peroxidic material has been carried out, will be published later. In the experiments described here a relatively mild heat treatment of 10 min. at 120° was used. This reduced the initial relaxation rate by approximately 50 %. The hatched area I shown in Fig. 2 covers the relaxation curves (on a linear scale) of eight vulcanizates of modulus varying from 2.4 to 4.7 kg/cm² (at an extension ratio of 1.25), the upper curve corresponding to the higher modulus. Relaxation was carried out in dry oxygen at 90°. Without preheating, a modulus of 2.7 gives the relaxation curve II. Several factors play a part in determining the dependence of the curves I on modulus: The dependence of the concentration of the peroxidic material on the degree and conditions of vulcanization, the dependence of the oxidation rate on this concentration, and the consumption of natural antioxidant as a function of degree and mode of vulcanization.

The curve III represents a preheated sample of modulus 2.7 kg/cm² relaxed at 100°. The activation energy calculated from the relaxation curves differs little from the value obtained for the inhibited relaxation of the whole crepe vulcanizates described above. In accordance with the network theory the amount of oxygen absorbed (preliminary gravimetric measurements) at 99 % relaxation is proportional to the degree of vulcanization. There was no measurable crosslinking at 90°.

Fig. 3 shows the relaxation (at 90°) of samples vulcanized a) in water and with 5 % stearic acid in the rubber, b) in 2 N NaOH, and c) in pure DTBP vapour. (The ranges of moduli covered by the areas shown are not as large as in Fig. 2.) Apparently the stearic acid promotes the deactivation of the proteins, giving a less inhibited reaction, whereas the addition of NaOH to the water mainly serves to reduce the water concentration in the rubber, thus reducing the rate of deactivation by the DTBP. So efficient is this osmotic effect that a dry sample vulcanized in a press gave exactly the same relaxation curve.

It is interesting to observe the effect of incorporating 0.2 % copper stearate in the samples of a) before preheating. There is a marked reduction in the initial relaxation rate, presumably because of a more complete destruction of the peroxidic material*. In the

later stages, however, the catalytic and antioxidant-inactivating effect of the copper stearate becomes apparent, giving a much faster rate than uncatalyzed samples.

By the vulcanization in DTBP vapour for 30 min. at 150°, the rubber became seriously overvulcanized at the surface. As should be expected, the curves c) in Fig. 3 show that the antioxidant-free stage is reached comparatively late in this case.

This investigation is being completed and extended to comprise accurate oxygen absorption measurements in order that the relation between this pure scission reaction and the detailed shape of the relaxation curve may be studied.

This work has been made possible by the kind assistance and financial support from *The British Council, The Rubber Development Board, The Rubber Producers Research Association, The Norwegian Council for Scientific and Industrial Research* and the "*Fridtjof Nansens Fond*".

1. Farmer, E. H. and Moore, C. G. *J. Chem. Soc.* **1951** 142.
2. Bloomfield, G. F. and Farmer, E. H. *Trans. Inst. Rubber Ind.* **16** (1940) 69.

Received July 14, 1955.

Separation of Corticosteroids by Countercurrent Distribution

HANS CARSTENSEN

Institute of Physiology, University of Upsala, Upsala, Sweden

The separation of adrenal cortical steroids by physical methods has been attempted mainly by paper chromatography^{1,2}. Countercurrent distribution has been applied by Voigt³ in order to fractionate some nitrogen-containing, highly polar substances in adrenocortical extracts, supposed to be of steroidal nature. Talbot *et al.*⁴ have recently reported the use of countercurrent distribution for fractionation of urinary corticosteroids. They seem, however, not to have achieved a satisfactory separation. Engel *et al.*⁵ found that a 150 transfer countercurrent distribution was insufficient to separate urinary metabolites of corticosterone. The present experiments show that most corticosteroids may be

* Note added in proof: This marked effect of copper stearate indicates that these peroxidic materials are rubber hydroperoxides. They may possibly be formed at the moment the cooled sample (with a certain amount of free radicals) is removed from the press or the autoclave and exposed to the air.

