ficult to assess in this situation until further study.

Summary. Benzylpenicillin, 2,6-dimethoxyphenylpenicillin, and 6-aminopenicillanic acid were compared with respect to several biochemical activities. 2,6-Dimethoxyphenylpenicillin and 6-aminopenicillanic acid were hydrolyzed at very low rates, compared to the rate for benzylpenicillin, by partially purified concentrated cell-free preparations of penicillinase derived from *B. cereus,* and at still lower rates by analogous preparations from *S. aureus.* For *S. aureus,* but not *B. cereus,* the growth-inhibitory concentrations of the two penicillinase-resistant compounds, particularly 2,6-dimethoxyphenylpenicillin, were much lower than that of benzylpenicillin. 2,6-Dimethoxyphenylpenicillin was a very effective inducer of penicillinase formation in *B. cereus* and in *S. aureus.* 6-Aminopenicillanic acid and benzylpenicillin were considerably less active as inducers of penicillinase in *S. aureus* than in *B. cereus,* as measured by effective inducing concentrations and by the amounts of enzyme formed. A mutant of a penicillinase-producing strain of *S. aureus* of increased resistance to 2,6-dimethoxyphenylpenicillin was obtained by successive subculture in increasing concentrations of the antibiotic. Its resistance did not appear to be due to the increased amount of its penicillinase content.

It is a pleasure to acknowledge the skillful assistance of Miss Emmy Lou Denny.

ADDENDUM: Examination of the manometric assay of the rate of hydrolysis of 6-APA has revealed that this method yields artifactually low results with this particular compound because the product of enzymatic hydrolysis combines with some of the CO_2 liberated from the $NaHCO_3$ buffer system. Iodometric assay of this reaction shows the real rates of hydrolysis to be several-fold higher than given in Table I.

1. Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., Rolinson, G. N., *Nature,* 1959, v183, 257.

2. Bunn, P. A., Knight, R., Amberg, J., *N. Y. State J. Med.,* 1960, v60, 3074.

3. Douthwaite, A. H., Trafford, J. A. P., *Brit. Med. J.,* 1960, v2, 687.

4. Henry, R. J., Housewright, R. D., *J. Biol. Chem.,* 1947, v167, 559.

5. Steinman, H. G., *Fed. Proc.,* 1960, v19, 347.

6. Foster, J. W., *J. Biol. Chem.,* 1942, v144, 285.

7. Steinman, H. G., *J. Bact.,* 1961, in press.

8. Demerec, M., *Proc. Nat. Acad. Sci. U.S.,* 1945, v31, 16.

9. Pollock, M. R., *Biochem. J.,* 1957, v66, 419.

10. Demerec, M., *J. Bact.,* 1948, v56, 63.

11. Rolinson, G. N., Stevens, S., Batchelor, F. R., Cameron-Wood, J., Chain, E. B., *Lancet,* 1960, v2, 564.

12. Newton, G. G. F., Abraham, E. P., *Biochem. J.,* 1956, v62, 651.

13. Rifkind, D., Knight, V., in *A Symposium on a New Synthetic Penicillin,* P. A. Bunn, Ed., Syracuse University Press, Syracuse, 1961, in press.

14. Geronimus, L. H., *New Eng. J. Med.,* 1960, v263, 349.

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Biological Activities of 16α, 17α Dihydroxyprogesterone Derivatives. (26296)

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Since the demonstration by Inhoffen and Holweg that ethisterone (17α-ethinyl testosterone) is an orally active progestational agent(1), a wide variety of steroidal compounds possessing progestational activity have been reported(2-12). Fried *et al.* have recently reported on the synthesis and progestational activity of 16α, 17α ketal derivatives of 16α, 17α-dihydroxyprogesterone (DHP), including those with acetophenone (P-DHP) and acetone (A-DHP), as well as their 6-methyl, and 6-fluoro analogues(13,

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TABLE I. Comparative Activities of 16α, 17α-Dihydroxyprogesterone and Certain of Its Derivatives.*

Compound	Total dose in mg													
	.125		.25		.5		1.0		2.0		5.0		25.0	
	P†	O‡	P	O	P	O	P	O	P	O	P	O	P	O
Standard§	0	0	1	1	3	2	4	3		3		4		
16α, 17α-Dihydroxyprogesterone (DHP)							0	0			0	0	0	0
16α, 17α-Acetophenone of DHP	1	1	2	2	3	2	4	3	4	4		4		
16α, 17α-Acetonide of DHP	1		2		3	0			4	1		2		

* Numerical values of the McPhail grades are listed under the respective compounds. Each value represents mean response of at least 6 animals.

† Subcut. administration.

‡ Oral " .

§ Progesterone was parenteral standard and Norethisterone was oral standard.

13a). The biological activities of these derivatives of DHP are presented in this report.

Methods. New Zealand white rabbits weighing between 800 and 1200 g were used in all the progestational studies. These animals were primed with a total of 15 μg estradiol benzoate, given in 3 divided doses on alternate days for a 6-day period, following which the steroids were administered in solution, subcutaneously or orally, daily for the next 5 days. All compounds were dissolved in sesame oil and given once daily in .2 ml doses. Animals were autopsied 24 hours following the final dose, uteri removed and weighed, and sections taken for histological evaluation according to the method of McPhail(14).

