ENERGY BALANCE, METABOLISM & NUTRITION

Energy Metabolism

• the amount of energy liberated per unit time is the ***metabolic rate***
• energy liberated by catabolic processes in the body appears as,

Energy Output = Work + Stored Energy + Heat

• isotonic muscle contractions perform work at a peak efficiency **~ 50%**
• where,

Efficiency = Work / Total Energy Expended

• essentially all of the energy of ***isometric*** contractions appears as ***heat***, because little or no external work is done
• the standard unit of heat energy is the ***calorie***,

Def'n: 1 calorie is the heat energy required to raise the temperature of 1 gram of pure water, 1 degree, from 15 to 16 °C

1 cal. = 4.186 joule
= 0.738 lb.ft

→ termed the gram calorie, small calorie, or standard calorie

• too small for routine use, therefore use the ***Calorie*** (kcal) = 1000 cal.
• the energy liberated by foodstuffs can be measured by ***direct calorimetry***, measuring the heat liberated during oxidation in a bomb calorimeter
• the ***caloric values*** of the common foodstuffs are,

a. CHO ~ 4.1 kcal/g
b. fat ~ 9.3 kcal/g
c. protein ~ 5.3 kcal/g

• similar values are found for the former in the body
• however, the oxidation of ***protein*** is incomplete and the *in vivo* value **~ 4.1 kcal/g**
• the energy liberated can also be measured by ***indirect calorimetry***, measuring either the oxygen consumed, or the total end products of catabolism
• complicated as the amount of O_2 consumed varies slightly with the energy source being oxidized
• the approximate energy liberation is 4.82 kcal/l of O_2
• more accurate measurements require information on the ***respiratory quotient*** and nitrogen excretion

Def'n: ***respiratory quotient*** (RQ) = $\frac{\text{volume of } CO_2 \text{ produced}}{\text{volume of } O_2 \text{ consumed}}$ per unit time

note that this is different to the ***respiratory exchange ratio***, which the ratio of the excretion of CO_2 to the uptake of O_2 by the lung

• the RQ's for the various substrates are,

a. CHO = 1.0 - ratio of O_2:H in CHO ~ water

b. fat ~ 0.7 - extra O_2 required to form water

c. protein ~ 0.82 - varies with metabolism

• measurement of the RQ and nitrogen excretion may give an indication of substrate oxidation in the body, however this is only an approximation as other factors may influence the RQ,

a. hyperventilation → R rises due to removal of CO_2

b. exercise → RQ rises due to (a) and O_2 debt

c. metabolic acidosis → RQ rises

d. metabolic alkalosis → RQ falls

• using the Fick principle, O_2 consumptions for individual organs can be calculated and give some indication of their substrate utilisation
• whole body O_2 consumption is measured using a Benedict-Roth spirometer in a closed circuit with a CO_2 absorber (see Ganong fig. 17-1)
• O_2 consumed is corrected to STP and multiplied by **4.82 kcal/l**
• the metabolic rate is affected by many factors, the most important of which is muscular activity
• ingested foods also increase the MR, because of the obligatory energy expenditure in their assimilation into the body = ***specific dynamic action***
• the SDA required for the assimilation of 100 kcal of the major substrates is,

a. CHO ~ 6 kcal / 100 kcal

b. fat ~ 4 kcal / 100 kcal

c. protein ~ 30 kcal / 100 kcal

• the exact cause of SDA is uncertain, but may relate to,

a. increased SNS activity postprandially

b. deamination of constituent AA's from protein in the liver

c. direct stimulation of metabolism by FFA's

d. energy expended in the formation of glycogen

• the stimulating effects of food last up to 6 hrs
• another factor affecting the MR is environmental temperature
• the curve relating MR to temperature is U-shaped, with the trough at ~ 20°C
• the ***basal metabolic rate*** **BMR** is usually taken as the VO_2,

a. 12-14 hrs after the last meal

b. at complete mental & physical rest

c. in a temperature adjusted room

NB: this is not truly basal, as the VO_2 during sleep is lower

the BMR for the ubiquitous 70 kg male **~ 2000 kcal/d**

• larger animals have higher absolute BMR's, however the ratio of BMR to weight is higher in smaller animals
• body surface area provides a closer correlation with BMR
• however, the production of heat still increases at a slightly greater rate (Exp: 0.67 vs. 0.75)
• normograms are available for such estimations
• the average BMR in an adult male **~ 960 kcal/m^2/d**
• factors influencing the BMR include,

a. sex - F < M at all ages
b. age - highest in children and decreases with age
c. temperature- BMR increases 14% per °C fever
d. anxiety & tension
e. starvation - BMR falls
f. SNS & thyroid hormones

Intermediary Metabolism

• the end products of digestion are mostly AA's, fat derivatives, and the hexoses fructose, galactose and glucose
• the short-chain fragments from catabolism of these products are very similar and enter a common metabolic pool of intermediates
• from here CHO, fat and protein are synthesised, or the fragments enter the ***citric acid cycle***, in which they are completely oxidised to CO_2 & H atoms
• the H atoms are oxidised to form water in the ***flavoprotein-cytochrome system***
• the energy liberated by such oxidations is stored in high energy ester bonds between phosphoric acid residues of a number of compounds,

a. adenosine triphosphate ATP
b. guanosine triphosphate GTP (a+b = purine bases)
c. cytidine triphosphate CTP
d. uridine triphosphate UTP (c+d = pyrimidine bases)
e. inosine triphosphate ITP (hypoxanthine derivative)
f. muscle creatine phosphate

• these bonds yield **~ 10-12 kcal/mol**, in comparison to the low energy phosphate bonds of compounds like glucose-6-P ~ 2-3 kcal/mol
• another group of high energy compounds are the ***thioesters***, the acyl derivatives of mercaptans
• coenzyme A (CoA) is a common ***mercaptan***, condensation with acetic acid yielding acetyl-CoA, which has a far higher energy than acetic acid and therefore is able to participate in a number of reactions = "***active acetate***"

NB: the energy yield from acetyl-CoA ≡ ATP

Biological Oxidation

• oxidation of a compound may be achieved by any of the following,

a. combination with O_2
b. loss of hydrogen
c. loss of electrons

• a number of coenzymes serve as H-receptors, especially NAD^+ and $NADP^+$ which accept hydrogen to form NADH & NADPH
• a common example is the ***dehydrogenation*** of R-OH to R=O
• the hydrogen is then transferred to the flavoprotein-cytochrome system, reoxidizing the NAD^+ & $NADP^+$
• the ***flavoprotein-cytochrome*** system (FCS) is the chain of enzymes which transfers hydrogen to oxygen in the mitochondria
• each of the enzymes is a protein, with a non-protein prosthetic group
• the flavoprotein prosthetic group is a derivative of B complex riboflavin
• the cytochrome prosthetic group is a Fe-porphyrin complex similar to haem

Oxidative Phosphorylation

• the transfer of hydrogen from NADH to flavoprotein generates ATP and further transfer along the cytochrome chain generates a further 2 ATP per proton pair
• this production of ATP associated with oxidation → ***oxidative phosphorylation***
• the mechanism is ***chemiosmotic***, involving the transfer of protons across the impermeable inner cristae of the ***mitochondria***
• the chemical gradient is maintained by oxidation in the respiratory chain, the movement of H across the inner cristal membrane driving a reversible ATPase
• the process thus depends upon an adequate supply of ADP and this allows feedback control
• the more rapidly ATP is used, the more ADP will be available and the faster will be oxidative phosphorylation
• (see Ganong fig. 17-6 & 17-7)

CARBOHYDRATE METABOLISM

• dietary CHO is for the majority polymers of ***hexoses***, of which the most important are ***glucose***, ***galactose*** & ***fructose***
• most of the monosaccharides occurring in the body are the ***d-isomers***
• the principal product of CHO metabolism and the main circulating sugar is glucose,

→ fasting venous levels ~ 3.9-5.6 mmol/l (~ 70-110 mg/dl)

• the levels in arterial blood are 15-30 mg/dl higher

• on entering the cells, glucose is phosphorylated to G-6-phosphate by ***hexokinase***
• in the liver there is an additional, more specific enzyme, ***glucokinase*** which is increased by insulin and reduced in starvation or diabetes
• the resulting G-6-P is then either,

a. polymerized to glycogen, or
b. catabolised to CO_2 and water (see Ganong fig. 17-9)

• glycogen, the storage form of glucose, is present in most body tissues but the major stores are in liver & skeletal muscle
• glycolysis is the catabolism of glucose to lactate ± pyruvate
• this may proceed by one of two pathways,

1. the ***Embden-Meyerhof pathway*** EMP
   → breakdown to trioses, then to pyruvate
2. the ***hexose-monophosphate shunt*** HMPS (direct oxidative pathway)
   → conversion to 6-phophogluconate, then the pentoses

• pyruvate is converted to acetyl-CoA in the mitochondrion and can then enter the citric acid cycle, or be converted into fats
• through the short-chain intermediates of the EMP, interconversions between CHO, fat and protein can be achieved
• as most of these reactions are reversible, non-glucose molecules can be converted to glucose, ***gluconeogenesis***

NB: glucose can be converted to fats through ***acetyl-CoA***,
however, as this reaction is ***irreversible***,
fats are not converted to glucose through this pathway

• there is therefore very little net conversion of fats to glucose in the body because, except for the small production of glycerol, there is no pathway for conversion

Citric Acid Cycle (CAC)

• also known as the Kreb's cycle, or tricarboxylic acid cycle, is the pathway by which acetyl-CoA is catabolised to CO_2 & H-atoms
• the essential reactions of the cycle are,

a. acetyl-CoA + oxaloacetate $\rightarrow$ citrate + HS-CoA (reduced CoA)

b. citrate $\rightarrow$ seven subsequent reactions
+ 2 molecules of CO_2 regenerating oxaloacetate
+ 4 pairs of H-atoms are transferred to the FCS
$\rightarrow$ 12 ATP + $4H_2O$ (two water molecules are used)

• the CAC is the final common pathway for the oxidation to CO_2 & H_2O of CHO, fat and some amino acids
• the major entry is through ***acetyl-CoA***, though, pyruvate may also enter by taking up CO_2 to form oxaloacetate
• the CAC requires the presence of O_2 and ***does not*** function anaerobically
• glycolysis to pyruvate occurs outside the mitochondrion
• the conversion of pyruvate to acetyl-CoA $\rightarrow$ CO_2 + $2H^+$ and therefore another 3ATP

Energy Production

- ***Anaerobic***

• metabolism of glucose or glycogen to pyruvate depends upon whether the EM pathway, or the HMPS is used
• the two reactions generating ATP are,

a. *3-phosphoglycerate $\rightarrow$ phosphoglycerate + ATP

b. phophoenolpyruvate $\rightarrow$ pyruvate + ATP

$\rightarrow$ 1 mol of 3-PG $\rightarrow$ 2 mol ATP

• each mol of glucose produces 2 mol of 3-PG via the EMP, but only 1 mol of 3-PG by the HMPS
• however, in the EM pathway, 1 mol of ATP is used to phosphorylate glucose when it enters the cell, and another mol in the conversion of fructose-6-phosphate to fructose-1,6-diphosphate
• therefore, under ***anaerobic*** conditions, oxidation of,

a. blood glucose $\rightarrow$ 2 mol ATP

b. glycogen $\rightarrow$ 3 mol ATP

• reaction (a) above* requires a supply of NAD^+ to accept protons, this is normally regenerated under aerobic conditions by oxidative phosphorylation
• under anaerobic conditions the reaction is allowed to proceed as NAD^+ is regenerated by the reaction,

pyruvate + NADH + H^+ $\rightarrow$ ***lactate*** + NAD^+

• this allows anaerobic metabolism to continue with the continuing production of lactate until the O_2 supply is restored

- ***Aerobic***

• the production of ATP is 19 times that produced anaerobically [2:38]
• the net production of ATP via the EM pathway is as follows,

Reaction			Product	Net ATP	
glucose	→	2 pyruvate	4ATP	2	mol
3-PG	→	phosphoglycerate	+ NADH[1]	2 x 3	mol
pyruvate	→	acetyl-CoA	+ NADH[1]	2 x 3	mol
acetyl-CoA	→	the CAC	+ 8H[1]	2 x 12	mol
			Total	**38 mol**	

[1] each mol of glucose results in 2 mol of substrate

• oxidation via the ***HMPS*** produces large amounts of NADPH, which is an essential coenzyme for a number of reactions
• the pentoses formed are the precursors of the ***nucleotides***
• the amount of ATP generated depends upon the amount of NADPH converted to NADH and oxidised to CO_2 & water

Regulation of Intermediary Metabolism

• most of the reactions of intermediary metabolism are freely reversible, however there are a number of "directional flow valves", reactions which proceed in one direction under the influence of one enzyme and in the other direction under the influence of another (see Ganong fig. 17-12)
• these include,

Catabolism	Anabolism
phosphorylase	glycogen synthase
hexokinase/glucokinase	glucose-6-phosphatase[1]
phosphofructokinase	fructose-1,6-diphosphatase
pyruvate kinase	phosphoenolpyruvate carboxykinase

[1] liver only

Phosphorylase

• glycogen is synthesised from G-1-phosphate, via ***uridine-diphosphoglucose***, by the enzyme ***glycogen synthase***
• cleavage of the straight chain, 1-4α-linkage is catalyzed by ***phosphorylase***
• phosphorylase activity in the liver is increased by the action of adrenaline on β_2 receptors (see Ganong fig. 17-14)
• this activates cAMP → protein kinase → phosphorylase kinase
→ phosphorylase → ***glycogenolysis***

• activation of ***cAMP-dependent protein kinase*** not only enhances glycogenolysis, but also inhibits glycogen synthesis as glycogen synthase is inactive in the phosphorylated form
• glycogenolysis is also increased by CA action on α-receptors; this is mediated by a phosphoinositol and a **Ca^{++}-*dependent phosphorylase kinase***, independent of the action of cAMP
• large doses of angiotensin II & ADH can also activate this later system

Glucose-6-Phosphatase

• this enzyme is present in the ***liver*** but not in skeletal muscle
• it catalyses the conversion of G-6-P to glucose and hence allows the ***exit*** of glucose from cells
• in skeletal muscle, where this enzyme is absent, G-6-P enters the EM pathway and HMPS with subsequent conversion to pyruvate ± lactate
• therefore, skeletal muscle glycogen is ***not*** a source of plasma glucose
• adrenaline activates phosphorylase in both the liver and skeletal muscle, hence causes an increase in both plasma glucose & lactate concentrations
• glucagon activates liver phosphorylase only, thus elevates plasma glucose and not lactate

McArdle's Disease

• also known as myophosphorylase deficiency glycogenesis
• due to a deficiency of ***muscle phosphorylase***, glycogen accumulates and patients develop muscle pain and stiffness, especially on exertion
• they have a greatly reduced exercise tolerance
• however, they show a normal rise in plasma glucose to adrenaline or glucagon, indicating their hepatic phosphorylase is intact

Hepatic Handling of Glucose

• the liver effectively act as a "glucostat" having profound control over plasma glucose levels
• see notes on endocrine pancreatic function and CHO metabolism

Renal Handling of Glucose

• glucose is freely filtered at the glomerulus, however at normal serum levels is completely reabsorbed
• the renal Tm for glucose is ~ 375 mg/min for males and ~ 300 mg/min for females
• the former is ~ to a plasma glucose level of 180-200 mg/100ml
• this compares with a maximal intestinal absorption of ~ 120 g/hr!

Determinants of Plasma Glucose Concentration

• the blood glucose at any given time is the algebraic sum of,

a. intestinal absorption
b. entry into cells - brain, muscle, adipose and other tissues
c. the glucostatic function of the liver

• the fate of ingested glucose in the resting state is approximately,

a. ~ 5% of the converted into glycogen in the liver
b. ~ 30-40% into fat
c. the remainder is metabolized by muscle and other tissues

• during fasting, ***glycogenolysis*** in the liver adds glucose to the bloodstream
• with more prolonged fasting, ***gluconeogenesis*** from amino acids and glycerol occurs in the liver, and to a small extent in the kidney
• the is a moderate decrease in the plasma glucose levels to ~ 65 mg/dl (M) and 40 mg/dl (F), but more severe hypoglycaemia is prevented

CHO Homeostasis in Exercise

• in a 70 kg male, stored CHO amounts to ~ 1900 kcal,

a. 350 g of muscle glycogen
b. 85 g of liver glycogen
c. 20 g of extracellular glucose

• this contrasts to fat, which constitutes 80-85% of the bodies stored fuel supply ~ 140,000 kcal
• resting skeletal muscle utilizes fatty acids for energy requirements
• in the fasting human at rest, the ***brain*** accounts for ~ 70-80% and the RBC's the remainder of the basal glucose consumption
• during exercise, the caloric needs of muscle are initially met by breakdown of muscle glycogen and increased uptake of glucose from plasma
• the blood glucose initially rises with an increase in hepatic glycogenolysis but this may fall with prolonged exercise and there is a switch to hepatic gluconeogenesis as hepatic glycogen is depleted
• the plasma level of insulin falls and glucagon rises
• after exercise, gluconeogenesis continues and liver glycogen is replenished
• plasma insulin levels rise, especially in hepatic portal blood and exit of glucose from the liver is prevented, aiding liver glycogenesis

Metabolism of Other Hexoses

• ***galactose*** is liberated from the digestion of lactose and, after phosphorylation, is converted to uridine-diphosphogalactose
• this later metabolite is then converted to uridine-diphosphoglucose, which is used for ***glycogenesis***
• this second reaction is freely reversible, and in this way the galactose necessary for the formation of glycolipids and mucoproteins is formed when the dietary intake of galactose is insufficient
• the utilisation of galactose is ***insulin dependent***, as for glucose
• ***galactosaemia*** is an inborn error of metabolism, where the enzyme phosphogalactose uridyl transferase is deficient, resulting in an excessive accumulation of galactose in the circulation

• ***fructose*** is in part converted to F-6-phosphate, then F-1,6-diphosphate which enters the EM pathway
• the first reaction is catalysed by hexokinase, as for glucose; however, a larger amount of the fructose is converted to F-1-P by ***fructokinase***
• the majority of F-1-P is cleaved into dihydroxyacetone-P and glyceraldehyde
• the later is phosphorylated and both fragments enter the EM pathway
• since these reactions through F-1-P can proceed in the ***absence*** of insulin, fructose has been recommended for the replacement of CHO in diabetics
• however, most of the fructose is metabolized in the intestines and liver, little reaching the peripheral tissues

• F-6-P can be phosphorylated in the 2-position to ***fructose-2,6-diphosphate***
• this is an important regulator of hepatic gluconeogenesis
• F-2,6-diP facilitates the reaction F-1-P $\rightarrow$ F-1,6-diP and thus enhances the breakdown of glucose to pyruvate
• low levels of F-2,6-diP facilitate the reverse reaction and thus enhance gluconeogenesis

NB: this is one of the actions of the ***glucagon***-dependent protein kinase,
to ***decrease*** hepatic F-2,6-diP $\rightarrow$ ***gluconeogenesis***

PROTEIN METABOLISM

Proteins

• these are formed from the linkage of amino acids by ***peptide bonds***
• where the carboxyl group of one protein is joined to the amino group of the next
• some proteins contain CHO, ***glycoproteins***, while others contain lipids, ***lipoproteins***
• smaller chains of AA's are termed either peptides (2-10 AA's), or polypeptides (10-100 AA's), but the boundaries are somewhat arbitrary
• proteins are complex molecules and their structures subdivided into,

a. primary structure = the AA sequence
b. secondary structure = spatial twisting & folding
c. tertiary structure = arrangement of twisted chains
d. quaternary structure = applies to proteins with subunits

Amino Acids (AA's)

• there are **24 AA's** found in proteins, though, there are only tRNA's for 20 of these, the other four being formed by post-transcriptional modification
• (see Ganong table 17-3)
• there are other important AA's not found in proteins, these include,

a. ornithine
b. 5-HT
c. l-DOPA
d. taurine
e. thyroxine (T_4)

• in the higher animals the ***l-isomers*** are the only naturally occurring form and for those that act as hormones are significantly more active than the d-isomers

■ ***The Amino Acid Pool***

• small amounts of protein are absorbed from the GIT of infants and even less in adults
• most ingested proteins are digested and their constituent AA's absorbed
• the bodies own proteins are continually being hydrolyzed and resynthesised
• the turnover of endogenous protein is ~ 80-100 g/d, being highest in the intestines and lowest in collagen
• these two sources contribute AA's to a common pool from which the bodies needs are met
• AA's are freely filtered at the glomerulus but are normally almost totally reabsorbed in the proximal tubules
• the Fanconi syndrome, and other proximal tubule disorders may result in the urinary excretion of large amounts of AA's

Specific Metabolic Functions of Amino Acids

• methionine, cysteine, and cystine provide ***sulphur*** necessary for various proteins, HS-CoA, taurine and others
• methionine is converted to ***S-adenosylmethionine*** which a the major active donor of biologically active methyl groups
• another source of active methyl groups is from folic acid derivatives and cyanocobalamin (see N_2O induced megaloblastic anaemia & marrow depression)

