considerable overlap between the groups. Thyroid weights of intoxicated males were similar to the controls; the same was true of the females.

Discussion. These findings show that lead intoxication can impair the uptake of I^{131} by the thyroid gland *in vivo* and are in agreement with Slingerland's(7) *in vitro* demonstration that lead salts impair the uptake of iodine by thyroid slices. Studies in the human(2) have shown that the decreased I^{131} uptake can be increased in some individuals by administration of thyroid-stimulating hormone (TSH). In addition, pituitary TSH reserve has been found to be decreased. These findings suggest that the abnormalities are secondary to toxic effects of lead on the metabolic processes of cells, both at the pituitary and thyroid level. Studies of the effect of lead on sulfhydryl enzymes in the Krebs cycle are compatible with this idea. Lead apparently combines with sulfhydryl groups, thus inhibiting the enzymes(8). In addition to blocking sulfhydryl enzymes, lead may also displace iodine from a protein sulfenyl iodide carrier proposed by Cunningham(9). This would effectively inhibit the uptake of iodine by the gland and impair the iodination of thyrogloblin.

Summary. *In vivo* depression of I^{131} uptake by the rat thyroid, and the conversion ratio has been produced by chronic lead intoxication. Female rats are more susceptible than male rats.

The authors would like to thank Dr. William J. Darby for his encouragement and suggestions.

The opinions are those of the authors and do not necessarily reflect the opinion of the U.S. Navy.

1. Crutcher, J. C., Ann. Int. Med., 1963, v59. 707.
2. Sandstead, H. H., Brill, A. B., Arias, L., Terry, R. T., Tinnell, L. E., Fed. Proc., 1966, v25, 688.
3. Porritt, N., Brit. Med. J., 1931, v2, 92.
4. Kremer, H. V., Martin, F. N., Ann. Int. Med., 1955, v42, 1130.
5. Prasad, A. S., Doscherholmen, A., Flink, E. B., Am. J. Clin. Path., 1957, v28, 50.
6. Parker, R. H., Beirwaltes, W. H., J. Clin. Endocrinol., 1961, v21, 21.
7. Slingerland, W., ibid., 1955, v15, 131.
8. Bair, H., Bassler, K. H., Lang, K., Arch. Exp. Path, Pharm., 1956, v229, 495.
9. Cunningham, L. W., Biochemistry, 1964, v3, 1629.

Received June 15, 1966. P.S.E.B.M., 1967, v124.

Renal Excretion of Some Isomeric Hexoses in the Dog.* (31657)

R. WOOSLEY† AND K. C. HUANG
Department of Pharmacology, University of Louisville School of Medicine, Louisville, Ky.

It is well known that D-glucose and D-galactose are actively reabsorbed by the renal tubules and that this reabsorption can be blocked by phloridzin(1). The renal excretion of other hexoses, especially the L-isomers, has not been studied. The literature reveals that L-glucose and L-galactose may behave quite differently from the D-isomers of these hexoses. The L-forms were not transported across the intestinal mucosa(2,3) and moved across the membranes of human erythrocytes by a process of simple diffusion (4).

The glucose analogue, 3-O-methylglucose (3MG), has been found to be actively absorbed from the intestinal lumen by the same mechanism as D-glucose(3). The behavior of this compound with respect to renal excretion has not been studied. It is the purpose of this investigation to study the renal excretion of some hexose isomers in an effort to determine: 1) if the renal tubules treat these compounds in the same manner as D-glucose; 2) and if processes other than filtration are involved in the excretion of these compounds.

Experimental. Technique. I. The close arterial injection technique of Chinard(5) was

* This work was supported in part by USPHS grant AM-02217-08 and Nat. Science Foundation grant GB 4774.

† Predoctoral fellow of USPHS (1-Fl-GM-31, 135-01).

chosen: a) to reduce the amounts of the compounds used because of their limited supply; b) to minimize the biotransformation, if any, of the injected sugar in the body. Mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg. After the ureters were catheterized and the external jugular vein cannulated, an infusion of Ringer's solution was begun. Diuresis was induced by adding a 20% mannitol solution to the infusion fluid until the urine flow reached 5 ml/kidney/min. Then, the left kidney and its renal artery were exposed by a lateral incision. Two to three ml of a solution containing 100 mg/ml each of inulin and test sugar were injected directly into the renal artery. After the injection urine samples of 20 seconds duration were collected from each ureter for 5 consecutive minutes. Volume was determined by weighing the tared collection vials. The detail of the technique was the same as described by Chinard(5).