Duration of activity was determined in ovariectomized rabbits. The animals were rested for 2 weeks post operation and then estrogen-primed as described above. A single 10 mg dose of progestogen was administered intramuscularly on the day after the last priming dose was given. A maintenance dose of .5 μg estradiol benzoate was administered every other day throughout the study. Animals were killed at 5 day intervals beginning 5 days after administration of progestogens and their uteri removed and treated as above.

P-DHP was assayed for androgenic and anti-androgenic activity according to the method of Hershberger *et al.*(15), and for uterotrophic and estrogen antagonistic properties by a modification of the method of Rubin *et al.*(16). Allen and Doisy's test for vaginal cornification in the ovariectomized rat was used as a specific end point for estrogenicity(17). The steroid was tested for glucocorticoid activity by a modification of the rat liver glycogen deposition test of Pabst *et al.*(18), and its effect on urinary sodium and potassium was determined using a modification of the method of Kagawa *et al.*(19).

Results and discussion. The results of the progestational studies with DHP, P-DHP and A-DHP are summarized in Table I. DHP was inactive at a total dose of 25 mg when administered parenterally or orally. P-DHP and A-DHP were equal in progestational activity when parenterally administered, each being 1-2 times as potent as progesterone. However, when given orally, P-DHP was 1-2 times as active as norethisterone. (17α-ethinyl-19 nortestosterone), the oral standard, while A-DHP was only one-eighth as active as norethisterone.

Parenteral and oral activities of the 6β-methyl homologue of P-DHP were similar to and those of the 6α-methyl homologue were less than those of the parent compound. No significant change in parenteral activity of A-DHP was observed following 6α or 6β-methylation, but both the 6α-methyl and 6β-methyl homologues appeared to be approximately 5 times more potent orally than A-DHP. It is interesting to note here that enhancement of progestational activity by 6-methylation parallels that observed with 6α-methylation of 17-acetoxyprogesterone (8) only in the case of the oral activity of the 6-methyl homologues of A-DHP.

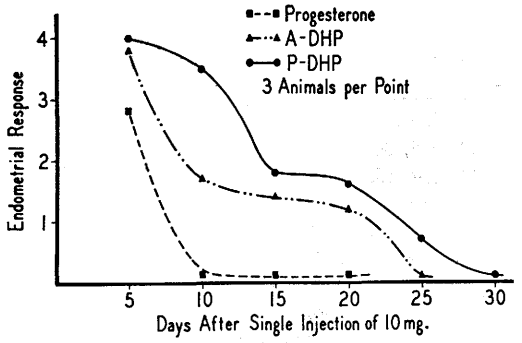

FIG. 1. Duration of progestational response following administration of progesterone, A-DHP, and P-DHP.

6α-fluorination of the P-DHP caused a 1-2 fold increase in parenteral activity but reduced oral potency. Parenteral activity of A-DHP was unaltered by 6α-fluorination, however, oral activity was reduced. 6β-fluorination abolished activity by either route of administration.

Duration studies demonstrated that P-DHP and A-DHP maintain a progestational effect for 25 and 20 days, respectively, whereas a comparable dose of progesterone shows no residual luteal effect 10 days following injection (Fig. 1). P-DHP appears to act somewhat longer than A-DHP, since some activity may still be observed on the twenty-fifth day, whereas following injection of the A-DHP endometrial gland development is absent at this time.

When administered to castrated immature male rats, a 5 mg daily dose of P-DHP failed to have any effect on the weights of ventral prostate, seminal vesicles or coagulating glands, nor did it show any anti-androgenic properties when 1 mg was administered daily with 25 μg testosterone propionate. Total doses of 100 μg and 1 mg of this steroid exhibited moderate uterotrophic effects in the immature female mouse, and when given simultaneously with .1 μg estradiol benzoate showed anti-estrogenic activity equivalent to that elicited by similar doses of progesterone. A 5 mg dose of the compound produced no vaginal cornification in ovariectomized rats, indicating the absence of estrogenicity. This steroid does not appear to possess glucocorticoid-like activity, as demonstrated by the absence of glycogen deposition in the liver of the adrenalectomized rat following a 2 mg dose. When tested for electrolyte excretion, 625 μg of P-DHP produced very slight sodium excretion and potassium retention, and when administered simultaneously with 5 μg desoxycorticosterone acetate (DCA), no anti-DCA effect was evident.

Results of these studies demonstrate that the formation of cyclic ketals of DHP yields progestationally active derivatives from the inactive parent compound. A similar phenomenon has been previously observed with the 17α esterification of 17α-hydroxyprogesterone (4). However, 6α-methylation does not enhance progestational potency in the 16α, 17α cyclic ketals of DHP with the same consistency as is seen with 17α-acetoxy progesterone(8). Thus, in contrast to the additive corticoid effects of various enhancing groups introduced into the hydrocortisone molecule, such as 9α-fluoro, Δ^1, 16α-methyl, 6α-fluoro, and 16α, 17α cyclic ketals and acetals(11), there appears to be no general additive effect of the groups which enhance progestational activity. From the wide range of active structures reported, it would seem that a more complex relationship exists between chemical structure and progestational activity than is seen between structure and activity in the corticoid series.