Urinary Sulphates

• S-containing AA's are the source of urinary sulphate
• some nonoxidised S-containing compounds are excreted in the urine as neutral sulphur
• however the vast majority are excreted as $SO_4^{=}$ accompanied by corresponding of cation
• the ethereal sulphates in the urine are organic sulphate esters, $R\text{-}O\text{-}SO_3H$ formed in the liver from endogenous and exogenous phenols
• the later include oestrogens, other steroids, indoles and drugs

Deamination, Amination & Transamination

• interconversions between AA's and the short chain products of CHO and fat catabolism at the level of the common metabolic pool & the CAC, involve the formation, removal and transfer of amino groups
• transamination reactions involve the following,

$$AA_1 + \alpha\text{-keto-}A_2 \rightarrow \alpha\text{-keto-}A_1 + AA_2$$

• these occur in many tissue and transaminases are also present in the circulation
• however, during tissue injury serum transaminase levels often rise, eg. SGOT after an AMI
• oxidative deamination of AA's occurs in the liver as follows,

$AA + NAD^+$	$\rightarrow$	imino acid + NADH	(dehydrogenation)
$IA + H_2O$	$\rightarrow$	α-keto-A + NH_4^+	(hydrolysis)

• AA's can also take up NH_4^+ forming the corresponding amide
• an example is the binding of NH_4^+ in the brain by glutamine with the reverse reaction occurring in the renal tubules
• interconversions with the common metabolic pool → Ganong fig. 17-22
• leucine, isoleucine, tyrosine and phenylalanine are ***ketogenic*** as they are converted to ***acetoacetate***

▪ *Urea Formation*

• most of the NH_4^+ formed by deamination in the liver is converted to urea
• excepting the brain, the ***liver*** is probably the only site of urea synthesis, and this is achieved through the urea cycle, involving,

a. ornithine + $\mathbf{NH_4^+}$ → citrulline (in mitochondrion)
b. 2 step conversion → arginine
c. arginine → ornithine + ***urea***

• in severe hepatic disease urea synthesis falls and the serum NH_3 levels rise with ensuing nervous system dysfunction

▪ *Creatine & Creatinine*

• ***creatine*** is synthesised in the liver and phosphorylated in skeletal muscle to ***phosphorylcreatine*** by ATP
• this then acts as a high energy phosphate reserve during exercise
• the creatinine in the urine is formed from phosphorylcreatine; there being no direct conversion from creatine
• daily production of creatinine is relatively constant for a given individual and thus renal excretion is a measure of GFR
• creatinuria occurs normally in children, pregnancy, and rarely in nonpregnant women
• abnormal creatinuria occurs in,

a. starvation
b. thyrotoxicosis
c. poorly controlled diabetes mellitus
d. various primary & secondary myopathies

Purines & Pyrimidines

• the functionally important members of these two groups are,

a. purines
- adenine
- guanine
- hypoxanthine
- xanthine

b. pyrimidines
- cytosine
- uracil
- thymine

• ***nucleosides***, combinations of these compounds with ***ribose***, are the precursors of a number of coenzymes and related substances,

a. NAD^+, $NADP^+$, ATP, UDPG, etc

b. RNA and DNA

• nucleic acids in the diet are digested and their component purines & pyrimidines are absorbed
• however, most of the bodies requirement is met by *de novo* synthesis from AA's in the liver
• RNA is in dynamic equilibrium with the AA pool, however, DNA is stable throughout life
• excess amounts are catabolised,

a. purines $\rightarrow$ ***uric acid***

b. pyrimidines $\rightarrow$ CO_2 and NH_3

• minor amounts are excreted unchanged in the urine

Uric Acid

• formed from the breakdown of ***purines*** and by direct synthesis from 5-phosphoribosyl pyrophosphate (5-PRPP) and glutamine
• in humans, uric acid is excreted in the urine (mammals $\rightarrow$ allantoin)
• normal SUA ~ 4 mg/dl (~ 0.24 mmol/l)
• uric acid is filtered, secreted and reabsorbed by the kidney
• 98% of the filtered load is reabsorbed, the remaining 2% constitutes ~ 1/5 of the excreted mass, the remainder coming from tubular secretion

Primary & Secondary Gout

• there are two forms of primary gout,

a. increased production due to enzyme abnormalities
b. decreased renal tubular secretion

• in secondary gout the increased SUA is the result of either increased production or decreased excretion due to another process, eg,

a. thiazide diuretics → decreased secretion
b. renal disease → decreased secretion
c. leukaemia → WBC breakdown increased SUA
d. pneumonia → WBC breakdown increased SUA

• ***cholchicine*** does not affect UA metabolism but appears to prevent leukocyte phagocytosis and degranulation
• ***phenylbutazone*** and ***probenacid*** decrease proximal tubular UA reabsorption
• ***allopurinol*** inhibits xanthine oxidase, normally responsible for the conversion of xanthine to uric acid

Nitrogen Balance

• loss of protein and its derivatives in the stools is very small, thus the excretion of nitrogen in the urine is a reliable indicator of irreversible protein and AA breakdown
• when urinary nitrogen is equal to the nitrogen intake in the diet , the individual is said to be in nitrogen balance
• nitrogen balance is negative with,

a. increased secretion of the catabolic hormones of the adrenal cortex
b. decreased secretion of insulin
c. starvation
d. absence of an essential AA from the diet
e. forced immobilisation

• nitrogen balance is positive with,

a. normal growth
b. recovery from severe illnesses
c. administration of anabolic steroids

Essential Amino Acids

• in humans, nitrogen balance is not maintained unless 8 AA's, the essential amino acids, are added to the diet,

i. phenylalanine
ii. tryptophan
iii. lysine
iv. leucine
v. isoleucine
vi. valine
vii. threonine
viii. methionine

• ***histidine & arginine*** are not essential for nitrogen balance, however they are essential for normal growth
• when any one AA required for the synthesis of a protein is absent, protein synthesis is ceased and the remaining AA's are deaminated and excreted as urea nitrogen
• thus, nitrogen balance becomes negative whenever an essential AA is absent from the diet

Response to Starvation

• a low protein, adequate calorie diet leads to,

a. a decrease in the excretion of ***urea***, inorganic and ethereal ***sulphates***
b. ***uric acid*** excretion falls by ~ 50%
c. however, creatinine excretion is unaffected

• thus, the urinary creatinine and ~ 50% of the normal uric acid must result from tissue breakdown unaffected by protein intake
• under these conditions, nitrogen excretion is up to ~ 3.6 g/d, as a result of essential AA absence
• if the diet is inadequate in calories as well, excretion may reach 10 g/d, as proteins are catabolised for energy
• small amounts of glucose counteract this effect, the protein sparing effect of glucose
• due mostly to the increased secretion of ***insulin***, with resulting decreased protein breakdown
• fats also spare nitrogen, keto-acids being used by the brain and other tissues
• the branched chain AA's, leucine, isoleucine and valine share cofactors for metabolism with the short chain products of fat catabolism; thus, these AA's are spared by fat administration
• most of the protein burned during total starvation comes from the muscles, liver and spleen
• negligible amounts coming from the heart & brain
• blood glucose falls a little after depletion of liver glycogen, but is then maintained by ***gluconeogenesis***
• ***ketosis*** is present, and neutral fat is rapidly catabolised

FAT METABOLISM

Lipids

• the biologically important fats are the,

a. triglycerides - TG, or neutral fats
b. phospholipids - PL and related compounds
c. sterols

• the TG's are made up of 3 ***fatty acids*** bound to ***glycerol***
• naturally occurring fatty acids contain ***even*** numbers of carbon atoms, and may be either saturated, or unsaturated by dehydrogenation with various numbers of double bonds

■ ***Fatty Acid Oxidation & Synthesis***

• FA's are broken down to ***acetyl-CoA*** which then enters the citric acid cycle
• FA oxidation begins with activation of the acid, which occurs both inside and outside the mitochondrion
• active FA's move across the mitochondrial membrane in a process which requires the lysine derivative ***carnitine***, which greatly stimulates FA oxidation
• the remaining steps occur in the mitochondrion and 2-carbon fragments are sequentially split from the molecule, ***beta-oxidation***, with the formation of NADH + H^+ and acetyl-CoA
• the energy yield is great, β oxidation of 1 mol of a 6-C FA $\rightarrow$ 44 mol ATP, compared with 6-C glucose $\rightarrow$ 38 ATP

• many tissues can synthesise FA's from acetyl-CoA and long chain FA synthesis can occur in the mitochondrion by direct reversal of β oxidation
• however, most FA synthesis occurs outside the mitochondrion from acetyl-CoA by a different pathway is microsomes, utilizing acyl carrier protein
• FA synthesis stops in virtually all cells when the chain length $\rightarrow$ 16-C
• only small amounts of 12 & 14-C FA's are made and none > 16-C
• the combination of FA's with glycerol to form neutral fats occurs in the mitochondria

■ ***Ketone Bodies***

• in many tissues acetyl-CoA molecules condense to form ***acetoacetyl-CoA***
• the liver possesses ***deacylase*** and free ***acetoacetate*** is formed
• this β-keto acid is then converted to b-***hydroxybutyrate*** and ***acetone***
• these two are metabolised poorly and diffuse into the circulation
• together with acetoacetate → ***ketone bodies***

• acetoacetate is also formed from β-hydroxy-β-methylglutaryl-CoA, HMG-CoA, and this is quantitatively more significant
• tissues ***other*** than the liver transfer CoA from succinyl-CoA to acetoacetate and metabolise the "active" acetoacetate to CO_2 & H_2O via the citric acid cycle
• ketones are normally metabolised as rapidly as they are formed
• therefore, serum concentrations are low ≤ 1 mg/dl
• acetyl-CoA accumulates and conversion to ketone bodies in the liver increases if,

1. the entry of acetyl-CoA into the CAC is depressed due to a decreased supply of the products of glucose metabolism, or
2. the entry does not increase when acetyl-CoA concentrations rise

• the capacity of tissues to oxidise ketones is soon exceeded → ketosis
• acetoacetate & β-hydroxybutyrate are anions of moderately strong acids and their buffering in plasma is exceeded in a number of conditions resulting in a metabolic acidosis
• 3 conditions lead to deficient ***intracellular glucose*** supplies,

1. starvation
2. diabetes mellitus
3. a high fat - low CHO diet

• the later is due to the absence of a quantitatively significant path for the conversion of fat to glucose
• small amounts of glucose will abolish this ketosis and glucose is antiketogenic

■ ***Cellular Lipids***

• the lipids in cells are of two main types, structural lipid & neutral fat
• depot fat is metabolised during starvation but structural lipid remains intact
• storage fat is not stable but continually being recycled
• in storage sites glucose is metabolised to FFA's and neutral fats are synthesised
• neutral fats are broken down and FFA's released into the circulation

■ ***Brown Fat***

• a third and different type of lipid makes up a small percentage of total body fat
• levels are somewhat higher in ***infants***, being located between the scapulas, at the nape of the neck, and along the great vessels in the thorax and abdomen
• the differences from normal adipocytes are,

a. these fat cells have a SNS innervation
b. multiple small droplets of fat, c.f. one large droplet
c. contain abundant mitochondria
d. the mitochondria posses a second proton conductance which does not generate ATP

• this later mechanism is associated with a 32,000 MW membrane protein and effectively uncouples metabolism and ATP production, resulting in the generation of heat

→ ***non-shivering thermogenesis***

• SNS tone acts via NA and b_1-***adrenergic*** receptors, increasing lipolysis, FA metabolism and heat production
• brown fat is believed to function in this way in two situations,

a. adaptation to cold
b. after eating, increasing heat production

• thus, there are two components of heat production after eating,

1. the prompt specific dynamic action of food, and
2. the slower increase in brown fat thermogenesis

■ ***Plasma Lipids & Transport***

• the major lipids in the plasma do not circulate in the free form

a. FFA's are bound to plasma albumin
b. triglycerides, phospholipids and cholesterol are transported in ***lipoprotein*** complexes

• there are six families of lipoproteins (Ganong tab. 17-7 & fig. 17-31)
• there general structure is a hydrophobic core composed of TG's and cholesterol esters, surrounded by PL's and protein
• the protein constituents are ***apoproteins***, types B-48, B-100, E & C
• ***chylomicrons*** are formed in the intestinal mucosa during absorption of the products of fat digestion and they enter the circulation via the lymphatic ducts
• these are cleared from the circulation by the action of ***lipoprotein lipase***, which is located on the surface of the endothelium of capillaries and catalyses the breakdown of TG's in the chylomicrons to FFA's and glycerol
• these products then enter adipocytes and are reesterified; some FFA remains in the circulation bound to albumin
• lipoprotein lipase, which requires ***heparin*** as a cofactor, also removes TG from VLDL

• both chylomicrons and VLDL contain apoprotein C, which is a complex, one component of which is apolipoprotein CII, and this activates lipoprotein lipase
• chylomicrons depleted of their TG remain in the circulation as cholesterol rich chylomicron remnants, which bind to liver receptors, are internalised by receptor-mediated endocytosis and degraded in lysosomes
• these systems transport exogenous lipids
• endogenous TG's and cholesterol are transported throughout the body by VLDL, IDL, LDL and HDL
• VLDL formed in the liver transports TG to the tissues where this is largely removed by the action of lipoprotein lipase, forming IDL
• IDL provides phospholipids, and through the action of ***lethecin-cholesterol acyl transferase***
• LCAT picks up cholesterol esters formed in HDL
• this IDL may then either return to the liver and be internalised, or remain in the circulation, lose more TG & protein and become LDL
• LDL provides cholesterol to the tissues, where it forms an integral part of cell membranes and is used by gland cells in hormone synthesis
• LDL is taken up by the liver and most extrahepatic tissues by receptor mediated endocytosis, after which it is broken down in lysosomes
• the released cholesterol has a number of actions,

a. inhibits cellular cholesterol synthesis by inhibiting HMG-CoA reductase
b. stimulates esterification of any excess cholesterol, and
c. inhibits synthesis of new LDL receptors

• there is also a lower affinity uptake of LDL in macrophages and some other cells
• when serum LDL levels are elevated, the "foamy macrophages" seen in atherosclerotic lesions may be formed
• cholesterol leaving cells is absorbed into HDL, lipoproteins synthesised in the liver and intestine, the chief function of which is cholesterol exchange and esterification
• HDL is the source of the LCAT above which provides the cholesterol esters that are transferred to IDL and thence to LDL

Free Fatty Acid Metabolism

- FFA's are transported to the tissues by chylomicrons and VLDL
- the are also synthesised in adipocytes where they are stored
- they circulate bound to plasma ***albumin*** and are the major energy source of most tissues
- they are used extensively by the heart, ~ 55-60% basal O_2 consumption
- probably all tissues, including the brain, can oxidise FFA's to CO_2 & H_2O
- the supply of FFA's is regulated by 2 enzymes;

a. lipoprotein lipase → TG & glycerol from neutral fat

b. hormone-sensitive lipase → intracellular liberation of FFA

- the later is converted from an inactive form via a cAMP dependent protein kinase
- this is activated by both ***glucagon*** and circulating ***catecholamines*** (β_1)
- GH, glucocorticoids and T_3 produce a slower increase in hormone sensitive lipase by a slower mechanism which requires protein synthesis
- GH produces a protein which enhances CA increases of cAMP
- cortisol produces a protein which enhances the action of cAMP
- ACTH, TSH, LH, ADH, and serotonin can also increased cAMP dependent lipolysis, but their role in physiological regulation is uncertain

- insulin and PGE_2 decrease the formation of hormone sensitive lipase, possibly by inhibiting the formation of cAMP

Cholesterol Metabolism

- cholesterol is the precursor of the ***steroid hormones*** and ***bile acids***, and is an essential component of cell membranes
- found only in animals, related sterols are found in plants but these are not normally absorbed from the GIT
- most dietary cholesterol is contained in egg yolks and animal fat
- after absorption across the intestine, chylomicrons are formed in mucosa and transported in the lymph to the thoracic duct
- TG is removed from chylomicrons in the tissues and the cholesterol laden remnants are absorbed by the liver
- the liver and other tissues also synthesise cholesterol
- some of the cholesterol in the liver is secreted in the bile, both in the free form and as bile acids, most of which is then reabsorbed in the terminal ileum
- most of the cholesterol in the liver is incorporated into VLDL
- cholesterol feeds-back inhibiting its own synthesis, by inhibiting the conversion of β-hydroxy-β-methylglutaryl-CoA to ***mevalonic acid***; 6 molecules of which condense to form ***squalene***, which is then hydroxylated and converted to cholesterol
- the feedback compensation is incomplete, in that a diet low in cholesterol and saturated fats leads to a modest decline in plasma cholesterol

• the plasma cholesterol level is ***decreased*** by,

a. thyroid hormones, which decrease LDL receptors

b. oestrogens, which increase hepatic catabolism of LDL (rats)

• the plasma cholesterol level is ***increased*** in,

a. untreated diabetes mellitus

b. biliary obstruction

• if bile acid reabsorption in the terminal ileum is decreased by resins, such as cholestyramine, more cholesterol is diverted to bile acid formation
• however, the plasma level drops minimally as there is a compensatory increase in liver cholesterol synthesis
• if inhibitors of ***HMG-CoA reductase***, such as compactin or mevinolin, are given concurrently, then there is an appreciable fall in the plasma cholesterol and an increase in LDL receptors

■ ***Relationship To Atherosclerosis***

• of the cholesterol in the body, ~ 93% is within the cells and only ~ 7% in the plasma
• familial hypercholesterolaemia can be caused by a number of defects in cholesterol metabolism and leads to rapidly advancing athersclerosis
• further, recent studies have demonstrated that lowering the plasma cholesterol in normal individuals slows the development of atherosclerosis and lowers the incidence of AMI's & CVA's
• the plasma concentrations of LDL & HDL also need to be considered, as elevations of the former are associated with more severe disease, whereas elevations of HDL are associated with a lower incidence of complications
• HDL levels are elevated in individuals who exercise and in those who drink 1-2 alcoholic drinks per day
• HDL levels are decreased in the obese, sedentary individuals and in smokers
• elevated ***LDL*** and ***chylomicron remnant*** levels predispose to athersclerosis
• whereas chylomicron and VLDL levels have ***no*** association

■ ***Essential Fatty Acids***

• animals fed a fat free diet fail to grow, develop skin and kidney lesions and become infertile

1. ***linolenic acid*** restores normal growth, and
2. ***linoleic & arachidonic acids*** restore all abnormalities

• thus these three acids are termed essential FA's
• dehydrogenation of FA's does occur in the body but there appears to be no means of synthesis of the double bond arrangements found in these acids

■ ***Prostaglandins***

• FA's are the precursors of PG synthesis; a series of closely related 20-C unsaturated FA's containing a ***cyclopentane ring***
• they are divided into groups depending upon the configuration of the cyclopentane ring
→ PGA, PGD, PGE, PGF, PGG, PGI (Ganong fig. 17-35)
• the number of double bonds in the side chains is given by the subscript
• PG's are synthesised via endoperoxides (PGG_2) from arachidonic acid and other essential FA's
• after conversion to ***endoperoxide*** **PGG_2**, there are 3 pathways,

a. to prostacycline PGI_2
b. to prostaglandins PGE_2, PGF_2 and PGD_2
c. to thromboxanes A_2 and B_2

• arachidonic acid is also converted to 5-HPETE and the ***leukotrienes***
• four of the leukotrienes are ***aminolipids*** which contain AA's
• the steroidal anti-inflammatory agents such as cortisol inhibit the release of arachidonic acid from phospholipid stores, thus block the synthesis of both the PG's and leukotrienes
• the NSAID's, such as aspirin and indomethacin, inhibit ***cyclooxygenase***, leaving the leukotriene pathway intact
• all of these agents have short plasma half-lives and are inactivated in a number of tissues
• therefore they act as ***local hormones***
• the leukotrienes are probably mediators of allergic and inflammatory responses, being released by Ag-IgE mediated mast cell degranulation →

a. vasoconstriction
b. bronchoconstriction
c. increased vascular permeability, and
d. PMN chemotaxis

• thromboxane A_2 is synthesised in platelets and promotes platelet aggregation & vasoconstriction
• thromboxane B_2 is predominantly a metabolite of TXA_2
• ***prostacyclin***, PGI_2, is synthesised by endothelial and smooth muscle cells in arterial walls
→ inhibits platelet aggregation and promotes vasodilation

• small doses of aspirin inhibit platelet cyclooxygenase without significant effects on the formation of prostacyclin by vessels

• other effects of the PG's include,

a. modification of RBC plasticity
b. decrease gastric acid secretion and promote ulcer healing
c. cause luteolysis and regulate the female reproductive cycle
d. modify pituitary response to hypothalamic hormones
e. induction of labor near term
f. stimulate renin secretion and regulate medullary blood flow
g. mimic the effects of ACTH, TSH and other hormones
h. others

• interestingly cAMP is involved in most of the actions of the PG's; and they may act by adjusting the generation of cAMP to a variety of stimuli

NUTRITION

• the essential dietary components include,

a. water
b. adequate calories
c. protein
d. fat
e. minerals & vitamins

Caloric Intake & Distribution

• caloric intake must be sufficient to support the BMR plus additional energy expenditure
• intake of less than this amount results in protein and fat catabolism, whereas excesses result in obesity
• a daily protein intake of 1g/kg body weight is considered desirable to supply the 8 essential AA's and others
• the source of the protein is important,

a. grade I protein - animal protein, minimal plant
   - AA ratios ~ requirement
b. grade ii protein - most plant proteins
   - AA ratios differ & lack 1 or more essential AA's

