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Conversion of Steroids by Microorganisms[1]

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To begin with, reference should be made to the existing recent and very valuable *reviews* on microbiological reactions in the steroid field, e.g. the appropriate chapter in DORFMAN and UNGAR's monograph[3], the papers by PETERSON[4], FLOREY[5], FINCH[6], and HANČ[7], and – last but not least – by FRIED *et al.*[8]. Furthermore, PERLMAN[9] delivered at the same meeting a lecture on the entire field of the modification of organic compounds by microorganisms. I shall not try, therefore, to give you another complete review but rather to present the actual position of microbiological processes, a field which is still in full development.

We cannot here go into the normal origin of sterols in natural metabolism, for example ergosterol and related substances occuring in ergot and yeast. We have to limit ourselves to the *transformation of steroids added to microorganisms* by means of the enzymes contained in the latter. One should realize, therefore, that we are dealing with artificial, more or less unphysiological systems that have nevertheless acquired great importance for preparative purposes. In particular, they permit the introduction of functional groups typical of the steroid hormones, and crucial for the biological effect, into natural or synthetic starting materials, i.e. the substrates. They also enable us to prepare analogs of the natural hormones, which may lead to interesting conclusions about the relation of chemical constitution and physiological activity.

The *method* of microbiological conversion has to compete with the chemical synthetic methods, and with the conversion by animal tissue enzymes. In comparison with pure chemistry, microbiology can, in certain cases, be employed very economically, since it often allows the performance in a single but rather complicated process of one or more changes in the molecule for which quite a number of chemical reactions are required. Compared with the use of animal tissue enzymes, that is to say perfusion of intact organs *in situ* or *in vitro*, and incubation of tissue slices, homogenates or fractions thereof, the microbiological method has great advantages, but also some disadvantages. It is much simpler practically, but not physiologically specific in its results. One will therefore have recourse to animal tissue enzymes for the elucidation of problems of natural biosynthesis in the organism, while for the *preparation* of hormones and especially their analogs, it is advantageous to use, whenever possible, enzymes of microbiological origin.

Our knowledge concerning the general *reaction mechanism* of microbiological conversions is still very meagre. It may be assumed, in analogy with the current conception of enzymatic reactions, that in this case too the attacking enzyme of protein character forms intermediate complexes with the steroidal substrates. The shape of the surface of the substrate molecule is thus of primary importance, such that it allows contact at three points at least, as required by the polyaffinity theory. The linkages may consist in anything from normal valence or coordination bonds, hydrogen bonds, to such as caused by van der Waal's forces. For a sufficiently close approach so that the latter can operate, the large almost planar rear side of the steroid molecule ougth to be especially suitable. This could explain the somewhat preferential attack of the microorganism enzymes in the α-positions, although the adrenal enzymes attack primarily in the β-positions. The chemical activation of the reactive bond, e.g. by its being allyl to a carbon-carbon double bond or vicinal to a carbonyl group, seems to be only of secondary importance. Because of this, a distinct though limited substrate specificity is exhibited. Certain substituents may sterically hinder the microbiological reaction, or at least influence its steric course.

The *principle of operation* of the microbiological conversions of steroids derives from known methods of mold fermentation. These are the surface culture processes, and, especially, submerged fermentations in shaking flasks or, on an industrial scale, in deep culture fermenters with aeration and stirring as used

[1] From a lecture at the 128th National Meeting of the American Chemical Society, Minneapolis, Minn.; Symposium on Metabolic Conversions of Steroids; September 16, 1955. Communication No. 136 "On Steroids". No. 135 compare E. VISCHER, J. SCHMIDLIN, and A. WETTSTEIN, Exper. (in press).

[2] Research Laboratories, CIBA Ltd., Basle, Switzerland.

[3] R. I. DORFMAN and F. UNGAR, *Metabolism of Steroid Hormones* (Minneapolis, 1953), p. 34.

[4] D. H. PETERSON, Research *6*, 309 (1953). – D. H. PETERSON in: S. A. WAKSMAN, *Perspectives and Horizons in Microbiology* (New Brunswick, N. J., 1955), p. 121.

[5] K. FLOREY, Chimia *8*, 81 (1954).

[6] C. A. FINCH, Manufact. Chemist *25*, 247, 548 (1954): *26*, 120 (1955).

[7] O. HANČ and E. RIEDL-TUMOVÁ, Pharmazie *9*, 877 (1954).

[8] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 149 (1955).

[9] D. PERLMAN, Conference 128th Nat. Meet. Amer. Chem. Soc., Minneapolis, Minn., September 14, 1955.

in the manufacture of antibiotics, riboflavin, citric acid etc.

In CIBA's pilot plant just coming into operation in Basle we are using a series of 10 gallon stainless steel fermenters (Figure 1) with built in partly automatic and registering controls of experimental conditions (temperature, pH, air-flow, etc.). Larger tanks (100 gallons and 1000 gallons) have also been installed, the design being such that by means of the smallest tanks (Figure 2, on the gallery, upper right), the middle-sized ones and from these the large tanks can be directly inoculated in sterile conduits by gravity-feed, maximal sterile functioning being of the greatest importance.

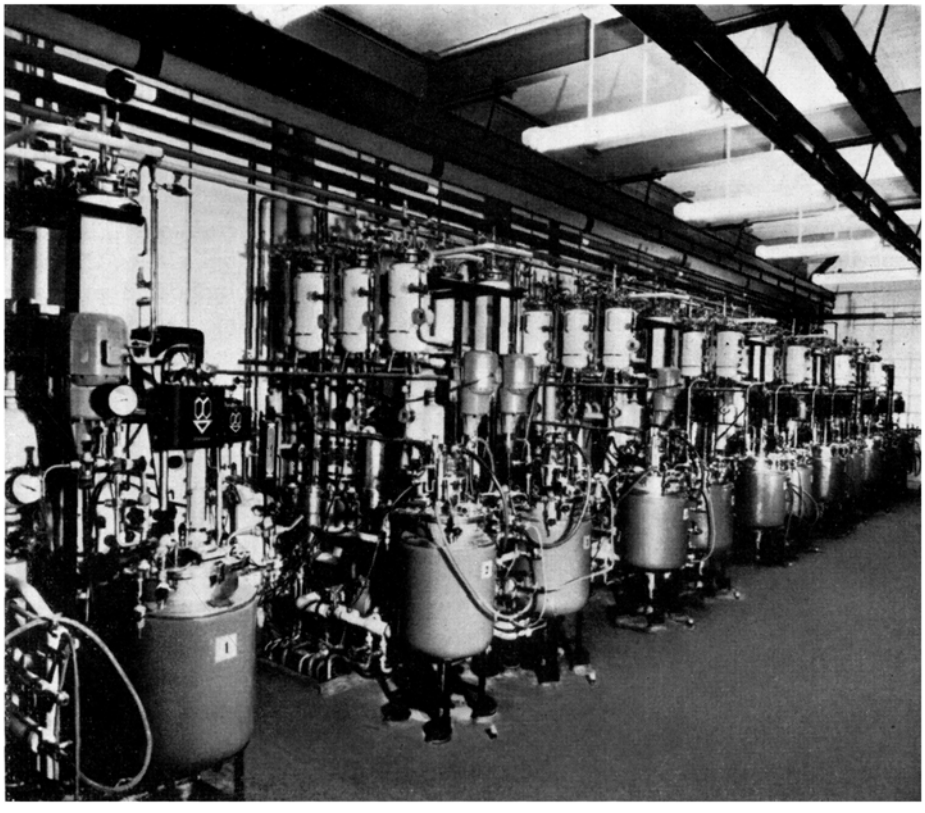


Fig. 1.

The *operational procedure* is briefly as follows: inoculation cultures of the microorganisms, mostly *Phycomycetes*, *Fungi imperfecti* or bacteria are prepared, and inoculated into a suitable sterile nutrient solution.

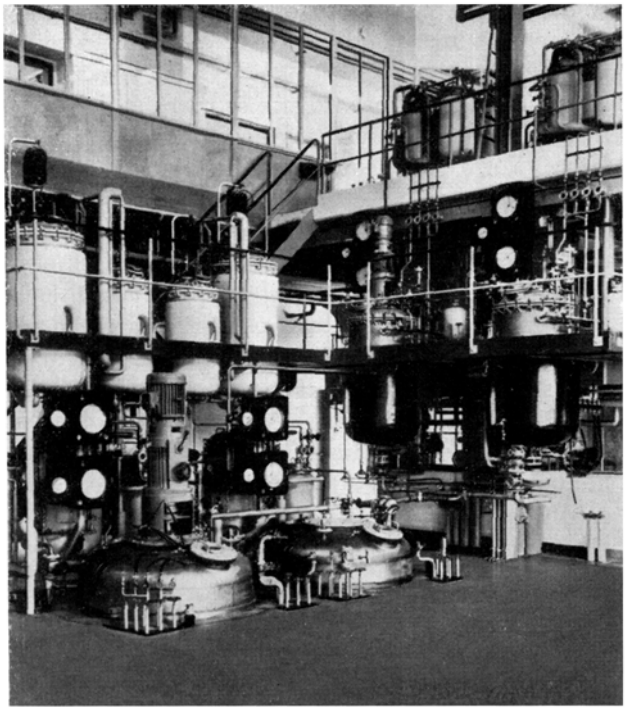


Fig. 2.

After 1–2 days' development of the organisms, a solution or a very fine suspension of the steroid precursor in a water-miscible solvent such as acetone, alcohol or dioxane is added under sterile conditions. The mixture is now incubated aerobically for 1–10 days, the mycelium removed by filtration, and the solution and/or the mycelium extracted with an organic solvent. Recently, ROLAND and WEINER[1] and our group[2] have also used *Basidiomycetes* – fungi in common language – with submerged fermentation conditions according to HUMFELD[3]. Cell-free enzyme solutions from microorganisms have only been used exceptionally (in the patent literature and by STADTMAN *et al.*[4] working with *Mycobacteria*); such experiments are interesting because they should permit the purification and finally the isolation of the enzyme systems (see also TALALAY *et al.*[5]). Conversely, it has been found advantageous to filter the mycelium from the microorganism

[1] J. F. ROLAND jr. and B. A. WEINER, Science *121*, 803 (1955).