II. The steady-state technique was used for L-glucose and 3MG. A small dog was anesthetized with 25 mg/kg of sodium pentobarbital. Inulin, mannitol and test sugar (with corresponding ^{14}-C labelled sugar added as a tracer) were given intravenously in a priming dose followed by a sustaining infusion at a constant rate. Ten minutes were allowed for the compounds to approach equilibrium in the body and urine from both ureters was collected for 15-minute periods. Blood samples were taken from a leg artery at the beginning and end of each urine collection. Details of the experiment were the same as described elsewhere(6).

Materials. L-glucose, L-fucose and their D-isomers were purchased from Nutritional Biochem. Corp. (Cleveland). L-galactose was kindly supplied by Dr. H. S. Isbell of the U. S. Department of Commerce. 3MG and ^{14}-C-fucose were purchased from Calbiochem. Co. (Calif.). The ^{14}-C-L-glucose and ^{14}C-3MG were prepared by New England Nuclear Corp. (Mass.).

Analysis. All sugars were determined by the method of Nelson(7). The glucose oxidase method was used to detect the amount of D-glucose which might have appeared in the urine samples after the arterial injection of another sugar or after the infusion of phloridzin. Inulin was determined by the method of Roe *et al*(8) and in several experiments ^{14}-C-inulin was used as tracer. Results from the radioactive analysis corresponded very closely with the chemical determination. The determination of radioactivity was made by adding 0.2 ml of either urine sample or plasma to 10 ml of scintillation fluid(9) which was then counted with a Nuclear-Chicago Liquid Scintillator unit. The efficiency of the counting was calculated by the channel ratio method. The data were calculated according to Chinard as follows:

$$W_c = \text{corrected percent excretion} = \frac{\text{Amt of substance excreted in the experimental kidney} - \text{Amt excreted in control kidney}}{\text{Amount of substance injected}} \times 100$$

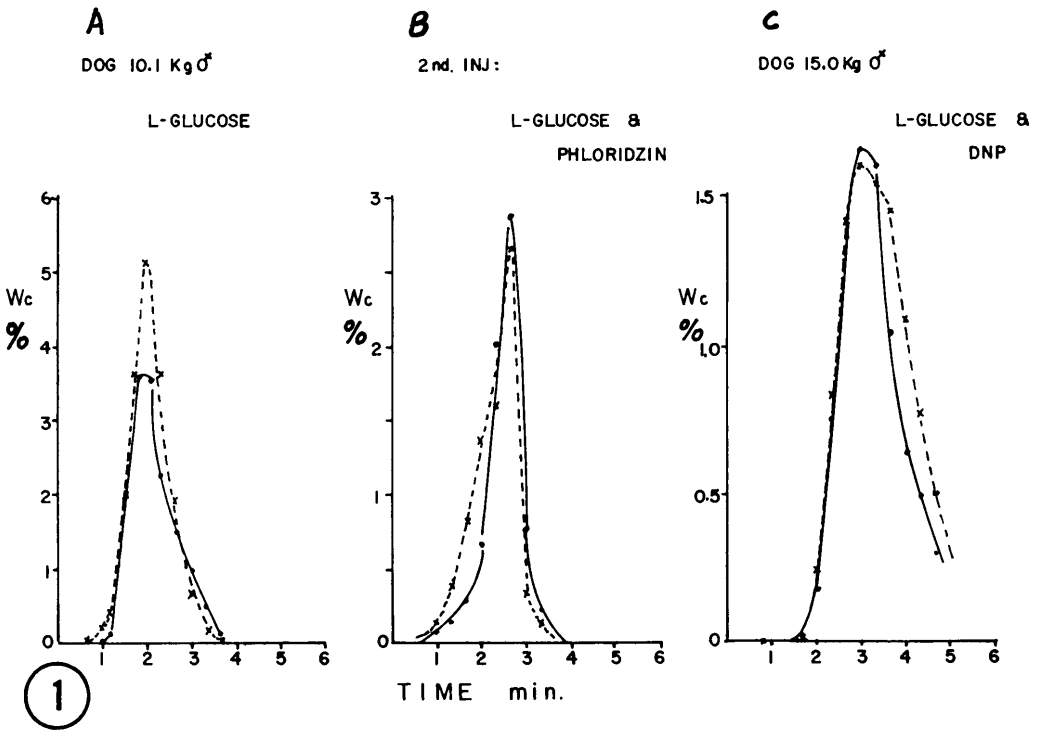
$$\Sigma W_c = \sum_{n=1}^{\infty} W_{c_1} + W_{c_2} + W_{c_3} + \cdots W_{c_n}$$

