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Advances in the Field of Adrenal Cortical Hormones[1]

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The *literature* on adrenal cortical hormones has recently become so voluminous that it is no longer possible in a paper of this kind to give a detailed summary of the publications which have appeared even during the last two years. In addition, a number of excellent reviews of the subject are available.

In the purely chemical field there is that of SHOPPEE in RODDS' "Chemistry of Carbon Compounds"[3] and in the "Annual Review of Biochemistry"[4], of HEMS[5] and particularly of DJERASSI in "Vitamins and Hormones"[6] and of ROSENKRANZ and SONDHEIMER in "Progress in the Chemistry of Organic Natural Products"[7]. Concerning chemistry and metabolism there are available the reviews of SAMUELS and REICH[8], of STAUDINGER[9], of ZANDER[10], and the particularly useful monograph by DORFMAN and UNGAR[11]. Finally, the last volumes of "Recent Progress in Hormone Research"[12] and of the "Ciba Foundation Colloquia on Endocrinology"[13] are mines of compressed information. It is not attempted, therefore, to give an all-round survey on the subject, but to choose somewhat arbitrarily certain aspects of the chemistry and biochemistry of the adrenal cortical hormones and to report in more detail on aldosterone.

The adrenals are the only endocrine glands in which all groups of steroid hormones, namely cortical, progestational, androgenic and estrogenic, have been detected with certainty. Up to the present there have been isolated seven highly active *crystalline hormones* with the typical action of the adrenal cortex, namely 17-hydroxy-corticosterone (that is hydrocortisone, REICHSTEIN's substance M or KENDALL's compound F), cortisone (KENDALL's compound E), corticosterone, 11-dehydro-corticosterone (compound A of KENDALL), 17-hydroxy-cortexone (substance S of REICHSTEIN), cortexone (or 11-desoxy-corticosterone), and aldosterone (or electrocortin).

The other 23 steroids, predominantly also pregnane derivatives, which have also been obtained from the adrenals, cannot be discussed here. Their number will doubtless considerably increase as a result of the recent new investigations of adrenal extracts in connection with the isolation of aldosterone. Thus, for instance, we just obtained for the first time very considerable quantities of 11β-hydroxy-androstenedione from hog adrenals, and identified this substance chemically and physically (IR)[1]. BUSH[2] had provisionally identified this substance in the adrenal venous blood of other mammalians and SALAMON and DOBRINER[3] isolated it from the urine of patients treated with ACTH.

Occurrence. The already classical isolation work goes to the credit particularly of REICHSTEIN, of WINTERSTEINER and PFIFFNER, KENDALL and MASON, CARTLAND and KUIZENGA, of whom the last-named also worked out an especially suitable method for the preparation of adrenal extracts. Hog *adrenals* contain considerably more hormones than beef adrenals, and according to DOBRINER *et al.*[4] in descending quantities hydrocortisone, cortisone, 11-dehydro-corticosterone, substance S and corticosterone; the quantities determined by measuring the UV extinctions lie between 2 and 0·5 mg per kilo gland. Recently we have demonstrated in our own investigations[5] the presence of particularly large quantities of corticosterone, namely about 9 mg per kilo hog gland followed by 7 mg

[1] Based on a talk at the Gordon Research Conference on the Chemistry of Steroids, organized by the American Association for the Advancement of Science, New Hampton N.H., August 4, 1954. Communication No. 125 "On Steroids". No. 124 compare K. HEUSLER and A. WETTSTEIN, Chem. Ber. *87*, 1301, 1954.

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[12] G. PINCUS, Rec. Progr. Hormone Res. *7–9* (New York, 1952–1954).

[13] Ciba Foundation Colloquia on Endocrinology, 4: *Water Metabolism*, 5: *Bioassay*, 6: *Carbohydrate Metabolism*, 7: *Synthesis and Metabolism of Adrenocortical Steroids* (London, 1952–1953).

[1] R. NEHER and A. WETTSTEIN, unpublished data.

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hydrocortisone and 3·5 mg cortisone; the latter figures fit best with those of HAINES[1], whereas the high corticosterone content is at variance with the findings of DOBRINER *et al.*[2].

As the adrenals are among the organs with the best blood supply, it is not surprising that the venous *blood* carries off larger quantities of corticosteroids per minute than corresponds to the content of the whole gland. The fairly troublesome isolation, or estimation by extraction, dialysis, chromatography, polarography in whole blood or in plasma has recently been studied intensively (for example by LEVY *et al.*[3]), NELSON and SAMUELS[4], BUSH[5] and very elegantly by MORRIS and WILLIAMS[6]. In peripheral human blood the last-named authors showed on an avarage 80γ each of hydrocortisone and corticosterone and 37γ each of 11-dehydrocorticosterone and cortisone to be present in 1 liter of plasma; these values increase 2–8 times after stimulation by ACTH. According to BUSH, corticosterone would occur only in small quantities relative to hydrocortisone. This author identified in adrenal venous blood of various kinds of animal over 74–85% of the Δ^4-3-ketones as being hydrocortisone and corticosterone. He refers especially to the characteristic ratio of these two hormones in each animal species, the human being to be classed with the sheep and monkey, and to the very high total excretion per unit body weight in the rat. Of late, interesting diurnal variations of 17α-hydroxy corticoids (e.g. hydrocortisone and cortisone in particular) have been noted in human blood[7]. The most common type has the highest content in the early morning; the level drops to about half during the forenoon, rises again somewhat towards evening, and reaches the previous maximum in the course of the night. In human *placenta*, which is known to contain large quantities of progesterone and its metabolites, two groups of workers[8] detected the presence of adrenocortical hormones by their glucocorticoid activity, or by paper chromatography. Recently the *binding* of steroids *to protein* has been investigated[9], which may give an interesting insight into the form of the hormones as circulating in the organism. Cortisone[1] and hydrocortisone[2] together with many metabolites are also excreted unchanged in the urine.

Detection, Concentration and Purification. Only a selection of the large number of publications can be briefly mentioned. The *detection*[3] is possible specially by paper chromatography and most suitably with the systems of ZAFFARONI[4] or BUSH[5] and others[6] in combination with color reactions, for example with phosphoric acid[7], or with dinitrophenylhydrazine[8]; further by utilizing the reducing properties[9], by paper electrophoresis[10], by estimation of the formaldehyde formed on oxydation[11], a method which easily gives too high values, by using the sensitivity to alkali[12], and by oxydation to 17-keto-steroids and estimation of the latter[13]; finally by comparing the specific optical rotation[14], the UV absorption, also after the action of sulfuric acid[15], and the IR spectra[16], if desired direct on

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[2] COPE and HURLOCK, loc. cit.; For guinea pigs compare lately: S. BURSTEIN and R. I. DORFMAN, J. biol. Chem. *206*, 607 (1954); Feder .Proc. *13*, 188 (1954).

[3] Reviews see: W. J. HAINES, Rec. Progr. Hormone Res. *7*, 268 (1952). – A. ZAFFARONI, Rec. Progr. Hormone Res. *8*, 51 (1953). – K. SAVARD, Rec. Progr. Hormone Res. *9*, 185 (1954). – K. H. PFEFFER and HJ. STAUDINGER, Z. Vit. Horm. Fermentforsch. *5*, 51 (1952).

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the paper chromatogram[1]. For the *concentration* and *purification*, again partition chromatography[2], also on filter paper (see above), counter current or ordinary distribution processes, on occasion paper electrophoresis[3] and lately also dialysis[4] are used in particular.

Biological Testing. We have to limit ourselves to referring to the most recent reviews[5] which describe the very varied test methods: assay of the electrolyte metabolic activity, survival growth methods, liver glycogen methods, muscle work and stress tests, assay measuring the involution of thymus gland, eosinophil test, cytocidal action on lymphocytes, anti-inflammation tests etc. For the first isolation of aldosterone, in particular, the highly sensitive method of SIMPSON and TAIT[6] was used, determining the urinary ratio of $^{24}Na/^{42}K$, then a modified Kagawa test[7] determining Na and K (with the flame photometer) as well as the water excretion, and further the maintenance test in the adrenalectomized dog[8].

Isolation of Aldosterone. The isolation of the first 6 adrenocortical hormones in the thirties does not fall within the scope of this report. After their separation from adrenal extracts the investigators mentioned earlier found that the remaining amorphous fraction had a disproportionally strong action in survival tests using adrenalectomized animals. This action was, in fact, more pronounced than that of the most effective compound known at the time, cortexone, which was first synthesized by REICHSTEIN, but was later also detected in glandular extracts and in the blood. The crystallized hormones, were, it is true, able to explain the carbohydrate and protein activity of the adrenals; if the unlikely possibility of a synergistic effect of hormone mixtures were excluded, a hormone particularly active in the mineral metabolism was still missing. The recent isolation of this hormone should be reported here.

SIMPSON and TAIT prepared the way: with their test already mentioned, and, using the paper chromatographic method, they detected in concentrates from beef adrenals[1], then also from hog adrenals[2] and from the venous blood of the adrenals of monkeys and dogs[3], a single new substance which they called electrocortin. It was possible to differentiate this compound clearly from the similarly running cortisone[4] and to characterize it in a preliminary manner by some physico-chemical and chemical properties[5]. Of the latter the assumption that it contains an α,β-unsaturated keto group (UV) and an α-ketol (not a dioxy-acetone) side chain (negative Porter-Silber reaction[6]) was confirmed later on.

In the meantime the past-master of adrenal research, Prof. REICHSTEIN in Basle, had taken up the hunt for the new hormone, which was actively supported and supplemented by parallel investigations in Ciba. A free exchange of information took place, joint publications[7] being made with the SIMPSON and TAIT group, and the desirable mutual control of the progressive concentration was thereby guaranteed. The previously mentioned beef adrenal extracts prepared according to CARTLAND and KUIZENGA, and which were defatted in aqueous methanol by means of petroleum ether, proved to be the most valuable starting material in the beginning. The main purification consisted of a large-scale chromatography (Fig. 1) lasting more than one month, using Kieselguhr and water (1:1) as the stationary phase, and petroleum ether with increasing additions of benzene and then chloroform as the mobile phase. Adrenosterone, 11-dehydro-corticosterone and corticosterone in crystalline form were mainly obtained at first, then fractions containing cortisone and later also aldosterone and finally crystalline hydrocortisone. The fractions containing aldosterone were again purified by partition chromatography, this time in a B2 column of BUSH[8], with cellulose and aqueous methanol as the stationary and toluene-petroleum ether as the mobile phase. Some of the eluates obtained in this way from completely independent batches run in Prof. REICHSTEIN's and in our place, crystallized directly within a short interval, the presence of traces of

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moisture proving to be essential. At this point, it should be remembered that the earlier, considerably purified but very sensitive concentrates of aldosterone had defied attempts at crystallization for almost 20 years.

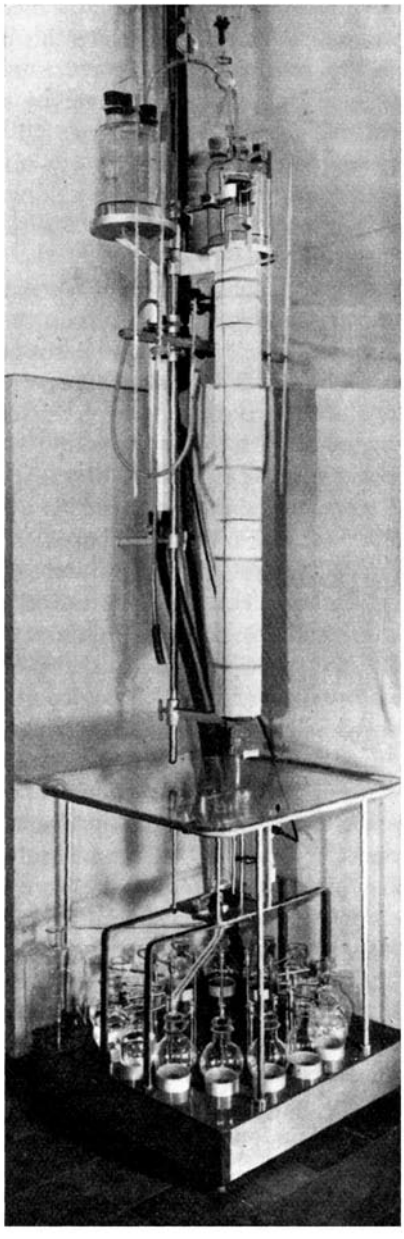
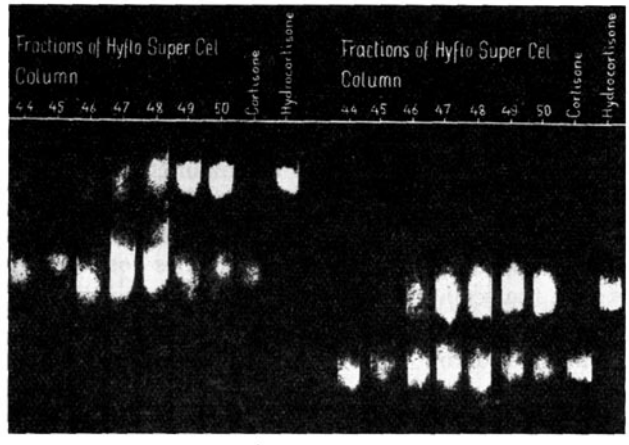


Fig. 1.

The main difficulty consisted, of course, in the ever-recurring detection of aldosterone. This was carried out, apart from biological testing, by paper chromatography (Fig. 2). In the ZAFFARONI system (propyleneglycol/toluene)[1], aldosterone travels at about the same rate, or very slightly more quickly than cortisone, but hydrocortisone considerably more slowly. On the other hand aldosterone behaves similar to hydrocortisone in BUSH's system C, whereas cortisone runs much faster[1]; in BUSH's B 5 system[2], aldosterone lies between the other two glucocorticoids. For the identification in the chromatograms the ultraviolet absorption, the power of reduction, the yellow fluorescence with sodium hydroxide and the absence of color reactions with phosphoric acid and with antimonytrichloride[3] were used in particular. About 40–95 γ aldosterone were present per kilo of fresh beef adrenals and could be largely isolated.



System: ZAFFARONI (Prop./Tol.) System: BUSH C
UV-Photocopies

Fig. 2.

As we found on an average 3·5γ aldosterone per liter of beef blood, the quantity circulating in the blood of this animal is about 70 times greater than the total content of its adrenals. In the perfusate of isolated monkey adrenal glands, aldosterone, cortisone and hydrocortisone were shown to be present in the ratio of about 1:10:1000[4] under the action of ACTH. More interesting are the values given by SIMPSON[5] for human peripheral blood without stimulation by ACTH: As she estimates the levels of hydrocortisone, determined by the H^3 and C^{14} method, at 25 γ per liter, and that of aldosterone, determined by her bioassay method, at 0·4 to 1 γ per liter, there results a ratio of aldosterone to hydrocortisone of about 1:35.

Shortly after us, MATTOX *et al.*[6] reported the successful crystallization of a strong mineralocorticoid hormone

[1] A. ZAFFARONI, R. B. BURTON, and E. H. KEUTMANN, Science *111*, 6 (1950); J. biol. Chem. *188*, 763 (1951); *193*, 749 (1951).

