mit Reserpin geprüft, wobei sich eine eindeutige qualitative und quantitative Übereinstimmung der depressorischen und bradykarden Wirksamkeit der beiden Alkaloide ergab. Die durchschnittlich ermittelte Reaktion des Blutdrucks bei Anwendung des einen Alkaloids bewegt sich stets innerhalb der Variationsbreite der Wirkung des anderen Alkaloids. Auch die blutdruckwirksamen Schwellendosen sind im Durchschnitt der Fälle für beide Alkaloide gleich gross. Wie Reserpin hemmt auch das Canescin die pressorische Adrenalinwirkung nicht, sondern steigert sie sogar gelegentlich. Auf die Atmung wirkt Canescin ebenso wie Reserpin deutlich hemmend, was besonders bei narkotisierten Tieren schon nach kleinen Dosen der Fall sein kann. Diese weitgehende pharmakodynamische Identität von Canescin und Reserpin bestätigt sich auch darin, dass die Wirkung beider Alkaloide durch Decerebrierung sowie durch Dekapitierung (Spinaltier) in gleicher Weise beeinflusst wird. Am Spinaltier fehlt die typische Kreislaufwirkung vollständig, während sie beim decerebrierten Tier weitgehend erhalten bleibt.

Nach diesen Ergebnissen konnte es nicht mehr überraschen, dass für das Canescin auch eine verengernde Wirkung auf die Pupille sowie ein allgemein sedativer Effekt nachweisbar sind, wie das in charakteristischer Weise beim Reserpin der Fall ist. In unseren diesbezüglichen Untersuchungen an Mäusen und Hunden lassen sich weder für die miotische noch für die sedative Wirkung signifikante Unterschiede zwischen Canescin und Reserpin finden.

Zusammenfassend ergibt sich also die interessante Tatsache, dass dem Canescin, das heisst dem 11-Desmethoxyreserpin, in jeder Beziehung die Eigenschaften des Reserpins zukommen. Dieser Befund ist nicht zuletzt deshalb von Bedeutung, weil er zeigt, dass gewisse Veränderungen am Reserpinmolekül ohne Verlust der spezifischen Wirksamkeit möglich sind.

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*Pharmakologisches Laboratorium der Sandoz AG., Basel, den 7. Februar 1955.*

*Summary*

Canescine or 11-Desmethoxyreserpine, a new alkaloid isolated by Stoll and Hofmann from *Rauwolfia canescens* has been submitted to a comparative pharmacological study with Reserpine. The circulatory effects obtained with these two alkaloids can practically not be distinguished from each other. Like Reserpine also Canescine exhibits a sedative action and produces pupillary constriction.

## 19-Hydroxylation of $\Delta^4$-Androstene-3,17-dione and Dehydroepiandrosterone by Bovine Adrenals

Recently, it has been reported[1] that incubations with dehydroepiandrosterone (I) and $\Delta^4$-androstene-3,17-dione (II) with bovine adrenal homogenate preparations produced a wide galaxy of conversion products. The most abundant ones have been isolated and characterized as 11-$\beta$-hydroxyandrostenedione (IV), 6-$\beta$-hydroxyandrostenedione (V), 6-$\alpha$-hydroxyandrostenedione (VII), compound VIII tentatively identified as 6-$\alpha$,11-$\beta$-dihydroxyandrostenedione, and $\Delta^4$-androstene-3,11,17-trione (III). Furthermore, compound VI, a substance migrating as a single band in various paper chromatographic systems, has been characterized as follows: the elemental composition corresponded to a monohydroxylated androstenedione ($C_{19}H_{26}O_3$); m.p. 168–170°; $[\alpha]_D^{30}$ + 178° ± 4° (c. 0·56 in chloroform); $M_D$ + 538; $\tilde{\nu}_{min}^{solid}$ 3400 cm$^{-1}$ (O–H), 1725 cm$^{-1}$ (pentacyclic C = O), 1650 and 1620 cm$^{-1}$ (C = O, conjugated) (see Fig. 1); $\lambda_{max}^{MeOH}$ 242 m$\mu$ (log$\varepsilon$ 4·18), but no end absorption near 200 m$\mu$ in oxygen-free absolute ethanol[1]; *Rtol*[2] 1·35 cm/h visualized e.g. by the purple Zimmermann color characteristic of 17-keto-steroids. These data are compatible with the assumption

