Relationship Between R_m Values and Protein Binding of Corticosteroids and Androgens

The usefulness of R_m values for structure/activity studies in a series of testosterone esters was previously pointed out[1,2]. The purpose of the present work was to show a relationship between R_m values and albumin-binding of corticosteroids and androgens. The R_m values of the test compounds were determined by means of a reversed-phase TLC method, the details of which have already been described[1].

The mobile phase consisted of water in various mixture (v/v) with acetone. The stationary phase was represented by a Silica Gel G layer impregnated with Silicone oil. In the case of corticosteroids, the acetone concentrations in the mobile phase ranged from 16 to 23.5%; in that of androgens from 24 to 52%. Because of a linear relationship between R_m values and acetone concentrations in the mobile phase[1], it was possible to calculate, for each compound, a theoretical R_m value corresponding to a 0% acetone concentration (Table).

In the Table are reported some data of CHEN et al.[3] regarding the protein-binding of the test compounds. Log BR indicates the logarithm of the percentage ultrafilterable fraction. Since the ultrafilterable fraction is inversely related to the bound fraction, steroids with high log BR values are bound to the least extent. The best rationalization of the relationship between log BR and R_m values is provided by Eq. 1, with a correlation coefficient of 0.964.

$$\log BR = 2.290 - 0.672\, Rm \quad (n = 9,\ r = 0.964,\ s = 0.094) \qquad (1)$$

The negative sign associated with the R_m term shows that protein-binding increases with the R_m values. Since higher R_m values indicate compounds more lipophilic, protein-binding depends linearly on the lipophilic character of molecules.

This result is in agreement with those of HANSCH et al.[4,5], FUJITA et al.[6] and BIRD et al.[7], who showed that protein-binding depends mainly on lipophilicity in several series of drugs. In the case of acids or bases, also the electronic character of molecules can play an important role in protein-binding. KLOTZ et al.[8] showed that the pK_a values, which can be considered as an expression of the electronic character of molecules, were inversely related with the protein-binding of sulfonamides. FUJITA[9] showed that uncoupling activity of phenols is mainly dependent on their pK_a values. However, in a series of uncharged molecules, such as steroid compounds, the most important factor seems to be the lipophilic character, which can be usefully expressed by the chromatographic R_m values. The TLC technique has its major limit in the adsorption phenomena which can affect the distribution of the compounds between the polar phase and the non-polar one. Therefore the direct measurement of the partition coefficient seems still to provide the most reliable results. However, the advantages of the chromatographic technique, as pointed out by BOYCE and MILBORROW[10], are noteworthy. The method is rapid and simple, and requires a very small amount of material; this does not need to be very pure because impurities are separated during the migration. The detection of spots by unspecific methods avoids the need for specific quantitative analytical procedures. Finally, in an earlier paper[1] a very good correlation was shown between the R_m values of testosterone esters and their π values obtained from the partition data in an octanol/water system.

Riassunto. Un metodo cromatografico su strato sottile a fasi invertite ha permesso di ottenere valori R_m altamente correlati con il legame con l'albumina in due serie di corticosteroidi e androgeni.

O. GANDOLFI, A. M. BARBARO and G. L. BIAGI

Cattedra di Farmacologia e Farmacognosia dell'Università di Bologna, Via Irnerio, 48, I-40126 Bologna (Italy),
20 November 1972.

Albumin binding and R_m values of various steroids

Steroid	log BR[3]	R_m
Androsterone	0.74	2.391
Deoxycorticosterone	0.90	2.057
Dehydroisoandrosterone	0.95	1.790
Corticosterone	1.25	1.447
Dehydroandrosterone	1.28	1.607
Cortisone	1.50	1.121
Hydrocortisone	1.49	1.214
11 β Hydroxyandrostenedione	1.60	0.940
Prednisolone	1.69	1.141

The log BR values indicate the logarithm of the percentage ultrafilterable fraction.

[1] G. L. BIAGI, M. C. GUERRA and A. M. BARBARO, J. med. Chem. *13*, 944 (1970).
[2] G. L. BIAGI, A. M. BARBARO and M. C. GUERRA, Experientia *27*, 918 (1971).
[3] P. S. CHEN JR., J. H. MILLS and F. C. BARTTER, J. Endocr. *23*, 129 (1961).
[4] C. HANSCH and A. R. STEWARD, J. med. Chem. *7*, 691 (1964).
[5] C. HANSCH, K. KIEHS and G. L. LAWRENCE, J. Am. chem. Soc. *87* 5770 (1965).
[6] T. FUJITA and C. HANSCH, J. med. Chem. *10*, 991 (1967).
[7] A. E. BIRD and A. C. MARSHALL, Biochem. Pharmac. *16*, 2275 (1967).
[8] I. M. KLOTZ and F. M. WALKER, J. Am. chem. Soc. *70*, 943 (1948).
[9] T. FUJITA, J. med. Chem. *9*, 797 (1966).
[10] C. B. C. BOYCE and B. V. MILBORROW, Nature, Lond. *208*, 537 (1965).

Failure of Hemicholinium-3 to Inhibit the Uptake of ^{3}H-Choline in Mouse Brain in vivo

Tritium-labelled choline and acetylcholine have been detected in mouse brain almost immediately after i.v. injection of ^{3}H-choline of high specific activity[1]. Thus choline is able to pass the blood brain barrier in spite of being a polar quaternary ammonium compound. This uptake of choline may therefore be due to an active mechanism of the same type as that operating in the cholinergic nerve terminals. Since the neuron uptake is inhibited by hemicholinium-3[2] and troxopyrrolidinium[3], we considered it of interest to investigate the effect of these compounds on the uptake of ^{3}H-choline into mouse brain in vivo.

[1] J. SCHUBERTH, B. SPART and A. SUNDVALL, J. Neurochem. *16*, 695 (1969).
[2] F. C. MACINTOSH, Can. J. Biochem. *37*, 343 (1959).
[3] S. P. BHATNAGAR, A. LAMB and J. O. MCCOLL, Biochem. Pharmac. *14*, 421 (1965).