acid were initially more effective than p-aminobenzoic acid in allowing *L. mesenteroides* to proliferate in the absence of serine. However, it is clear that with prolonged incubation a maximal level of growth was attained with p-aminobenzoic acid. The simplest explanation is that *L. mesenteroides* possesses the necessary pathways for converting p-aminobenzoic acid to an active form of folic acid.

The studies with washed suspensions indicated more directly that the folic acid compounds actually affected the synthesis of serine by *L. mesenteroides*. In addition, the results shown in Table II substantiated the growth studies in that leucovorin and tetrahydrofolic were found to be more effective than p-aminobenzoic acid. This discrepancy with respect to the finding of Cross(3) that leucovorin and p-aminobenzoic are of equal activity for *L. mesenteroides* (ATCC 7881) may be the result of his having used a longer incubation period (24 hr). During this period, p-aminobenzoic acid could be converted to an actvie form of folic acid. In the present study, the use of a short incubation period in conjunction with C^{14}-labeled substrate allowed for a minimum exposure of compounds to the action of enzymes present in the cells.

Summary. *Leuconostoc mesenteroides* (ATCC 8042) required a member of the folic acid group of compounds for growth in the absence of serine. p-Aminobenzoic acid, or more efficiently, 5-formyltetrahydrofolic acid and tetrahydrofolic acid, met this requirement. With washed cell suspensions, serine synthesis was followed by incorporation of C^{14}-labeled formate into serine. Over a relatively short incubation period p-aminobenzoic acid had little effect, while the reduced derivatives of folic acid effectively catalyzed the synthesis of serine.

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Potency Relationships of Various C-21 Steroids as Measured by Two Methods of Liver Glycogen Deposition. (27676)

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In the area of glucocorticoid research numerous observations have been made showing the disparity between anti-inflammatory activity in humans and laboratory animals(1, 2,3). Even more prevalent in the bioassay literature is disagreement between the estimates of adrenocortical steroid efficacy assessed by different criteria of response. One response which has shown wide divergence in steroid potency estimates from different laboratories is liver glycogen deposition(2,3,4). To determine if a relationship exists between liver glycogen deposition potencies assessed by 2 different bioassay methods, a series of 15 substituted adrenocortical steroids was evaluated at Lederle and Upjohn Laboratories.

Materials and methods. Steroids* were ad-

* Steroids kindly supplied by the Organic and Biochemical Research Sections, Lederle Laboratories, and by the Chemistry Dept., Upjohn Co.

ministered as suspensions in carboxymethylcellulose vehicle, as previously described (2,5). Suspension was accomplished by grinding steroid and vehicle together in a ground glass tissue homogenizer. Minimum of 2 assays for each steroid was performed in each laboratory. Liver glycogen deposition bioassay methodology employed in each laboratory is presented below.

A. *Upjohn Laboratories.* Male rats from the Upjohn colony (Sprague-Dawley) were adrenalectomized at a body weight of 140-160 g and fed a stock laboratory diet (Archer dog pellets) and 1% sodium chloride drinking fluid. On the morning of the fourth postoperative day, food was withdrawn. On the morning of the fifth day the drinking fluid was withdrawn and the animals were given the test compound by a single subcutaneous injection of 0.5 ml of suspended steroid in appropriate dosage. Seven hours later the rats were anesthetized by intraperitoneal injection of 1 ml of 1% sodium cyclopal, livers removed, weighed, and digested. The anthrone color reaction(6) was used to estimate glycogen.

B. *Lederle Laboratories.* Immature male rats of the Sherman strain, weighing 40-60 g, were bilaterally adrenalectomized. Animals were maintained with Purina Lab Chow and 1% sodium chloride drinking fluid *ad libitum.* Twenty-four hours following adrenalectomy, the rats were injected subcutaneously with graded doses of steroid suspended in 0.2 ml of vehicle and received daily injections for 4 days subsequently. They were fasted 15 hours prior to and 7 hours after the last injection to insure maximum depletion of liver glycogen. Rats were anesthetized with sodium pentobarbital, livers excised and hydrolyzed in 30% KOH. Liver glycogen was measured by the anthrone method(6).

Statistical treatment of data for calculation of potency estimates was similar in the 2 laboratories. Geometric average of the several estimates of relative potency along with the combined 95% confidence limits were calculated for each compound. The normal regression line and 95% confidence limits were calculated by the method outlined in Snedecor(7).

