Table I. Ileal absorption of disodium ethane-1-hydroxy-1,1-diphosphonate (^{14}C-EHDP) and disodium dichloromethylene diphosphonate (^{14}C-Cl_2MDP) in the chick

	^{14}C-EHDP	^{14}C-Cl_2MDP
No. of chicks	6	6
Body weight (g)	170 ± 15.0[a]	192 ± 7.5
Length of ileal segment (cm)	19.3 ± 0.9[a]	22.7 ± 1.4
Absorption (% dose)	28.1 ± 1.4[b]	43.3 ± 2.3
In gut tissue (% dose)	15.4 ± 1.9[a]	11.8 ± 2.9
Into body (% dose)	12.7 ± 2.1[b]	31.5 ± 2.5

Values are means ± SE
[a] Difference between EHDP and Cl_2MDP groups not significant ($p > 0.05$). [b] Difference between EHDP and Cl_2MDP groups significant at $p < 0.001$.

Table II. Comparison of absolute transfer of ^{14}C-EHDP and ^{14}C-Cl_2MDP across chick ileum in situ

	Absorption (μmoles/cm/h)	Into body
EHDP (8 m*M*)	0.46	0.21
Cl_2MDP (8 m*M*)	0.61	0.44

were added to a small screw-cap bottle, dried at 65 °C, and solubilized with Soluene 100 (Packard®) at 65 °C. A 0.1 ml aliquot of the solubilized material was added to 10 ml of liquid scintillation solution made from 600 ml toluene and 400 ml ethylene-glycomonoethylether in which were dissolved 80 g Naphtalene (Merck® Nr. 6200) and 7.0 g Butyl-PBD (Ciba-Geigy®). The radioactivity was measured in a Packard Tricarb scintillation spectrometer. The luminal solution (0.2 ml aliquot from 40 ml) was counted in the same fashion. Quench corrections were made by the use of internal standards.

Results and discussion. The values, given in Table I, indicate that both diphosphonates are absorbed from chick ileum. Within the 15 minute absorption period, about 28% and 43% of the injected EHDP and Cl_2MDP, respectively, left the intestinal lumen. About 15% and 12% of EHDP and Cl_2MDP, respectively, remained in the intestinal tissue, whereas 14% and 32% of these compounds were tranferred to the body, i.e., left the intestinal region. Cl_2MDP was absorbed and was transferred into body to a significantly greater degree than EHDP ($p < 0.001$).

In Table II, the amount of the diphosphonates that was transferred out of the lumen or entered the plasma per unit time and per unit length as calculated from the specific activity of the labelled dosing solution and the amount of radioactive label that was transferred are given.

As before, the rate of translocation of Cl_2MDP was somewhat greater than that for EHDP. It is interesting to compare the tranfer rate of diphosphonates to that of inorganic phosphate (P_i). In a different but comparable study in chicks[18], the percentage of the dose of P_i absorbed from ileal segment into the body was found to be 27.6% and 24.5% for luminal concentrations of 5 m*M* and 20 m*M* respectively. Assuming a linear relation between these 2 concentrations, the interpolated percentage for 8 m*M* would be 27%, and the transfer rate of P_i into the body would be 0.55 μmoles/cm/h. This value for P_i transfer is greater than that for EHDP, but not too dissimilar from the value for Cl_2MDP.

These results indicate that the diphosphonates can cross the intestinal epithelium, and that the intestinal tract might represent a significant route of entrance of these compounds into the body. However, it should be recalled that absorption was occurring from a washed intestinal loop containing little or no residual ingesta. The previous suggestion that diphosphonates are little absorbed from the gastrointestinal tract of mammals was based on feeding experiments or the administration of the compounds per os to the fasted animal[16]. It was proposed by MICHAEL et al.[16] that this low absorption rate might be due to binding of the diphosphonates to some endogenously secreted or dietary substances. Also the study of MICHAEL et al.[16] was done with mammals only, whereas the current results were obtained in birds and therefore might reflect species variability in the absorption of EHDP and Cl_2MDP. The present experimental protocol does provide a defined system for the systematic assessment of the effect of various factors on diphosphonate translocation across the intestine.

Résumé. Une proportion non négligeable de disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP) et de disodium dichloromethylenediphosphonate (Cl_2MDP) peut être absorbée au niveau de l'ileum de poulet. L'absorption du Cl_2MDP est significativement supérieure à celle de l'EHDP.

R. H. WASSERMAN[19], J.-P. BONJOUR[20] and H. FLEISCH[20,21]

Department of Physical Biology, New York Veterinary College, Cornell University, Ithaca (N.Y., USA); and Department of Pathophysiology, University of Berne, Hügelweg 2, CH-3012 Bern (Switzerland), 24 April 1973.