[2] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, unpublished work.

[3] H. HUMFELD, Science *107*, 373 (1948). – H. HUMFELD and T. F. SUGIHARA, Mycologia *44*, 605 (1952).

[4] T. C. STADTMAN, A. CHERKES, and CH. B. ANFINSEN, J. biol. Chem. *206*, 511 (1954).

[5] P. TALALAY, M. M. DOBSON, and D. F. TAPLEY, Nature *170*, 620 (1952). – P. TALALAY and PH. I. MARCUS, Nature *173*, 1189 (1954). – P. TALALAY and M. M. DOBSON, J. biol. Chem. *205*, 823 (1953). – P. TALALAY, F. A. LOEWUS, and B. VENNESLAND, J. biol. Chem. *212*, 801 (1955).

after its development, transfer it to a new nutrient or to water, and only then to incubate it with the steroid (WELSH and HEUSGHEM[1], and lately SHULL and KITA[2]). The amount of the added steroid (mostly not over 1 g per l) is naturally of significance; higher concentrations, desirable from an economic point of view, hardly suit the solubility conditions, and can, as in the case of the bile acids, retard the growth of the microorganisms. Authors of the UPJOHN group have been able to improve biological conversions very much by the addition of alcohols[3] or of certain fatty acid ester compounds[4]. Recently, the addition of zinc ions also proved advantageous[5].

The onset and nature of the transformation is followed throughout by means of paper chromatography, for example according to ZAFFARONI or BUSH. Besides the preparation of derivatives, physical methods such as IR. and UV. spectra (the latter also after the action of H_2SO_4), as well as the molecular rotation differences serve to identify and prove the constitution of the compounds isolated. Paper chromatographic and IR. measurements may be carried out, if necessary, with amounts down to 1 or 5 μg.

The *individual reactions* carried out on steroids by means of microorganisms and dealt with in this talk are illustrated in Table I. The dehydrogenation to C=C double bonds will be considered in conjunction with side chain degradations and ring cleavage to lactones, since these reactions are frequently effected by the same microorganisms.

Chronologically speaking (Table II), the first of these microbiological transformations of steroids, apart from hydrolyses, were oxido-reductions, carried out in 1937 by MAMOLI and VERCELLONE. In 1947 and 1948 followed the first microbiological side chain degradations and in 1949 a hydroxylation in the 7-position. In 1952 PETERSON and MURRAY made the extremely important discovery of hydroxylation in the 11α-position. Shortly afterwards a spate of papers appeared.

Table I. — Conversion of steroids added to microorganisms

Hydrolysis:	of esters, of ethers
Hydrogenation:	of oxo-compounds, of $\overset{|}{C}=C\overset{|}{}$
Dehydrogenation:	of secondary alcohols, of $\diagdown CH-CH\diagup$
Hydroxylation:	of $-CH_2-$, $-CH_3$, $\diagup CH-$
Epoxydation:	of $C=C$
Side-chain degradation:	of $-CO-CH_3$, $-CO-CH_2OH$
Ring cleavage to lactones	

Hydrolysis

The *hydrolysis* of steroid esters or ethers cannot be discussed here in detail. In this context belong, for example, the saponification of ketol-21-acetates and 3-acetates, occuring nearly always during other transformations, the cleavage of estrogen sulfates, steroid glucuronidates and glycosides such as the cardiac active principles and the saponins.

Hydrogenation

Hydrogenations too belong to the common side-reactions during other microbiological processes, even in the presence of oxygen, and are naturally often coupled with dehydrogenation reactions. Some examples of reductions of *ketones and aldehydes* are given in Table III. The complete classical work of MAMOLI, VERCELLONE, ERCOLI and others is amply dealt with in the existing reviews. According to their findings, yeast will not in general reduce the $\alpha\beta$-unsaturated 3-keto-group (Δ^4-3-ketones). Androstane-3,17-dione

Table II

Year	Authors	Conversion	Microorganism
1937	MAMOLI and VERCELLONE[1]	oxido-reductions	yeast and bacteria
1947	HORVÁTH and KRÁMLI[2]	Side-chain degradation	*Azotobacter*
1948	TURFITT[3]	Side-chain degradation	*Proactinomyces*
1949	KRÁMLI and HORVÁTH[4]	Hydroxylation in 7	*Proactinomyces*
1952	PETERSON and MURRAY[5]	Hydroxylation in 11α	*Rhizopus*
1952	PERLMAN, TITUS and FRIED[6]	Hydroxylation in 16α	*Actinomyces*
1952	many additional papers		

[1] M. WELSH and C. HEUSGHEM, C. r. Soc. biol. *142*, 1074 (1948).
[2] G. M. SHULL and D. A. KITA, J. Amer. chem. Soc. *77*, 763 (1955).
[3] K. M. MANN, F. R. HANSON, P. W. O'CONNELL, H. V. ANDERSON, M. P. BRUNNER, and J. N. KARNEMAAT, J. appl. Microbiol. *3*, 14 (1955).
[4] P. W. O'CONNELL, K. M. MANN, E. D. NIELSON, and F. R. HANSON, J. appl. Microbiol. *3*, 16 (1955).
[5] E. L. DULANEY, E. O. STAPLEY, and CH. HLAVAC, Mycologia *47*, 464 (1955).

[1] L. MAMOLI and A. VERCELLONE, Z. physiol. Chem. *245*, 93 (1937); Ber. dtsch. chem. Ges. *70*, 470, 2079 (1937).
[2] J. HORVÁTH and A. KRÁMLI, Nature *160*, 639 (1947).
[3] G. E. TURFITT, Biochem. J. *42*, 376 (1948).
[4] A. KRÁMLI and J. HORVÁTH, Nature *162*, 619 (1948); *163*, 219 (1949).
[5] D. H. PETERSON and H. C. MURRAY, J. Amer. chem. Soc. *74*, 1871 (1952).
[6] D. PERLMAN, E. TITUS, and E. FRIED, J. Amer. chem. Soc. *74*, 2126 (1952).

Table III. — Reductions of Ketones and Aldehydes

Substrate		Product	Microorganism	Authors
3-CO:	androstane-3,17-dione	3β,17β-diol	yeast	MAMOLI and VERCELLONE[1]
	androstane-3,17-dione	3α-ol	*Pseudomonas*	TALALAY *et al.*[2]
	pregnane-3,20-dione	unchanged	yeast	MAMOLI[3]
	allopregnane-3,20-dione . . .	unchanged	yeast	MAMOLI[3]
	pregnane-3,11,20-trione . . .	3α-ol	yeast	CAMERINO *et al.*[4]
	allopregnane-3,11,20-trione. .	3α-ol	yeast	CAMERINO *et al.*[4]
	pregnane-11α-ol-3,20-dione . .	unchanged	yeast	CAMERINO *et al.*[4]
	allopregnane-11α-ol-3,20-dione	3β-ol	yeast	CAMERINO *et al.*[4]
	3-keto-cholanic acid	unchanged	yeast	ERCOLI and DE RUGGIERI[5]
	3,6-diketo-cholanic acid . . .	3α-ol	yeast	ERCOLI and DE RUGGIERI[5]
7-CO:	dehydrocholic acid	7-ol (slow!)	B. coli	FUKUI[6]
17-CO:	dehydroisoandrosterone . . .	17β-ol	yeast	MAMOLI and VERCELLONE[1]
	dehydroisoandrosterone . . .	17β-ol	*Pseudomonas*	TALALAY *et al.*[2]
	Δ^4-androstene-3,17-dione. . .	17β-ol	yeast	MAMOLI and VERCELLONE[7]
	Δ^4-androstene-3,17-dione. . .	17β-ol	*Pseudomonas*	TALALAY *et al.*[2]
	Δ^1-androstene-3,17-dione. . .	17β-ol	yeast	BUTENANDT[8]
	estrone	17β-ol	yeast	WETTSTEIN[9]
	estrone-acetate	17β-ol	yeast	MAMOLI[10]
20-CO:	progesterone	20β-ol	*Streptomyces lavendulae*	FRIED *et al.*[11]
	17α-hydroxy-cortexone . . .	20β-ol	*Streptomyces coelicolor*	VISCHER *et al.*[12]
22-CHO:	Δ^4-bisnorcholene-3-one-22-al .	22-ol (+11α-hydroxy) (+6β,11α-dihydroxy)	*Rhizopus nigricans*	MEISTER *et al.*[13]

[1] L. MAMOLI and A. VERCELLONE, Z. physiol. Chem. *245*, 93 (1937); Ber. dtsch. chem. Ges. *70*, 470, 2079 (1937). – L. MAMOLI, Ber. dtsch. chem. Ges. *71*, 2278 (1938).

[2] P. TALALAY, M. M. DOBSON, and D. F. TAPLEY, Nature *170*, 620 (1952). – P. TALALAY and PH. I. MARCUS, Nature *173*, 1189 (1954). – P. TALALAY and M. M. DOBSON, J. biol. Chem. *205*, 823 (1953). – P. TALALAY, F. A. LOEWUS, and B. VENNESLAND, J. biol. Chem. *212*, 801 (1955).

[3] L. MAMOLI, Ber. dtsch. chem. Ges. *71*, 2701 (1938).

[4] B. CAMERINO, C. G. ALBERTI, and A. VERCELLONE, Helv. chim. Acta *36*, 1945 (1953).

[5] A. ERCOLI and P. DE RUGGIERI, Boll. Soc. Ital. Biol. Sper. *28*, 611 (1952).

[6] T. FUKUI, J. Biochem. (Tokyo) *25*, 61 (1937).

[7] L. MAMOLI and A. VERCELLONE, Ber. dtsch. chem. Ges. *70*, 470 (1937).

[8] A. BUTENANDT and H. DANNENBERG, Ber. dtsch. chem. Ges. *71*, 1681 (1938).

[9] A. WETTSTEIN, Helv. chim. Acta *22*, 250 (1939).

[10] L. MAMOLI, Ber. dtsch. chem. Ges. *71*, 2696 (1938).

[11] J. FRIED, R. W. THOMA, and A. KLINGSBERG, J. Amer. chem. Soc. *75*, 5764 (1953).