• the caloric values of the foodstuffs are,

a. CHO ~ 4.1 kcal/g
b. fat ~ 9.3 kcal/g
c. protein ~ 5.3 kcal/g(*in vivo* ~ 4.1 kcal/g)

• thus fat is the most compact form of calories
• evidence suggests that a high unsaturated:saturated fat ratio is of value in the prevention of atherosclerosis
• provided the needs for essential FA's are met, the dietary intake may be very low without ill effects, and may in fact be desirable
• CHO is the cheapest form of calories and in the average "Western" diet, approximate proportions are,

a. CHO ~ 50%
b. fat ~ 35%
c. protein ~ 15%

• when calculating caloric requirements it is usual to meet the protein requirements first, then split the remaining calories between CHO & fat

Mineral Requirements			
sodium	potassium	calcium	iron
phosphorus	zinc	magnesium	iodine

Trace Elements			
arsenic	manganese	chromium	cobalt
copper	fluorine	molybdenum	nickel
selenium	silicon	vanadium	

Vitamins

Def'n: the term vitamin has come to refer to any organic dietary constituent necessary for life, health and growth, which ***does not*** function by supplying ***energy***

• most vitamins have important functions in intermediary metabolism, or in the special metabolism of various organ systems
• they are divided into,

a. fat soluble vitamins — A, D, E, and K
b. water soluble vitamins — B-group and C

• the fat soluble group are poorly absorbed in the absence of bile or pancreatic lipase, and require the intake of some dietary fat for their absorption
• very large doses of the ***fat soluble*** group are toxic, the ***hypervitaminoses***,

a. Vit.A → anorexia, headache, hepatosplenomegaly
dermatitis, hair loss, bone pain and hyperostosis
b. Vit.D → weight loss, ectopic calcification, renal disease
c. Vit.K → anaemia and GIT disturbances

• large doses of the water soluble group are less likely to cause problems as they are rapidly eliminated through the kidney
• however, megadoses of pyridoxine B_6 can cause a peripheral neuropathy

PARENTERAL NUTRITION

NB: whenever possible the route of dietary support should be enteral

- ***Indications for TPN***

1. patients unable to eat or absorb normally
2. in the well nourished who are temporarily unable to eat
3. patient's with Crohn's disease, intestinal fistulas, & pancreatitis
4. subjects in prolonged coma
5. nutritional support in patients with marked hypercatabolism or protein loss, ie. severe trauma & burns even if some oral intake is possible
6. nutritional support during therapy for malignant disease
7. occasionally, in malnourished patients likely to undergo surgery

Nutritional Requirements During TPN

- ***Energy & Fluid***

• the Harris-Benedict equation provides a good guide to total energy requirements,

a. For Men
   - Energy (kcal/24h) = 66.473 + (13.7516 x kg.wt) + (5.0033 x cm.ht) - (6.775 x age)

b. For Women:
   - Energy (kcal/24h) = 655.0955 + (9.5634 x kg.wt) + (1.8496 x cm.ht) - (4.6756 x age)

• these predict the requirements for weight maintenance in afebrile patients and there are a number of exceptions,

a. for weight increase → 30% increase
b. for septic patients → 30% increase
c. burns patients > 40% → ~ 100% increase

• in general, ~ 32 kcal/kg/d is sufficient for weight maintenance, and
~ 40 kcal/kg/d is sufficient for weight gain or septic patients

• basal fluid infusion should ~ 1-1.2 ml/kcal, plus the volume of any losses from diarrhoea, stomal losses, fistula drainage, N-G suction etc.
• in oliguric patients ~ 750-1000 ml, plus volume of urine output and other losses
• in patients with cardiac failure ~ 40 ml/kg can be infused providing sodium is restricted to 20-40 mmol/d

Amino Acids

• normal function requires visceral & musculoskeletal integrity, plus normal levels of enzymes, hormones and plasma proteins
• all of these are dependent upon new protein synthesis and provision of adequate AA's is a major objective of TPN
• although the requirement is influenced by a number of factors, nitrogen balance and protein synthesis are proportional to the amount of AA infused between the range 0-2 g/kg body weight/d
• the pattern is important as unbalanced mixtures do not support protein synthesis
• enrichment of mixtures with branch-chain AA's or keto-acids may aid protein synthesis in septic patients
• AA's are more efficiently utilised when infused with adequate nonprotein energy to meet caloric requirements
• a positive nitrogen balance is achieved in most malnourished patients byinfusing 0.5-1.0 g/kg ideal body weight of AA, together with optimal nonprotrein calories
• as the input of nonprotein energy is increased, nitrogen retention is augmented at all levels of AA intake, the most marked effects seen between the range of zero calories and an amount = the BMR
• beyond 50-60 kcal/kg, additional calories do not significantly improve nitrogen balance

Relation of Nitrogen Retention to Nonprotein Energy

• both CHO and lipids can be used and are of equal efficacy in malnourished or septic patients after an initial 3-4 day period of adaptation to the energy source
• thus, the factors governing the choice of calories are other than the effects on nitrogen balance,

a. osmotic pressure
b. CHO requirement for insulin
c. CHO may increase BMR and CO_2 production, thus ventilation

• concentrated glucose solutions are hyperosmolar and will cause thrombosis of peripheral veins, thus necessitating an SCV line
• obviously CHO loads are not ideal for diabetic individuals and the use of lipid infusions reduces the requirement for frequent BSL monitoring and additional insulin
• glucose infusion mixtures consist of 25% dextrose, 2% AA's, plus vitamins and minerals
• lipid infusions are mixtures of TG's, phospholipid as an emulsifying agent, and glycerol to maintain isotonicity, therefore, may be given peripherally
• these can be administered concurrently using a Y-connector
• insulin is not required for fat metabolism and plasma levels are low, and those of FFA's and ketones high, when lipid is the major nonprotein source
• also, lipid infusions can be ceased abruptly without the danger of hypoglycaemia
• essential FFA's are met if as little as 500 ml of "intralipid" is given daily

Recommendations for Nonprotein Energy

• lipid free systems are only required in patients with hyperchylomicronaemia
• infusions of ~ 80% lipid can be given peripherally, thereby minimising the treat of catheter sepsis and other complications
• Harrison's recommends a 1:1 ratio through a CV line as this approximates the normal dietary ratios of CHO & fat and cause neither hyperglycaemia or hyperinsulinaemia

Other Requirements

• vitamins must be added to the administered solution
• excessive amounts of the fat soluble group should be avoided because of the danger of hypercalcaemia and other toxic effects
• a combination of 5 ml Multivitamin Infusion (MVI) + 10 ml Soluzyme + Vit C on alternate days meets most requirements
• these should be supplemented with Vit K (5 mg) and Vit B_{12} (200 µg), initially at intervals of 3 weeks

• electrolytes are an essential component of TPN
• potassium, magnesium and phosphorus are necessary for optimal nitrogen retention and tissue formation
• sodium and chloride are essential to maintain osmolality and acid-base balance
• calcium is required to prevent demineralization of bone

• trace elements are only needed if TPN is to exceed 2 weeks
• these include Zn, Cu, Mn, Cr, Se

Routes of Administration

Central Venous Line

• has the advantages that fluids may be infused irrespective of osmolality and the need for repeated venipuncture is obviated, however, carries the risks of septicaemia and thrombosis
• the basic principles of insertion are as follows,

a. aseptic technique
b. position documented radiologically
c. introduced via a large central vein, not peripherally
d. the catheter should not be used to withdraw blood or measure the CVP
e. barium impregnated silicon rubber catheters are less likely to be surrounded with fibrin clot and are relatively atraumatic

Peripheral Venous Infusion

• this route is safe and unlikely to be associated with sepsis or thrombosis
• however, the infused fluids must be isotonic or only mildly hypertonic
• therefore, the majority of nonprotein calories must be lipid

Complications

Technical Complications

• most relate to placement of the CV line,

a. injury to the lung & pleura with pheumothorax or haemothorax
b. catheter embolism due to shearing off of the tip
c. brachial plexus injury

• incorrect placement can result in infusion of nutrients into the pleura or mediastinum, these can be avoided by infusing only saline until placement is confirmed radiologically
• late problems which can arise include thrombosis within the catheter and air embolism

Septic Complications

• the presence of a foreign body within the central veins provides considerable risk of sepsis, thus insertion and regular cleansing and dressing of the site should be done under strict aseptic technique
• sepsis in a patient with a central line is often not due to catheter sepsis and other causes should be excluded prior to the catheter being removed
• on removal there should be prompt defeveresence if the catheter was in fact the origin of the sepsis
• a new catheter may be inserted 48 hrs after the fever has subsided
• it is important not to withhold TPN from such patients as further malnutrition will further predispose them to sepsis

Metabolic Complications

• in septic patients, hyperglycaemia may occur owing to insulin resistance and high levels of CA's and cortisol
• management is to replace CHO calories with lipid, and/or add insulin
• during TPN the blood glucose levels should not be allowed to fall below 150 mg/dl due to the danger of hypoglycaemia
• the sudden onset of hyperglycaemia may herald the onset of sepsis
• hypoglycaemia is apt to occur when hypertonic glucose infusions are ceased, or rarely when a patient receiving TPN and insulin has their sepsis removed
• hyperammonaemia and a picture resembling hepatic encephalopathy may occur in patients receiving a mixture of AA's deficient in arginine
• hypertriglyceridemia may occur with overfeeding

Electrolyte Disturbances

• anabolism is associated with cellular uptake of phosphorus, magnesium and potassium
• this can result in low plasma levels of any or all of these
• hypophosphataemia results in low RBC levels of 2,3-DPG and thus reduced oxygen transfer to the tissues
• in the brain this may result in disorientation, convulsions and/or coma

- ***Acid-Base Balance***

• the metabolism of the basic AA's in their chloride form produces both chloride ions & protons, which, if unbuffered, can result in a hyperchloraemic acidosis
• for this reason all current AA mixtures contain sodium acetate, the conversion of acetate to bicarbonate serve to buffer the protons produced by the metabolism of the basic AA's

- ***Liver Disease***

• minimal elevations of ALP and AST (70-90%) are common in TPN but rarely associated with jaundice
• only in the occasional patient, ~ 1.5-2.0%, does cholestasis develop and this is only associated with minimal hepatocellular dysfunction
• hyperbilirubinaemia is common in septic patients
• "sludge" accumulates in the gallbladder and may lead to obstructive changes in the biliary tract
• the liver may become fatty, enlarged and tender if excess calories are given as CHO

- ***Hypercalcaemia And Pancreatitis***

• pancreatitis associated with hypercalcaemia can occur during TPN and this may be relieved by removing Vit. D from the supplementation

- ***Metabolic Bone Disease***

• in some patients receiving home TPN, osteomalacia & osteoporosis have occurred, leading to bone pain and fractures
• the mechanism for these changes is unclear

ENDOCRINE PANCREATIC FUNCTION

• at least 4 hormones are secreted by the islets of Langerhans in the pancreas,

1. insulin
2. glucagon
3. somatostatin
4. pancreatic polypeptide

Islet Cell Structure

• the islets of Langerhans are ovoid structures ~ 75 x 175 um, scattered throughout the pancreas but are more plentiful in the tail and body
• they comprise ~ 1-2% of the total weight of the pancreas and each has a copious blood supply
• unlike other endocrine glands, blood drains from these into the hepatic portal circulation
• the cells of the islets are divided into 4 types,

1. A(α) cells → glucagon
2. B(β) cells → insulin
3. D(δ) cells → somatostatin
4. F cells → pancreatic polypeptide

• the B cells, which are the most plentiful, are generally located at the centres of the islets and contain large numbers of granules containing insulin
• within the granules insulin is polymerised and also combined with zinc

Structure & Synthesis of Insulin

• insulin is a polypeptide containing 2 chains of AA's linked by disulphide bridges
• there are minor differences in the AA sequence from species to species and this has resulted in the antigenic behavior of the older porcine and bovine insulins, pork insulin is only 1 AA different and has the lowest Ag activity
• human insulin is now prepared with recombinant DNA techniques
• as for other proteins, insulin is synthesised in the endoplasmic reticulum, then transported to the Golgi apparatus and packaged into granules which are later secreted by exocytosis
• the neighboring capillaries are fenestrated and allow insulin to diffuse across the capillary wall
• like other polypeptide hormones, insulin is synthesised as a part of a larger preprohormone, the gene for which is located on chromosome 11 and has 2 introns
• preproinsulin has a 23 AA leader sequence which is removed as it enters the endoplasmic reticulum, the molecule is then folded and the disulphide bridges formed to make proinsulin
• proinsulin may be secreted after prolonged stimulation and in some islet cell tumors, however the chain connecting the A & B chains, the C peptide, is normally detached before secretion
• the removed connecting peptide has ~ 10% of the biological activity of insulin
• radioimunoassay of the C peptide provides an index of B cell function in patients receiving exogenous insulin
• tissue kallikrien may play a role in the conversion of proinsulin to insulin

The Fate Of Secreted Insulin

• the plasma half-life of insulin, $t_{½\beta}$ ~ 5 mins
• almost all tissues have the ability to metabolise insulin but normally over 80% is metabolised in the liver and kidneys
• three systems have been described, two break the disulphide bridges, one enzymatically the other nonenzymatically, and the other cleaves the peptide chains
• the enzymatic cleavage of the disulphide bridges is by hepatic glutathione insulin transhydrogenase, resulting in free A & B chains

• the plasma contains a number of substances with "insulin-like" activity in addition to insulin
• if insulin-like activity is measured by glucose uptake and gas exchange in adipose tissue, then only ~ 7% of the plasma insulin activity is suppressible by anti-insulin Ab's
• the remaining 93% is termed nonsuppressible insulin like activity NSILA
• much of the NSILA persists after pancreatectomy and ~ 5% is due to polypeptide growth factors, somatomedins, with insulin like activity
• these include insulinlike growth factors, IGF-I & IGF-II
• the remaining NSILA is high MW material sometimes referred to as nonsuppressible insulinlike protein NSILP
• the main point is that despite this activity, the presence of insulin is required for normal CHO metabolism

Insulin Deficiency & Action of Insulin

Diabetes Mellitus

• characterised by polyuria, polydipsia, weight loss, hyperglycaemia, glycosuria, ketosis, acidosis and coma
• there are widespread biochemical abnormalities, however the two fundamental defects are ,

a. reduced entry of glucose into peripheral tissues
b. increased liberation of glucose into the circulation from the liver, ie. increased hepatic gluconeogenesis

• there is also decreased entry of AA's into muscle and increased lipolysis
• there is an either an absolute or a relative hypersecretion of glucagon
• this is true even when the pancreas is removed, as glucagon is also secreted by the GIT
• thus, some of the hyperglycaemia is due to elevated glucagon, however the hyperglycaemia persists even when the glucagon secretion is reduced to zero

Glucose Tolerance

• the response to a standard oral dose of glucose is used in the diagnosis of diabetes, where the plasma level rises above the normal range and the return to normal is considerably prolonged
• the impaired tolerance is in part due to decreased peripheral utilisation of glucose as insulin is required for cellular uptake
• most cells require insulin for this purpose, however cells which utilize glucose independent of insulin are,

a. the brain

b. the RBC's
c. the tubules of the kidney - reabsorption
d. the intestinal mucosa - absorption

• glucose uptake by the liver is also reduced, however this is an indirect effect
• the second major cause of the impaired tolerance is the deranged function of the hepatic glucostat
• as the liver contains G-6-phosphatase, glucose formed from glycogenolysis and gluconeogenesis can be liberated into the blood stream
• insulin facilitates glycogenesis and inhibits hepatic glucose output
• glycogen has the opposite effects, inhibiting glycogenesis and enhancing glycogenolysis
• glucagon achieves this via action on a cAMP dependent protein kinase (see Ganong fig. 19-8 and Part 1 notes)
• CA's, cortisol, and GH can also increase hepatic glucose output when circulating levels are elevated by stress or illness

Distribution Of Endogenous & Exogenous Insulin

• endogenously secreted insulin enters the portal vein, so that the liver is normally exposed to concentrations of insulin 3-10 times those of the peripheral tissues
• the liver binds ~ 1/2 the insulin injected into the portal vein but only ~ 1/4 of a peripherally injected dose
• in addition the liver is more sensitive to insulin than are the peripheral tissues

Effects Of Hyperglycaemia

• alone this can result in symptoms from the hyperosmolality of blood
• in addition, there is glycosuria and osmotic diuresis with appreciable urinary losses of water, sodium and potassium
• for every gram of glucose lost in the urine, 4.1 kcal are lost from the body
• increased caloric intake to cover this simply raises the blood sugar further and does not prevent mobilization of protein and fat stores with subsequent weight loss

The Hypoglycaemic Action Of Insulin

• in normal or diabetec individuals, following IV administration of insulin, the decline in plasma glucose is maximal at ~ 30 mins, whereas following s.c. administration maximal effects are seen at 2-3 hrs
• insulins may be complexed with protamine to delay absorption and provide prolonged effects

Regulation Of Plasma Potassium

• insulin causes K^+ to enter cells and thus lowers its extracellular concentration
• infusions of insulin and glucose are very effective for the temporary relief of hyperkalaemia
• hypokalaemia often develops in patients with diabetic ketoacidosis treated with insulin
• the exact mechanism for the intracellular shift is still uncertain, however, insulin increases the activity of the membrane bound Na-K-ATPase

• this activation may be secondary to activation of the Na-H-ATPase responsible for the movement of H^+ out of the cells, the subsequent rise in the intracellular Na^+ stimulating the pump
• insulin has been reported to increase the intracellular pH
• K^+ depletion decreases insulin secretion, and patients with primary hyperaldosteronism display diabetic glucose tolerance curves which are corrected by K^+ replacement
• the thiazide diuretics, which cause renal loss of K^+ & Na^+, decrease glucose tolerance and make diabetes worse

Exercise

• the entry of glucose into skeletal muscle is increased during exercise in the absence of insulin, probably due to a relative O_2 deficiency as uptake is also increased under anaerobic conditions
• exercise may precipitate hypoglycaemia in a diabetic taking insulin for three reasons,

a. exercise increases the affinity of insulin receptors
b. the production of a relative O_2 deficit, and
c. increased blood flow increases the rate of absorption

• thus diabetics should decrease their dose of insulin and increase their caloric intake prior to exercise

Effects Of Intracellular Glucose Deficiency

• the energy for intracellular processes must be met by protein and fat catabolism, with the increase in fat catabolism leading to ketosis
• deficient glucose utilisation by the cells of the ventromedial nuclei of the hypothalamus (the satiety centre) probably causes the hyperphagia in diabetes, due to the unopposed action of the lateral appetite centre
• glycogen depletion is a common consequence of insulin deficiency, especially in the liver and skeletal muscle
• insulin increases glycogenolysis in skeletal muscle and also in the liver providing the resulting hypoglycaemia is not sufficient to activate glycogenolysis

Changes In Protein Metabolism

• both the rate at which AA's are catabolised to CO_2 & H_2O, and the rate of gluconeogenesis in the liver are increased (see Ganong fig. 19-9)
• in the fasting state, with liver glycogen depleted, the only major source of plasma glucose is protein, (FFA's $\rightarrow$ glycerol insignificant)
• thus an indication of the rate of gluconeogenesis can be obtained from the ratio of glucose (dextrose) to nitrogen in the urine, D/N ratio
• for each 1 g of urinary nitrogen ~ 8.3 g of glucose can be formed
• consequently the D/N ratio of ~ 3 seen in fasting diabetics, indicates the conversion of ~ 33% of protein carbon to glucose, the remainder being fully catabolised
• the causes of increased gluconeogenesis include,

a. increased glucagon
b. adrenal glucocorticoids

c. increased supply of AA's due to decreased protein synthesis

d. increased activity of enzymes concerned

• insulin deficiency decreases lipogenesis, thus increases the supply of acetyl-CoA, this stimulates pyruvate carboxylase which converts pyruvate to oxaloacetate, which is then converted to phosphoenolpyruvate etc. (fig. 17-9)
• when the plasma glucose is episodically raised small amount of HbA are nonenzymatically glycosylated to HbAIc, thus giving a guide to control of plasma glucose over the preceding 4-6 week period
• albumin is also glycosylated in this manner, however it turns over more rapidly and is an index of more rapid fluctuations
• the net result is a markedly negative nitrogen balance, protein depletion and wasting
• protein depletion from any cause is associated with increased susceptibility to infections → decreased PMN function & Ab formation
• this together with the glucose rich body fluids places diabetics at significantly increased risk of infection

■ ***Insulin & Growth***

• not only is protein catabolism increased but synthesis is also decreased
• the anabolic effect of insulin, increased cellular uptake of AA's & protein synthesis, is aided by the protein-sparing action of adequate intracellular glucose
• failure to grow is seen in children with diabetes, and insulin increases the growth of immature hypohpysectomised rats to almost the same degree as GH

