TABLE II. Adrenal Depressant Activity of 6-Methylated Steroids in Rats.

Compound	Test	Dose (mg)	N	Adrenal wt (mg)
Oil	1	—	8	50.
	2	—	6	59.1
Progesterone	1	1	8	53.8
6α-CH_3-Progesterone	2	1	5	33.4*
6-CH_3-6-Dehydroprogesterone	2	1	6	53.5
17-Acetoxyprogesterone	1	1	8	57.4
6α-CH_3-17-OAc-Progesterone	1	.3	9	34.8*
	1	1	8	23.2*
6-CH_3-6-Dehydro-17-OAc-prog.	1	1	8	23.2*
6-CH_3-6-Dehydro-17-OAc-21-fluoroprog.	1	1	9	20.6*

* Mean differs significantly from mean of simultaneous oil-treated controls ($P < .01$; Wilcoxon Rank Sum Test).

pound reduced activity while having no effect on activity of the 17-acetoxyprogesterone form. Since several active progestins lack adrenal depressant activity it appears that the 2 activities are not directly related.

Summary. Progestational, estrogen-antagonistic and adrenal depressant activities of a series of 6-methylated steroids was determined. Generally, introduction of a 6α-methyl group increased potency over the parent compound; 6β-methylation of 17-acetoxyprogesterone increased potency to a lesser extent. When the configuration of the 6-methyl group was neither α nor β, potencies generally were about the same as or slightly less than the 6α-methylated form. Exception to this was 6 - methyl - 6-dehydro-17-acetoxyprogesterone which had high oral potency. Adrenal depression was noted following 6-methylation.

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Serum Levels After Single Oral Doses of 6-(α-Phenoxypropionamido) Penicillanate and Penicillin V.* (25450)

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Isolation of 6-aminopenicillanic acid by Bachelor *et al.*(1) has made possible the preparation of new penicillins and related compounds, not easily accessible in other ways. One such new synthetic penicillin is 6 - (α-phenoxypropionamido) penicillanate.†

* Aided by grant from Nat. Insts. Health.

† Also designated α-phenoxyethyl penicillin (Syncillin, Maxipen); both the powder and the tablets consisted of a mixture of the d- and l- isomers.

The present paper deals with controlled comparison of levels of antibacterial activity resulting in serum from equivalent oral doses of this compound and of phenoxymethylpenicillin (penicillin V).

Materials and methods. The synthetic penicillin, which will be referred to in the Tables as S, was supplied as potassium salt by Bristol Labs as P152, both as sterile powder and as tablets, each containing 268 mg. Potassium phenoxymethyl penicillin (penicillin V) was supplied as powder and as tablets containing 250 mg of penicillin V by Eli Lilly & Co. Each tablet contained the equivalent of 144 mg of 6-aminopenicillanic acid. The 10 subjects of this study were healthy young men weighing 64-95 (av. 75) kg. Each was given, in rotation, 2 tablets of each of these forms of penicillin before or immediately after breakfast with interval of 3 or 4 days between doses. Because of an error in coding, all of subjects repeated the first 2 rotations so that each subject received 6 doses, of which 2 were duplicates; the 2 forms were thus given before or after breakfast twice to each of 5 subjects. Venous blood was obtained before, and again 1, 2, 4, 8 and 12 hours after each dose; those given the dose on a fasting stomach took breakfast after the 2-hour blood. Serum was separated as soon as possible and each specimen was stored in 2 tubes, kept frozen at −20°C until assayed. All specimens in each patient were assayed simultaneously by the 2-fold dilution method in beef heart infusion broth (pH 7.2), using both *Streptococcus 98* and *Staphylococcus aureus 209P*.‡ Sensitivity of these strains to 3 forms of penicillin is shown in Table I. Equal parts of a 10^{-4} dilution of an 18 hr broth culture were added to serum dilutions and incubated at 37°C for 24 hours. Results of these tests are expressed as maximum number of 2-fold dilutions of serums which completely inhibited the test organism. Total activity resulting from each dose was estimated from the area under the curve of penicillin levels and expressed as dilution hours. All second tubes of serum were delivered under code and in the frozen state to the Bristol Labs, where they were assayed by the cup-plate, agar-diffusion method using *Sarcina lutea* (ATCC 9341) as assay organism(2). Results of these tests were compared with standards of both penicillin V and of P152 and expressed as μg/ml of each agent, and total activity from each dose was estimated from the "area" under the blood level curve and expressed as μg-hrs.

