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Molecular structure in relation to oestrogenic activity. Compounds without a phenanthrene nucleus

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In earlier publications the oestrogenic activity of a number of synthetic substances has been discussed. The first compounds to be tested contained the phenanthrene ring system, and it was shown (Cook, Dodds and Hewett 1933; Cook and Dodds 1933; Cook, Dodds, Hewett and Lawson 1934; Cook, Dodds and Greenwood 1934) that a high degree of activity is possessed by 9 : 10-dihydroxy-9 : 10-di-*n*-propyl-9 : 10-dihydro-1 : 2 : 5 : 6-dibenzanthracene. Potency was also observed in the highly unsaturated aliphatic acid, clupanodonic acid, whilst a feeble but definite oestrogenic action was found to be possessed by calciferol. Since neither of these compounds contains the phenanthrene ring system, it was considered that this was not essential for oestrogenic properties. A further interesting observation was made in the partial activity of 1-keto-1 : 2 : 3 : 4 : 5 : 6 : 7 : 8-octahydroanthracene. This substance produced an advanced pro-oestrus, but full cornification could not be produced no matter how much of the material was administered.

A series of derivatives of acenaphthene was studied because these compounds contain a three-ring system arranged in a manner different from that in phenanthrene. These were tested on ovariectomized rats in the manner described in the above-mentioned publications. Table I shows the

Table I

Derivatives of acenaphthene	Method of preparation	Dose in mg.	% positive
1 : 2-Dihydroxy-1 : 2-di-*n*-propyl acenaphthene	Maxim (1929)	100	Nil
1 : 2-Dihydroxy-1 : 2-diphenyl acenaphthene	Beschke (1909)	100	Nil
1 : 2-Dihydroxy-1 : 2-dibenzyl acenaphthene	Maxim (1929)	100	Nil
1 : 2-Dihydroxy-1 : 2-di-α-naphthyl acenaphthene	—	100	100
		10	100
		1	20
1 : 1-Diphenyl acenaphthenone	Beschke (1909)	100	Nil
1 : 1-Di-α-naphthyl acenaphthenone	—	100	100

results obtained with this group of substances. It will be observed that the most potent member of the series is 1 : 2-dihydroxy-1 : 2-di-α-naphthyl acenaphthene. In doses of 10 mg. this compound produces prolonged oestrus. This new substance confirmed our assumption that the phenanthrene ring system was unnecessary for oestrogenic activity and led to experiments with a number of aromatic carbinols (Table II). This series of compounds showed surprising differences in behaviour. Thus, whilst diphenyl-α-naphthyl carbinol possesses full activity in doses of 100 mg. the corresponding diphenyl-β-naphthyl carbinol and triphenyl carbinol were

TABLE II

Substance	Method of preparation	Dose mg.	% positive
Diphenyl carbinol	—	100	Nil
Triphenyl carbinol	—	100	Nil
Phenyl-α-naphthyl carbinol	Acree (1904)	100	Nil
Di-α-naphthyl carbinol	Schmidlin and Massini (1909)	100	Nil
Diphenyl-α-naphthyl carbinol	Acree (1904)	100	100
Diphenyl-β-naphthyl carbinol	Ullmann and Mourawiew-Winigradoff (1905)	100	Nil
1 : 8-Di-α-naphthoyl naphthalene	—	100	Nil
Benzil	—	100	Nil
Hydrobenzoin	—	100	Nil
Phenyl hydrobenzoin	Acree (1904)	100	Nil
α-Naphthyl benzoin	Acree (1904)	100	60
α-Naphthyl hydrobenzoin	Acree (1904)	100	80
Benzpinacone	—	100	Nil
Benzpinacoline	—	100	Nil
9-Hydroxy-9-α-naphthyl fluorene	Ullmann and Wurstemberger (1905)	100	Nil

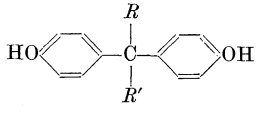
both without action. The effect of hydroxy groups in the aromatic nucleus was then studied and several phenolic diphenyl methane derivatives were investigated with results as shown in Table III. 3 : 3′- and 4 : 4′-dihydroxy-diphenyl methane both showed activity, the latter much greater than the former, but the corresponding dicarboxylic acids were without effect. Many potent substances were found in derivatives of 4 : 4′-dihydroxy-diphenyl methane. From this series it appears that the alkyl substituents on the central carbon atom have no influence on the efficacy of the substance. A single phenyl nucleus has a distinct depressing action, and the diphenyl derivative is completely inert. The introduction of methyl groups in the 3 : 3′ positions appears to diminish the potency in some cases.