In the steady-state experiment urine samples and plasma filtrate of cadmium sulfate were subjected to thin-layer chromatographic separation, with butanol-acetic acid-water (60:20:20) as solvent(10). A single spot for each test sugar was observed, of Rf value identical with the standards. In order to be counted the spot was scraped from the plate and delivered carefully into 10 ml of scintillation fluid.

Results. A total of 28 experiments on the renal excretion of hexoses isomers was performed by the close arterial injection technique. Fig. 1 presents a typical result showing the renal excretory pattern after injection of L-glucose in the absence or presence of inhibitors. It can be seen here that the fraction of the injected L-glucose that appeared in the urine from the left kidney was greater than the fraction of inulin. Infusion of either 5 μmol/kg phloridzin or 2,4-dinitrophenol (DNP) reduced the amount of the injected L-glucose excreted in the urine to a level close to that of inulin.

Fig. 2 presents a typical result from studies of the renal excretory pattern of L-galactose and L-fucose. Both were excreted in greater amounts than was inulin.

Fig. 3 presents a typical result from ex-


A
DOG 10.1 Kg ♂
L-GLUCOSE
B
2nd. INJ:
L-GLUCOSE &
PHLORIDZIN
C
DOG 15.0 Kg ♂
L-GLUCOSE &
DNP
Wc
%
TIME min.
1

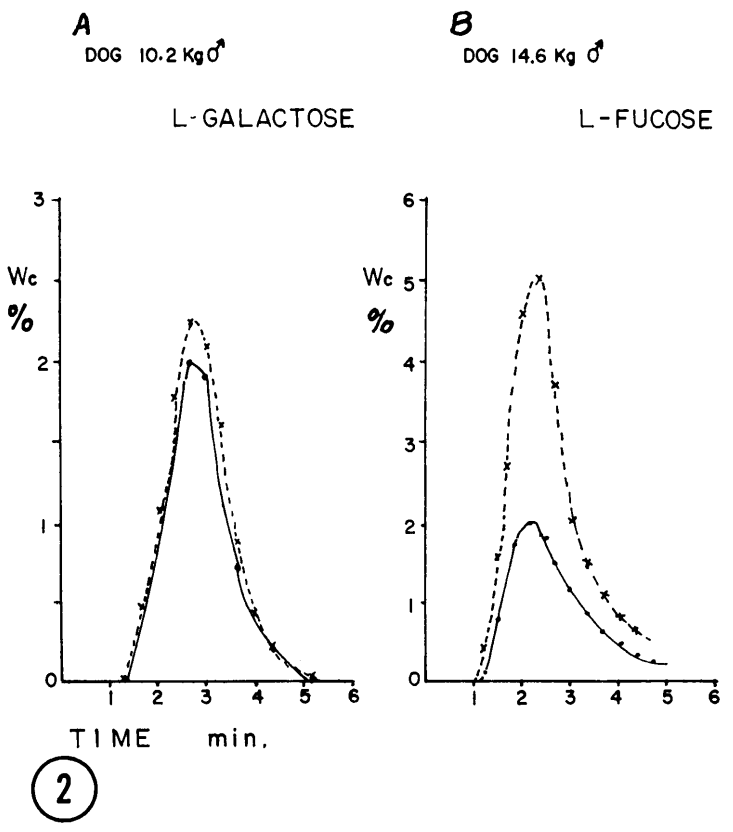

A
DOG 10.2 Kg ♂
L-GALACTOSE
B
DOG 14.6 Kg ♂
L-FUCOSE
Wc
%
TIME min.
2

FIG. 1. A. Renal excretion pattern of L-glucose (dotted line) and inulin (solid line); B. During infusion of phloridzin (5 μmol/kg given within 20 min); C. During infusion of 2,4 DNP (5 μmol/kg given within 20 min). Each sample represents a collection of 20 sec.

