Quantitative traits and Human Disease

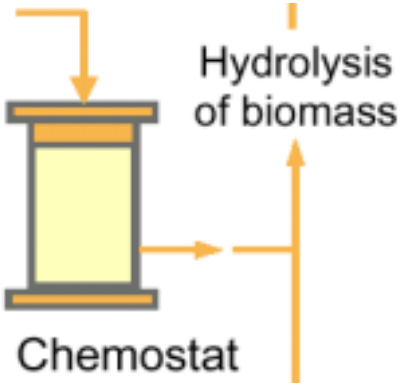
How have models been used here to describe physiology?

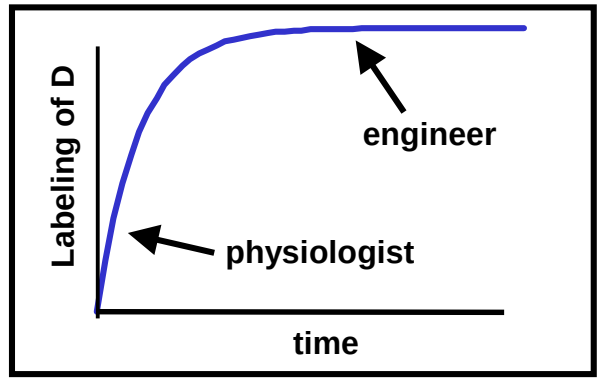
How to apply genetic linkage information to find causes of polygeneic diseases ?

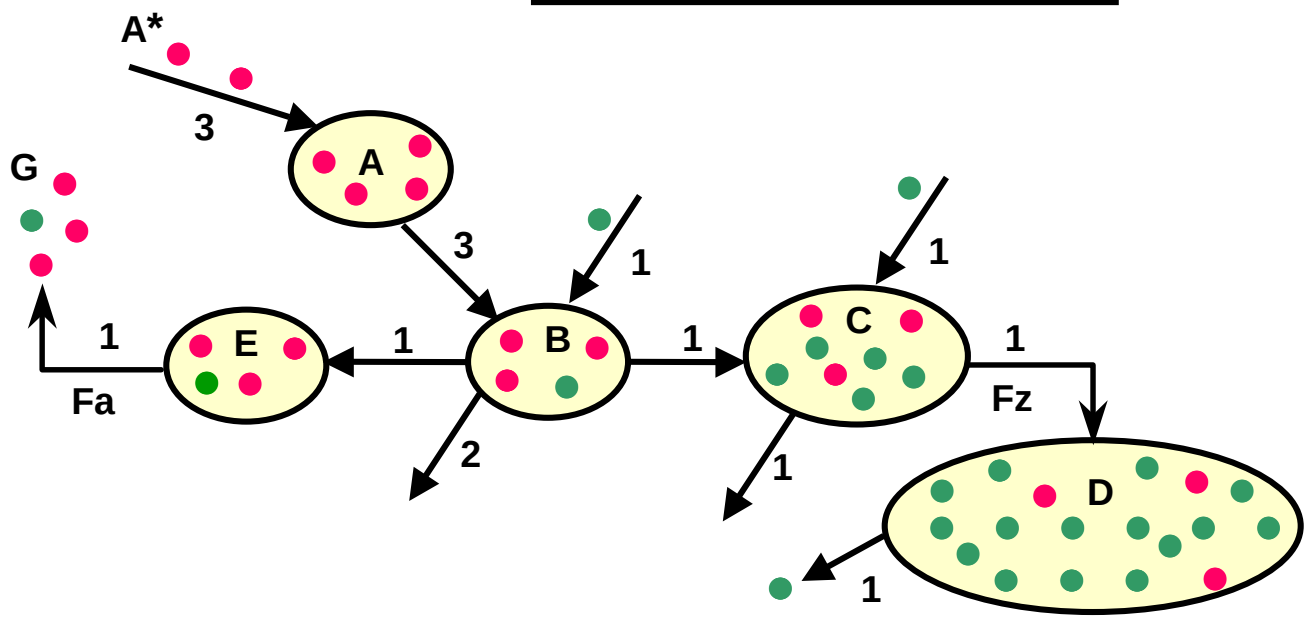
Animal studies -

a special case of congenic rats and blood pressure.

jkk@mit.edu

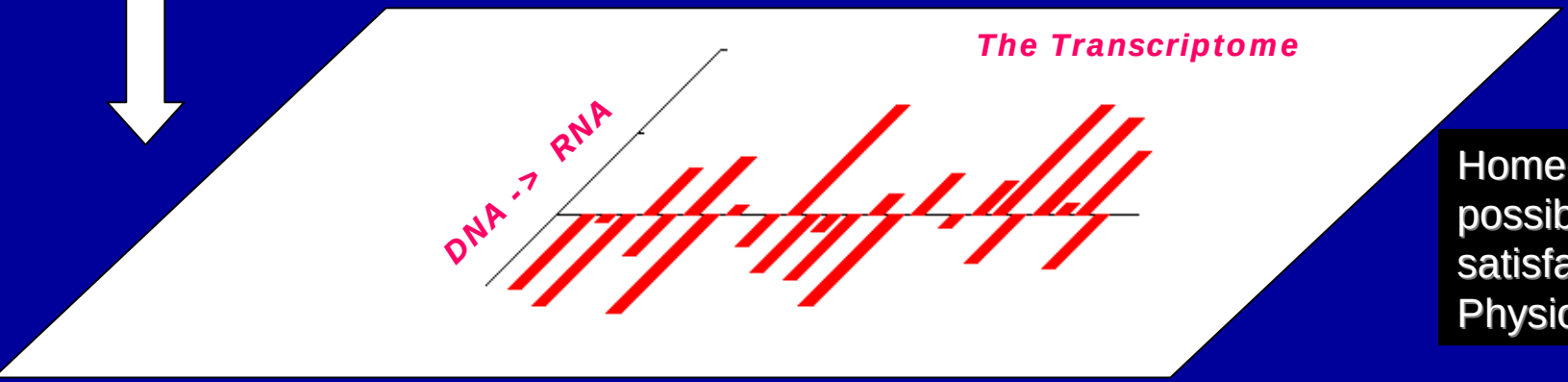




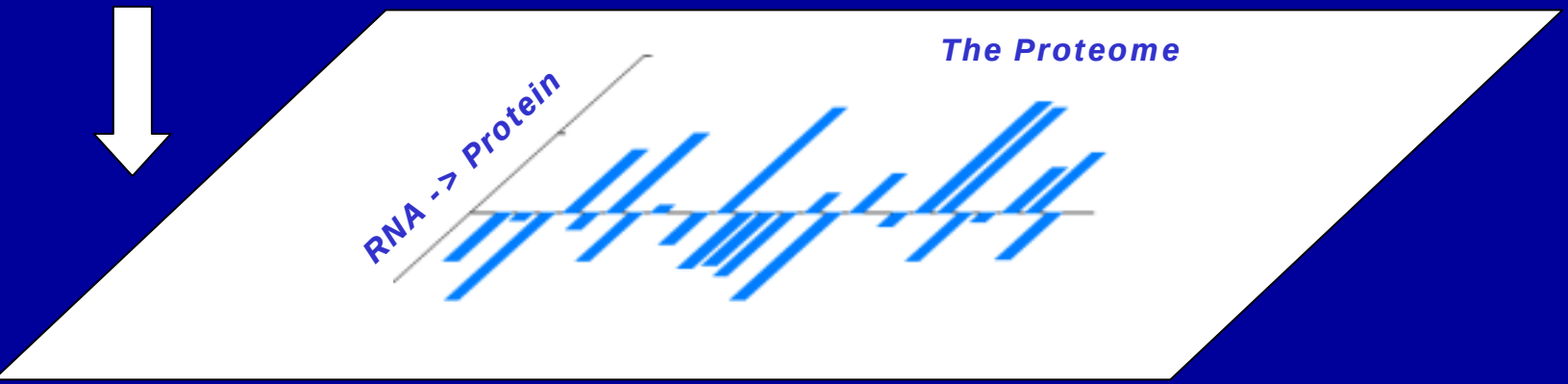


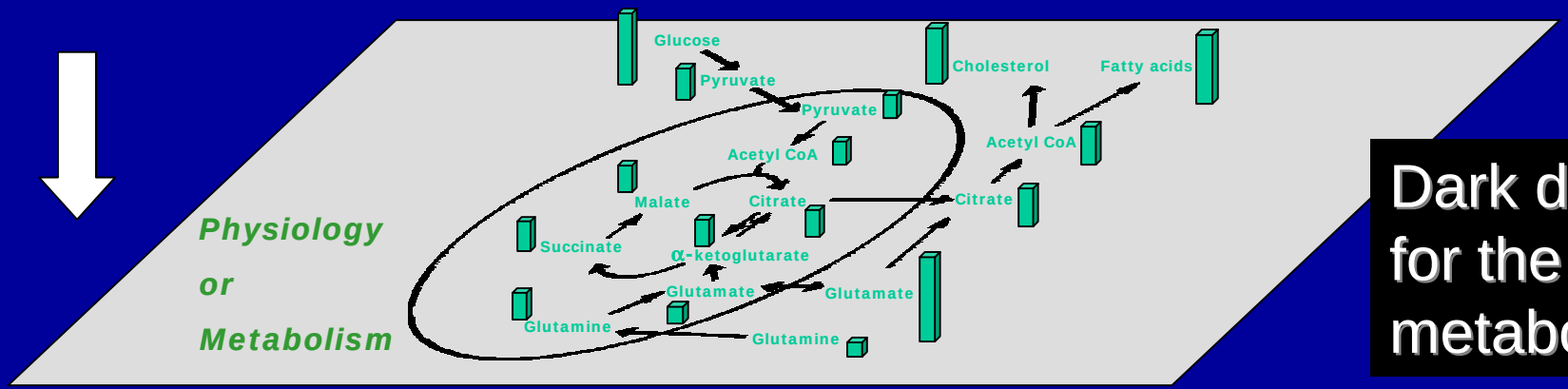
Sample problems: Whole body protein synthesis.
Rate of proliferation of specific cells (DNA synthesis).
Rate of glucose synthesis by the liver.
Rates of lipogenesis.

GENOME

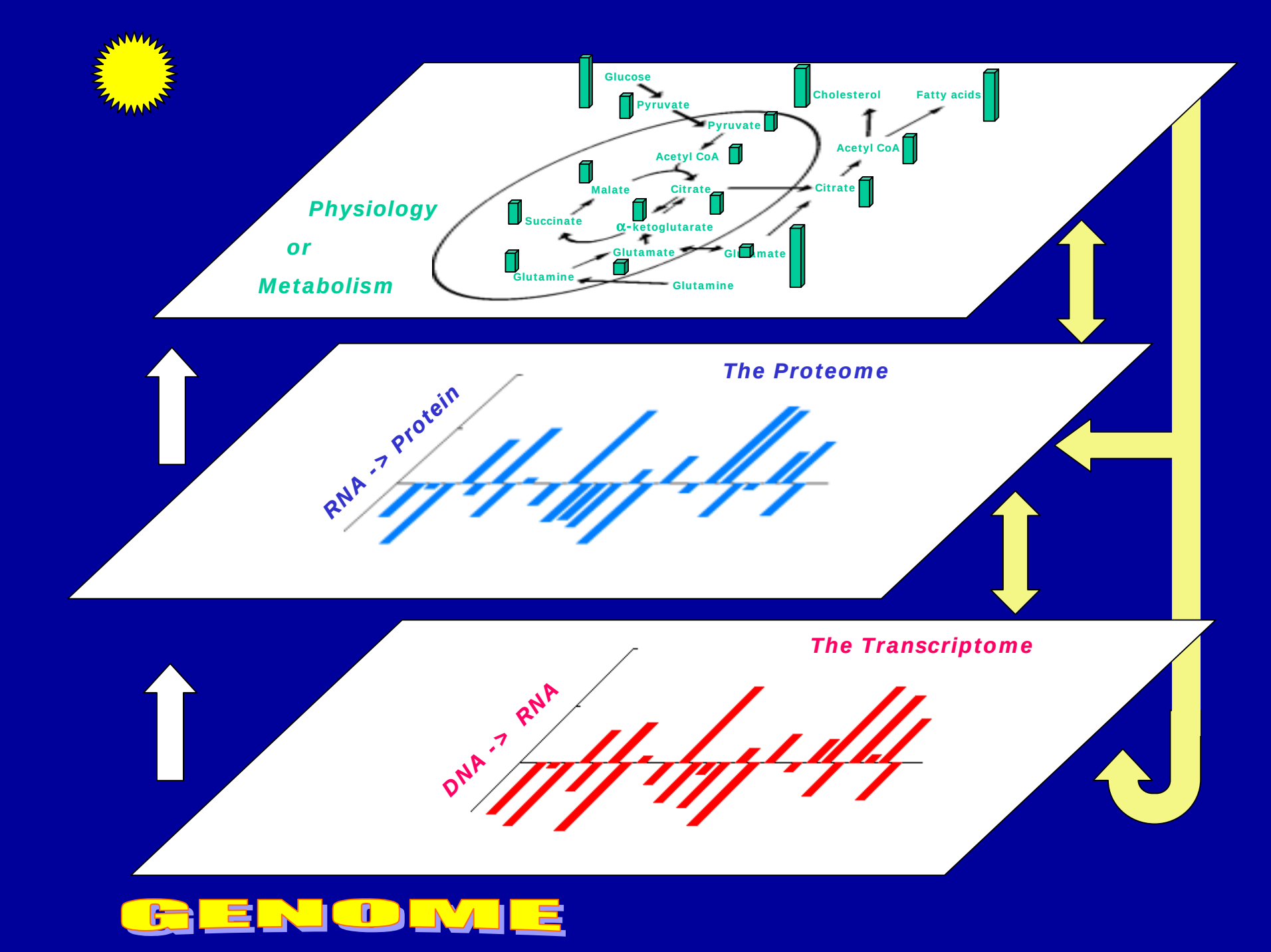


Homeostasis not possible. Not satisfactory to Physiologist.