Dihydroxy benzophenone was less powerful than the corresponding diphenyl methane compound. It is interesting to note the surprising effect of reducing 4-hydroxy-triphenyl carbinol to 4-hydroxy-triphenyl methane,

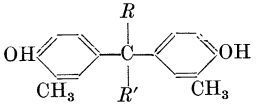
Table III

	Method of preparation	Dose (mg.)	% positive
Derivatives of diphenyl methane:			
4-Hydroxy-diphenyl methane	Clemmensen (1914)	100	Nil
3 : 3′-Dihydroxy-diphenyl methane	Auwers and Rietz (1907)	100	40
4 : 4′-Dihydroxy-diphenyl methane	Eberhardt and Welter (1894)	100	100
Diphenyl-methane-3 : 3′-dicarboxylic acid	Schöpf (1894)	100	Nil
Diphenyl-methane-4 : 4′-dicarboxylic acid	Schöpf (1894)	100	Nil
4 : 4′-Dihydroxy-diphenyl-methane-3 : 3′-dicarboxylic acid	Kahl (1906)	100	Nil
2 : 5-Dihydroxy-diphenyl-methane-carboxylic acid lactone	—	100	Nil
α-(2-Hydroxy-5-methyl-phenyl) phthalide	—	100	Nil
Benzilic acid	—	100	Nil
2 : 2′-Dihydroxy-di-α-naphthyl methane	Fries and Hübner (1906)	100	Nil
α : α-Diphenyl ethylene	Klages (1902)	100	Nil

Derivatives of 4 : 4′-dihydroxy diphenyl methane (Easson, Harrison, McSwiney and Pyman 1934):

R	*R′*			
Methyl	Methyl		100	100
Methyl	Ethyl		100	100
Methyl	*n*-Propyl		100	100
Ethyl	Ethyl		100	100
H	*n*-Hexyl		100	100
H	Phenyl	Russanow (1889)	100	100
			10	20
Methyl	Phenyl	—	100	60
Phenyl	Phenyl	—	100	Nil

Derivatives of 4 : 4′-dihydroxy-3 : 3′-dimethyl-diphenyl methane (Easson, Harrison, McSwiney and Pyman 1934):



R	*R′*			
Methyl	Methyl	100	100	
Methyl	Ethyl	100	100	
H	*n*-Hexyl	100	Nil	
Methyl	Phenyl	100	Nil	
Di-(4-hydroxyphenyl)-1 : 1-*cyclo*hexane		100	100	
Di-(4-hydroxy-3-methyl phenyl)-1 : 1-*cyclo*hexane		100	100	

Derivatives of benzophenone:

4-Hydroxy-benzophenone	Heller (1913)	100	Nil
4 : 4′-Dihydroxy-benzophenone	Baeyer and Burkhardt (1880)	100	80
2 : 3 : 4 : 4′-Tetrahydroxy-benzophenone	—	100	60
2 : 3 : 4 : 3′ : 4′ : 5′-Hexahydroxy-benzophenone	Bleuler and Perkin (1916)	100	20

Derivatives of triphenyl methane:

4-Hydroxy-triphenyl acetic acid	Bistrzycki and Novakowski (1901)	100	Nil
4-Hydroxy-triphenyl carbinol	Bistrzycki and Herbst (1901)	100	Nil
4-Hydroxy-triphenyl methane	Bistrzycki and Herbst (1902)	100	100
Phenolphthalein	—	100	Nil
2-Hydroxy-5-methoxy-tri-phenyl-methane carboxylic acid lactone	Easson, Harrison, McSwiney and Pyman (1934)	100	Nil
2 : 4-Dihydroxy-4′ : 4″-dimethoxy-tri-phenyl-methane carboxylic acid lactone	Easson, Harrison, McSwiney and Pyman (1934)	100	Nil
2 : 4-Dihydroxy-triphenyl-methane carboxylic acid lactone	Easson, Harrison, McSwiney and Pyman (1934)	100	80
4-Hydroxyphenyl-di-α-naphthyl methane	Schmidlin and Massini (1909)	100	100
		10	100
4-Hydroxy-tetraphenyl methane	Baeyer and Villiger (1902)	100	Nil

and finally the increased activity obtained by substitution of α-naphthyl for phenyl in this molecule.