Table 1. Partition coefficients of corticosteroids in different solvent systems evaluated from counter-current distribution data.

- A: Benzene/Water (22.0° C)
 B: Benzene/Water (27.0° C)
 C: Benzene/50 % Methanol + 50 % Water (22.0° C)
 D: Benzene/50 % Ethanol + 50 % Water (22.0° C)
 E: n-Hexane/Water (22.0° C)
 F: 20 % Ethanol + 80 % Water; 0.05 % (w/v) NaCl/50 % Chloroform + 50 % n-Hexane (21.0° C)
 G: 60 % n-Hexane + 40 % Benzene/5 % Ethanol + 95 % Water (22.0° C)
 H: 20 % Ethanol + 80 % Water; 0.05 % (w/v) NaCl/70 % Carbon tetrachloride + 30 % n-Hexane (22.0° C)
 I: 20 % Ethanol + 80 % Water; 0.05 % (w/v) NaCl/Carbon tetrachloride (23.0° C)

Corticosteroid	Solvent system *								
	A	B	C	D	E	F	G	H	I
Δ^4 -Pregnene-11 β , 17 α , 21-triol-3,20-dione	0.32	0.39	0.14	0.30		2.57			
Δ^4 -Pregnene-17 α , 21-diol-3,11, 20-trione	0.92	1.01	0.26	0.64		0.54			
Δ^4 -Pregnene-11 β , 21-diol-3,20-dione	10.1		1.57	1.27			1.08	4.26	1.86
Δ^4 -Pregnene-17 α , 21-diol-3,20-dione	9.0	10.2	1.22	1.63			1.13	1.94	0.79
Δ^4 -Pregnene-21-ol-3,11,20-trione			2.6	2.23	0.03		6.1		
Δ^4 -Pregnene-21-ol-3,20-dione			16	7	2.45				

* The composition of the biphasic systems is described by the volumes of the pure components used for each phase.

separated in suitable biphasic systems by 24 to 48 transfers. Only the six conventional corticosteroids are included in this investigation. Aldosterone was not available.

The partition coefficients (K) for different systems of solvent pairs were calculated from the countercurrent distribution data according to the procedure of Way and Bennett⁶ (Table 1). A fairly good separation is obtained if the separation factor, β , *i. e.* the ratio of higher to lower K of a pair of solutes, exceeds 3 in a 24 transfer countercurrent distribution and if it exceeds 2.6 in a 48 transfer distribution. No single system is efficient enough in separating all six corticosteroids investigated. The separation of Δ^4 -pregnene-11 β , 21-diol-3,20-dione and Δ^4 -pregnene-17 α , 21-diol-3,20-dione did not fulfil the above-mentioned demands. The most favourable β value was obtained in system H (Table 1), $\beta = 2.35$. However, a reasonably simple differentiation is obtained in this case by applying the Porter-Silber reac-

tion^{7,8} — in which Δ^4 -pregnene-17 α , 21-diol-3,20-dione reacts and Δ^4 -pregnene-11 β , 21-diol-3,20-dione does not.

1. Bush, I. E. *Biochem. J. London* **50** (1951) 370.
2. Zaffaroni, A. and Burton, R. B. *J. Biol. Chem.* **193** (1951) 749.
3. Voigt, K. D., Schroeder, W., Beckmann, I. and von der Werth, H. *Acta endocrinol.* **14** (1953) 1.
4. Talbot, N. B., Ulick, S., Koupreianow, A. and Zygmuntowicz, A. *J. Clin. Endocrinol. and Metabolism* **15** (1955) 301.
5. Engel, L. L., Carter, P. and Fielding, L. L. *J. Biol. Chem.* **213** (1955) 99.
6. Way, E. L. and Bennett, B. M. *J. Biol. Chem.* **192** (1951) 335.
7. Porter, C. C. and Silber, R. H. *J. Biol. Chem.* **185** (1950) 201.
8. Wilson, H. and Fairbanks, R. *Arch. Biochem. and Biophys.* **53** (1954) 71.

Received July 22, 1955.