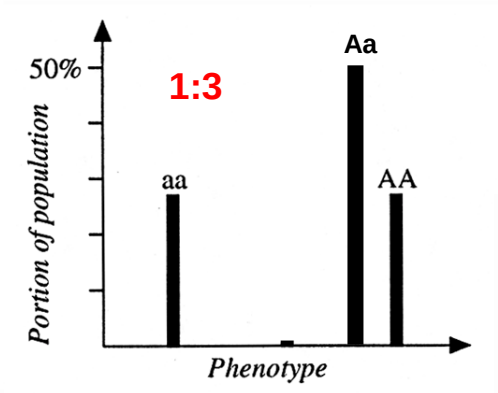


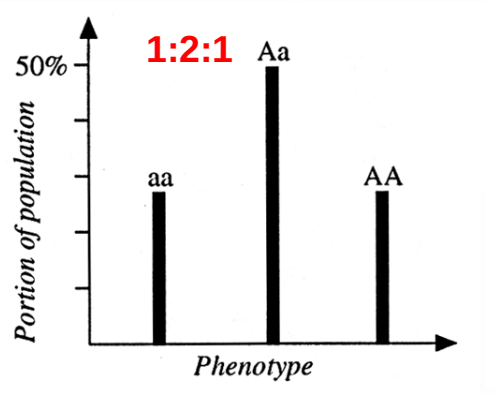
Dark days for the metabolites

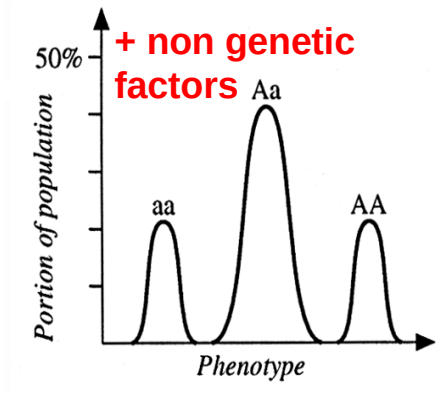

Physiology
or
Metabolism
Glucose
Pyruvate
Pyruvate
Acetyl CoA
Malate
Citrate
Succinate
α-ketoglutarate
Glutamate
Glutamine
Cholesterol
Fatty acids
Acetyl CoA
Citrate
Glutamate
Glutamine
The Proteome
RNA -> Protein
The Transcriptome
DNA -> RNA
GENOME

Quantitative traits are important for many human diseases. These cannot be described by simple Mendelian ratios

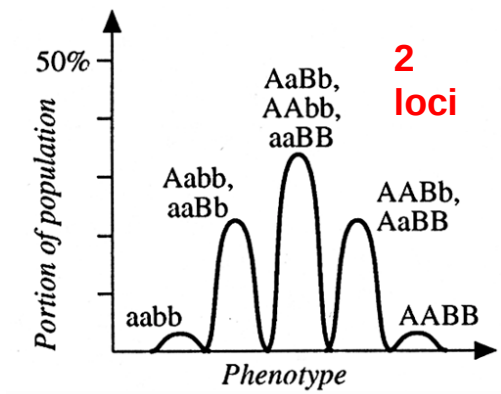
A) single genetic locus

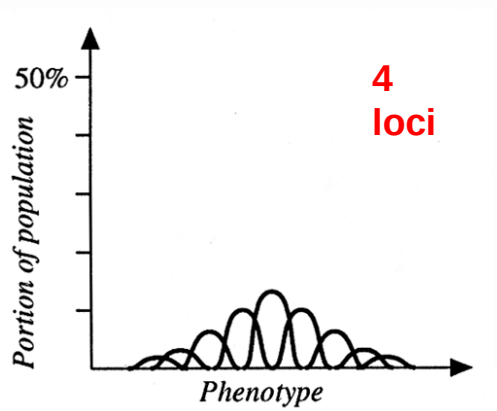


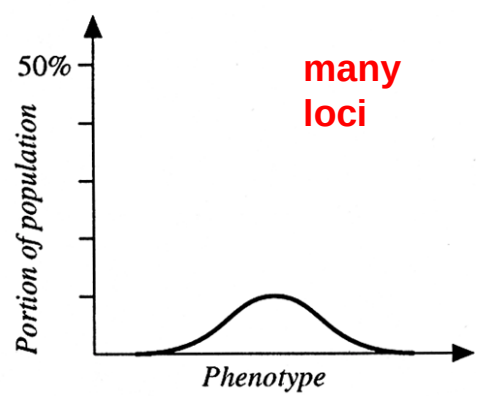




B) two and more unlinked genetic loci



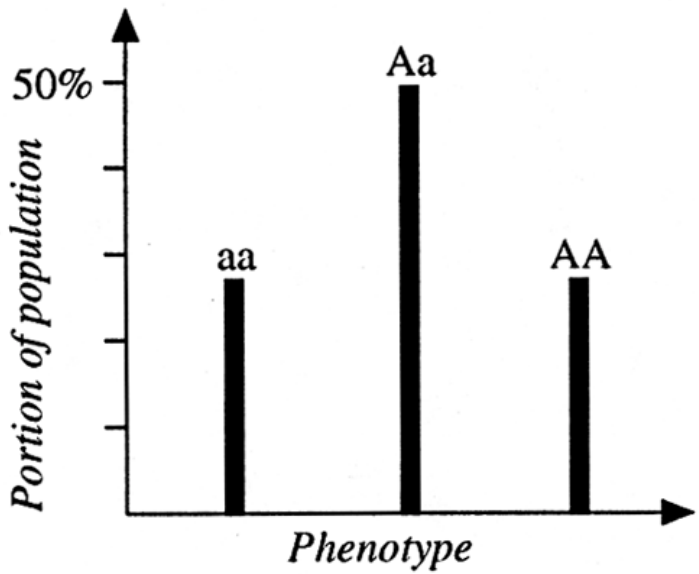




Quantitative traits and disease: A grand challenge

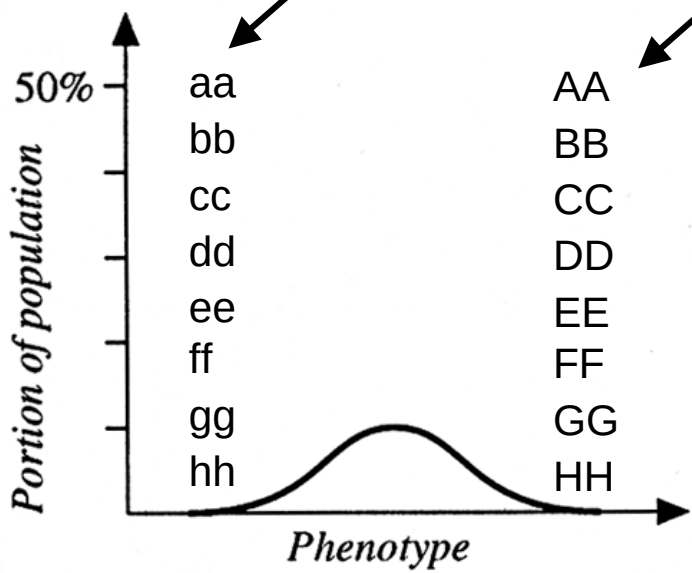
Quantitative traits and human disease: example hypertension

Single gene Medelian trait



Good alleles

Bad alleles



Blood Pressure

? Can one refine the phenotype ?

Hypertension

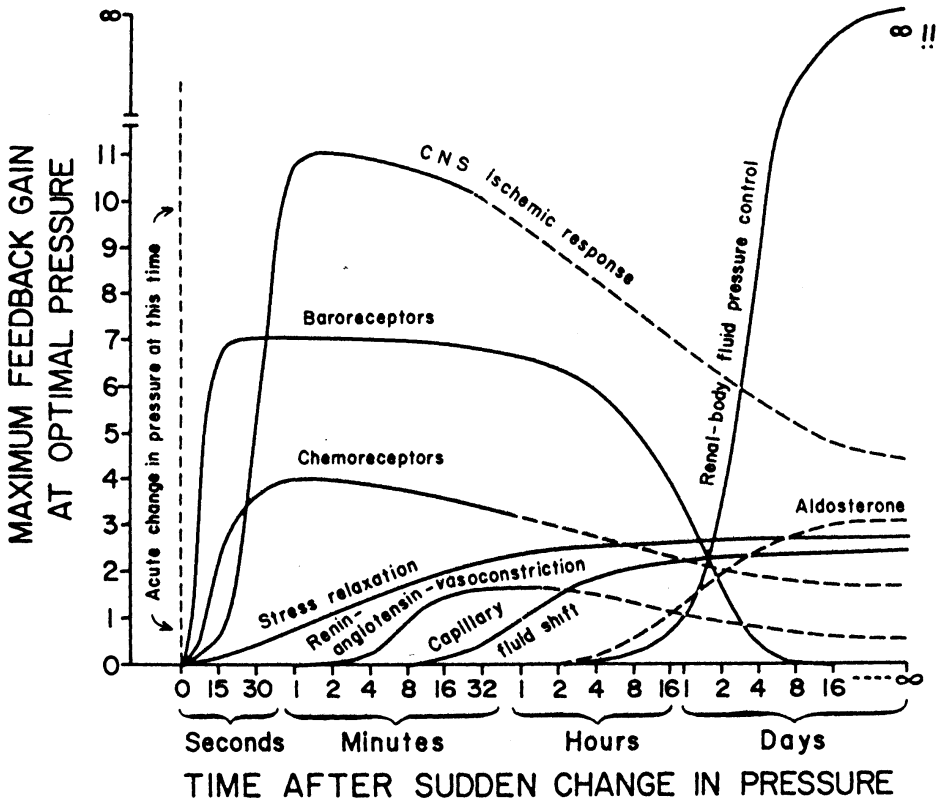
Some of the causes

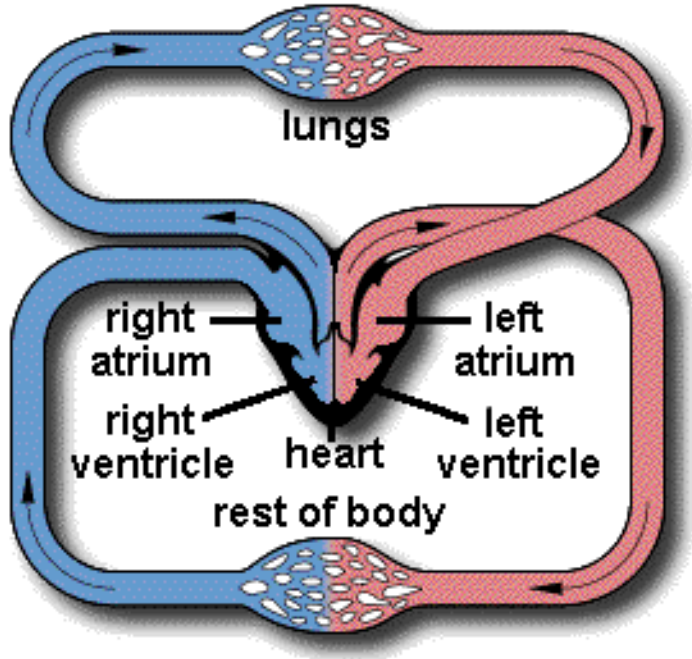
Increased peripheral resistance

- Baroreceptor settings
- Sympathetic tone

Increased blood viscosity

Local vasodilation /constriction

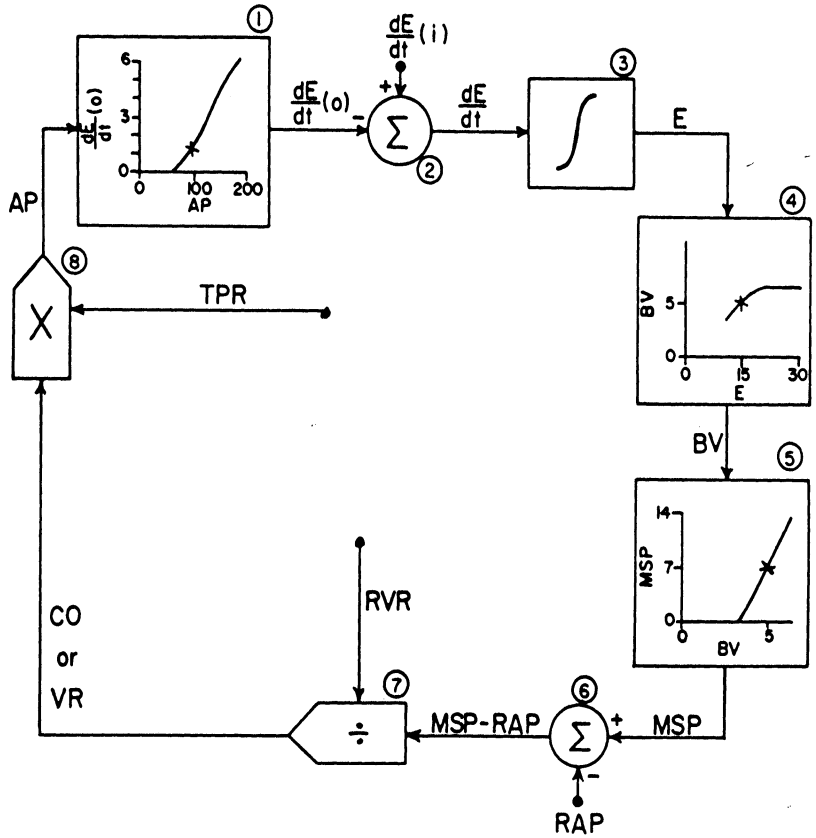


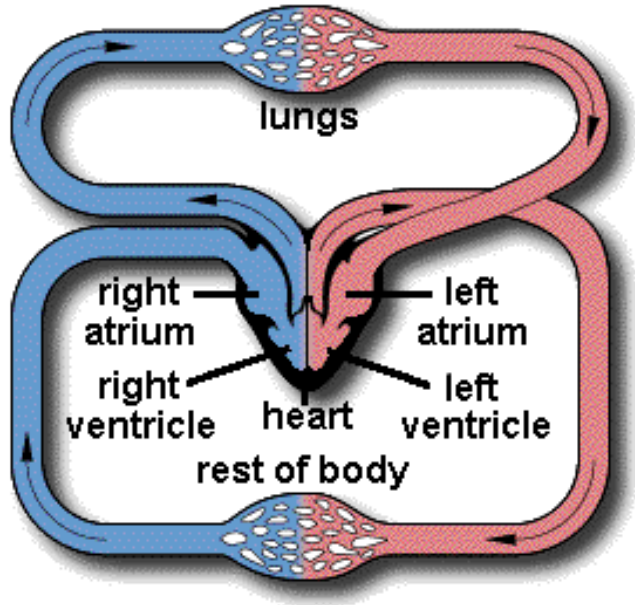


Heart plays important role in physiology

But is it in control?

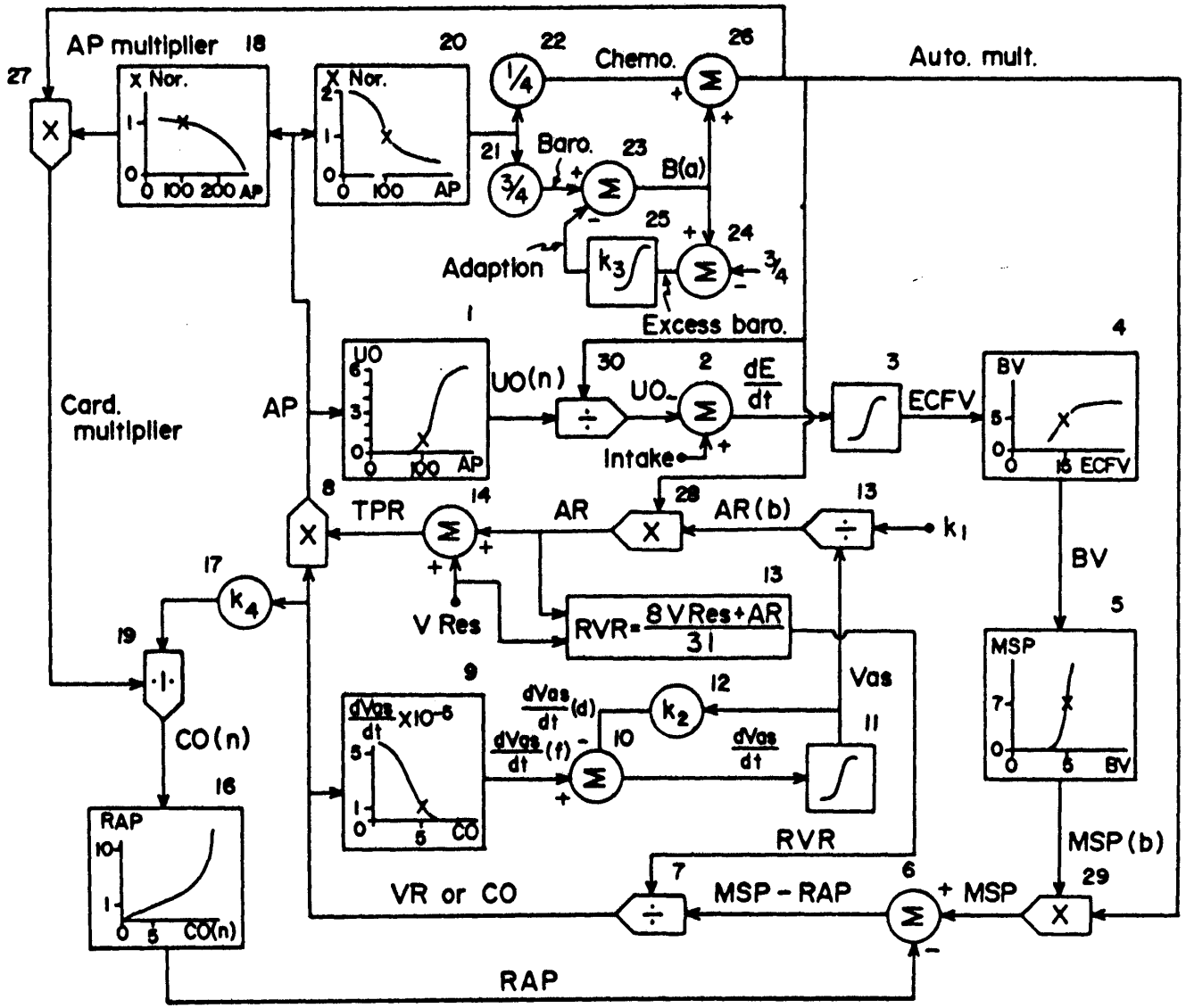
Quantitative pathophysiology of Hypertension A. C Guyton