Investigation of a group of substances in which the linkage between the two phenol nuclei was varied provided many powerful agents which are given in Table IV.

TABLE IV

	Method of preparation	Dose (mg.)	% positive
Derivatives of diphenyl:			
2-Hydroxy-diphenyl	—	100	Nil
3-Hydroxy-diphenyl	—	100	Nil
4-Hydroxy-diphenyl	Schlenk (1909)	100	Nil
2 : 2′-Dihydroxy-diphenyl	—	100	Nil
3 : 3′-Dihydroxy-diphenyl	Häussermann and Teichmann (1894)	100	Nil
4 : 4′-Dihydroxy-diphenyl	Hirsch (1889)	100	100
4 : 4′-Dihydroxy-3 : 3′-dimethyl diphenyl	Hobbs (1888)	100	Very slight activity
Diphenyl-4 : 4′-dicarboxylic acid	—	100	Nil
2-Hydroxy fluorene	Diels (1901)	100	Nil
4 : 4′-Dihydroxy-1 : 1′-dinaphthyl	—	100	Nil
Derivatives of α : β-diphenyl ethane:			
α-Hydroxy-diphenyl ethane	Schmidlin and Garcia-Banùs (1912)	100	Nil
2-Hydroxy-diphenyl ethane	Kostanecki, Rost, Szabránski (1905)	100	Nil
4-Hydroxy-diphenyl ethane	Stoermer and Kippe (1903)	100	Nil
4-4′-Dihydroxy-diphenyl ethane	Heumann and Wiernik (1887)	100	100
Stilbene	Wislicenus and Endres (1903)	100	100
		25	100
2-Hydroxy stilbene	Kostanecki and Tambor (1909)	100	Nil
4-Hydroxy stilbene	Hewitt, Lewcock and Pope (1912)	100	100
		10	100
		5	40
3′-Hydroxy-stilbene-α-carboxylic acid	Werner (1895)	100	Nil
4 : 4′-Dihydroxy stilbene	Auwers (1903)	100	100
		10	100
		5	80
4 : 4′-Dimethoxy stilbene	Elbs (1893)	100	Nil
Tolane	Fittig and Ammand (1873)	100	Nil
4 : 4′-Dihydroxytolane	Zincke and Münch (1904)	10	100
		5	80
Triphenyl ethylene	Klages and Heilmann (1904)	10	100
		5	50
Tetra-phenyl ethylene	Norris, Thomas and Brown (1910)	100	Nil
Derivatives of α : γ-diphenyl propane:			
4-Hydroxy-phenyl-styryl ketone	Kostanecki and Tambor (1899)	100	Nil
Phenyl-4-hydroxy-styryl ketone	Bablich and Kostanecki (1896)	100	Nil
4 : 4′-Dihydroxy-diphenyl propane	—	100	100
2 : 4 : 6 : 4′-Tetrahydroxy-diphenyl propane	—	100	100
Phloretin	Schiff (1874)	100	100
Phloridzin	—	100	80
α : δ-Diphenyl butadiene	Thiele and Schleussner (1899)	25	100
4 : 4′-Dihydroxy-dibenzylidene acetone	Zincke and Muhlhausen (1903)	100	Nil
4 : 4′-Dihydroxy-α : ε-diphenyl pentane	Borsche (1919)	80	100

After the publication of the activity of diphenyl ethylene (Dodds and Lawson 1937*a*), Robson and Schönberg (1937) described the activity of triphenyl ethylene. The full series was described later (Dodds, Fitzgerald and Lawson 1937).