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[19] Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca (N.Y., USA).
[20] Department of Pathophysiology, University of Berne (Switzerland)
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Correlation of Physicochemical Parameters and Biological Activity in Steroids. 9α-Substituted Cortisol Derivatives[1]

The nature of the relationship between chemical constitution and biological activity in steroids is of both practical and theoretical importance. Recently, we demonstrated that a given molecular modification in a

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steroid may exert its effect through a variety of steric[2] or inductive mechanisms[3]. Such complex relationships must be common to structure-function analyses in many drugs, and the separation of some of these variables has been greatly facilitated by the multiple parameter approach[4]. We now describe the application of this approach to steroids, for the first time. In this report we consider the anti-inflammatory activity[5a-d] of 9α-substituted cortisol derivatives (Table). Previous workers[5b] attempted to account for the biological influence of 9α-substituents on the basis of their inductive effects, but this factor explains only a portion of the variation (cf. equation 3).

Methods. Rather than attempting to derive de novo constants[6a-d], we utilized the stochastic method using known physicochemical parameters[4] for the inductive effect (σ_I)[7], the hydrophobic bonding power (π)[8], and the size of substituents (molar refractivity, P_E)[9] and (E_S)[10]. From the data in the Table we derived via the method of least squares equations (1–7). In the above equations, n represents the number of data points used in the regression, r is the correlation coefficient and s is the standard deviation.

Results. Of the single-parameter equations (1–3), the one with π gives the poorest result. Independently σ_I and P_E account for only 23% and 30%, respectively, of the variance in the data. Together they account for 73% of the variance. In the two-parameter equations, little, if anything, is gained using π.

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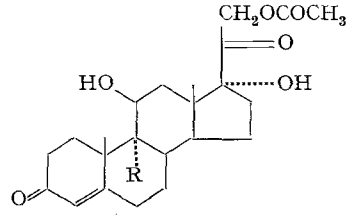
	n	s	r^2	r
(1) $\log A = -0.1799 - 0.2248\pi$	7	0.8708	0.0378	0.1943
(2) $\log A = 0.3219 - 0.0959 \log P_E$	7	0.7433	0.2989	0.5467
(3) $\log A = -0.7408 + 1.7707\,\sigma_I$	7	0.7791	0.2296	0.4792
(4) $\log A = 0.5992 + 0.5821\pi - 0.1623 \log P_E$	7	0.7634	0.4084	0.6391
(5) $\log A = -0.7256 - 0.2965\pi + 1.8857\,\sigma_I$	7	0.8337	0.2943	0.5425
(6) $\log A = -0.2385 - 0.1298 \log P_E + 2.5396\,\sigma_I$	7	0.5120	0.7339	0.8567
(7) $\log A = 0.0726 + 0.7642\pi - 0.2202 \log P_E + 2.7794\,\sigma_I$	7	0.3267	0.9187	0.9585

Liver glycogen deposition activity and substituent constants for 9α-substituted cortisol derivatives



R	Obsd.[a] relative Activity	Calcd. relative Activity	Obsd. log A	Calcd.[b] log A	σ_I[c]	π[d]	Log Es[e]	Log P_E[f]
F	10.7	13.3	1.03	1.124	0.52	−0.17	0.78	1.20
Cl	4.7	2.3	0.67	0.364	0.47	0.39	0.27	5.96
Br	0.3	0.7	−0.52	−0.169	0.45	0.60	0.08	8.86
I	0.1	0.1	−1.00	−1.168	0.38	1.00	−0.16	13.90
OH	0.2	0.2	−0.70	−0.696	0.25	−1.16	0.69	2.62
H	1.0	0.7	0.00	−0.170	0.0	0.0	1.24	1.10
CH_3	0.1	0.2	−1.00	−0.805	0.0	0.50	0.0	5.72
OMe	0.0γ	0.1	—	−1.186	0.25	−0.47	0.69	7.24
OEt	0.0γ	0.0	—	−1.821	0.25	0.03	0.69	11.86
SCN	0.0	0.0	—	−1.864	0.55	0.03	0.17	15.84

[a] Relative activity (cortisol acetate = 1) from ref. [5]. [b] Calcd. using equation (7). [c] From ref. [7]. [d] From ref. [8]. [e] From ref. [10]. [f] From ref. [9].

γ This compound was resynthesized and the biological activity remeasured by the same technique by Endocrine Laboratories, Madison, Wisconsin. We thank Dr. WINSTON HO for the synthetic work.

