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.

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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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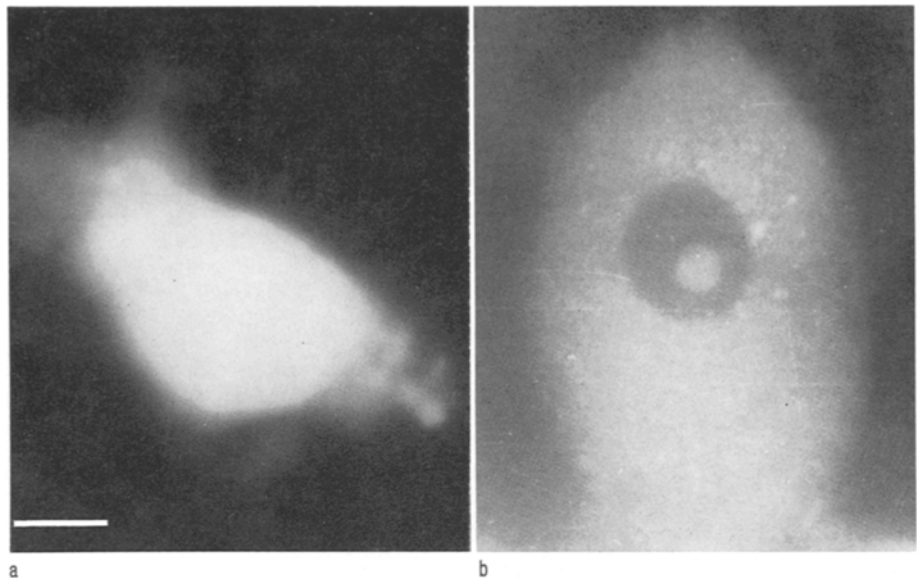

Fig. 1. Fluorescence of 2 neurons isolated using methylene blue, incubated in '199' medium[8]; a) a cell fluorescing 30 min after placing in the medium; b) another cell after 6 weeks' incubation. Note that the nucleolus is fluorescing. The bar is 20μm long in all figures.

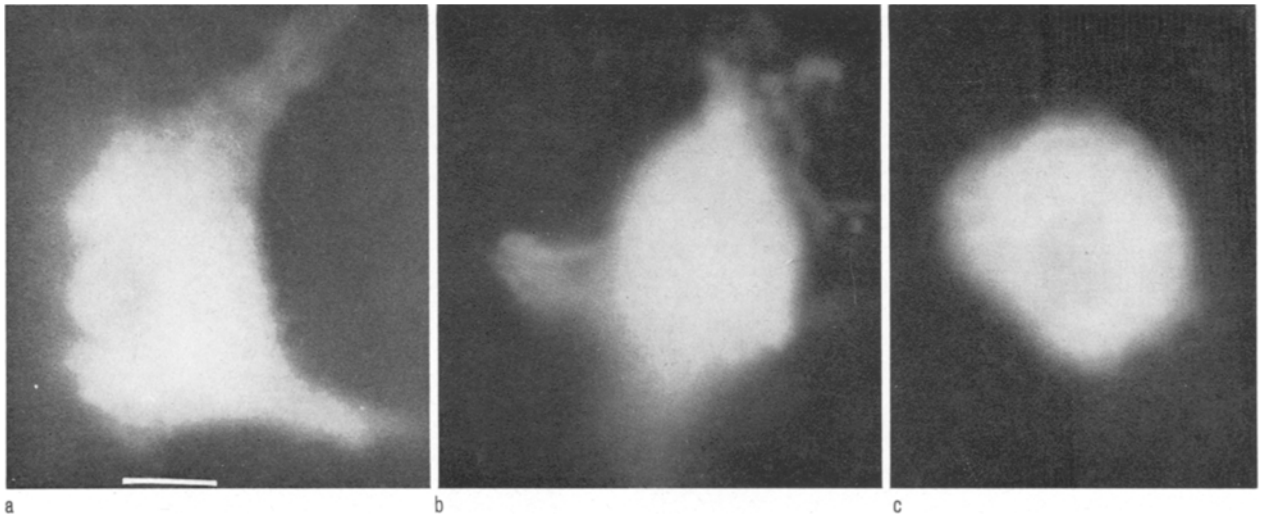

Fig. 2. Fluorescence of 3 neurons, isolated in, and placed in 0.9% NaCl solution. The nucleus of one cell (a) has been partially separated and is seen to fluoresce less than the cytoplasm. Cell (a) had been placed in 0.9% NaCl solution immediately after isolation, while cells (b) and (c) had been kept in saline for 6 weeks at 4 °C.

increased to a maximum intensity after 30–45 min continuous exposure (Figure 2); the image intensity of 11 neurons at this time was 0.22 ± 0.08, and for 13 neuroglial 'clumps' was 0.23 ± 0.09. It did not change in intensity for at least 6 weeks if the cells were kept in the medium at 4 °C. It was always present uniformly throughout the cytoplasm, and usually in the nucleolus, but rarely in the nucleoplasm (Figure 2). It did not diffuse out. Whereas the neurons took 30–45 min for the full intensity of the fluorescence to develop, the neuroglial clumps fluoresced immediately at their maximum intensity and with the same wave length of emission. The fluorescence of both neurons and neuroglia was present without added fluorochomes, in unfixed cells, and in aqueous media. Its wavelength was examined using either a Leitz or a Browning microspectroscope, and the yellow green emission was of peak wavelength 5400–5700 Å. It was concluded to arise from the lipofuscin[8], which has been detected previously in sections of fixed paraffin-embedded nervous tissue[9–12]. However, the present findings also