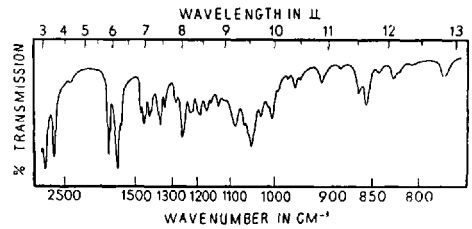



Fig. 1–Infrared spectrum of 19-hydroxyandrostenedione (VI) as solid film on a Perkin-Elmer 12-C spectrometer with a sodium chloride prism.

that during the bioconversion of II to VI the steroid nucleus was not altered, and the immediate problem was therefore to assign the correct position to the newly introduced hydroxyl group. Compound VI was not identical with any of the above isolated and the 11-$\alpha$-[3], 12-$\alpha$-[4], or 16-$\alpha$-[5]-monohydroxy-$\Delta^4$-androstene-3,17-dione. This communication will advance evidence for the elucidation of the structure of *compound VI as being 19-hydroxy-$\Delta^4$-androstene-3,17-dione* (see formulae).

Compound VI yielded upon exposure to acetic anhydride and pyridine or phosphoric acid at room temperature a syrupy acetate VI*a*:

*Rtlg* 0·7 cm/h; $\tilde{\nu}_{min}^{CS_2}$ 1737 cm$^{-1}$ (pentacyclic and acetate C = O), 1680 cm$^{-1}$ (conjugated C = O), 1225 cm$^{-1}$ ("acetate C = O stretch"); $\lambda_{max}^{EtOH}$ 239 m$\mu$; $[\alpha]_D^{26}$ ~ + 185° (chloroform).

The specific rotation decreased to ~ + 130° after high vacuum distillation of VI*a*, whereas the infrared spectrum remained almost identical; this would indicate that during the distillation a small amount of a strongly negative rotating substance was formed. Oxidation of

[1] A. S. Meyer, M. Hayano, M. C. Lindberg, M. Gut, and O. G. Rodgers, Acta Endocrin. *18*, 148 (1955).

[1] A. S. Meyer, H. Rosenkrantz, and G. Pincus, Amer. Chem. Soc. 126th Meeting, Abstract p. 53-0, New York, 1954. – The absence of an isolated olefinic bond in compound VI has been kindly confirmed by Dr. R. Norman Jones who examined the infrared absorption of the compound in chloroform solution with a Perkin-Elmer double-beam spectrophotometer with a sodium chloride prism (R. N. Jones, P. Humphries, E. Packard, and K. Dobriner, J. Amer. Chem. Soc. *72*, 86 [1950]), and was unable to detect any evidence of an unsaturation other than the $\Delta^4$-bond.

[2] The *R*-values indicate the mobilities of the substances in the ligroin- and toluene-propylene glycol paper chromatographic system respectively as defined previously (A. S. Meyer, M. Hayano, M. C. Lindberg, M. Gut, and O. G. Rodgers, Acta Endocrin. [l. c.]). For compound II for instance a *Rtlg* 2·2 cm/h and for compound IV a *Rtol* 4·0 cm/h have been determined.

[3] S. H. Eppstein, P. D. Meister, H. M. Leigh, D. H. Peterson, M. C. Murray, L. M. Reineke, and A. Weintraub, J. Amer. Chem. Soc. *76*, 3174 (1954).

[4] Ch. Meystre and A. Wettstein, Helv. chim. Acta *32*, 1978 (1949).