FIG. 2. A. Renal excretion pattern of L-galactose (dotted line) and inulin; B. Renal excretion pattern of L-fucose and inulin. Each sample represents a collection of 20 sec.

periments on the renal excretion of 3MG and L-mannose. The result obtained using ^{14}C-3MG was identical to that obtained by chemical analysis. These studies showed that the excretion of 3MG and L-mannose was greater than the excretion of inulin.

Table I summarizes the studies on the renal excretion of 3MG and the L-hexoses as well as their D-isomers by the close arterial injection technique. The results are expressed as the ratio of the sum of fractions of test sugar excreted to the sum of fractions of inulin excreted. The ratio is greater than unity for all L-hexoses and 3MG. The ratio is less than unity for D-glucose, D-galactose and D-fucose.

Four experiments were done on the renal excretion of L-glucose and 3MG by the steady-state technique. T.L.C. analysis showed no trace of metabolic product being formed. The results, summarized in Table II, show that both compounds gave a positive T value, indicating tubular secretion. The clearance ratio (C_{Hexose}/C_{IN}), was above one but less

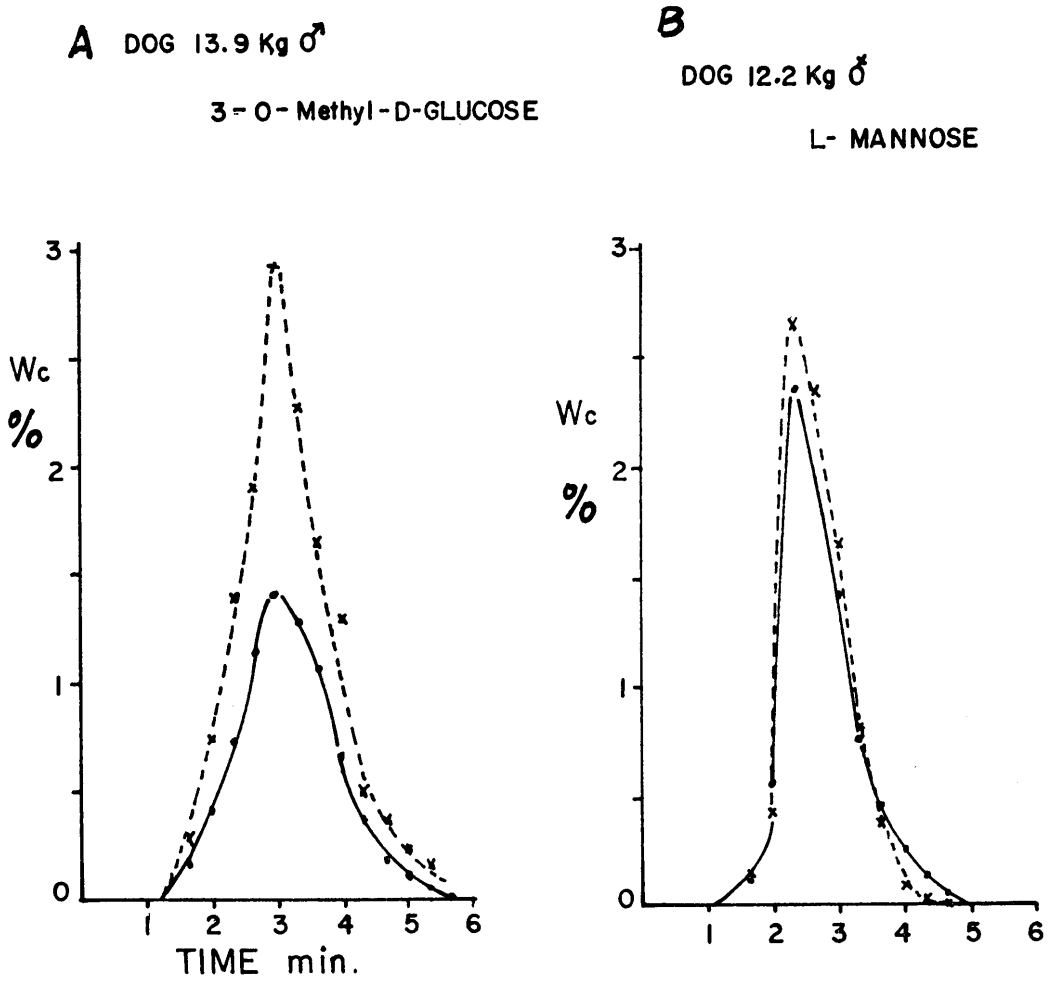


FIG. 3. A. Renal excretion pattern of 3MG (dotted line) and inulin. B. Renal excretion pattern of L-mannose and inulin. Each sample represents a collection of 20 sec.