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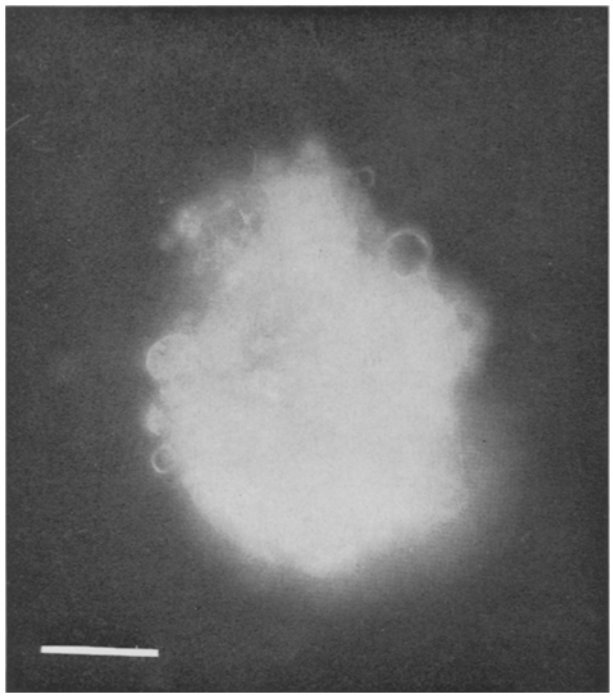

Fig. 3. Fluorescence of a neuroglial 'clump' isolated in, and immediately examined in, 0.9% NaCl solution.

differed from these quoted, in that 1. the present cells, the whole of the cytoplasm fluoresced, compared with less than 18% in the latter, 2. the lipofuscin in our cells was not confined to granules, 3. the present neurons and neuroglia were unfixed and isolated, 4. in the animals were generally younger, and 5. the preparation carried out in the present experiments was much simpler than that in the experiments quoted.

Résumé. Nous avons démontré l'autofluorescence dans des neurones et neuroglies détachées du noyau de Deiters du lapin. Les cellules furent isolées et examinées dans une solution de 0.9% NaCl, sans fixation, deshydration ou addition de fluorochromes. La longueur d'onde de la fluorescence était 5400–5700 Å, et la fluorescence apparaissait d'habitude dans le cytoplasme et le nucléole. On a conclu qu'elle provient de la lipofuscine.

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[13] We wish to thank the Handicapped Children's Aid Committee of London for a studentship to T.H. and substantial equipment.

Cytochemical Changes of a Glycocalyx of Human Placenta with Maturation

Diastase-resistant, PAS-positive material was found in the free surface of the syncytiotrophoblast of the chorionic villi of the immature human placenta[1]. Electron microscopy[2,3] and further characterization of carbohydrates by light microscopic histochemical techniques[3] indicated the presence in the syncytiotrophoblast of the human immature placenta of a cell coat or glycocalyx which contained carbohydrates. Thus, it was shown that this surface coat contained sialic acid and that it was fluffy in appearance under the electron microscope[3]. We shall report now cytochemical variations of plasmalemma-bound carbohydrates of the syncytiotrophoblast of human placenta with age.

Materials and methods. Light microscopy. Tissue blocks of placenta of the 2nd, 5th and 9th month of pregnancy were fixed in 10% formalin containing 2% calcium acetate, dehydrated and embedded in paraffin. Sections were stained with the following procedures: a) Periodic acid-Schiff, with and without prior diastase digestion[4]. b) Colloidal iron[5]. c) Alcian blue (pH 1.0 and 2.5)[5]. d) Alcian blue (pH 1.0 and 2.5)-PAS sequence[5]. e) Aldehyde fuchsin[6]. f) Aldehyde fuchsin-alcian blue sequence[7]. g) Alcian blue-safranin[8]. h) Periodic acid-*p*-diamine procedure[9]. i) Periodic acid-phenylhydrazine-Schiff[8]. j) Alcian blue with graded increases in magnesium chloride (0.1, 0.2, 0.5, 0.8 and 1.0 *M*)[10,11]. k) Azure A at pH 1.0, 2.0, 3.0, 4.0 and 5.0[8]. l) Methylation at 37 °C and 60 °C[12–14]. m) Methylation (37 °C and 60 °C)-saponification sequence[8]. n) Sialidase digestion and AB procedure[15]. o) Two-step PAS[16].

Electron Microscopy. Tissue blocks were fixed in glutaraldehyde-Ruthenium red and osmium tetroxide-Ruthenium red[17]. Sections were stained with lead citrate and uranyl acetate.

Results and discussion. As shown in the Table, the PAS reaction of the syncytiotrophoblast free surface, less intense at the 2nd month, increased progressively, reaching greatest intensity at the 9th month, in contrast with the basement membrane in which no significant changes were noted (Figure 1–3).

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