resonance results from the association of a positively charged resonating group (arginine's guanidinium) and a negatively charged resonating group (carboxylate or phosphate) and this increases the stability of the ionic bond.

From the above considerations and from knowledge of the respective pK values I have computed the following order of strength of ionic linkages in relation to the carboxylate side chain (glutamic acid or aspartic acid) in neutral aqueous solution as:

arginine	>	lysine	>	terminal	>	histidine
(guanidin-		(ϵ -ammon-		(ammon-		(imin-
ium)		ium)		ium)		azole)

A parallel and more detailed order with respect to diesterified phosphate negatively charged group has already been computed (Lewin, 1969). Evaluation of the relative strength of the ϵ -ammonium-carboxylate and ϵ -ammonium-phosphate (diesterified) linkages indicates that the latter is likely to be somewhat stronger than the former.

Addition of increasing amounts of foreign electrolytes, e.g. NaCl, should therefore rupture the above linkages in accordance with the computed order. These expectations are consistent with several experimental observations including those in which additions of increasing NaCl concentrations to deoxyribonucleohistone remove lysine-rich histone fractions first followed by the arginine-rich fractions (for extensive references see Bonner & Ts'o, 1964; Busch, 1965; de Reuck & Knight, 1966).

Examination of a number of carboxylate groups in proteins suitably located near guanidinium (arginine) groups indicates that the formation of strong ionic linkages can take place, and these could affect the relevant pK values and may also make them appear partially masked over their normal titration ranges. In some cases it is feasible to consider that some time may elapse before such ionic bonds are opened. This could explain the time requirement to attain equilibrium such as that noted in histone associations.

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Effect of Intravenous Infusion of L-Tryptophan on the Cerebral Metabolism of 5-Hydroxyindoles and Dopamine (3,4-Dihydroxyphenethylamine)

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An intravenous injection of L-tryptophan transiently increased concentrations of 5-hydroxyindoles in dog brain without altering the normal regional pattern of metabolites (Moir & Eccleston, 1968), and alterations in the concentration of 5-hydroxyindol-3-ylacetic acid were reflected by corresponding concentration changes in cerebrospinal fluid from cisterna magna (Eccleston *et al.* 1968). The present experiments have made an attempt to study the cerebral metabolism of a raised concentration of the 5-hydroxyindoles in a steady state by following an intravenous injection of L-tryptophan (50mg./kg.) by an infusion (20mg./kg./hr.). Plasma, erythrocytes and cisternal cerebrospinal fluid were sampled hourly for 4 hr., and the dogs were then killed and various regions of brain examined. Although the rise in concentration of tryptophan in erythrocytes was well maintained, the concentration in the plasma declined from its 1 hr. value. The concentration in cerebrospinal fluid rose to a maximum at 2 hr. and then declined.

In cerebrospinal fluid the main acid metabolites of 5-hydroxytryptamine and dopamine (3,4-dihydroxyphenethylamine), namely 5-hydroxyindol-3-ylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid, both reached maximal values that were maintained for the 3-4 hr. period. The 5-hydroxyindol-3-ylacetic acid concentration was three times its control value; however, the concentration of 4-hydroxy-3-methoxyphenylacetic acid was 14 times the control. Despite this marked change in the concentration of 4-hydroxy-3-methoxyphenylacetic acid in cerebrospinal fluid, normal concentrations of dopamine, methoxydopamine (4-hydroxy-3-methoxyphenethylamine), 4-hydroxy-3-methoxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid were found in the caudate nucleus. Similarly concentrations of tyrosine throughout the brain and in all samples taken during the infusion period were normal.