[12] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, unpublished work.

[13] P. D. MEISTER, D. H. PETERSON, S. H. EPPSTEIN, H. C. MURRAY, L. M. REINEKE, A. WEINTRAUB, and H. M. LEIGH OSBORN, J. Amer. chem. Soc. *76*, 5679 (1954).

gives the 3β, 17β-diol. TALALAY concentrated and studied more thoroughly the dehydrogenase systems of a *Pseudomonas* species that act with diphosphopyridine nucleotide. He demonstrated, amongst other conversions, the reversible change of androstane-dione to androsterone (3α-ol), but established also a 3β-dehydrogenase. In the pregnane series, other than in chemical reduction, it is not the configuration at C-5 that determines the occurrence and course of reduction with yeast at the 3-carbonyl, but the presence and nature of a substituent in the 11-position; thus, without an 11-substituent no reduction takes place, in the presence of an 11-keto-group reduction to the 3α-hydroxy-group occurs, and with an 11β-hydroxy group, reduction, if occurring, gives the 3β. Hydroxyl groups in the 17α and/or the 21 positions hinder reduction[1].

The 17-keto group is reduced every time to the 17β-hydroxyl group. In this way, for example the hormones testosterone and estradiol are obtained by selective hydrogenation.

FRIED *et al.* have observed reduction of the 20-carbonyl group to a 20β-hydroxyl group with a *Streptomyces* species as a side-reaction. In the case of REICHSTEIN's substance S, we obtained this conversion specifically as the main reaction.

Hydrogenation of an aldehyde group was arrived at in the course of 11α- and 6β,11α-hydroxylation with *Rhizopus*.

From the earlier examples of hydrogenations of a *carbon-carbon double bond* in conjugation with a keto-group (Table IV), I should just like to mention the transformation of androstenedione and testosterone to the 5β-dihydro-compounds. The hydrogenation of progesterone to the 5β- or 5α-dihydro-derivative was observed as a side-reaction in connection with hydroxylation in the 16α- or 11α-position. The saturation of the 16-double bond during the 11α-hydroxylation, forming the 17α-progesterone derivative, is theoretically interesting, in that a side-chain in the sterically "unnatural" configuration is obtained.

[1] B. CAMERINO, C. G. ALBERTI, and A. VERCELLONE, Helv. chim. Acta *36*, 1945 (1953).

Tabelle IV. — Hydrogenations of $\diagdown C{=}C \diagup$

Substrate	Product	Microorganism	Authors
$\Delta^{4,5}$: Δ^4-androstene-3,17-dione	testane-3,17-dione	*Clostridium*	ERCOLI and MAMOLI[1]
testosterone	testane-17β-ol-3-one	*Clostridium*	MAMOLI *et al.*[2]
progesterone	pregnane-dione-16α-ol	*Actinomycete*	PERLMAN *et al.*[3]
progesterone	allopregnane-dione-11α-ol	*Rhizopus nigricans*	PETERSON *et al.*[4]
$\Delta^{16,17}$: 16-dehydro-progesterone	17α-progesterone-11α-ol	*Rhizopus nigricans*	MEISTER *et al.*[5]

[1] A. ERCOLI and L. MAMOLI, Ber. dtsch. chem. Ges. *71*, 156 (1938).
[2] L. MAMOLI, R. KOCH, and H. TESCHEN, Z. physiol. Chem. *261*, 287 (1939).
[3] D. PERLMAN, E. TITUS, and E. FRIED, J. Amer. chem. Soc. *74*, 2126 (1952).
[4] 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).
[5] 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).

Table V. — Dehydrogenations of Secondary Alcohols

Substrate	Product	Microorganism	Authors
3-CH(OH)– cholesterol	Δ^4-3-ketone	*Proactinomyces roseus*	KRÁMLI and HORVÁTH[1]
cholesterol	Δ^4-3-ketone	*Mycobacterium* (cell-free extract!)	STADTMAN *et al.*[2]
coprosterol	3-ketone	*Proactinomyces erythropolis*	TURFITT[3]
dehydroisoandrosterone .	Δ^4-3-ketone	*Corynebacterium mediolanum*	MAMOLI and VERCELLONE[4]
dehydroisoandrosterone .	Δ^4-3-ketone	*Pseudomonas*	TALALAY *et al.*[5]
dehydroisoandrosterone .	Δ^4-3-ketone	*Proactinomyces erythropolis*	TURFITT[3]
Δ^5-androstene-3β,17β-diol	Δ^4-3-ketone	*Proactinomyces erythropolis*	TURFITT[3]
Δ^5-androstene-3β,17β-diol	Δ^4-3-ketone	*Flavobacterium dehydrogenans*	ERCOLI[6]
17-methyl-androstene-diol	Δ^4-3-ketone	*Corynebacterium mediolanum*	MAMOLI[7]
17-ethinyl-androstene-diol	Δ^4-3-ketone	*Micrococcus dehydrogenans*	ERCOLI[8]
pregnenolone	Δ^4-3-ketone	*Corynebacterium mediolanum*	MAMOLI[9]
pregnenolone	Δ^4-3-ketone	*Streptomyces*	PERLMAN[10]
21-acetoxy-pregnenolone	cortexone	*Corynebacterium mediolanum*	MAMOLI[11]
3+17-CH(OH)– Δ^5-androstene-3β,17β-diol	androstene-dione	*Corynebacterium mediolanum*	MAMOLI and VERCELLONE[4]
17-CH(OH)– Δ^5-androstene-3β,17β-diol	dehydroisoandrosterone	*Pseudomonas*	TALALAY *et al.*[5]
testosterone	androstene-dione	*Pseudomonas*	TALALAY *et al.*[5]
estradiol	estrone	*Micrococcus dehydrogenans*	ARNAUDI[12]
estradiol	estrone	*Actinomyces albus* (mycelium!)	WELSH and HEUSGHEM[13]
3+7+12-CH(OH)– cholic acid	triketocholanic acid	*Alcaligenes faecalis*	HOEHN *et al.*[14]
HO, OH, OH; 17 CH–CH$_2$ (20, 21)	4 possible stereoisomers	unchanged	*Acetobacter suboxydans*	LARDON and REICHSTEIN[15]

[1] A. KRÁMLI and J. HORVÁTH, Nature *162*, 619 (1948); *163*, 219 (1949).
[2] T. C. STADTMAN, A. CHERKES, and CH. B. ANFINSEN, J. biol. Chem. *206*, 511 (1954).
[3] G. E. TURFITT, Biochem. J. *40*, 79 (1946).
[4] L. MAMOLI and A. VERCELLONE, Ber. dtsch. chem. Ges. *71*, 154, 1686 (1938).
[5] P. TALALAY, M. M. DOBSON, and D. F. TAPLEY, Nature *170*, 620 (1952). – P. TALALAY and PH. I. MARCUS, Nature *173*, 1189 (1954). – P. TALALAY and M. M. DOBSON, J. biol. Chem. *205*, 823 (1953). – P. TALALAY, F. A. LOEWUS, and B. VENNESLAND, J. biol. Chem. *212*, 801 (1955).
[6] A. ERCOLI, Z. physiol. Chem. *270*, 266 (1941).
[7] L. MAMOLI, Gazz. chim. Ital. *69*, 237 (1939).
[8] A. ERCOLI, Biochem. Terap. sper. *28*, 125 (1941).
[9] L. MAMOLI, Ber. dtsch. chem. Ges. *71*, 2701 (1938).
[10] D. PERLMAN, Science *115*, 529 (1952).
[11] L. MAMOLI, Ber. dtsch. chem. Ges. *72*, 1863 (1939).
[12] C. ARNAUDI, Boll. Istit. Sieroterap. Milanese *21*, 1 (1942).
[13] M. WELSH and C. HEUSGHEM, C. r. Soc. biol. *142*, 1074 (1948).
[14] W. M. HOEHN, L. H. SCHMIDT, and H. B. HUGHES, J. biol. Chem. *152*, 59 (1944).
[15] A. LARDON and T. REICHSTEIN, Helv. chim. Acta *34*, 760 (1951).

Dehydrogenation

Concerning *oxidations*, only certain functional conversions are within the scope of this talk, not the total oxidative degradation, which occurs in the sterols generally by the opening of ring A to a ketoacid and by side-chain degradation[1]. Here, I shall only touch on the much-observed dehydrogenation of sec-

[1] G. E. TURFITT, Biochem. J. *42*, 376 (1948).

ondary alcohols to ketones by aerobic incubation (Table V). The first of these reactions, apart from the oxidation of coprosterol, represent the familiar transformation of a Δ^5-3β-hydroxy-compound to a Δ^4-3-ketone, which is normally carried out chemically by an Oppenauer oxidation. Especially worth mentioning to-day is probably only the partial dehydrogenation of androstenediol to testosterone, besides the use of cell-free extracts of a *Mycobacterium* by STADTMAN referred to previously. One of these transformations represents an example of the simultaneous hydrolysis of a 21-acetate. Several dehydrogenations of secondary carbinols in other positions of the steroid nucleus are shown also in Table V. Under this heading come again TALALAY's reversible interconversions with the DPN-linked dehydrogenases from *Pseudomonas*. The conversion of estradiol into estrone was carried out by WELSH in a remarkable way with filtered mycelium of an *Actinomyces*. During the dehydrogenation of cholic acid, HOEHN *et al.* determined long ago that first the 7-, then the 12-, and lastly the 3-hydroxyl reacted, the same sequence as observed during the chemical oxidation.

A theoretically interesting oxidation experiment was carried out by LARDON and REICHSTEIN. They subjected to the action of *Acetobacter suboxidans* the four possible stereoisomeric allopregnane- and 17α-allopregnane-3β,17,20,21-tetrols, the partial formulas of which are shown. It was surprising that all four remained practically unchanged, none of them giving the desired dihydroxyacetone side chain structure by oxidation at carbon atom 20. This negative result probably is caused by the fact, that the steroid tetrols, in contrast to the oxidizable sugar alcohols, have no hydrogen atom on the third carbon atom, i.e. in position 17.

