of the tetracycline is some 1/2 Å out of contact with the 2 position of the purine. The OH in this position in oxytetracycline, methacycline and deoxycycline is not within H-bonding distance from the guanine 2 HN-group and the CH in this position in tetracycline, rolitetracyline, chlortetracycline and demeclocycline is likewise not in lipophilic contact with the 2CH group of adenine.

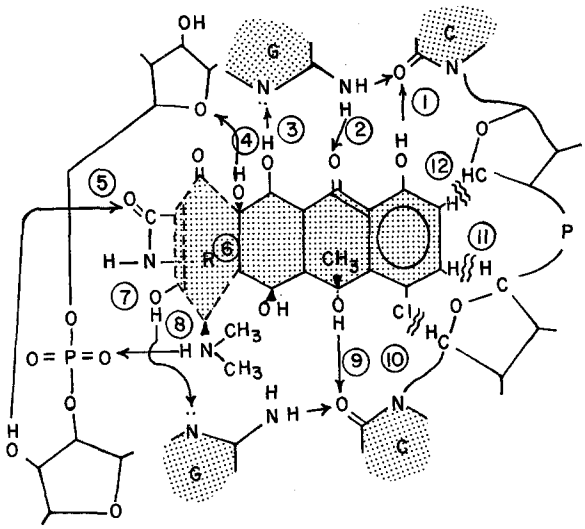

Fig. 1. A composite tetracycline (including all the active groups of the tetracyclines listed in GOODMAN and GILLMAN[4]. The suggested mode of binding is indicated as numbered in the text. The molecule is turned over relative to its normal presentation in line formulae.

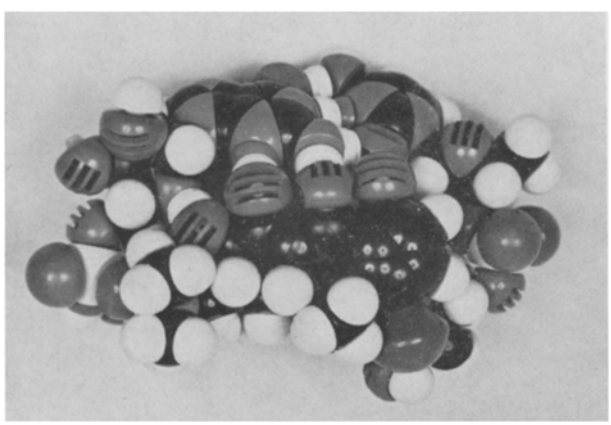

Fig. 2. A CPK model of the proposed mechanism of binding of tetracyclines to the ribosome.

The general 'fit' is extremely precise with correct bond angles maintained in all cases. Every OH group is used as an H-bond donor. The only groups not actively involved in hydrogen bond or ionic interactions are the $C_1 = 0$ and the amide group on 2 (these groups make a van der Waals' contact with the purine π clouds). Thus the tetracycline binds by 6 hydrogen bonds, 1 ionic bond and various lipophilic bonds and weak interactions.

An alternative site is provided by a strand of RNA in the fully extended conformation (2Gs) bound to protein (-Glu-x-Glu-) by 2 G-Glu ion-dipole bonds. In this case the 10 OH and 6 OH bind to Glu Os and C7 and 9 lipophilic bonds are to Glu methylenes and the C8 lipophilic bond to the α CH of x.

A complex protein structure could be derived based on 2 parallel β-pleated chains cross linked by one Arg-Glu link plus one or more additional chains to provide binding sites for the 12–1 OH, the basic NH^+ and the 6 OH groups which cannot be accommodated on the first structure.

Discussion. This precise fit described is not attainable on any other structure involving RNA. Amanatin has a similar precise stereochemical relationship to a segment of RNA (or complexed individual nucleotides) bound to protein[3]. The RNA sequence required is 3 adenines and the protein sequence is -Gln-x-Gln-x-Gln-. In this case double-stranded RNA will not do. Ribosomes could also have a segment of RNA bound to protein in a comparable manner (G-Glu in place of A-Gln). Alternatively the 'receptor' for tetracyclines could be constructed entirely from RNA (double stranded GC:GC or GC:AU). This structure could be located in or adjacent to the binding site for aminoacyl t-RNA so that occupancy by the tetracycline blocks the binding of the latter. It would be of interest to explore the relationship between the structures we have described and t-RNA.