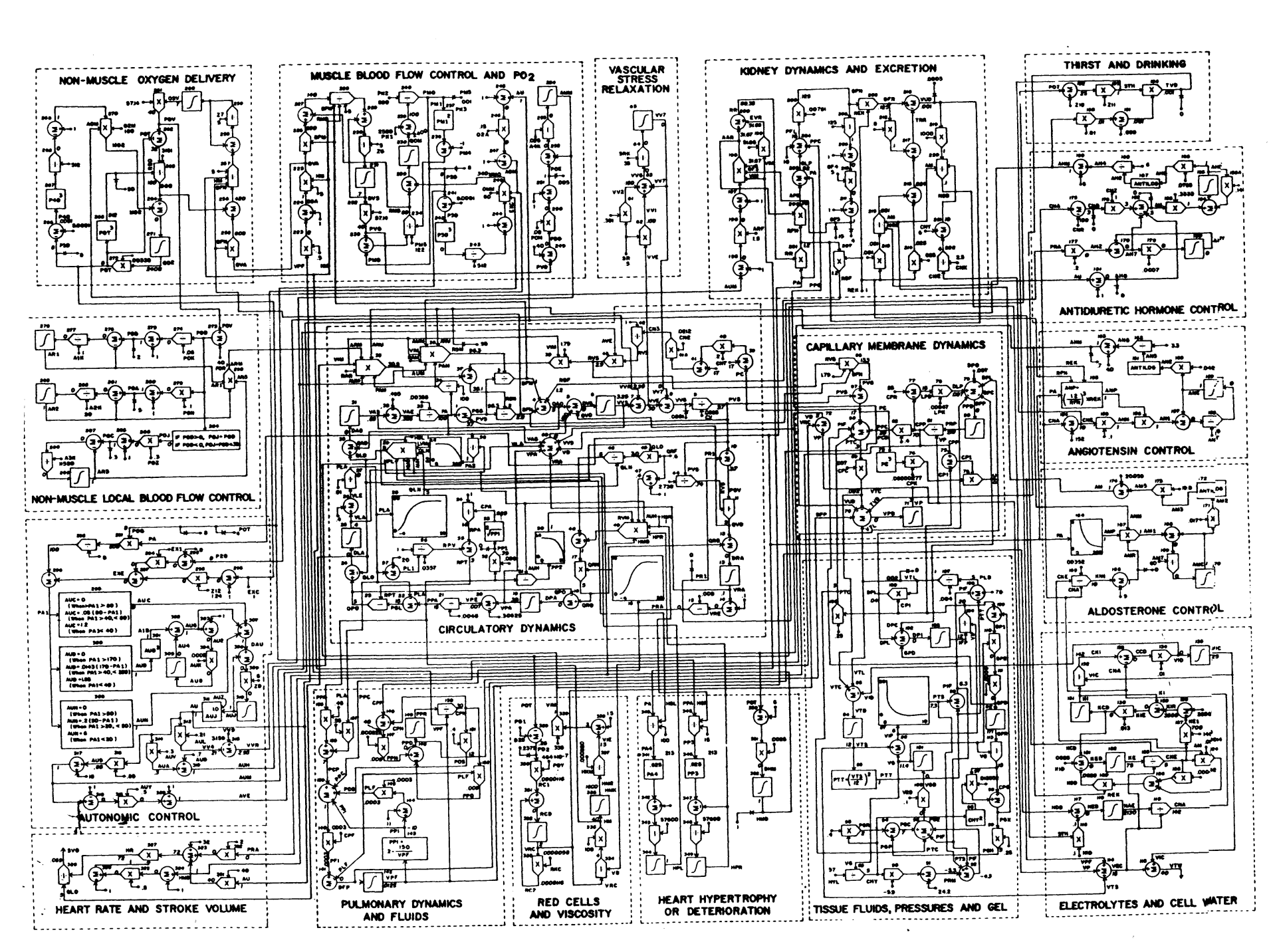


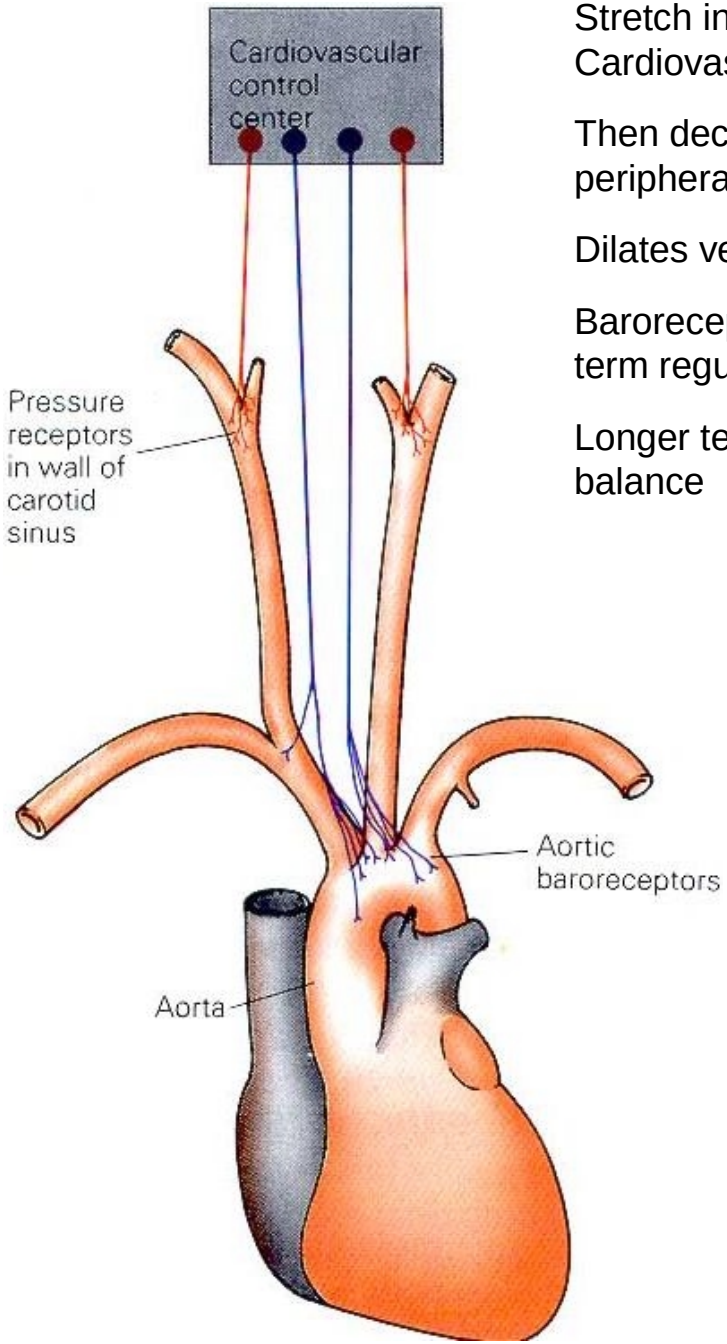


Guyton, et al., 1972. Systems Analysis of Arterial Pressure Regulation and Hypertension. Annals of Biomedical Engineering 1: 254-281

1. Effect of arterial pressure on Urine output
2. Intake of water and salts. dE/dt rate of change of ECFV
3. Integral Determines extracellular fluid volume (ECFV)
4. Relationship between ECFV and Blood volume
5. Relationship between blood volume and mean systemic pressure (MSP)
6. Subtract Right Atrial pressure (RAP) from MSP, proportional to venous return
7. Calculation of venous return (VR) which equals cardiac output (CO)
8. Calculate arterial pressure as CO * total peripheral resistance


AP multiplier
18
27
X Nor.
0 100 200 AP
20
X Nor.
0 100 AP
22
1/4
Chemo.
26
Auto. mult.
21
3/4
Baro.
23
B(a)
25
Adaption
k3
24
3/4
Excess baro.
1
UO
0 3 6
0 100 AP
UO(n)
30
2
UO-
dE/dt
3
ECFV
4
BV
0 5
0 15 ECFV
Card. multiplier
AP
Intake
8
TPR
14
AR
28
AR(b)
13
k1
BV
17
k4
V Res
13
RVR = 8 VRes + AR / 31
5
MSP
0 7
0 5 BV
19
CO(n)
9
dVas/dt x10-6
0 1 5
0 5 CO
dVas/dt (d)
dVas/dt (f)
12
k2
10
11
Vas
dVas/dt
RVR
16
RAP
0 1 10
0 5 CO(n)
VR or CO
7
MSP - RAP
6
+ MSP
29
MSP(b)
RAP


NON-MUSCLE OXYGEN DELIVERY
MUSCLE BLOOD FLOW CONTROL AND PO_2
VASCULAR STRESS RELAXATION
KIDNEY DYNAMICS AND EXCRETION
THIRST AND DRINKING
ANTIDIURETIC HORMONE CONTROL
ANGIOTENSIN CONTROL
ALDOSTERONE CONTROL
NON-MUSCLE LOCAL BLOOD FLOW CONTROL
CAPILLARY MEMBRANE DYNAMICS
CIRCULATORY DYNAMICS
AUTONOMIC CONTROL
HEART RATE AND STROKE VOLUME
PULMONARY DYNAMICS AND FLUIDS
RED CELLS AND VISCOSITY
HEART HYPERTROPHY OR DETERIORATION
TISSUE FLUIDS, PRESSURES AND GEL
ELECTROLYTES AND CELL WATER



Stretch increases impulses to Cardiovascular Control Center

Then decreased sympathetic outflow to peripheral vessels

Dilates vessels, decreases pressure

Baroreceptors are important for short term regulation of pressure

Longer term is determined by fluid balance

Arterioles are under resting sympathetic tone.

Increasing the level of sympathetic tone constricts vascular smooth muscle.

Reducing the level of sympathetic tone reduces the level of vascular smooth muscle constriction.

Most sympathetic fibres release noradrenaline that acts on a-receptors on the smooth muscle producing contraction.

a-receptors are stimulated by phenylephrine and blocked by phentolamine.

Coronary circulation is little altered by sympathetic nerves.

Hypertension

Some of the causes

Increased peripheral resistance

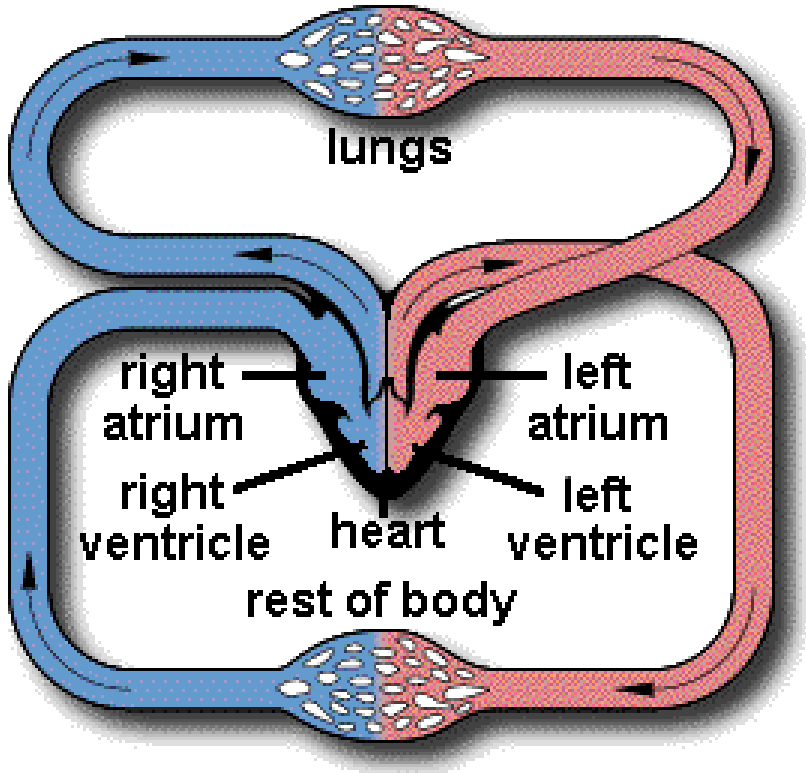
- Baroreceptor settings
- Sympathetic tone

Increased blood viscosity

Local vasodilation /constriction

Inappropriate high fluid volume

Poor Na balance



ANP secreted when blood pressure high (28 amino acid peptide hormone)

Decreases peripheral resistance

Increase Na and water excretion by kidney

GFR = Glomerular filtration rate = fluid processed by kidney = 160 L/day.