Hydroxylation

The biological *hydroxylations* which have been observed using adrenals or adrenal preparations are compared with those which have been effected by microorganisms in Figure 3. Of the former, only this year a 6α-hydroxylase has been found by MEYER *et al.*[1],

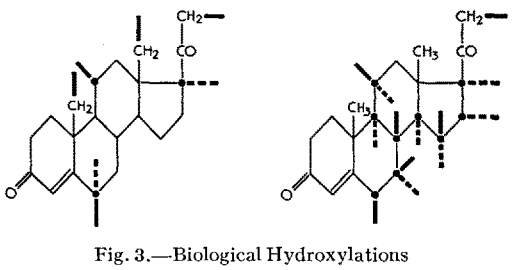


Fig. 3.—Biological Hydroxylations

Adrenals:	Microorganisms:
6α, 6β, 11β, 17α, 18, 19, 21	6β, 7α, 7β, 8β or 9α, 14α, 15α, 15β, 16α, 11α, 11β, 17α, 21

[1] A. S. MEYER, M. HAYANO, M. C. LINDBERG, M. GUT, and O. G. RODGERS, Acta endocrinol. *18*, 150 (1955).

an 18-hydroxylase by us[1], which is interesting in connection with the biosynthesis of aldosterone, and a 19-hydroxylase, which attacks the other angular methyl group, by no less than four teams, namely, besides us[1], by MEYER[2], by HAYANO and DORFMAN[3], by ZAFFARONI *et al.*[4], and by LEVY and KUSHINSKY[5]. The three "natural" points of attack named are, at the same time, precisely those for which a microbiological method has not yet been found. Microorganisms produce, apart from the other hydroxylations effected also by adrenals, i.e. those in the 6β-, 11β-, 17α-, and 21-position, a whole galaxy of such reactions in other, "unnatural" positions. These are, up till now, the 7α-, 7β-, 8β- or 9α-, 14α-, 15α-, 15β-, 16α- and the very important 11α-position. This is an illustration of what was mentioned in the introduction, that a wide variety of possibilities exists with microbiological enzymes in comparison with those of animal tissues.

Microbiological hydroxylations have been extensively studied with progesterone and cortexone, but also with quite a number of other precursors. Most of them give in this way analogous conversions, but a certain substrate specificity is nevertheless apparent; thus dihydroxylated derivatives have been observed particularly with progesterone. Very little is yet known about the mechanism. Since FRIED *et al.*[6] could not bring about such transformations on 9,11-dehydro- or 16,17-dehydro-starting material with microorganisms hydroxylating other precursors in the 11α- or 16α-positions, these unsaturated compounds are ruled out as intermediate products (*cf.* also the microbiological epoxydation below). From the assumption that microbiological hydroxylations do not, on the whole, proceed *via* an intermediate double bond, STONE *et al.*[7] suggest that the hydroxyl replaces directly a hydrogen atom of the precursor, the hydroxyl then arising in the spatial position of the hydrogen.

Let us consider briefly hydroxylations in individual positions. Table VI shows some examples of a favored side reaction of many microbiological conversions, *6β-hydroxylation*. It always takes place on Δ^4-3-ketones (I), i.e. in the allyl position. In progesterone, FRIED *et al.* have described exclusive 6β-hydroxylation. The UPJOHN group found in *Rhizopus arrhizus* a micro-

[1] F. W. KAHNT, R. NEHER, and A. WETTSTEIN, Helv. chim. Acta *38*, 1237 (1955).

[2] A. S. MEYER, M. HAYANO, M. C. LINDBERG, M. GUT, and O. G. RODGERS, Acta endocrinol. *18*, 150 (1955). – A. S. MEYER, Exper. *11*, 99 (1955).

[3] M. HAYANO and R. I. DORFMAN, Arch. Biochem. Biophys. *55*, 289 (1955).

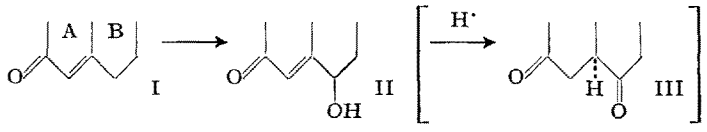
[4] A. ZAFFARONI, V. TRONCOSO, and M. GARCIA, Chem. Ind. *1955*, 534.

[5] H. LEVY and S. KUSHINSKY, Arch. Biochem. Biophys. *55*, 290 (1955).

[6] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 164 (1955).

[7] D. STONE, M. HAYANO, R. I. DORFMAN, O. HECHTER, C. R. ROBINSON, and C. DJERASSI, J. Amer. chem. Soc. *77*, 3926 (1955).

Table VI. — 6β-Hydroxylations



Substrate	Product hydroxylated in	Microorganism	Authors
progesterone	6β	*Streptomyces aureofaciens*	FRIED *et al.*[1]
progesterone	6β, 11α (+ much 11α)	*Rhizopus arrhizus*	PETERSON *et al.*[2]
progesterone	6β, 11α	*Rhizopus cambodjae*	CAMERINO *et al.*[3]
progesterone	6β, 11α	*Aspergillus niger*	FRIED *et al.*[4]
progesterone-17α-ol	6β	*Rhizopus arrhizus*	MEISTER *et al.*[5]
progesterone-21-ol	6β	*Rhizopus arrhizus*	EPPSTEIN *et al.*[6]
progesterone-21-ol	6β (+ much 17α)	*Trichothecium roseum*	MEYSTRE *et al.*[7]
progesterone-21-ol	6β	*Lenzites abietina* (*Basidiomycetes*)	VISCHER and WETTSTEIN[8]
progesterone-21-ol	6β, 17α	*Cephalothecium roseum*	MEISTER *et al.*[9]
progesterone-17α, 21-diol	6β	*Rhizopus arrhizus*	PETERSON *et al.*[10]
progesterone-17α, 21-diol	6β	*Helicostylum piriforme*	MEISTER *et al.*[11]
progesterone-16α-ol	6β	*Aspergillus nidulans*	FRIED *et al.*[1]
androstene-3, 17-dione	6β (+ 11α)	various *Rhizopus* sp.	EPPSTEIN *et al.*[12]
androstene-3, 17-dione	6β	*Aspergillus niger*	FRIED *et al.*[1]
testosterone	6β (+ 11α) (+ III)	various *Rhizopus* sp.	EPPSTEIN *et al.*[12]
testosterone-17α-methyl	6β (+ 11α)	various *Rhizopus* sp.	EPPSTEIN *et al.*[12]

[1] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 155 (1955).

[2] 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).

[3] B. CAMERINO, C. G. ALBERTI, A. VERCELLONE, and F. AMMANNATI, Gazz. chim. Ital. *84*, 301 (1954).

[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); for configuration compare L.F. FIESER, J. Amer. chem. Soc. *75*, 4378 (1953).

[5] 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).

[6] 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).

[7] CH. MEYSTRE, E. VISCHER, and A. WETTSTEIN, Helv. chim. Acta *37*, 1548 (1954).

[8] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, unpublished work.

[9] P. D. MEISTER, L. M. REINEKE, R. C. MEEKS, H. C. MURRAY, S. H. EPPSTEIN, H. M. LEIGH OSBORN, A. WEINTRAUB, and D. H. PETERSON, J. Amer. chem. Soc. *76*, 4050 (1954).

[10] D. H. PETERSON, S. A. 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).

[11] P. D. MEISTER, S. H. EPPSTEIN, D. H. PETERSON, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, Abstr. 123rd Meet. Amer. Chem. Soc., Los Angeles, March 1953, p. 5*c*.

[12] S. H. EPPSTEIN, P. D. MEISTER, H. M. LEIGH, D. H. PETERSON, H. C. MURRAY, L. M. REINEKE, and A. WEINTRAUB, J. Amer. chem. Soc. *76*, 3174 (1954).

organism which introduced a hydroxyl specifically in the 6β-position of progesterone derivatives already hydroxylated in the side chain. We have ourselves recently established the occurrence of such a reaction with a member of the *Basidiomycetes*. 6β-Hydroxylations have been performed also in the androstene series. Characteristic for the reaction products, the Δ^4-3-keto-6β-hydroxy steroids (II), is the UV. spectrum which exhibits a shift to lower wave-length and a decrease of the maximum absorption intensity. A further characteristic is the allylic rearrangement in mineral acid to the saturated 3,6-diketo-derivatives of the 5α-series (III), according to EHRENSTEIN[1]. In the case of 6β-hydroxy-testosterone this change has also been observed under the influence of the hydroxylating microorganism (EPPSTEIN *et al.*). Very interesting, biologically, are the discoveries of FIESER[2] concerning the ability of 6β-hydroxy-cholestenone, and, to a much greater extent, 6β-hydroperoxy-cholestenone, to cause fibrosarcomas when injected in oil into mice.

Only comparatively few observations on *hydroxylation* of carbon atoms 7, 8, and 9 are known (Table VII). The previously mentioned hydroxylation of KRÁMLI and HORVÁTH in the sterically undefined 7-position, the first hydroxylation of any kind, belongs here. The introduction of a 7α-hydroxyl was described recently by us, that of the 7β-hydroxyl into a saturated precursor some time ago. The 7-hydroxylation of MURRAY *et al.* observed at that time was, not long ago, assigned as attack also of the 7β-position. The 7-hydroxy group in Δ^4-3-ketones is very easily split off to form the $\Delta^{4;6}$-dienes.

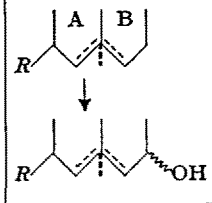
With hydroxylation in the *8- or 9-position*, we come for the first time to an attack on a tertiary carbon atom (Table VIII). An adequate constitutional and configurational elucidation of the resulting compounds is still not available, positions 8 or 9 being assigned on the whole by exclusion of other possibilities. From their reaction mechanism theory of microbiological

[1] P. T. HERZIG and M. EHRENSTEIN, J. org. Chem. *16*, 1050 (1951).

[2] L. F. FIESER *et al.*, J. Amer. chem. Soc. *75*, 4377, 4386, 4395 (1953); *77*, 3928 (1955).

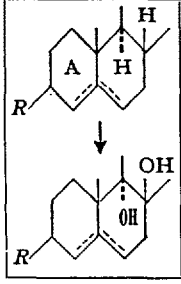
hydroxylations, STONE *et al.* have recently deduced the formulation 8β- or 9α- as the most probable, corresponding to the positions of the hydrogen atoms in the precursors.

