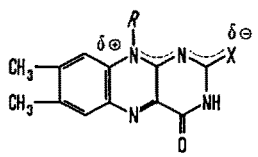
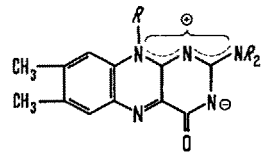
3. Die Thione I, welche durch Einwirkung von überschüssigem H_2O_2 schon bei Raumtemperatur zu IV entschwefelt werden, lassen sich bei Behandlung mit nur 2–3 Äquivalenten stark verdünnter Peroxyessigsäure in die Desoxyflavine VIII überführen. Diese sehr empfindlichen Körper[7] lassen sich auf diesem Wege rein erhalten, sie autoxydieren jedoch in wässrig-alkalischer Lösung augenblicklich unter Bildung von IV.

Der Vergleich der Spektren zeigt, dass Flavoxime III (λ_{max}[8]: 294 mμ, 526 mμ), Flavohydrazone II (λ_{max}: 296 mμ, 512 mμ) als echte (Aza)Chinon-Derivate in der Form IIa vorliegen, während für die Amine VI (λ_{max}: 276 mμ, 366 mμ, 452 mμ) die dipolare Form VIa am wahrscheinlichsten ist.



IIa



VIa

Die Amine VI, $R = CH_3$, sind dementsprechend als einzige Derivate dieser Reihe in $CHCl_3$ unlöslich.

Experimentelle Details, physikochemische, koordinationschemische und biologische Eigenschaften sollen demnächst an anderer Stelle näher beschrieben werden.

Summary. Starting from 2-thioisoalloxazines, a synthetic route to the formerly inaccessible 2-(alkyl)imino, 2-oximino, 2-hydrazono and 2-azino derivatives of vitamin B_2 was developped.

F. Müller, P. Hemmerich und H. Erlenmeyer

Institut für Anorganische Chemie der Universität Basel (Schweiz), 18. Juni 1962.

[7] Vgl. P. Hemmerich und H. Erlenmeyer, Helv. chim. Acta *40*, 180 (1957); P. Hemmerich, Helv. chim. Acta *41*, 514 (1958).

[8] λ_{max} bezieht sich auf die Neutralmoleküle in $CHCl_3$ (II, III) resp. Äthanol (VI).

Arachnoidal Cell Clusters in the Lizard *Lacerta lepida*

Arachnoidal cell clusters were first described as postmortem findings in the meninges of patients suffering from mental disease[1]. In general these cell clusters have been accepted as a manifestation of advancing age. Cushing, Weed and Essick observed that the arachnoidal cell clusters occurred not only in man but also in various laboratory animals[2]. The association of the cell clusters with advancing age can be attributed largely to the work of Weed[3], who in a series of cats of different ages found that in very young animals the cell clusters were never present, while they were always observed in very old animals. According to Watt[4], in man arachnoidal cell clusters can be observed even in the unborn foetus and consequently factors other than those of old age must be considered in the aetiology.

We observed arachnoidal cell proliferations in a series of very young and adult specimens of the lizard *Lacerta lepida*. These proliferations appeared to be present in nearly 70% of all very young lizards. As in man[4] the histological features varied from typical arachnoidal cell clusters to a more fibrous structure, which often showed degenerative changes and sometimes contained capillaries. The significance of the arachnoidal cell clusters is uncertain. Chornyak[5] demonstrated that under conditions of anoxaemia the cells of the arachnoid do proliferate and form typical cell clusters as well as freely wandering macrophages. Such a mechanism could certainly operate *in utero*, but on the strength of the occurrence of arachnoidal cell clusters in *Lacerta lepida* it is likely that other factors are also involved. As our results in *Lacerta lepida* are in good agreement with those obtained in man[4], a general significance should be attached to these structures.

Zusammenfassung. Beschreibung von Zellanhäufungen in der Arachnoidea bei der Eidechse *Lacerta lepida*.

A. Stolk

Department of Histology, Free University, Amsterdam (The Netherlands), July 3, 1962.

[1] L. Meyer, Virchows Arch. *17*, 209 (1859).

[2] H. Cushing and L. H. Weed, Johns Hopkins Hosp. Bull. *26*, 297 (1915). – C. R. Essick, Contr. Embryol. *110*, Pub. 394, 377 (Carnegie Inst., Washington 1920).

[3] L. H. Weed, Johns Hopkins Bull. *31*, 343 (1920).

[4] J. Watt, Nature (London) *194*, 880 (1962).