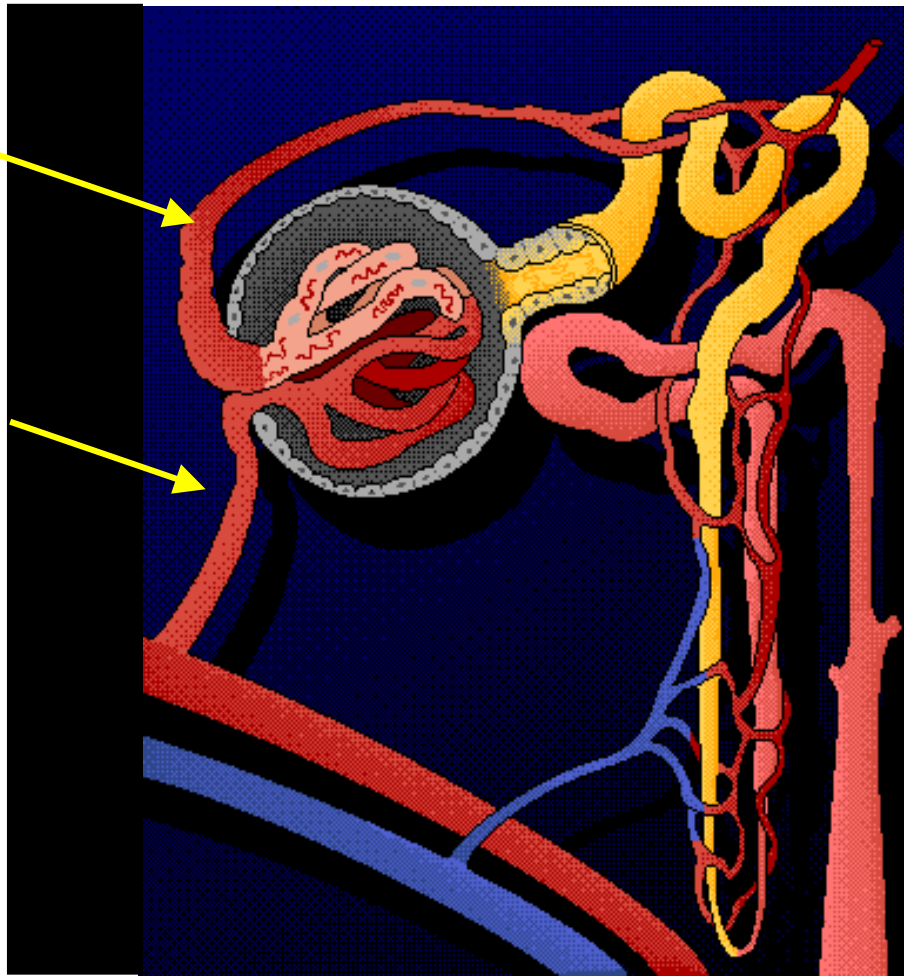
urine is produced at rate about 1.5 L/day

Constrict efferent arteriole
to increase GFR
(Glomerular filtration rate)

ANP

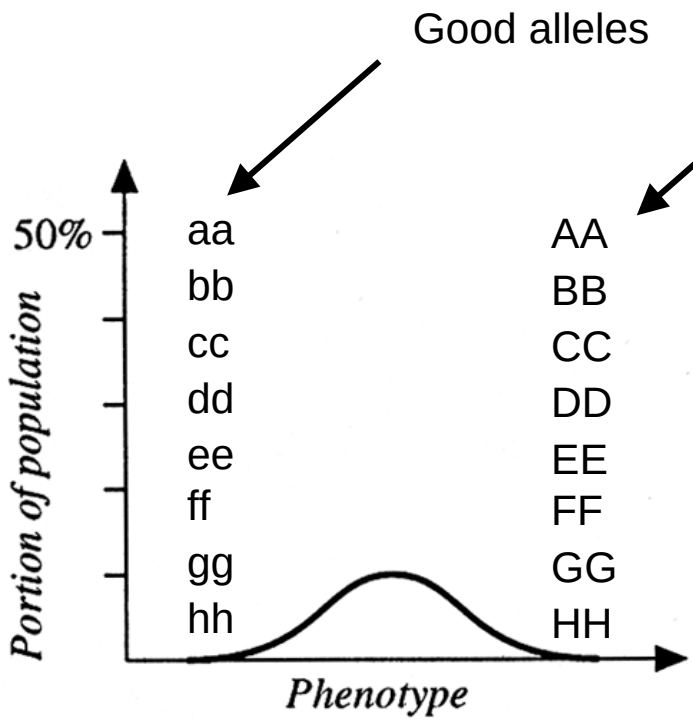
Constrict afferent arteriole
to decrease GFR
(Glomerular filtration rate)

adenosine



Kidney filters about 2500 meq Na/day
and loses in urine only about 150 meq.

Return to Quantitative traits



Blood Pressure

A Genomic-Systems Biology Map for Cardiovascular Function

M. Stoll, A.W. Cowley Jr., P.J. Tonellato, A.S. Greene, M.L. Kaldunski, R.J. Roman, P. Dumas, N.J. Schork, Z. Wang, and H.J. Jacob

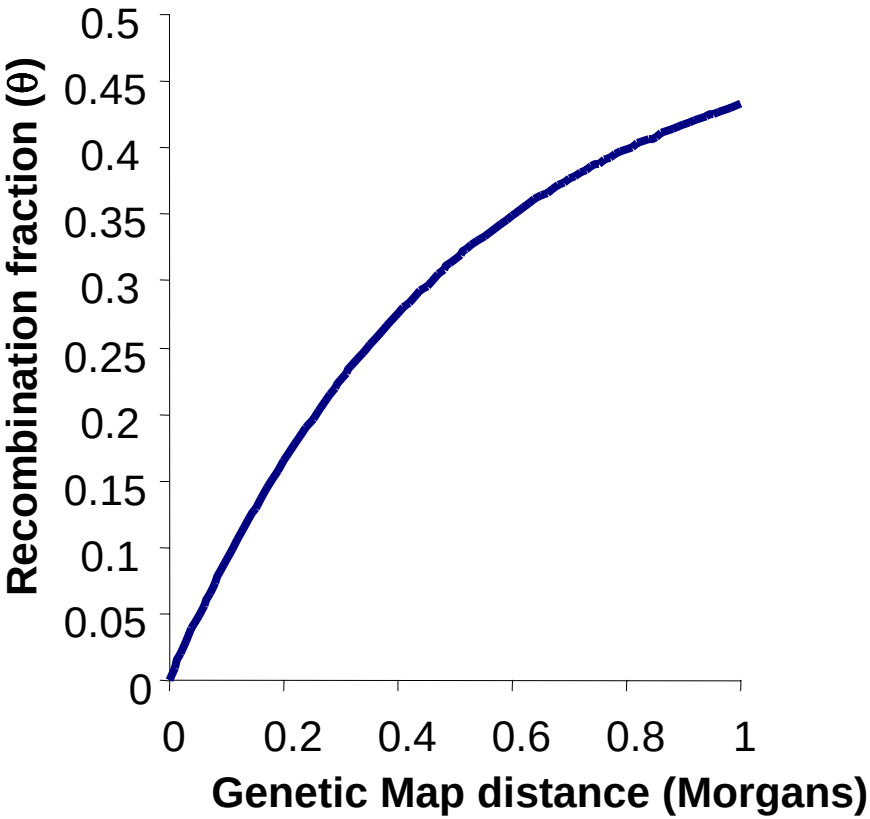
Science 2001 November 23; 294: 1723-1726.

Geography of hypertensive rats

New Zeeland, genetically hypertensive rat	GH
USA, Dahl salt Sensitive rat	SS
Japan, spontaneously hypertensive rat	SHR
Israel, DOCA salt sensitive rat	SBH
Italy, Milan hypertensive strain	MHS
Netherlands, fawn hooded hypertensive	FHH
Russia, inherited stress induced arterial hypertensive	ISIAH
Czech Republic Prague hypertensive rat	PHR

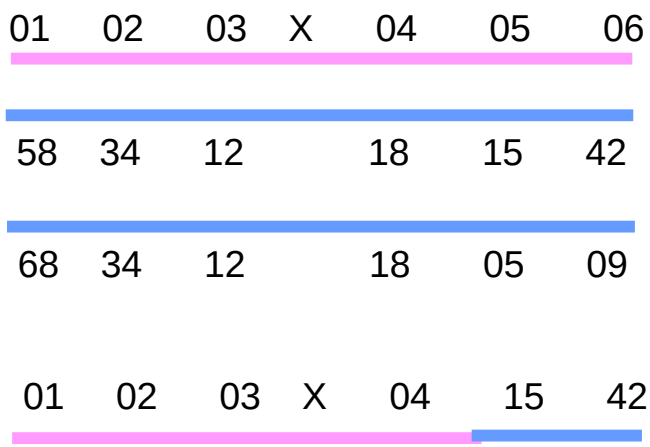
Analysis of these strains has indicated that high blood pressure is associated with linkage groups on almost every chromosome

Normal lab rat, not hypertensive = BN



Haldane map function

Recombination fraction = (θ)

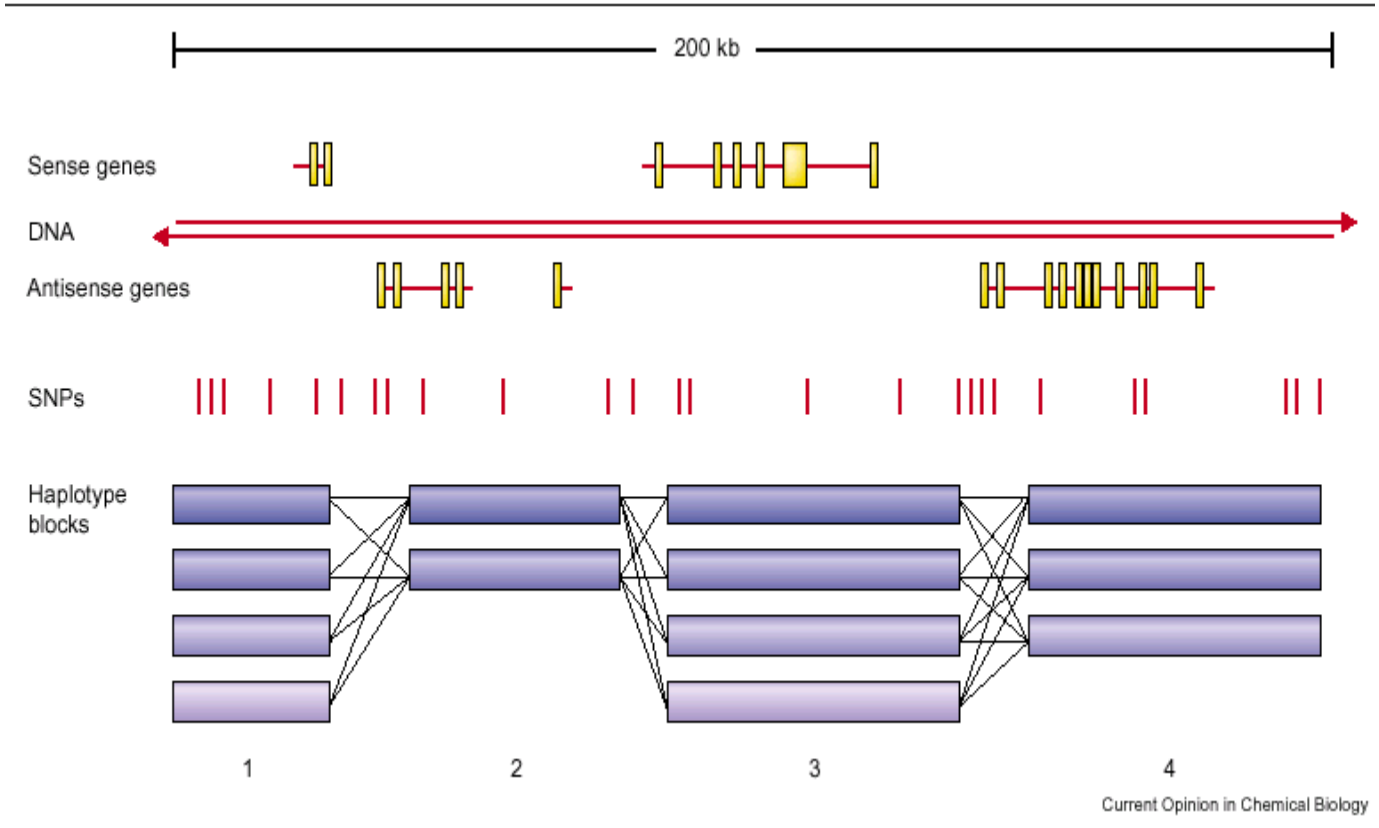


Note: Map distances are additive recombination fractions are not additive

(Morgans) = Expected number of crossovers per meiosis

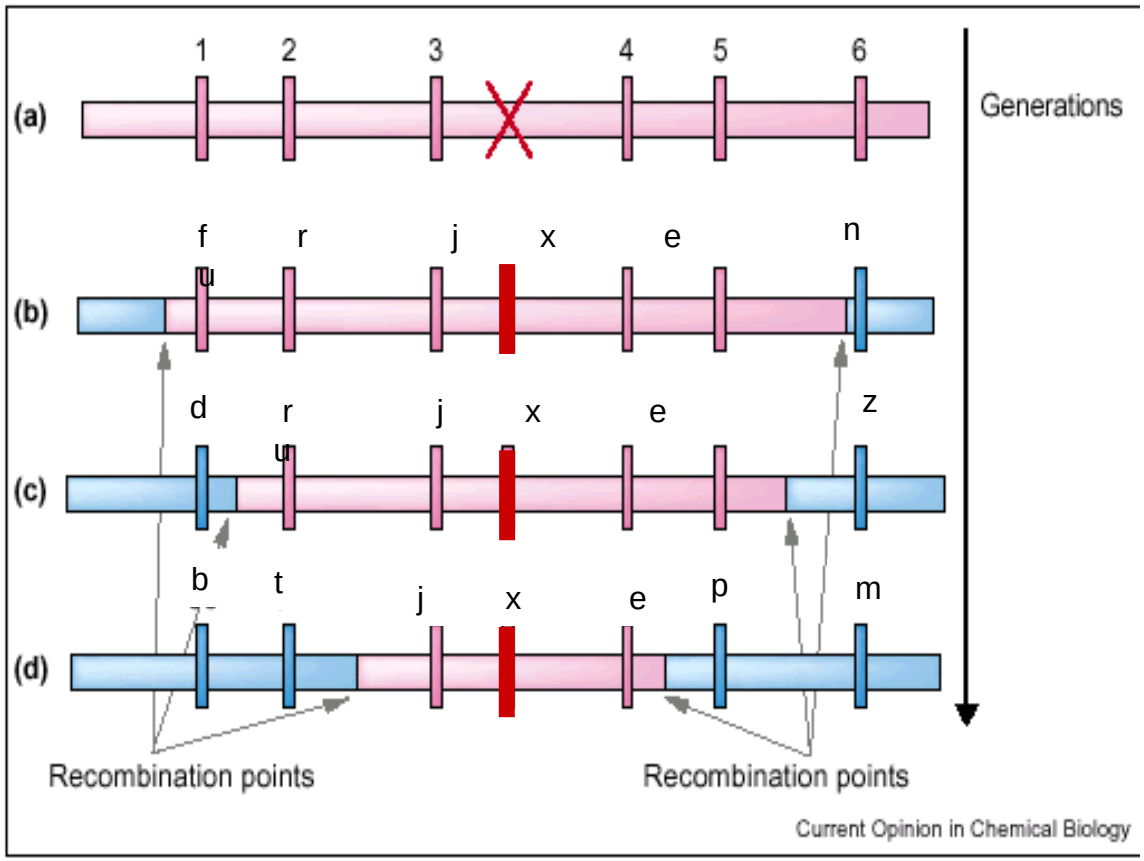
$$\theta = \frac{1 - e^{-2m}}{2}$$

$$m = -1/2 \ln(1-2\theta)$$



Haplotype blocks. This figure illustrates the characteristic pattern of SNPs in the human genome. Within haplotype blocks, the diversity is low; 2–4 variants are typically representing 90–95% of population. Haplotype blocks are interrupted with regions of frequent recombination, which are responsible for ‘shuffling’ of blocks between chromosomes.