Fat Metabolism

• the principal abnormalities of fat metabolism in insulin deficiency are,

a. increased lipolysis

b. formation of ketone bodies

c. decreased synthesis of FFA's and triglycerides

• these effects are as, or more prominent than the effects on CHO metabolism
• of a normal CHO load,

a. 50% → oxidised to CO_2 & H_2O

b. 5% → glycogen synthesis

c. 30-40% → FFA & TG synthesis

• in the diabetic,

a. << 50% → oxidised to CO_2 & H_2O

b. < 5% → FFA & TG synthesis

• thus, glucose accumulates in the plasma and is lost in the urine
• insulin inhibits hormone sensitive lipase and, in its absence, the plasma levels of FFA's is more than doubled
• the relative glucagon excess also contributes to this effect
• plasma FFA levels parallel blood glucose levels in diabetics and in some ways are a better indicator of the severity of the disease
• in the liver & other tissues, FFA's are catabolised to acetyl-CoA
• some of the acetyl-CoA is catabolised through the CAC, however this pathway is overloaded and the excess results in ketone body formation in the liver by the action of deacylase
• circulating ketone bodies are an important source of energy in the fasting state and the rate of utilisation in diabetics is also considerable
• it has been calculated that the maximum rate of fat catabolism without significant ketosis is ~ 2.5 g/kg body weight/d
• this is greatly exceeded in uncontrolled diabetes and there is some evidence that ketone utilisation is decreased in insulin deficiency due to decreased uptake into skeletal muscle
• in uncontrolled diabetes the plasma levels of chylomicrons, FFA's & TG's are increased, and the plasma is often lipaemic
• this is contributed to by the decreased activity of lipoprotein lipase

Acidosis

• most of the H^+ ions liberated from acetoacetate & β-hydroxybutyrate are buffered, but severe metabolic acidosis still develops with compensatory respiratory stimulation
• the urine becomes acidic, however, eventually the kidneys ability to replace plasma cations with NH_4+ & H^+ is exceeded and significant quantities of Na^+ & K^+ are lost in the urine with organic anions
• the electrolyte and water losses lead to hypovolaemia and hypotension which may proceed to coma

• in severe diabetic acidosis, the total body sodium is markedly low, and when sodium losses exceed water loses the plasma Na^+ is also not infrequently low
• total body K^+ is also low, however plasma K^+ may be sustained by contraction of the ECF and a shift of K^+ out of the cells due to the acidosis
• sudden correction of the acidosis and hypovolaemia may result in profound hypokalaemia

Coma

• in diabetes may be due to either,

a. ketoacidosis and dehydration
b. hyperosmolality of plasma
c. lactic acidosis due to tissue hypoxia

• brain oedema may be seen with diabetic coma, the cause is strictly unknown but it carries a bad prognosis

Cholesterol Metabolism

• the plasma cholesterol is usually elevated and this, in part, is responsible for the accelerated atherosclerosis seen in diabetes
• this is caused by elevated levels of VLDL & LDL, due to increased hepatic production and/or decreased peripheral removal

Principal Actions of Insulin		
	Increased	Decreased
Adipose Tissue	• entry of glucose • FFA synthesis • glycerol phosphate synthesis • triglyceride deposition • activity of lipoprotein lipase • cellular K^+ uptake	• activity of hormone sensitive lipase
Skeletal Muscle	• entry of glucose • glycogen synthesis • amino acid uptake • protein synthesis in ribosomes • uptake of ketone bodies lipase • cellular K^+ uptake	• protein catabolism • release of gluconeogenic AA's
Liver	• protein synthesis in ribosomes • lipid synthesis • glycogen synthesis	• cyclic AMP • ketogenesis • gluconeogenesis • output of glucose

Insulin Excess

• all of the known consequences of excess insulin are manifestations of hypoglycaemia on the CNS
• except for individuals who have been fasting for some time, glucose is the only fuel used appreciably by the brain
• CHO reserves in neural tissues are limited, and normal function depends on a continuous supply of glucose
• the cortex and areas with higher metabolic rates are the first affected, followed by the vegetative centres in the diencephalon and hindbrain
• if hypoglycaemia is prolonged, irreversible changes develop in the same cortical-diencephalic-medullary sequence, and death results from depression of the respiratory centre
• hypoglycaemia is a potent stimulus to the SNS with increased secretion of CA's, especially adrenaline
• the tremors, palpitations and sweating seen in hypoglycaemia are manifestations of this SNS activity
• the exact level at which hypoglycaemia appears is variable, depending upon the preexisting state of the individual
• an average value is 45 mg/dl, however those with insulin secreting tumors may adapt to levels of 20 mg/dl, and diabetics may develop symptoms at levels of 100 mg/dl

• hypoglycaemia stimulates the release of 5 counter-regulatory hormones,

a. adrenaline
b. noradrenaline
c. glucagon

d. cortisol

e. growth hormone

• the first three stimulate glycogenolysis and enhance hepatic glucose output
• the later two decrease peripheral utilisation of glucose
• the two key hormones are adrenaline & glucagon, the actions of the other hormones are simply complementary

Mechanism of Action of Insulin

• insulin receptors are tetramers, made of 2 α and 2 β glycoprotein subunits, the former are extracellular and linked by disulphide bridges to the later, which traverse the membrane
• each β subunit has tyrosine hydroxylase activity at its intracellular end, and the proteins that are phosphorylated are responsible for insulins actions
• these receptors are found on virtually all cells of the body, even those where insulin has no known action
• binding of insulin results in an increased number of glucose carrier proteins in the cell membrane, the rate of transport/carrier is the same but the net flux increased
• when insulin binds to receptors, they aggregate and are taken up by receptor mediated endocytosis, eventually entering lysosomes and being broken down
• the half-life of an insulin receptor is ~ 7 hrs
• exposure to increased concentrations of insulin decreases the number of receptors = ***downregulation***, and exposure to decreased levels of insulin has the opposite effect
• the number of receptors is also increased in starvation and decreased in obesity
• the affinity of the receptors is increased in adrenal insufficiency and decreased by excess glucocorticoids
• in muscle, adipose, and connective tissues, insulin facilitates the entry of glucose by an action on the cell membrane, however, once inside the cell, the subsequent phosphorylation is regulated by other hormones
• GH and cortisol have both been reported to inhibit phosphorylation in certain tissues
• however, this process is normally so rapid that the rate limiting step is entry into the cells
• because of this rapid phosphorylation, the free glucose concentration in the cell is kept low maintaining a concentration gradient for facilitated diffusion, this occurs in the absence of insulin but only at a much slower rate
• insulin does not affect the rate of glucose entry into hepatic cells directly, but by its actions increasing glycogenesis and decreasing gluconeogenesis, lowers both the free & phosphorylated glucose concentrations and increases glucose uptake
• the suppression of synthesis of key gluconeogenic enzymes and the induction of synthesis of glycolytic & glycogenic enzymes seen with insulin, have not been demonstrated to be direct effects of the hormone

Regulation of Insulin Secretion

• normal secretion requires Ca^{++} and K^{+}, and there is evidence that raised intracellular Ca^{++} triggers exocytosis in a similar fashion to neurons and other endocrine cells
• the amount of insulin secreted in normal humans has been calculated to be about 40 units

- ***The Blood Glucose Level***

• the major control of insulin secretion is via direct feedback control by glucose on the pancreatic B cells
• glucose penetrates B cells readily and the rate of entry is independent of insulin
• when the blood [glucose] exceeds 100-110 mg/dl insulin secretion is enhanced, whereas at low levels secretion is virtually abolished
• fructose has a moderate stimulatory effect, but is converted to glucose intracellularly
• the stimulatory effect of glucose depends upon its metabolism, as agents such as 2-deoxyglucose and mannoheptulose which inhibit glucose metabolism, also inhibit insulin secretion
• the action of glucose is biphasic, there is an initial, rapid phase of secretion triggered by Ca^{++}, followed by a slowly developing prolonged phase
• these are due to release of stored insulin and synthesis of new insulin respectively, the later being blocked if protein synthesis is inhibited

- ***Protein & Fat Derivatives***

• arginine, leucine, and other AA's stimulate insulin secretion, as do β-keto acids such as acetoacetate
• the exact mechanism is uncertain

- ***Cyclic AMP***

• any stimuli which increases intracellular cAMP increase secretion, probably due to raised intracellular Ca^{++}
• these include,

a. β adrenergic stimulation
b. glucagon
c. phosphodiesterase inhibitors

• CA's have a dual action, as α adrenergic stimulation is inhibitory, the net effect of both AD & NA are inhibitory

- ***Autonomic Nervous System***

• branches of the right vagus innervate the islets and enhance insulin secretion, the response being blocked by atropine
• increased SNS tone to the pancreas inhibits secretion by release of NA, this may be converted to excitation by prior infusion of an α blocking agent
• the pancreas will maintain homeostasis in the denervated state but the ANS modulates the sensitivity of the islets to glucose

- ***Intestinal Hormones***

• orally administered glucose produces greater insulin release than does IV administration, the same being true for AA's
• the major factor in this response is gastric inhibitory peptide GIP

• however, glucagon, secretin, CCK, and gastrin also influence insulin secretion but the effects are minimal at physiological levels

Oral Hypoglycaemic Agents

• sulphonylurea derivatives, such as tolbutamide, chlorpropamide, glipizide, and glyburide, lower the blood glucose initially by stimulating insulin secretion, but subsequently by enhancing the effects of endogenous insulin, either at the receptor, or the postreceptor level
• thus, they are of value in diabetics with some residual pancreatic function
• the biguanides, such as phenformin, do not affect insulin secretion but enhance glucose utilisation, apparently by inhibition of the oxidative metabolism of glucose $\rightarrow$ increased anaerobic glycolysis with the threat of lactic acidosis
• they also decrease absorption of glucose from the GIT
• diazoxide, initially developed for hypertension, has been shown to inhibit insulin secretion and to be diabetogenic, some of the thiazide diuretic have a similar action

Long Term B Cell Responses

• the magnitude of the insulin response to a given stimulus depends upon the recent secretory history of the B cells $\rightarrow$ high CHO diets lead to higher fasting levels and greater responses
• although the B cells respond with hypertrophy, as do other endocrine cells, the may become exhausted and cease secretion = B cell exhaustion, when stimulation is marked and prolonged
• the cells will recover if stimulation is stopped, however after a certain point they atrophy and die
• such "exhaustion atrophy" is not seen in any other endocrine gland
• pancreatic reserve is large and this is difficult to produce in normal animals, however is more likely when the reserve is reduced as in partial pancreatectomy with concomitant administration of diabetogenic hormones, such as T_3, GH, or anterior pituitary extracts

Effects Of Exogenous Insulin

• exogenous administration results in a fall in blood glucose and thus a fall in endogenous insulin secretion, both due to direct and indirect feedback
• remaining endogenous secretion may be measured by radioassay of the C peptide
• when insulin treatment is stopped the B cells are hyperresponsive, as though they have "rested", this is believed to be the origin of the decrease in insulin requirements seen early in the course of treatment of juvenile diabetics

Glucagon

Chemistry

• glucagon is a linear polypeptide, MW = 3485, containing 29 AA residues which is produced by the A cells of the islets
• all mammalian glucagons appear to have the same structure
• glicentin is a larger molecule also found in the intestinal mucosa which possesses some glucagon activity, actually consists of glucagon extended at the C terminus
• human proglucagon is a 179 AA polypeptide that contains glicentin near its N-terminus, followed by glucagon, then 2 glucagon-like peptides of unknown function

Mechanism Of Action

• glucagon is glycogenolytic, gluconeogenic, lipolytic & ketogenic
• it achieves these effects by increasing cAMP in hepatocytes and the study of the mechanism of action of glucagon, was the origin of the discovery of the membrane bound adenylate cyclase system
• the subsequently activated glucagon dependent protein kinase phosphorylates a number of proteins, including,

a. phosphorylase - activated (glucagon → liver only)
b. glycogen synthase - inactivated
c. decreased synthesis of fructose-2,6-diphosphate
d. decreased conversion phosphoenolpyruvate to pyruvate

• glucagon does not cause glycogenolysis in muscle (a)
• it increases gluconoegenesis from available AA's in the liver (c & d)
• increases ketone body formation by decreasing malonyl-CoA in the liver and increasing lipolysis
• the calorigenic action of glucagon is not due to the elevated blood glucose per se, but probably due to the increased hepatic deamination of AA's
• large doses of exogenous glucagon exert a (+)'ve inoptropic effect on the heart, without increasing myocardial excitability, presumably by increasing cAMP
• also stimulates secretion of GH, insulin, and pancreatic somatostatin

Metabolism

• the circulation half-life of glucagon ~ 5-10 mins and the principal site of degradation is the liver, though, many tissues can metabolise glucagon
• as it is released into the portal system, peripheral levels are significantly lower, as are those of insulin

Regulation Of Secretion

• secretion is decreased by a rise in the plasma [glucose], but this effects requires insulin → the A cells are an insulin dependent tissue

• secretion is also increased by increased SNS tone, mediated by β receptors and cAMP, α receptors being inhibitory
• the net effect of increased SNS tone being stimulation as the β receptor effects are predominant
• increased vagal tone also increased glucagon output
• both a protein meal and infusions of various AA's stimulate secretion, particularly the gluconeogenic AA's
• the increase following a meal is greater, due to the action of either CCK or gastrin as both enhance glucagon secretion
• the increase in glucagon following a protein meal is valuable, as AA's also stimulate insulin secretion and the glucagon prevents hypoglycaemia, while the insulin promotes storage of absorbed CHO & fat
• secretion increases in fasting and reaches a maximum by the 3rd day when gluconeogenesis is maximal, thereafter the level declines as FFA's and ketones become the major energy source
• during exercise glucagon increases & insulin falls, there is increased glucose production and increased utilisation
• the increase in utilisation is due to,

a. hypoxia
b. increased affinity of insulin receptors
c. increased muscle blood flow and insulin supply

• the increase in glucose production is due to,

a. the decrease in plasma insulin
b. primarily the increase in plasma glucagon

Insulin-Glucagon Molar Ratios

• when energy is needed, as during starvation, the ratio is low
• when the need for energy mobilization is low, the ratio is high, favoring the storage of CHO & fat
• when energy is needed, as during exercise, the ratio is reduced but not to the same degree as in starvation

Other Pancreatic Hormones

Somatostatin

• exists in 2 forms, the 14 AA version and the N-terminal extended 28 AA version
• both forms inhibit the secretion of insulin, glucagon, and pancreatic polypeptide and may act locally within the pancreas in a paracrine fashion
• secretion is increased by many of the stimuli which increase insulin secretion: AA's, arginine & leucine, CCK
• somatostatin is released from the pancreas and the intestinal mucosa, its main actions being inhibition of gastric acid secretion and gastric emptying, and inhibition of gallbladder contraction
• may be more effective in the inhibition of gastric acid secretion than H_2 antagonists (G&G)
• effective in the treatment of bleeding varicies

Pancreatic Polypeptide

• a linear polypeptide containing 36 AA's which is secreted in response to ingestion of protein, fasting, exercise, and acute hypoglycaemia
• its secretion is decreased by IV glucose and somatostatin
• infusions of AA's do not have any effect, thus the response to a protein meal may be indirect
• it appears to slow absorption from the GIT and may act to average absorption peaks & troughs

Functional Organization Of The Islets

• there are gap junctions between A, B, & D cells which allow the passage of ions between cells and may act to coordinate secretion
• there are clearly two types of islets, glucagon-rich & PP-rich, but the functional significance is uncertain but the interrelated reciprocal stimulation/inhibition displayed points to a paracrine regulation of secretion

Other Hormones in Carbohydrate Metabolism

Catecholamines

• CA's activate phosphorylase in the liver, probably via α receptors, increasing intracellular Ca^{++}, and β_2 receptors, increasing cAMP
• according to G & G the exact α/β responses in man not been determined
• in any case, hepatic glucose output is increased, raising the blood glucose
• in muscle, phosphorylase is activated by β_2 receptors and cAMP, however the G-6-Phosphate formed can only be catabolised due to the absence of the enzyme glucose-6-phosphatase
• for unknown reasons, a large amount of the pyruvate formed is converted to lactate, which is later converted in the liver to glycogen
• thus, the response to administered adrenaline is an initial glycogenolysis with raise blood glucose and lactate levels, followed by a later increase in hepatic glycogen
• both AD & NA increase lipolysis and elevate plasma FFA levels
• AD also decreases the peripheral utilisation of glucose

Thyroid Hormones

• thyrotoxicosis exacerbates clinical diabetes, and metathyroid diabetes can be produced in animals with limited pancreatic reserves
• the principal diabetogenic effect is to increase intestinal absorption, but these hormones also cause some degree of hepatic glycogen depletion
• glycogen depleted hepatocytes are easily damaged, with a resultant diabetic tolerance curve due to decreased glucose uptake
• they may also accelerate the degradation of insulin

Adrenal Glucocorticoids

• these elevate the blood glucose level and produce a diabetic type glucose tolerance curve
• in humans this may only occur in susceptible individuals, and the GTT is reduced in ~ 80% of patients with Cushing's syndrome
• they are also necessary for glucagon to exert its gluconeogenic effects in fasting, they are gluconeogenic themselves, however their main action is permissive
• in adrenal insufficiency, the blood glucose is normal providing food intake is maintained, however, fasting produces hypoglycaemia and collapse
• the hypoglycaemic effects of insulin are greatly enhanced in patients with adrenal insufficiency
• the following are actions of the glucocorticoids,

a. increased peripheral protein catabolism & plasma AA's
b. increased hepatic uptake of AA's
c. increased hepatic transamination & deamination of AA's
d. increased hepatic conversion oxaloacetate → P-enolpyruvate
e. increased fructose diphosphatase activity: F-1,6-dP → F-6-P
f. increased glucose-6-phosphatase activity: G-6-P → Glucose
g. decrease in peripheral glucose utilisation
h. increased blood lactate & pyruvate
i. decreased hepatic lipogenesis
j. increased plasma FFA's and ketone body formation
k. increased glycogen synthase activity

Growth Hormone

- the diabetogenic effects of anterior pituitary extract are due to ACTH & TSH, but also to GH
- GH exacerbates clinical diabetes and ~ 25% of patients with GH secreting tumors have diabetes
- hypophysectomy ameliorates diabetes and increases the sensitivity to insulin to an even greater extent than adrenalectomy
- GH mobilizes FFA's from adipose tissue and favors ketogenesis
- it also decreases glucose uptake in some cells, increases hepatic glucose output and may decrease tissue binding of insulin
- there is evidence that GH decreases the number of insulin receptors and that the glucocorticoids decrease their affinity
- however, the decreased glucose utilisation produced by these hormones is more due to inhibition of glucose phosphorylation, than the entry of glucose into cells

THE ADRENAL MEDULLA & ADRENAL CORTEX

• there are two endocrine organs in the adrenal gland, one surrounding the other,

a. adrenal medulla → adrenaline, noradrenaline, dopamine

b. adrenal cortex → glucocorticoid & mineralocorticoids

• the medulla is in effect a sympathetic ganglia, in which the postganglionic neurons have become secretory cells
• the medullary hormones are not essential for life but they greatly assist the body in function & homeostasis during periods of stress
• the glucocorticoids have widespread effects on protein & CHO metabolism and a mineralocorticoid is essential for the maintenance of sodium and ECF volume
• the cortex also secretes sex steroids with a minor effect on reproductive function
• the absence of the corticoids results in collapse & death

Adrenal Morphology

• the medulla comprises ~ 28% of the adrenal mass, and is composed of interlacing cords of densely innervated granule containing cells which abut the venous sinuses
• two types of cell can be distinguished,

a. AD secreting 90% - larger cell with less dense granules

b. NA secreting 10% - smaller cell, dense granules

• the type of cell which secretes dopamine is unknown
• paraganglia, small groups of cells resembling those of the adrenal medulla, are found near the thoracic and abdominal sympathetic ganglia

• the cortex is divided into three zones,

1. the outer zona glomerulosa, whose cells are continuous with
2. the zona fasciculata formed from columns of cells, joining
3. the inner zona reticularis

• these three regions approximate 15%, 50% and 7% of the adrenal mass respectively
• all the cells contain abundant lipid stores and all three zones secrete corticosterone, however, aldosterone is limited to the outer glomerulosa, while cortisol & the sex hormones are limited to the inner two zones
• arterial blood reaches the adrenal from many small branches of the phrenic & renal arteries, and from the aorta
• from an arterial plexus in the capsule blood flows inwardly through to the venous sinuses of the medulla
• the medulla is also supplied by a few arterioles which pass directly through the cortex
• there is usually a single large adrenal vein and the adrenal blood flow is large
• during foetal life, these 3 zones ~ 20% of the gland, the remainder is the large foetal adrenal cortex which secretes sulphate conjugates of androgens, which are converted in the placenta to oestrogens
• this is under control of the pituitary and undergoes rapid degeneration after birth

• another function of the zona glomerulosa is the supply of new cortical cells, small remnants of the capsule left at adrenalectomy will grow into substantial adrenal cortical tissue
• like other tissues of neural origin the medulla does not regenerate
• immediately after hypophysectomy the inner 2 cortical zones begin to degenerate, whereas the zona glomerulosa remains unchanged (angiotensin II)
• increased exogenous or endogenous ACTH similarly result in hypertrophy of only the inner 2 zones

