

Studies in Diuresis with Isomannide. Sugar Alcohols XVIII.

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In previous communications the authors^{1, 2, 3} showed that isomannide (1,4,3,6 anhydromannitol) when fed or administered by stomach tube to the white rat failed to give rise to additional liver glycogen. In the feeding experiments large quantities of unchanged isomannide were observed to crystallize from the evaporated urine of animals, besides the compound was strikingly innocuous. The non-toxic nature of isomannide and its physical and physiological properties suggested its use as a diuretic. The work of Bullock, *et al.*,⁴ on the use of intravenous sucrose as a diuretic in the treatment of intracranial pressure and the work of West and Burget⁵ on the use of sorbitol as a diuretic prompted the study of the effect of isomannide on urine flow. Isomannide (Fauconnier⁶), a white crystalline solid, melts between 86° and 87°C. The compound is dextrorotatory; $[\alpha]^{20} + 91.36$. At room temperature 900 g may be dissolved in sufficient water to make a liter. In aqueous solution the molecules are not appreciably hydrated or associated as evinced by cryoscopic determinations. The pH of a 10% solution in boiled distilled water is approximately 7.2.

Diuresis in Dogs Intravenously. Animals anesthetized with nembutal (32 experiments) were prepared for urine flow determinations by cannulating the ureters with a V-shaped cannula. Urine volumes were measured. Isomannide was injected in a 50% solution on the basis of 2.5 cc per kg after a uniform flow had been established. The total volume was injected within a period of 5 minutes. Graph 1 shows the average diuresis effect from the injection of isomannide intravenously in successive doses.

Diuresis in Dogs Orally. The foregoing experiment was repeated excepting that isomannide was injected through the stomach wall

¹ Carr, C. J., Musser, R., Schmidt, J. E., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, **102**, 721.

² Carr, C. J., Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, **124**, 221.

³ Krantz, J. C., Jr., Evans, W. E., Jr., and Carr, C. J., *Quart. J. Pharm. and Pharmacol.*, 1935, **8**, 213.

⁴ Bullock, L. T., Gregersen, M. I., and Kinney, I., *Am. J. Physiol.*, 1935, **112**, 82.

⁵ West, E. S., and Burget, G. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 105.

⁶ Fauconnier, A., *Bull. Soc. Chim. Paris*, 1884, **41**, 119.

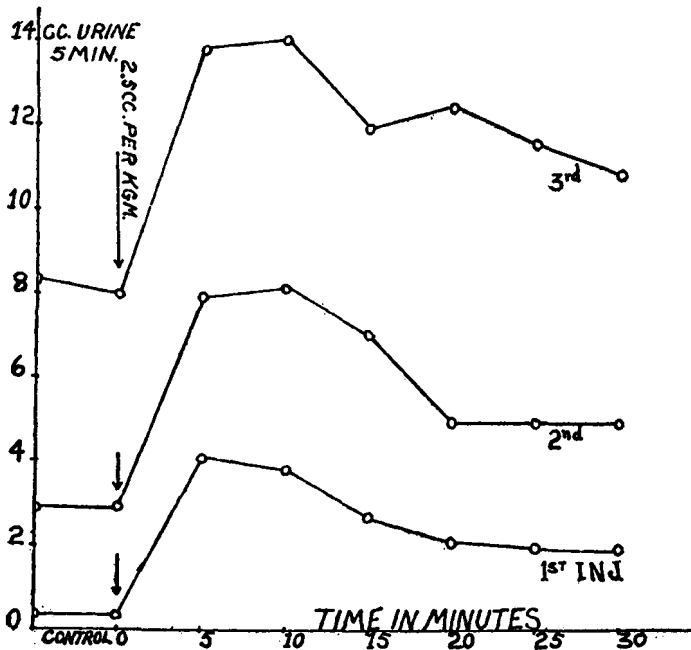


FIG. 1.
Diuresis in Dogs by Isomannide (Intravenously).

instead of intravenously. The effect of an equal volume of water administered in the same manner served as a control. Definite diuresis was produced by isomannide in 6 experiments.

Isomannide Excretion. The strongly dextrorotatory properties of isomannide permit accurate determinations polarimetrically in optically inactive urine. It was found that standard solutions of ceric sulfate in the presence of sulfuric acid quantitatively oxidized isomannide. The excess ceric sulfate reacts with potassium iodide and the residual oxidizing reagent can be titrated as free iodine with standard thiosulfate solutions. The organic non-protein constituents in the tungstic acid blood filtrate reduce also the ceric sulfate solution and corrections must be made for this factor on each animal. In the present state of refinement of this method the authors are able to determine 0.1 mg of isomannide in 0.5 cc of blood within an accuracy of $\pm 2.5\%$ of the concentration of isomannide present. The foregoing intravenous experiment on the dog was repeated (5 experiments) and the urinary and blood isomannide concentrations determined at various intervals. Graph 2 shows 2 typical experiments on the rate of clearance from the blood of isomannide by intravenous injection in the dog (2.5 cc of a 50% solution per kg).