Summary. The 16α, 17α acetophenone (P-DHP) and acetone (A-DHP) derivatives of 16α, 17α-dihydroxyprogesterone (DHP) and their 6α-methyl, 6β-methyl, 6α-fluoro and 6β-fluoro analogues were assayed for parenteral and oral progestational activity. P-DHP and A-DHP are equal in parenteral activity, each being about twice as potent as progesterone. P-DHP is strongly active orally, being 1 to 2 times as active as norethisterone, whereas A-DHP has only weak oral activity. Parenteral activity of P-DHP or A-DHP was not altered by 6-methylation. Oral activity of 6β-methyl-P-DHP was similar to P-DHP but both 6α and 6β-methyl-A-DHP were more potent orally than A-DHP. 6α fluorination had little or no enhancing effect on either parent compound when parenterally administered but did re-

duce oral potency. 6β fluorination made both parent steroids inactive by either route of administration. P-DHP and A-DHP have longer duration of activity than progesterone, moderate progestational effects still being present 25 days and 20 days, respectively, following a single 10 mg intramuscular injection. P-DHP is devoid of inherent androgenic, anti-androgenic, estrogenic, anti-DCA or corticoid activities.

1. Inhoffen, H. H., Holweg, W., *Naturwissenschaften,* 1938, v26, 96.

2. Hertz, R., Tullner, W., Raffelt, E., *Endocrinology,* 1955, v54, 228.

3. Saunders, F. J., Edgren, R. A., Drill, V. A., *ibid.,* 1957, v60, 804.

4. Junkmann, K., *Arch. Exp. Pathol. u. Pharmakol.,* 1954, v223, 244.

5. Kessler, W. B., Borman, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 820.

6. Fried, J., *Ann. N. Y. Acad. Sci.,* 1955, v61, 573.

7. Fried, J., Kessler, W. B., Borman, A., *ibid.,* 1958, v71, 444.

8. Babcock, J. C., Gutsell, E. S., Herr, M. E., Hogg, J. A., Stucki, J. C., Barnes, L. E., Dulin, W. E., *J. Am. Chem. Soc.,* 1958, v80, 2904.

9. Ringold, H. J., Batres, E., Bowers, A., Edwards, J., Zderic, J., *ibid.,* 1959, v81, 3485.

10. Bergstrom, C. G., Nicholson, R. T., Elton, R. L., Dodson, R. M., *ibid.,* 1959, v81, 4432.

11. Fried, J., Borman, A., *Vitamins and Hormones,* 1958, v16, 303.

12. Reerink, E. H., Schöler, H. F. L., Westerhof, P., Kuerido, A., Kassenaar, A. A. H., Diczfalusy, E., Tillinger, K. C., Nature, 1960, v186, 168.

13. Fried, J., Sabo, E. F., Grabowich, P., Lerner, L. J. Kessler, W. B., Brennan, D. M., Borman, A., *Chemistry and Industry,* 1961, in press.

13a. Fried, J., Guiducci, M. A., Diassi, P. A., Sabo, E. F., Basco, I., Grabowich, P., *ibid.,* 1961, in press.

14. McPhail, M. K., *J. Physiol.,* 1934, v83, 145.

15. Hershberger, L. G., Shipley, E. G., Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 175.

16. Rubin, B. L., Dorfman, A. S., Black, L., Dorfman, R. I., *Endocrinology,* 1951, v49, 429.

17. Allen, E., Doisy, J., *J. Am. Med. Assn.,* 1923, v81, 819.

18. Pabst, M. L., Sheppard, R., Kuizenga, M. H., *ibid.,* 1947, v81, 55.

19. Kagawa, C. M., Shipley, E. G., Meyer, R. K., PROC. SOC EXP. BIOL. AND MED., 1952, v80, 281.

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Absorption of Endogenous Pyrogens by Bentonite.* (26297)

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Animals made febrile with bacterial endotoxins and myxoviruses have a substance in the circulation which is capable of producing fever when passively transferred to normal and tolerant animals(1,2). This substance which has been termed endogenous pyrogen (E.P.), is biologically distinct from the initial pyrogenic stimulus(3). E.P. has also been demonstrated in serum of animals with experimental pneumococcal(4), streptococcal(4), and staphylococcal infections(5), and in sensitized animals challenged with tuberculoprotein(6). The E.P. found in serum shares many of the biological properties of peritoneal exudates or leukocytic extracts which are also capable of producing fever (7). On this basis it has been suggested that E.P. is a product of injury to leukocytes by pyrogenic stimuli(3).

The chemical constitution of E.P. has not been determined, although recent evidence suggests that it is a protein(8). It is also not known whether endogenous pyrogens found following a variety of thermogenic stimuli are biochemically identical. One of the difficulties in answering these questions is that identification of E.P. rests solely in its ability to produce fever in animals. It seemed reasonable therefore to attempt to correlate the appearance of E.P. with the ap-

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