[1] From the differences in the area of the spots in the lower and the upper series of these two systems shown in Figure 2, it followed that in this example aldosterone was contained primarily in fractions 46–49.

[2] I. E. BUSH, Biochem. J. *50*, 370 (1952); Rec. Progr. Hormone Res. *9*, 453 (1954).

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from hog adrenals, by enzymatic hydrolysis of its acetate; on later direct comparison it proved to be identical with aldosterone. HAINES *et al.*[1] obtained a considerable concentration from the same starting material, FARREL and RICHARDS[2] from adrenal venous blood of dogs and MAJNARICH and DILLON[3] apparently from placental extracts. The crystallizate which VOIGT *et al.*[4] obtained from beef adrenal extracts by paper electrophoresis, amongst other methods, is doubtless, in view of its properties, different from aldosterone. LUETSCHER *et al.*[5] and also SINGER *et al.*[6] have obtained concentrates of a sodium retaining corticoid, which might be identical with aldosterone, from the urine of nephrotic and edematous patients, a particularly rich source[7]; recently it was detected biologically in the urine of normal people and of patients with cirrhosis[8]. In the case of a normal young man who received 1 mg of aldosterone by injection daily, we were able to find, by paper chromatography, about 1·5γ per 24 h in the urine; in a human adrenal tumor (CUSHING syndrome) we detected 285γ aldosterone per kilo[9].

Physiological Actions of Aldosterone. These will only be summarized here: Whereas a strict separation of the adrenocortical hormones into mineralocorticoids and glucocorticoids was more practical than correct even in earlier days (VERZÀR had already referred to the connection between potassium and carbohydrate metabolism), it has become completely untenable after the findings with crystallized aldosterone (Table I). The latter is, it is true, more than 100 times as active as cortexone[10] in SIMPSON and TAIT's test (as the earlier name "electrocortin" indicates) and possesses an extraordinary sodium retaining activity and a still somewhat greater potassium excreting activity than cortexone, but the effect on water retention is wholly lacking[1]. The permeability of muscle to the two alkali ions[2] mentioned, is strongly influenced. Aldosterone also proved correspondingly highly active in the survival test in the adrenalectomized dog[3]. Qualitative differences in action as compared with cortexone were also observed. On the other hand, however, contrary to the provisional findings of HAINES *et al.*[4], aldosterone shows some quite considerable cortisone-like actions, particularly in the liver glycogen[5], in the cold stress[6] and in the eosinophil depletion[6,7] tests, and in its ability to suppress the ACTH release[8]. Its potency here is equal to or about ⅓ of that of cortisone.

Table I.–Biological Activities of Crystalline Aldosterone (compiled by R. GAUNT[8])

Test	Potency of Aldosterone Relative to:	
	Cortexone	Cortisone
Test in which Cortexone is more potent than Cortisone		
Sodium Retention	25×	–
Potassium Excretion . . .	5×	–
Na/K Excretion	120×	–
Maintenance Adx. Dog . .	25–30×	500×
Tests in which Cortisone is more potent than Cortexone		
Cold Stress	>	= or >
Liver Glycogen	30×	1/3
Eosinophil Depletion . . .	–	1/2–1/4
ACTH Suppression	8×	1/2–1/3

Starting from her ratio of about 1:35 for the levels of aldosterone and hydrocortisone in human peripheral blood and from the potency ratio in SPEIRS' eosinophil test of 1:3, SIMPSON[9] calculates the share of gluco-

[1] R. E. KNAUFF, E. D. NIELSON, and W. J. HAINES, J. Amer. chem. Soc. *75*, 4868 (1953).

[2] G. L. FARRELL and J. B. RICHARDS, Fed. Proc. *12*, 41 (1953); Proc. Soc. exp. Biol. Med. *83*, 628 (1953); *84*, 89 (1953). According to a personal communication, Dr. FARRELL and coworkers have made it highly probable that their product is essentially homogeneous and identical with aldosterone.

[3] J. J. MAJNARICH and R. N. DILLON, Northwest Med. *50*, 677 (1951); Arch. Biochem. Biophys. *49*, 247 (1954).

[4] K. D. VOIGT and W. SCHROEDER, Naturwissenschaften *40*, 485 (1953). – W. SCHROEDER, K. D. VOIGT, and H. v. D. WERTH, Acta Endocrinol. *14*, 12 (1953).

[5] J. A. LUETSCHER, A. B. DEMING, and B. B. JOHNSON, Ciba Found. Colloquia on Endocrinol. *4*, 530 (1952). – J. A. LUETSCHER and B. B. JOHNSON, Amer. J. Med. *15*, 417 (1953); J. clin. Invest. *32*, 585 (1953); *33*, 276 (1954). Other literature is given herein. See also: J. Clin. Endocrinol. *14*, 812, 1086 (1954). Test procedure compare B. B. JOHNSON, Endocrinology *54*, 196 (1954).

[6] B. SINGER and E. H. VENNING, Endocrinology *52*, 623 (1953). – B. SINGER and J. WENER, Amer. Heart. J. *45*, 795 (1953); M. F. MCCALL and B. SINGER, J. Clin. Endocrinol. *13*, 1157 (1953).

[7] Added when reading proofs: From a concentrate of this type, kindly made available to us by Dr. LUETSCHER, we[9] were in fact able, by further chromatographic purification, to obtain considerable quantities of aldosterone in crystalline form. (Paper Exper. in press.)

[8] J. J. CHART and E. S. SHIPLEY, J. clin. Invest. *32*, 560 (1953). – C. L. COPE and J. GARCIA, Brit. med. J. *1954*, 1290. – E. S. GORDON, J. J. CHART, D. HAGEDORN, and E. G. SHIPLEY, Obstetr. and Gynecol. *4*, 39 (1954).

[9] R. NEHER and A. WETTSTEIN, Unpublished data.

[10] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, and T. REICHSTEIN, Exper. *9*, 333 (1953). – S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, Helv. chim. Acta *37*, 1163 (1954).

[1] P. DESAULLES, J. TRIPOD, and W. SCHULER, Schweiz. med. Wschr. *83*, 1088 (1953); several papers on hormonal influences in water metabolism see Ciba Found. Colloquia on Endocrinol. *4*, 414 (1952).

[2] E. FLÜCKIGER and F. VERZÀR, Exper. *10*, 259 (1954).

[3] F. GROSS, Rec. Progr. Hormone Res. *10* (in press). – F. Gross and H. GYSEL, Acta Endocrinol. *15*, 199 (1954). – W. W. SWINGLE, R. MAXWELL, M. BEN, C. BAKER, S. J. LEBRIE, and M. EISLER, Proc. Soc. exper. Biol. Med. *86*, 147 (1954); Endocrinology *54*, 698 (1954); see also Endocrinology *54* (1954), in press.

[4] R. E. KNAUFF, E. D. NIELSON, and W. J. HAINES, J. Amer. chem. Soc. *75*, 4868 (1953).

[5] W. SCHULER, P. DESAULLES, and R. MEIER, Exper. *10*, 142 (1954).

[6] R. GAUNT, A. S. GORDON, A. A. RENZI, J. PADAWER, G. J. FRUHMAN, and M. GILMAN, J. Clin. Endocrinol. *14*, 711 (1954); Endocrinology *55*, 236 (1954).

[7] R. S. SPEIRS, S. A. SIMPSON, and J. F. TAIT, Endocrinology *55*, 233 (1954).

[8] R. GAUNT, Ciba Found. Colloquia Endocrinol. *8* (1954) (in press). – A. A. RENZI, M. GILMAN, and R. GAUNT, Proc. Soc. Exper. Biol. Med. (in press).

[9] S. A. SIMPSON and J. F. TAIT, Ciba Found. Colloqu. on Endocrinol. *8* (1954), in press.

corticoid effect of aldosterone in this blood as one hundredth of that of hydrocortisone. Assuming an opposite potency ratio of about 1000:1 for aldosterone and hydrocortisone in her labelled alkali ion test, she arrives at a ratio of 30:1 for the mineralocorticoid effect produced by the levels of these hormones in the blood.

Clinically, in ADDISON's disease, aldosterone was effective in doses of 100–200 γ per day, i.e. about 20–30 times as potent as cortexone[1]. It produced at the same time typical effects on carbohydrate metabolism and apparently caused neither an increase in blood pressure nor a pathological water retention.

Elucidation of the Constitution of Aldosterone. The noticeably broad spectrum of activity of aldosterone is readily explained by its chemical constitution. This constitution was resolved within a short time by REICHSTEIN *et al.*[2] essentially on the basis of the following findings:

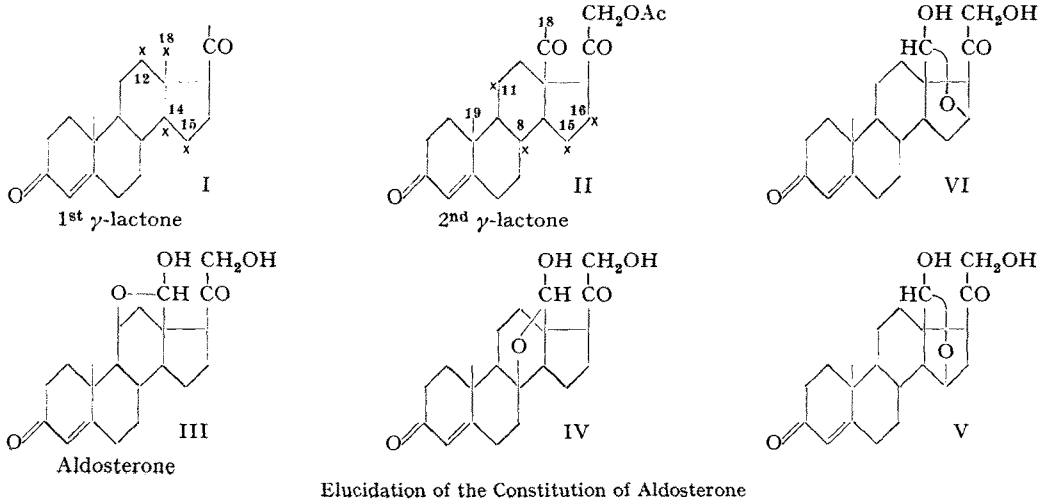
Most important, at the beginning was the microanalysis of aldosterone, one of a total of 3 determinations which it was possible to make during the entire degradation studies. It gave an empirical formula of $C_{21}H_{30}O_5 \pm H_2$, H_{28} later proving to be correct. The presence of *5* oxygen atoms has to be emphasized! On account of the ultraviolet spectrum, the reducing properties, and the formation of 1 mole formaldehyde with sodium bismuthate the working hypothesis that it is a cortexone derivative with 2 additional oxygen atoms then suggested itself. One of these must, on account of the formation of a diacetate, which in the IR no longer showed a hydroxyl group, be present as a secondary or primary hydroxyl. An indication of its position was given by degradation with $NaIO_4$, which led to a γ-lactone (Chart 1; formula I) easily detectable in the IR. This compound proved to be extraordinarily useful, as it had a very high melting point, crystallized perfectly, sublimated without decomposition in high vacuum and could thus be obtained in perfect purity even from very impure mother liquors of aldosterone. It was only this which made possible degradation in very small but yet sufficient quantity.

The hydroxyl group used in the formation of the lactone must be, we have stated, in the γ-position to the carboxyl group of the etio acid, produced by the side chain degradation, i.e. in one of the positions 12, 14, 15 or 18 marked with a cross. A 12-hydroxyl can be eliminated for steric reasons, as the 17β-oriented carboxyl group cannot react with such a hydroxyl in the presence of the also β-oriented angular 18-C atom. The 14-position can be excluded, as such a tertiary hydroxyl would have to be present in the diacetate in the free form. The choice between the 15 and 18 positions was facilitated by the fact that aldosterone-21-monoacetate again formed a γ-lactone (IR) with CrO_3, the ketol side chain with its reducing properties remaining intact. The carbonyl group of this lactone, corresponding to the original second hydroxyl, can only be in the 18 position, as all hydroxyls which are possible on the basis of the formation of the first lactone (positions 12, 14, 15, 18) lie too far from C_{19}. For the second γ-lactone, formula II thus results, with possible oxygen bridges to position 8, 11, 15 or 16 marked with a cross. As, however, aldosterone contains only *2* and not 3 free hydroxyl groups, and the first gamma lactone none at all, this oxygen ring must already have been present in aldosterone. Since a hydroxyl group has been established in position 18, this leads to one of the 4 cyclic hemiacetal structures III–VI.

[1] R. S. MACH, J. FABRE, A. DUCKERT, R. BORTH, and P. DUCOMMUN, Schweiz. med. Wschr. *84*, 407 (1954). – L. SOFFER, personal communication; G. W. THORN, Colloquium on *The Human Adrenal Cortex* II, The Ciba Foundation, London, June 1954. – A. KEKWICK and G. L. S. PAWAN, Lancet *267*, 162 (1954). – F. T. G. PRUNTY, R. R. MCSWINEY, and M. A. SMITH, Lancet *267* (1954), in press.

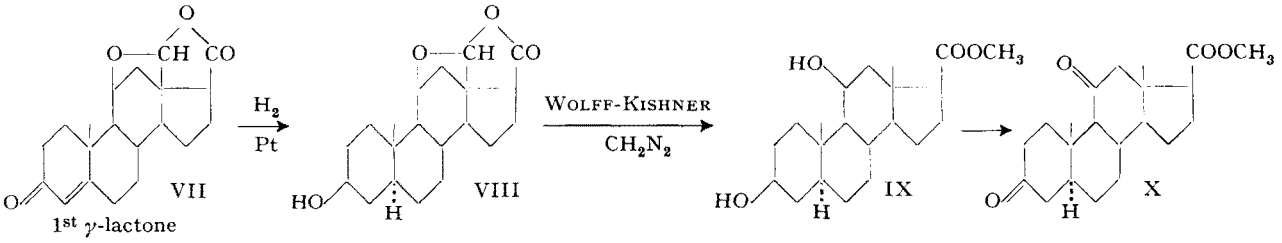
[2] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, Exper. *10*, 132 (1954); Helv. chim. Acta *37*, 1200 (1954).

Chart 1



Elucidation of the Constitution of Aldosterone

Chart 2



Elucidation of the Constitution of Aldosterone

Prof. REICHSTEIN's chemical instinct instantly chose formula III for the immediate working hypothesis. The establishment of this formula and thus the proof that aldosterone is a steroid at all was made in principle in the following way (Chart 2); the first γ-lactone to which formula VII would have to be attributed, was converted, by means of hydrogenation, WOLFF-KISHNER reduction, methylation of the acid fraction obtained, and oxidation, into compound X, which proved to be identical with authentic methyl 3,11-diketo-etio-allocholanic acid. For steric reasons aldosterone must then be an 11β-hydroxy derivative which can be designated 18-oxo-corticosterone; in solution it is eminently present as the cyclohemiacetal of formula III, which we saw on chart 1.

This structure, which is in strong contrast to all previous speculations, readily explains the biological properties which, as stated, include most marked mineralocorticoid actions and strong glucocorticoid actions, attributable to the 11-oxygen.