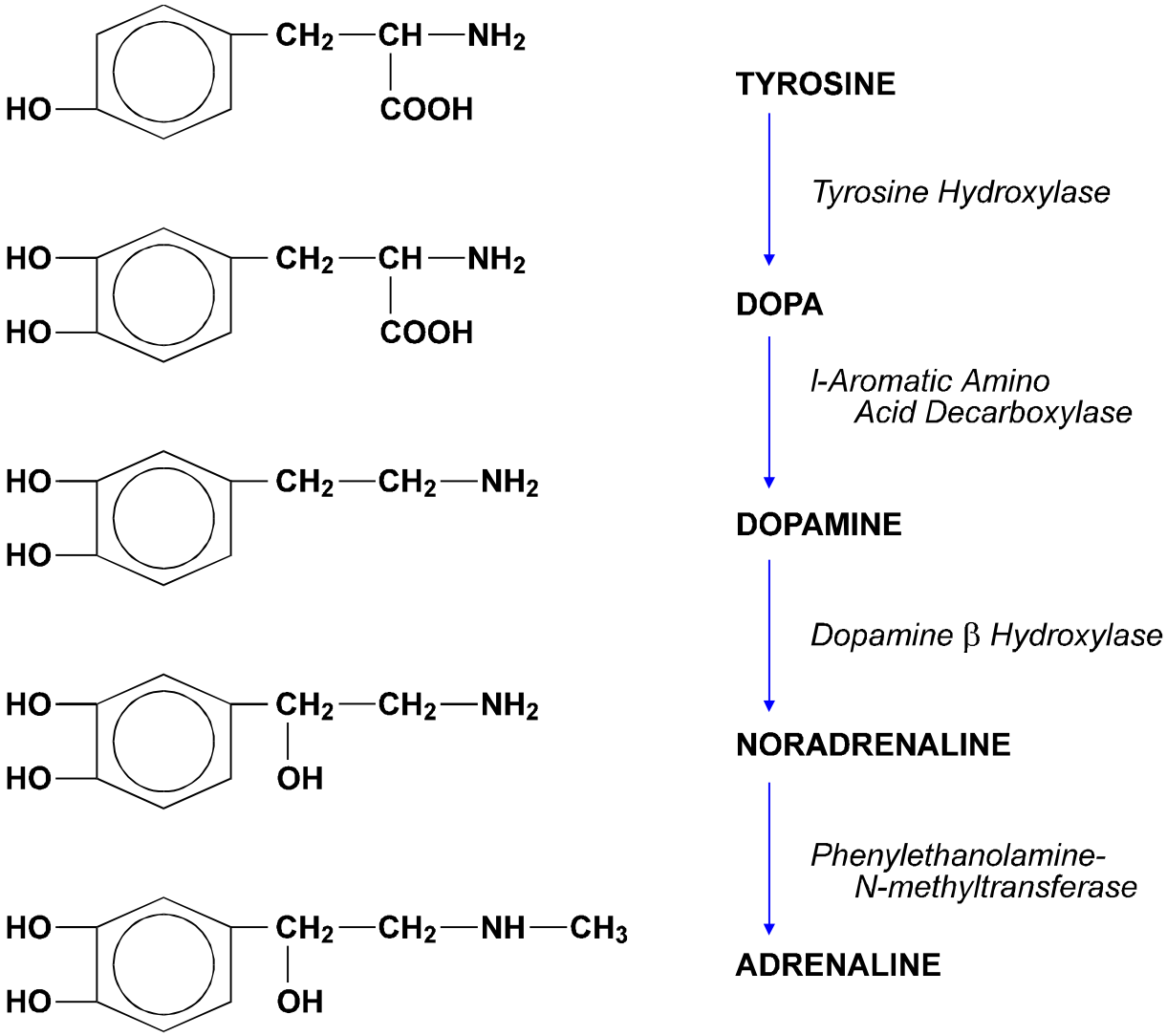
THE ADRENAL MEDULLA

Structure & Function of Adrenal Hormones

Def'n: CA's, by definition, all possess the ***catechol nucleus*** = a ***benzene ring*** with adjacent hydroxyl substitutions, ***O-dihydroxybenzene*** known as catechol

• β-phenylethylamine can be viewed as the parent compound, consisting of a benzene ring and an ***ethylamine*** side chain
• optical ***isomerism*** is conferred by substitution on either of the ethyl C atoms
• levorotatory substitution at the β-C atom produces naturally occurring NA & AD, both of which are over 10 times as potent as their *d*-isomers
• the following steps in the synthesis of AD were proposed by Blaschko (1939)

Biosynthesis of Catecholamines



NB: 1. (-)'ve feedback is via CA competition with the ***tetrahydrobiopterin*** cofactor of tyrosine hydroxylase
2. the enzymes tyrosine hydroxylase, dopamine-β-hydroxylase, and phenylethanolamine-N-methyl transferase share AA homology and may be under common genetic control

• none of these enzymes is highly specific, and a variety of endogenous substances and drugs are acted upon at the various steps, eg. the conversion of methyldopa to α-methyldopamine then the false transmitter α-methyl-NA
• ***tyrosine hydroxylase*** is activated following stimulation of adrenergic nerves or the adrenal medulla
• this enzyme is a substrate for cAMP-dependent protein kinase, and kinase-catalyzed phosphorylation is associated with increased enzyme activity
• this is also a substrate for Ca^{++}-activated protein kinase

• CA's act by direct binding to membrane bound receptors,

a. β_1 & β_2 → largely activation of ***adenylate cyclase*** & cAMP

b. α → mobilization of Ca^{++}
± α_1 → formation of ***inositol triphosphate***
α_2 → inhibition of adenylate cyclase

• the relationship between electrical events, ion fluxes and contraction of smooth muscle is complex and varies between tissues
• visceral smooth m. contractions are generally associated with ***slow waves*** of partial depolarisation and in some muscles superimposed AP's
• in muscles inhibited by β receptors, the membrane becomes hyperpolarised and AP's become less frequent, or may disappear, apparently due to a reduction in cytosolic free Ca^{++} and both enhanced efflux & sequestration may be involved
• relaxation of some smooth m. due to polariz activation may be due to hyperpolarisation due to increased g_K & g_{Cl}, in addition to the presynaptic inhibition of ACh release
• contraction of the intestinal sphincter smooth m. by α agonists appears to result from a generalised increase in ion permeability (Na^+, K^+, Cl^- and possibly Ca^{++})
• adrenergic receptors are well localised to the vicinity of the nerve terminals in peripheral target organs, though, there are other receptors located distant to these sites, possibly preferentially stimulated by circulating CA's from the medulla
• chronic exposure of CA sensitive tissues to the CA's results in a diminution of their response

→ ***tachyphylaxis***, down-regulation, refractoriness, or desensitisation

• there is evidence for multiple points for this phenomenon,

1. β receptors become phosphorylated and inactive
2. receptor & adenylate cyclase become uncoupled (G-protein)
3. the number of receptors declines

• sympathomimetic effects can also be obtained by elevation of the concentration of cAMP by inhibition of the enzyme ***phosphodiesterase***
• the ***methylxanthines***, such as caffeine & aminophylline act via this mechanism, and their effects are synergistic to the CA's
• about a half of the plasma DA comes from the adrenal medulla, the remaining half presumably from sympathetic ganglia
• average plasma levels (recumbent) are,

a.	adrenaline	30	pg/ml	0.16	nmol/l
b.	noradrenaline	300	pg/ml	1.8	nmol/l
c.	dopamine	35	pg/ml	0.23	nmol/l

• there is an increase of up to 50-100% upon standing
• in the plasma ~ 95% of the dopamine and ~ 70% of the NA are conjugated to ***sulphates***, which are inactive and their function is unsettled
• the CA's have a circulating half life of ~ 2 mins
• metabolism is primarily by ***methoxylation*** & ***oxidation***, the chief metabolite being ***3-methoxy-4-hydroxymandelic acid***, called vanillylmandelic acid (VMA)
• only small amounts of free NA & AD are excreted, average 24 hr values,

a.	adrenaline	6 µg
b.	noradrenaline	30 µg
c.	VMA	700 µg

• in the medulla, the CA's are stored bound to ATP in granules along with ***chromogranins***
• release is triggered by an ACh mediated increase in intracellular Ca^{++} which initiates exocytosis
→ thus all of the contents of the granules are released together
• the AD containing cells of the medulla also secrete ***opioid peptides***, the precursor molecule being ***proenkephalin*** which contains a number of AA sequences for met-ENK and one for leu-ENK
• most of the circulating ***met-ENK*** comes from the adrenal medulla
• however, these peptides cannot cross the BBB and their peripheral function is unsettled

Effects of Circulating Catecholamines

NB: for the effects of AD & NA see G.& G. table 4-1 and pharmacology notes

• the physiological function of circulating DA in unknown
• administered DA causes renal and mesenteric vasodilation probably by a direct action on dopamine receptors
• elsewhere it produces vasoconstriction due to release of NA and direct α adrenergic effects
• it produces a positive inotropic effect on the heart via β_1 receptors

Regulation of Adrenal Medullary Secretion

• certain drugs directly affect medullary release of CA's, however, physiologic stimuli all affect secretion via the autonomic nervous system
• CA secretion is low in the resting state, though, secretion of AD and, to a lesser extent, NA is reduced further during sleep
• release is a part of the diffuse activation of the SNS seen in the classical "fight or flight" preparation and, also, as a part of specific homeostatic reflexes
• in the presence of increased SNS discharge, the role of the circulating CA's in augmenting the vascular effects of neurally released NA is slight

> ***NB:*** injected CA's producing only 10-20% of the response of increased SNS tone, therefore, the major effects of the circulating CA's are probably ***metabolic***
> eg. the responses to cold and hypoglycaemia

• when secretion is increased as a part of general SNS discharge the ratio of AD:NA in the adrenal vein is usually unchanged, however, both asphyxia & hypoxia decrease the ratio of AD:NA

THE ADRENAL CORTEX

Structure & Function of Adrenocortical Hormones

• the hormones of the adrenal cortex are derivatives of ***cholesterol***
• like cholesterol, the bile acids, vit. D, and the ovarian & testicular steroids, they contain a ***cyclopentanoperhydrophenanthrene*** nucleus
• they are of 2 structural types, according to the number of carbon atoms,

a. C_{21} steroids - a 2-C side chain at C_{17} of the ring
b. C_{19} steroids - a keto or hydroxyl group at C_{17}

• most of the C_{19} steroids have a keto group at C_{17} and are thus called the ***17-ketosteroids***
• the C_{21} steroids with an -OH group at C_{17} in addition to the 2-C chain are often termed the ***17-hydroxycorticosteroids***

a. C_{19} steroids → androgenic activity
b. C_{21} steroids → either mineralocorticoid or glucocorticoid acticity

• for the purposes of nomenclature,

a. d → indicates a double bond
b. b and a solid line → the group is above the plane of the ring
c. a and a dotted line → the group is below the plane of the ring

• in most naturally occurring adrenal steroids, the 17-OH groups are α configured, whereas the 3-, 11-, and 21-OH groups are in the β configuration

Secreted Steroids

• the only steroids secreted in physiologically significant amounts are,

a. mineralocorticoid - aldosterone
b. glucocorticoid - cortisol
- corticosterone
c. androgen - dehydroepiandrosterone
- androstenedione

• deoxycorticosterone is a mineralocorticoid which is secreted in ~ the same amount as aldosterone, however possesses only 3% of the activity
• the adrenals may also secrete small amounts of oestrogen, however the vast majority is formed in the ovaries from androstenedione

Steroid Biosynthesis

• cholesterol is the precursor of all of the steroid hormones
• some is synthesised from acetate but the majority is derived from LDL in the circulation, LDL receptors are abundant on adrenocortical cells

• cholesterol is converted to pregnenolone which is the main precursor of corticosterone and aldosterone (see Ganong fig. 20-8 & 20-9)

- ***Action Of ACTH***

• ACTH binds to high affinity receptors on the plasma membrane which activate adenylate cyclase / cAMP / protein kinases
• the phosphorylated proteins increase the conversion of cholesterol esters to free cholesterol, with a subsequent increase in pregnenolone
• other effects of ACTH include,

a. increased binding of cholesterol to cytochrome P450 in the mitochondria
b. increased uptake of LDL from the circulation
c. increased metabolism of other phospholipids

- ***Enzyme Deficiencies***

• blockade of the synthesis of various of the steroids results in the syndrome of congenital adrenal hyperplasia (CAH)
• the hyperplasia being due to increased secretion of ACTH
• depending upon the enzyme deficiency, one of several types may be produced,

a. ***virilizing*** form 3-β-dehydrogenase
11-β-hydroxylase
21-β-hydroxylase
   - glucocorticoid secretion is deficient increasing ACTH
   - precursors are converted to androgens via remaining pathways

b. ***salt-losing*** form of virilizing CAH
   - 21-β-H deficiency incomplete with enough glucocorticoid & mineralocorticoid to sustain life, but results in salt loss

c. ***hypertensive*** form of virilizing CAH
   - 11-β-H deficiency increases 11-deoxycortisol and deoxycorticosterone
   - both are mineralocorticoids which lead to salt & water retention

• ***metyrapone*** can inhibit 11-β-hydroxylase and is used clinically to test pituitary reserve by producing a transient cortisol deficiency
• this results in ACTH secretion with increased 11-deoxycortisol in direct proportion to the pituitary response
• ***amphenone*** blocks the synthesis of all steroids with adrenal hyperplasia
• mitotane (o,p-DDD) produces adrenocortical cell necrosis and is used in the treatment of adrenal cancers

Transport, Metabolism & Excretion

• cortisol is transported bound to an α globulin ***transcortin***, or corticosteroid binding globulin CBG, and to a small extent bound to plasma albumin
• the plasma half-life for cortisol, $t_{½\beta}$ ~ 60-90 mins

• the bound steroid is inactive
• CBG is synthesised in the liver and its production is increased by oestrogen
• thus, levels are increased during pregnancy and decreased in cirrhosis, multiple myeloma, and renal failure
• changes in protein binding lead to transient changes in the free steroid concentration, ensuing feedback control leads to a proportionate change in the total plasma concentration and a normal free steroid level
• thus pregnant women have high total cortisol levels but no effects of glucocorticoid excess, the opposite is true in nephrosis

• cortisol is metabolised in the liver, as are other glucocorticoids
• the majority is reduced to dihydrocortisol and then tetrahydrocortisol
• the later is conjugated with glucuronic acid and then excreted in the urine
• the glucuronyl transferase system is responsible for the conjugation of a number of other drugs and also bilirubin and these substrates display competition for the enzyme system
• some of the cortisol is converted to cortisone, which, like other steroids with an 11-keto group are metabolites of the secreted glucocorticoids
• little, if any, of the cortisone formed in the liver enters the systemic circulation, being rapidly metabolised and excreted
• approx. 10% of the secreted cortisol is converted in the liver to the 17-ketosteroid derivatives
• these are conjugated with sulphate and excreted in the urine
• there is enterohepatic circulation of the glucocorticoids, and ~ 15% of the secreted cortisol is excreted in the stools
• the metabolism of corticosterone in similar, though, it does not form the 17-ketosteroid derivatives

• the hepatic inactivation of the steroids is decreased by liver disease, post-surgical and other stresses
• thus, in stressed individuals the level of free cortisol rises higher after ACTH stimulation than in non-stressed individuals

• aldosterone is only slightly protein bound and has a short plasma half-life of ~ 20 mins
• the amount secreted is small and the plasma levels are only ~ 0.006 µg/dl, compared to 13.5 µg/dl for cortisol
• most of the aldosterone is converted in the liver to the tetrahydroglucuronide derivative and excreted in the urine

• the major adrenal androgen is the 17-ketosteroid, dehydroepiandrosterone, though androstenedione is also secreted
• around 2/3 of the urinary ketosteroids in men are secreted by the adrenal or formed from cortisol in the liver, the remainder are of testicular origin

Physiological Effects

Androgens

• these exert masculinizing effects, promote protein anabolism and growth
• testosterone from the testes is the most active androgen, the adrenal androgens possessing less than 20% of its activity

• secretion is under the control of ACTH
• the amount of adrenal androgen secreted is ~ equal in normal men, women and castrated men, thus, they exert very little physiological effect
• excess secretion may result in the following syndromes,

a. precocious pseudopuberty - in prepubertal males
b. adrenogenital syndrome - in females
c. pseudohermaphroditism - female embryo's < 12/52

- ***Oestrogens***

• the adrenal androgen, androstenedione is converted to oestrogens in the peripheral circulation, predominantly in adipose tissue
• the quantity formed is generally to small to exert any physiological effect
• however, feminizing oestrogen secreting tumors of the adrenal have been described

Physiological Effects of The Glucocorticoids

• the multiple effects of the glucocorticoids are due to an action on the genetic mechanism controlling protein synthesis
• like other steroids, they act by stimulating DNA-dependent synthesis of specific mRNA's in the nuclei of target cells, leading to the formation of enzymes which alter cellular function
• the effects on intermediary metabolism include,

a. increased protein catabolism
b. increased hepatic glycogenesis and gluconeogenesis
c. G-6-phosphatase activity is increased, as is the BSL
d. opposition to the peripheral actions of insulin
   → diabetogenic, but the heart & brain are spared
e. increase lipolysis & ketogenesis in diabetics
   • not seen usually due to increased insulin secretion

• in addition, there are a number of reactions which require the presence of the glucocorticoids, though, they are not directly responsible for the effects,

a. calorigenic effects of glucagon
b. calorigenic effects of the catecholamines
c. lipolytic effects of the catecholamines
d. catecholamine induced vasoconstriction & bronchodilation

→ so called permissive actions

• glucocorticoids inhibit the secretion of ACTH
• the effects on vascular reactivity are such that in adrenal insufficiency, vascular smooth muscle become unresponsive to the effects of NA & AD, the capillaries dilate and, terminally, become permeable to colloidal dyes
• these effects contribute to the hypovolaemia and vascular collapse seen in adrenal insufficiency
• they are also important in the compensation for blood loss, the decreased end-capillary permeability favoring movement of fluid into the intrvascular compartment, the exact mechanism for this being unsettled

• effects in the CNS seen in adrenal insufficiency include,

a. slowing of the normal α-wave activity of the EEG

b. personality changes

c. increased sensitivity to gustatory & olfactory stimuli

• adrenal insufficiency is also characterised by an inability to excrete a water load, and only the glucocorticoids repair the defect
• the load is eventually excreted, however at a much slower rate and there is considerable danger of water intoxication
• similarly, such patients receiving a glucose infusion may develop a high fever, followed by collapse & death = glucose fever
• the infused glucose is metabolised to CO_2 & water, with subsequent hypo-osmolar disruption to the thermoregulatory centres in the hypothalamus
• the cause for the defective water excretion is unsettled
• plasma ADH levels are elevated and are reduced by glucocorticoid administration, also, the GFR is low due to the relative hypovolaemia, and this probably contributes
• even though the mineralocorticoids restore the plasma volume and improve the GFR, they do not elevate GFR to the same degree as the glucocorticoids

• glucocorticoids decrease the circulating levels of eosinophils by increasing their sequestration in the spleen and lungs
• they also lower the number of basophils, and increase the numbers of rbc's, platelets and neutrophils
• they decrease the circulating lymphocyte count and decrease the size of lymhpoid tissues by inhibiting lymphocyte mitotic activity
• this is achieved by inhibition of the synthesis of IL-2 by T-cells, thus effectively stopping lymphocyte proliferation

• most stressful stimuli that increase the secretion of ACTH also activate the sympathoadrenal system and increase the levels of circulating CA's
• the role of cortisol under these circumstances may be to maintain the vascular reactivity to the CA's, in addition to the other permissive effects above
• another role of cortisol may be the intravascular shift of fluid

Cushing's Syndrome

- the clinical picture of glucocorticoid excess, characterised by,

a. protein depletion due to excess catabolism
   - wasted muscles, especially the limbs
   - poor wound healing
   - thin skin and subcutaneous tissues
   - bruise easily
   - thin & scraggy hair

b. increased adrenal androgen secretion
   - facial acne
   - increased facial hair
   - frontal baldness

c. redistribution of body fat
   - thinning of the extremities
   - fat deposition on the trunk, abdomen, upper back
   - "buffalo hump" & abdominal striae

d. metabolic effects
   - diabetic GTT
   - hyperlipidaemia and ketosis but acidosis is not severe

e. mineralocortocoid actions
   - salt & water retention, volume expansion
   - may be K^+ depletion and weakness
   - hypertension ~ 85%
   - oedema + facial obesity $\rightarrow$ "moon facies"

f. effects on bone homeostasis
   - demineralisation of bone with osteoporosis ± fractures

g. central nervous system effects
   - acceleration of the basic EEG rhythm
   - personality changes from increased appetite, insomnia through to euphoria and frank psychoses

Anti-Inflammatory Actions

• in large doses the glucocorticoids inhibit the tissue response to injury and also suppress the manifestations of allergic disorders
• neither of these effects is produced at physiological levels
• large therapeutic doses inhibit secretion of ACTH, such that severe adrenal insufficiency may be seen upon withdrawal
• the decreased response is due, in part, to inhibition of phospholipase A_2, with a consequent reduction in the release of arachidonic acid from tissue phospholipids
• thus, the production of leukotrienes, thromboxanes, prostaglandins & prostacyclin is reduced
• other effects produced include,

a. reduce the effects of collagenase
b. inhibition of fibroblastic activity
c. decreased release of IL-1 (endogenous pyrogen) from PMN's
d. initially elevate then depress Ab production
e. prevent the release of histamine from mast cells