Equation (7) gives the best correlation. This equation satisfies the 'F-test'[11] and is statistically significant at the 0.95 confidence level, $F_{3,3} = 11.3$ ($F_{3,3}$ at the 0.95 level is 9.6). Equation (7) is a significant improvement over the two-parameter equation (6); ($F_{1,3} = 6.8$, $F_{1,3} = 6.8$, $F_{1,3\ \alpha=0.1} = 5.5$). Ideally, one would like to have 15 data points for a three variable equation. In effect, there are 10 data points in the present case, but only 7 have been used in deriving the equation. Although inactive compounds cannot be used to fit the regression, nevertheless, the fact that equation (7) predicts the SCN, OET and OMe compounds to have little or no activity, gives confidence in its correctness.

The positive coefficient with σ_I and with π indicates activity is promoted by electron-withdrawing groups and by groups of high hydrophobicity. The negative coefficient with P_E indicates an increasing activity with decreasing size of the substituent.

Equation (8), having a form comparable to equation (7), compares the quality of fit with another parameter for size, E_S.

$$\log A = -1.6416 + 1.4777\,E_s + 0.3920\pi + 2.5271\,\sigma_I \quad (8)$$

n	s	r^2	r
7	0.5950	0.7305	0.8547

It gives the same qualitative answer but the correlation is poor. The steric parameter E_S is directional in nature. The fact that E_S gives poorer results than P_E indicates a bulk tolerance problem rather than a steric effect in the Taft sense.

Discussion. Recently, we found through X-ray crystallographic studies[2] and CNDO/2 calculations[3] that the conformation and electron density distribution of the 9α-substituted cortisol derivatives is markedly affected by the nature of the 9α-substituent. Major changes are seen in the conformation of the A-ring, and in the electron density on the 11β-OH and at C-4. These effects offer an explanation for the high dependence on steric and electronic terms in equation (7). Clearly, the inductive nature of the 9α-substituent will affect its influence on the electron density distribution in the substituted compound, whereas the steric influence will have conformational and electronic consequences. The use of equation (7) to evaluate the biological effect of a substituent is, of course, far easier and more direct than the CNDO/2 approach.

The significance of the π parameter is more difficult to delineate. The increase in activity with increasing hydrophibicity could be due to better transport to the site of action for more lipophilic compounds and/or to hydrophobic interaction at the active site[12]. The fact that large groups (as measured by P_E) are only weakly active indicates that α-substituents may have to fit the receptor site, a situation in harmony with a positive role for π for 9α-functions. Although it has been suggested that corticoids interact with the receptor on the β-face[13], this result could be evidence that the 9α-substituent does in fact interact with the receptor.

Equation (7) offers the chance to predict more active corticoids but few substituents meet the requirements. A 9α-CF_3 group should have activity of about 4.0 relative to hydrocortisone, but most groups with the required hydrophobicity are too large to be active.

Resumen. Por primera vez se han analizado las relaciones estructura-función de diez derivados del 9α-cortisol substituido usando técnicas de regresión de parámetros múltiples. Se concluye que los factores electrónicos y estéricos son de mayor importancia al determinar el efecto del substituyente, y que la unión hidrofóbica también es de importante consideración. Se discute el significado de estos resultados.

M. E. Wolff and C. Hansch

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco (California 94122, USA) and Department of Chemistry, Pomona College, Claremont (California 91713, USA), 19 February 1973.

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Autofluorescence of Isolated Unfixed Rabbit Deiters' Neurons and Surrounding Neuroglial Clumps

Neurons were isolated from the Deiters' nuclei of young rabbits weighing 2–3 kg by the method of Hyden[1,2]. Groups of small neuroglia, of similar volumes to which Hyden has given the name 'clumps'[3] were also separated from the adjacent neurons. The single neurons or neuroglial 'clumps' were incubated in '199' culture medium[4] in parallel-walled chambers made from microscope slides[5]. Over 150 neurons and 50 neuroglial clumps were examined with mercury vapour illumination, at an overall magnification of ×600, using Leitz filters BG 38 and UG 1, under either a Beck 48 or a Leitz Orthoplan microscope; the exciting wavelength was approximately 3650 Å. Both the neurons (Figure 1) and the neuroglial 'clumps' fluoresced. The intensity of this fluorescence in a random selection of these cells was measured using an EEL microphotometer; the image contrast was calculated using the formula of Young[6], as

$$\frac{\text{intensity of object} - \text{intensity of background}}{\text{intensity of object.}}$$

For 11 neurons and 9 neuroglial 'clumps', the contrast was 0.28 ± 0.07, and 0.34 ± 0.22, respectively. The relatively larger variation in respect of the neuroglial clumps probably reflects greater variability of their sizes.

Methylene blue causes amino acids to fluoresce[7], and '199' medium contains several fluorochromes[4]. Therefore, it was decided to isolate the same cells without methylene blue in isotonic NaCl only, and to examine them for fluorescence immediately. As soon as they were exposed, a weak applegreen fluorescence was detected, and then

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