMENSE UNDERLYING COMPLEXITY, CHARACTERISTICS OF BACTERIAL GROWTH OBEY SIMPLE 'LAWS'.

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BALANCED GROWTH:

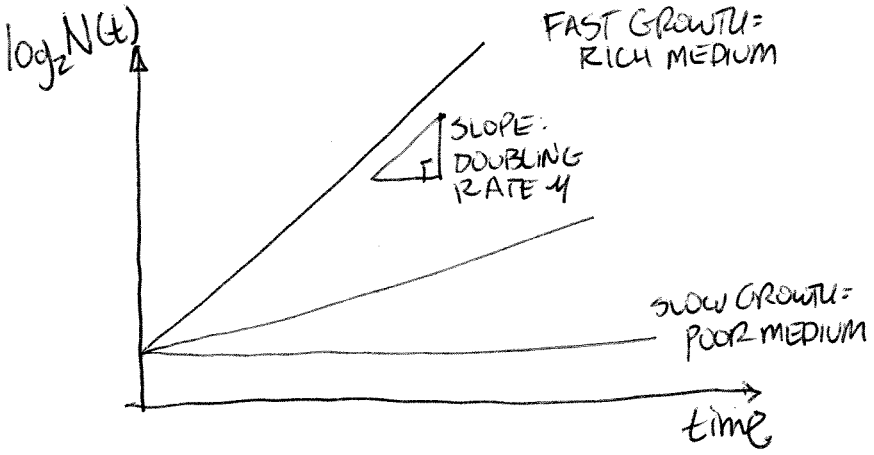
UP TO ABOUT THE MID-1950s, IT WAS THE BACTERIAL GROWTH 'CYCLE' THAT WAS THE FOCUS OF STUDY. SCIENTIST SAW IN IN THESE SIMPLE 'PHASES' ECHOS OF STAGES IN HUMAN DEVELOPMENT. BUT IN 1957, ELEVATED ONE PHASE OF THE GROWTH CYCLE ABOVE 'ALL OTHERS.

CAMPBELL DEFINED 'BALANCED GROWTH' AS THAT STATE WHERE ALL CELLULAR CONSTITUENTS DOUBLE AT THE SAME RATE DURING EXPONENTIAL GROWTH $N(t) = N_0 2^{\mu t}$. THIS BECAME THE MOST IMPORTANT 'STEADY STATE' IN BACTERIOLOGY AND COULD BE MAINTAINED ALMOST INDEFINITELY VIA DILUTION

IN EXPONENTIAL GROWTH $N_0 2^{\mu t}$, A LOG-LINEAR PLOT OF THE NUMBER DENSITY $N(t)$ AGAINST TIME PROVIDES THE GROWTH RATE:

$$N(t) = N_0 2^{\mu t} \Rightarrow \log_2 N = \log_2 N_0 + \mu t$$

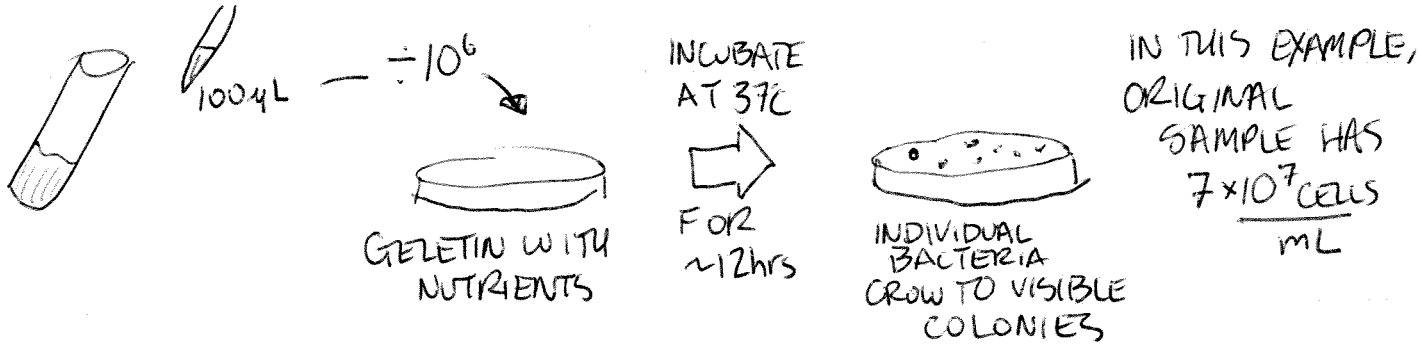
SLOPE: DOUBLING RATE



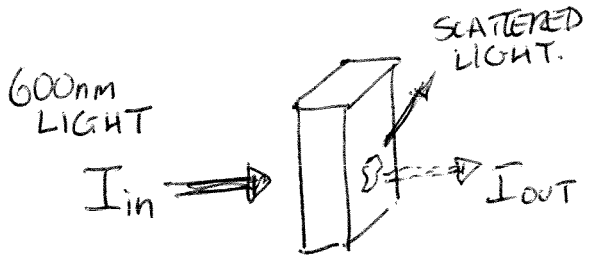
BY ADJUSTING THE NUTRIENT QUALITY, (eg. USE RAPIDLY-METABOLIZED GLUCOSE AS A CARBON SOURCE INSTEAD OF ~~FUE~~ SLOWLY-METABOLIZED SUCCINATE) THE GROWTH RATE CAN BE MODULATED FROM TENS OF HOURS PER DOUBLING DOWN TO ABOUT 20 min DOUBLINGS IN E. COLI. DOUBLING RATE IN A GIVEN MEDIUM, WITH A GIVEN STRAIN, IS INCREDIBLY ROBUST – ALMOST NO VARIATION (<5%) DAY-TO-DAY, DECADE-TO-DECADE, LAB-TO-LAB.

TWO METHODS FOR COUNTING CELLS $N(t)$:

1. PLATING VIABLE COLONIES. AN ALIQUOT OF CULTURE (eg 100 μL) IS TAKEN & RAPIDLY DILUTED (eg ABOUT 10^6-FOLD) AND SPREAD ON A NUTRIENT AGAR PLATE



2. TURBIDITY: AN ALIQUOT IS PUT IN A SPECTROPHOTOMETER AND THE 'ABSORBANCE' AT 600nM IS RECORDED. THIS IS A MEASURE OF THE LIGHT SCATTERED BY THE BACTERIA, AND (OVER A NARROW RANGE) PROPORTIONAL TO THE NUMBER DENSITY IN THE ORIGINAL SAMPLE.



$$A_{600} = \ln\left(\frac{I_{in}}{I_{OUT}}\right)$$

$$\propto N(t) \cdot Q(\lambda)$$

NUMBER DENSITY. SHAPE FACTOR

THE CONSTANT OF PROPORTIONALITY, HOWEVER, DEPENDS UPON CELL SIZE & SHAPE, AS WELL AS THE MECHANICAL PROPERTIES OF THE INSTRUMENT, AND SO MUST BE CALIBRATED AGAINST CELL COUNTS FOR DIFFERENT BACTERIAL STRAINS & GROWTH CONDITIONS.

WITH A STANDARD REFERENCE STATE (BALANCED GROWTH) BACTERIAL PHYSIOLOGY ENTERED A GOLDEN AGE FROM 1958-1968.