High-density genotyping and linkage disequilibrium in the human genome using chromosome 22 as a model, Current Opinion in Chemical Biology, 6, 24-30 February 2002, Pages 24-30

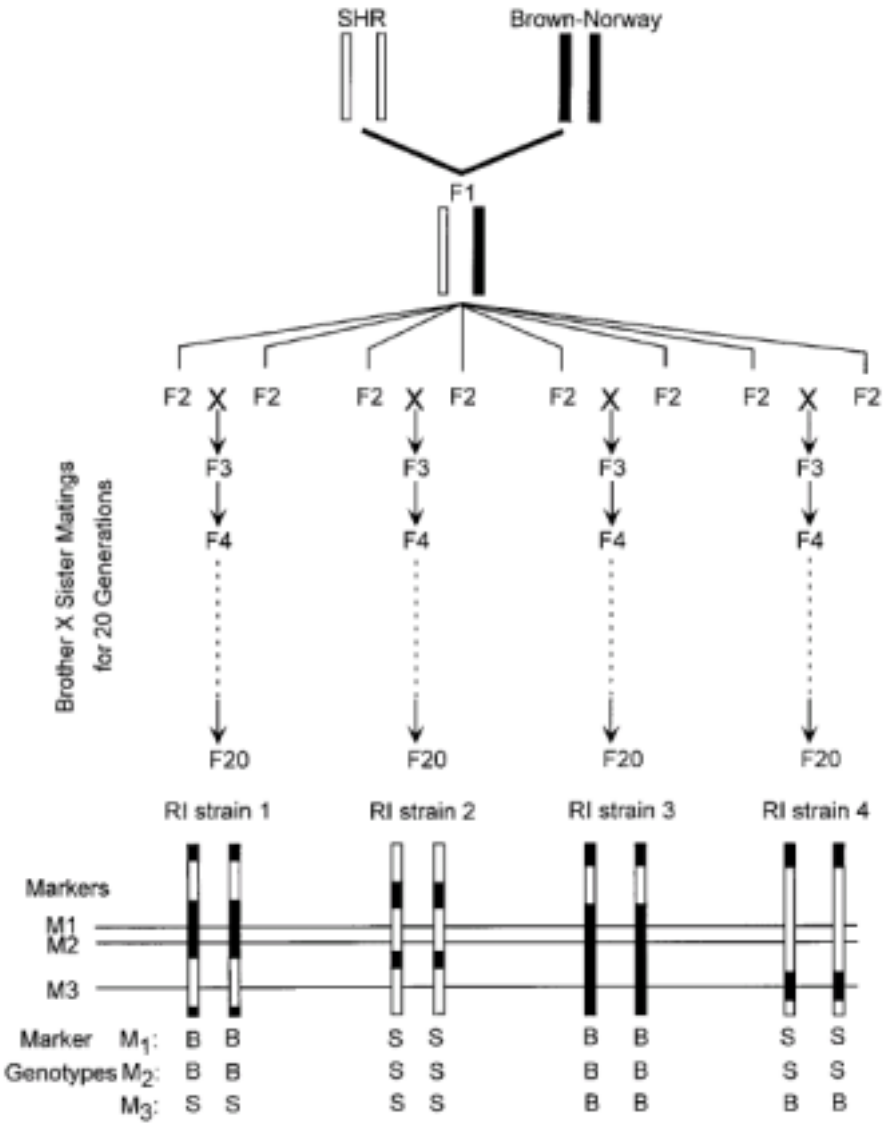


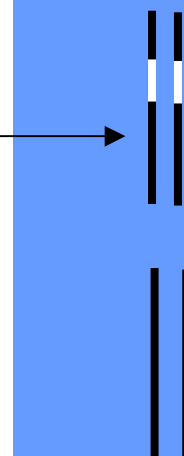
Description of LD. (a) Situation shortly after a mutation event (shown as a red cross) has generated a new SNP. At this point, the new allele is in complete LD with other marker alleles 1–6 on the same chromosome. (b,c) The situation changes as generations pass by. The extent of LD decays because of recombination between chromosomes. The recombination shuffles marker alleles and they loose their association with mutation allele (x). (d) The LD between mutation (x) and nearby markers is observed only in a short region around the mutation.

High-density genotyping and linkage disequilibrium in the human genome using chromosome 22 as a model, Current Opinion in Chemical Biology, Volume 6, February 2002, Pages 24-30

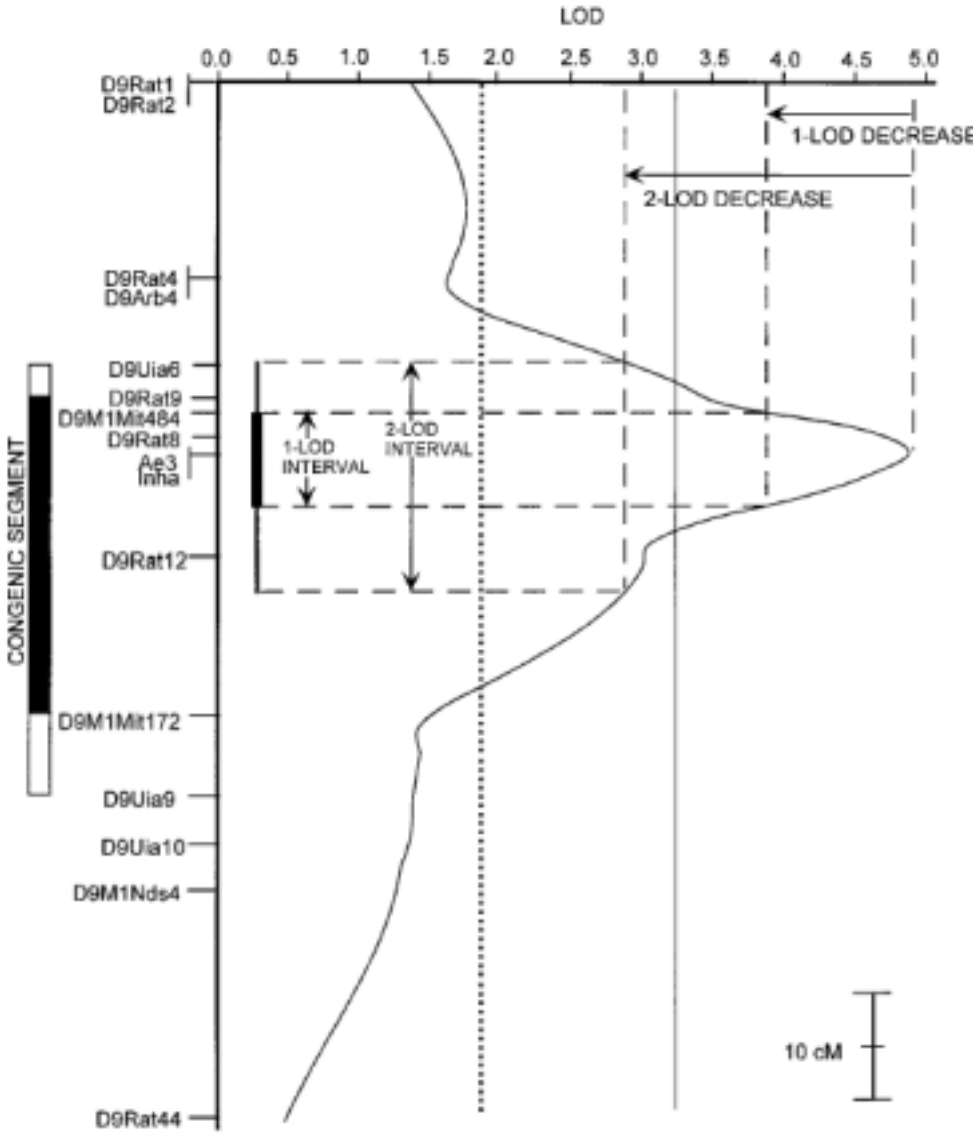
Creating consomic and congenic rat strains.

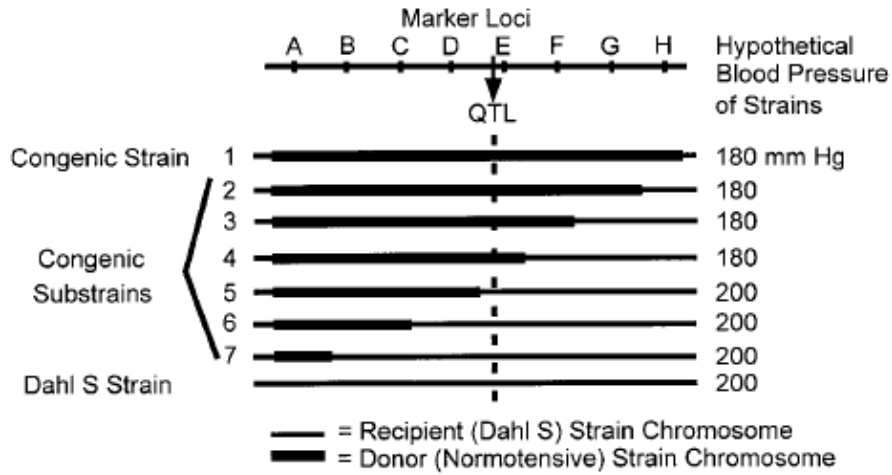
Consomic rats have one entire chromosome moved from one background to another





back cross to BN to obtain line with one piece of SHR DNA on one chromosome.
All other chromosomes are BN.

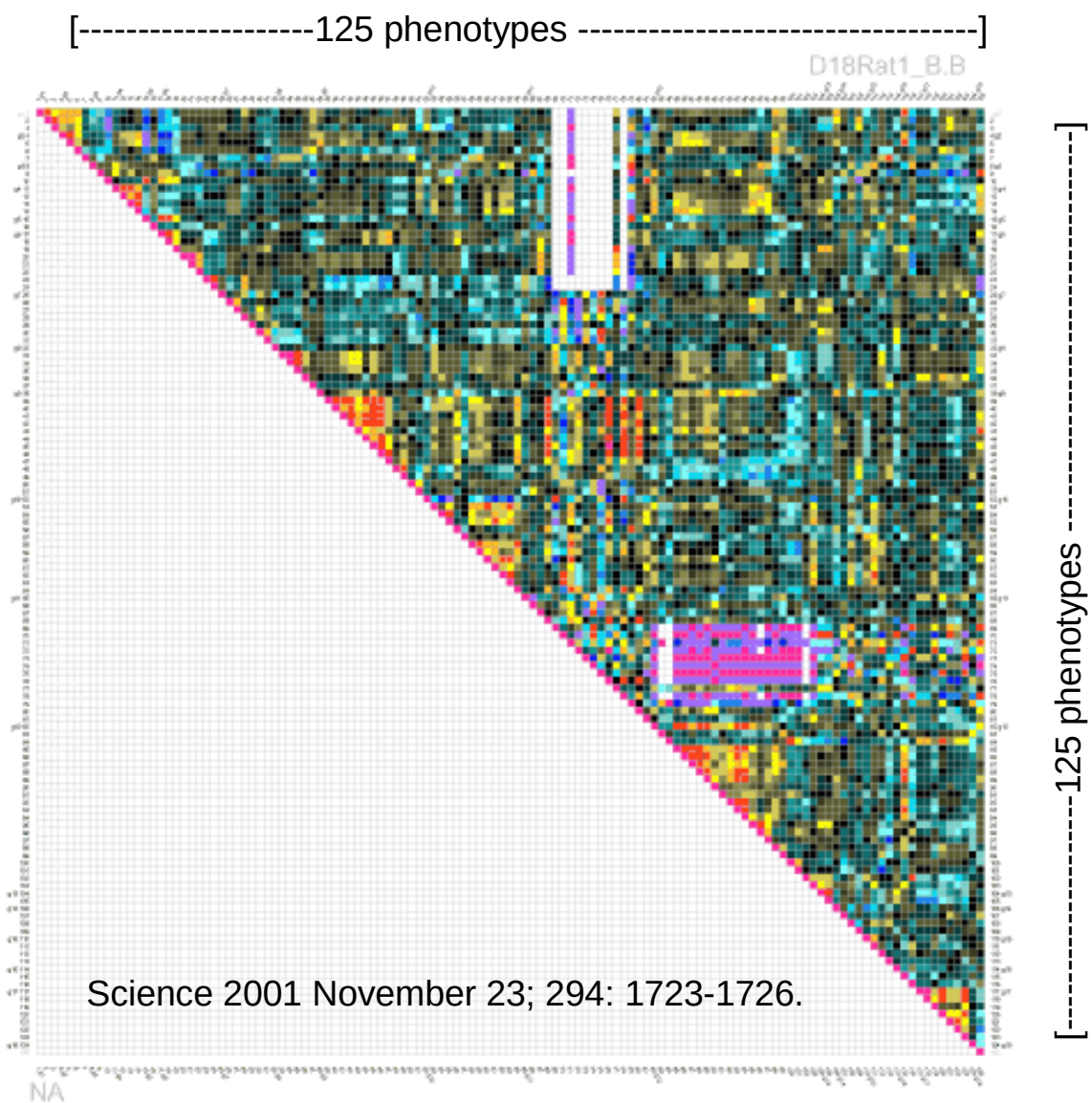




1 cMorgan = 1 recombination in 100 meioses
1 cMorgan ≈ 2X106 base pairs
1 cMorgan ≈ 40 genes

Resolution to 1 cM or less is required for positional cloning

A Genomic-Systems Biology Map for Cardiovascular Function



Physiological profiles

A correlation matrix with phenotypic ordering of 125 likely determinants of arterial blood pressure, based on Guyton's model of blood pressure control, on each axis.

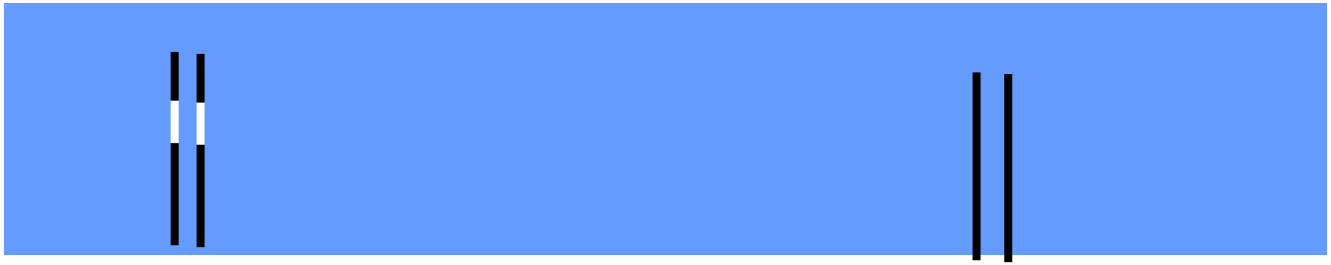
EXAMPLES

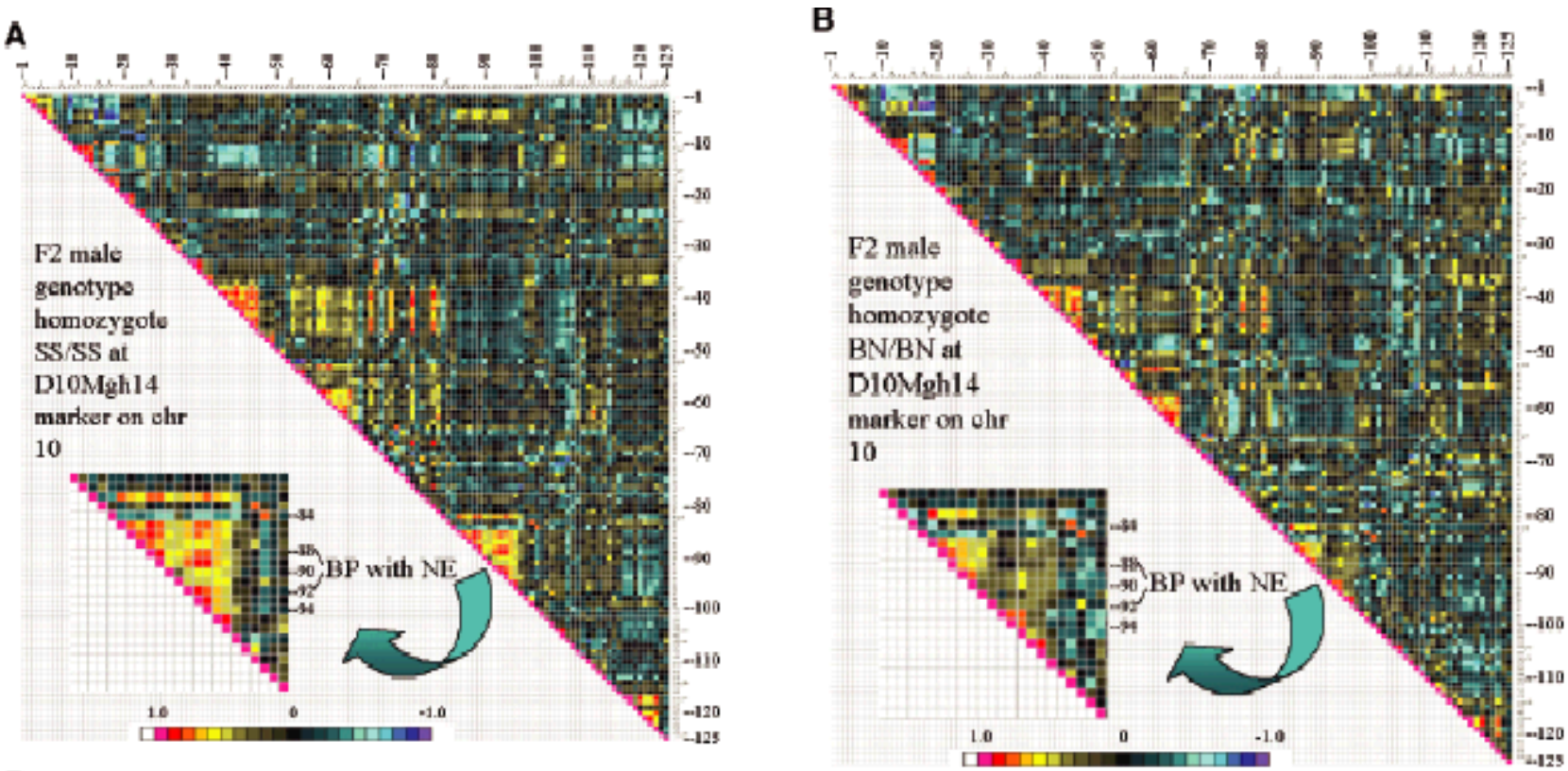
47 = systolic blood pressure, high salt diet