Chemical Constitution and Physiological Action. Even after the elucidation of the constitution of aldosterone the fact remains that all the highly active typical hormones of the adrenal cortex represent oxygenated progesterones, i.e. cortexone (21-hydroxy-progesterone) and its further oxygenated derivatives. The additional oxygen functions enter in position 11 and/or 17, and in the case of aldosterone—for the first time—in position 18. It was, of course, of particular interest to investigate compounds representing structural or stereo-isomers of true hormones, all the more so since compounds of such a structure had been considered when discussing the possible formula of aldosterone. A group of such substances and their close relations will thus be considered before discussing new chemical methods (Chart 3).

Chart 3

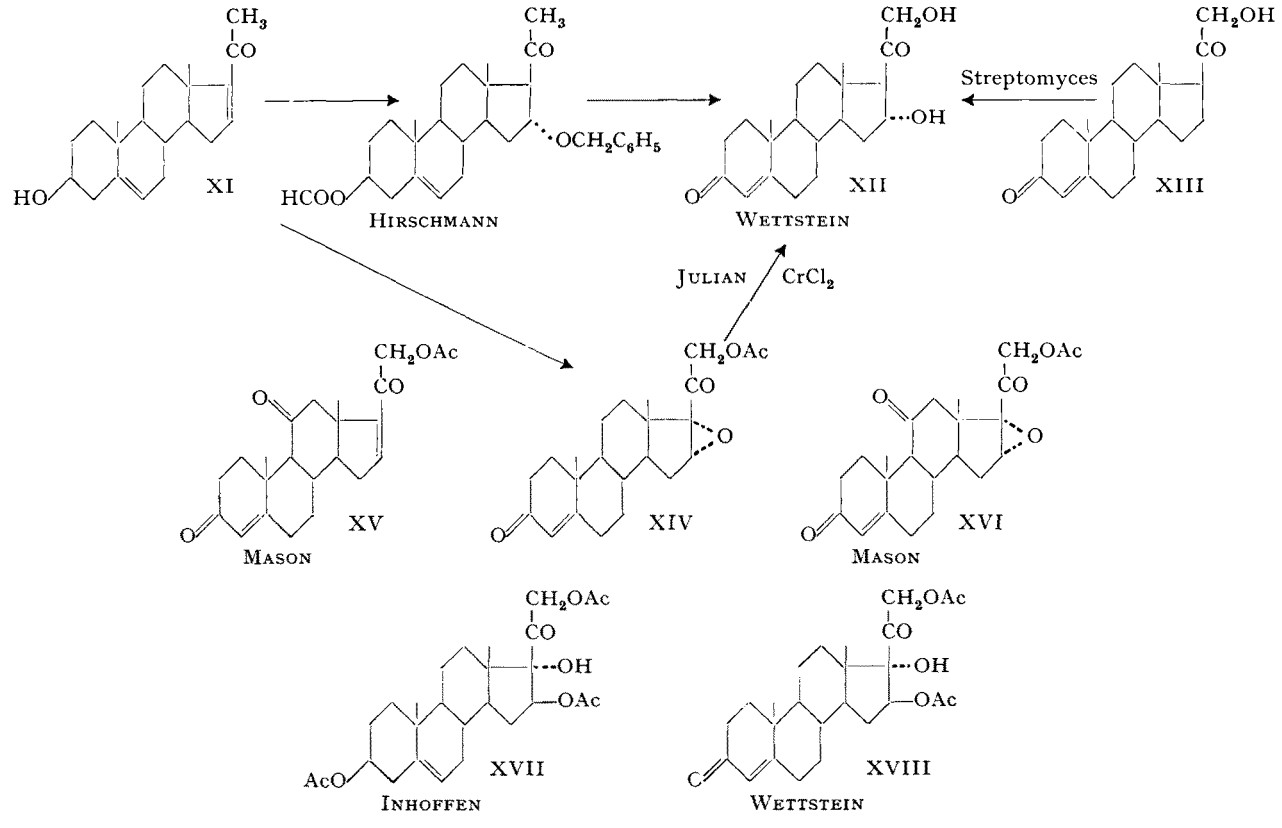
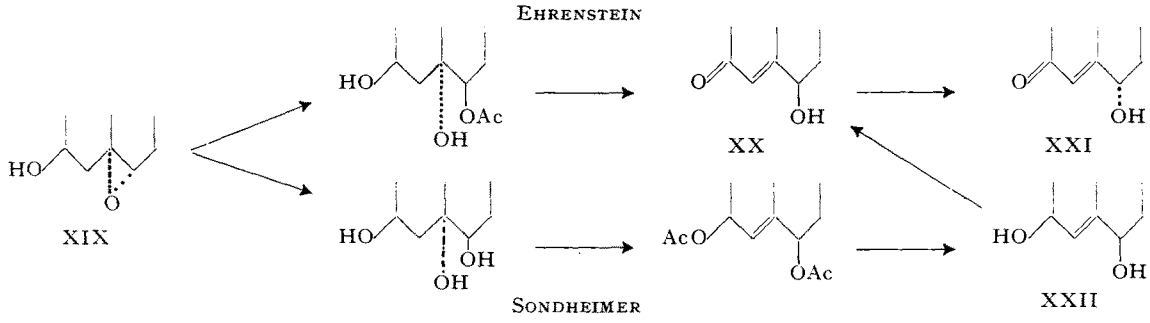


Chart 4



In this connection it was *16α-hydroxy*-cortexone (XII) which recently aroused some interest, also in view of the discovery of various 16α-hydroxylated steroid metabolites[1]. We were able to obtain it direct from cortexone (XIII) by means of the microbiological method to be discussed in detail later, involving the action of some streptomyces species[2]. Its diacetate was identical with a compound obtained by HIRSCHMANN[3] in a chemical multistage synthesis, starting from 16-dehydro-pregnenolone (XI). The new substance XII, however, proved inactive in the glycogen and the KAGAWA test and its activity in the survival test in the adrenalectomized dog was infinitesimal. The same compound was obtained simultaneously by COLE and JULIAN[4], also from 16-dehydro-pregnenolone (XI), via 16,17α-epoxy-cortexone acetate (XIV), using a novel reduction method with chromous salts. Speaking of *16-dehydro-* and *16,17α-epoxy* compounds, both substances of the 11-dehydro-corticosterone acetate series (XV and XVI) were found inactive as glucocorticoids and as cortisone antagonists[5]. INHOFFEN[6], when synthesizing an acetylated *16β,17α-diol* of the Δ^5-21-hydroxy-pregnenolone series (XVII), also started with the 16,17α-epoxide and subjected this to acetolytic cleavage. Our findings show[7] that this compound has the constitution given by the author, while his corresponding conversion products of the pregnenolone series represent rearranged compounds of the D-Homo-series. The 16β,17α-dihydroxy-cortexone diacetate (XVIII), the 16β-acetoxy derivative of substance S now produced by us[7], proved about $^1/_{10}$ as active as free cortexone as regards sodium retention, but, in contrast to this, also caused retention of potassium, which may perhaps be the result of the relatively strong water retention observed.

Considerable work has been done on cortexones hydroxylated in position 6 (Chart 4), all the more so since, as will be discussed later, the adrenal is able to introduce a 6β-hydroxyl. This is the method by which HAYANO and DORFMAN[1] obtained *6β-hydroxy*-hydrocortisone, a compound which proved, however, definitely less active than hydrocortisone, and failed to show any mineralocorticoid effect. By purely chemical means 6β- and *6α-hydroxy*-cortexone (XX and XXI) were produced by EHRENSTEIN *et al.*[2], using acetolysis of the 5,6α-epoxide (XIX) and rearrangement of the 6β- into the 6α-compound, while SONDHEIMER *et al.*[3], when preparing 6β-hydroxy-cortexone, oxidized the allyl alcohol XXII with manganese dioxide. 6α-Hydroxy-cortexone caused weak sodium retention, about 4% of that of cortexone, whereas the 6β-hydroxy compound showed no detectable activity. Similar biological results have been obtained with the 6α- and 6β-hydroxy derivatives of substance S[4] (Chart 4).

The Mexican group mentioned[5] recently identified a compound (Chart 5), obtained 15 years ago by EHRHART *et al.* from progesterone with lead tetra-acetate, as the diacetate of *2α-hydroxy*-cortexone (XXIV). This compound could now also be obtained by rearrangement of 6-bromo-cortexone acetate (XXIII) by means of potassium acetate in glacial acetic acid. Nothing appears to have been published about the biological activity of this compound.

The *19-hydroxy*-cortexone (XXV), which is of particular interest in connection with aldosterone since it is oxygenated at the other angular methyl group—as well as the corresponding progesterone derivative—were described only recently by BARBER and EHRENSTEIN[6]. The former produces only about

[1] See e.g. S. LIEBERMANN, B. PRAETZ, PH. HUMPHRIES, and K. DOBRINER, J. biol. Chem. *204*, 491 (1953).

[2] E. VISCHER, J. SCHMIDLIN, and A. WETTSTEIN, Helv. chim. Acta *37*, 321 (1954).

[3] H. HIRSCHMANN, F. B. HIRSCHMANN, and G. L. FARRELL, J. Amer. chem. Soc. *75*, 4862 (1953).

[4] W. COLE and P. L. JULIAN, J. org. Chem. *19*, 131 (1954).

[5] W. F. MCGUCKIN, H. L. MASON, and G. M. HIGGINS, Abstr. 125th Meeting Amer. chem. Soc. *1954*, 4C.

[6] H. H. INHOFFEN, F. BLOMEYER, and K. BRÜCKNER, Ber. dtsch. chem. Ges. *87*, 593 (1954).

[7] K. HEUSLER and A. WETTSTEIN, Chem. Ber. *87*, 1301, (1954).

[1] M. HAYANO and R. I. DORFMAN, Arch. Biochem. Biophys. *50*, 218 (1954).

[2] P. TH. HERZIG and M. EHRENSTEIN, J. org. Chem. *16*, 1050 (1951). – CH. P. BALANT and M. EHRENSTEIN, J. org. Chem. *17*, 1587 (1952).

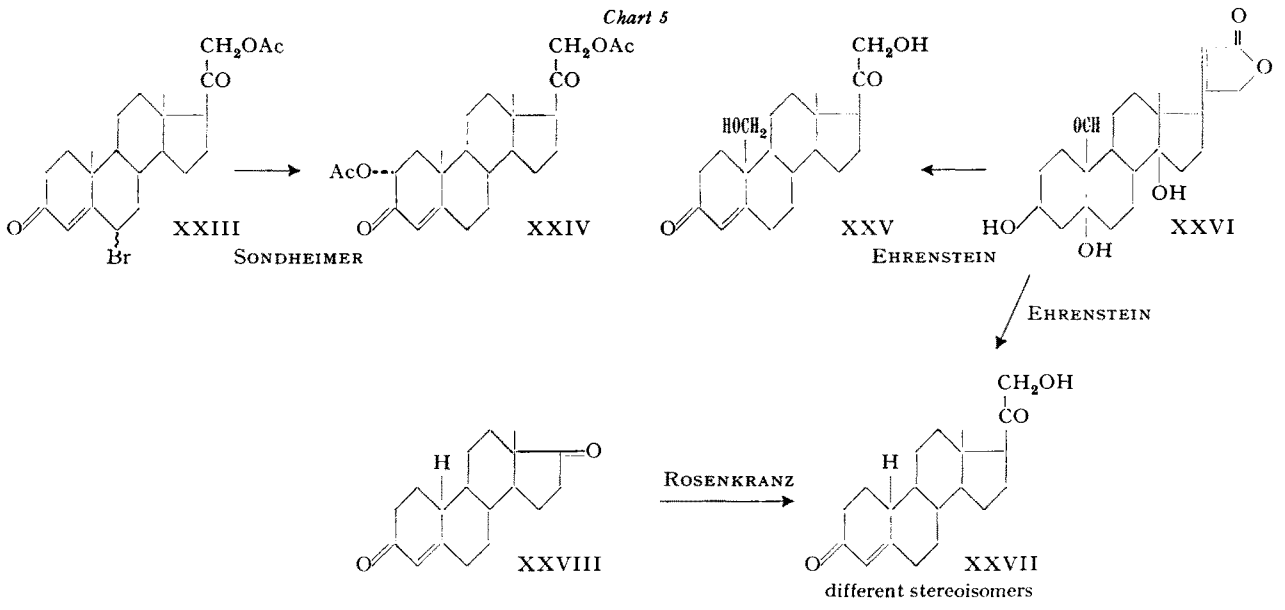
[3] C. AMENDOLLA, G. ROSENKRANZ, and F. SONDHEIMER, J. chem. Soc. *1954*, 1226; J. Amer. chem. Soc. *75*, 5930 (1953). – F. SONDHEIMER and G. ROSENKRANZ, Exper. *9*, 62 (1953).

[4] K. FLOREY and M. EHRENSTEIN, J. org. Chem. *19*, 1331 (1954).

[5] F. SONDHEIMER, ST. KAUFMANN, J. ROMO, H. MARTINEZ, and G. ROSENKRANZ, J. Amer. chem. Soc. *75*, 4712 (1953).

[6] G. W. BARBER and M. EHRENSTEIN, J. Amer. chem. Soc. *76*, 2026 (1954).

Chart 5



4% of the sodium-retaining action of cortexone acetate and less than 2% of the glucocorticoid activity of hydrocortisone. It was obtained be means of diazoketone synthesis from a 19-acetoxy-etienic acid[1], the acid itself being available from strophanthidine (XXVI). The latter is also a possible starting material for *19-nor*-cortexone (XXVII)[2]. This cortexone derivative might have the wrong configuration in 14- and 17-position. Another 19-nor-cortexone has been obtained from estradiol after Birch-reduction according to ROSENKRANZ *et al.*[3] via 19-nor-androstenedione (XXVIII). This lower homologue (XXVII) of cortexone proved to be about twice as active as the parent hormone, while 19-nor-progesterone apparently is the most active gestagen known[3].

The microbiological hydroxylation in 11α-position will be considered later. All that need be said here is that 11-epicorticosterone (= *11α-hydroxy*-cortexone)[4,5] and also 11-epi-hydrocortisone[4,6] were very much less active[4] than cortexone in the survival test using epinephrectomized dogs (assuming there was any activity at all) while 11-epi-hydrocortisone was in addition without effect in the glycogen test[6]. As is known, the *12α-hydroxy* and *12-keto*-cortexone acetates produced earlier by REICHSTEIN were also without effect. The 12α,17α-dihydroxy derivative, a hydroxyl position isomer of hydrocortisone, is said to be a cortisone antagonist[1].

The cortexone derivatives showing an additional double bond in ring C (Chart 6), and in particular the products derived from them, were recently the source of most interesting results. Some time ago we had already noted that *Δ^{11}-anhydro*-corticosterone acetate (XXIX) resembled cortexone quantitatively in its effect in survival tests (quoted in[2]). *$\Delta^{9,11}$-Anhydro*-corticosterone acetate (XXX), which was first produced by REICHSTEIN and more recently obtained by SHOPPEE[2], by partial synthesis from desoxycholic acid, also has an effect which resembles that of cortexone in the survival test, but is two or three times as active in the Everse-de Fremery test. Various teams of workers[3] prepared $\Delta^{9,11}$-anhydro-hydrocortisone acetate (XXXII) by splitting off the 11-hydroxyl group in hydrocortisone or 11-epi-hydrocortisone derivatives (XXXI).