Regulation of Glucocorticoid Secretion

• both the basal release and the secretion in response to stress are mediated by ACTH from the anterior pituitary
• angiotensin II also stimulates the adrenal cortex, but its effects are mainly on aldosterone secretion
• a large number of endogenous substances will stimulate secretion, including ADH & VIP, but they play no role in physiological regulation
• ACTH is a single polypeptide (39AA), originating from POMC
• the first 23 AA's are the same in all species and this fragment has full activity in vivo
• its plasma half-life, t½ ~ 10 mins, but the site of inactivation is uncertain
• after hypophysectomy secretion falls to very low levels within 1 hr
• with low doses of ACTH there is a linear relationship between log dose and the increase in secretion, however an "output ceiling" is soon reached
• in patients with hypopituitarism, single doses of ACTH do not restore adrenal responsiveness, and repeated injections or infusions are required
• similar derceases in responsiveness may be seen after prolonged glucocorticoid administration
• normal secretion follows a circadian rhythm, such that secretion is highest in the early mornings and least in the evenings
• regulation of this rhythm appears to be in the suprachiasmic nuclei of the hypothalamus
• the response to stress is mediated through the hypothalamus by the release of CRH from the median eminence, which reaches the anterior pituitary via the hypothalamo-hypophyseal portal circulation
• afferent nerve pathways from many regions of the brain converge on the median eminence
• fibres from the amygdaloid nuclei mediate responses to fear, emotional stress, anxiety and apprehension, while fibres from the nociceptive pathways and ARAS mediate responses to injury
• there is also inhibitory input from the baroreceptors via the NTS and contrary to previous beliefs, NA and AD do not decrease ACTH secretion
• high levels of glucocorticoids inhibit ACTH secretion in a linear fashion

• the inhibition is exerted at both pituitary and hypothalamic levels and is due to an action on DNA, maximal inhibition taking several hours to develop
• the ACTH-inhibiting activity of the various hormones parallels their glucocorticoid potency

NB: thus ACTH secretion is the sum of two opposing influences,

i. the sum of neural input to the median eminence → CRH
ii. the direct inhibition by plasma glucocorticoids

Physiological Effects of The Mineralocorticoids

• aldosterone and other hormones with mineralocorticoid action increase the reabsorption of Na^+ from the urine, sweat, saliva and gastric juice
• in the kidney they act on the distal tubule and collecting duct, effectively exchanging Na^+ for K^+ & H^+, producing a K^+ diuresis and increased urine acidity
• they may also increase the K^+ and decrease the Na^+ in muscle and brain cells
• like other steroids, they combine with a cytosolic receptor which migrates to the nucleus to stimulate DNA-dependent mRNA synthesis, the mRNA then stimulating protein synthesis at the ribosomal level
• aldosterone fails to exert any effect for 10-30 mins, representing the time required for increased protein synthesis
• the amount of Na^+ retained is directly proportional to the rate of active transport, the energy being derived from the ATPase pump and the CAC
• there are, however, 3 hypotheses regarding the mechanism of action of aldosterone,

a. permease hypothesis → increased luminal gNa^+
b. metabolic hypothesis → increased substrate oxidation
c. Na^+ pump hypothesis → direct action on the Na-K-ATPase

• in any case the net effect is increased activity of the basal pump with removal of Na^+ from the tubular lumen
• aldosterone is the principal mineralocorticoid secreted by the adrenal, however, corticosterone is secreted in sufficient quantities to exert a minor action
• deoxycorticosterone is secreted in appreciable amounts only in abnormal situations and has ~ 3% of the activity of aldosterone
• its synthetic acetate, desoxycorticosterone acetate DOCA, is used clinically as it is cheaper and more readily available than aldosterone

▪ ***Effects Of Adrenalectomy***

• Na^+ is lost in the urine with commensurate loss of water, K^+ is retained in the plasma with resulting hyperkalaemia
• when insufficiency appears rapidly the decline in ECF Na^+ exceeds the urinary losses, indicating that Na^+ must also be entering the cells
• when the posterior pituitary is intact, salt loss exceeds water loss with resulting hyponatraemia
• the loss of ECF volume is manifest by hypotension, circulatory insufficiency and eventually shock

Effects of Excess Mineralocorticoids

• K^+ diuresis and hypokalaemia are prominent features of prolonged mineralocorticoid excess
• when K^+ depletion is marked, intracellular K^+ is replaced by Na^+, and if dietary intake is unrestricted the total body Na^+ increases
• the plasma Na^+ remains stable as water is retained along with the excess Na^+ ions, the ECF volume is expanded and hypertension may result
• when ECF expansion passes a certain point, Na^+ excretion is usually enhanced despite the action of the mineralocorticoids → escape phenomenon, probably due to the action of atrial natriuretic factor
• due to this escape, oedema rarely develops in patients with primary hyperaldosteronism, though, in the disease states of secondary aldosteronism this escape is not observed and there is continued expansion of the ECF

Primary Aldosteronism

• usually due to aldosterone secreting tumors of the adrenal cortex, Conn's Syndrome
• they are severely K^+ depleted, volume expanded and hypertensive, however they are not markedly hypernatraemic or oedematous
• the prolonged hypokalaemia results in,

a. damage to the kidney, hypokalaemic nephropathy
   - resulting in loss of concentrating ability and polyuria
b. muscle weakness
c. alkalosis, to the extent where plasma Ca^{++} may be reduced to the point of frank tetany
d. a minor but detectable decrease in glucose tolerance

Regulation of Aldosterone Secretion

• four direct inputs to the adrenal regulate aldosterone secretion,

a. plasma $[Na^+]$
b. plasma $[K^+]$ |
c. angiotensin II | → increased cytosolic Ca^{++}
d. ACTH → cAMP

• the adrenal responds directly to the $[K^+]$ and inversely to the $[Na^+]$ in perfusing blood
• the effects of $[Na^+]$ are minor in humans, the plasma Na^+ having to fall acutely by ~ 20 mmol/l to produce stimulation
• the plasma K^+, being more important, results in stimulation with changes of less than 1 mmol/l
• ACTH stimulates release transiently, or when present in high concentrations, but more importantly is permissive for other factors within the physiological range, however, ACTH secretion is not keyed to Na^+ homeostasis
• other possible factors in aldosterone release include β-endorphin, β-lipotropin and dopamine, the first two are secreted with ACTH as products of POMC

• the octapeptide angiotensin II is by far the most important controller of aldosterone secretion in Na^+ regulating reflexes
• accordingly, aldosterone secretion is largely determined by the release of renin which is determined by,

a. intrarenal baroreceptors
b. macula densa
c. renal sympathetic NS
d. angiotensin II

• the actions of angiotensin II are both early and late in the steroid biosynthetic pathway,

a. conversion of cholesterol to pregnenolone
b. conversion of corticosterone to aldosterone

• Na^+ depletion increases the binding of angiotensin II to the adrenal cortex, by increasing both the affinity, and the number of A-II receptors
• at the same time there is a decrease in the binding of A-II to blood vessels
• individuals who are confined to bed display a circadian rhythm of renin and aldosterone secretion, with the peak occurring in the early a.m., prior to waking

■ ***Regulation Of Salt Balance***

• aldosterone in only one of the factors maintaining salt and ECF balance, others include,

a. GFR
b. ANF
c. glomerulotubular balance
d. direct tubular effects of catecholamines
e. direct tubular effects of angiotensin II
f. distribution of intrarenal blood flow

THE THYROID GLAND

Anatomy

- originates from an evagination in the floor of the pharynx
- occasionally a ***thyroglossal duct*** persists marking the tract from the tongue to the neck
- the gland consists of two main ***lobes***, connected by a bridge of tissue, the ***thyroid isthmus***
- there is occasionally a ***pyramidal lobe*** arising from the isthmus, situated in the front of the larynx
- the gland is well vascularised and has one of the highest blood flows per gram of tissue
- the gland is composed of multiple ***acini*** (follicles), each spherical follicle being surrounded by a single layer of cells and being filled with ***colloid***
- when inactive, the colloid is abundant, the follicles are large and the cells lining them are flat
- when active, the follicles are small, the cells are cuboidal or columnar, the edge of the colloid is scalloped, forming many small "reabsorption lacunae"
- microvilli project into the colloid from the lining cells
- there is prominent endoplasmic reticulum, as for other gland cells
- the endothelial cells of adjacent capillaries are fenestrated

Formation & Secretion of Thyroid Hormones

- the principal hormones secreted by the thyroid are,

    i. thyroxine **T_4**

    ii. thiiodothyronine **T_3**

- the later also being produced in the peripheral tissues by deiodination of T_4 (~ 80% of T_3)
- both hormones are iodine containing ***amino-acids*** (Ganong fig. 18-4)
- T_3 is more active than T_4 and reverse T_3 (rT_3) is inactive
- the naturally occurring forms of T_4 and its cogners are the ***l-isomers***

▪ ***Thyroglobulin***

- T_3 & T_4 are synthesised in the colloid by iodination and condensation of ***tyrosine*** molecules, bound in a peptide linkage in thyroglobulin
- this glycoprotein is synthesised in the thyroidal cells and secreted into the colloid by exocytosis, along with ***thyroid peroxidase***
- the hormones remain bound to ***thyroglobulin*** until their secretion
- thyroid cells thus have three functions,

    1. to collect and transport iodine
    2. synthesise thyroglobulin and secrete it into the colloid
    3. removal of thyroid hormones from thyroglobulin and their secretion into the circulation

- thyroglobulin is also secreted into the circulation, however no physiological role is known

Iodine Metabolism

- ingested iodine is converted to ***iodide***, I^- and absorbed
- the minimum adult daily requirement to maintain thyroid function ~ 100-150 µg
- the thyroid secretes ~ 80 µg of I^- per day as T_3 & T_4
- of which ~ 60 µg is metabolised in the liver with release of I^- into the ECF
- thus, the total I^- added to the plasma per day is approximately,

a. 500 µg - average "Western" dietary intake

b. 60 µg - from the liver metabolism of T_3 & T_4

c. 40 µg - diffusion from the thyroid to the ECF

→ **~ 600 µg** of iodide is distributed throughout the ECF daily and is taken up by the thyroid and kidney

- the thyroid takes up ~ 20% of this, or **~ 120 µg/d**
- the remaining 80% is taken up by the kidney and excreted in the urine

■ ***Iodide Trapping***

- the thyroid concentrates I^- by actively transporting it from the plasma into the colloid, the mechanism for which is termed iodide trapping
- the thyroid cells have a resting E_m ~ -50 mV
- I^- is transported ***against*** this electrical gradient by an active basal pump
- once intracellular, it then diffuses down its δEC gradient into the colloid
- in the gland the I^- is rapidly oxidised and bound to tyrosine
- despite this binding, the ratio of thyroid:plasma [free I^-], or ***T:S ratio*** is normally > 1.0
- if I^- binding is blocked by drugs, such as ***propylthiouracil***, then the ratio is markedly increased
- the active transport is stimulated by ***TSH***, and is dependent upon the Na^+/K^+-ATP'ase, therefore is inhibited by ***ouabain***
- other glands which transport I^- against a δEC gradient include,

a. salivary glands

b. gastric mucosa

c. placenta

d. ciliary body of the eye

e. choroid plexus

f. mammary glands

NB: however, these are ***insensitive*** to TSH and their role is uncertain

- iodine deficiency ***goitre*** results from TSH induced hypertrophy of the gland

Thyroid Hormone Synthesis & Secretion

• I^- is oxidised to iodine & bound to tyrosine (3-position) within seconds of entering the thyroid
• this reaction is catalysed by ***thyroid peroxidase*** and hydrogen peroxide acts as the electron receptor
• ***monoiodotyrosine*** (MIT) is next iodinated at the 5-position forming DIT
• two ***DIT*** molecules then undergo ***oxidative condensation***, leaving dehydroalanine on one residue and thyroxine T_4 on the other, both still in peptide linkage to ***thyroglobulin***
• T_3 is probably formed by the condensation of MIT & DIT
• a small amount of rT_3 is also formed, probably by the condensation of DIT & MIT
• the condensation reaction is an aerobic, energy requiring event, and like the oxidation and binding appears to be catalyzed by thyroid peroxidase
• in the normal human thyroid, the normal distribution is,

a. MIT ~ 23%
b. DIT ~ 33%
c. T_4 ~ 35%
d. T_3 ~ 7%

• the average daily secretion is,

a.	T_4	~ 80	µg	103	nmol
b.	T_3	~ 4	µg	7	nmol
c.	rT_3	~ 2	µg	3.5	nmol

• colloid is absorbed by ***endocytosis***, the peptide linkages are broken-down by proteases in lysosomes, and the products are released into the cytoplasm
• the iodinated tyrosines (MIT & DIT) are deiodinated by ***iodotyrosine dehalogenase***
• the iodinated ***thyronines*** (T_3 & T_4) are released into the circulation

▪ *Transport & Metabolism*

• both hormones are bound to plasma proteins and are measured directly by radioimmunoassay, thus replacing the protein-bound iodine as an index
• the proteins which bind thyroid hormones include,

a. albumin
b. thyroxine-binding prealbumin (TBPA)
c. thyroxine binding globulin (TBG)

• TBG resides between α_1 & α_2-globulin
• of the three proteins, albumin has the highest capacity to bind T_4, however the affinities are such that most circulating T_4 is bound to ***TBG***

Thyroid Hormone Binding				
Protein	Concentration (mg/dl)	Half-life (days)	**T_4** binding %	**T_3** binding %
Albumin	3,500	13	13	53
TBG	2	5	**67**	46
TBPA	15	2	10	1
Normal Plasma level:			8.0 µg/dl	0.15 µg/dl
Total percentage bound:			99.98%	99.8%
Plasma free hormone concentration:			2 ng/dl	0.3 ng/dl

- as these hormones are highly protein bound, thus,
    a. their Vd's are less than ECF ~ 10 l, or ~ 15% body weight
    b. their plasma half-lives are long, T_4 ~ 6-7 days ($T_3 \rightarrow$ less)
    c. minimal amounts are found in the urine

- it is the ***free fractions*** which are physiologically active, being in equilibrium with protein bound hormones in the tissues and being responsible for inhibition of pituitary TSH secretion
- as with other protein bound entities, alterations in the levels of the carrier proteins results in only a transitory change in the levels of free agent
- a new equilibrium being achieved at euthyroid free hormone levels
- TBG levels are elevated in pregnancy, hepatic disease and in oestrogen treated patients
- levels of TBG are decreased by,
    a. glucocorticoids
    b. androgens
    c. danazol - antioestrogen
    d. l-aspariginase - cytotoxic

- decreased binding to TBG, producing effects similar to above, is seen with,
    a. salicylates
    b. phenytoin
    c. mitotane & 5-fluorouracil

- changes in total T_3 & T_4 can also be caused by changes in plasma albumin and prealbumin

Metabolism Of Thyroid Hormones

• both hormones are normally deiodinated in the liver, kidneys and other tissues
• of the circulating T_4,

i. ~ 33% is converted to T_3
ii. ~ 45% is converted to rT_3

• thus, far more T_3 is formed by deiodination than is secreted by the thyroid
• during foetal life, far more rT_3 is formed and the ratio shifts to the adult pattern by ~ 6 weeks after birth
• because T_3 acts more rapidly and is ~ 3-5 times as potent on a molar basis, T_4 is believed to be metabolically inert until deiodinated→ ***prohormone***
• additional evidence for this is that T_3 binds nuclear receptors with far greater affinity
• however, a substantial portion of the T_3 in the pituitary is formed from intracellular deiodination, thus the negative feedback on TSH is mediated predominantly by T_4
• in the liver they are conjugated with sulphate and glucuronide and then enter the bile, from where they are hydrolyzed and undergo ***enterohepatic circulation***
• the total daily iodide loss in the stools ~ 4%

• a number of commonly used drugs inhibit the ***5'-deiodinase*** which converts T_4 to T_3,

i. glucocorticoids - liver
ii. propylthiouracil - liver & kidney
iii. propranolol - liver & kidney

• ***5'-deiodinase*** also catalyses the conversion of rT_3 to 3,3'-DIT, therefore, plasma T_3 levels fall and rT_3 levels increase
• none of these inhibit conversion in the ***anterior pituitary*** → no reflex ↑ TSH
• ***amiodarone***, and cholecystographic radiocontrast dyes also inhibit conversion in the pituitary

→ ↑ TSH levels

• a wide variety of nonthyroidal illnesses also depress the activity of 5'-deiodinase and result in a reversible low T_3 state,

i. severe burns or trauma
ii. advanced malignancies
iii. cirrhosis
iv. renal failure
v. AMI
vi. febrile states

• ***fasting*** affects deiodination,

i. ↓ T_3 levels ~ 10-20%in 24 hrs
~ 50% in 3-7 days
ii. free and bound T_4 levels remain normal
iii. ↓ BMR and urinary nitrogen conserving calories and protein

• overfeeding has the opposite effects

Effects of Thyroid Hormones

- most of the widespread effects are secondary to ↑ O_2 consumption, the ***calorigenic effect***
- other effects include,
    a. growth and maturation
    b. regulation of lipid metabolism
    c. increase absorption of CHO from the GIT
    d. increase 2,3-DPG & shift Hb-O_2 dissociation curve to the ***right***

- oddly, the iodine atoms are not essential for physiological activity, and a number of synthetic analogues are free of iodine

▪ ***Calorigenic Action***

- T_3 & T_4 increase the VO_2 in all tissues, except,
    a. adult brain
    b. testes
    c. uterus
    d. spleen & lymph nodes
    e. anterior pituitary (actually decreases)

- anterior pituitary VO_2 actually decreases, presumably due to decreased secretion of TSH
- a single dose has a latency of several hours and the effects are evident for 6 or more days
- the magnitude of the calorigenic response is dependent upon,
    a. the level of circulating CA's
    b. the basal VO_2 - greatest effects are seen at ***low*** BMR's

- doses of T_4 ~ 2.2 µg/kg produce near normal plasma TSH, T_3 & T_4 post-thyroidectomy

▪ ***Effects Secondary to Calorigenesis***

• when the BMR is increased nitrogen excretion is also increased
• if total caloric intake is not increased proportionately, protein and fat stores are catabolised and weight is lost
• in hypothyroid children, small doses of T_4 may result in a (+)'ve nitrogen balance as growth in stimulated, however, large doses result in a similar pattern to that seen in adults
• large doses may raise the core temperature, activating reflex mechanisms,

a. decrease in TPR with cutaneous vasodilation
b. CO rises due to direct effects & those of the CA's
   → pulse pressure & HR are increased and the circulation time shortened

• in the absence of thyroid hormones a ***mild anaemia*** develops due to decreased bone marrow metabolism and decreased absorption of B_{12} from the gut
• when the BMR is increased the requirement for all vitamins is increased and hyperthyroidism may unmask vitamin deficiencies
• thyroid hormones are necessary for the conversion of ***carotene*** to vitamin A in the liver
• accumulation in hypothyroidism is responsible for the yellow discoloration of the skin
• thyroid hormones are also essential for normal menstrual cycles and fertility

▪ ***Effects on the Nervous System***

• hypothyroidism results in slowed mentation and elevated CSF protein levels
• CBF, C-VO_2 and glucose consumption are ***unaffected*** by thyroid hormones
• T_4 crosses the BBB and is found in the gray matter in a number of locations
• the brain actively converts T_4 to T_3 and 5'-deiodinase activity is increased after thyroidectomy
• some of the CNS effects are secondary to increased responsiveness to the CA's, with subsequent increased activation of the ARAS
• thyroid hormones have marked effects on brain ***development***,

i. hypothyroid infants develop synapses abnormally
ii. myelination is defective
iii. mental development is seriously retarded

• they also affect the peripheral nervous system, decreasing the reaction time for stretch reflexes

▪ ***Effects on Skeletal Muscle***

• hyperthyroidism results in muscle wasting and weakness, ***thyrotoxic myopathy***
• this is in part due to protein catabolism but also due to alterations in the myosin chains
• hypothyroidism results in weakness, stiffness and cramps

- ***Effects on the Heart***

• these hormones increase the number & affinity of β-adrenergic receptors in the heart, thus increasing the actions of the CA's
• there are 2 myosin heavy chain isoforms in the heart, α-MHC predominating in the adult ventricles and its proportion increased by thyroid hormone
• expression of the α-MHC gene is depressed and the β-MHC gene enhanced in hypothyroidism, the later having less myosin ATP'ase activity than the former

- ***Other Effects***

a. increased absorption of CHO from the GIT
b. increased number of LDL receptors and a decrease in circulating cholesterol levels
c. required for normal bone growth & epiphyseal closure
d. increase the secretion and potentiate the effects of GH

- ***Mechanism Of Action***

• T_3 binds with a ***nuclear receptor***, the nonhistone proteins in the chromatin and acts on DNA to increase the synthesis of ***mRNA*** and ribosomal RNA
• the mRNA dictates the formation of proteins in the ribosomes, and these thyroid induced proteins act as enzymes which modify cellular function
• the calorigenic action of thyroid hormones is blocked by agents which inhibit protein synthesis
• the proteins increase the activity of the membrane bound Na-K-ATP'ase in many tissues, and this in part accounts for the increased VO_2
• inhibition of the pump with ***ouabain*** reduces, but does not abolish the increase