Résumé. L'usage de modèles moléculaires CPK permet de comparer la structure des molécules de la tétracycline à celle des molécules du RNA. Il est suggéré que le site récepteur de la tétracycline sur le ribosome comprend soit du RNA à double tresse, dont les radicaux formés par GC/GC ou GC/AU, soit un complexe RNA/protéine stéréochimiquement analogue au précedent ou à la portion CC (ou CU) du RNA. Il peut enfin être remplacé par une portion isomorphique de protéine ayant une séquence aminoacidique telle que Glu-x-Glu (ou Glu-x-Gln).

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A Model For Investigating Erythropoiesis[1]

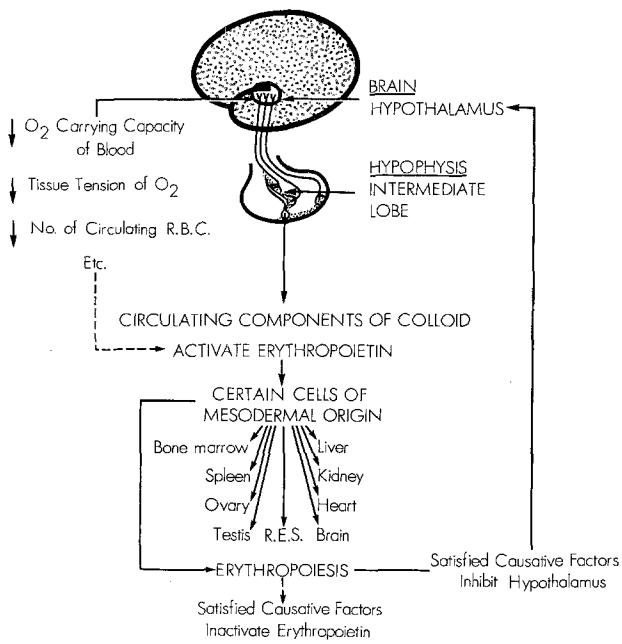
Basic models showing the feedback circuit responsible for the regulation of red cell production, have been constructed from the results of investigations concerning erythropoiesis. The models are continually being modified[2–4], since certain factors, once thought to be the source of erythropoietin have no erythropoietic activity[5].

Although the role of erythropoietin in the regulation of red cell development is well established, the processes of production and release remain unclear. The results of early investigations suggested that erythropoietin is produced by the kidney[6,7], and that its site of action is the bone marrow stem cell[8,9]. It is now thought that erythro-

poietin formed in the kidney is in a lipid bound inactive form and released by a serum factor[10]. Furthermore, it seems that the action of erythropoietin is only on erythroid committed stem cells[11]. The present study by BOYD[12–16], strongly suggests that erythroid committed cells are mesodermal in origin and are present in many organs (liver, heart, kidney, testis, ovary, spleen, bone marrow, brain, walls of blood vessels, etc.).

Two schools of thought can be culled from the literature. One suggests that the central nervous system provides a rich network of autonomic fibers to haemopoietic organs[17–21]; the other associates hormonal factors with erythropoiesis[22–24]. Each school supports its case with critical evidence; for example, certain studies show that hypothalamic stimulation[22–25]; as well as stimulation of the midbrain[23], increases erythropoietic activity. Although disputed by some investigators[17,18], others indicate that sera from hypothalamic stimulated animals produce an increase in red cell mass and radioiron incorporation into newly formed red blood cells[22–24].

Recent papers by BOYD are the first to associate the hypophyseal intermediate lobe with erythropoiesis, and to suggest that the relationship of the hypothalamus to the intermediate lobe plays a leading role in this phenomenon. Hypothalamic intermediate lobe fibers are readily demonstrable in the bovine gland and the literature cites many important references pointing out this relationship in many other animals[26–29]. One interesting report[30] suggests that the erythropoietic effect by hypothalamic stimulation is mediated by way of the pituitary relase of ACTH. This suggestion seems noteworthy since it has been discovered that the intermediate lobe contains ACTH producing cells[31–33], and that intermediate lobe MSH, a polypeptide, has inherent ACTH activity. These data are significant for ACTH is a known erythropoietic stimulant[28,34–37], and evidence shows that the corticotropin releasing activity of the hypothalamus[38] stimulates ACTH production[39]. The contention that intermediate lobe cells produce ACTH or ACTH-like substances, is in sharp contrast to the well accepted idea that ACTH production is associated with the cells in the anterior lobe. The confusion concerning the location of ACTH producing cells in the hypophysis may well stem from studies of early investigators who made no clear distinction between the anterior and intermediate lobe. Other pertinent data show that intermediate lobe cells and intraglandular colloid give a positive PAS-reaction, indicative of glyco-



A shift in oxygen sensors may either directly activate erythropoietin or excite the hypothalamo-hypophyseal complex to activate erythropoietin.