The free alcohol of XXXII was found to be inactive in the rat liver glycogen test but quite active as an antiinflammatory by local application in the granuloma test[4]. According to FRIED and SABO[3] the acetate can serve as a suitable intermediate in the conversion of

[1] P. TH. HERZIG and M. EHRENSTEIN, J. org. Chem. *17*, 713 (1952). – M. EHRENSTEIN, Chimia *6*, 287 (1952).

[2] M. EHRENSTEIN, J. org. Chem. *9*, 435 (1944). – G. W. BARBER and M. EHRENSTEIN, J. org. Chem. *19*, 365 (1954).

[3] A. SANDOVAL, L. MIRAMONTES, G. ROSENKRANZ, C. DJERASSI, and F. SONDHEIMER, J. Amer. chem. Soc. *75*, 4117 (1953). – C. DJERASSI, L. MIRAMONTES, and G. ROSENKRANZ, J. Amer. chem. Soc. *75*, 4440 (1953).

[4] F. W. KAHNT, CH. MEYSTRE, R. NEHER, E. VISCHER, and A. WETTSTEIN, Exper. *8*, 422 (1952).

[5] S. H. EPPSTEIN, P. D. MEISTER, D. H. PETERSON, H. C. MURRAY, H. M. LEIGH, D. A. LYTTLE, L. M. REINEKE, and A. WEINTRAUB, J. Amer. chem. Soc. *75*, 408 (1953).

[6] J. FRIED, R. W. THOMA, J. R. GERKE, J. E. HERZ, M. N. DONIN, and D. PERLMAN, J. Amer. chem. Soc. *74*, 3962 (1952). – D. H. PETERSON, S. H. EPPSTEIN, P. D. MEISTER, B. J. MAGERLEIN, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, J. Amer. chem. Soc. *75*, 412 (1953).

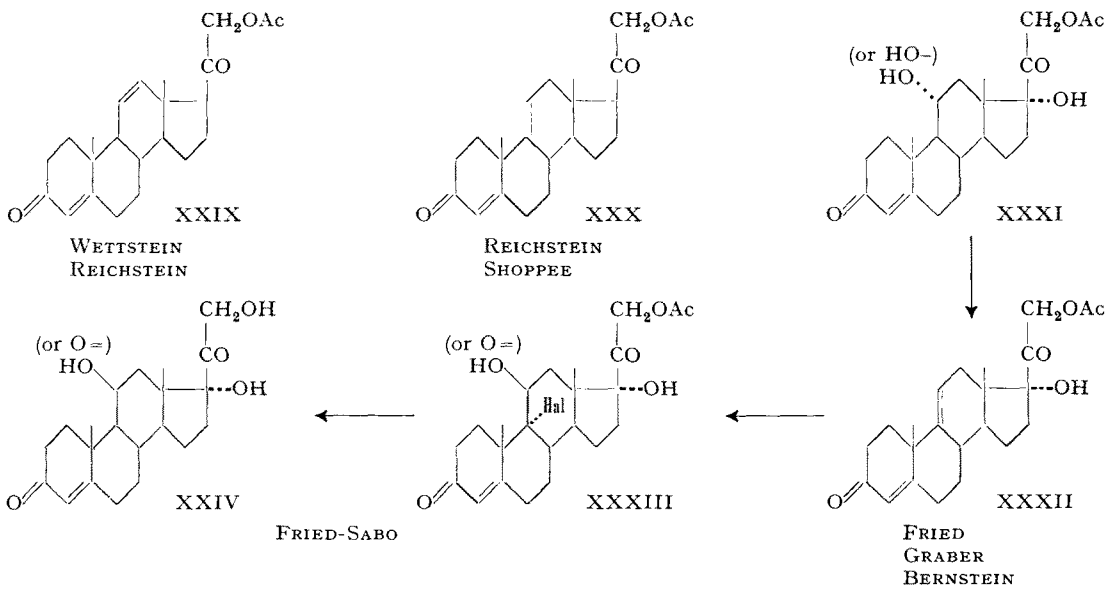
[1] W. J. ADAMS, B. G. CROSS, A. DAVID, F. HARTLEY, D. PATEL, V. PETROW, and I. A. STUART, J. Pharmacy Pharmacol. (Brit.) *5*, 861 1953).

[2] R. CASANOVA, C. W. SHOPPEE, and G. H. R. SUMMERS, J. chem. Soc. *1953*, 2983. – R. CASANOVA, A. RUFF, and C. W. SHOPPEE, Ciba Found. Colloquia on Endocrinol. *7*, 59 (1953).

[3] J. FRIED and E. F. SABO, J. Amer. chem. Soc. *75*, 2273 (1953). – R. P. GRABER, A. C. HAVEN, and N. L. WENDLER, J. Amer. chem. Soc. *75*, 4722 (1953). – S. BERNSTEIN, R. LITTELL, and J. H. WILLIAMS, J. Amer. chem. Soc. *75*, 4830 (1953).

[4] Merck Institute for Therapeutic Research. Personal commun - cation from Dr. N. L. WENDLER.

Chart 6



11-epi-hydrocortisone (XXXI) into hydrocortisone or cortisone (XXXIV); during this process it appeared that their *9α-halogenated* derivatives of formula XXXIII, which are obtained by addition of hypohalic acid to the 9,11-double bond, are yet much more interesting than the true hormones; while the latter were still superior to the acetylated 9α-iodo-hydrocortisone, 9α-bromo-cortisone and -hydrocortisone as regards glucocorticoid effect, the corresponding 9α-chloro- and in particular the 9α-fluoro-derivatives[1] proved to be 3 to 11 times more potent glucocorticoids than cortisone acetate. Moreover, the 9α-bromo and chloro derivatives in adrenalectomized rats produced a survival which was 2–20 times as long as that brought about by cortexone acetate, while the 9α-fluoro derivative was less effective than this standard substance in this respect[2]. Promising clinical results appear obtainable in addition (THORN[3]). Partly inhibited metabolic transformation and excretion of these "unnatural" hormonal substances has been suggested as an explanation for their protracted and enhanced activity, which is found even when they are given by the oral route.

The 20-keto-*21-aldehydes* corresponding to the true hormones are also active compounds, being obtained via the nitrones, some of which are also active. While REICHSTEIN's aldehyde of cortexone retained only a small fraction of the original activity, presumably as a result of dimerisation, MIESCHER's aldehyde of substance S had roughly the same activity as the basic substance. The latter is also applicable to the 21-aldehydes of cortisone and hydrocortisone[1], presumable because of the ready reducibility which was observed, e.g. with rat-liver enzymes[2], and also to the aldehyde bisulfite addition products[1]. If oxygen substitution in 21-position is dispensed with, a very considerable loss of the antiarthritic effect is observed in the case of *21-desoxy*-cortisone and -hydrocortisone[3].

If one imagines the 4,5-double bond as being on the other side, but also in conjugation with the 3-keto group, isomeric cortisone acetates of the Δ^1-allopregnene-[4] or Δ^1-pregnene series[5], for instance, result. The first of these two still retains about 10% of the cortisone effect.

Introduction of an additional double bond, now in position 6, results in *6-dehydro*-cortisone[6], which is sometimes also found as an impurity of commercial cortisone preparations of which the double bond was introduced in the last step. Its effect upon the survival of adrenalectomized rats is similar to that of cortisone, but it brings about an additional gain in weight. In normal animals it exerts a mild lymphocytopoietic influence[7].

Enlargement of the ring D in the androstene series and construction of the side-chain resulted in the

[1] J. FRIED and E. F. SABO, J. Amer. chem. Soc. *76*, 1455 (1954).

[2] A. BORMAN and F. M. SINGER, Fed. Proc. *13*, 185 (1954). – A. BORMAN, F. M. SINGER, and P. NUMEROF, Proc. Soc. Exper. Biol. Med. *86*, 570 (1954). – See also: G. W. LIDDLE, M. M. PECHET, and F. C. BARTTER, J. Clin. Endocrinol. *14*, 813 (1954).

[3] G. W. THORN, Colloquium on *The Human Adrenal Cortex* II, The Ciba Foundation, London, June, 1954.

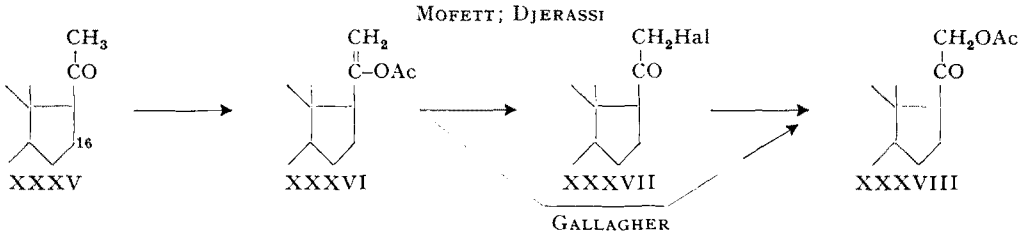
[4] Sr. KAUFMANN and J. PATAKI, Exper. *7*, 260 (1951). – E. WILSON and M. TISHLER, J. Amer. chem. Soc. *74*, 1609 (1952). – E. P. OLIVETO, C. GEROLD, and E. B. HERSHBERG, J. Amer. chem. Soc. *74*, 2248 (1952).

[5] V. R. MATTOX and E. C. KENDALL, J. biol. Chem. *188*, 287 (1951).

[6] V. R. MATTOX, E. L. WOROCH, G. A. FLEISHER, and E. C. KENDALL, J. biol. Chem. *197*, 261 (1952).

[7] G. M. HIGGINS, K. A. WOODS, and E. C. KENDALL, Endocrinology *48*, 175 (1951).

Chart 7



production of *D-homo*-cortexone acetate[1], but this possessed only about 10% of the survival effect of cortexone acetate.

A very recent synthesis was that of *17α-methyl*-cortexone acetate[2] from 17α-methyl-etio-acid. In contrast to the analogous highly active progesterone derivative, it possesses only about $^1/_5$ of the survival effect of cortexone acetate.

Methods of Partial Synthesis. The comment at the beginning of this paper about the volume of literature and of new findings in the field is particularly applicable to this chapter of adrenal research.

(a) *Side-chain degradation.* MIESCHER's by now already classical side-chain degradation of bile acids was recently again simplified: When working with two moles of N-bromosuccinimide in allylbromide, we[3] were able to combine into a single step three of the usual steps, i.e. α-bromination of the diphenyl-cholenes, splitting off of hydrogen bromide, and renewed bromination of the diene in position 21. The degradation of the bisnor-aldehydes, which are obtained from ergosterol or stigmasterol in particular, is suitably accomplished via their 20,22-unsaturated enol, enamine, or dehydro-nitril derivatives[4].

(b) *Ketol and dioxyacetone grouping.* The synthesis of the 20,21-ketol group according to REICHSTEIN from the corresponding acid chlorides via the diazo-ketones is now also possible with the 11α-hydroxy- and even the 11β-hydroxy derivatives provided one protects these hydroxyl groups by converting them into the 11-formates or -trifluoroacetates, which are readily saponifiable again[5]. By this method, it was possible to synthesise, amongst others, corticosterone and 11-epi-corticosterone. RUSCHIG's oxalic ester method for the selective halogenation and acetoxylation of methyl-ketones in position 21 was employed by SARETT[6] in the total synthesis of cortisone, and by ERCOLI[1] in the conversion of progesterone into cortexone. A new approach (Chart 7) was opened by MOFFETT[2] when he observed that the reaction of methyl-ketones (XXXV) with isopropenyl acetate did not produce 17,20- but 20,21-enol-acetates (XXXVI). With bromine these provide the 21-bromo-20-ketones (XXXVII) or, according to DJERASSI[3], with N-iodo-succinimide, the 21-iodo-20-ketones, which are readily converted into the ketol-acetates (XXXVIII)[4]. The procedure is also applicable to 16-dehydro-20-ketones yielding 16-dehydro-20,21-ketols[5], which, according to JULIAN, can be converted to dihydroxy-acetone compounds via the epoxides.

The latter, one might mention, are usually still produced according to GALLAGHER's excellent method. This method now also permits one to obtain $\Delta^{17,20}$-monoenol-acetates from 11,20-diketones[6]. A number of teams succeeded in producing 17α-mono- and also 17,21-diacetates from dihydroxy-acetone compounds[7].

(c) *Displacement of the oxygen from position 12 to 11.* The methods of GALLAGHER via the 11,12-ketols (e.g. MARKER-LAWSON acid) and of REICHSTEIN via the 11,12-unsaturated compounds by addition of hypohalic acid are nowadays more or less of historical interest because of their poor yields. KENDALL's process via the Δ^{11}-3α,9α-oxides is of lasting technical importance in the bile-acid series and is to be prefered to the method of HEYMANN and FIESER, using 3β-hydroxy-3α,9α-oxido compounds, i.e. hemiketals. A novel method (Chart 8), which for the time has been described in the hecogenin series, was recently published by us[8] and

[1] R. M. DODSON, P. B. SOLLMAN, and B. RIEGEL, J. Amer. chem. Soc. *75*, 5132 (1953).

[2] CH. R. ENGEL and G. JUST, J. Amer. chem. Soc. *76* (1954) in press. – H. HEUSSER, private communication.

[3] J. HEER and A. WETTSTEIN, Helv. chim. Acta *36*, 891 (1953).

[4] M. E. HERR and F. W. HEYL, J. Amer. chem. Soc. *72*, 2617 (1950); *74*, 3627 (1952). – A. F. B. CAMERON, J. S. HUNT, J. F. OUGHTON, P. A. WILKINSON, and B. M. WILSON, J. chem. Soc. *1953*, 3864.

[5] F. REBER, A. LARDON, and T. REICHSTEIN, Helv. chim. Acta *37*, 45 (1954). – A. LARDON and T. REICHSTEIN, Helv. chim. Acta *37*, 388, 443 (1954).

[6] L. H. SARETT, G. E. ARTH, R. M. LUKAS, R. E. BEYLER, G. I. POOS, W. F. JOHNS, and J. M. CONSTANTIN, J. Amer. chem. Soc. *74*, 4974 (1952).

[1] A. ERCOLI and P. DE RUGGIERI, Gazz. chim. ital. *84*, 312 (1954).

[2] R. B. MOFFETT and D. I. WEISBLAT, J. Amer. chem. Soc. *74*, 2183 (1952).

[3] C. DJERASSI and C. T. LENK, J. Amer. chem. Soc. *75*, 3493 (1953); *76*, 1722 (1954).

[4] According to GALLAGHER* the latter are also obtained from the 20,21-enol-acetates XXXVI with perbenzoic acid and silica gel under rearrangement. – * H. VANDERHAEGHE, E. R. KATZENELLENBOGEN, K. DOBRINER, and T. F. GALLAGHER, J. Amer. chem. Soc. *74*, 2810 (1952). – A. H. SOLOWAY, W. J. CONSIDINE, K. D. FUKUSHIMA, and T. F. GALLAGHER, J. Amer. chem. Soc. *76*, 2941 (1954).