Tryptophan concentrations of approx. 40 $\mu\text{g./g.}$ were found in all brain regions. The concentration of 5-hydroxytryptamine in all regions was between two and three times the control values, and the concentration of 5-hydroxyindol-3-ylacetic acid was increased to a slightly, but significantly, greater extent. As the elevated plateau of 5-hydroxyindol-3-ylacetic acid concentration in cerebrospinal fluid

reflects an elevated steady state in the cerebral metabolism of the 5-hydroxyindoles, the decreased [5-hydroxytryptamine]/[5-hydroxyindol-3-ylacetic acid] ratios may indicate an increased bypassing of 5-hydroxytryptamine 'stores'. However, the fact that the total 5-hydroxyindoles ([5-hydroxytryptamine] + [5-hydroxyindol-3-ylacetic acid]) retained, for the most part, the normal pattern of distribution suggests that regional intracerebral hydroxylation was still the controlling factor.

It is of interest that the evidently important change in the metabolism of dopamine caused by tryptophan was detectable only from the study of body fluids, since the metabolite concentrations in the brain were unaltered.

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Isotopic Exchange between Acetylcholine and [*Me*-¹⁴C]Choline Catalysed by Human Placental Choline Acetyltransferase

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Choline acetyltransferase catalyses the reversible transfer of acetyl groups between acetyl-CoA and choline. A marked difference in the kinetic behaviour of the partially purified enzymes from rat brain (Potter & Glover, 1968) and from human placenta (Schuberth, 1966) has been reported. Analysis of double-reciprocal plots of initial velocity versus the concentration of one substrate at different fixed concentrations of the second substrate indicate a 'sequential' ternary-complex mechanism (Cleland, 1963) for the brain enzyme; in contrast, a 'ping-pong' mechanism was indicated for the placental enzyme. Although such a fundamental interspecies difference in mechanism is not impossible it must *a priori* be considered unlikely.

Partially purified human placental choline acetyltransferase (Morris, 1966) was incubated with acetylcholine (10 μ moles) and [*Me*-¹⁴C]choline (0.5 μ C, 54 μ C/ μ mole). The incubation was stopped by addition of 0.2M-HClO₄, and unlabelled choline (10 μ moles) was added as a carrier. The acetylcholine and choline were isolated via their reineckate salts (Hemsworth & Morris, 1964) and separated by paper electrophoresis (7kv for 1hr.) in 0.1M-pyridine-acetic acid buffer, pH 4. The well-separated spots were located with either I₂ vapour or Dragendorff's reagent and cut out and the radio-

activity was measured in a scintillation spectrometer. Recovery of radioactivity was between 95 and 100% and acetylcholine recovery, estimated by biological assay, was 90%.

A fairly rapid exchange of radioactivity occurred, 90% of the total being present in the acetylcholine after 30min. incubation. The exchange, however, was absolutely dependent on the presence of CoA; various control experiments gave exchange of 1% or less. This result rules out the 'ping-pong' mechanism and is good presumptive evidence for a ternary-complex mechanism. It is very probable that acetyl-CoA is first formed, and this is then able to incorporate labelled choline into acetylcholine by reversal of the initial step. That acetyl-CoA formation rather than just the presence of CoA is required is rendered more likely by the observation that addition of oxaloacetate and citrate synthase inhibited the exchange to an appreciable extent. Further evidence was provided by the marked inhibition obtained in the presence of chloroacetylcholine; this compound has been shown (Morris & Grewaal, 1969) to be a potent specific inhibitor of acetylcholine synthesis.

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Isolation of a Mucoprotein from Porcine Gastric Mucosa

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Of the various muco-secretions from the gastric mucosa (Glass, 1962), blood-group substances (Kabat, 1956), a sulphated mucoprotein (Pamer, Glass & Horowitz, 1968) and sulphated uronic acid-containing mucopolysaccharides (Kimura, Watanabe & Nagai, 1964) have been well characterized, after isolation by enzymic digestion. To study the major mucoprotein secretion of the surface mucosa without previous enzymic digestion, the gastric mucoproteins were radioactively labelled by incubation of porcine gastric mucosal scrapings with [¹⁴C]glucose *in vitro* by the method of Allen & Kent (1968). Acid hydrolysis and chromatography of the non-diffusible material showed that radioactivity had been incorporated (14.8% of the total added d.p.m.) into mainly galactose (77%) with smaller amounts in fucose (5%) and the amino sugars, glucosamine and galactosamine (8%).