into competitive reactions to prevent the formation of essential metabolites from specific substrates; and, (4) the activity may be influenced by the formation of split products not containing the sulfonamide radical such as succinate from succinyl derivatives. Since the antibacterial activity of members of an homologous series of these compounds does not parallel the ease with which sulfathiazole, for example, is formed by simple hydrolysis of the succinyl, phthalyl, quinolinyl, malonyl, oxalyl, and maleyl derivatives of sulfathiazole, it seems highly probable that the biologic activity is not explained entirely if it be assumed that such activity is due entirely to the sulfathiazole present.

Similarly, the high toxicity of maleylsulfanilamide as compared to the relatively low toxicity of maleylsulfathiazole cannot be explained upon the assumption that it is due to the split products, because sulfanilamide does not possess any such high degree of toxicity when compared to sulfathiazole.

Conclusion. Much of the data presented here cannot be correlated to show a relationship between antibacterial activity and rate of deacylation of the conjugated compounds, and it seems clear that the antibacterial activity of these compounds is not due solely to the liberation of the free sulfonamides. A considerable part of the antibacterial activity of these substances must, therefore, reside in the conjugated molecule. The node of action is complex and cannot be defined at this time.

The toxicity of the various compounds, likewise, does not necessarily parallel the chemical instability of the several conjugated sulfonamides.

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Effect of Steroid Substances on Synthesis of Acetylcholine.*

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Previous investigations have shown that male and female sex hormones may exert a vasomotor effect on the small vessels;[1-17] hormones of the adrenal cortex may change the size of the vessels due to changes induced in water and ion metabolism of the body;[18-20] estrogenic substances may increase the amount

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1 Carloni, E., *Arch. di ostet. e ginec.*, 1930, **17**, 327.

2 Markee, J. E., *Am. J. Physiol.*, 1932, **100**, 32.

3 Valle, G., *Ann. di ostet. e ginec.*, 1934, **56**, 1011.

4 Mortimer, H., Wright, R. P., Bachman, C., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 535.

5 Reynolds, S. R. M., *Am. J. Obstet. and Gynec.*, 1938, **36**, 437.

6 Reynolds, S. R. M., and Foster, F. I., *Am. J. Physiol.*, 1939, **128**, 147.

7 Reynolds, S. R. M., and Foster, F. I., *J. Clin. Invest.*, 1939, **18**, 649.

8 Reynolds, S. R. M., and Foster, F. I., *J. Pharm. Exp. Therap.*, 1940, **68**, 173.

9 Reynolds, S. R. M., *J. Invest. Dermatology*, 1940, **4**, 7.

10 Hamblen, E. C., Cuyler, W. K., and Hirst, D. V., *Endocrinology*, 1940, **27**, 172.

11 Reynolds, S. R. M., Kaminester, S., Foster, F. I., and Schloss, S., *Am. J. Obstet. and Gynec.*, 1941, **41**, 1022.

12 Edwards, E. A., Hamilton, J. B., Duntley, S. Q., and Hubert, G., *Endocrinology*, 1941, **28**, 119.

13 Reynolds, S. R. M., Kaminester, S., Foster, F. I., and Schloss, S., *Surg., Gynec., and Obstet.*, 1941, **73**, 206.

14 Hirst, D. V., and Hamblen, E. C., *J. Clin. Endocrinology*, 1942, **2**, 664.

15 Reynolds, S. R. M., Hamilton, J. B., di Palma, J. R., Hubert, G. R., and Foster, F. I., *J. Clin. Endocrinology*, 1942, **2**, 228.

16 Hirst, D. V., Hamblen, E. C., and Cuyler, W. K., *J. Clin. Endocrinology*, 1942, **2**, 442.

17 Walker, J. C., *J. Clin. Endocrinol.*, 1942, **2**, 560.

of acetylcholine in certain tissues such as uterus[6,21,22] and nasal mucosa;[23,24] estrogenic hormones may have a cholinergic effect;[25,26] and some of the steroid hormones modify the activity of choline esterase,[27] and the sensitivity of effector cells to acetylcholine.[27] In the following investigation it was ascertained whether or not steroid substances modify the synthesis of acetylcholine.

Method. The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[28] with minor modifications.[29] Varying amounts of the steroid substances were added to mixtures containing 100 mg minced fresh frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, and 3 cc Ringer's solution. The pH of the mixtures was adjusted to 7.4. Identical mixtures without the steroid substances served as controls. When oil solutions of the steroid substances were used the controls contained an equal amount of the solvent. The mixtures were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of free and total acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog. The amount of acetylcholine synthesized was calculated by subtracting from the acetylcholine content of the incubated mixtures the acetylcholine content of identical non-incubated mixtures. By adding the steroid substances in varying concentrations to incubated control mixtures after incubation it was ascertained whether or not the substances modified the sensitivity of the rectus abdominis muscle to the acetylcholine content of the mixtures during the 2 minutes of immersion for biological assay. If so, the potentiation was taken in account by the calculation.