Table VII. — Hydroxylations in 7-Position

Reaction	Substrate	Product hydroxylated in	Microorganism	Authors
	cholesterol	7ξ	*Proactinomyces roseus*	KRÁMLI and HORVÁTH[1]
	progesterone	7ξ (+15β)	*Phycomyces blakesleeanus*	FRIED *et al.*[2]
	cortexone	7α	*Peziza* and *Curvularia* sp.	MEYSTRE *et al.*[3]
	allopregnane-3β,21-diol-20-one	7β	various *Rhizopus* sp.	KAHNT *et al.*[4]
	allopregnane-3β-ol-20-one .	7β	*Rhizopus arrhizus*	MURRAY *et al.*[5]

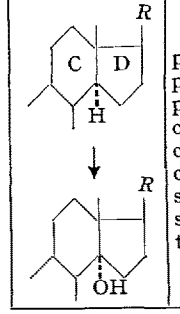
[1] A. KRÁMLI and J. HORVÁTH, Nature *162*, 619 (1948); *163*, 219 (1949).
[2] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 157 (1955).
[3] CH. MEYSTRE, E. VISCHER, and A. WETTSTEIN, Helv. chim. Acta *38*, 381 (1955).
[4] F. W. KAHNT, CH. MEYSTRE, R. NEHER, E. VISCHER, and A. WETTSTEIN, Exper. *8*, 422 (1952).
[5] H. C. MURRAY, H. CORNERS, and D. H. PETERSON, US. Pat. 2 602 769. – P. D. MEISTER, L. M. REINEKE, R. C. MEEKS, H. C. MURRAY, S. H. EPPSTEIN, H. M. LEIGH OSBORN, A. WEINTRAUB, and D. H. PETERSON, J. Amer. chem. Soc. *76*, 4050 (1954).

Table VIII. — Hydroxylations in 8- or 9-Position

Reaction	Substrate	Product hydroxylated in	Microorganism	Authors
	Δ⁵-pregnene-3β-ol-20-one .	8ξ, 11α (+3-one)	*Rhizopus arrhizus*	MURRAY *et al.*[1]
	progesterone	8ξ or 9ξ? (+6β)	*Streptomyces aureofaciens*	FRIED *et al.*[2]
	cortexone	8β?	*Curvularia pallescens*	VISCHER *et al.*[3]
	cortexone	8β? identical	*Mucor parasiticus*	MEISTER[4]
	cortexone	8β or 9α	*Neurospora crassa*	STONE *et al.*[5]
	substance S	8ξ (+11α, +14α)	*Helicostylum piriforme*	MURRAY *et al.*[1]

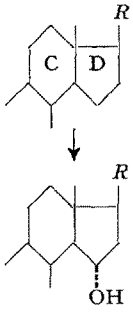
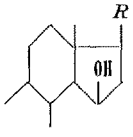
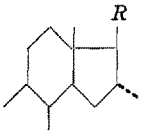
[1] H. C. MURRAY, H. CORNERS, and D. H. PETERSON, US. Pat. 2 602 769.
[2] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 156 (1955).
[3] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, unpublished work.
[4] P. D. MEISTER, cited in[5].
[5] D. STONE, M. HAYANO, R. I. DORFMAN, O. HECHTER, C. R. ROBINSON, and C. DJERASSI, J. Amer. chem. Soc. *77*, 3926 (1955).

Table IX. — Hydroxylations in 14α-Position

Reaction	Substrate	Product hydroxylated in	Microorganism	Authors
	progesterone	14α	various *Mucor* sp.	MEISTER *et al.*[1]
	progesterone	14α	*Bacillus cereus*	FRIED *et al.*[2]
	progesterone	14α, 6β	*Mucor corymbifer*	CAMERINO *et al.*[5]
	cortexone or acetate . .	14α	*Mucor griseocyanus*	MEISTER *et al.*[1]
	cortexone	14α	various *Curvularia* sp.	VISCHER *et al.*[3]
	cortexone or acetate . .	14α	*Helicostylum piriforme*	MEISTER *et al.*[1]
	substance S	14α (+11α, +6β, +8ξ)	*Helicostylum piriforme*	MEISTER *et al.*[1]
	substance S	14α, 11β	*Curvularia lunata*	AGNELLO *et al.*[4]
	testosterone	14α	*Mucor* sp.	MEISTER *et al.*[1]

[1] P. D. MEISTER, S. H. EPPSTEIN, D. H. PETERSON, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, Abstr. 123rd Meet. Amer. Chem. Soc., Los Angeles, March 1953, p. 5c.
[2] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 157 (1955).
[3] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, unpublished work.
[4] E. J. AGNELLO, B. L. BLOOM, and G. D. LAUBACH, J. Amer. chem. Soc. *77*, 4684 (1955). – G. M. SHULL and D. A. KITA, J. Amer. chem. Soc. *77*, 763 (1955).
[5] B. CAMERINO, C. G. ALBERTI, and A. VERCELLONE, Gazz. Chim. Ital. *83*, 684 (1953).

Table X. — Hydroxylations in 15α-, 15β- or 16α-Positions

Reactions	Substrate	Product hydroxylated in	Microorganism	Authors
	progesterone	15α	*Colletotrichum antirrhini*	FRIED *et al.*[1]
	cortexone	15α	*Gibberella baccata*	MEYSTRE *et al.*[2]
or				
	progesterone	15β (+7ξ)	*Phycomyces blakesleeanus*	FRIED *et al.*[1]
	cortexone	15β	*Lenzites abietina*	MEYSTRE *et al.*[2]
or	progesterone	16α (+5β-hydrogenation)	*Actinomyces* sp.	PERLMAN *et al.*[3]
	cortexone	16α	*Streptomyces* sp.	VISCHER *et al.*[4]
	cortexone	16α	*Didymella vodakii*	VISCHER *et al.*[5]
	cortexone	16α	*Streptomyces roseochromogenus*	FRIED *et al.*[6]
	Δ^4-androstene-3,17-dione	16α	*Streptomyces roseochromogenus*	FRIED *et al.*[6]
	testosterone	16α	*Streptomyces roseochromogenus*	FRIED *et al.*[6]

[1] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 157 (1955). – J. FRIED, R. W. THOMA, P. GRABOWICH, and J. R. GERKE, Chem. Ind., in press.

[2] CH. MEYSTRE, E. VISCHER, and A. WETTSTEIN, Helv. chim. Acta *38*, 381 (1955).

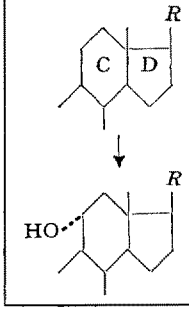
[3] D. PERLMAN, E. TITUS, and E. FRIED, J. Amer. chem. Soc. *74*, 2126 (1952). – D. PERLMAN, E. O'BRIEN, A. P. BAVAN, and R. B. GREENFIELD jr., J. Bact. *69*, 347 (1955).

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

[5] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, unpublished work.

[6] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 153 (1955).

Table XI. — Hydroxylations in 11α-Position

Reaction	Substrate	Product hydroxylated in	Yield	Microorganism	Authors
	progesterone	11α	10%	*Rhizopus arrhizus*	PETERSON and MURRAY[1]
	progesterone	11α	45%	*Rhizopus* sp.	MANCERA *et al.*[2]
	progesterone	11α	40%	*Rhizopus* sp.	KAHNT *et al.*[3]
	progesterone	11α	35%	*Aspergillus niger*	FRIED *et al.*[4]
	progesterone	11α	~95%	*Rhizopus nigricans*	PETERSON *et al.*[5]
	progesterone	11α	~82%	*Aspergillus ochraceus*	DULANEY *et al.*[6]
	Many examples with other precursors				

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

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

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

[6] E. L. DULANEY, E. O. STAPLEY, and C. HLAVAC, Mycologia *47*, 464 (1955).

A better supported tertiary hydroxylation is the one in the *14α-position* (Table IX), because the products may be identified by degradation to synthetic 14α-hydroxy-androstene-3,17-dione[1]. These conversions take place quite specifically, except for the one on Reichstein's substance S. The latter, hydroxylated in 14α- and 11β-position, shows activity as a glucocorticoid[1].

[1] A. F. ST. ANDRÉ, H. B. MCPHILLAMY, J. A. NELSON, A. C. SHABICA, and C. R. SCHOLZ, J. Amer. chem. Soc. *74*, 5506 (1952).

[1] E. J. AGNELLO, B. L. BLOOM, and G. D. LAUBACH, J. Amer. chem. Soc. *77*, 4684 (1955).

Hydroxylations in the *15α-* and *15β-positions* have been found independently by FRIED *et al.* and by us with progesterone and cortexone (Table X). In view of the evidence brought forward by the first group, the more easily esterified, more dextrorotatory compounds ought to be associated with the 15α-configuration, so that for *our* derivatives, the configurations now assigned in the Table result.

Progesterone was substituted in the *16α-position* by PERLMAN *et al.* as one of the first microbiological hydroxylations; cortexone has been similarly substituted by us. This reaction, previously carried out by *Actinomycetes*, we have now been able to effect by means of a *Didymella* species.

The hydroxylations in the 11-, 17α- and 21-positions about to be described are of interest particularly in view of these substituents being met with in the adrenal cortical hormones. The practical significance of the microbiological hydroxylation in the *11α- and 11β-positions*, especially in connection with the production of cortisone and hydrocortisone, cannot be overestimated. In this lecture, I shall have to limit myself to citing a few of the more important contributions (Table XI), and for the rest refer to the earlier reviews (especially FRIED *et al.*[1]). The merit of having been the first to describe an 11α-hydroxylation (by means of *Rhizopus* species) belongs to PETERSON and MURRAY[2]. Their U.S. Patent[3] is a mine of fundamental information, and their discovery has been developed along the most diverse ways by the UPJOHN group[4]. The initial yield of 10% obtained from progesterone with *Rhizopus arrhizus*[5], has been raised to about 40% by three other research groups using other *Rhizopus* species and *Aspergillus niger*, and finally has been brought up to nearly quantitative by PETERSON *et al.*[1] by means of *Rhizopus nigricans*.