[5] Dr. Josef Fried, private communication.

*Table I.*–Rate of Formaldehyde Elimination as Estimated by the Chromotropic Acid Color Formation

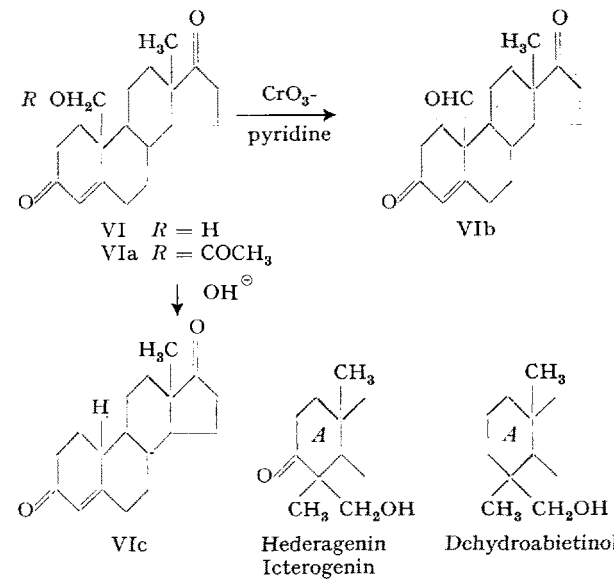
| Compound | Conditions* | Formaldehyde in per cent of theoretical after | | | |
|---|---|---|---|---|---|
| | | 10 min | 1·5 h | 3·0 h | 11·0 h |
| 19-Hydroxy-$\Delta^4$-androstene-3,17-dione (VI) . . . . | 3·4 N KOH | | | 46 | |
| | 2·3 N KOH | | 28 | 34 | 47 |
| | 1·3 N KOH | 5 | 13 | 21 | 32 |
| | 0·15 N KOH | | | | 11 |
| | 2·0 N $H_2SO_4$ | | | 0 | |
| 19-Hydroxy-$\Delta^4$-pregnene-3,20-dione . . . . . | 3·4 N KOH | | | 46 | |
| | 2·3 N KOH | | | 32 | 49 |
| | 1·3 N KOH | | | 18 | |
| 19-Hydroxy-3-oxo-$\Delta^4$-etienic acid . . . . . | 2·3 N KOH | | 18 | 26 | 45 |
| | 1·3 N KOH | | 9 | 14 | |
| Methyl hederagonate . . . . . | 1·3 N KOH | 14 | 45 | 66 | |
| | 0·4 N KOH | 8 | 26 | 37 | |
| | 0·03 N KOH | | 0 | | |
| Icterogenin . . . . . | 1·3 N KOH | 17 | 49 | 65 | |
| | 0·15 N KOH | | | | 36 |

* ~ 80 $\mu$g of substance were reacted at 24°C in 0·2 ml ethanol with 1·0 ml aqueous potassium hydroxide solution of various strength (or acid) and then neutralized with phosphoric acid; further treatment essentially as described by BARTON and DE MAYO, J. Chem. Soc. 887 (1954).

compound VI with chromium trioxide in acetic acid did not result in a product with the expected mobility for the corresponding tricarbonyl derivative. However, the desired tricarbonyl compound (VIb) was obtained in approximately 40% yield when the chromium trioxide-pyridine complex[1] (20 mg $CrO_3$ in 1 ml of pyridine for 5.7 mg of compound VI) was allowed to react at room temperature for 15 min. Besides VIb, some $\Delta^4$-androstene-3,11,17-trione and two slower migrating non-acidic substances were recognized in the oxidation mixture. Compound VIb showed

$R_{lig}$ 0·65 cm/h; $\tilde{\nu}^{CS_2}_{min}$ 1735 cm$^{-1}$ (pentacyclic C = O), 1723 cm$^{-1}$ (C = O), 1680 cm$^{-1}$ (conjugated C=O). Reaction with pyridine alone did not alter the mobility of VI.