Results. The amounts of acetylcholine synthesized in the presence of 23 steroid substances are given in Table I. Within the range of concentrations used only the estrogenic substances and pregnenolone increased the synthesis of acetylcholine. These results offer some explanation of the mechanism of the synergistic action of physostigmine with estrogenic substances in inducing menstruation.[30,31] The failure of Emmens, MacIntosh, and Richter[32] to demonstrate changes in the synthesis of acetylcholine in the presence of estradiol was probably due to the use of relatively high concentrations of estradiol.

The other substances either did not modify the synthesis of acetylcholine (cholesterol), or did not modify it in low concentrations and depressed the synthesis in higher (bile salts, vitamin D), or depressed the synthesis in low and increasing concentrations. Therefore, should these substances have any acetylcholine-like effect it is not because of the increase of synthesis of an acetylcholine-like agent in the body.

Summary and Conclusions. 1. The effect of steroid substances on the synthesis of acetylcholine was investigated. 2. Cholesterol did not modify the synthesis of acetylcholine. 3. Bile salts and vitamin D did not modify the synthesis in low concentrations and depressed it in higher. 4. Hormones of the adrenal cortex, male sex hormones, and corpus luteum hormones, except pregnenolone, depressed the synthesis in low and increasing concentrations. 5. Estrogenic hormones and

18 Grollmann, A., *The Adrenals,* Baltimore, Williams & Wilkins, 1936.

19 Thaddea, S., *Die Nebennierenrinde,* Leipzig, Thieme, 1936.

20 Sirota, H. H., *J. Clin. Endocrinol.,* 1943, **3**, 141.

21 Reynolds, S. R. M., *J. Physiol.,* 1939, **95**, 258.

22 Reynolds, S. R. M., *Science,* 1938, **87**, 537.

23 Reynolds, S. R. M., and Foster, F. I., *Am. J. Physiol.,* 1940, **131**, 422.

24 Reynolds, S. R. M., and Foster, F. I., *Endocrinology,* 1940, **27**, 841.

25 Pompen, A. W. M., *Die Invloed van Menformon op de Baarmoeder,* Thesis, Amsterdam, 1933.

26 Reynolds, S. R. M., *Physiology of Uterus,* Hoeber, 1939.

27 Torda, C., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 121.

28 Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Bioch. J.,* 1936, **30**, 1668.

29 Torda, C., and Wolff, H. G., *J. Clin. Invest.,* 1944, **23**, 649.

30 Soskin, S., Wachtel, H., and Hechter, O., *J. A. M. A.,* 1940, **114**, 2090.

31 *Roche Review,* 1942-3, **7**, 293, 292, 21; 1943-4, **8**, 330, 340; 1941-2, **6**, 275, 288.

32 Emmens, C. W., MacIntosh, F. C., and Richter, D., *J. Physiol.,* 1943, **101**, 460.

TABLE I.
Effect of Steroid Substances on the Synthesis of Acetylcholine.

Substance	Solvent	No. of series of exper.	Amt of acetylcholine synthesized in % of control*: Amts of substances added to 100 mg frog brain (in mg) — Free acetylcholine					Total acetylcholine				
			3	0.3	0.03	0.003		3	0.3	0.03	0.003	
Cholesterol		10	108	107	102	105		98	99	97	107	
Bile salts†		8	25	83	97	96		31	85	90	94	
Vitamin D‡		8		30	75	97			32	77	95	
Desoxycorticosterone acetate (Ciba)		6	55	67	78	85		42	61	77	76	
" " (Schering)		6	50	60	69	83		55	59	68	82	
4,5-dihydro-17-hydroxy-11-dehydro-corticosterone (Upjohn)		3	39	57	81	83		38	65	71	80	
Androstenedione (Schering)		8	27	40	64	90		29	35	59	88	
Dehydroandrosterone acetate (Schering)		8	60	79	89	95		60	71	85	91	
Testosterone (Schering)		8	55	77	81	89		47	71	86	90	
" propionate (Ciba)		8	53	66	75	85		57	74	81	86	
Methyltestosterone (Ciba)		8	28	48	72	96		35	50	70	94	
Δ^5-pregnenolone (Schering)		8	124	127	130	120		122	125	127	119	
Progesterone (Ciba)		10	62	68	84	91		61	71	82	87	
Pregnandiol (Ayerst, McKenna, & Harrison)		8	75	83	87	91		73	80	85	91	
Sodium pregnandiol glucoronidate (same)		5	53	64	75	80		56	60	73	82	
Allo-pregnanedione-11,20-diol-3,21 (Upjohn)		3	31	37	76	85		30	37	65	80	
Etiochilonol-3α-11-one acetate (Ayerst, McKenna, & Harrison)		5	65	80	100	110		73	86	115	121	
Estrone (Upjohn)		3	160	155	150	140		157	153	149	137	
Estradiol (Ciba)		8	103	110	125	130		102	111	127	129	
			Amts of substances added to 100 mg frog brain (in cc)									
			0.5	0.1	0.05	0.01	0.001	0.5	0.1	0.05	0.01	0.001
Doca (Roche-Organon) (1)§	Sesame oil	12	40	45	51	57		34	50	55	58	
Oreton (Schering) (2)§	" "	12	77	77	75	74		70	72	74	76	
Progestin (Roche-Organon) (3)§	Peanut "	12	63	65	71	77		63	69	74	76	
Estrone suspension (Abbott) (4)§	Acacia	12	100	122		138	137	110	130		139	141