Still more elegant than the introduction of the 11α-hydroxyl group, which occurs in the "unnatural" configuration when compared to adrenal hormones, and which must therefore be subsequently oxidized or converted to an *11β-hydroxyl group*, is the *direct* hydroxylation in the 11β-position (Table XII). COLINGSWORTH *et al.*[2], also with UPJOHN, realized this reaction for the first time in 1952 by the use of *Streptomyces fradiae* in a low percent yield. The same group succeeded in converting 35% of substance S into hydrocortisone using a *Cunninghamella* species. According to the latest results, as I already indicated, a yield of up to 64% of hydrocortisone may be obtained with addition of alcohol to the incubation mixture, and even up to 77% using a modified Czapek-Dox medium with dextrin and a monoglyceride of castor oil. This illustrates the possibilities of improving the yield merely by systematic variation of the culture medium. Recently SHULL and KITA (PFIZER) have described 11β-hydroxylation of various starting materials with a maximum yield of 41% using the watery suspension of a damp mycelium of *Curvularia lunata* obtained by filtration of the initial broth.

Hydroxylation of the important tertiary carbon atom 17, and indeed in the *17α-position* was published within a short interval by our group (MEYSTRE *et al.*[3]) as well as by MEISTER *et al.*[4] (Table XIII). It is an amusing coincidence that not only do the names of the authors in the first place both mean "master" though with different spelling, but that the designation of the different species used by them could also serve as synonyms, although the cultures showed a difference in their action on cortexone especially.

An interesting fact is that the presence of a 21-hydroxyl group in the starting materials does not prevent the microbiological introduction of the 17α-hydroxyl group, as it usually does in the adrenal enzyme reaction.

At the same time we[3] described the microbiological introduction, in high yield and quite specifically, of the *21-hydroxyl group*, a characteristic of all adrenal hormones. This was also carried out not long ago by another team[5] utilizing an apparently less specific species of the very versatile *Aspergillus niger*.

[1] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 160 (1955).

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

[3] H. C. MURRAY, H. CORNERS, and D. H. PETERSON, US. Pat. 2 602 769.

[4] 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). – 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). – 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). – D. H. PETERSON, S. A. 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). – 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). – 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). – S. H. EPPSTEIN, D. H. PETERSON, H. M. LEIGH, H. C. MURRAY, A. WEINTRAUB, L. M. REINEKE, and P. D. MEISTER, J. Amer. chem. Soc. *75*, 421 (1953). – S. H. EPPSTEIN, P. D. MEISTER, H. M. LEIGH, D. H. PETERSON, H. C. MURRAY, L. M. REINEKE, and A. WEINTRAUB, J. Amer. chem. Soc. *76*, 3174 (1954). – P. D. MEISTER, D. H. PETERSON, S. H. EPPSTEIN, H. C. MURRAY, L. M. REINEKE, A. WEINTRAUB, and H. M. LEIGH OSBORN, J. Amer. chem. Soc. *76*, 5679 (1954).

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

[1] 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).

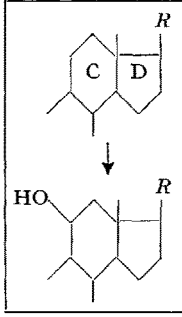
[2] D. R. COLINGSWORTH, M. P. BRUNNER, and W. J. HAINES, J. Amer. chem. Soc. *74*, 2381 (1952). – D. R. COLINGSWORTH, J. N. KARNEMAAT, F. R. HANSON, M. P. BRUNNER, K. M. MANN, and W. J. HAINES, J. biol. Chem. *203*, 807 (1953).

[3] CH. MEYSTRE, E. VISCHER, and A. WETTSTEIN, Helv. chim. Acta *37*, 1548 (1954).

[4] P. D. MEISTER, L. M. REINEKE, R. C. MEEKS, H. C. MURRAY, S. H. EPPSTEIN, H. M. L. OSBORN, A. WEINTRAUB, and D. H. PETERSON, J. Amer. chem. Soc. *76*, 4050 (1954).

[5] A. ZAFFARONI, C. C. CAMPILLO, F. CORDOBA, and G. ROSENKRANZ, Exper. *11*, 219 (1955).

Table XII. — Hydroxylations in 11β-Position

Reaction	Substrate	Product hydroxylated in	Yield	Microorganism	Authors
	cortexone	11β	few %	*Streptomyces fradiae*	COLINGSWORTH *et al.*[1]
	cortexone	11β (+ various products)	?	*Curvularia lunata*	SHULL and KITA[2]
	substance S	11β	few %	*Streptomyces fradiae*	COLINGSWORTH *et al.*[1]
	substance S	11β (+11 CO)	35%	*Cunninghamella blakesleeana*	HANSON *et al.*[3]
	substance S (+ethanol)	11β (+11 CO)	64%	*Cunninghamella blakesleeana*	MANN *et al.*[4]
	substance S (in modified Czapek-Dox)	11β (+11 CO)	77%	*Cunninghamella blakesleeana*	O'CONNELL *et al.*[5]
	substance S	11β	41%	*Curvularia lunata* (mycelium suspension)	SHULL and KITA[2]
	substance S	11β	?	*Coniothyrium* sp.	FRIED *et al.*[6]
	other conversions				SHULL and KITA[2]

[1] D. R. COLINGSWORTH, M. P. BRUNNER, and W. J. HAINES, J. Amer. chem. Soc. *74*, 2381 (1952). – 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).

[2] G. M. SHULL and D. A. KITA, J. Amer. chem. Soc. *77*, 763 (1955).

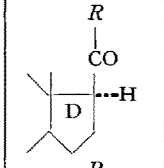
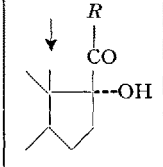
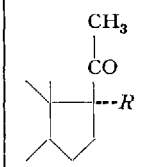
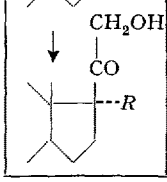
[3] F. R. HANSON, K. M. MANN, E. D. NIELSON, H. V. ANDERSON, M. P. BRUNNER, J. N. KARNEMAAT, D. R. COLINGSWORTH, and W. J. HAINES, J. Amer. chem. Soc. *75*, 5369 (1953).

[4] K. M. MANN, F. R. HANSON, P. W. O'CONNELL, H. V. ANDERSON, M. P. BRUNNER, and J. N. KARNEMAAT, J. appl. Microbiol. *3*, 14 (1955).

[5] P. W. O'CONNELL, K. M. MANN, E. D. NIELSON, and F. R. HANSON, J. appl. Microbiol. *3*, 16 (1955).

[6] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 164 (1955).

Table XIII. — Hydroxylations in 17α- or 21-Positions

Reactions	Substrate	Product hydroxylated in	Microorganism	Authors
	progesterone	17α, 11α	*Cephalothecium roseum*	MEISTER *et al.*[1]
	cortexone	17α (+6β)	*Trichothecium roseum*	MEYSTRE *et al.*[2]
	cortexone	17α, 11α+17α, 6β	*Cephalothecium roseum*	MEISTER *et al.*[1]
	corticosterone	17α (+11 CO)	*Trichothecium roseum*	MEYSTRE *et al.*[2]
	corticosterone	17α (+11 CO)	*Cephalothecium roseum*	MEISTER *et al.*[1]
	corticosterone-11-dehydro	17α	*Cephalothecium roseum*	MEISTER *et al.*[1]
	corticosterone-11-dehydro	17α	*Trichothecium roseum*	MEYSTRE *et al.*[2]
	progesterone	21	*Ophiobolus herpotrichus*	MEYSTRE *et al.*[2]
	progesterone	21	*Aspergillus niger*	ZAFFARONI *et al.*[3]
	progesterone-11-one	21	*Ophiobolus herpotrichus*	MEYSTRE *et al.*[2]
	progesterone-17α-ol	21	*Ophiobolus herpotrichus*	MEYSTRE *et al.*[2]
	cp. also other conversions		*Aspergillus niger*	ZAFFARONI *et al.*[3]

[1] P. D. MEISTER, L. M. REINEKE, R. C. MEEKS, H. C. MURRAY, S. H. EPPSTEIN, H. M. L. OSBORN, A. WEINTRAUB, and D. H. PETERSON, J. Amer. chem. Soc. *76*, 4050 (1954).

[2] CH. MEYSTRE, E. VISCHER, and A. WETTSTEIN, Helv. chim. Acta *37*, 1548 (1954).

[3] A. ZAFFARONI, C. C. CAMPILLO, F. CORDOBA, and G. ROSENKRANZ, Exper. *11*, 219 (1955).

By exclusive use of the microbiological hydroxylations it is now possible in principle, to obtain adrenal cortical hormones in relatively few steps. Thus one might, for instance, obtain from the readily available progesterone, hydrocortisone in three reactions, through 21-, 17α- and 11β-hydroxylation. Connected to the very smooth hydroxylation of progesterone in the 11α-position, a number of technically significant syntheses have been worked out for cortisone, hydrocortisone and its 9α-fluorinated derivative which we

cannot go into here (*cf.* amongst others WETTSTEIN and ANNER[1], FRIED *et al.*[2], FINCH[3], DJERASSI[4]).

Epoxydation

A theoretically most interesting transformation with certain fungi was announced recently for the first time by SHULL and BLOOM[5] at the International Congress of Biochemistry in Brussels, epoxidation of steroids containing an isolated double bond (Table XIV). 14-Dehydro- or 9,11-dehydro-substance S were incubated with a culture capable of introducing in corresponding saturated compounds an axial hydroxyl group at the site of the unsaturation. In this way the 14α, 15α- and 9β,11β-epoxy derivatives respectively were obtained, with none of the corresponding mono-hydroxylation products, let alone dihydroxylation products, e.g. such as were observed with aliphatic olefines (*cf.* PERLMAN[6]). This result shows once more that microbiological hydroxylation obviously does not go by way of these unsaturated compounds.