Results and discussion. Each of the 15 steroids possessing varying potencies was compared with hydrocortisone for liver glycogen deposition at both laboratories (Table 1). With the exception of prednisolone, the relative steroid potencies recorded by Upjohn exceeded those recorded by Lederle. Why liver glycogen deposition potencies for prednisolone compare favorably from different laboratories, regardless of methodology, remains unresolved. Tolksdorf has claimed (8) that "among synthetic steroids, only prednisolone and prednisone maintain perfect correlation between all test parameters and therapeutic activity."

Regression of relative steroid liver glycogen deposition potency values determined in the 2 laboratories is shown in Fig. 1. The only point which falls beyoand the 95% confidence limits is that recorded for 6α-methyl-9α-fluorohydrocortisone. Reason for this discrepancy is not known. Potency as determined by the method used at the Upjohn Laboratories is 1.3 times the value found in the method used at the Lederle Laboratories raised to the 1.4 power, or potency (Upjohn) $=$ 1.3 (potency [Lederle])$^{1.4}$. The 1.4 power differs very significantly from 1.0 (P $=$

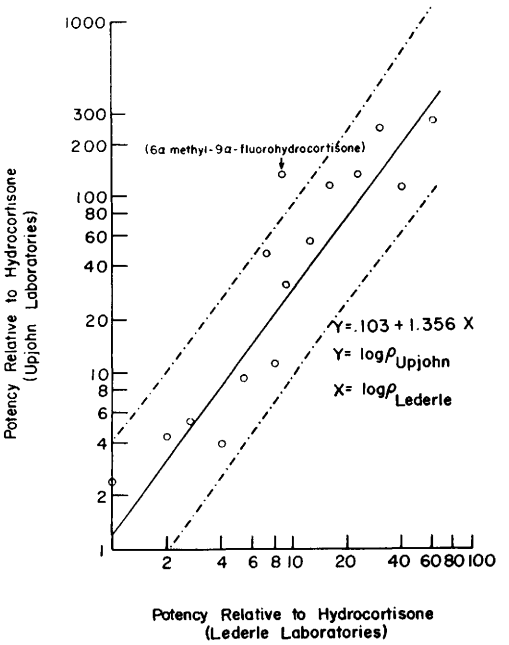

FIG. 1. Relationships of adrenocorticosteroid liver glycogen deposition potencies.

TABLE I. Comparative Liver Glycogen Deposition Relative Potencies.

Steroid	Potency relative to hydrocortisone		Order of activity	
	Lederle	Upjohn	Lederle	Upjohn
Prednisolone	4.0 (2.9-5.7) [2]	3.9 (3.7-4.8) [4]	4	2
9α-fluorohydrocortisone acetate	6* (5-8) [2]	9 (8-11) [3]	5	5
9α-fluoroprednisolone	13 (8-22) [2]	55 (46-65) [3]	10	9
16α-hydroxy-9α-fluoroprednisolone	7 (7-8) [12]	47 (38-59) [2]	6	8
16α-hydroxy-9α-fluoroprednisolone acetonide	32 (16-39) [5]	242 (197-294) [2]	13	14
16α-methyl-9α-fluoroprednisolone	63 (49-81) [3]	265 (231-307) [2]	15	15
2α-methyl-9α-fluorohydrocortisone acetate	10 (8-11) [2]	31 (26-38) [2]	9	7
2α-methyl-16α-hydroxy-9α-fluorohydrocortisone	2.7 (2.2-3.3) [3]	5 (4-7) [2]	3	4
6α-methyl-9α-fluorohydrocortisone	9 (7-12) [2]	134 (114-158) [2]	8	13
6α-methyl-16α-hydroxy-hydrocortisone	1.0 (.8-1.5) [3]	2.3 (1.8-2.9) [2]	1	1
6α-methyl-prednisolone	8 (5-10) [2]	11 (11-12) [8]	7	6
6α-methyl-16α-hydroxy-prednisolone	2.0 (1.2-3.4) [2]	4.3 (3.5-5.3) [2]	2	3
6α-methyl-16α-hydroxy-prednisolone acetonide	24 (20-30) [2]	132 (103-167) [2]	12	12
6α-methyl-9α-fluoroprednisolone acetate	16 (13-20) [2]	115 (94-140) [2]	11	11
6α-9α-difluoro-16α-hydroxy-prednisolone	42 (32-55) [5]	112 (88-143) [2]	14	10

[ ] No. assays. ( ) 95% confidence limits. * Free alcohol.