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NB: the old theory that these hormones increase the VO_2 by uncoupling oxidative phosphorylation has now been largely ***abandoned***

Regulation of Secretion

• the primary control is via circulating pituitary ***TSH***, under the control of hypophyseotrophic ***TRH*** and the feedback from circulating T_3 & T_4
• TSH secretion is also inhibited by stress and warmth, and in increased by cold
• human TSH is a 211 AA glycoprotein made of 2 subunits, α & β
• each subunit is synthesised from a separate precursor molecule and the TSH-α unit is identical to the α-subunit of FSH & LH and differs only slightly from hCG-α
• function specificity is thus conferred by the ***b-subunit***
• the biological half life, $t_{½\beta}$ ~ 60 mins, and degradation is predominantly in the kidney and to a lesser extent in the liver
• secretion is pulsatile and increases around 2100 hrs, reaching a maximum at 2400 hrs and declining throughout the day

• in the absence of TSH the thyroid atrophies, iodine trapping falls and blood flow decreases
• all of these are reversed by TSH administration, however, this growth response requires the presence of GH, corticosteroids and insulin
• TSH alone will not result in thyroid hypertrophy in hypophysectomised animals
• the TSH receptor is made of a glycoprotein linked to a ganglioside, which acts by activating adenylate cyclase and increasing cAMP
• Ab's against the receptor are responsible for Graves' disease, most are IgG type and are collectively called ***thyroid stimulating immunoglobulins TSI***
• Ab's are both inhibitory and stimulatory,

a. the former are formed against the glycoprotein component of the receptor
b. the later may actually be Ab's against Ab's to the ganglioside component, ie. ***anti-idiotope Ab's***

• cAMP is responsible for most TSH effects, however it also increases the metabolism of cell membrane phospholipids and this later effect is responsible for the hypertrophy of the gland
• the (-)'ve feedback on TSH secretion is mainly at the anterior pituitary level as T_3 & T_4 block the TSH response to TRH administration
• the thyroid hormones inhibit secretion before they inhibit synthesis, and their administration results in an initial increase in TSH content in the pituitary
• the role of the hypothalamus appears to be limited to special situations such as cold & warmth
• the rise in TSH produced by cold in adult humans is negligible, thus thyroid hormone thermogenesis plays a minimal role in the adult response to cold
• the inhibition seen in stress is probably due to glucocorticoid induced inhibition of TRH
• dopamine & somatostatin inhibit pituitary TSH secretion but their physiological role is uncertain

Radioiodine Uptake

• hyperthyroidism → rapid uptake & clearance of ^{131}I activity
• levels at 24 hrs may be falling whereas in euthyroid individuals the gland is still taking up iodine at this stage
• the uptake is low in hypothyroidism, however may also be low in individuals on a high iodine diet
• this is the basis of iodine treatment of individuals in radioactive fallout regions
• large doses may be used for effective ablation of the gland in thyrotoxicosis

Antithyroid Drugs

• most act by either blocking the I^- trapping mechanism, or by blocking organic iodine binding
• TSH secretion is induced and hypertrophy of the gland results
• a number of ***monovalent ions*** compete with iodide for uptake into the thyroid and thus reduce the T/S ratio to around unity, these include,

a. chlorate & perchlorate
b. pertechnetate
c. periodate & bi-iodate
d. nitrate

• the ***thiocarbamides*** inhibit the iodination of MIT and block the coupling reaction
• the two clinically used members are ***propylthiouracil & methimazole***
• these compete with tyrosine for iodine and are themselves iodinated
• propylthiouracil, but not methimazole, also inhibits the peripheral conversion of T_4 to T_3
• both drugs may also ameliorate thyrotoxicosis by suppressing the immune system and reducing the formation of TSI, and by inhibiting the biosynthesis of thyroglobulin
• the effects inhibiting coupling are produced at lower doses than the effects on iodination
• the drugs do not block iodide trapping and due to the increased TSH, early in treatment the ^{131}I uptake may be increased, with the T/S ratio becoming as high as 250:1
• large doses of iodide itself act directly to produce a mild transient inhibition of organic binding and hormone synthesis, the ***Wolff-Chaikoff effect***
• this effect is greater when iodide transport in high, thus those with thyrotoxicosis are more susceptible to iodide than normals
• there are two other mechanisms by which iodide inhibits thyroid function,

a. reduces the cAMP response to TSH
b. inhibits proteolysis of thyroglobulin

• in thyrotoxicosis, iodides cause colloid to accumulate, and the vascularity of the gland is decreased, both of which are valuable for preoperative preparation
• there are a number of naturally occurring goitrogens, particularly vegetables of the Brassicaceae family (cabbage & turnips), which contain progoitrin and a substance which converts this to goitrin, an active antithyroid agent

CALCIUM METABOLISM

- elemental metal, AN = 20, divalent cation, MW = 40
- total body content ~ 380 mmol/kg, distributed as follows,

a.	bone	~ 99%
b.	ICF	~ 0.004%
c.	ECF	~ 0.01%
d.	exchangeable	~ 1%

- this equates to ~ 1100 g/average adult, ~ 27.5 mol of Ca^{++}
- the daily requirement in the adult ~ 0.11 mmol/kg
- Ca^{++} is the fifth most plentiful cation in the body
- concentration ranges vary between tissues;

a. ECF **~ 2.2-2.8 mmol/l**
   - i. 45% - ionised Ca^{++}
   - ii. 15% - complexed to low MW anions (citrate, $HPO_4^{=}$)
   - iii. 40% - reversibly bound to plasma proteins (alb, glob.)
     - non-filterable fraction

b. ICF ~ 1 mmol/l total
   ~ 10^{-4} mmol/l as free ionised Ca^{++}
   ~ 99% bound to enzymes in SR, cisternae, & tubules

- only plasma ionised Ca^{++} is biologically active
- the most important influence on protein binding is ***plasma pH***
- an increase of pH increasing the binding of Ca^{++} due to the exposure of more anionic sites

→ decreased ionised Ca^{++}

Important Functions of Calcium

a. ***cytoplasm***
   i. excitation contraction coupling in all muscle
   ii. enzyme cofactor
   iii. regulation of mitotic activity

b. ***cell membrane***
   i. excitability of nerve / muscle membrane
      • setting the threshold V_m for excitation
   ii. automaticity - smooth muscle
      - SA & AV nodes
   iii. neurotransmitter release at nerve terminals (NMJ)
   iv. neuro-hormonal release & activity
      • 1. α-adrenergic - smooth muscle
      - hepatic glycogenolysis
      - salivary secretion
      • 2. ACh - smooth muscle
      - GIT, GB, bladder contraction
      • 3. ADH - smooth muscle (V_1)
      • 4. oxytocin - uterine & myoepithelial
      • 5. angiotensin II - aldosterone secretion from Z.G.
      • 6. CCK - pancreatic secretion
      - GB contraction
      • 7. histamine (H_1) - bronchial contraction
      - GIT smooth muscle contraction

c. ***extracellular***
   i. coagulation cascade - I, II, VII, IX, X
   ii. complement cascade
   iii. bone & teeth formation - Ca^{++} hydroxyapetite

- ***Effector Sites for Calcuim Homeostasis***

a. **GIT**

- the major variable under control for homeostasis is GIT absorption of Ca^{++}
- ***absorption*** ~ 1000 mg with typical daily intake
  ~ 10% absorption
- GIT secretes up to 600 mg/d which is reabsorbed along with the above 10%
- Ca^{++} is actively transported from the brush border by a Ca^{++} dependent ATPase, the activity of which is regulated by 1,25-$(OH)_2$-D_3
- thus, by altering GIT absorption, Ca^{++} balance can be achieved and this is quantitatively the most important variable

b. **kidney**

- ~ 60% of plasma Ca^{++} is ultrafilterable
- reabsorption throughout the nephron, except in the ***DLH***, similar to Na^+
- ~ 60% is reabsorbed in the PT, the remainder in the ALH and DT
- ~ 98-99% of filtered mass is reabsorbed
- the kidneys are involved in Ca^{++} balance, but only ~ 5% of an increment in dietary Ca^{++} appears in the urine
- reabsorption is under control of ***PTH***, however is also affected by large number of other inputs, especially Na^+ and acid-base changes
- there is some coupling of Na^+/Ca^{++} in the PT and ALH
- however this is lost in more distal segments,

i. ***aldosterone*** & ***PTH*** do not affect distal handling of both ions

ii. ***thiazides*** inhibit distal Na^+ reabsorption, however enhance Ca^{++} reabsorption, cf. proximal or loop diuretics which increase excretion of both ions

- chronic metabolic ***acidosis*** markedly ***increases*** Ca^{++} excretion with subsequent loss from bone
- alkalosis produces the opposite

c. **bone**

- ~ 99% of total body Ca^{++} held as ***hydroxyapetite***
- calcium in bone is of two types,

i. readily exchangeable Ca^{++}

ii. the larger pool of stable, slowly exchangeable Ca^{++}

- two systems affect calcium movement to & from bone,

i. regulation of plasma Ca^{++} $\rightarrow$ movement of ~ 500 mmol/d

ii. ***remodelling*** of bone $\rightarrow$ over 95% of new bone formation

- interchanges of Ca^{++} between ECF and bone affect the internal distribution, not the body mass of Ca^{++}
- this acts as an enormous sink for exchange of Ca^{++} with the ECF

- ***Control Mechanisms***

  a. $[Ca^{++}].[HPO_4^{=}]$ solubility product
     - product > 6 increases the likelihood of ***ectopic calcification***

  b. parathyroid hormone

  c. vitamin D - 1,25 dihydroxycholecalciferol

  d. calcitonin

- ***Secondary Influences***

  a. steroids - decrease Ca^{++}

  b. growth hormone - increase Ca^{++}

  c. albumin levels ~ 0.02 mmol Ca^{++} / gram albumin (**0.2 mmol/10g**)

  d. acid-base status
     i. acidosis - increases Ca^{++}
     ii. alkalosis - decreases Ca^{++}

  e. renal function - GFR
     - tubular excretion
     - hydroxylation of 1-(OH)-D_3

  f. thyroid hormones - increase Ca^{++}

  g. glucagon - decrease Ca^{++}

- ***Hormonal Control of Effector Sites***

a. **parathyroid hormone**

i. increases movement of Ca^{++} and $HPO_4^{=}$ out of bone
ii. increases renal tubular reabsorption of Ca^{++}
iii. reduces renal tubular reabsorption of $HPO_4^{=}$
iv. stimulates production of Vit. D $\rightarrow$ ***indirect effects***

- inhibits proximal tubular H^+ secretion & HCO_3^- reabsorption
- the decrease in plasma pH displaces Ca^{++} from plasma protein and bone
- increased $HPO_4^{=}$ excretion aids further reabsorption from bone due effect on $[HPO_4^{=}].[Ca^{++}]$ solubility product

- ***NB:*** ***hyperparathyroidism*** causes,

i. an elevated plasma calcium with a low to normal phosphate
ii. enhanced bone reabsorption with cysts
iii. ectopic calcification
iv. renal stones
  - renal Ca^{++} excretion increases, despite the elevated PTH, as the filtered mass increases >> the reabsorptive increase

b. **vitamin D**

- actually a group of closely related ***sterols***,

7-dehydrocholesterol	+ UV light		$\rightarrow$	D_3
D_3	+ liver	25-hydroxylation	$\rightarrow$	25-(OH)-D_3
25-(OH)-D_3	+ kidney	**1-hydroxylation**	$\rightarrow$	1,25-$(OH)_2D_3$

- by definition this is a ***hormone*** not a vitamin
- also absorbed from the GIT, the plant form differing only slightly
- **1-*hydroxylation*** is increased by PTH and a low plasma $HPO_4^{=}$
- also increased by oestrogen and prolactin (pregnancy)
- the major actions of vitamin D are,

i. enhance GIT absorption of Ca^{++} and $HPO_4^{=}$
ii. enhance the reabsorption of Ca^{++} and $HPO_4^{=}$ from bone
iii. stimulates the renal tubular reabsorption of Ca^{++} (the significance of this is unsettled)

- ***NB:*** ***hypervitiminosis D***, results in an elevated Ca^{++} and $HPO_4^{=}$

c. **calcitonin**

- secreted by the ***parafollicular cells*** of the thyroid gland in response to a raised plasma Ca^{++}
- lowers the plasma calcium principally by inhibiting bone reabsorption
- overall contribution to homeostasis is very ***minor***

PHYSIOLOGY OF BONE

Structure

• bone is a living tissue with a collagenous protein matrix, impregnated with mineral salts, especially phosphates of calcium
• it functions to,

1. support the body
2. provide a store for Ca^{++} and other minerals, and
3. aid the lungs & kidneys in acid-base balance by providing additional phosphate and bicarbonate buffer

• the protein in bone is predominantly ***type I collagen***
• the mineral predominantly hydroxyapetites
• histologically there are 3 types of bone,

1. compact bone - in shafts of long bones & on outer surfaces
2. cancellous, or trabecular bone- in the marrow cavities
3. woven bone - an immature form found at fracture sites

• compact bone is organised into ***Haversian systems***, or osteons, where cylinders of consolidated bone are formed around central blood vessels
• bone is cellular and well vascularised, total ***blood flow*** **~ 200-400 ml/min**
• the calcium in bone turns-over at a rate of 18%/year in the adult and nearly 100% /yr in infants
• cells present in bone include,

a. osteoblasts - secreting collagen which is then calcified
   - derived from osteoprogenitor cells of ***mesenchymal*** origin
b. osteocytes - bone cells surrounded by calcified matrix
c. osteoclasts - multinuclear cells eroding bone
   - derived from the ***haemopoietic*** stem cells via monocytes

• osteocytes remain in contact with each other and active osteoblasts by tight cellular junctions on long protoplasmic processes which run through channels in the bone

▪ ***Bone Formation & Reabsorption***

NB: the exact mechanism by which new calcification occurs is uncertain

• osteoblasts form a partial membrane separating bone fluid from the ECF, thus allowing regulation of Ca^{++} & P_i concentrations
• whether calcium salts precipitate depends on the product of the concentrations of Ca^{++} and PO_4^{3-}, above a certain value of this ***solubility product*** the solution is saturated
• associated with the osteoblasts is an ***alkaline phosphatase*** which hydrolyzes phosphate esters, thus increasing the local $[P_i]$
• bone also contains a protein with a large number of gamma-carboxyglutamic acid residues, which bind Ca^{++}, and a high concentration of this protein is associated with ongoing calcification
• however, deficiency of vitamin K which catalyzes gamma-carboxylation, only results in skeletal malformation in the foetus
• resorption is effected by both osteocytes and osteoclasts which increase their permeability to Ca^{++} in response to ***PTH***

▪ ***Bone Growth***

• the bones of the skull are formed by ossification of membranes

→ ***intramembranous bone formation***

• the long bones are first modelled in cartilage, ossification beginning in the shaft and the ends,

→ ***endochondral bone formation***

• specialised areas at each end, the ***epiphyses*** are separated from the shaft of the bone by actively proliferating cartilage, the ***epiphyseal plate***
• growth in bone length occur as new bone is laid down on the end of the shaft, the rate of growth being proportional to the width of the plate
• the width is most markedly affected by pituitary GH via somatomedins
• linear bone growth can occur so long as the epiphyses are separated from the shaft of the bone, ceasing with epiphyseal closure

▪ ***Uptake Of Other Minerals***

• a number of other minerals can be taken-up and incorporated into the skeleton, rapid bone uptake effectively removing these from the circulation
• these include,

i. lead
ii. plutonium
iii. strontium
iv. fluoride - stimulates new bone formation

Metabolic Bone Disease

• ***osteosclerosis*** refers to the presence of increased amounts of calcified bone
• this occurs in,

1. metastatic tumors
2. lead poisoning
3. hypoparathyroidism

• ***osteomalacia*** refers to disorders where the amount of mineral accretion in bone, per unit of bone matrix, is deficient
• osteoporosis is a decrease in bone mass with a normal mineral:matrix ratio
• this is caused by increased bone resorption, or decreased new bone formation
• postmenopausal bone loss is increased due to oestrogen deficiency, however the mechanism involved in uncertain, possible mechanisms being,

a. decreased renal α-1-hydroxylase and D_3 activation

b. decreased calcitonin secretion → increased bone resorption
increased plasma Ca^{++}
decreased PTH secretion
decreased levels of 1,25-$(OH)_2$-D_3

• osteoporosis also develops with disuse, as in immobilization or weightlessness, and with excess glucocorticoids

Vitamin D & The Hydroxycholecalciferols

▪ ***Chemistry***

• actually a group of closely related sterols
• vitamin D_3, cholecalciferol, is formed by the action of UV light on 7-dehydrocholesterol in the skin
• vitamin D is also absorbed from the GIT and the plant form differs only slightly from the endogenous variety
• vitamin D_3 and its hydroxylated derivatives are transported in the plasma bound to a specific globulin, vitamin-D-binding protein DBP, which moves D_3 from the skin to the circulation
• vitamin D_3 is hydroxylated in the liver at the 25-position and activated by the kidney by further hydroxylation at the 1-position to 1,25-$(OH)_2$-D_3, or calcitriol, which by definition is a hormone not a vitamin
• final hydroxylation in the kidney is stimulated by PTH and a low plasma phosphate []n, also, formation is increased by oestrogen and prolactin, as in pregnancy
• in normal humans the kidney is the only site of 1-hydroxylation, however this may occur extrarenally in some disease states such as sarcoid

- ***Actions***

• in the intestinal epithelial cells it binds to a cytoplasmic receptor, is translocated to the nucleus and stimulates the formation of specific mRNA, which dictates the formation of calcium binding protein
• how this protein increases absorption is unsettled, but the major action of vitamin D is to enhance the absorption of calcium and phosphate from the GIT
• D_3 does increase the basolateral pumping of Ca^{++} from intestinal cells
• the Ca^{++} binding proteins have been isolated and are of two types, with MW's of 9700 & 28,000 respectively
• 1,25-$(OH)_2$-D_3 receptors have been located in a number of tissues in addition to the intestine, including,

a. brain
b. kidney
c. various endocrine glands
d. monocytes

• many of these contain only one type of binding protein, however the physiological function is uncertain
• 1,25-$(OH)_2$-D_3 also enhances the reabsorption of calcium and phosphate from bone, possibly by an interaction with PTH, and can stimulate the renal tubular reabsorption of calcium, but the significance of this is unsettled

- ***Regulation Of Synthesis***

• formation of 25-hydroxycholecalciferol does not appear to be regulated and is substrate dependent
• however, 1-α-hydroxylase activity is regulated in a feedback fashion by plasma Ca^{++} & PO_4^{3-}
• formation is facilitated by PTH, which is increased when the plasma Ca^{++} is low
• when the plasma Ca^{++} is high, little 1,25-$(OH)_2$-D_3 is formed, the kidney producing the inactive metabolite 24,25-di-OH-D_3
• the production of 1,25-$(OH)_2$-D_3 is also increased by a low, and decreased by a high plasma PO_4^{3-}
• this is due to a direct inhibitory effect of $PO_4$3- on 1-α-hydroxylase
• oestrogens and prolactin increase 1-α-hydroxylase activity and circulating 1,25-$(OH)_2$-D_3 levels are increased during pregnancy
• 1,25-$(OH)_2$-D_3 production is decreased during metabolic acidosis and insulin deficiency

THE PARATHYROID GLANDS

Anatomy

• usually number 4, 2 in the superior poles and 2 in the inferior poles of the thyroid, though, considerable variation exists
• parathyroid tissue is occasionally found in the mediastinum
• each gland is a richly vascularised disc, ~ 3 x 6 x 2 mm, containing 2 distinct types of cells,

a. chief cells - abundant, clear cytoplasm → PTH
b. oxyphil cells - larger, less in number ? function

Parathyroid Hormone (PTH)

▪ *Synthesis & Metabolism*

• a linear polypeptide hormone, MW = 9500 containing 84 AA's
• synthesised as prepro-PTH of 115 AA's, which upon entry to the endoplasmic reticulum has 25 AA's removed from the N-terminal forming pro-PTH
• the remaining 6 AA's are removed in the golgi apparatus
• PTH is the main secretory product of the chief cells, released in direct response to a lowered serum Ca^{++} level
• the plasma half-life is ~ 20 mins, being rapidly cleaved by the Kupffer cells of the liver

▪ *Physiological Actions*

• PTH exerts at least four distinct effects on Ca^{++} homeostasis,

a. increases movement of Ca^{++} and phosphate out of bone
b. stimulates 1-α-hydroxylase activity → indirect effects
c. increases renal tubular reabsorption of Ca^{++}
d. decreases renal tubular reabsorption of phosphate

• when PTH induces bone reabsorption both Ca^{++} and phosphate are released, similarly, the vitamin D induced GIT absorption increases phosphate also
• the action of PTH on the renal tubules, increasing phosphate excretion, prevents elevation of the plasma phosphate
• maximal amounts of PTH can reduce phosphate reabsorption from 80% to 15%
• plasma phosphate may actually decrease in response to hypocalcaemic induced increases in PTH
• this aids in further reabsorption of bone due to local interactions between Ca^{++} and phosphate
• PTH also inhibits proximal tubular H^+ secretion and, therefore, bicarbonate reabsorption resulting in a decrease in plasma pH which displaces Ca^{++} from plasma protein and bone
• hyperparathyroidism causes enhanced bone resorption with cysts, osteitis fibrosa cystica, an elevated plasma calcium and lowered phosphate, ectopic calcification and renal stones
• renal calcium excretion increases, despite the elevated PTH, as the filtered mass increases more than the reabsorptive increase