Only the 4 : 4′-dihydroxy derivative of diphenyl was found to have oestrogenic properties, which were not possessed by compounds having the hydroxy groups in other positions. Introduction of methyl groups in the adjacent position to the hydroxyl in this substance considerably decreased its activity. This was confirmed by Grant (1937) who found that 4 : 4′-dihydroxy diphenyl promoted mammary growth in young adult male guinea-pigs, while the 4 : 4′-dihydroxy-3 : 3′-dimethyl diphenyl was without effect. Dihydroxy-diphenyl ethane was found to possess full activity in doses of 100 mg. and the introduction of an ethylenic linkage between the α and β carbon atoms was accompanied by a great increase in potency, which further unsaturation appears not to affect. Thus 4 : 4′-dihydroxy tolane possesses considerable oestrogenic power in doses of 5 mg. It is interesting to note that the naturally occurring glucoside phloridzin possesses considerable activity whilst phloretin, obtained by hydrolysis of phloridzin, is more powerful. Increase in length of the connecting carbon chain appears not to affect the action of the substances since 2 : 4 : 6 : 4′-tetra-hydroxy-α : γ-diphenyl propane (from phloretin by reduction of the carbonyl group) and 4 : 4′-dihydroxy-α : ϵ-diphenyl pentane both gave positive results in doses of 100 mg.

The hydrocarbon stilbene itself possessed considerable activity which is increased by the introduction of a further phenyl nucleus (triphenyl ethylene), whilst tetra-phenyl ethylene is without action. In Table V is shown the effect of linking two molecules of phenol by oxygen or nitrogen. Full activity was shown by 4 : 4′-dihydroxy diphenyl ether in doses of 100 mg. and slightly less by the mono-hydroxy compound. Similar derivatives of diphenylamine were inert. The properties of stilbene and its

TABLE V

	Method of preparation	Dose mg.	% positive
Derivatives of diphenyl ether:			
Diphenyl ether	—	100	Nil
2-Hydroxy-diphenyl ether	Ullmann and Stein (1906)	100	Nil
4-Hydroxy-diphenyl ether	Häussermann and Bauer (1896)	100	80
2 : 2′-Dihydroxy-diphenyl ether	Ullmann and Stein (1906)	100	Nil
2 : 4′-Dihydroxy-diphenyl ether	—	100	Nil
4 : 4′-Dihydroxy-diphenyl ether	Häussermann (1897)	100	100
4-(*p*-Hydroxyphenoxy) benzoic acid	—	100	Nil
Thyronine	—	90	Nil
Derivatives of diphenylamine:			
4-Hydroxy diphenylamine	Schneider (1899)	100	Nil
4 : 4′-Dihydroxy diphenylamine	Knoevenagel (1914)	100	Nil

hydroxy derivatives led us to examine similarly constructed compounds containing only a single aromatic ring. *p*-Propenyl phenol induced full oestrus in doses of 100 mg. but the isomeric *p*-allyl phenol proved completely inactive as did also the hydrocarbon propenyl benzene. It is interesting to note that methylation or the presence of a methoxyl in the 2 position renders such feebly active substances inert, as anethole and *iso*-eugenol were both without effect. All the *p*-*n*-alkyl phenols from *p*-cresol to *p*-*n*-octyl phenol, and also *p*-*n*-hexadecyl phenol, were tested, but only the propyl derivative had oestrogenic properties. Other phenols and their derivatives are found in Table VI.

TABLE VI

Derivatives of phenol	Method of preparation	% positive with 100 mg. dose
2-Allyl phenol	—	Nil
4-Allyl phenol (chavicol)	—	Nil
4-Propenyl phenol (anol)	—	100
4-*tert*-Butyl phenol	—	Nil
2-*n*-Amyl phenol	—	Nil
4-*tert*-Amyl phenol	—	80
4-*n*-Amyl-2-methyl phenol	—	Nil
2-Methoxy-4-*n*-butyl phenol	—	Nil
2-Methoxy-4-allyl phenol (eugenol)	—	Nil
2-Methoxy-4-propenyl phenol (*iso*-eugenol)	—	Nil
4-*iso*-Propyl-3-methyl phenol	—	Nil
4-*n*-Hexyl resorcinol	—	Nil
4-*cyclo*-Pentyl phenol	—	Nil
4-*cyclo*-Hexyl phenol	Bartlett and Garland (1927)	80
3 : 4 : 5-Triphenyl phenol	Smith (1893)	Nil
Hydroquinone	—	Nil
4-Hydroxybenzaldehyde	—	Nil
4-Hydroxy benzoic acid	—	Nil
4-Hydroxy-phenyl acetic acid	—	Nil
4-Hydroxy-β-phenyl ethyl alcohol	v. Braun (1912)	30
4-Hydroxy hydrindene	Mills and Nixon (1930)	Nil
5-Hydroxy hydrindene	Mills and Nixon (1930)	Nil
5-Hydroxy-α-hydrindone	Auwers and Hilliger (1916)	Nil
7-Hydroxy-α-hydrindone	Auwers and Hilliger (1916)	Nil