<0.01). Thus, the degree of divergence is a direct function of the steroid liver glycogen deposition potency; the greater the potency, the greater the difference in the activity estimated by the 2 assay methods. For example, steroids with potencies of 1 relative to hydrocortisone (by the Lederle assay) are approximately 1.3 times more active when tested by the Upjohn Laboratories method, and those with a potency of 15 by the Lederle test are about 50 when tested by the Upjohn assay.

Qualitatively, the liver glycogen deposition responses to various steroid modifications are comparable. When a certain structural modification caused potency to increase or decrease as measured in one laboratory, it was found to change in the same direction in the other laboratory.

These data show that relative liver glycogenic potency values vary with the test method. Several differences exist between the 2 methods(2,5), any one or combination of which could contribute to the disparity in results. It is not unlikely that the strain and weight of rats, as well as the number and time sequence of steroid injection, are important factors in accounting for this difference.

It has been shown, therefore, that even though potencies determined by 2 different assay procedures were widely divergent, it has been possible to show a consistent mathematical relationship between the sets of values. It is also possible that by studies of this nature predictable interrelations of potencies found between different endpoints as measured in one species, as well as differences between species, may be realized.

Summary. Each of 15 steroids possessing various chemical modifications were compared with hydrocortisone for liver glycogen

deposition activity in both Lederle and Upjohn Laboratories. Variations in potency relative to hydrocortisone can be reconciled by the formula: potency (Upjohn) = 1.3 (potency [Lederle])$^{1.4}$. Thus, results obtained in one laboratory become meaningful when compared to those in the other. Though the steroid potencies differ from laboratory to laboratory, the qualitative changes in activity induced by a given structural permutation are consistent in each laboratory.

The authors gratefully acknowledge the assistance of members of the Dept. of Metabolic Chemotherapy, Lederle Laboratories and the Dept. of Endocrinology, Upjohn Co., in the conduct of these studies. Appreciation is extended to Mr. J. Haynes, Lederle Laboratories, for statistical evaluation of these data.

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Renal Clearance of Polythiazide. (27677)

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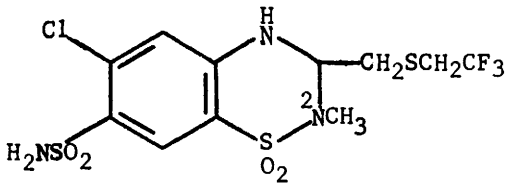
Polythiazide, 2-methyl-3-(β,β,β-trifluoroethyl-thiomethyl) - 6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine, 1, 1-dioxide is one of the recently developed benzthiadiazine type diuretics(1,2,3). In rats and dogs this drug was found to be a highly potent diuretic and antihypertensive agent with prolonged duration of action. Clinical studies confirmed these observations(4,5,6). Further studies were recently undertaken to investigate the mechanism of renal excretion of polythiazide. The results of these studies are described here.



Methods. Polythiazide was labeled with C^{14} in 2 position as described by Pinson *et al.* (7). Renal clearance of C^{14} labeled polythiazide was studied in dogs anesthetized with sodium pentobarbital (25 mg/kg i.v.). The animals were infused with either 5% dextrose or 0.9% NaCl or 0.15M sodium bicarbonate at the rate of 0.4 ml/kg/min. The 5% dextrose and 0.9% NaCl solution was infused for an hour and one-half prior to the creatinine prime (0.2 g/kg, i.v. followed by creatinine infusion at 1.2 mg/kg/min). One hour later, 2 control creatinine clearance periods, 20 minutes each, were collected. C^{14} labeled polythiazide was then given intravenously followed by an infusion of solution containing drug. Two or more renal clearance periods of 20 minutes duration followed administration of the drug. The infusion of 0.15M sodium bicarbonate solution differs only from that of the 5% dextrose infusion in that a priming dose (10 ml/kg/min for 10 minutes) of 0.15M sodium bicarbonate was given at the beginning of experiment and was immediately followed by creatinine prime and infusion at the same dose (0.2 g/kg and 1.2 mg/kg/min respectively) and the same rate (0.4 ml/kg/min) as in experiments with dextrose solution. The concentration of C^{14} labeled polythiazide