Mechanism Of Action

• PTH acts by increasing the intracellular cAMP levels, but how this affects calcium handling in bone is unsettled
• it does result in increased calcium permeability in osteocytes, osteoclasts and osteoblasts, the later then pumping this Ca^{++} into the ECF
• the pump is stimulated by 1,25-$(OH)_2$-D_3, thus the action of PTH is augmented by 1,25-$(OH)_2$-D_3 without any direct effect of the later on cAMP
• on a long-term basis, PTH increases osteoclastic activity and numbers, while inhibiting the formation of osteoblasts
• it appears that all of the hormone receptors, except those for calcitonin, are located on osteoblasts, the regulation of osteoclasts thus involving some form of intercellular second messenger
• in pseudohypoparathyroidism there is a failure of recognition of PTH, due to either,

a. a congenital 50% reduction in the membrane Gs protein
b. a normal cAMP response but defective phosphaturic action

Regulation Of Secretion

• the circulating level of ionised calcium acts by direct negative feedback on parathyroid cells
• magnesium appears to have a similar direct effect, an acute fall in the plasma Mg^{++} stimulating PTH release
• another factor maintaining the plasma Ca^{++} is the bone readily exchangeable pool, however this can only maintain a level of ~ 7 mg/dl, the normal level of 10 mg/dl can only be achieved in the presence of PTH
• secretion of PTH is increased by β-adrenergic discharge and cAMP
• in conditions such as chronic renal disease or rickets, the chronic hypocalcaemia and excess PTH cause secondary hyperparathyroidism
• the cause in renal disease is deficient α-1-hydroxylase activity

Calcitonin

• calcitonin is a 32 AA polypeptide hormone of MW = 3500
• as for most polypeptide hormones it is synthesised as a preprohormone
• the mRNA transcribed from the calcitonin gene is processed to a different mRNA in the brain, such that calcitocin gene related peptide is formed, rather than calcitonin
• the hormone is also secreted by a number of extrathyroidal sites, however the significance of this is unsettled
• secreted by the parafollicular cells of the thyroid gland, derived from the last branchial pouch, in response to a raised plasma Ca^{++}
• secretion does not begin until the plasma level exceeds ~ 9.5 mg/dl, and is proportional thereafter
• the plasma half-life is ~ 10 mins
• calcitonin receptors are found in the kidneys and in bone
• calcitonin lowers the plasma calcium, principally by inhibiting bone reabsorption by direct inhibition of the Ca^{++} permeability of osteoclasts and (?) osteoblasts
• it also increases Ca^{++} excretion in the urine
• overall contribution to Ca^{++} homeostasis is very minor c.f. PTH & Vit.D
• it appears to be more active in young individuals and may play some role in skeletal development
• it may also protect against excessive calcium loss during pregnancy due to the elevated $1,25\text{-}(OH)_2\text{-}D_3$ levels
• it has been synthesised and is useful in the treatment of Paget's disease, where increased osteoclastic activity triggers compensatory disorganised new bone formation

> ***NB:*** remember that the patient with medullary carcinoma of the thyroid has a 1/3 chance of also having a ***phaeochromocytoma*** (MEN IIa)

Other Hormones Affecting Calcium Homeostasis

• high levels of cortisol can induce a negative calcium balance by decreasing GIT absorption and increasing renal excretion
• over prolonged periods glucocorticoids also decrease bone formation and increase reabsorption by inhibiting cellular replication and protein synthesis
• growth hormone also increases urinary excretion of calcium but simultaneously increases GIT absorption, the net effect usually being (+)'ve
• thyroid hormones may also cause hypercalcaemia, hypercalcuria and result in osteoporosis in some instances
• oestrogens prevent osteoporosis, E_2 receptors ? being located on osteoblasts
• insulin increases bone formation and there is significant bone loss in untreated diabetics
• an osteoclast activating factor is secreted by B & T lymphocytes and appears to be the cause of the hypercalcaemia in patients with haematological cancers

THE PITUITARY GLAND

• the anterior, intermediate, and posterior lobes of the pituitary are effectively 3 separate endocrine organs, secreting 14 or more hormonally active substances
• the 6 established hormones secreted by the anterior pituitary are,

a. thyroid stimulating hormone TSH*
b. adrenocorticotropic hormone ACTH
c. growth hormone GH
d. follicle stimulating hormone FSH*
e. leutinizing hormone LH*
f. prolactin PRL

NB: * these are ***glycoproteins***, c.f. simple polypeptides

• prolactin acts on the breast, the remaining five are tropic hormones, stimulating secretion of other endocrine glands
• the anterior pituitary also secretes,

a. β-lipotropin (β-LPH), a linear polypeptide of 91 AA's, which contains the sequences for the endorphins and enkephalins, though, the physiological role of this is unclear
b. γ-melanocyte stimulating hormone (γ-MSH)

• the hormones secreted by the intermediate lobe are α-MSH & β-MSH

• the hormones secreted by the posterior lobe are vasopressin & oxytocin

Anatomy

• the posterior pituitary is largely comprised of endings on blood vessels, of nerve fibres originating in the supraoptic and paraventricular nuclei of the hypothalamus
• there are also neuroglial cells and pituicytes, believed to be modified astroglial cells
• the intermediate lobe is formed in the embryo from the dorsal half of Rathke's pouch, an evagination of the roof of the pharynx, but is closely adherent to the intermediate lobe
• it is rudimentary in humans and most of the cells are agranular, a few containing basophilic elements similar to the anterior pituitary
• the anterior pituitary is connected functionally to the brain via the hypothalamohypophyseal portal vessels
• it is composed of interlacing cords of cells and an extensive sinusoidal capillary network
• the capillary endothelium is fenestrated and the cells contain numerous secretory granules which store hormones, which are then released by exocytosis
• the cells are classically divided according to their staining reactions,

a. acidophils (40%) | → ***granular chromophobes***
b. basophils (10%) |
c. agranular chromophobes

- the acidophils → PRL & GH
- the basophils → TSH, LH, FSH *the glycoprotein hormones
- those secreting ACTH are variously classified as chromophobic or basophilic

NB: with EM techniques, 5 types of cells can be identified,

i.	thyrotropes	TSH
ii.	somatotropes	GH
iii.	corticotropes	ACTH
iv.	gonadotropes	FSH & LH
v.	mammotropes	PRL

FSH, LH & TSH

- these 3 glycoprotein hormones are composed of 2 subunits, designated α & β
- the placental glycoprotein hCG has a similar structure
- the AA subunits of the α-subunits of all three are identical, though, the CHO residues may differ
- the β-subunits differ in structure and confer hormonal specificity

Melanocyte Stimulating Hormones

- mammalian pituitaries contain 3 hormones called melanotropins, or melanocyte stimulating hormones

a. α-MSH - made up of AA's 1-13 of the ACTH molecule

b. β-MSH - made up of the 17 C-terminal AA's of -LPH

c. γ-MSH - present in the highest concentration

- thus, ACTH has considerable MSH activity
- γ-MSH is also present in the anterior lobe and is secreted along with ACTH

Pro-Opiomelanocortin (POMC)

• synthesised in the intermediate lobe and in the corticotropes of the anterior lobe
• POMC is also synthesised in the hypothalamus, other parts of the CNS, the lungs, the GIT and in the placenta
• in the corticotropes this is hydrolyzed to,

a. ACTH
b. β-LPH
c. β-END (small amount only)

• in the intermediate lobe, POMC is further hydrolyzed to,

a. α-MSH
b. CLIP - corticotropin-like intermediate lobe peptide
c. γ-LPH
d. β-END - appreciable amounts

• the intermediate lobe in humans is rudimentary and neither α-MSH or β-MSH are secreted in the adult
• mammals do possess melanocytes, which contain melanosomes which synthesise melanins
• these are then transferred to the keratinocytes of the skin, and are responsible for the pigmentation of the hair and skin
• administration of MSH accelerates melanin production and results in appreciable darkening of the skin within 24 hrs
• pigmentation changes in a number of endocrine disorders are due to changes in either ACTH, as this has MSH activity,

a. pituitary insufficiency → pallor
b. Addison's disease → hyperpigmentation

Growth Hormone

• a linear polypeptide, containing 2 disulphide bridges, of MW = 21,500
• human GH has a marked structural resemblance to prolactin and human chorionic somatomammotropin (hCS)
• hGH has intrinsic lactogenic activity
• basal plasma levels are normally < 3 ng/l
• metabolised rapidly in the liver and has a plasma half life ~ 20-30 mins
• the effects of GH on growth, cartilage, and protein metabolism are not directly mediated
• rather, GH stimulates the liver, plus some other tissues, to synthesise polypeptide growth factors, somatomedins
• the primary somatomedins are the insulin-like growth factors IFG-I & IGF-II
• another insulin-like factor is relaxin which acts on the ligaments of the female prior to parturition
• other growth factors are,

a. neural growth factor NGF
b. epidermal growth factor EGF
c. ovarian growth factor OGF
d. fibroblast growth factor FGF

• IGF-I is induced by GH, and its plasma concentration parallels the postnatal growth rate
• IGF-II is induced by hCG during prenatal development
• both increased glucocorticoids and protein deficiency decrease the plasma activity of somatomedins
• the secretion of somatomedins is reduced in untreated diabetes, an effect restored by insulin therapy

▪ *Effects on Growth*

• prior to epiphyseal fusion, chondrogenesis is accelerated and the width of the growth plate of long bones widened
• after fusion, linear growth is no longer possible, and GH produces the pattern of bone and soft tissue deformities known as acromegaly
• these effects are mediated through the somatomedin IGF-I

▪ ***Effects on Protein, CHO and Electrolyte Metabolism***

• GH is a protein anabolic hormone which produces a positive nitrogen & phosphorus balance
• it also stimulates erythropoiesis
• GIT absorption of Ca^{++} is increased
• Na^{+} & K^{+} excretion are reduced by a mechanism independent of the adrenal cortex
• hydroxyproline excretion is increased in disorders where there is increased breakdown of collagen, however it is also increased when there is increased synthesis of soluble collagen, as occurs with GH

• GH is diabetogenic because it increases hepatic glucose output and exerts anti-insulin effects in skeletal muscle
• it is also ketogenic because it increases circulating FFA
• GH does not stimulate the B cells of the pancreas directly, but increases the response to other stimuli, such as arginine or glucose
• this adds to the anabolic effects of GH

NB: effects which ***do not*** appear to be mediated by somatomedins include,

i. lipolysis, in the presence of cortisol
ii. stimulation of erythropoiesis
iii. increased B-cell responsiveness to stimuli
iv. increased cellular uptake of AA's

▪ ***Hypothalamic Control Of Secretion***

• the hypothalamus secretes two controlling hormones,

a. GH-releasing hormone - GHRH
b. somatostatin - inhibitory

• hypothalamic lesions or section of the stalk result in inhibition of secretion
• secretion is pulsatile and shows some diurnal rhythm
• stimuli which increase secretion include,

a. deficiency of energy substrate - hypoglycaemia, exercise
b. increased levels of certain AA's - arginine
c. stressful stimuli - pyrogen, lysine vassopressin, psych.
d. glucagon
e. α-adrenergic agonist which cross the BBB & l-DOPA
f. dopaminergic agonists - apomorphine
g. oestrogens

• stimuli which decrease secretion include,

a. glucose
b. cortisol
c. FFA's
d. GH
e. REM sleep
f. medroxyprogesterone

Growth

• normal growth also requires thyroid hormones, androgens, oestrogens, glucocorticopids and insulin
• it is also affected by genetic and external factors, the most important of which is the food supply
• injury and disease stunt growth because they increase protein catabolism, following which there is usually a period of "catch-up growth"
• in humans there are 2 periods of rapid growth, the first in infancy and the second in late puberty
• the first is a continuation of intrauterine growth and is associated with elevated plasma GH levels
• the second is due largely to the effects of androgens and oestrogens, and is independent of the actions of somatomedins
• thyroid hormones alone have no effect on growth, their action is permissive for the effects of GH, possibly via potentiation of the action of somatomedins
• other adrenocortical hormones exert a permissive action on growth, however, glucocorticoids are potent inhibitors of growth due to their direct cellular actions
• children treated with pharmacologic doses of steroids fail to grow normally for the duration of therapy
• although androgens & oestrogens stimulate growth, they may ultimately limit growth due to premature fusion of the epiphyses

Pituitary Insufficiency

• the adrenal cortex atrophies, with minimal secretion of glucocorticoid and sex steroids
• stress induced rises in aldosterone are absent, but basal secretion is normal
• thus, as there is no mineralocorticoid deficiency, salt depletion and hypovolaemia do not develop, however these individuals are more susceptible to stress
• growth is inhibited, thyroid function is depressed and the gonads atrophy with loss of the secondary sexual characteristics
• there is a tendency to hypoglycaemia, especially during fasting, and hypophysectomy ameliorates diabetes mellitus, markedly increasing the hypoglycaemic effect of insulin
• although the removal of the supraoptic-posterior pituitary results in diabetes insipidus, removal of both anterior & posterior usually causes only transient polyuria
• the amelioration of the diabetes insipidus in this circumstance is probably due to the decrease in osmotic load presented for excretion, being due to the decreased rate of tissue catabolism
• GH increases the GFR and RPF in humans, thus its deficiency further decreases the osmotic load
• thus the "diuretic" activity of the anterior pituitary can be explained in terms of TSH, ACTH, and GH
• ACTH & MSH deficiency leads to pallor of the skin
• there may be some protein loss, however, most animals with hypopituitarism appear well nourished
• causes include,

a.	anterior pituitary tumours	- acidophil - basophil - chromophobe
b.	suprastellar cysts	
c.	Sheehan's syndrome	- post-partum necrosis

Pituitary Hyperfunction

• acidophil cell tumors secreted large amounts of GH and result in either gigantism, or acromegally
• many patients with Cushing's syndrome have bilaterally hyperplastic adrenals and small ACTH-secreting pituitary tumors, microadenomas
• a significant number of such patients who have their adrenals removed develop rapidly growing ACTH-secreting pituitary tumors, Nelson's syndrome
• these cause hyperpigmentation of the skin and local neurological signs
• the majority are made of chromophobe cells, not basophils, and a few are malignant

OTHER ENDOCRINE ORGANS

Endocrine Functions of the Kidney

▪ *Renin & Angiotensin*

• renin is an acid protease secreted by the kidney into the bloodstream
• it is a glycoprotein hormone of MW = 37,326, which has 2 lobes, or domains between which the active site resides
• the active site is formed by 2 adjacent aspartic acid residues, thus it is an aspartyl protease
• like other protein hormones, it is synthesised as a larger preprohormone which is processed to active renin, which contains 340 AA's
• prorenin has negligible biological activity and is converted to active renin by tissue kallikrein
• renin has a circulation half-life of ~ 80 mins and acts on a glycoprotein in the ~2-globulin fraction, angiotensinogen to form the decapeptide angiotensin I
• angiotensonogen is synthesised in the liver and its levels are increased by glucocorticoids and oestrogens
• angiotensin converting enzyme ACE is a dipeptidyl-carboxypeptidase which splits 2 AA's from angiotensin I to form the octapeptide angiotensin II
• most of the ACE is located on the surfaces on endothelial cells, especially in the calveoli in the lung, however conversion occurs in a number of other tissues
• angiotensin II is rapidly destroyed in the circulation, t½ ~ 1-2 mins
• metabolism is by a number of enzymes, including an aminopeptidase which splits Asp from the N-terminal end yielding angiotensin III, which has some biological activity
• in addition A-II appears to be remover from the circulation by some trapping mechanism in the vascular beds other than the lungs
• the actions of angiotensin II include,

a. arteriolar vasoconstriction (4-8 x NA)
b. elevated systolic and diastolic blood pressures
c. increased secretion of aldosterone from the adrenal cortex
d. facilitation of NA release from sympathetic nerve terminals
e. acts on the CNS* to:
   - raise BP (area postrema)
   - increase water intake (SFO & OVLT)
   - increase secretion of ACTH
   - increase secretion of ADH

NB: A-II does not cross the BBB, these actions are on the circumventricular organs (in brackets)

• A-III has ~ 40% of the pressor activity of A-II, but the same effect in stimulating aldosterone secretion
• the juxtaglomerula cells are epithelioid cells located in the media of the afferent arterioles as they enter the glomeruli
• they contain numerous secretory granules containing renin
• the junction of the ascending loop and the distal convolution are in direct contact with the afferent-efferent arterioles at this point, and the tubular cells are modified to form the macula densa
• the entire arrangement is termed the juxtaglomerula apparatus

• renin secretion is increased by,

a. lowered afferent arteriolar pressure (baroreceptor)

b. decreased filtered mass of Na-Cl reaching the macula densa

c. increased sympathetic tone & CA's (β_1-receptors)

d. increased renal prostaglandins (PGI^2)

• secretion appear to vary inversely with the serum K^+, however this may be indirect due to effects on Na-Cl
• renin secretion is decreased by feedback inhibition by A-II

• inhibitors of PG synthesis, such as the NSAID's, or β-blocking agents reduce the rate of renin secretion
• captopril, and the other ACE inhibitors, reduce the formation of A-II
• saralasin and some other analogues of A-II act as competitive antagonists at peripheral A-II receptors

■ ***Role In Hypertension***

• Goldblatt demonstrated that constriction of one renal artery led to sustained hypertension
• postulated that hypertension was in fact due to elevated renin and angiotensin
• later found that elevations were often transient and that a significant number of such individuals had normal renin levels despite continued hypertension
• although these individuals do have low, or normal renin levels, they still respond to treatment with ACE-I agents, possibly due to an increased sensitivity to A-II
• the decline in BP seen in these patients may be due to a rise in the plasma bradykinin, as this is usually broken down by ACE in the lung
• patients with idiopathic hypertrophy and hyperplasia of the JGA, Bartter's syndrome, have persistent hypokalaemia, elevated A-II & aldosterone, however, their blood pressure is usually normal (GOK)

■ ***Erythropoietin***

• this is a circulating glycoprotein of MW = 23,000, secreted principally by the epithelial cells of the glomeruli, but also by the liver and some other organs
• it acts on erythropoietin sensitive stem cells in the marrow, resulting in their conversion to proerythroblasts
• this action is mediated by altered mRNA synthesis
• its presence is required for maintenance of the normal red cell mass, as anti-erythropoietin Ab's result in anaemia
• the usual stimulus for secretion is hypoxia, but secretion can also be stimulated by cobalt salts, androgens, and possibly some other hormones
• secretion is facilitated by CA's acting on β-adrenergic receptors
• postulated that hypoxia results in the production of PG's and these activate adenylate cyclase, which then triggers erythropoietin production
• the hormone has a plasma half life of ~ 5 hrs and is inactivated in the liver
• however, any increase in the RBC mass is not seen for 2-3 days, due to the slow maturation process

• hypophysectomised animals become anaemic, and pituitary extracts stimulate RBC production
• however, the response to hypoxia or haemorrhage is not blocked, and the kidneys secrete erythropoietin
• glucocorticoids, androgens and T_4 stimulate erythropoiesis
• oestrogens inhibit erythropoiesis

Endocrine Functions of the Heart

■ *Atrial Natriuretic Peptide*

• the muscle cells of the atria contain secretory granules that increase in number when the intake of NaCl is increased, or the ECF expanded
• several different natriuretic peptides have been isolated, the principal peptide in humans designated ~-hANP, containing 28 AA's
• this, and related polypeptides are formed from a large precursor polypeptide of 151 AA's
• all of the various ANF's have a ring structure with disulphide bridges between cysteine residues
• this results in a marked natriuresis, generated by an increase in GFR
• ANP receptors exist in the glomeruli and no direct effect has been demonstrated on the tubules
• other effects of ANP include,

a. decreased responsiveness of vascular smooth muscles to vasoconstrictor substances
b. decreased responsiveness of the zona glomerulosa
c. inhibition of renin secretion
d. inhibition of vasopressin secretion

• ANP is also found in neurons in the brain, and pathways from the preoptic area project to the BP regulating areas in the hypothalamus

The Pineal

Anatomy

• the pineal arises from the roof of the third ventricle, under the posterior end of the corpus callosum, and is connected by a stalk to the posterior commissure and habenular commissure
• the stalk contains nerve fibres but these don't reach the gland
• the gland contains neuroglia and parenchymal cells, which have features consistent with a secretory function
• the gland is larger in children and involutes after puberty
• often develops small concretions of calcium salts, pineal sand, which are visible on X-ray
• like the circumventricular organs, the pineal has fenestrated capillaries and lies outside the BBB

Function

• possibly has a role in inhibiting the onset of puberty
• contains the indole, N-acetyl-5-methoxytryptamine, named melatonin
• this lightens the skin of tadpoles by an action on melanophores, however, has no such function in humans
• synthesis is decreased in the light and increased in darkness
• this circadian rhythm is regulated by sympathetic innervation
• the entrainment of SNS tone to the light-dark cycle is via the retinohypothalamic nerve fibres and the suprachiasmic nuclei
• melatonin is present in the plasma and to a greater extent in the CSF
• actual functions unknown but proposed as an antigonadotropin