It would appear from a study of this table that the most interesting compounds are those with the 3-carbon atom chain para to the hydroxyl group. Thus both *p*-hydroxy-propyl benzene and *p*-hydroxy-propenyl benzene are active whilst *p*-hydroxy-allyl benzene is inactive. A very complex situation was encountered with certain specimens of *p*-hydroxy-

propenyl benzene or anol. This compound was made by demethylation of anethole with strong alkali and alcohol. Certain specimens were found to possess a very high degree of activity (Dodds and Lawson 1937*a*), and later it was shown that this activity was due to contamination with some substance, possibly a polymer of anol. It was shown that substances of high activity could always be detected in the mother liquor obtained from crystallizing anol from ether or chloroform (Dodds and Lawson 1937*b*). From these experiments it is obvious that either a polymeride or derivative of anol must possess very great activity. In this connection it is interesting to note that Serini and Steinruck (1937) have obtained highly active crystalline substances as by-products in the demethylation of anethole.

Preparation of materials

Most of the substances were prepared by methods already described, to which references are given in the tables.

4-*cyclo*-Pentyl phenol, which is described by v. Braun (1929), is more conveniently prepared from *cyclo*-pentyl bromide and phenol in the presence of zinc chloride by the method of Bartlett and Garland (1927) for the *cyclo*-hexyl derivative.

The following compounds are new:

1 : 2-*Di-hydroxy*-1 : 2-*di*-α-*naphthyl acenaphthene.* To an ice-cold Grignard solution (4 mols) from α-bromonaphthalene, was added in portions, with stirring, finely powdered acenaphthene quinone (1 mol). After 1 hr. the mixture was heated on the water bath for 4 hr. after which the whole was decomposed with ice and ammonium chloride. The ethereal solution was dried over sodium sulphate, the ether removed and the resinous residue dissolved in 10 parts of benzene from which it crystallized slowly, giving an almost pure product which on recrystallization from benzene gave a colourless, microcrystalline powder, m.p. 142°.

(Found, after air drying: C, 88·1, 88·6; H, 5·4, 5·5; loss when dried in a vacuum at 90°, 15·0 %. $C_{32}H_{22}O_2$, C_6H_6 requires C, 88·35; H, 5·45; C_6H_6, 15·1 %.)

1 : 1-*Di*-α-*naphthyl acenaphthenone.* To a solution of the di-α-naphthyl-acenaphthene diol (5 g.) in acetic acid (50 c.c.) at the boiling-point, concentrated hydrochloric acid was added drop by drop. The solution became bright yellow and crystals appeared. After cooling, these were filtered off and washed with alcohol. The substance is sparingly soluble in organic solvents and crystallised from 100 parts of xylene in pale yellow needles, m.p. 289°. (Found: C, 91·1; H, 4·9. $C_{32}H_{20}O$ requires C, 91·4; H, 4·8 %.)

1 : 8-*Di-α-naphthoyl naphthalene.* A solution of di-α-naphthyl acenaphthene diol (3 g.), chromic anhydride (0·75 g.) in acetic acid (30 c.c.) was boiled for 5 min. Water was then added till a turbidity appeared and the solution was cooled. The crystals were separated and washed with alcohol. 1 : 8-Di-α-naphthoyl naphthalene crystallized from toluene in prisms, m.p. 227–228°. (Found: C, 88·1; H, 4·8. $C_{32}H_{20}O_2$ requires C, 88·0; H, 4·6 %.)

2 : 4 : 6 : 4′-*Tetra-hydroxy-α-γ-diphenyl propane.* Phloretin (3 g.) was boiled under reflux with amalgamated zinc wool (10 g.) and hydrochloric acid (15 c.c. diluted with 15 c.c. water) for 18 hours. On cooling the solution gave crystals which after two recrystallizations from water melted at 158–159° and contained one molecule of water of crystallization. (Found: C, 64·9; H, 6·6. $C_{15}H_{16}O_4$, H_2O requires C, 64·7; H, 6·5 %.)