[5] J. Chornyak, Bull. U.S. Army Med. Dept. *8*, 695 (1948).

On the Specific Inhibition of Adrenal Steroid Biosynthesis

Since the discovery that amphenone[1] inhibits adrenal steroidogenesis, steadily growing interest in this special type of action resulted in the elaboration of a large number of substances[2–5] with similar activity. Some of these were found to produce quite specific inhibitions of steroidal 11β-hydroxylation, such as e.g. Su-4885 (Metopirone®)[6,7], or of 17α-hydroxylation, e.g. Su-8000, Su-9055 and Su-10'603[7,8]; these properties are of

[1] R. Hertz, W. W. Tullner, J. A. Schricker, F. G. Dhyse, and L. F. Hallman, Recent Progr. Hormone Res. *11*, 119 (1955).

[2] W.L. Bencze and M. J. Allen, J. med. pharm. Chem. *1*, 395 (1959).

[3] J. J. Chart and H. Sheppard, J. med. pharm. Chem. *1*, 407 (1959).

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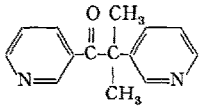
[5] R. Gaunt, J. J. Chart, and A. A. Renzi, Science *133*, 613 (1961).

[6] J. J. Chart, H. Sheppard, M. J. Allen, W. L. Bencze, and R. Gaunt, Exper. *14*, 151 (1958).

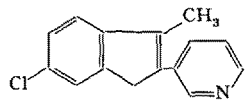
[7] J. J. Chart, H. Sheppard, T. Mowles, and N. Howie, Endocrinology *71*, 479 (1962).

[8] H. Sheppard and J. J. Chart, Biochem. Pharmacol. *8*, 128 (1961).

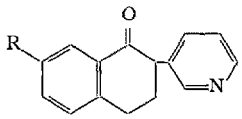
considerable interest in experimental and clinical endocrinology[9].



Su-4885
(Metopirone®)



Su-8000



R = H Su-9055
R = Cl Su-10′603

In the course of our own studies directed towards a specific inhibition of aldosterone biosynthesis, results with other substances prompted us to investigate the above-mentioned Su-compounds in our experimental setup.

This preliminary report[10] deals with the influence of these substances on the *in vitro* formation of aldosterone, 18-hydroxycorticosterone, and 19-hydroxycorticosterone in comparison to that of cortisol, cortisone, 11-desoxycortisol, corticosterone, 11-dehydrocorticosterone, and 11-desoxycorticosterone (DOC) from endogenous precursors under various conditions. The results are supplemented by some *in vitro* experiments using exogenous steroid precursors and by *in vivo* experiments.

The incubation procedures using beef adrenal cortex homogenate, sucrose, phosphate buffer, fumarate, $Mg^{\cdot\cdot}$, nucleotides, and oxygen will be reported on in detail elsewhere[11]. In each series of 12 incubations (containing 5 g tissue/34 ml medium) two served as controls and the others for the investigation of substances in different concentrations. Following the usual extraction and defatting steps, the extracts were submitted to a complex paper-chromatographic separation procedure to be described later, which made it possible to determine the above-mentioned steroids with the use of blue tetrazolium and soda fluorescence.

The incubation, without the addition of exogenous precursor, give rise to a 5–15 fold increase in corticosteroids. The optimal conditions for the biogenesis of the individual steroids in the control experiments were found to be different; the formation of corticosterone, 11-dehydrocorticosterone, cortisol and cortisone was markedly increased, that of 18- and 19-hydroxycorticosterone not significantly so by the addition of nicotinamide, whereas that of aldosterone was usually decreased. Thus, two series of inhibitory experiments have been performed, one without nicotinamide and the other with. Inhibition of the steroidogenesis is regarded as significant if the yield decreases to 50% or less of the mean control values (in at least duplicate experiments). In the experiments with exogenous precursors, pregnenolone, progesterone, or corticosterone was added in concentrations of 0.1 to 0.01% of the adrenal tissue.

In the *in vivo* experiments adrenal vein cannulation of male rats was used[12], blood being collected in periods of 30 min each; the first period served as control, the following 3–4 periods for the study of the intravenously administered substances. Whole blood was analysed for corticosterone and 18-hydroxycorticosterone by paper chromatography and for aldosterone by a double isotope dilution derivative method[13].