76 = Set point for baro-receptor response (mechanistic model)

121 =left ventricle weight in grams

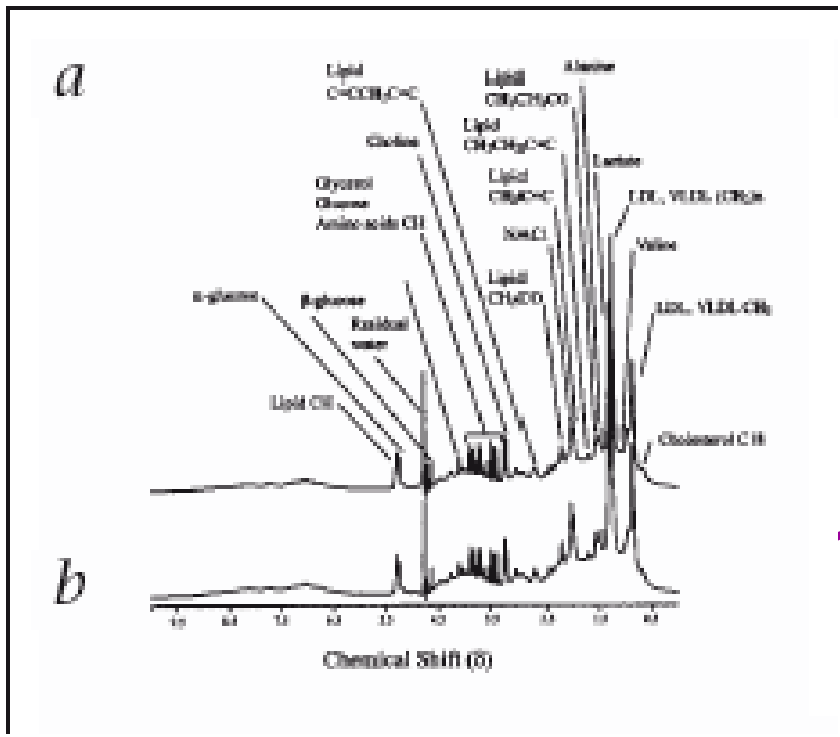
Data for one of 82 rat genotypes with possible high blood pressure traits





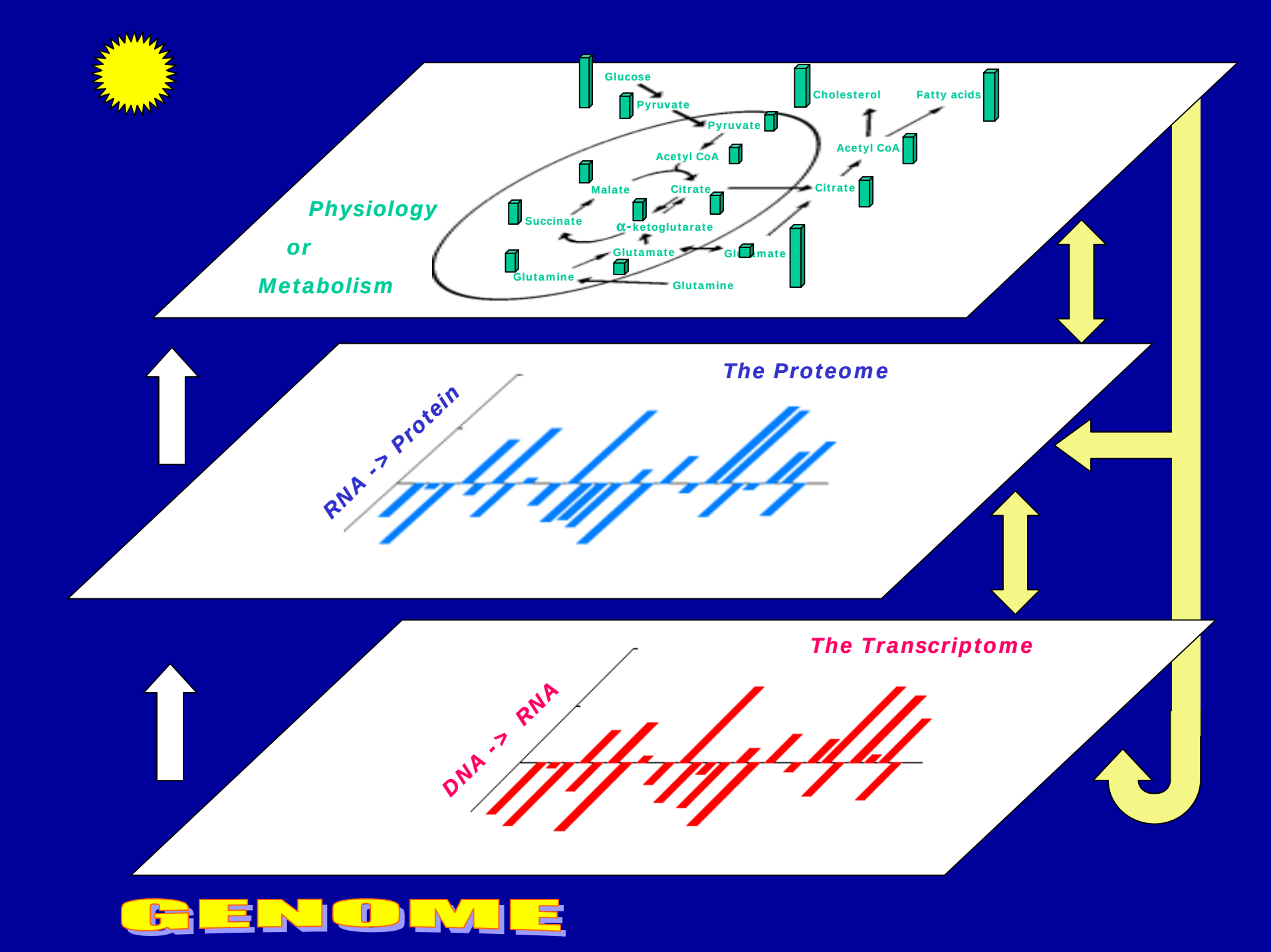
shown by the expanded insert, there was a high degree of correlation between blood pressures determined immediately before, during, and after the short-term infusions of norepinephrine (NE), angiotensin II, and acetylcholine in F2 rats homozygous for insert

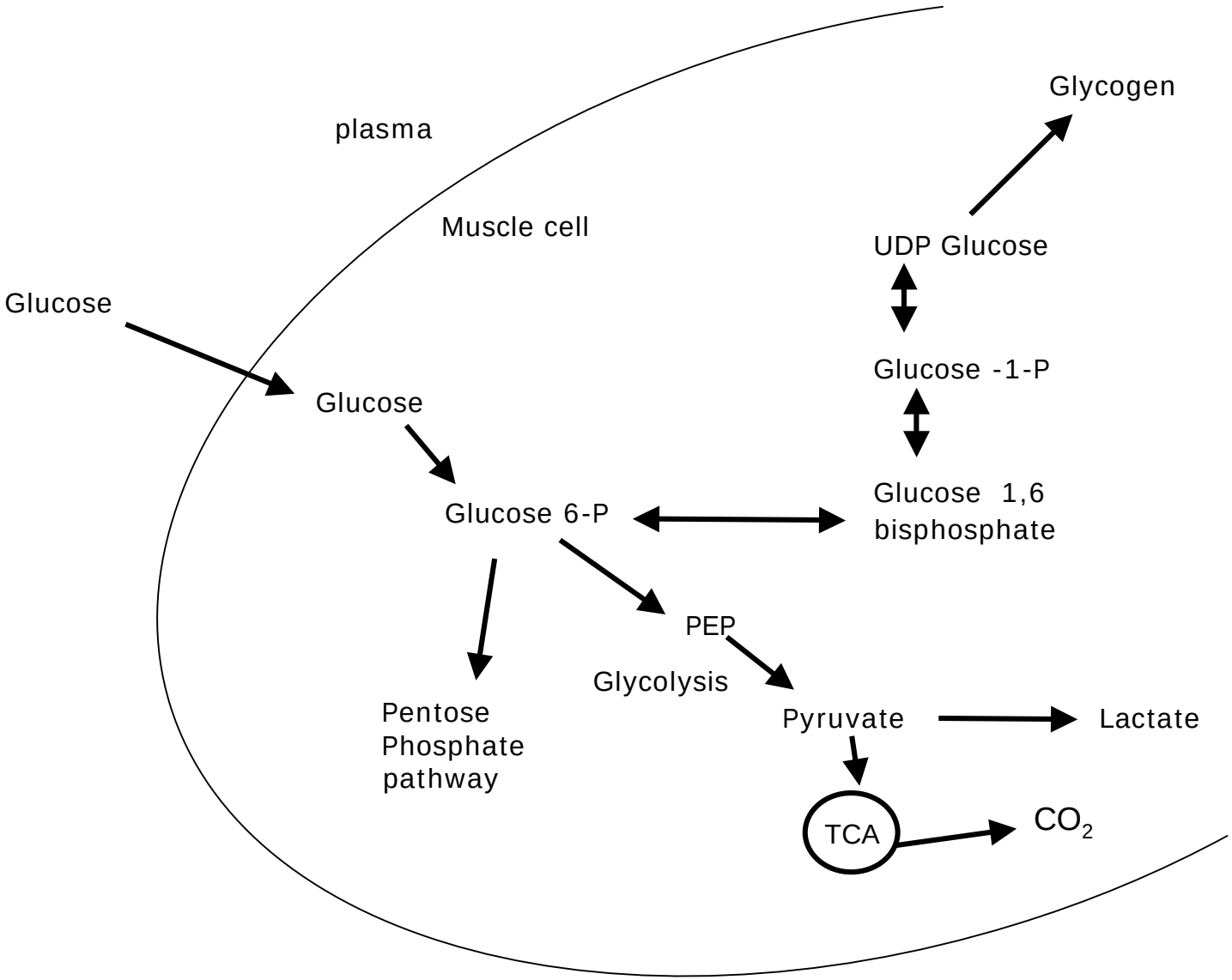
Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based **metabonomics**. Nat. Med. 8: 1439-1444.
Brindle, J. T., et al., 2002.



Ideas to develop

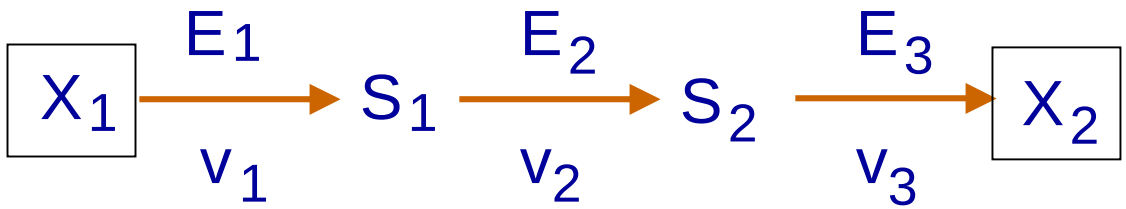
Fig. 1 Comparison of patients with severe atherosclerosis (TVD) and patients with normal coronary arteries (NCA). The 600 MHz 1H-NMR spectra of serum samples from a typical NCA patient (a) and a TVD patient (b) are shown. The chemical shifts of a selection of major metabolites are indicated (based on assignments from 2D-TOCSY spectra), although these metabolites do not contribute all (or in some cases, even most) of the signal at the indicated chemical shift.


Physiology
or
Metabolism
Glucose
Pyruvate
Pyruvate
Acetyl CoA
Malate
Citrate
Succinate
α-ketoglutarate
Glutamate
Glutamine
Cholesterol
Fatty acids
Acetyl CoA
Citrate
Glutamate
Glutamine
The Proteome
RNA -> Protein
The Transcriptome
DNA -> RNA
GENOME


plasma
Muscle cell
Glycogen
UDP Glucose
Glucose
Glucose -1-P
Glucose
Glucose 6-P
Glucose 1,6
bisphosphate
PEP
Glycolysis
Pentose
Phosphate
pathway
Pyruvate
Lactate
TCA
CO_2

Metabolic Control Analysis

Understanding the Control of Metabolism by David Fell. Portland Press, London, 1997.