‡ These assays were carried out by Clare Wilcox and Joan H. Yarrows.

TABLE I. Sensitivity of Assay Organisms to 3 Forms of Penicillin.

Penicillin	Minimum inhibiting conc., μg/ml* Strep. 98	Staph. 209P
Penicillin G	.008	.031
" V	.013	.026
P152	.031	.063

* Determined by 2-fold dilution method in broth. Each value is avg of 7 determinations done on different days; results were all either identical or varied within a single 2-fold dilution. The minimum concentration detectable by the *S. lutea* cup-plate method was 0.02 μg/ml of both penicillin V and P152.

Results of all assays (excluding those of the second of the same doses given each subject) are summarized in Table II. All control serums obtained before the doses and all serums drawn 8 and 12 hours after the doses yielded no detectable levels. Average levels obtained by any of the 3 methods with each form of penicillin given after breakfast did not differ significantly from those obtained when the same tablets were given on a fasting stomach. Likewise average peak levels obtained from nearly all doses were quite similar, the only statistically significant differences being in the antistaphylococcal and antistreptococcal activity derived from penicillin V taken fasting, which were higher than those obtained from P152 taken after breakfast. Total antistreptococcal and antistaphylococcal activity produced in serum by penicillin V after a meal was also significantly greater statistically than that of the synthetic form given either before or after breakfast, and antistaphylococcal activity of serum resulting from penicillin V after the meal was significantly greater than that obtained from P152 after the meal. The 4-hr levels after the fasting dose of P152 were significantly lower than at the same interval after penicillin V given either fasting or after

TABLE II. Serum Levels after Oral Doses of Penicillin V and 6-(α-Phenoxypropionamido) Penicillanate in Relation to Food.

A. Antistreptococcal and antistaphylococcal activity

Assay organism	Dose*	Avg No. of 2-fold inhibiting dilutions of serum‡ 1 hr	2 hr	4 hr	Peak	Avg total 8 hr activity,† dilution-hr
Strep. 98	Vb	8.1	6.8	3.9	8.2	30.4
	Sb	7.7	6.5	2.8	7.7	25.9
	Va	7.5	6.9	4.6	7.7	32.3
	Sa	7.3	6.3	3.7	7.5	27.7
Staph. 209P	Vb	6.9	5.5	2.3	7.1	21.9
	Sb	6.8	5.1	.4	6.8	15.7
	Va	6.6	5.6	3.1	6.8	24.3
	Sa	6.4	5.2	1.7	6.4	19.2

Significant differences

Strep. 98	Peaks	Vb-Sa ($P < .05$)	Staph. 209P	Peaks	Vb-Sa ($P < .02$)
	Total activity	Va-Sb ($P < .01$)		Total activity	Vb-Sb ($P < .02$)
		Va-Sa ($P < .05$)			Va-Sb ($P < .01$)
					Va-Sa ($P < .05$)
	4 hr level	Vb-Sb ($P < .05$)		4 hr level	Vb-Sb ($P < .02$)
		Va-Sb ($P < .01$)			Va-Sb ($P < .01$)

B. Serum levels based on penicillin standards in cup-plate diffusion method

Penicillin standard*	Dose*	μg/ml of serum (avg)‡ 1 hr	2 hr	4 hr	Peak	Avg total 8 hr activity,† μg-hr
V	Vb	3.0	1.0	.2	3.1	5.1
	Sb	2.8	1.1	.1	2.8	4.6
	Va	2.6	1.3	.3	2.7	5.2
	Sa	2.3	1.1	.2	2.4	3.9
S	Vb	3.2	1.2	.2	3.3	5.4
	Sb	3.0	1.2	.1	3.0	5.1
	Va	2.9	1.3	.2	2.8	5.1
	Sa	2.4	1.1	.2	2.5	4.7

None of differences are significant.

* V = penicillin V; S = 6-(α-phenoxypropionamido) penicillanate; dose was 500 mg and 536 mg, respectively, given orally as the potassium salt; b = dose given on fasting stomach, and breakfast taken after 2 hr blood; a = dose given immediately after breakfast.