The Table shows the mean concentration of the different substances necessary to decrease the formation of various corticosteroids to 25–50% of the control values. The effective inhibiting concentration of Su-4885 is markedly less for aldosterone than for the other 11-oxygenated steroids; one could thus postulate that the aldosterone inhibition comprises an additional blocking effect. The figures for both 18- and 19-hydroxy-corticosterone do not necessarily suggest a blocking effect other than that on 11β-hydroxylation. When progesterone or pregnenolone was used as exogenous precursor (with or without nicotinamide), Su-4885 blocked very effectively the formation of aldosterone, 18-hydroxycorticosterone, and 19-hydroxycorticosterone in a concentration of 100 μg, whereas no significant influence was observed even with 200 μg on the biosynthesis of the other 11-

[9] A. WETTSTEIN, Exper. *17*, 329 (1961).
[10] A short account of some of these results was given in the course of a discussion (on the paper by J. J. CHART) at the International Congress on Hormonal Steroids, Milan, 14–19th May (1962).
[11] Similar conditions have been used earlier by F. W. KAHNT, R. NEHER, and A. WETTSTEIN, Exper. *11*, 446 (1955), and have now been modified according to present needs.
[12] This cannulation was done by Dr. DESAULLES of our Biological Department.
[13] R. NEHER, in press. This method represents a modification of that of B. KLIMAN and R. E. PETERSON, J. biol. Chem. *235*, 1639 (1960), using T-aldosterone and 4-C^{14}-acetic anhydride.

Mean concentration of Su-substances (in μg/34 ml incubation mixture) effecting an inhibition of adrenal steroidogenesis down to 25–50% of control values

		Aldosterone	18-Hydroxy-corticosterone	19-Hydroxy-corticosterone	Cortisol + cortisone	Corticosterone + 11-dehydrocorticosterone	DOC and 11-desoxycortisol
Su 4885 (Metopirone®)	[a]	80	200	150	200	300	accumulation
	[b]	100	200	150	300	200	
Su 8000	[a]	80	600	>5000	80	∅	no accumulation observed
	[b]	200	>400	>400	80		
Su 9055	[a]	20	>200	>500	200	∅	no accumulation observed
	[b]	80	>200	>1000	100		
Su 10′603	[a]	100	200	>200	20	∅	no accumulation observed
	[b]	>300	>300	>300	20		

[a] *without* nicotinamide in the medium; [b] *with* nicotinamide in the medium; ∅ increase (1,5–3 fold) instead of inhibition

oxygenated steroids (cortisol, cortisone, corticosterone, 11-dehydrocorticosterone). Though the effective 11β-hydroxylation inhibiting concentration of Su-4885 could depend on the substrate, our observations regarding the blockage of the 19-hydroxylation are supported by the findings of GRANT[14] and GRIFFITHS[15]. A more direct approach was studied using 4-C^{14}-corticosterone as precursor. A relatively small but significant transformation of corticosterone into 19-hydroxycorticosterone and aldosterone was shown to occur in the control experiments. Both transformations were strongly inhibited by 50–100 μg of Metopirone.

Our *in vivo* experiments in rats seem to confirm that Su-4885 has strong additional inhibiting properties regarding aldosterone formation and 18-hydroxylation. Inhibition was observed with 0.1–0.3 mg/kg i.v. for aldosterone, whereas 1.0 mg/kg i.v. was needed for 18-hydroxy-DOC inhibition and even 3.0 mg/kg i.v. for corticosterone inhibition.

As far as the blockers of 17α-hydroxylation are concerned, the Table shows that Su-10'603, Su-8000 and Su-9055 were found to be strong inhibitors of cortisol and cortisone formation in this order of decreasing activity. On the other hand, they have no appreciable effect on 11β- or 19-hydroxylation as judged by the respective steroidogeneses from endogenous precursors. However, there was a very strong additional inhibition of aldosterone formation, in particular by Su-9055, nicotinamide having a marked quantitative influence. The biosynthesis of 18-hydroxycorticosterone, as one of the possible aldosterone precursors, was much less inhibited, if at all. Similar results were obtained using pregnenolone or progesterone as exogenous precursor; using 4-C^{14}-corticosterone the formation of aldosterone was inhibited by 40 μg Su-9055 and even that of 19-hydroxycorticosterone by 200 μg.

In conclusion, these substances have been found to possess characteristic spectra of blocking activities; in terms of decreasing activity, the following order of inhibitions is suggested:

Su-4885: aldosterone, 19-hydroxylation, 11β-hydroxylation,
Su-8000: 17α-hydroxylation, aldosterone, 18-hydroxylation,
Su-9055: aldosterone, 17α-hydroxylation, 19-hydroxylation,
Su-10'603: 17α-hydroxylation, aldosterone, 18-hydroxylation[16].