These findings permitted the exclusion of a number of positions as likely points of attachment for the hydroxyl group of VI. A tertiary position ($C_{(8)}$, $C_{(9)}$, or $C_{(14)}$) was precluded by the demonstrated conversion of VI to the acetate VIa and the tricarbonyl derivative VIb. Any secondary hydroxyl group on the steroid nucleus not influenced by another functional grouping (as in position $C_{(12)}$) would have been oxidized by the acidic chromium trioxide to the tricarbonyl derivative. The hydroxyl group in compound VI was not located at $C_{(15)}$ and $C_{(16)}$, since the additional infrared carbonyl frequency of VIb was of the hexacyclic or aliphatic type. Other positions for the hydroxyl group were excluded by the following considerations. In the ultraviolet, 4-hydroxy-$\Delta^4$-3-ketosteroids absorb, as enolized $\alpha$-diketones, most intensely near 280 m$\mu$[2] in contrast to compound VI. When exposed to alkaline ethanol, 2- and 7-hydroxylated $\Delta^4$-3-ketosteroids produce characteristic changes in their light absorption spectra[3] different from those observed with compound VI[4]. The latter data were also distinct from those of $\Delta^{1,4}$-androstadiene-3,17-dione[1] which would undoubtedly have been formed from a 1-hydroxylated derivative under those alkaline conditions[2]. Thus positions $C_{(18)}$ and $C_{(19)}$ remained for




Partial formulae

further examination. A vague indication which might have been interpreted in favor of a hydroxyl group at $C_{(19)}$ was the hypsochromic shift of 3 m$\mu$ observed in the ultraviolet[3] when compound VI was converted to the acetate VI*a*.

[1] G. I. POOS, G. E. ARTH, R. E. BEYLER, and L. H. SARETT, J. AMER. Chem. Soc. *75*, 422 (1953).
[2] L. DORFMAN, Chem. Revs. *53*, 82 (1953).
[3] A. S. MEYER, manuscript in preparation.
[4] A. S. MEYER et al., Acta Endocrin. l. c.

[1] A. S. MEYER, manuscript in preparation.
[2] *Cf.* P. STIEBEL and CH. TAMM, Helv. chim. Acta *37*, 1094 (1954); see also [1] for a vinylogous instance.
[3] G. W. BARBER and M. EHRENSTEIN, J. Org. Chem. *19*, 1758 (1954).

The interesting observation by BARTON and DEMAYO[1], that relatively mild basic conditions lead to formaldehyde elimination with methyl hederagonate[2] and icterogenin[1], opened a new approach to our problem. Both these triterpenes possess a primary $\beta$-ketol grouping at ring *A* comparable to a 18-hydroxy-17-keto moiety at ring *D* of the $C_{19}$-steroid series. Since 19-hydroxy-$\Delta^4$-3-ketosteroids represent a vinyl analogue, 19-hydroxy-$\Delta^4$-pregnene-3,20-dione (19-hydroxy-progesterone)[3] and 19-hydroxy-3-oxo-$\Delta^4$-etienic acid[4] were tested under similar alkaline conditions; in these instances, as well as with compound VI, formaldehyde was also eliminated (Table I)[5]. Subsequently, compound VI was submitted to the alkaline treatment on a preparative scale (13·6 mg, 3·2 N in potassium hydroxide for 2·5 h). From the complex reaction mixture, an approximately equivalent quantity of the formaldehyde-dimedone compound (m.p. 188–189·5°) and the main steroidal alteration product VIc ($R_{lig}$ 1·65 cm/h)[6] were isolated in a 20% yield. After rechromatography and repeated crystallization from ether-pentane, 0·75 mg of slightly impure 19-nor-$\Delta^4$-androstene-3,17-dione (VIc) were secured; m.p. 168–171°; $\tilde{\nu}_{min}^{solid}$ 1725 $cm^{-1}$ (pentacyclic C = O), 1670 and 1620 $cm^{-1}$ (C = O, conjugated); $\lambda_{max}^{MeOH}$ 240 m$\mu$ (log $\varepsilon$ 4·21).