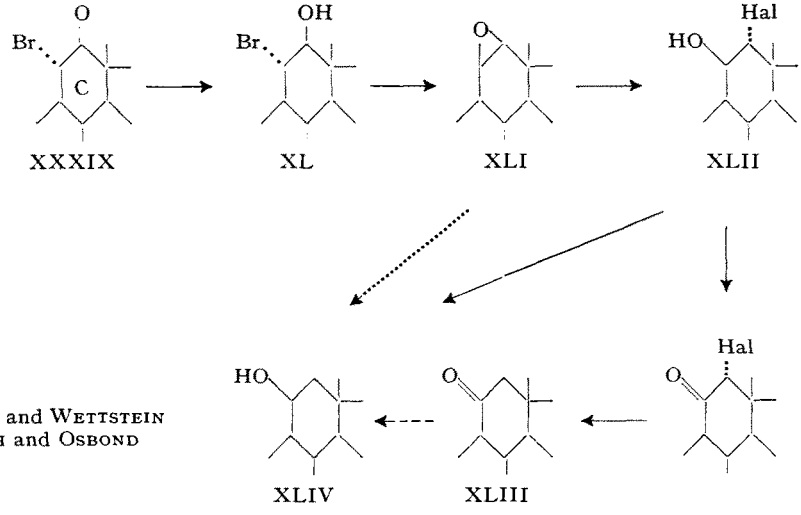
[5] Compare also: F. B. COLTON, W. R. NES, D. A. VAN DORP, H. L. MASON, and E. C. KENDALL, J. biol. Chem. *194*, 235 (1952). – F. B. COLTON and E. C. KENDALL, J. biol. Chem. *194*, 247 (1952).

[6] H. V. ANDERSON, E. R. GARRETT, F. H. LINCOLN, A. H. NATHAN, and J. A. HOGG, J. Amer. chem. Soc. *76*, 743 (1954). – D. H. R. BARTON, R. M. EVANS, J. C. HAMLET, P. G. JONES, and T. WALKER, J. Chem. Soc. *1954*, 747.

[7] R. B. MOFFETT and H. V. ANDERSON, J. Amer. chem. Soc. *76*, 747 (1954) and literature cited therein.

[8] J. SCHMIDLIN and A. WETTSTEIN, Helv. chim. Acta *36*, 1241 (1953).

Chart 8



Transformation of 12- into 11-Oxygenated Compounds

almost simultaneously by CORNFORTH *et al.*[1]: The 11α-bromo-12-ketone (XXXIX)[2] can be reduced with alkali-borohydrides while retaining the halogen atom, in good yield, to the 11α-bromo-12β-hydroxy compound XL. The 12β-configuration is of importance, because this compound, with alkali, or almost quantitatively with silver oxide in pyridine, now yields the 11β,12β-epoxide XLI. The latter is readily split by means of hydrohalic acids to the 11β-hydroxy-12α-halogen derivative XLII[3]. 11α,12α-Epoxides yield, as is known, only the inverse 11β-halogen-12α-hydroxy-compounds. XLII is analogous to the compounds prepared by REICHSTEIN in poor yield by the addition of hypohalic acids to 11,12-unsaturated steroids. On the one hand it can be dehydrogenated, of course, with chromium trioxide and dehalogenated with zinc to the 11-ketone XLIII, thus providing a smooth transition from 12-keto to 11-keto steroids. More interesting on the other hand is the new, direct dehalogenation of XLII, which, particularly in the case of the iodo derivative, took us almost quantitatively to the biologically important 11β-hydroxy derivative (XLIV). This is thus available from the 12-ketone in only 5 steps and in excellent yield.

(d) *The reduction of the 11-keto to the 11-hydroxy group* received very detailed attention because of the special properties of hydrocortisone as compared to cortisone. This reaction requires that other keto groups, i.e. those in position 3 and 20, are initially present in preformed form only, or that they are protected intermediately (Chart 9). WENDLER *et al.*[1] thus reduced 11-ketones with alkali-borohydrides to 11β-hydroxy derivatives, while the side-chain still contained the 17,20-dehydro-20-cyano group and the 3-keto group was protected by ketalization or semicarbazone formation (XLV). These authors were also able to produce *20*-semicarbazones from *free* 20,21-ketols (XLVI) thus achieving a protection of the 20-keto group against reduction. As is known, this is the method with which they were able for the first time to achieve a purely chemical synthesis of hydrocortisone. The protection of the 3- and also the 20-keto group by transformation into cyclic diketals (XLVII) is now frequently used; this results in Δ^5-3-alkylenedioxy derivatives from Δ^4-3-ketones, while substitution in 20-position takes place only with the free 20,21-ketols. By this means ANTONUCCI *et al.*[2] were able to convert cortisone even with the highly reactive $LiAlH_4$ into hydrocortisone plus a little 11-epi-hydrocortisone. The employment of $NaBH_4$[3] has the advantage over this that it is possible to work in the presence of water or alcohols; there is further the possibility of obtaining selective reductions of saturated, free 3,20-di- or

[1] J. W. CORNFORTH and J. M. OSBOND, Chem. and Ind. *1953*, 919. – J. W. CORNFORTH, J. M. OSBOND, and G. H. PHILIPPS, J. chem. Soc. *1954*, 907.

[2] Compare G. P. MUELLER and L. L. NORTON, J. Amer. chem. Soc. *76*, 749 (1954).

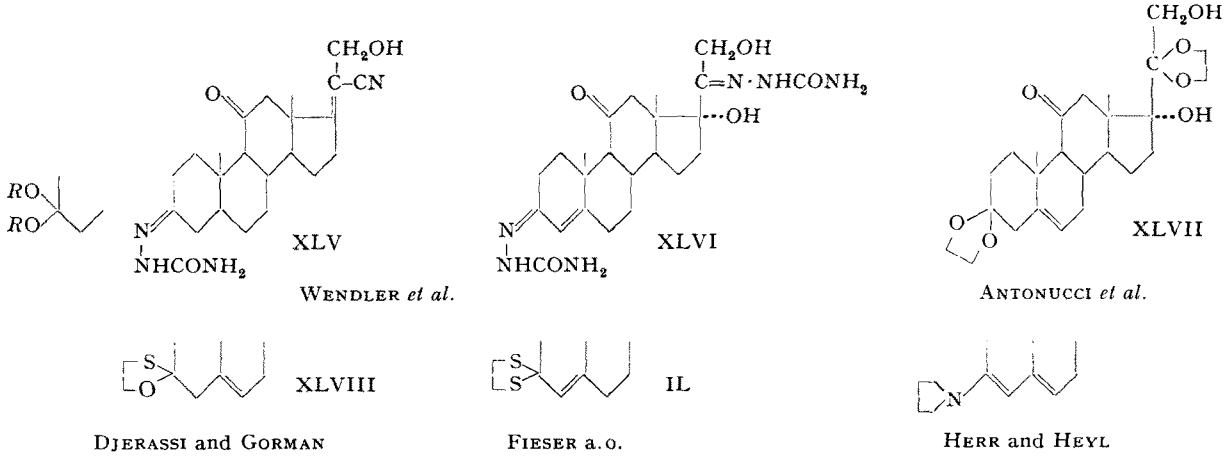
[3] Compare A. FÜRST and R. SCOTONI, Helv. chim. Acta *36*, 1410 (1953).

[1] N. L. WENDLER, R. B. GRABER, R. E. JONES, and M. TISHLER, J. Amer. chem. Soc. *72*, 5793 (1950); *74*, 3630 (1952). – N. L. WENDLER, Huang-Minlon, and M. TISHLER, J. Amer. chem. Soc. *73*, 3818 (1951).

[2] R. ANTONUCCI, S. BERNSTEIN, M. HELLER, R. LENHARD, R. LITTELL, and J. H. WILLIAMS, J. org. Chem. *18*, 70 (1953); see also R. ANTONUCCI, S. BERNSTEIN, and R. LENHARD, J. Amer. chem. Soc. *76*, 2956 (1954), furthermore R. H. LEVIN, B. J. MAGERLEIN, A. V. MCINTOSH, A. R. HANZE, G. S. FONKEN, J. L. THOMPSON, A. M. SEARCY, A. M. SCHERI, and E. S. GUTSELL, J. Amer. chem. Soc. *75*, 502 (1953); *76*, 546 (1954). – A. R. HANZE, G. S. FONKEN, A. V. MCINTOSH, A. M. SEARCY, and R. H. LEVIN, J. Amer. chem. Soc. *76*, 3179 (1954).

[3] E. P. OLIVETO, T. CLAYTON, and E. B. HERSHBERG, J. Amer. chem. Soc. *75*, 486 (1953).

Chart 9



3,11,20-triketones in position 3[1] or of Δ^4-unsaturated 3,11,20-triketones in position 20[2]. If 11α-hydroxy derivatives are desired as the main products it is expedient to reduce with sodium and propanol or with lithium, ammonia and alcohol[3].

The preparation of the ketals is best carried out, according to SARETT, by exchange dioxolanation using the ethylenedioxy derivative of mesityl oxide[4], a reaction which was recently studied in much detail by DAUBEN[5]. For certain syntheses, or for the elucidation of the constitution, cyclical alkylene hemithioketals (XLVIII)[6] or thioketals (IL)[7] should be considered, since the carbonyl functions may be regenerated therefrom with RANEY nickel alone, without the use of acids, or since they may be reduced to methylene groups.

An elegant method, also for the partial protection of carbonyl groups in polycarbonyl steroids against reduction by a metal hydride, consists in their transformation into enamines according to HERR and HEYL[8]. Pyrrolidine derivatives of Δ^4-3-ketones, e.g., are not reduced by $LiAlH_4$.

(e) *The chemical introduction of 11-oxygen into steroids with ring C unsubstituted* is fundamentally based on advancing a conjugated double bond system from ring B into C, later removing this auxiliary system once more by means of reduction (Chart 10). The most important starting materials for this are ergosterol and diosgenin, both of which permit simple sidechain degradation. The conversion of the two, or of their degradation products, into the corresponding 7,8; 9,11-dienes (L) with trans-configuration of the rings A/B (allo-series) is known. The various methods of converting these dienes into 11-ketones or 11-hydroxy compounds have been described particularly clearly in the review by ROSENKRANZ and SONDHEIMER[1] and cannot be gone into within the framework of this paper[2]. The latest publications, alone, concerning these methods would provide sufficient material for a separate review. Because of the difficulties of the eventual introduction of the 4,5-double bond into the allo-series, such conversions, according to JONES, are now carried out with 7,8; 9,11-dienes in which the original 5,6-double bond is not hydrogenated but converted into a potential double bond in the form of a 5α-hydroxyl. For this one converts the triene LI via 5,8-peroxides (formulated as LII according to FIESER) by reduction and water removal into LIII[3]. In JONES' 63rd communication[4], which appeared only recently the complete synthesis of cortisone (Chart 11) via such a 5α-acetoxy triene LIV was described.

[1] O. MANCERA, A. ZAFFARONI, B. A. RUBIN, F. SONDHEIMER, G. ROSENKRANZ, and C. DJERASSI, J. Amer. chem. Soc. *74*, 3711 (1952). – A. H. SOLOWAY, A. S. DEUTSCH, and T. F. GALLAGHER, J. Amer. chem. Soc. *75*, 2356 (1953).

[2] J. K. NORYMBERSKI and G. F. WOODS, Chem. and Ind. *1954*, 518.

[3] H. HEUSSER, R. ANLIKER, and O. JEGER, Helv. chim. Acta *35*, 1537 (1952). – F. SONDHEIMER, R. YASHIN, G. ROSENKRANZ, and C. DJERASSI, J. Amer. chem. Soc. *74*, 2696 (1952). – H. L. HERZOG, E. P. OLIVETO, M. A. JEVNIK, and E. B. HERSHBERG, J. Amer. chem. Soc. *74*, 4470 (1952). – S. BERNSTEIN, R. LITTELL, and J. H. WILLIAMS, J. Amer. chem. Soc. *75*, 1481 (1953). – E. P. OLIVETO, H. L. HERZOG, and E. B. HERSHBERG, J. Amer. chem. Soc. *75*, 1505 (1953).

[4] J. M. CONSTANTIN, A. C. HAVEN, and L. H. SAERTT, J. Amer. chem. Soc. *75*, 1716 (1953).

[5] H. J. DAUBEN, B. LÖKEN, and H. J. RINGOLD, J. Amer. chem. Soc. *76*, 1359 (1954).

[6] C. DJERASSI and M. GORMAN, J. Amer. chem. Soc. *75*, 3704 (1953).

[7] L. F. FIESER, J. Amer. chem. Soc. *76*, 1945 (1954).

[8] M. E. HERR and F. W. HEYL, J. Amer. chem. Soc. *74*, 3627 (1952); *75*, 1918, 5927 (1953).

[1] G. ROSENKRANZ and F. SONDHEIMER, Progr. Chem. Org. Natur. Prod. *10*, 274 (1953.

[2] Compare however: H. HEUSSER and O. JEGER, Ciba Found. Colloquia on Endocrinol. *7*, 46 (1953). – C. DJERASSI and G. ROSENKRANZ, Ciba Found. Colloquia on Endocrinol. *7*, 79 (1953). – F. S. SPRING *et al.*, Ciba Found. Colloquia on Endocrinol. *7*, 96 (1953). – G. ROSENKRANZ *et al.*, Rec. Progr. Hormone Res. *8*, 1 (1953).

[3] H. B. HENBEST and E. R. H. JONES, Ciba Found. Colloquia on Endocrinol. *7*, 39 (1953). – H. H. INHOFFEN and W. MENGEL, Chem. Ber. *87*, 146 (1954). – A. ZÜRCHER, H. HEUSSER, O. JEGER, and P. GEISTLICH, Helv. chim. Acta *37*, 1562 (1954).

[4] P. BLADON, H. B. HENBEST, E. R. H. JONES, B. J. LOVELL, and G. F. WOODS, J. chem. Soc. *1954*, 125, and literature cited therein.

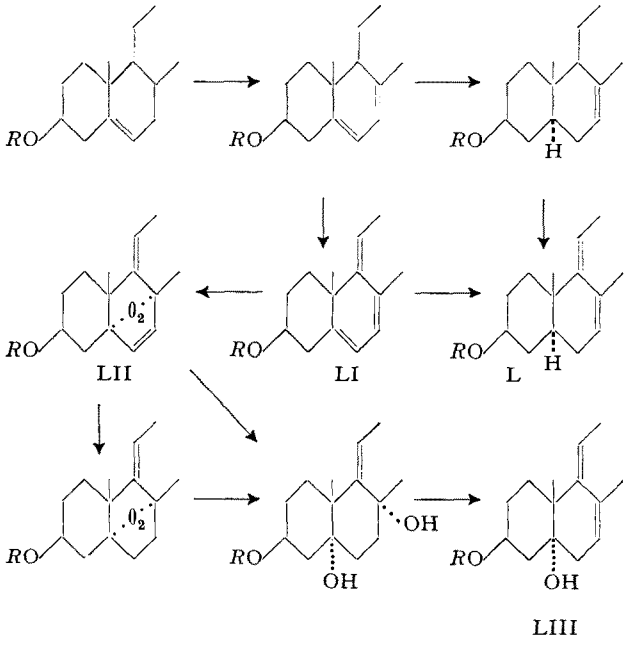
This is converted by means of what is probably the best of the currently available methods, using perbenzoic acid, then boron trifluoride, followed by acid isomerization as described by us[1] into the Δ^8-11-ketone LV, which is reduced to the saturated ketone LVI by means of lithium metal in liquid ammonia in accordance with the method of TISHLER as well as SONDHEIMER[2]. In this step the side-chain degradation to the methylketone LVII takes place, as stated earlier[1]. Since the usual process of GALLAGHER's for the introduction of the 17α-hydroxyl is too drastic for 5α-hydroxy-compounds, SARETT's method via the 17,20-dehydro nitril LVIII was chosen. Finally one produces the Δ^4-3-keto-group (LIX).