Zusammenfassung. Der Einfluss von Su-4885 (Metopiron®), Su-8000, Su-9055 und Su-10'603 auf die Corticosteroid-Biosynthese wurde *in vitro* und teils *in vivo* untersucht. Ausser den bekannten Hemmeffekten auf die 11β- und 17α-Hydroxylierung wurden davon unabhängig auch solche auf die Bildung von Aldosteron, 18-Hydroxy- und 19-Hydroxycorticosteron beobachtet.

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Forschungslaboratorien der CIBA Aktiengesellschaft, Pharmazeutische Abteilung, Basel (Switzerland), August 10, 1962.

[14] J. K. GRANT, in discussion on the paper by J. J. CHART at the International Congress on Hormonal Steroids, Milan, May 14–19th (1962).
[15] K. GRIFFITHS (1960), quoted by J. K. GRANT in Brit. Med. Bull. *18*, 104 (1962).
[16] *Acknowledgments.* Our thanks are due to Dr. R. GAUNT for the Su-substances, to Dr. P. A. DESAULLES and Dr. K. SCHMID for valuable collaboration, and to Dr. A. WETTSTEIN for his encouraging interest.

Répression par l'oxygène de la biosynthèse de la thiosulfate-réductase de *Proteus vulgaris*

TARR[1] a observé en 1933 que les suspensions cellulaires de *Proteus vulgaris* produisent de l'hydrogène sulfuré en présence de thiosulfate. MITSUHASHI et MATSUO[2,3] ont montré que les extraits du même organisme catalysent la réduction de $S_2O_{3=}$ en $SO_{3=}$ et H_2S. Travaillant avec les bactéries sulfato-réductrices, ISHIMOTO, KOYAMA et NAGAI[4] ont abouti à des conclusions identiques. Les recherches de ces derniers auteurs établissent que les extraits de *Desulfovibrio desulfuricans* acquièrent la faculté de consommer l'hydrogène en présence de thiosulfate lorsqu'on leur ajoute un indicateur chimique d'oxydoréduction. Nous verrons qu'une méthode basée sur l'emploi du benzyl-viologène permet de mesurer l'activité thiosulfate-réductase des extraits bruts de *P. vulgaris*. A l'aide de la technique ainsi mise au point, nous avons étudié l'influence de l'oxygène sur la biosynthèse de l'enzyme par les cultures sur milieu complexe.

Nous avons employé la souche 41 qui nous a été procurée par M. ROUX. La souche est conservée par repiquage sur gélose nutritive. On utilise pour les cultures un milieu liquide complexe dont la composition est la suivante: Na_2HPO_4. 12 H_2O, 3.575 g; KH_2PO_4, 0.98 g; $MgSO_4$, 0.03 g; NH_4Cl, 0.5 g; $FeSO_4$ et $CaCl_2$, traces; extrait de levure Difco, 0.25 g; bacto-peptone Difco, 0.25 g; eau, 1000 ml; pH 7. Le glucose (2 g/1000 ml) stérilisé à part est ajouté au moment de l'ensemencement. La solution de $Na_2S_2O_3$. 5 H_2O (1 g/1000 ml) est stérilisée par filtration. Les cultures aérobies sont réalisées dans des fioles à toxine agitées. Les cultures anaérobies sont placées dans des ballons sous vide. On récolte les bactéries par centrifugation après 16 h d'incubation à 32°. On ajoute du chloramphénicol (25 μg/ml) aux cultures aérobies pour éviter qu'elles ne synthétisent la thiosulfate-réductase pendant la centrifugation. A la concentration employée, cet antibiotique n'exerce aucune action inhibitrice à l'égard de l'enzyme. Les extraits sont préparés par traitement sonique (10 min) des suspensions bactériennes dans un appareil Raytheon. Ils sont séparés des débris cellulaires par centrifugation (15000 tours/min; 20 min). Leur teneur en azote protéique est évaluée par micro-Kjeldahl. La préparation d'hydrogénase est obtenue par traitement sonique d'une suspension de *Desulfovibrio desulfuricans*. Toutes les expériences sont réalisées à 37° en tampons phosphates pH 7. Les $-Q_{H_2}$ représentent les vitesses de consom-

[1] H. L. A. TARR, Biochem. J. *27*, 1869 (1933).
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[3] S. MITSUHASHI et Y. MATSUO, J. exp. Med. *23*, 1 (1953).
[4] M. ISHIMOTO, J. KOYAMA et Y. NAGAI, J. Biochem. *42*, 41 (1955).