TABLE I. Summary of Studies on Renal Excretion of Sugar Analogues by the Close Arterial Injection Technique.

D-hexose	Ratio ($\Sigma WC_H/\Sigma WC_{IN}$)	L-hexose	Ratio ($\Sigma WC_H/\Sigma WC_{IN}$)
D-glucose	.49	L-glucose	1.25 ± .18 (5)*
D-galactose	.92 ± .06 (4)	L-glucose + phloridzin	1.00, 1.02
D-galactose + phloridzin	.9 ± .1 (4)	L-glucose + DNP	1.15, 1.03
D-fucose	.93	L-galactose	1.08
3-O-methyl-D-glucose	1.78, 1.39	L-fucose	1.69 ± .42 (3)
		L-mannose	1.05

* Mean ± S.D., number in parentheses indicates number of experiments.

TABLE II. Summary of Renal Excretion of L-Glucose and 3MG by Steady-State Technique.

Hexose	Dog wt, kg	Dose given: Prime, mg/kg	Dose given: Inf., mg/min	Urine flow, ml/min	C_{IN}, ml/min	Hexose*: C_H/C_{IN}	Hexose*: UV, mg/min	Hexose*: P, mg/ml	Hexose*: T,† mg/min
L-glucose	8.0 f	10‡	1.6	1.8	16.1	1.24	1.10	.055	+.22
		20	2.4	4.47	19.1	1.13	2.74	.128	+.30
		Phloridzin 1 μmol/kg and 1% of dose/min infused							
			2.4	4.87	21.0	.95	2.41	.12	—.014
"	8.2 f	10	1.64	2.2	18.6	1.14	.932	.044	+.112
		20	3.6	3.05	11.0	1.19	2.00	.153	+.34
		Phloridzin 1 μmol/kg and 1% of that dose/min infused							
			3.6	2.05	10.8	1.03	1.61	.146	+.03
3MG	8.2 f	10	1.64	2.8	28.0	1.13	1.55	.05	+.16
		20	3.6	2.6	21.9	1.16	3.67	.144	+.51
		30	7.4	2.55	16.7	1.10	5.64	.307	+.52
		Phloridzin 1 μmol/kg and 1% of that dose/min infused							
			7.4	2.8	16.2	.99	4.60	.288	—.07
"	9.5 f	20	3.8	2.53	27.3	1.20	2.52	.077	+.426
		40	5.7	4.15	26.7	1.07	6.65	.232	+.45
		Phloridzin 1 μmol/kg and 1% of that dose/min infused							
			5.7	3.61	25.4	.94	4.48	.188	—.29

* The concentration of injected sugar in urine and plasma was analyzed by Tracer technique. TLC analysis indicated there was only one spot corresponding to standard solution.

† $T = UV - P.C_{IN}$.

‡ For each dose given 10 min were allowed to approach an equilibrium, then urine from both ureters was collected for 15 min. Arterial blood samples were taken at beginning and end of each urine collection.

than two, even at a low plasma concentration. This suggests that the tubular secretory capacity for L-glucose and 3MG is relatively small. When phloridzin was infused into the animals, the T value was reduced to a minimum or changed from positive to negative.