A mixed melting point with authentic material[7] did not show a depression, and identity was substantiated by the infrared spectra. It was anticipated that formaldehyde elimination of 19-hydroxy-$\Delta^4$-androstene-3,17-dione (VI) would lead to the same 19-nor-$\Delta^4$-androstene-3,17-dione (VIc) as obtained by alkaline[8] or acid[7] rearrangement of 19-nor-$\Delta^{5(10)}$-androsten-17-$\beta$-ol-3-one and subsequent oxidation of the 17-hydroxyl group, since both reactions would proceed by way of a common carbanion at $C_{(10)}$ to the thermodynamically favored configuration. Compound VI was compared with 19-hydroxyprogesterone and corresponded well in the following tests: molecular rotatory contribution ($\Delta M_D$) of the 19-hydroxyl group (Table II), the rates of the formation of "blue formazan"[9], and the changes in the ultraviolet spectra when these compounds were exposed to alkaline ethanol[10]; (in all these measurements 19-hydroxy-3-oxo-$\Delta^4$-etienic acid behaved irregularly[1]).

According to reports by G. PINCUS, R. I. DORFMAN, F. ELMADJIAN, and E. ROSEMBERG of this Institute, compound VI was not found to be markedly active in the following bioassays: modified myotrophic assay on rats[2], androgenic assay on chicks[3], electrolyte excretion assay (ratio of K/Na) on rats[4], and antitraumatic shock assay on rats[5].

Naturally occurring substances with a steroid skeleton oxygenated in $C_{(19)}$ are certain plant cardiac glycosides and possibly toad poisons[6]. 19-Hydroxyprogesterone and 19-hydroxy-desoxycorticosterone, prepared from the cardiac aglycone strophanthidin, did not have pronounced progestational and mineralocorticoid properties, respectively[7], paralleling the result of the androgenic assay on compound VI. However, 19-nortestosterone, 19-norprogesterone, and 19-nordesoxycorticosterone exhibited the basic bioactivity of the same order of magnitude as their corresponding 19-methylated compounds and in addition appeared to have activities of a different nature[8].

*Table II.*–Rotatory Contribution for the Introduction of the 19-Hydroxyl Group in $\Delta^4$-3-Ketosteroids

| Parent compound | $M_D$[1] | $M_D$ for the 19-hydroxylated cpd[2] | $\Delta M_D$ |
|---|---|---|---|
| $\Delta^4$-Androstene-3,17-dione (MW 286·4) | +573 | +538 | − 35 |
| $\Delta^4$-Pregnene-3,20-dione (MW 314·4) | +632 | +612[3] | − 20 |
| $\Delta^4$-Pregnen-21-ol-3,20-dione (MW 330·4) | +647 | +624[3] | − 23 |
| 3-Oxo-$\Delta^4$-etienic acid (MW 316·4) | +497 | (+356[4]) | (− 141) |
| Methyl-3-oxo-$\Delta^4$-etienate (MW 330·4) | +534 | (+385[4]) | (− 149) |

[1] All rotations were determined in a ~ 1% chloroform solution (accuracy of $M_D$ values ± 7). [2] In chloroform solution. [3] G. W. BARBER and M. EHRENSTEIN, J. Org. Chem. *19*, 1758 (1954). [4] P. TH. HERZIG and M. EHRENSTEIN, J. Org. Chem. *17*, 713 (1952).