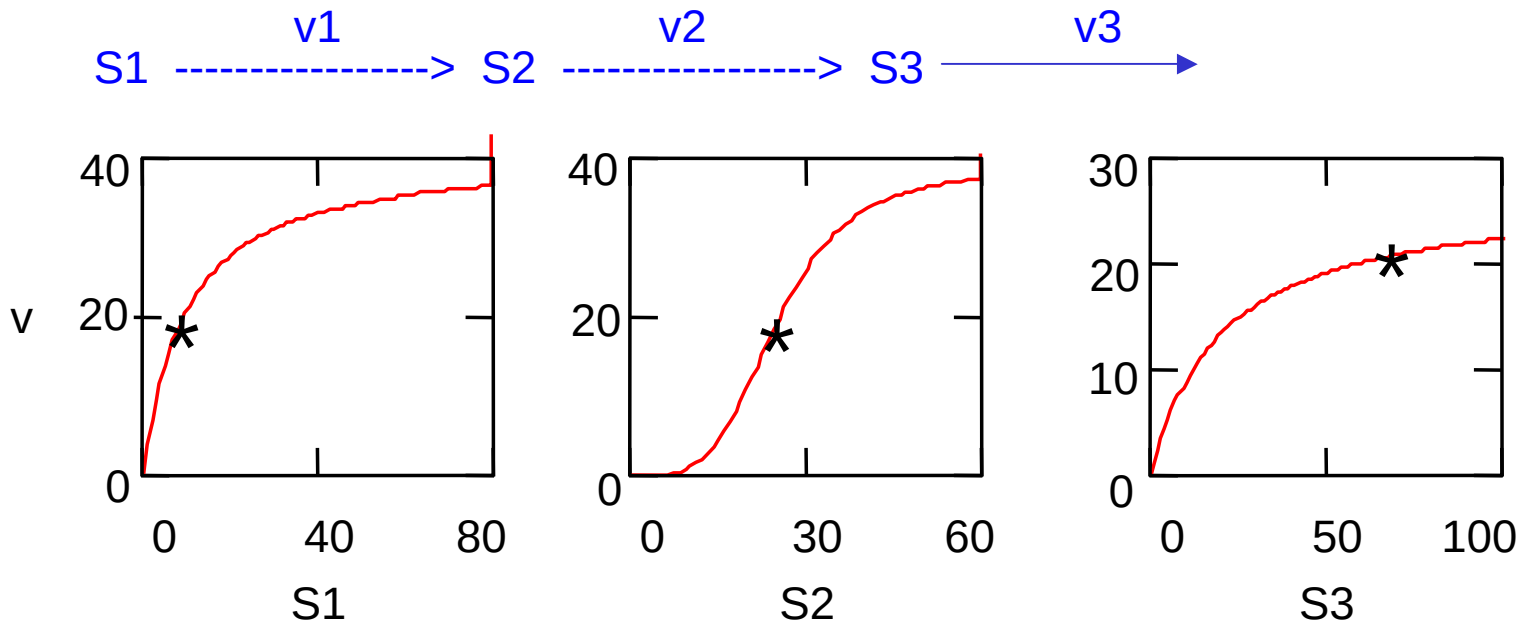
Variables

1. Concentrations of Molecular Species
2. Fluxes

Parameters

1. Enzyme Levels
2. Kinetics Constants
3. Boundary Conditions

MCA investigates the relationship between the variables and parameters in a biochemical network.



v1 =v2 = v3 = Flux

$$vf = \left[\frac{(Vmf \cdot S)}{(Kms + S)} \right]$$

$$v_f = V_{max\,F} / (1 + K_{m,S} / [S])$$

$$v_f = V_{max\,F} / (1 + K_{m,S} / [S])$$

$$v_r = V_{max\,R} / (1 + K_{m,P} / [P])$$

$$v_f = v_r \quad \text{and} \quad v = 0$$

$$K' = (V_{max\,F} \times K_{m,P}) / (V_{max\,R} \times K_{m,S})$$

$$v = \frac{V_{max\,F} / K_{m,S} ([S] - [P] / K')}{1 + [S] / K_{m,S} + [P] / K_{m,P}} = \frac{V_{max\,F} [S] / K_{m,S} - V_{max\,R} [P] / K_{m,P}}{1 + [S] / K_{m,S} + [P] / K_{m,P}}$$

$$v_f = V_{max\,F}/(1+K_{m,S}/[S]) \qquad v_r = V_{max\,R}/(1+K_{m,P}/[P])$$

$$v_f = v_r \quad \text{and} \quad v = 0 \qquad K' = (V_{max\,F} \times K_{m,P})/(V_{max\,R} \times K_{m,S})$$

$$v = \frac{V_{max\,F}/K_{m,S}([S]-[P]/K')}{1+[S]/K_{m,S}+[P]/K_{m,P}} = \frac{V_{max\,F}[S]/K_{m,S} - V_{max\,R}[P]/K_{m,P}}{1+[S]/K_{m,S}+[P]/K_{m,P}}$$

$$C^{J}_{E_i} = \lim_{\delta e_i \to 0} \frac{\delta J/J}{\delta e_i/e_i} = \frac{\delta J}{\delta e_i} \cdot \frac{e_i}{J}$$

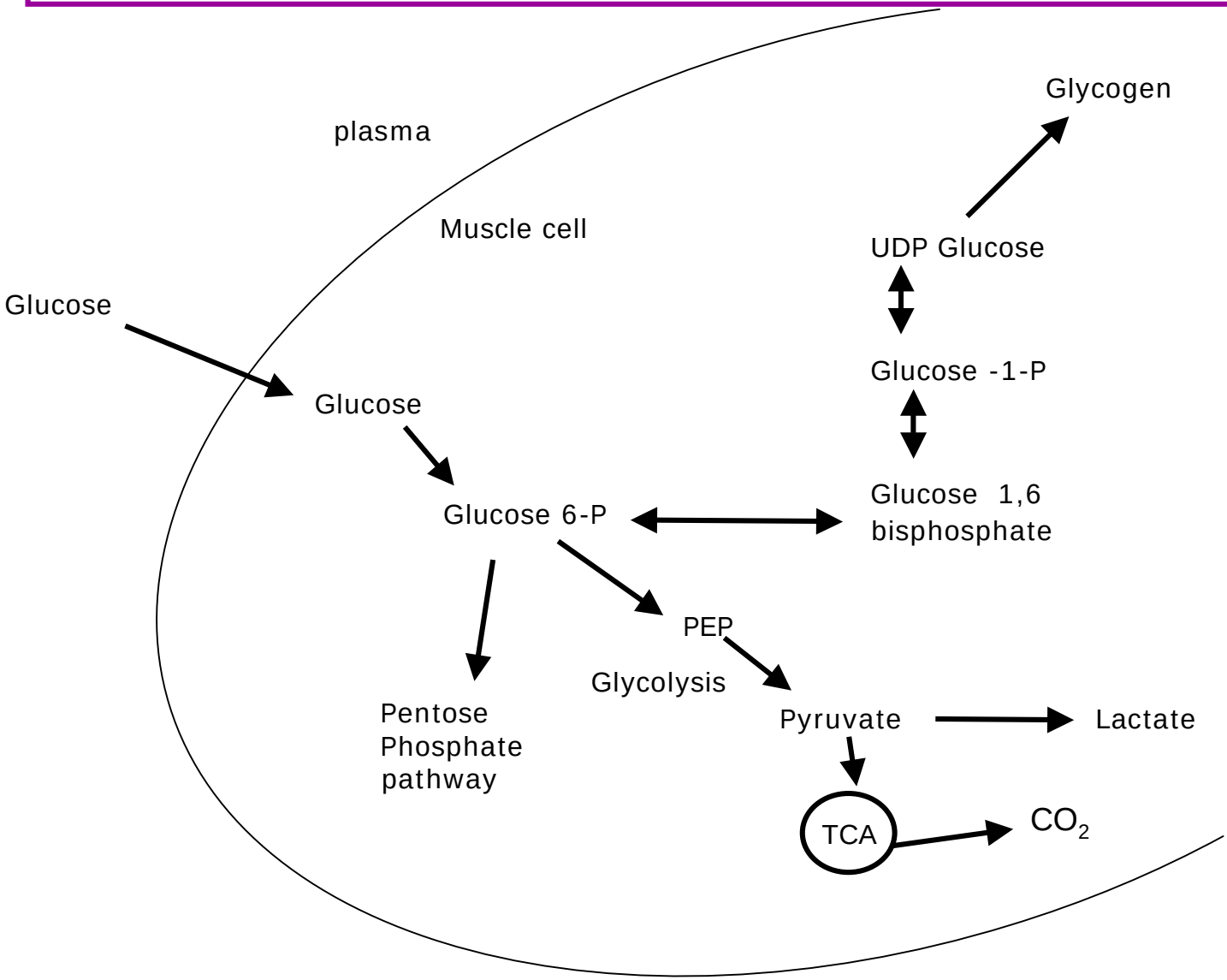
$$\varepsilon^{E_i}_{X_{i-1}(\text{substrate})} = \lim_{\delta[S]\to 0} \frac{\delta v_i/v_i}{\delta[S]/[S]} = \frac{\delta v_i}{\delta[S]} \cdot \frac{[S]}{v_i} \qquad \varepsilon^{E_i}_{X_i(\text{product})} = \lim_{\delta[P]\to 0} \frac{\delta v_i/v_i}{\delta[P]/[P]} = \frac{\delta v_i}{\delta[P]} \cdot \frac{[P]}{v_i}$$

$$\varepsilon^{E_i}_{S} = \frac{1}{1-\Gamma/K'_{E_i}} - \frac{[S]/K_{m.S(E_i)}}{1+[S]/K_{m,S(E_i)}+[P]/K_{m,P(E_i)}} = \frac{1}{1-\Gamma/K'_{E_i}} - \frac{v_{f,E_i}}{V_{max\,F,E_i}}$$

$$\varepsilon^{E_i}_{P} = \frac{-\Gamma/K'_{E_i}}{1-\Gamma/K'_{E_i}} - \frac{[P]/K_{m.S(E_i)}}{1+[S]/K_{m,S(E_i)}+[P]/K_{m,P(E_i)}} = \frac{-\Gamma/K'_{E_i}}{1-\Gamma/K'_{E_i}} - \frac{v_{r,E_i}}{V_{max\,R,E_i}}$$

An example of a practical use of MCA to determine flux control in the heart.

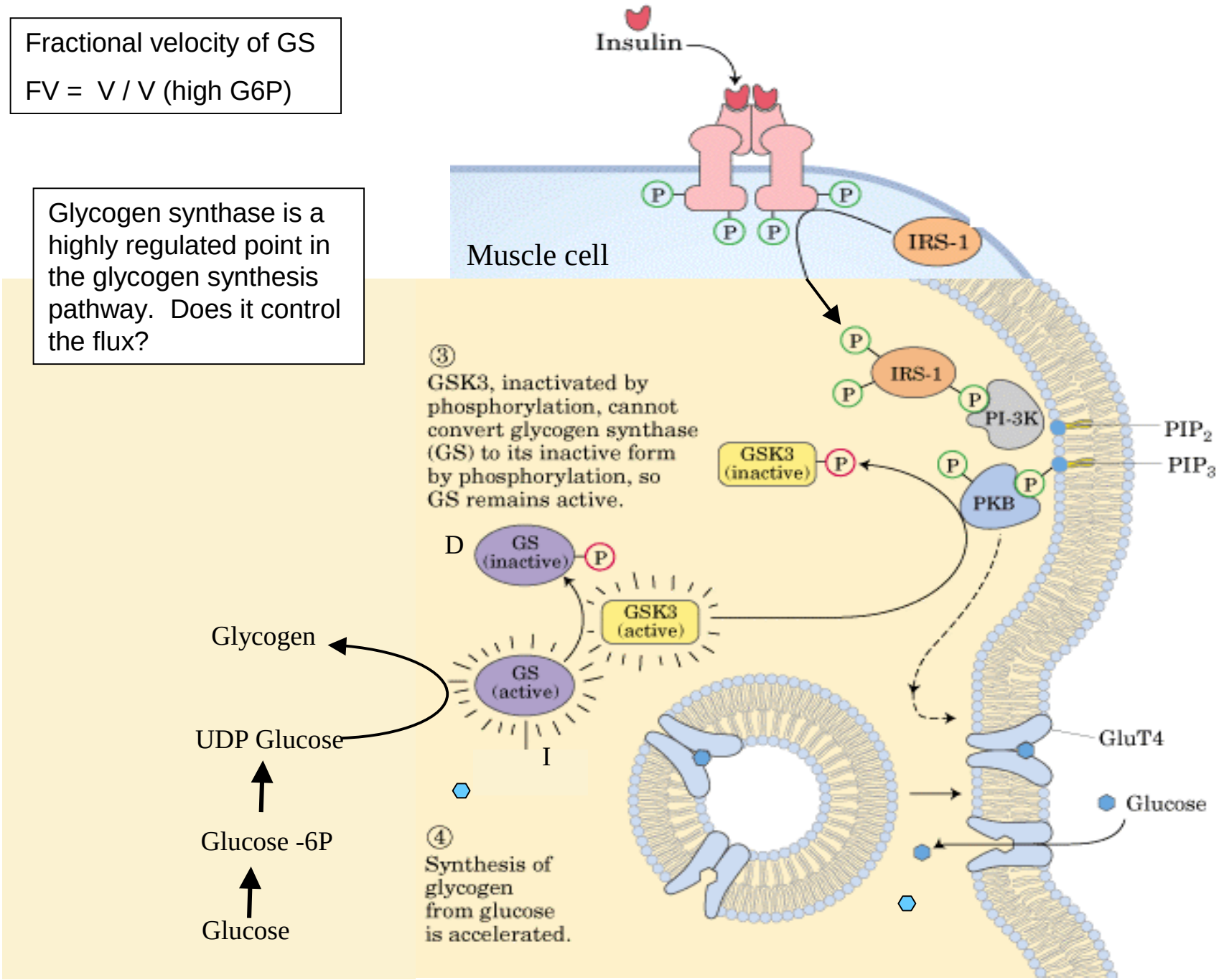
Kashiwaya, Y., K. Sato, N. Tsuchiya, S. Thomas, D. A. Fell, *R. L. Veech*, and J. V. Passonneau. 1994. Control of glucose utilization in working perfused rat heart. J Biol Chem 269: 25502-25514 (available on line)



Fractional velocity of GS

FV = V / V (high G6P)

Glycogen synthase is a highly regulated point in the glycogen synthesis pathway. Does it control the flux?



Rat hearts were perfused under different conditions. Basic metabolic parameters ere measured and flux control coefficients were calculated from elasticities using the bottom up approach based on enzyme kinetics.

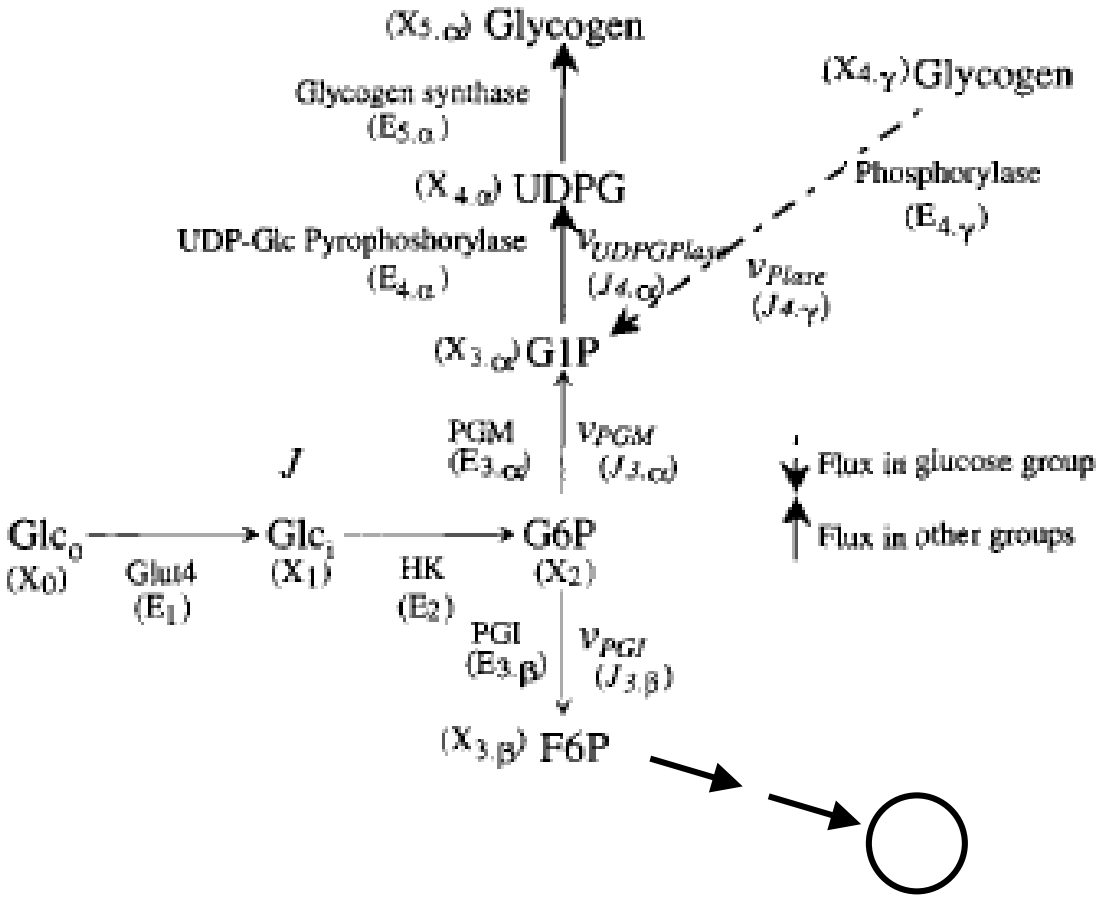
	Glucose	Glucose + ketones	Glucose + insulin	Glucose + ketones + insulin
Hydraulic work (J/min/g wet weight)	0.30 ± 0.01 (8)	0.37 ± 0.01* (8)	0.34 ± 0.01* (4)	0.32 ± 0.01 (4)
^3H_2O production from [2-^{3}H]glucose	4.58 ± 0.75 (3)	2.37 ± 0.69* (3)	6.36 ± 0.32* (3)	4.66 ± 0.5 (3)
Glycogen synthesis	−0.463 ± 0.12	0.673 ± 0.14	2.56 ± 0.43	2.46 ± 0.15
Net glycolytic flux through P-glucoisomerase	5.04 ± 0.76	1.70 ± 0.70	3.80 ± 0.53	2.20 ± 0.52
L-Lactate exiting heart	0.030 (2)	0.050 (2)	0.400 (2)	0.066 (2)