Side Chain Degradation and Dehydrogenation in Ring A

With this we come to the *side chain degradation* of steroids under the action of microorganisms. The very early work showing that sterols and especially cholesterol are degraded in the metabolism of *Mycobacteria, Proactinomycetes, Azotobacter, Flavobacteria* and even molds cannot be discussed here (*cf.* the surveys of HANČ[7] and ARNAUDI[8]), even moreso, since only quite exceptionally were intermediate products identified. TURFITT[1] was able, for instance, to isolate Δ^4-3-keto-etienic acid. Of greater practical interest was the announcement in 1953 of three groups of work (Table XV). We were able to show at that time[2], that by the action of *Fusarium* species, the acetyl and hydroxyacetyl side chain e.g. in progesterone and cortexone respectively (I) is degraded quite smoothly to the 17-ketone, when *dehydrogenation in ring A* to the $\Delta^{1;4}$-3-keto-diene (II) follows simultaneously. Both reactions took place, though more slowly, with pregnenolone (III), and with saturated pregnane-20-ketones (IV), in which the side chain is primarily degraded (V). In androstene-3,17-dione (VI) and in dehydroisoandrosterone where the side chain is lacking from the beginning, only dehydrogenation of ring A to the diene takes place. By prolonged action, a product of further oxidation (VII) instead of androstadienedione (II) was obtained from the unsaturated starting materials. When incubating 17α-hydroxy-20-ketopregnenes, like cortisone or substance S, we found that the side chain remained intact but even so a transformation occurred, which we shall come back to. Androstadiene-dione (II) is obtained in this way in only a single microbiological operation from progesterone, instead of the large number of reactions needed chemically. By aromatization according to INHOFFEN, this may be converted in only one step to estrone, which is therefore obtainable by quite an easy route.

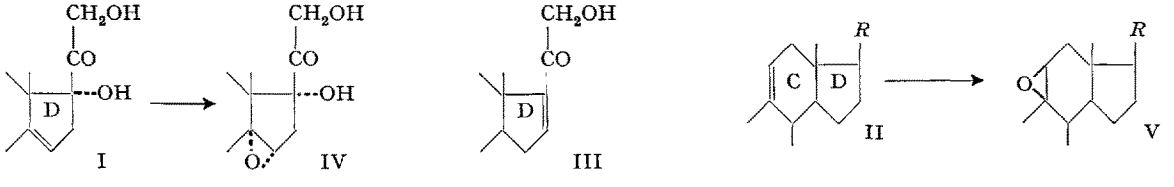
Shortly afterwards the teams of FRIED[3] and PETERSON[4], using other microorganisms, published independently results similar to ours but extending them considerably (Table XVI). FRIED also obtained in this manner from progesterone but in low yield the $\Delta^{1;4}$-

[1] A. WETTSTEIN and G. ANNER, Exper. *10*, 410 (1954).
[2] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 149 (1955).
[3] C. A. FINCH, Manufact. Chemist *25*, 247 (1954).
[4] C. DJERASSI, Vitamines and Hormones *11*, 230 (1953).
[5] G. M. SHULL and B. M. BLOOM, Communication 3rd Internat. Congress for Biochemistry, Brussels 1955, p. 48; J. Amer. chem. Soc. *77*, 5767 (1955).
[6] D. PERLMAN, Conference 128th Nat. Meet. Amer. Chem. Soc., Minneapolis, September 14, 1955.
[7] O. HANČ and E. RIEDL-TUMOVÁ, Pharmazie *9*, 877 (1954).
[8] C. ARNAUDI, J. appl. Microbiol. *2*, 274 (1954).

[1] G. E. TURFITT, Biochem. J. *42*, 376 (1948).
[2] E. VISCHER and A. WETTSTEIN, Exper. *9*, 371 (1953).
[3] J. FRIED, R. W. THOMA, and A. KLINGSBERG, J. Amer. chem. Soc. *75*, 5764 (1953).
[4] D. H. PETERSON, S. H. EPPSTEIN, P. D. MEISTER, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, J. Amer. chem. Soc. *75*, 5768 (1953).

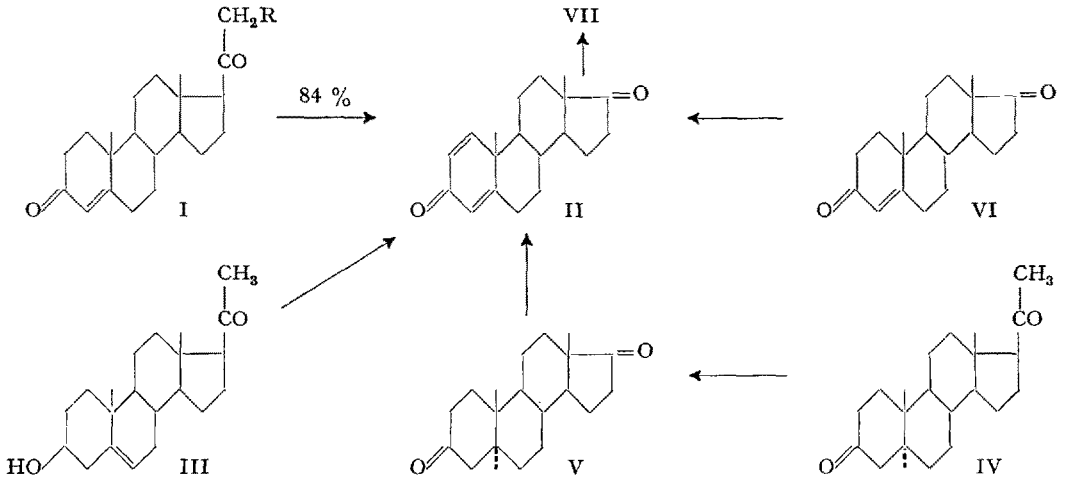
Table XIV. — Epoxydation

Substrate	Product	Microorganism	Authors
I substance S-14-dehydro	IV 14α, 15α-epoxido	*Curvularia lunata* and others	SHULL and BLOOM[1]
II substance S-9,11-dehydro	V 9β, 11β-epoxido	*Cunninghamella blakesleeana*	SHULL and BLOOM[1]
substance S-9,11-dehydro	V 9β, 11β-epoxido	*Curvularia lunata*	SHULL and BLOOM[1]
III substance S-16-anhydro	unchanged	*Curvularia lunata*	SHULL and BLOOM[1]



[1] G. M. SHULL and B. M. BLOOM, Communication 3rd International Congress for Biochemistry, Brussels 1955, p. 48; J. Amer. chem. Soc. *77*, 5767 (1955).

Table XV. — Side chain-Degradation and Dehydrogenation by *Fusarium* sp. (VISCHER and WETTSTEIN[1])



[1] E. VISCHER and A. WETTSTEIN, Exper. *9*, 371 (1953).

androstadiene-3,17-dione (II), besides the corresponding 17β-alcohol and compound (IX), the latter through simple reduction in the 20β-position. The main reaction, however, was elimination of the side chain with simultaneous *cleavage of ring D* to testololactone (VIII), with incidental dehydrogenation to (VII). PETERSON described simple side chain degradation to androstene-3,17-dione (VI), apart from lactone formation giving VIII, but in contrast, without any dehydrogenation in ring A. Corresponding conversions have been observed, amongst others, from testosterone to VII, from cortexone to VI, and contrary to our results, from substance S by way of side chain degradation to VI, VII, and VIII. 17α-Hydroxy-progesterone also gave VIII, while 14α-hydroxy-progesterone was degraded to 14α-hydroxy-androstenedione[1], and 16α-hydroxy-progesterone among other products to 16α-hydroxy-testosterone[2]. Lately, the conversion of progesterone into VI and VIII by means of a *Cephalosporium* sp. has been described[3].

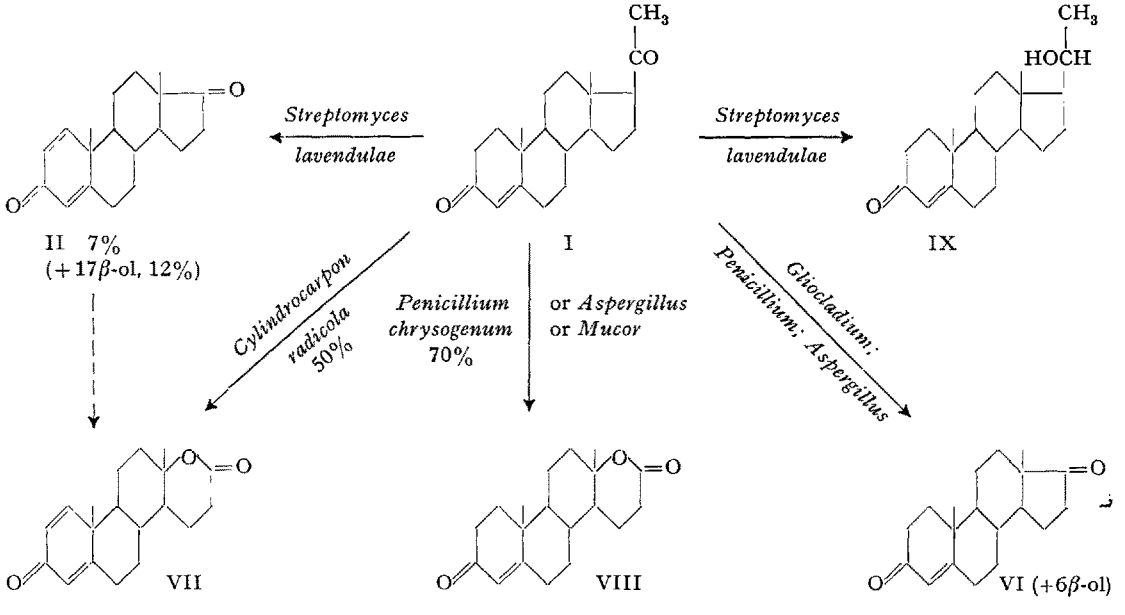
The highly active synthetic glucocorticoides, 1-dehydro-cortisone and 1-dehydro-hydrocortisone, like-

[1] P. D. MEISTER, S. H. EPPSTEIN, D. H. PETERSON, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, Abstr. 123rd Meet. Amer. Chem. Soc., Los Angeles, March 1953, p. 5*c*.