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Summary

1. The phenanthrene ring system is not necessary for oestrogenic activity.

2. Substances containing two phenol groups joined by a carbon chain are active. The number of carbon atoms, the position of double bonds and of substituent groups attached to the carbon chain all vary the activity.

3. Substituents in the aromatic nucleus apart from the hydroxyl group appear to lessen activity.

4. It is possible to obtain activity in compounds containing only one benzene ring. Thus both para-hydroxy *n*-propyl and propenyl benzene are active. The corresponding allyl compound is without activity. The propenyl compound (anol) readily undergoes polymerization under certain conditions to form very highly active substances.

References

Acree, S. F. 1904 *Ber. dtsch. chem. Ges.* **37**, 2760.
Auwers, K. 1903 *Ber. dtsch. chem. Ges.* **36**, 1887.
Auwers, K. and Hilliger, E. 1916 *Ber. dtsch. chem. Ges.* **49**, 2410.
Auwers, K. and Rietz, E. 1907 *Liebigs Ann.* **356**, 157.
Bablich, H. and Kostanecki, S. 1896 *Ber. dtsch. chem. Ges.* **29**, 236.
Baeyer, A. and Burkhardt, J. B. 1880 *Liebigs Ann.* **202**, 126.
Baeyer, A. and Villiger, V. 1902 *Ber. dtsch. chem. Ges.* **35**, 3018.
Bartlett, J. F. and Garland, C. E. 1927 *J. Amer. Chem. Soc.* **49**, 2098.
Beschke, E. 1909 *Liebigs Ann.* **369**, 200.
Bistrzycki, A. and Herbst, C. 1901 *Ber. dtsch. chem. Ges.* **34**, 3073.
— — 1902 *Ber. dtsch. chem. Ges.* **35**, 3137.
Bistrzycki, A. and Novakowski, L. 1901 *Ber. dtsch. chem. Ges.* **34**, 3063.
Bleuler, H. and Perkin, A. G. 1916 *J. Chem. Soc.* **109**, 541.
Borsche, W. 1919 *Ber. dtsch. chem. Ges.* **52**, 2078.
Braun, J. v. 1912 *Ber. dtsch. chem. Ges.* **45**, 1283.
— 1929 *Liebigs Ann.* **472**, 1.
Clemmensen, E. 1914 *Ber. dtsch. chem. Ges.* **47**, 682.
Cook, J. W. and Dodds, E. C. 1933 *Nature, Lond.*, **131**, 205.
Cook, J. W., Dodds, E. C. and Greenwood, A. W. 1934 *Proc. Roy. Soc.* B, **114**, 286.
Cook, J. W., Dodds, E. C. and Hewett, C. L. 1933 *Nature, Lond.*, **131**, 56.
Cook, J. W., Dodds, E. C., Hewett, C. L. and Lawson, W. 1934 *Proc. Roy. Soc.* B, **114**, 272.
Diels, O. 1901 *Ber. dtsch. chem. Ges.* **34**, 1758.
Dodds, E. C., Fitzgerald, M. E. H. and Lawson, W. 1937 *Nature, Lond.*, **140**, 772.
Dodds, E. C. and Lawson, W. 1936 *Nature, Lond.*, **137**, 996.
— — 1937*a* *Nature, Lond.*, **139**, 627.
— — 1937*b* *Nature, Lond.*, **139**, 1068.
Easson, A. P. T., Harrison, J., McSwiney, B. A. and Pyman, F. L. 1934 *Quart. J. Pharm.* **7**, 509.
Eberhardt, C. and Welter, A. 1894 *Ber. dtsch. chem. Ges.* **27**, 1814.
Elbs, K. 1893 *J. prakt. chem.* (2), **47**, 68.
Fittig, R. and Ammand, H. 1873 *Liebigs Ann.* **168**, 74.
Fries, K. and Hübner, E. 1906 *Ber. dtsch. chem. Ges.* **39**, 439.
Grant, G. A. 1937 *Nature, Lond.*, **139**, 155.
Häussermann, C. 1897 *Ber. dtsch. chem. Ges.* **30**, 738.
Häussermann, C. and Bauer, E. 1896 *Ber. dtsch. chem. Ges.* **29**, 2085.
Häussermann, C. and Teichmann, H. 1894 *Ber. dtsch. chem. Ges.* **27**, 2108.
Heller, G. 1913 *Ber. dtsch. chem. Ges.* **46**, 1502.
Heumann, K. and Wiernik, J. 1887 *Ber. dtsch. chem. Ges.* **20**, 914.
Hewitt, J. T., Lewcock, W. and Pope, F. G. 1912 *J. Chem. Soc.* **101**, 605.
Hirsch, R. 1889 *Ber. dtsch. chem. Ges.* **22**, 335.
Hobbs, P. L. 1888 *Ber. dtsch. chem. Ges.* **21**, 1067.
Kahl, L. 1906 *Ber. dtsch. chem. Ges.* **31**, 148.
Klages, A. 1902 *Ber. dtsch. chem. Ges.* **35**, 2647.
Klages, A. and Heilmann, S. 1904 *Ber. dtsch. chem. Ges.* **37**, 1455.
Knoevenagel, E. 1914 *J. prakt. Chem.* (2), **89**, 24.
Kostanecki, S., Rost, A. and Szabránski, W. 1905 *Ber. dtsch. chem. Ges.* **38**, 943.
Kostanecki, S. and Tambor, J. 1899 *Ber. dtsch. chem. Ges.* **32**, 1924.
— — 1909 *Ber. dtsch. chem. Ges.* **42**, 825.
Maxim, N. 1929 *Bull. soc. chim. Fr.* (4), **45**, 1137.