The removal of the angular methyl group at $C_{(19)}$ is also of interest when one considers aromatization of ring *A* or *B*. Such elimination is considerably facilitated by the introduction of oxygen into $C_{(19)}$, and chemically those reactions have already been realized[9]. However, the

[1] D. H. R. BARTON and P. DEMAYO, J. Chem. Soc. *1954*, 887. – We are grateful to Dr. DEREK H. R. BARTON for having brought to our attention the formaldehyde elimination reaction and for the specimens of the triterpenes employed in this study.

[2] W. A. JACOBS, J. Biol. Chem. *63*, 631 (1925).

[3] G. W. BARBER and M. EHRENSTEIN, J. Org. Chem. *19*, 1758 (1954).

[4] P. TH. HERZIG and M. EHRENSTEIN, J. Org. Chem. *17*, 713 (1952). – We are indebted to Dr. MAXIMILIAN EHRENSTEIN and his co-workers for the two specimens of 19-hydroxylated steroids.

[4] Other tested hydroxylated $\Delta^4$-3-ketosteroids and dehydroabietinol (this latter substance kindly furnished by Dr. ROBERT P. JACOBSEN) did not give, as expected, positive chromotropic acid reaction. Corticosteroids, however, produced under the same conditions an unspecific reddish color of relatively faint intensity.

[6] The same ratio of mobility as for II and VIc (1:0·75) was also observed for testosterone and 19-nortestosterone when chromatographed on Whatman no. 1 paper with propylene glycol saturated ligroin. It is further worthy to note that the retardation of the mobility of VI by the primary hydroxyl group at $C_{(19)}$ is of the same order of magnitude like that of an equatorial hydroxyl group (e.g. at $C_{(6\alpha)}$).

[7] A. L. WILDS and N. A. NELSON, J. Amer. Chem. Soc. *75*, 5366 (1953). – 19-Nor-$\Delta^4$-androstene-3,17-dione (m.p. 171–173° [from methanol]),$[\alpha]_D^{22}$ + 146° ± 2° (chloroform), $\lambda_{max}^{MeOH}CH_3OH$ 240 m$\mu$ [log $\varepsilon$ 4·24]) was kindly supplied by Dr. MARCEL GUT.

[8] A. J. BIRCH, J. Chem. Soc. *367* (1950).

[9] A. S. MEYER and M. C. LINDBERG, Anal. Chem. (in press).

[10] A. S. MEYER, manuscript in preparation.

[1] These data would thus confirm that 19-hydroxyprogesterone and 19-hydroxydesoxycorticosterone, prepared by EHRENSTEIN and co-workers from strophanthidin, have the normal steroid configuration. (G. W. BARBER, M. EHRENSTEIN, J. Org. Chem. *19*, 1758 [1954]).

[2] E. EISENBERG and G. S. GORDAN, J. Pharmacol. Exper. Therapy *99*, 38 (1950).

[3] R. I. DORFMAN, Endocr. *42*, 1 (1948).

[4] M. R. COOK, Jr. and F. ELMADJIAN, J. Amer. Pharm. Assoc. *42*, 329 (1953).

[5] V. SCHENKER, Endocr. *53*, 345 (1953).

[6] *Cf.* reviews by L. F. FIESER and M. FIESER, *Natural Products Related to Phenanthrene*, 3rd ed. (New York, 1949); H. HEUSSER, Progr. Chem. Organic Natural Products *7*, 87 (1950); T. REICHSTEIN, Angew. Chemie *63*, 412 (1951); A. STOLL, Exper. *10*, 282 (1954).

[7] G. W. BARBER and M. EHRENSTEIN, J. Org. Chem. *19*, 1758 (1954).

[8] W. W. TULLNER and R. HERTZ, Endocr. *52*, 359 (1953). – L. G. HERSHBERGER, E. G. SHIPLEY, and R. K. MEYER, Proc. Exptl. Biol. Med. *83*, 175 (1953). – A. SANDOVAL, L. MIRAMONTES, G. ROSENKRANZ, C. DJERASSI, and F. SONDHEIMER, J. Amer. Chem. Soc. *75*, 4117 (1953). – Dr. R. I. DORFMAN informed me privately that 19-nortestosterone is a potent androgen when administered to chicks orally or by comb inunction.