* The S.E. of the mean for each value was less than $\pm 4\%$. The amounts of acetylcholine synthesized in μg per 100 mg frog brain, followed by the S.E. of the mean were: Free acetylcholine, 0.70 ± 0.023; total acetylcholine, 1.50 ± 0.044.

† Sodium glycocholate and sodium taurocholate from fresh ox gall (Fairchild Bros. & Foster).

‡ Each mg contained 400 I.U. vitamin D. The synthesis of acetylcholine was not modified in the presence of vitamin D in concentrations from 0.003 to 0.00001 mg.

§ One cc of the original solution contained (1) 5 mg desoxycorticosterone acetate; (2) 5 mg testosterone propionate; (3) 1 mg progesterone; (4) 2 mg estrone.

pregnenolone increased the synthesis of acetylcholine.

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Dietary Cirrhosis without Ceroid in Rats.

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Lillie, Daft, and Sebrell[1] among others have reported the production of hepatic cirrhosis in rats given a purified diet low in protein and low in choline. In their report and in the reports of other investigators[2,3] it was noted that the cirrhosis was always accompanied by the deposition of considerable amounts of a peculiar insoluble pigment in the fibrous trabeculations of the cirrhotic livers. The name "ceroid" has been given to this pigment.[4] Endicott and Lillie[5] have described in detail its staining properties and have noted that it is insoluble in all common solvents. Ceroid has not been reported in human liver cirrhosis nor in the experimental cirrhosis produced in the dog, the guinea pig, the pig, or the rabbit.

The question of whether or not ceroid is an essential part of the cirrhosis in rats has been the subject of much speculation. Ceroid was found to resemble the lipoid residues in cod liver oil pneumonia and similar substances were produced by chromate oxidation of cod liver oil and linseed oil but not by oxidation of various hydrogenated oils.[6] This suggested that dietary cirrhosis without ceroid might be produced by altering the dietary fat. Together with the work of Hass,[7] it suggested also the possibility that fatty acids found in both vegetable and animal oils and possessing more than one double bond might be involved in ceroid production. We have now achieved the production of cirrhosis without ceroid. Our results seem to indicate, however, that the most important dietary precursor of ceroid is not an ingredient of Wesson oil and that it is distinct from linoleic and linolenic as well as oleic, palmitic and stearic acids. It appears probable that the substance in question is present in cod liver oil but we have not as yet identified it further.

Weanling albino rats were given one of several purified diets containing leached and alcohol-extracted casein 4%, cystine 0.5%, and salt mixture No. 550[8] 4%. Diet No. 895 contained no fat or fatty acid, diet No. 954 contained 5% of Wesson oil, diet No. 953 contained 1% of a mixture of oleic, linoleic, linolenic, and saturated acids,* and

1 Lillie, R. D., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1941, **56**, 1255.

2 György, P., and Goldblatt, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 492.

3 Blumberg, H., and McCollum, E. V., *Science*, 1941, **93**, 598.

4 Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V., *Pub. Health Rep.*, 1942, **57**, 502.

5 Endicott, K. M., and Lillie, R. D., *Am. J. Path.*, 1944, **20**, 149.

6 Endicott, K. M., *Arch. Path.*, 1944, **37**, 49.

7 Hass, G. M., *Arch. Path.*, 1938, **26**, 956; 1939, **28**, 177.

8 Spicer, S. S., Daft, Floyd S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

9 Daft, Floyd S., Sebrell, W. H., and Lillie, R. D., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 228.

* Armour and Company's "Neo-Fat No. 23." The composition given by the Company is oleic acid 30%, linoleic acid 56%, linolenic acid 10%, and saturated acids 4%.