Discussion. Data presented here show that when L-hexoses and inulin are injected simultaneously into the renal artery, a greater fraction of injected L-hexose appears in the urine than does inulin, indicating that there is an outward transtubular movement of L-hexoses. This transtubular movement of L-hexoses, such as the movement of L-glucose, is in the direction opposite that observed for D-glucose and can be blocked by infusion of phloridzin. There are 3 possible interpretations of this result: 1) a passive exchange of the luminal endogenous D-glucose for the peritubular L-glucose; 2) an inhibition of tubular reabsorption of the endogenous glucose by the injected L-glucose; 3) an active tubular secretion. Since phloridzin did block the outward transtubular movement of L-glucose, the possibility of passive exchange seems unlikely. And since the analysis of all urine samples by the glucose oxidase method revealed that D-glucose was not present in the urines after the close arterial injection of

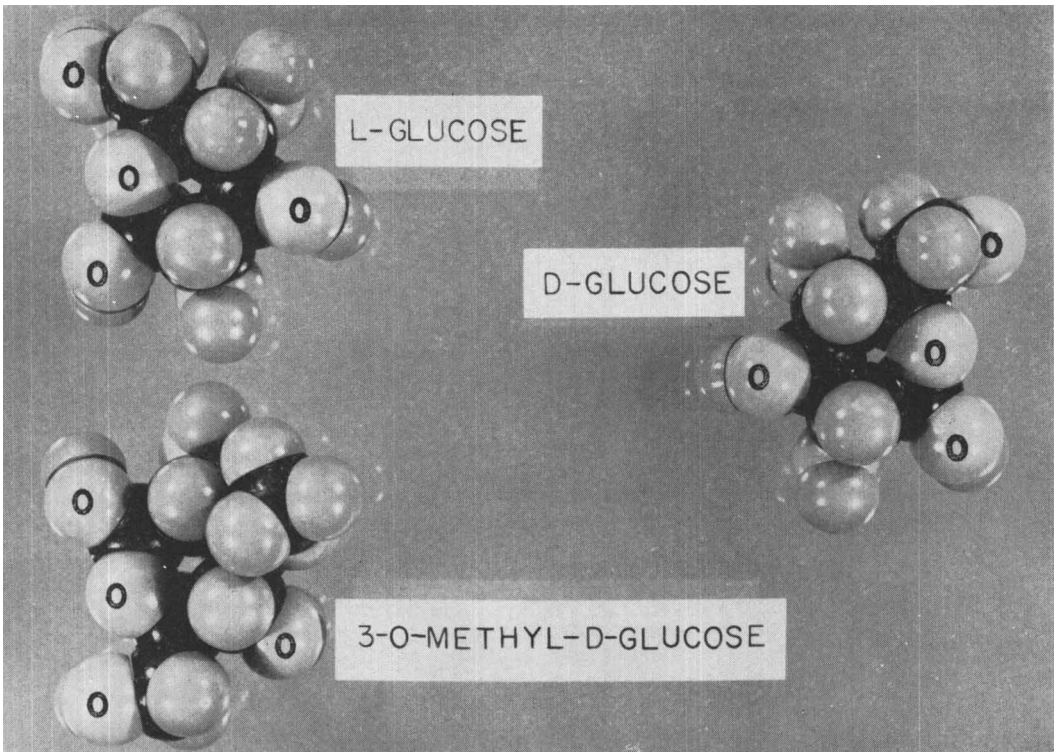


FIG. 4. Structural model of L-glucose. 3MG in comparison with that of D-glucose.

L-glucose, but was present only after phloridzin infusion, this rules out the second possibility.

There remains the possibility that 3MG, L-glucose and probably other L-hexoses such as L-galactose, L-mannose and L-fucose are actively secreted by the renal tubules.

The above conclusion on the tubular secretion of hexose isomers is further substantiated by the results from the studies with steady-state technique. Here the data show that both L-glucose and 3MG gave a positive T value, indicating that both were secreted by the renal tubules. Quantitatively, the tubular secretion of L-glucose and 3MG is relatively small in comparison with that of organic acids such as PAH or Phenol red(1). Preliminary studies with the stop-flow technique did show that the site of secretion is in the proximal tubules.‡ Furthermore, as shown in Table II, a maximum tubular transport rate for 3MG was indicated when the plasma concentration surpassed 0.1 mg/ml.

‡ Unpublished data.

The tubular secretion of L-glucose and 3MG was greatly reduced or abolished by phloridzin, resulting in a negative T value in 3 cases and suggesting that after the secretion was abolished, the compound partially diffused from the luminal fluid into the peritubular fluid due to a high U/P ratio.