[9] *Cf., inter al.*, M. EHRENSTEIN, A. R. JOHNSON, P. C. OLMSTED, V. I. VIVIAN, and M. A. WAGNER, J. Org. Chem. *15*, 264 (1950). – G. W. BARBER and M. EHRENSTEIN, J. Org. Chem. *19*, 363 (1954). – E. B. HERSHBERG, M. RUBIN, and E. SCHWENK, J. Org. Chem. *15*, 292 (1950).

physiological formation of estrone-type substances is still obscure. Nevertheless, the ability of mammalian tissue to introduce oxygen at $C_{(19)}$, described here, would tend to support the idea that removal of the angular group as a one-carbon fragment in the course of ring *A* aromatization is a possible pathway. Biochemical experiments to prove or disprove this hypothesis are now being initiated in our laboratory. In this connection it might also be recalled that estrone itself has been isolated from box adrenal extracts[1]. From structural considerations, the *in vitro* biohydroxylation of an angular methyl group, first demonstrated here, is unlikely to be preceded by dehydrogenation to an olefine since this would imply fission and re-formation of a C–C-linkage. The 11-$\beta$-hydroxylation of $\Delta^4$-pregnene-17-$\alpha$, 21-diol-3, 20-dione by a similar adrenal preparation does also not involve an olefinic intermediate with the subsequent addition of one mole of water; this has recently been shown by the use of deuterium oxide[2]. Whether these hydroxylations are effected as substitution reactions or a more complex oxidative sequence by way of either an ionic or a radical mechanism is still a matter of speculation.

The author is indebted for assistance in certain phases of the work to MARJORIE C. LINDBERG and PAUL SKOGSTROM. The investigation was supported by a grant of G. D. SEARLE and Company, Chicago, Illinois.

A. S. MEYER

*Worcester Foundation for Experimental Biology, Shrewsbury, Mass., January 2, 1955.*

*Zusammenfassung*

Die Aufklärung der Struktur von 19-Oxy-$\Delta^4$-androsten-3, 17-dion (VI), einer Substanz, isoliert nach Inkubation von $\Delta^4$-Androsten-3, 17-dion und von Dehydroepiandrosteron mit einem Rinder-Nebennieren-Homogenat, ist beschrieben. Dies ist unseres Wissens die erste Beobachtung der Hydroxylierung *in vitro* einer angulären Methylgruppe eines Steroids. Mehrere Folgerungen sind erörtert.

[1] D. BEALL, J. Endocr. *2*, 81 (1940).
[2] M. HAYANO and R. I. DORFMAN, J. BIOL. Chem. *211*, 227 (1954).

## Sul comportamento della sostanza fondamentale in membrane connettivali di ratto albino durante il passaggio di una soluzione salina

È noto che la diffusione di una soluzione acquosa nel derma è influenzata dalla presenza in esso di mucopolisaccaridi e dal loro grado di polimerizzazione (CHAIN e DUTHIE[1], DURAN-REYNALS[2], FAVILLI[3]. In un suo recente lavoro, DAY[4] ha provato che la velocità di passaggio di soluzione fisiologica attraverso membrane di connettivo sottocutaneo di topo albino aumenta grandemente sotto l'azione della jaluronidasi. Egli conclude pertanto che la sostanza interfibrillare può essere considerata come costituita da una microstruttura proteica, relativamente impermeabilizzata, in condizioni normali, da acido jaluronico.

La presente nota espone i risultati di ricerche eseguite per definire ulteriormente le particolarità del passaggio di una soluzione salina attraverso membrane connettivali, e per accertare se è possibile ottenere serie di valori relativamente omogenee, tali da permettere uno studio di eventuali modificazioni patologiche del connettivo anche sotto questo punto di vista e con questa tecnica, che presenta il vantaggio di poter essere seguita *in vitro*, al riparo da possibili interferenze di fattori estranei (regime del circolo locale, od altri).