[2] J. FRIED, R. W. THOMA, D. PERLMAN, J. E. HERZ, and A. BORMAN, Rec. Progr. Hormone Res. *11*, 160 (1955).

[3] A. BODANSZKY, J. KOLLONITSCH, and G. WIX, Exper. *11*, 384 (1955).

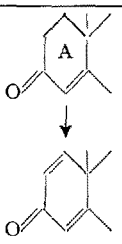
Table XVI. — Side Chain-Degradation, Lactone Formation and Dehydrogenation. (FRIED *et al.*[1] and PETERSON *et al.*[2]).



[1] J. FRIED, R. W. THOMA, and A. KLINGSBERG, J. Amer. chem. Soc. *75*, 5764 (1953).

[2] D. H. PETERSON, S. H. EPPSTEIN, P. D. MEISTER, H. C. MURRAY, H. M. LEIGH, A. WEINTRAUB, and L. M. REINEKE, J. Amer. chem. Soc. *75*, 5768 (1953).

Table XVII. — Further Dehydrogenations in 1-Position

Reaction	Products	Microorganism	Authors
	Without degradation of side chain also in 17-desoxy-20-ketones	*Calonectria* *Ophiobolus* *Alternaria*	VISCHER, MEYSTRE, and WETTSTEIN[1]
	Without degradation of side chain also in 17-desoxy-20-ketones	*Corynebacterium simplex*	NOBILE *et al.*[2]
	Without degradation of side chain also in 17-desoxy-20-ketones	*Didymella*	VISCHER, MEYSTRE, and WETTSTEIN[3]

[1] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, Helv. chim. Acta *38*, 835 (1955).

[2] A. NOBILE, W. CHARNEY, P. L. PERLMAN, H. L. HERZOG, C. C. PAYNE, M. E. TULLY, M. A. JEVNIK, and E. B. HERSHBERG, J. Amer. chem. Soc. *77*, 4184 (1955).

[3] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, Helv. chim. Acta *38*, 1502 (1955).

wise represent *$\Delta^{1;4}$-3-ketodienes*. Their properties were described in 1955 by HERZOG *et al.*[1], without any method of preparation being given. We therefore considered it advisable to publish the microbiological production of a number of such 1-dehydro-hormones[2]. We had been struck by the retention of the side chain in the "further substances" we had observed from 17α-hydroxy-20-keto-pregnenes in 1953 using a *Fusarium* species, dehydrogenating usually in the 1-position[3]; it was now apparent that the products represented the corresponding 1-dehydro compounds. In species of the genera *Calonectria*, *Ophiobolus* and *Alternaria* (Table XVII) we found microorganisms[2] that were similarly capable of smoothly introducing a 1,2-double bond in ring A, but, in contrast to *Fusarium solani*, did *not* degrade the side chain even in 17-desoxy-20-keto-steroids like cortexone, corticosterone and progesterone. Not long ago, NOBILE *et al.*[4] announced the preparation of these 1-dehydro-hormones by means of *Corynebacterium simplex* which is supposed to give excellent yields. In addition, 1-dehydro-cortisol and 1-dehydro-9α-fluoro-cortisol were obtained. The latter, representing until now the most active glucocorticoide, had been prepared by chemical means by TISHLER *et al.*[5] and by FRIED *et al.*[6] and, using a combination of microbiological and chemical methods, by HOGG *et al.*[7]. Practically quantitative yields are achieved in the 1-dehydrogenation, according to our new results[8], with strains of the genus *Didymella*, which we have employed recently to obtain the known 1-dehydro-compounds as well as a whole range of new ones; for example, the corresponding derivatives of 17α-methyl-testosterone, 17α-ethinyl-testosterone, 11-dehydro-progesterone and 11-dehydro-17α-methyl-corticosterone.

[1] H. L. HERZOG, A. NOBILE, S. TOLKSDORF, W. CHARNEY, E. B. HERSHBERG, P. L. PERLMAN, and M. M. PECHET, Science *121*, 176 (1955).

[2] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, Helv. chim. Acta *38*, 835 (1955).

[3] E. VISCHER and A. WETTSTEIN, Experientia *9*, 371 (1953).

[4] A. NOBILE, W. CHARNEY, P. L. PERLMAN, H. L. HERZOG, C. C. PAYNE, M. E. TULLY, M. A. JEVNIK, and E. B. HERSHBERG, J. Amer. chem. Soc. *77*, 4184 (1955); compare also ibid. pg. 4781.

[5] R. F. HIRSCHMANN, R. MILLER, R. E. BEYLER, L. H. SARETT, and M. TISHLER, J. Amer. chem. Soc. *77*, 3166 (1955).

[6] J. FRIED, K. FLOREY, E. F. SABO, J. E. HERZ, A. R. RESTIVO, A. BORMAN, and F. M. SINGER, J. Amer. chem. Soc. *77*, 4181 (1955).

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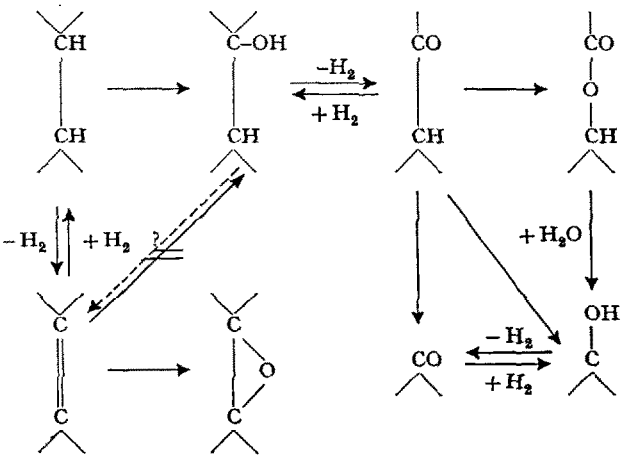
Table XVIII.

The Most Important Microbiological Conversions of Steroids.



Concerning the microbiological introduction of the 1,2-double bond there exist in principle two possibilities for the course of reaction: Hydroxylation in position 1 and subsequent loss of water from the resulting β-hydroxy-ketone[1], or the typical aerobic dehydrogenase mechanism. The analogy with the many known microbiological hydroxylations supports the first explanation. The second explanation would be more attractive if we were to consider the fact that this dehydrogenation, as we have seen, often but not always is associated with side chain degradation and ring cleavage to the lactone, reactions for which peroxides resulting from the dehydrogenase action might be responsible.

[1] E. VISCHER, CH. MEYSTRE, and A. WETTSTEIN, Helv. chim. Acta *38*, 835 (1955).

To sum up, the most important microbiological conversions of steroids, are listed in Table XVIII.

Zusammenfassung

Die methodischen Grundlagen der Umwandlung von Steroiden durch Mikroorganismen werden diskutiert, insbesondere die apparativen Erfordernisse, die Kulturbedingungen sowie der Nachweis und die Isolierung der Reaktionsprodukte. Die Umsetzungen mit Enzymen aus Mikroorganismen werden denjenigen mit Nebennieren-Enzymen gegenübergestellt.

Von mikrobiologischen Reaktionen stehen Hydrierungen, Dehydrierungen und besonders Hydroxylierungen an verschiedenen Stellen der Steroidmolekel im Vordergrund. Im weiteren wurde auch Abbau der Seitenkette von Pregnanderivaten, der mit Ringspaltung und/oder mit Dehydrierung in 1-Stellung einhergehen kann, festgestellt. Die Einführung dieser Doppelbindung wird speziell besprochen.

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Further Measurements on the Bioluminescence of the Seedlings

The introduction and the development of the photomultipliers in the technique of light detection has permitted the counting of individual photons corresponding to extremely feeble luminous fluxes[1].

This method of detection can be applied with advantage to the study of problems connected with feeble luminous emissions; photons come directly from molecules that take part in some reaction, chemical or biochemical for instance, and can be very useful in furnishing a clue to molecular processes.

By means of a very sensitive apparatus, some of us have detected recently the emission of light in the visible spectrum by various germinating plants[2]. The present work is concerned with specifying such preliminary results, discussing some further properties of luminescence, giving a quantitative comparison of the intensity of the emitted light for different plants and at various ages during the germination, and showing that the production of light is strictly connected with the vital functions of seedlings.

(1) The apparatus used in the present research work is the same described in previous papers[3]. It is possible with this dispositive to detect the light coming from a big emitting area or volume. The plants used for present experiments belong to graminaceous and leguminous families. The seedlings were grown in complete darkness in order to avoid the formation of chlorophyll which, by its luminescence, would have disturbed the measurement.

The seedlings grew in humid surroundings at a constant temperature of 25°C. Measurement were conducted both on whole plants and on cold water extract of the plants or of the separate organs.

The extract is made by grinding a constant quantity of seedlings (generally a few grams) or of their organs with a corresponding quantity of a phosphate buffer solution of pH 7·3 and centrifugating the ground mixture. This pH value corresponds to the optimum value of the luminescent intensity. The measurements are made on a constant volume (10 cm^3) of the transparent liquid obtained which is collocated very close to the photocathode of the phototube.

The absence of chlorophyll is checked by the lack of red fluorescence of chlorophyll in the extracts.

(2) A first measurement was made for the purpose of detecting the intensity of the light emitted by seedlings in well established physiological conditions.

For such purpose a few tenths of 8 days old seedlings were used, which were placed ordinately horizontally under the phototube in such a way as to cover a surface of about 100 cm^2.

Light intensity emitted by seedlings in physiological conditions

	Pulses/min
Phototube backgrounds . .	8 000
Wheat	41 000
Beans	38 000
Lentils	22 000
Corn	15 000
Beans cut into pieces . . .	84 000

In the Table, the results obtained are set down. Both the plant and the phototube were at room temperature (20°C) throughout the measurement.

The results show clearly the existence of bioluminescence, and the activity observed is much greater than the thermoelectronic background of the photomultiplier. We should remark that the background is very stable and reproducible for a period of months.

The measurements repeated many times show a good reproducibility in a factor 2, but a measurement of this kind does not make possible a precise comparison

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[3] L. COLLI and U. FACCHINI, Nuovo Cimento *12*, 150 (1954). – L. COLLI, U. FACCHINI, and A. ROSSI, Nuovo Cimento *11*, 255 (1954).