A modified route to cortisone from ergosterol under degradation of the side-chain in the 7-dehydro derivative, which takes one to the 3β-acetoxy-allopregnan-11,20-dione, was described by SPRING *et al.*[2] Unfortunately the final introduction of the Δ^4-3-keto system in this case is according to the "Standard Procedure", giving only yields of 0–10%.

A 5,8-maleic acid adduct of a 5,7;9,11-triene is said to prevent the hypertension induced by cortexone[3].

(f) The most direct method for the introduction of oxygen is the *microbiological hydroxylation*[4] (Chart 12). The starting material was chiefly progesterone (LX), which is available from cholesterol or lithocholic acid, better still from stigmasterol, and more recently again ergosterol[5], but particularly readily from sapogenins such as diosgenin. Other interesting starting materials are 17α-hydroxy-progesterone (produced according to GALLAGHER or JULIAN), cortexone (according to RUSCHIG or MOFFETT) and 17α-hydroxy-cortexone (LXIX) (REICHSTEIN's substance S; according to JULIAN). The fundamental work was done by PETERSON and MURRAY[6], who were the first to obtain from progesterone

Chart 10



[1] K. HEUSLER and A. WETTSTEIN, Helv. chim. Acta *36*, 398 (1953).

[2] E. SCHOENEWALDT, L. TURNBULL, E. M. CHAMBERLIN, D. REINHOLD, A. E. ERICKSON, W. V. RUYLE, J. M. CHEMERDA, and M. TISHLER, J. Amer. chem. Soc. *74*, 2696 (1952). – F. SONDHEIMER, R. YASHIN, G. ROSENKRANZ, and C. DJERASSI, J. Amer. chem. Soc. *74*, 2696 (1952). – Compare C. DJERASSI, W. FRICK, G. ROSENKRANZ, and F. SONDHEIMER, J. Amer. chem. Soc. *75*, 3496 (1953).

[1] M. E. HERR and F. W. HEYL, J. Amer. chem. Soc. *72*, 2617 (1950); *74*, 3627 (1952). – A. F. B. CAMERON, J. S. HUNT, J. F. OUGHTON, P. A. WILKINSON, and B. M. WILSON, J. chem. Soc. *1953*, 3864.

[2] D. MACLEAN, W. S. STRACHAN, and F. S. SPRING, Chem. and Ind. *1953*, 1259.

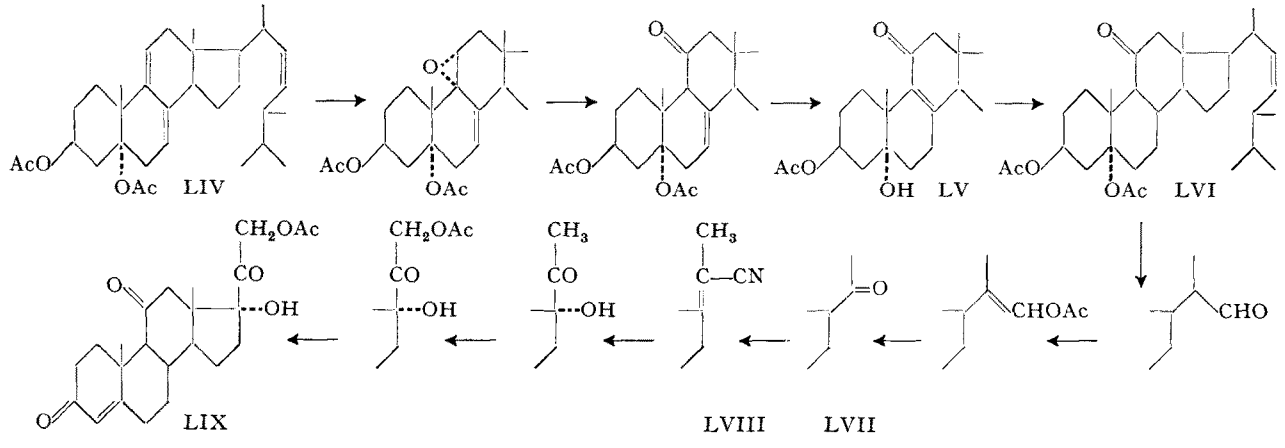
[3] R. O. STAFFORD, L. C. THOLE, and K. J. OLSON, Endocrinology *52*, 292 (1953).

[4] Reviews: D. H. PETERSON, Research *6*, 309 (1953). – K. FLOREY, Chimia *8*, 81 (1954). – R. I. DORFMAN and F. UNGAR, *Metabolism of Steroid Hormones* (Minneapolis, 1953).

[5] F. JOHNSON, G. T. NEWBOLD, and F. S. SPRING, J. chem. Soc. *1954*, 1302.

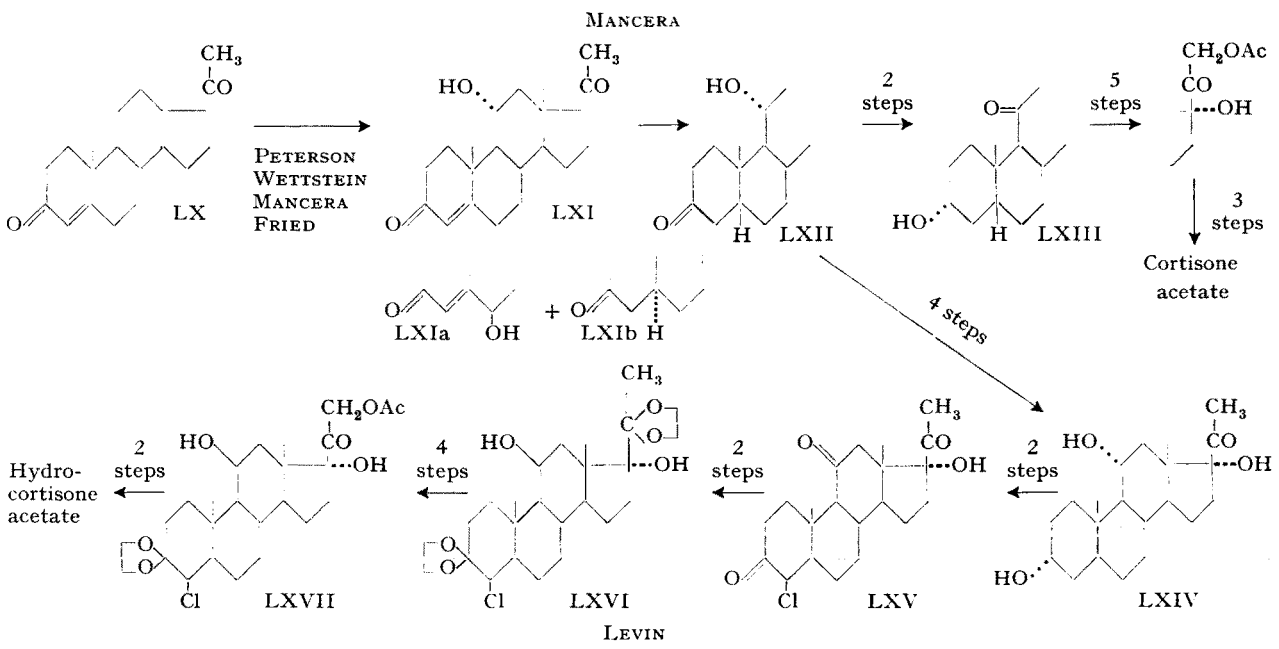
[6] D. H. PETERSON and H. C. MURRAY, J. Amer. chem. Soc. *74*, 1871 (1952).

Chart 11



Synthesis of Cortisone by JONES *et al.* from Ergosterol.

Chart 12



LX, with rhizopus species, a 10% yield of 11α-hydroxy-progesterone (LXI). We, ourselves[1], and also MANCERA *et al.*[2], were able to increase this yield to 40–45%, while PETERSON *et al.*[3] finally raised it to about 95%; FRIED *et al.*[4] achieved a 35% yield with an aspergillus strain. In addition, and in a secondary degree, a 6β-hydroxyl enters the molecule (LXIa) or else hydrogenation of the double bond to the 5α-configuration (LXI*b*)[3] takes place. Cortexone[1,4,5], substance S (LXIX)[1,4,6], and 17α-hydroxy-progesterone[4,7], as well as 6-dehydro-progesterone[8], and pregnane or allo-pregnane-3,20-dione[9], also readily provided the 11α-, and in part once more the 6β-hydroxylation products (e.g. LXXa). In the case of substance S, simultaneous reduction of the 4-double bond to the 5β-configuration (LXX*b*)[6] occurred in addition, while with 16-dehydro-progesterone there was always a simultaneous reduction of the 16-double bond to the unnatural 17α-configuration[1]. Our findings[2] show that 3β,21-dihydroxy-allopregnane-20-one is hydroxylated in 7β-position.

Because 11α-hydroxy-progesterone (LXI) is so readily accessible, it represents a suitable intermediate in the manufacture of cortisone and hydrocortisone. MANCERA *et al.*[3] have described the preparation of the former in 9 steps which are interesting in part in view of their steric course: The catalytic hydrogenation of the double bond in the presence of the 11α-hydroxy group provides the desired 5β-derivative LXII, which yields through oxidation and partial reduction with $NaBH_4$ the 3α-hydroxy-11-ketone LXIII, familiar from the bile-acid series (3β-hydroxyl thus preferentially results in the 5α-series). Cortisone acetate is obtained therefrom by means of the usual conversion of the side-chain and introduction of the double bond.

LEVIN *et al.*[4] reached hydrocortisone in about 14 steps, starting with the intermediate LXII: they obtain LXIV by partial reduction in position 3 and introduction of the 17α-hydroxyl (according to GALLAGHER),

[1] F. W. KAHNT, CH. MEYSTRE, R. NEHER, E. VISCHER, and A. WETTSTEIN, Exper. *8*, 422 (1952). – A. WETTSTEIN, in L. WEISSBECKER, *Probleme des Hypophysen-Nebennierenrindensystems* (Berlin, 1953), p. 33.

[2] O. MANCERA, A. ZAFFARONI, B. A. RUBIN, F. SONDHEIMER, G. ROSENKRANZ, and C. DJERASSI, J. Amer. chem. Soc. *74*, 3711 (1952).

[3] D. H. PETERSON, H. C. MURRAY, S. H. EPPSTEIN, L. M. REINEKE, A. WEINTRAUB, P. D. MEISTER, and H. M. LEIGH, J. Amer. chem. Soc. *74*, 5933 (1952).

[4] J. FRIED, R. W. THOMA, J. R. GERKE, J. E. HERZ, M. N. DONIN, and D. PERLMAN, J. Amer. chem. Soc. *74*, 3962 (1952).

[5] S. H. EPPSTEIN, P. D. MEISTER, D. H. PETERSON, H. C. MURRAY, H. M. LEIGH, D. A. LYTTLE, L. M. REINEKE, and A. WEINTRAUB, J. Amer. chem. Soc. *75*, 408 (1953).

[6] D. H. PETERSON, S. H. EPPSTEIN, P. D. MEISTER, B. J. MAGERLEIN, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, J. Amer. chem. Soc. *75*, 412 (1953).

[7] P. D. MEISTER, D. H. PETERSON, H. C. MURRAY, G. B. SPERO, S. H. EPPSTEIN, A. WEINTRAUB, L. M. REINEKE, and H. M. LEIGH, J. Amer. chem. Soc. *75*, 416 (1953).

[8] D. H. PETERSON, A. H. NATHAN, P. D. MEISTER, S. H. EPPSTEIN, H. C. MURRAY, A. WEINTRAUB, L. M. REINEKE, and H. M. LEIGH, J. Amer. chem. Soc. *75*, 419 (1953).

[9] S. H. EPPSTEIN, H. D. PETERSON, H. M. LEIGH, H. C. MURRAY, A. WEINTRAUB, L. M. REINEKE, and P. D. MEISTER, J. Amer. chem. Soc. *75*, 421 (1953).

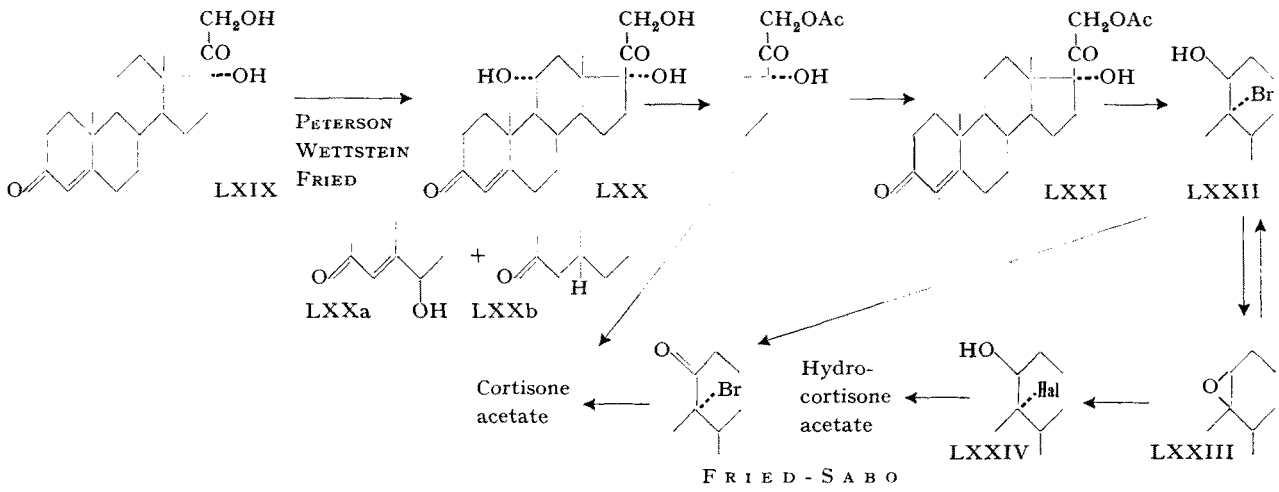
[1] P. D. MEISTER, D. H. PETERSON, H. C. MURRAY, S. H. EPPSTEIN, L. M. REINEKE, A. WEINTRAUB, and H. M. LEIGH, J. Amer. chem. Soc. *75*, 55 (1953).

[2] F. W. KAHNT, CH. MEYSTRE, R. NEHER, E. VISCHER, and A. WETTSTEIN, Exper. *8*, 422 (1952).