We submit the following hypothesis to explain the tubular secretion of L-glucose, and probably L-galactose and 3MG. There is a carrier system for the sugar transport, residing in the brush border of the tubular epithelial cells. This carrier has a complementary structure resembling that of the hexose. D-glucose reacts complementarily with the luminal facing site of the carrier and its mirror image; L-glucose reacts with the cytoplasmic facing site. The carrier moves in the membrane as would a revolving door, releases the D-glucose into the cells and the L-glucose into the lumen. Since phloridzin acts on the sugar carrier by fixation(11) and since both D- and L-sugars are transported by the same carrier system but in opposite

directions, it is not surprising that phloridzin not only blocks the tubular reabsorption of D-glucose, but also the secretion of L-glucose.

3MG, being an analogue of D-glucose, is considered in many respects to behave similarly to D-glucose. It is transported by the intestinal mucose actively against a concentration gradient(3), and it augments the sodium influx and short circuit current across the intestinal mucosa(12,13). However, as shown in the structural model constructed in Fig. 4, 3MG possesses a configuration similar to that of L-glucose. It is therefore reasonable to predict that 3MG be secreted by the renal tubules in a manner similar to L-glucose and that this secretion also be abolished by the infusion of phloridzin.

Summary. Studies with close arterial injection technique have demonstrated that the renal excretion of 3MG, L-glucose, L-galactose, L-fucose and L-mannose was greater than that of the simultaneously injected inulin, suggesting a renal tubular secretion of these compounds. Steady-state experiments also showed that a positive T value was obtained with L-glucose and 3MG. Phloridzin abolished the tubular secretion of these compounds. It is therefore postulated that a carrier system for sugar transport is located in the brush border of renal tubules. This carrier has a complementary structure resembling that of hexoses. Its luminal site reacts complementarily with D-hexoses and the cytoplasmic facing site of the carrier reacts with the 3MG and L-hexose.

1. Pitts, R. F., Physiology of the kidney and body fluids, Year Book Med. Publisher, Chicago, 1963, p71.
2. Wilson, T. H., Landau, B. R., Am. J. Physiol., 1960, v198, 99.
3. Csaky, T. Z., Fernald, G. W., ibid., 1960, v198, 445.
4. LeFevre, P. G., Marshall, J. K., ibid., 1958, v194, 333.
5. Chinard, F. P., ibid., 1955, v180, 617.
6. Huang, K. C., JPET, 1961, v134, 257.
7. Nelson, N. J., Biol. Chem., 1944, v153, 375.
8. Roe, J. H., Epstein, J. H., Goldstein, N. P., ibid., 1949, v178, 839.
9. Bray, G. A., Anal. Biochem., 1960, v1, 279.
10. Stahl, E., Thin-Layer Chromatography, Acad. Press Inc., New York, 1965, p461.
11. LeFevre, P. G., Marshall, J. K., J. Biol. Chem., 1959, v234, 3022.
12. Current, R. F., J. Gen. Physiol., 1959, v43, 1137.
13. Schultz, S. G., Zalusk, R., ibid., 1964, v47, 1043.

Received June 30, 1966. P.S.E.B.M., 1967, v124.

Further Studies on Effects of Anabolic Steroids on the Course of Murine Muscular Dystrophy.* (31658)

LEON ZUCKERMAN,† KATALIN ST. CLAIR, AND ROBERT M. DOWBEN
Children's Cancer Research Foundation, Boston, and Biology Department, Massachusetts Institute of Technology, Cambridge

The availability of a mutant strain of mice afflicted with an hereditary disease bearing some resemblance to human muscular dystrophy has made possible studies of drug effects. The administration of the anabolic steroid, 1-methyl-Δ^1-androsten-17β-ol-3-one and some other androgenic-anabolic steroids, but not testosterone propionate, has been reported to result in increased longevity accompanied by a slowing in the rate of loss of muscle strength(1-3).

Residual muscle from dystrophic mice, like muscle from patients with the Duchenne form of muscular dystrophy, contains less potassium, more sodium and more chloride than muscle from normal mice(3,4). Dystrophic muscle appears to be "leaky" to potassium and to soluble cytoplasmic enzymes such as

* This research was supported by grants AM-08096 and AM-09715 from Nat. Inst. Health and by a grant from National Foundation for Neuromuscular Diseases.

† Present address: Research Laboratories, Evanston Hospital, Evanston, Ill.