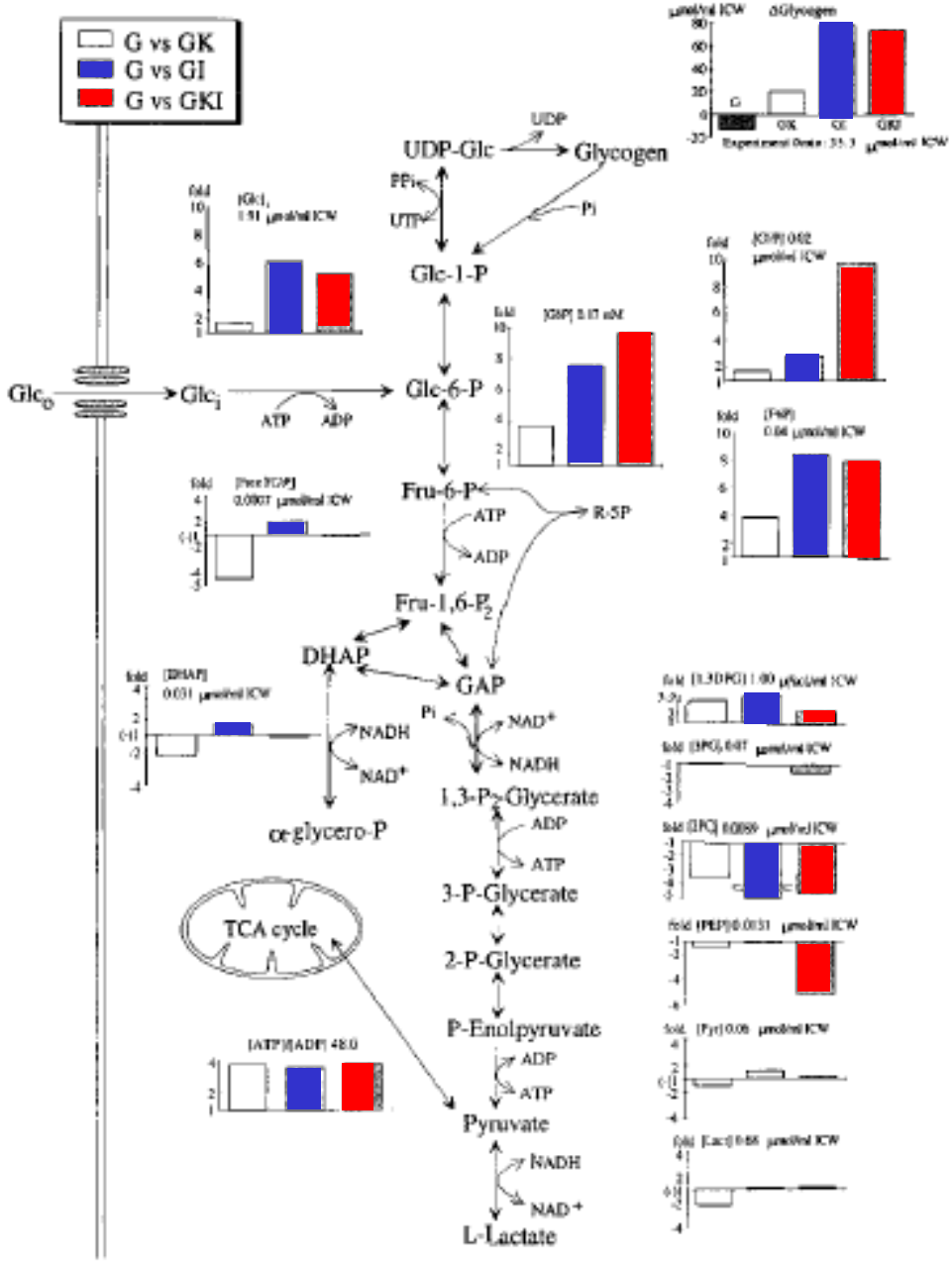
* $p < 0.05$, Mann-Whitney U test.

7. Branch Point Theorem

At a branch point under steady-state conditions, the sum of the flux control coefficients of enzymes in the branches is equal to the ratio of flux through the branches.

$$\frac{\sum C^{J}_{i,\alpha}}{\sum C^{J}_{i,\beta}} = \frac{v_{i,\alpha}}{v_{i,\beta}}, \qquad -v_{i,\alpha}\left(\sum C^{J}_{i,\beta}\right) + v_{i,\beta}\left(\sum C^{J}_{i,\alpha}\right) = 0$$





Determining amounts of metabolites in pathways are key to using kinetic approach to determining flux control coefficients.

This slide was omitted in lecture It shows the two important relationships for the sum of flux control coefficients and the sum or the products of control coefficients and elasticities. Also shown is example matrix to solve for C_i in a pathway

$$\sum_{i=1}^{n} C_{E_i}^{J} = 1$$

$$\sum_{i=1}^{n} C_{E_i}^{J} \varepsilon_{X}^{E_i} = 0$$

$$C_{Glut4}^{J} + C_{HK}^{J} + C_{PGM}^{J} + C_{PG1}^{J} + C_{Plase}^{J} = 1$$

$$C_{Glut4}^{J} \varepsilon_{Glc_i}^{Glut4} + C_{HK}^{J} \varepsilon_{Glc_i}^{HK} = 0$$

$$C_{HK}^{J} \varepsilon_{G6P}^{HK} + C_{PGM}^{J} \varepsilon_{G6P}^{PGM} + C_{PGI}^{J} \varepsilon_{G6P}^{PGI} = 0$$

$$C_{PGM}^{J} \varepsilon_{G1P}^{PGM} + C_{Plase}^{J} \varepsilon_{G1P}^{Plase} = 0$$

$$-C_{PGM}^{J} / v_{PGM} + C_{PGI}^{J} / v_{PGI} + C_{Plase}^{J} / v_{Plase} = 0$$

$$\begin{bmatrix} C_{Glut4}^{J} \\ C_{HK}^{J} \\ C_{PGM}^{J} \\ C_{PGI}^{J} \\ C_{Plase}^{J} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 1 & 1 & 1 \\ \varepsilon_{Glc_i}^{Glut4} & \varepsilon_{Glc_i}^{HK} & 0 & 0 & 0 \\ 0 & \varepsilon_{G6P}^{HK} & \varepsilon_{G6P}^{PGM} & \varepsilon_{G6P}^{PGI} & 0 \\ 0 & 0 & \varepsilon_{G1P}^{PGM} & 0 & \varepsilon_{G1P}^{Plase} \\ 0 & 0 & -1/v_{PGM} & 1/v_{PGI} & 1/v_{Plase} \end{bmatrix}^{-1} \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

TABLE VI

Flux control coefficients determined by bottom-up analysis

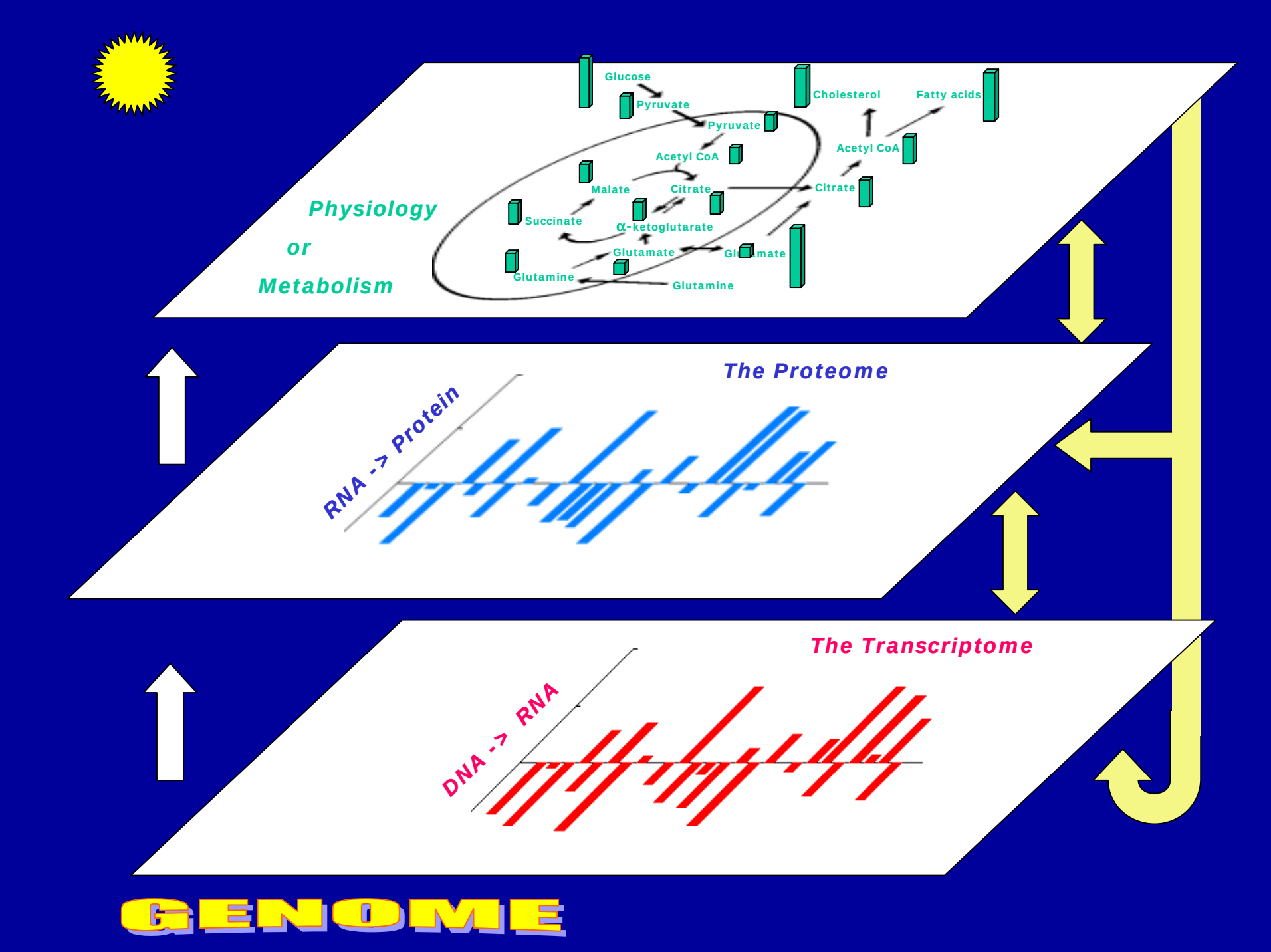
Standard errors and coefficients of variation were calculated using MetaCom (68). Errors of 10% were arbitrarily assigned to the elasticities and fluxes used in the calculation of flux control coefficients in order to calculate the sensitivity and error analysis. Values are given ± S.E.; the coefficient of variation (S.D. mean × 100) in percent is given in parentheses.

	Glucose	Glucose + ketones	Glucose + insulin	Glucose + ketones + insulin
1. Branched pathway from glucose transport to glycogen metabolism and P-glucoisomerase[a]				
$\varepsilon^{Glut4}_{Glc_i}$	−0.237	−0.516	−100	−100
$\varepsilon^{HK}_{Glc_i}$	0.159	0.248	0.164	0.224
ε^{HK}_{G6P}	−0.128	−0.232	−0.159	−0.218
ε^{PGM}_{G6P}	−1.22	12.9	3.18	100
ε^{PGM}_{G1P}	1.81	−12.5	−2.94	−100
ε^{PGI}_{G6P}	4.75	5.91	9.39	2.36
$\varepsilon^{UDPGPlase}_{G1P}$	NA[b]	100	100	100
$\varepsilon^{UDPGPlase}_{UDPGlc}$	NA[b]	−100	−100	−100
$\varepsilon^{GlycS}_{UDPGlc}$	NA	0.929	0.455	0.461
$\varepsilon^{Plase}_{G1P}$	−0.011	NA	NA	NA
C^J_{Glut4}	0.396 ± 0.34 (9.2)	0.314 ± 0.030 (9.6)	0.002 ± 0.0002 (10)	0.002 ± 0.0003 (15)
C^J_{HK}	0.590 ± 0.033 (5.6)	0.653 ± 0.029 (4.4)	0.972 ± 0.003 (0.31)	0.862 ± 0.017 (1.9)
C^J_{PGM}	<0.001	0.001 ± 0.0002 (20)	0.001 ± 0.0002 (20)	<0.001
C^J_{PGI}	0.016 ± 0.002 (13)	0.024 ± 0.003 (13)	0.016 ± 0.003 (19)	0.066 ± 0.008 (12)
$C^J_{UDPGPlase}$	NA	<0.001	<0.001	<0.001
C^J_{GlycS}	NA	0.009 ± 0.002 (22)	0.009 ± 0.002 (22)	0.069 ± 0.011 (16)
C^J_{Plase}	−0.001 ± 0.0001 (10)	NA	NA	NA

	Glucose	Glucose + ketones	Glucose + insulin	Glucose + ketones + insulin
2. Linear pathway from 3-P-glycerate kinase to pyruvate kinase[c]				
ε^{PGK}_{3PG}	−100	−100	−100	−100
$\varepsilon^{PGlyM}_{3PG}$	100	2.50	1.39	3.00
$\varepsilon^{PGlyM}_{2PG}$	−100	−1.82	−0.702	−2.24
ε^{Eno}_{2PG}	1.43	2.27	100	1.47
ε^{Eno}_{PEP}	−0.687	−1.45	−100	−0.577
ε^{PK}_{PEP}	0.859	0.929	0.981	1.23
C^J_{PGK}	0.008 ± 0.002 (25)	0.008 ± 0.001 (13)	0.008 ± 0.001 (13)	0.009 ± 0.002 (22)
C^J_{PGlyM}	0.008 ± 0.001 (13)	0.326 ± 0.036 (11)	0.575 ± 0.048 (8.3)	0.306 ± 0.31 (11)
C^J_{Eno}	0.547 ± 0.034 (6.2)	0.260 ± 0.019 (7.3)	0.004 ± 0.0004 (10)	0.466 ± 0.025 (5.4)
C^J_{PK}	0.438 ± 0.035 (8.0)	0.406 ± 0.039 (9.6)	0.412 ± 0.048 (12)	0.219 ± 0.029 (11)

[a] Scheme 1.
[b] NA, not applicable.
[c] Scheme 2.

These results illustrate that the control of the metabolic flux in glucose metabolism of rat heart is not exerted by a single enzyme but variably distributed among enzymes depending upon substrate availability, hormonal stimulation, or other changes of conditions


Physiology
or
Metabolism
Glucose
Pyruvate
Pyruvate
Acetyl CoA
Malate
Citrate
Succinate
α-ketoglutarate
Glutamate
Glutamine
Cholesterol
Fatty acids
Acetyl CoA
Citrate
Glutamate
Glutamine
The Proteome
RNA -> Protein
The Transcriptome
DNA -> RNA
GENOME