† Estimated for 8 hr as "area" under curve of serum levels.

‡ Each value is avg for same 10 subjects; all control seras taken before the doses and all those obtained 8 hr after the doses yielded no detectable levels.

breakfast. No differences in levels obtained by the cup-plate diffusion method, however, achieved statistical significance.

The validity of the comparisons is attested by results of duplicate tests done after the subjects had taken the same tablets on different occasions. Average results of these duplicate tests, each done in 5 subjects, are remarkably similar, and the same was true of assays of the corresponding individual serums.

Comment. These crossover studies indicate that the new synthetic penicillin and penicillin V are absorbed in a remarkably similar manner and degree and that these forms of penicillin yield quite comparable serum levels when given in the same manner to the same individuals at different times. The differences noted, although some of them were statistically significant, are not likely to be of clinical importance.

Other observations not detailed here have indicated some differences in susceptibility of a number of strains of staphylococci to these 2 forms of penicillin and to penicillin G, but these differences, too, are not large enough to be of clinical importance, and, for the most part, are of the same order of magnitude as those shown in Table I.

Synthetic penicillin is also inactivated by penicillinase. In our tests 194 strains of penicillinase-producing staphylococci and 35 which did not produce penicillinase were

streaked on the surface of agar inseminated with *Sarcina lutea* and containing P152 in excess of minimum inhibiting concentration of the latter, in a modified Gots test for penicillinase(3). Colonies of Sarcina developed around the surface growth of all the penicillinase producing strains of *Staphylococcus aureus* in these plates in the same manner as in similar plates in which penicillins V and G were used, indicating that all 3 of these penicillins are at least qualitatively similar in their susceptibility to penicillinase activity.

Conclusions. Potassium 6-(*a*-phenoxypropionamido) penicillinate and potassium phenoxymethyl penicillin are absorbed in essentially the same manner in normal men and produce comparable levels of antibacterial activity in the serum. Both of these penicillins are qualitatively similar to penicillin G in their susceptibility to penicillinase produced by *Staphylococcus aureus.*

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B_{12} Metabolism and Multiple Sclerosis.* (25451)

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Previous reports(1-5) indicate that the metabolic pattern as well as urinary and serum levels of Vit. B_{12} may differ according to the disease state in question, such as pernicious anemia(6-8), liver disease(9-11), myelogenous leukemia(12-13), diabetes mellitus (14-15) and others. Since Vit. B_{12} has been implicated in metabolism of proteins, carbohydrates, fats and nucleic acids, it is not too surprising that alterations in its disposition by the body in diverse disease states have been observed. Vit. B_{12} has been also implicated with multiple sclerosis (MS) because of the beneficial effect of Vit. B_{12} in preventing and even reversing the demyelinizing effects of pernicious anemia. For this reason, treatment of MS with large doses of Vit. B_{12} has been investigated in many clinics with variable results(16-18). It is, therefore, of interest to ascertain (1) whether there are biochemical signs of Vit. B_{12} deficiency in subjects with MS by measuring Vit. B_{12} serum levels and glutathione (GSH) contents in the erythrocytes and (2) to determine whether such patients can handle orally or parenterally administered Vit. B_{12} as the clinically healthy subjects.

Materials and methods. The Vit. B_{12} containing $Cobalt^{60}$ had a specific activity of approximately 800 μc/mg for urinary excretion studies. Preparation of "resting" cells of *Lactobacillus leichmannii* ATCC 4799 has been described(9). *Serum.* Whole blood was obtained by venipuncture from fasting patients. Serum obtained by centrifugation was stored in frozen state until assayed. Serum Vit. B_{12} levels were determined according to the method of Skeggs, *et al.*(20). *Vit. B_{12} absorption test.* To estimate absorption of orally administered Vit. B_{12}, the Schilling procedure(6) was modified. The details have been published elsewhere(19). *Glutathione in erythrocytes.* Determination of GSH in red blood cells was carried out in duplicate aliquots of 0.5 ml of blood cell suspension by the nitroprusside test as modified by Grunert and Phillips(21-22). *Urinary excretion test.* To determine amount of injected Vit. B_{12} re-

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