[3] O. MANCERA, A. ZAFFARONI, B. A. RUBIN, F. SONDHEIMER, G. ROSENKRANZ, and C. DJERASSI, J. Amer. chem. Soc. *74*, 3711 (1952). – A. H. SOLOWAY, A. S. DEUTSCH, and T. F. GALLAGHER, J. Amer. chem. Soc. *75*, 2356 (1953). – O. MANCERA, H. J. RINGOLD, C. DJERASSI, G. ROSENKRANZ, and F. SONDHEIMER, J. Amer. chem. Soc. *75*, 1286 (1953).

[4] R. H. LEVIN, B. J. MAGERLEIN, A. V. MCINTOSH, A. R. HANZE, G. S. FONKEN, J. L. THOMPSON, A. M. SEARCY, M. A. SCHERI, and E. S. GUTSELL, J. Amer. chem. Soc. *75*, 502 (1953); *76*, 546 (1954). – A. R. HANZE, G. S. FONKEN, A. V. MCINTOSH, A. M. SEARCY, and R. H. LEVIN, J. Amer. chem. Soc. *76*, 3179 (1954).

Chart 13



which is converted into LXV by coupled oxidation and chlorination by means of t-butyl hypochlorite and oxidation in position 11. The latter is hydrogenated as a diketal to the 11β-hydroxy compound LXVI, in which, after partial splitting of just the 20-ketal group, the dihydroxy-acetone side-chain is completed (LXVII). Upon finishing the Δ^4-3-keto group hydrocortisone acetate results.

It is unfortunately necessary in the two processes mentioned to saturate to the 3α-hydroxy-5β-derivative the Δ^4-3-keto group already contained in the starting material, and to reintroduce it in the end. A new, simple synthesis of hydrocortisone by FRIED and SABO[1] (Chart 13) avoids this disadvantage and also an intermediate protection of the 20-keto-group. It starts from 11-epihydrocortisone (LXX)[2], which is produced, as described, in the microbiological hydroxylation of substance S (LXIX) and comprizes 8 steps from the latter. By acetylating the 21- and esterifying the 11α-hydroxyl with p-toluenesulfonic acid, as well as splitting off the tosylate residue, one obtains 9,11-anhydro-hydrocortisone acetate (LXXI), to which is added hypobromous acid so as to obtain the 9α-bromo-11β-hydroxy derivative (LXXII). By splitting off HBr one obtains the 9β,11β-epoxide (LXXIII), which can be split up to any desired 9α-halogenated hydrocortisone acetate (LXXIV) with hydrohalic acid. The 9α-iodo derivative is particularly suitable for reduction to hydrocortisone acetate, while the 9α-chloro- and 9α-fluoro derivatives themselves represent the especially active cortical hormones mentioned earlier. The bromohydrin LXXII is converted into cortisone acetate by oxidation and debromination, but this product is, of course, also available direct by oxidizing epi-hydrocortisone-21-monoacetate. Analogous conversions were also carried out in the corticosterone series, starting with 11-epi-corticosterone.

Microorganisms, however, are able to hydroxylate steroids not only in the 11α-, but also in the 11β-position (Chart 14). HAINES *et al.*[1] were thus able to isolate hydrocortisone from the incubation of substance S with a maximum yield of about 23% when using a strain of Cunninghamella, while SHULL *et al.*[2] obtained about 40% with a Curvularia strain.

"Unnatural" microbiological hydroxylations in addition to the ones in 7β- and 11α-position were carried

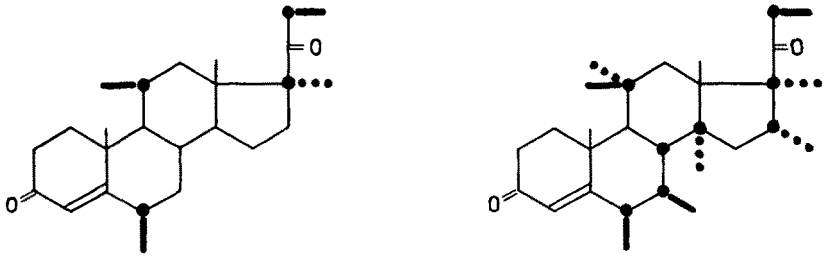
[1] J. FRIED and E. F. SABO, J. Amer. chem. Soc. *75*, 2273 (1953).

[2] Chemical syntheses see: J. ROMO, G. ROSENKRANZ, C. DJERASSI, and F. SONDHEIMER, J. Amer. chem. Soc. *75*, 1277 (1953). – E. P. OLIVETO, H. L. HERZOG, M. A. JEVNIK, H. E. JORGENSEN, and E. B. HERSHBERG, J. Amer. chem. Soc. *75*, 3651 (1953).

[1] D. R. COLINGSWORTH, J. N. KARNEMAAT, F. R. HANSON, M. P. BRUNNER, K. M. MANN, and W. J. HAINES, J. biol. Chem. *203*, 807 (1953). – F. R. HANSON, K. M. MANN, E. D. NIELSON, H. V. ANDERSON, M. P. BRUNNER, J. N. KARNEMAAT, D. R. COLINGSWORTH, and W. J. HAINES, J. Amer. chem. Soc. *75*, 5369 (1953).

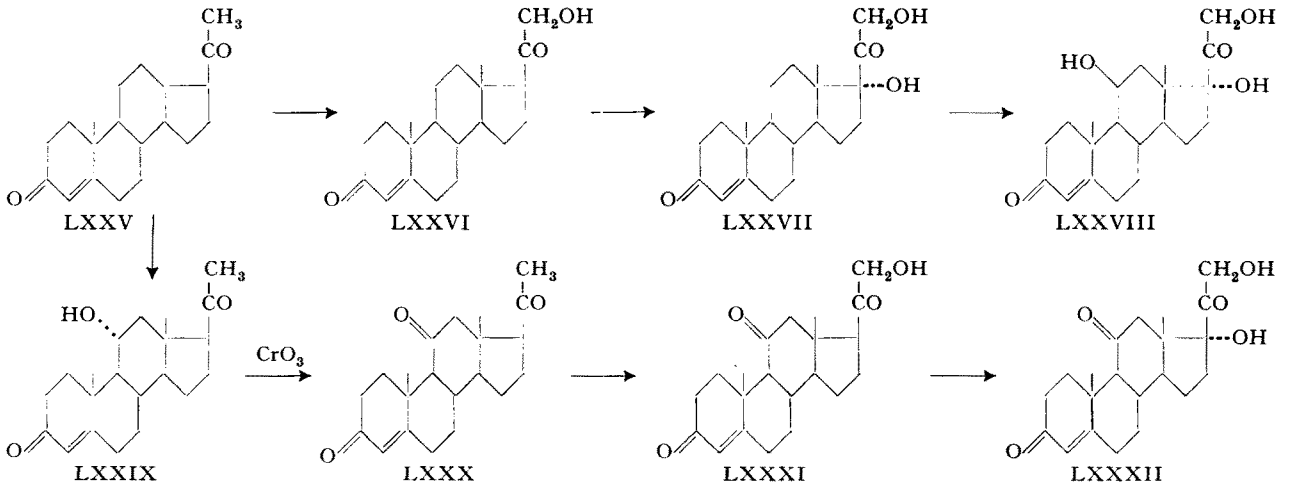
[2] G. M. SHULL, R. HEIGHTS, D. A. KITA, J. HEIGHTS, and J. W. DAVISSON, US. Pat. 2658023 (1953).

Chart 14



Adrenals: 21, 17 α, 11 β, 6 β Microorganisms: 6 β, 7 β, 8, 11 α, 11 β, 14 α, 16 α, 17 α, 21 Hydroxylations

Chart 15



out in 8-[1], 14α-[1,2], and 16α-[3,4] position. Of the "natural" hydroxyl groups in 11β-, 17α-, and 21-position, which are essential to the action of the adrenocortical hormones, and which the adrenal gland is able to introduce in the biosynthesis, only the first has been produced up to now by microbiological means.

While investigating a considerable series of strains of fungi we[5] recently found certain organisms which are able in submerged culture to hydroxylate the C_{21}-steroids in 17α- or 21-position as well (Chart 15). The first group are Moniliaceae, in particular the Trichothecia. From Cortexone (LXXVI), for instance, they produced, apart from 6β-hydroxy-cortexone, 17α-hydroxy-cortexone (LXXVII; substance S), from 11-dehydro-corticosterone (LXXXI) they produced cortisone (LXXXII) and from corticosterone, hydrocortisone in addition to cortisone. The reaction products were generally identified by means of mixed melting points, rotation, and IR-spectra.

Hydroxylation in position 21 was achieved, in particular with Ascomycetes, such as Ophiobolus strains. Progesterone (LXXV), for instance, was thus converted into cortexone (LXXVI) with a very good yield, 11-ketoprogesterone (LXXX) into 11-dehydro-corticosterone (LXXXI), and 17α-hydroxy-progesterone into substance S. Practically no secondary products were obtained here; cortexone was left completely unchanged under the influence of such cultures.

If one combines the familiar 11-hydroxylations with these new microbiological reactions, syntheses of adrenocortical hormones can be achieved in very few steps. One path, for instance, leads from progesterone to 11α-hydroxy-progesterone and 11-keto-progesterone, which is hydroxylated in position 21 and then in position 17α, thus yielding cortisone (LXXXII) in 4 steps. Still simpler, in only 3 microbiological steps, is the conversion of progesterone into hydrocortisone (LXXVIII) via cortexone and substance S.

The new enzymatic conversions by microbiological methods appear, at least as regards the final effect, to approximate those obtained in the biosynthesis of the hormones in the adrenal. It is clear, however, that these enzyme systems of the microorganisms are less specific than those of the endocrine mammalian glands, which has advantages as regards their preparative application. Thus, for instance, the mammalian 17α-hydroxylase, which is found in particular in the adrenals and testes, is hardly able to attack 11β-hydroxylated and also 21-hydroxylated pregnane compounds[1]. This limitation does not apply to our microbiological 17α-hydroxylation.

(g) *4-unsaturated 3-keto group.* As is known, this group, in addition to the ketol group in the side-chain, is an absolute prerequisite for the adrenocortical hormone action. A more recent development, going beyond the classical method of MATTOX and KENDALL for the introduction of the conjugated double bond, is the elegant procedure by LEVIN *et al.*[2], who simultaneously oxidize the 3-hydroxyl group by means of t-butyl hypochlorite and introduce chlorine in position 4 (LXIV → LXV). During this the usually readily oxidizable 11α-hydroxyl group remains unattacked. A specific oxidation is further possible according to

[1] H. C. MURRAY and D. H. PETERSON, US. Pat. 2602769 (1952).
[2] P. D. MEISTER *et al.*, Abstr. 123rd Meet. Amer. chem. Soc. *1953*, 5C.
[3] E. VISCHER, J. SCHMIDLIN, and A. WETTSTEIN, Helv. chim. Acta *37*, 321 (1954).
[4] D. PERLMAN, E. TITUS, and J. FRIED, J. Amer. chem. Soc. *74*, 2126 (1952).
[5] CH. MEYSTRE, E. VISCHER, and A. WETTSTEIN, Helv. chim. Acta *37*, 1548 (1954). Added when reading the proofs: In the meantime also P. D. MEISTER *et al.* (J. Amer. Soc. *76*, 4050 (1954) described the microbiological introduction of the 17α-hydroxyl group.

[1] J. E. PLAGER and L. T. SAMUELS, Feder. Proc. *12*, 357 (1953); Arch. Biochem. Biophys. *42*, 477 (1953); J. biol. Chem. 1954, (in press).
[2] R. H. LEVIN, B. J. MAGERLEIN, A. V. MCINTOSH, A. R. HANZE, G. S. FONKEN, J. L. THOMPSON, A. M. SEARCY, M. A. SCHERI and E. S. GUTSELL, J. Amer. chem. Soc. *75*, 502 (1953); *76*, 546 (1954). – A. R. HANZE, G. S. FONKEN, A. V. MCINTOSH, A. M. SEARCY, and R. H. LEVIN, J. Amer. chem Soc. *76*, 3179 (1954).

SONDHEIMER and ROSENKRANZ[1] in the case of allyl alcohols with Δ^4-3-hydroxy compounds, for instance, by the action of manganese dioxide. Mention has already been made of the again widely used method for the preparation of a Δ^4-3-keto-group from the Δ^5-3-hydroxy group via saturated 5α-hydroxy compounds (LIII)[2].

If no specifically acting oxidizing agent is used with polyhydroxy compounds, the hydroxyl groups, with the exception of the one in position 3, have to be protected intermediately. This is possible with the not readily reacting 11β-hydroxy derivatives, as previously discussed, for instance by converting into the readily resaponifiable formates[3,4] or trifluoro-acetates[3], of which even the former were able to withstand treatment with N-bromoacetamide, and were thus usable in a hydrocortisone synthesis[4]. Although it is possible to produce 11β-acetates with an acetylating agent in the presence of an acid catalyst – after the manner of the 17α-acetates earlier on[5] – and also to split partially acetate groups formed simultaneously in another position[6], the saponification of an 11β-acetate is no longer readily feasible.

Still lacking is a method, which in good yield permits the direct introduction of a double bond into 11-oxygenated, saturated 3-ketones of the 5α-(allo-)series, wherein rings A and B are in trans configuration.

Active derivatives for special purposes. In addition to the already mentioned 9α-chloro- and -fluoro-hydrocortisones and -cortisones reference should be made in particular to the relatively poorly saponifiable esters – this accounting for the protracted action – for instance the 21-trimethyl-acetate of cortexone and cortisone produced by MIESCHER *et al.*[7]. DESAULLES and MEIER[8] have thus been able to show, lately, that cortisone trimethyl-acetate in the rat has a much longer inhibiting action on the formation of the foreign body granuloma and a lower but protracted action on the wheight increase when compared with cortisone acetate of the same crystal size.

Total Synthesis. The chemically highly interesting and ever more important field of total synthesis[1] of hydrogenated steroids, and thus also of the corticoids, is too voluminous to be dealt with here, in view of the many formulae this would require. We shall therefore limit ourselves to listing the most important literature, with special reference to the names of ROBINSON[2], WOODWARD[3], SARETT[4], JOHNSON[5], MIESCHER[6], and WILDS[7].

Biosynthesis. This field, too, has developed into quite a specialty[8]. Only a certain chapter from it is therefore selected here, one to which we were recently able to make a contribution in connection with the isolation of aldosterone: *The formation of corticoids from pregnane compounds in the presence of tissue enzymes.* This work does not as yet cover the use of labeled pregnane derivatives such as progesterone, cortexone, cortisone, substance S[9] as these investigations are still in progress. Furthermore, we worked exclusively with total homogenates and not with enriched, position-specific enzyme preparations. Such were, however, used by SWEAT[10] and

[1] C. AMENDOLLA, G. ROSENKRANZ, and F. SONDHEIMER, J. chem. Soc. *1954*, 1226; J. Amer. chem. Soc. *75*, 5930 (1953). – F. SONDHEIMER and G. ROSENKRANZ, Exper. *9*, 62 (1953).