Mills, W. H. and Nixon, I. G. 1930 *J. Chem. Soc.* p. 2520.
Norris, J. F., Thomas, R. and Brown, B. M. 1910 *Ber. dtsch. chem. Ges.* **43**, 2958.
Robson, J. M. and Schönberg, A. 1937 *Nature, Lond.*, **140**, 196.
Russanow, A. 1889 *Ber. dtsch. chem. Ges.* **22**, 1944.
Schiff, H. 1874 *Liebigs Ann.* **172**, 357.
Schlenk, W. 1909 *Liebigs Ann.* **368**, 303.
Schmidlin, J. and Garcia-Banùs, A. 1912 *Ber. dtsch. chem. Ges.* **45**, 3199.
Schmidlin, J. and Massini, P. 1909 *Ber. dtsch. chem. Ges.* **42**, 2381.
Schneider, F. 1899 *Ber. dtsch. chem. Ges.* **32**, 689.
Schöpf, M. 1894 *Ber. dtsch. chem. Ges.* **27**, 2324.
Serini, A. and Steinruck, K. 1937 *Naturwissenschaften*, **25**, 682.
Smith, A. 1893 *Ber. dtsch. chem. Ges.* **26**, 68.
Stoermer, R. and Kippe, B. 1903 *Ber. dtsch. chem. Ges.* **36**, 4009.
Thiele, J. and Schleussner, K. 1899 *Liebigs Ann.* **306**, 198.
Ullmann, F. and Mourawiew-Vinigradoff, A. 1905 *Ber. dtsch. chem. Ges.* **38**, 2218.
Ullmann, F. and Stein, A. 1906 *Ber. dtsch. chem. Ges.* **39**, 624.
Ullmann, F. and Wurstemberger, R. 1905 *Ber. dtsch. chem. Ges.* **38**, 4105.
Werner, G. 1895 *Ber. dtsch. chem. Ges.* **28**, 1999.
Wislicenus, W. and Endres, A. 1903 *Ber. dtsch. chem. Ges.* **36**, 1194.
Zincke, T. and Münch, S. 1904 *Liebigs Ann.* **335**, 184.
Zincke, T. and Muhlhausen, G. 1903 *Ber. dtsch. chem. Ges.* **36**, 131.

611.013.16:611.013.9

The development *in vitro* of the mammalian gonad. Ovary and ovogenesis*

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[Plates 9–11]

Introduction

Several investigators have tried to cultivate *in vitro* the mammalian and avian gonad, usually with the object of obtaining the growth and differentiation of the germ cells under *in vitro* conditions. The results so far obtained, however, have been disappointing. Champy (1920) is the only

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