[1] This study was supported by a grant from the National Research Council of Canada.

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protein[40-42]. This is credible since it has been demonstrated that highly purified preparations, glycoprotein in nature, with no lipid fraction are capable of initiating the differentiation and subsequent maturation of red cell precursors from primitive mesenchyme.

The above mentioned facts are continually appearing in the literature, and correlate quite closely with the more pertinent features in the present study[12-16,43].

Although the intermediate lobe has not been implicated, heretofore, in the normal sequence of events associated with red blood cell production, this study places the lobe within a central nervous system hormonal complex. It is suggested that under the influence of the hypothalamus, the intermediate lobe undergoes degeneration and autolysis resulting in the formation of intraglandular colloid. The colloid passes into the venous circulation of the cavernous sinuses by way of well defined capsular clefts. Within the circulation, colloid components (possibly ACTH or ACTH-like substances) either directly induce red blood cell production, or activates erythropoietin which initiates this phenomenon. Properly stimulated erythroid committed cells differentiate and proliferate along the red blood cell line.

A working model (Figure) was constructed from these data. The present model is designed to investigate erythropoiesis and makes no attempt to replace any previous models for the regulation of red blood cell production. Although the process or processes concerning the production of erythropoietin are unknown, the model suggests that inactive erythropoietin either stored (kidney, liver, etc.) or circulating, is activated either by a sudden demand for an increased number of circulating red blood cells or by mechanisms regulating the normal turn-over rate of red blood cells.

The model is maintained in a steady state by oxygen sensors. They are sensitive, for example, to a decrease in circulating red blood cells, a decrease in oxygen carrying capacity of blood, a decrease in tissue tension of oxygen, etc. Thus, an immediate demand for circulating red blood cells caused by a rapid shift in the sensors, directly activates erythropoietin or like substances. The operation of the sensors during the normal course of regulating red blood cell production is thought to be through certain hypothalamic centres, which in turn control the cyclic behavior of the intermediate lobe. This results in the formation of intraglandular colloid. Components of the colloid within the circulation activate erythropoietin or like substances. In turn, erythroid committed cells within a large number of organs differentiate and proliferate along the red blood cell line, and at speeds necessary to satisfy the requirements of the sensors. Once accomplished, on the one hand, erythropoietin is inactivated, and on the other, the hypothalamus is inhibited.

Zusammenfassung. Ein Modell zur Untersuchung der Erythropoese zeigt zwei Hauptmerkmale. Erstens aktiviert eine schnelle Veränderung der Sauerstoffsensoren unmittelbar das gespeicherte und das im Umlauf befindliche Erythropoetin. Zweitens wird der normale Verlauf des Umsatzes von roten Blutkörperchen durch die Sauerstoffsensoren beeinflusst, die die Hypothalamuskontrolle des zyklischen Verhaltens des Hypophysenzwischenlappens und seiner innerdrüslichen Kolloidproduktion steuern.

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PRO EXPERIMENTIS

Separation of Fast and Slow Components of S-100 Protein by Column Electrophoresis with Continuous Buffer System and Application to Micro Electrophoresis

The S-100 protein is a defined and brain specific protein. Correlations between the protein and neural functions are supposed but not yet certain. For the physiochemical study of neural functions, micro analytical methods at the cellular level are valuable because of the highly differentiated structure and function in the central nervous tissue.

With regard to the separation of the S-100 protein, UYEMURA et al.[1] tried slab electrophoresis of mixed agarose acrylamide gel with continuous buffer system and obtained much better results in comparison with that of starch gel electrophoresis[2]. According to the former electrophoretic systems, brain S-100 was separated into a fast and a slow migrating fraction. Identifications of the fractions have been detailed[1,3-7]. In the present paper, an application of the method to micro disc electrophoresis is described.

Materials and methods. Cats were anesthetized with sodium pentobarbital and rats were killed by decapitation. The brains were rapidly obtained after extensive craniotomy and separated respectively into forebrain, brain stem and cerebellum. These brain samples were homogenized in a Teflon homogenizer with 2 volumes of *Tris*-phosphate buffer 5 m*M* (pH 7.1) and centrifuged at $10,000 \times g$ for 30 min. Before use the supernatant was colored with a small amount of bromphenol blue (BPB) and used as a sample for electophoresis. Extracts from other organs of cat were prepared in the same way. In order to obtain micro samples for micro electrophoresis fresh cells or small pieces (approximately 1 µg of wet wt.) of tissue in identified locations were dissected from brain slices (1.5 mm

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