[2] H. B. HENBEST and E. R. JONES, Ciba Found. Colloquia on Endocrinol. *7*, 39 (1953). – H. H. INHOFFEN & W. MENGEL, Chem. Ber. *87*, 146 (1954). – A. ZÜRCHER, H. HEUSSER, O. JEGER & P. GEISTLICH, Helv. *37*, 1562 (1954). – P. BLADON, H. B. HENBEST, E. R. H. JONES, B. J. LOVELL, & G. F. WOODS, J. Chem. Soc. *1954*, 125, and literature cited therein.

[3] F. REBER, A. LARDON, and T. REICHSTEIN, Helv. chim. Acta *37*, 45 (1954). – A. LARDON and T. REICHSTEIN, Helv. chim. Acta *37*, *388*, 443 (1954).

[4] E. P. OLIVETO, C. GEROLD, and E. B. HERSHBERG, Arch. Biochem. Biophys. *49*, 244 (1954).

[5] HUANG-MINLON, E. WILSON, N. L. WENDLER, and M. TISHLER, J. Amer. chem. Soc. *74*, 5394 (1952). – R. B. TURNER, J. Amer. chem. Soc. *75*, 3489 (1953).

[6] E. P. OLIVETO, C. GEROLD, and E. B. HERSHBERG, J. Amer. chem. Soc. *43*, 234 (1953). – E. P. OLIVETO, C. GEROLD, L. WEBER, H. E. JORGENSEN, R. RAUSSER, and E. B. HERSHBERG, J. Amer. chem. Soc. *75*, 5486 (1953).

[7] P. WIELAND, J. HEER, J. SCHMIDLIN, and K. MIESCHER, Helv. chim. Acta *34*, 354 (1951). – F. GROSS and E. TSCHOPP, Exper. *8*, 75 (1952).

[8] P. DESAULLES and R. MEIER, Schweiz. med. Wschr. *84*, 741 (1954).

[1] Review see: C. A. FINCH, Manufacturing Chemist *24*, 509 (1953).

[2] A. R. PINDER and R. ROBINSON, Nature *167*, 484 (1951). – H. M. E. CARDWELL, J. W. CORNFORTH, S. R. DUFF, H. HOLTERMANN, and R. ROBINSON, Chem. and Ind. *1951*, 389; J. chem. Soc. *1953*, 361.

[3] R. B. WOODWARD, F. SONDHEIMER, D. TAUB, K. HEUSLER, and W. M. MCLAMORE, J. Amer. chem. Soc. *73*, 2403 (1951); *74*, 4223 (1952). – R. B. WOODWARD, F. SONDHEIMER, and D. TAUB, J. Amer. chem. Soc. *73*, 3547, 3548, 4057 (1951); Ref. in Chemical Age *69*, 1222 (1953). – B. BARKLEY, M. W. FARRAR, W. S. KNOWLES, and H. RAFFELSON, J. Amer. chem. Soc. *75*, 4110 (1953).

[4] L. H. SARETT, R. M. LUKES, G. I. POOS, J. M. ROBINSON, R. E. BEYLER, J. M. VANDEGRIFT, and G. E. ARTH, J. Amer. chem. Soc. *74*, 1393 (1952). – R. E. BEYLER and L. H. SARETT, J. Amer. chem. Soc. *74*, 1397, 1406 (1952). – R. M. LUKES, G. I. POOS and L. H. SARETT, J. Amer. chem. Soc. *74*, 1401 (1952). – L. H. SARETT, G. E. ARTH, R. M. LUKES, R. E. BEYLER, G. I. POOS, W. F. JOHNS, and J. M. CONSTANTIN, J. Amer. chem. Soc. *74*, 4974 (1952). – G. I. POOS, G. E. ARTH, R. E. BEYLER, and L. H. SARETT, J. Amer. chem. Soc. *75*, 422 (1953). – R. M. LUKES, G. I. POOS, R. E. BEYLER, W. F. JOHNS, and L. H. SARETT, J. Amer. chem. Soc. *75*, 1707 (1953). – L. H. SARETT, W. F. JOHNS, R. E. BEYLER, R. M. LUKES, G. I. POOS, and G. E. ARTH, J. Amer. chem. Soc. *75*, 2112 (1953). – G. E. ARTH, G. I. POOS, R. M. LUKES, F. M. ROBINSON, W. F. JOHNS, M. FEURER, and L. H. SARETT, J. Amer. chem. Soc. *76*, 1715 (1954). – R. M. LUKES and L. H. SARETT, J. Amer. chem. Soc. *76*, 1178 (1954).

[5] W. S. JOHNSON, B. BANNISTER, B. M. BLOOM, A. D. KEMP, R. PAPPO, E. R. ROGIER, and J. SZMUSZKOVICZ, J. Amer. chem. Soc. *75*, 2275 (1953). – W. S. JOHNSON, R. PAPPO, and A. D. KEMP, J. Amer. chem Soc. *76*, 3353 (1954).

[6] P. WIELAND, H. ÜBERWASSER, G. ANNER, and K. MIESCHER, Helv. chim. Acta *36*, 1231 (1953).

[7] A. L. WILDS, J. W. RALLS, W. C. WILDMAN, and K. E. MCCALEB, J. Amer. chem. Soc. *75*, 5794 (1950). – A. L. WILDS, J. W. RALLS, D. A. TYNER, R. DANIELS, ST. KRAYCHY, and M. HARNIK, J. Amer. chem. Soc. *75*, 4878 (1953).

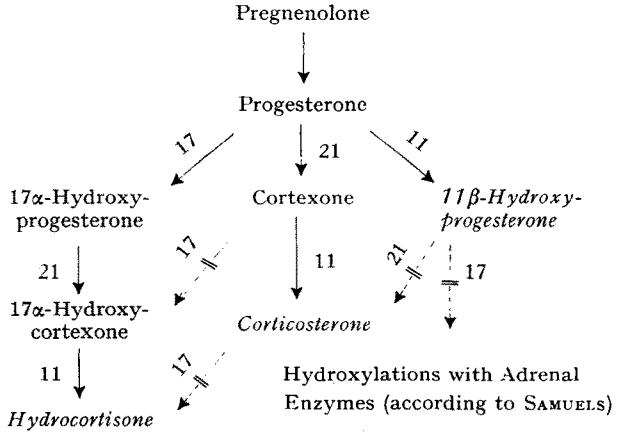
[8] Review see: O. HECHTER and G. PINCUS, Physiol. Rev. *34*, 459 (1954).

[9] Compare e.g.: M. GUT, Helv. chim. Acta *36*, 906 (1953). – G. I. FUJIMOTO and J. PRAGER, J. Amer. chem Soc. *75*, 3259 (1953). – L. M. THOMPSON, C. H. YATES, and A. D. ODELL, J. Amer. chem Soc. *76*, 1194 (1954). – E. SCHWENK, N. T. WERTHESSEN and A. F. COLTON, Arch. Biochem. Biophys. *48*, 322 (1954). – R. D. H. HEARD *et al.*, Rec. Progr. Hormone Res. *9*, 383 (1954). – T. F. GALLAGHER *et al.*, Rec. Progr. Hormone Res. *9*, 411 (1954).

[10] M. L. SWEAT, J. Amer. chem. Soc. *73*, 4056 (1951).

also HAYANO and DORFMAN[1] among others. These workers were able to show that enzymes in the adrenal mitochondria are responsible for the 11β-hydroxylation. As already mentioned, PLAGER and SAMUELS[2] then succeeded in demonstrating a 21-hydroxylase which proved precipitable with ammonium sulfate from the supernatant and was katalysed by ATP, and also a more unstable 17α-hydroxylase in the solution. The presence of an 11β-hydroxyl group in the reacting compound inhibited the entry of the 17- and 21-hydroxyl, while the presence of a 21-hydroxyl group prevented the entry of the 17-hydroxyl. In analogy to the earlier concepts of PINCUS-HECHTER regarding biosynthesis, SAMUELS[3] now arrives at the scheme shown in Chart 16. According to this, enzymatic hydroxylations in the series 17,21,11 are as yet possible only from left to right and not in the reverse order. As far as can be told this agrees with the earlier adrenal perfusion findings of the Worcester group[4].

Chart 16



Our experiments[5] were directed in particular to obtaining the greatest possible yields of aldosterone from adrenals. They find their early origin in the work of HAINES[6], who had shown that the glucocorticoid bioactivity in hog adrenal breis can be enhanced by incubation plus aeration. Many other investigators have since worked with this method. We, ourselves[1], had been able to obtain a very considerable activation of the 11β-hydroxylation during the aerobic incubation of adrenal homogenates upon addition of substrates of the citric acid cycle, such as for instance fumarate or pyruvate, but also of nicotinamide, ATP or ascorbic acid, amongst others. This made us assume that oxidative phosphorylation is involved in the steroid 11β-hydroxylation, an assumption which has just been proved elegantly by BROWNIE and GRANT[2]. These authors show that the role of members of the citric acid cycle might be that of oxidizable substrates for oxidative phosphorylations involved in the activation of the steroids before hydroxylation.

With our previous experimental setup, it was now possible for us[3] (Table II) to increase the aldosterone content of beef adrenal homogenates, measured by paper chromatography, seven-fold by aerobic incubation in the presence of the above supplements without the addition of ATP, and as much as nineteenfold when ATP was added. The content of hydrocortisone and corticosterone even increased by 131 and 45 times respectively through incubation. Hog adrenals per se contained about 12 times as much aldosterone as beef adrenals and their content of hydrocortisone and corticosterone was 30 and 14 times higher respectively. Whereas the latter two hormones increased further by about eight times by incubation of the hog homogenates, the aldosterone content was not much affected. A clear-cut species difference becomes evident: The enzyme systems and/or precursors contained in beef adrenals, and required for the formation of the corticoids during incubation, are contained in hog adrenals to a limited degree only; on the other hand, the latter have a much higher content of *finished* corticoids. Thus in the end incubated beef adrenals, and hog adrenals with or without incubation, contain about the same quantity of aldosterone, namely approximately 1 mg per kilo.

Table II contains finally some of the preliminary experiments which concerned themselves with possible precursors in this *in-vitro* biosynthesis of aldosterone. It is evident that the addition of cortexone had an unequivocally beneficial effect; yields two or three times as great as the former optimum, and thus a maximum of more than 3 mg of aldosterone per kilo of adrenals, were demonstrable, especially in the absence of nicotinamide. Corticosterone was similarly increased, whereas hydrocortisone remained at best equal. The addition of corticosterone reduced the aldosterone value to 1/6 of the comparative experiment. Progesterone also reduced the aldosterone yield but increased

[1] M. HAYANO and R. I. DORFMAN, Feder. Proc. *11*, 228 (1952); J. biol. Chem. *200*, 175 (1953).

[2] J. E. PLAGER and L. T. SAMUELS, Feder. Proc. *11*, 383 (1952); *12*, 357 (1953); Arch. Biochem. Biophys. *42*, 477 (1953); J. biol. Chem. (1954) in press.

[3] L. T. SAMUELS, *Endocrinological Symposium*, Marburg, June 1954.

[4] O. HECHTER, Ciba Found. Colloquia on Endocrinol. *7*, 204 (1953). – Dorfman now observes a certain degree of 21-hydroxylation in 21-desoxycortisone by adrenal homogenates (Discussion subsequent to [5]).

[5] A. WETTSTEIN, F. W. KAHNT, and R. NEHER, Ciba Foundation Colloquia on Endocrinol. *8* (1954), in press; compare literature cited therein.

[6] W. J. HAINES, Rec. Progr. Hormone Res. *7*, 255 (1952).

[1] F. W. KAHNT and A. WETTSTEIN, Helv. chim. Acta *34*, 1790 (1951). – F. W. KAHNT, CH. MEYSTRE, R. NEHER, E. VISCHER, and A. WETTSTEIN, Exper. *8*, 422 (1952).

[2] A. C. BROWNIE and J. K. GRANT, Biochem. J. *57*, 255 (1954).

[3] A. WETTSTEIN, F. W. KAHNT, and R. NEHER, Ciba Foundation Colloquia on Endocrinol. *8* (1954), in press.

Table II. Aerobic Incubation of Adrenal Homogenates (according to WETTSTEIN, KAHNT and NEHER)

Adrenals	Supplements: Nicotinamide (NA), Fumarate, Glucose		ATP in % of Adrenals	γ Corticoids per kg Adrenals: Aldosterone	Hydrocortisone	Corticosterone	Relative Yields: Aldosterone	Hydrocortisone	Corticosterone
Beef	no incubation		—	61	230	630	1	1	1
Beef	incubated		—	425	23,500	10,600	7	100	17
Beef	incubated		0·002	764	30,200	28,500	12·5	131	45
Beef	incubated		0·2	1,145	23,900	26,300	19	104	42
Beef	incubated	Cortexone 0·05 %	0·2	2,410	22,560	80,400	40	100	128
Beef	incubated (without NA)	Cortexone 0·05 %	0·2	3,360	3,760	53,000	55	16	84
Beef	incubated	Corticosterone 0·025%	0·2	190	13,650	—	3	60	—
Beef	incubated	Progesterone 0·08 %	0·04	<475	34,400	129,000	<8	150	205
Hog	no incubation		—	765	7,080	9,050	1	1	1
Hog	incubated		0·2	1,056	58,800	64,500	1·4	8·3	7·1

that of corticosterone to an absolute maximum of about 130 mg per kilo adrenals.

In general our experiments are in agreement with the schemes drawn up by HECHTER and by SAMUELS. They show furthermore that cortexone might be a precursor for aldosterone. In view of the results with progesterone, the 18-oxygenation should then occur after that in 21-position but, as the results with corticosterone indicate, before that in 11-position. The oxygenations leading to the formation of aldosterone would thus occur in the sequence 21-, 18-, and 11-position. This working hypothesis has to be confirmed, as said before, by tracer experiments and with purified enzymes.

Zusammenfassung

Aus den Arbeiten der letzten zwei Jahre auf dem Gebiet der Nebennierenrinden-Hormone stechen – was die Herstellung neuer, hochwirksamer Verbindungen anbetrifft – die Isolierung und Konstitutionsaufklärung des natürlichen Hormons Aldosteron und die synthetische Gewinnung der 9α-halogenierten Hydrocortisone hervor. Diese Ergebnisse haben den Weg zu weiteren wesentlichen Entwicklungen eines Gebietes geöffnet, das mit Cortison und Hydrocortison abgeschlossen schien.

Auf dem Gebiete der Teilsynthese wurde die Herstellung von 11-oxygenierten aus 12-oxygenierten Derivaten und insbesondere aus Verbindungen ohne Sauerstoff im Ring C stark ausgebaut. Bei der letzteren liegen die wichtigsten Befunde wohl in den mikrobiologischen Hydroxylierungen in 11-Stellung und den anschliessenden chemischen Umwandlungen. Erst kürzlich ist es möglich geworden, auch die typischen Seitenketten durch mikrobiologische Methoden zu gewinnen.

Die Totalsynthese der Hormone aus einfachsten chemischen Bausteinen wurde entwickelt und macht rasche Forschritte. Die Auffassungen über die Biosynthese haben konkretere Formen angenommen. Die unzähligen Arbeiten über Katabolismus und klinische Verwendung der Nebennierenrinden-Hormone konnten im Rahmen dieser Übersicht nicht einmal gestreift werden.