La tecnica è quella proposta da DAY[1]. Le membrane, prelevate dal fianco degli animali, erano fissate a mezzo di un anello elastico piuttosto alto ad un estremo di un tubo immerso in un beaker contenente NaCl 9 %° in $H_2O$ distillata e riempito della stessa soluzione fino ad una altezza di cm 4 sopra il livello del liquido nel beaker. Si cronometrava il tempo impiegato dal menisco della soluzione nel tubo a scendere fra due traguardi posti a mm 3 l'uno dall'altro; il volume della porzione di cilindro compresa fra questi due traguardi era pari a $mm^3$ 71,2. Si è curato di mantenere costante la temperatura, operando in termostato: generalmente sono state usate temperature tra 32° e 34°C.

Come anche DAY riconosce, non è possibile ottenere serie di membrane di spessore omogeneo; sì che i tempi di passaggio della soluzione clorurosodica variano grandemente tra loro, anche quando si confrontino membrane provenienti dallo stesso animale. Si poteva tuttavia pensare, supponendo che nel normale sia contenuta entro limiti non troppo larghi la quantità di mucopolisaccaridi presenti, che fossero confrontabili i valori, ottenuti da vari individui, del rapporto tra tempo di passaggio della soluzione salina e tempo di passaggio della stessa soluzione addizionata di jaluronidasi (S/J). È stata usata in queste ricerche jaluronidasi liofilizzata da testicolo di toro (Kinetin, della casa Schering di Berlino), in quantità di 4 U.R.V. Schering per ml. In numerose prove eseguite i valori di tale rapporto, in ratti normali giovani di peso tra 30 e 125 g, hanno variato essenzialmente fra 6 e 18. Si è inoltre potuto osservare, usando successivamente membrane provenienti dallo stesso ratto, che, se i tempi di passaggio della soluzione clorurosodica (S) così come quelli della soluzione clorurosodica più jaluronidasi (J) possono singolarmente presi differire moltissimo da una membrana all'altra, i rapporti S/J sono generalmente simili; il che porta a supporre che la quota di mucopolisaccaridi nel connettivo sottocutaneo di uno stesso animale sia sensibilmente del medesimo ordine di grandezza per le regioni prese in esame (vedi tabella).

Valori, in secondi, dei tempi di deflusso di soluzione clorurosodica 0,9 % e della stessa + jaluronidasi, e rapporti tra questi due valori per coppie di membrane connettivali provenienti dal medesimo animale

| Ratti e relativo peso | Tempo di deflusso della soluzione salina (S), in second | Tempo di deflusso della soluzione con enzima (J), in s | Rapporto S/J |
|---|---|---|---|
| 1) ♀ g 30 | 226 | 14,8 | 15 |
| | 1294 | 72 | 18 |
| 2) ♀ g 75 | 113 | 14 | 8,1 |
| | 217 | 31 | 7 |
| 3) ♀ g 83 | 668 | 40 | 16,7 |
| | 93 | 5,8 | 16 |
| 4) ♀ g 125 | 95 | 8 | 11,9 |
| | 66 | 4,4 | 15 |
| 5) ♂ g 110 | 95 | 14,6 | 6,5 |
| | 99 | 17 | 5,8 |
| 6) ♂ g 33 | 86,8 | 12,2 | 7,1 |
| | 54 | 9 | 6 |

[1] E. CHAIN e E. S. DUTHIE, Brit. J. exp. Path. *21*, 324 (1940).
[2] F. DURAN-REYNALS, Bact. Rev. *6*, 197 (1942).
[3] G. FAVILLI, *Fenomeni di diffusione nei tessuti nel loro aspetto fisiologico e patologico* (I.T.E.R. ed., Torino 1941).
[4] T. D. DAY, J. Physiol. *117*, 1 (1952).

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