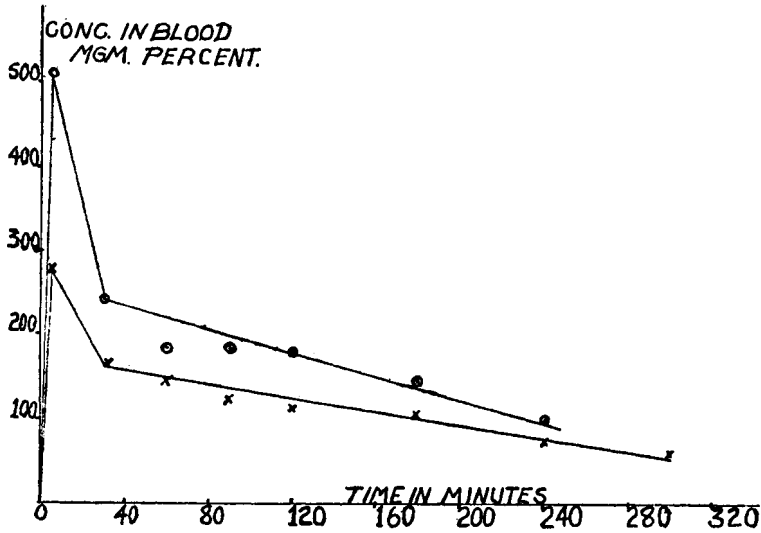


FIG. 2.

Excretion of Isomannide from Dog's Blood. Two Typical Experiments.

Man. Four normal males were given approximately the same food and water intake for 2 days. On the second day 10 g of isomannide were ingested dissolved in 50 cc of water. Urinary findings and isomannide excretion are shown in Table I. Two normal individuals were given accurately weighed diets which contained protein 75 g, fat 100 g, carbohydrate 250 g, salt 5 g, caloric value 2,500, and added water to make a total of 2 liters. Their emotional and physical behavior was similar on all 4 days. No precipitous changes in the temperature or humidity occurred during the experiment. On the third and fourth days 10 g of isomannide were administered in the morning and 3 g every 3 hours for 3 doses. Urinary findings and isomannide excretion are shown in Table I.

Toxicity. Dogs were given 2.5 cc per kg (15 experiments) of a 50% solution intravenously without demonstrable gross effects. An 11 kg monkey (*Rhesus macacus*) was given 20 cc of a 50% solution intravenously apparently without effect. In man (15 experiments) as much as 20 g have been administered by mouth without symptoms of gastrointestinal irritation. Two rats were fed a standard laboratory ration for 4 days and then 0.25 g of isomannide was added to the food of each animal each day. This was continued for 14 days. The animals did not lose weight or show toxic symptoms during this period. The animals were sacrificed and their kidneys and livers were examined grossly and histologically. There was no evidence of kidney or liver damage. The foregoing procedure was repeated

TABLE I.
Isomannide Diuresis in Man.

Subject	Urine volume, cc	Sp. Gr.	pH	Total NaCl g	Total N ₂ g	Isomannide ingested g	% excretion 24 hours
L.Y.	750	1.030	5.50	10.80	12.40	0	0
L.Y.	952	1.030	5.16	14.70	12.52	10	68.4
W.E.	805	1.024	5.78	9.20	10.17	0	0
W.E.	1000	1.022	5.85	12.20	9.95	10	57.7
H.W.	780	1.023	5.57	6.80	11.85	0	0
H.W.	1112	1.021	5.68	11.00	12.40	10	70.7
C.J.	835	1.022	6.20	10.50	8.95	0	0
C.J.	1551	1.015	6.03	11.00	8.80	10	90.0
L.Y.	840	1.024	5.91	10.81	10.53	0	0
L.Y.	620	1.026	5.42	5.10	10.45	0	0
L.Y.	886	1.028	5.48	6.05	12.95	19	74.1
L.Y.	677	1.035	5.42	4.20	11.40	19	94.5
H.W.	890	1.018	7.21	5.12	9.70	0	0
H.W.	920	1.022	6.32	5.71	9.60	0	0
H.W.	800	1.025	5.68	5.24	11.27	19	63.4
H.W.	1425	1.015	7.45	5.78	10.96	19	119.0*
Control Mean	805	1.024	5.97	8.01	10.45		
Isomannide Mean	1050	1.024	5.84	8.77	11.28		79.7

*Excretion from preceding day.

on 2 additional animals for 14 days and daily examinations were made of the urine for hemoglobin. All tests were negative.

Effect Upon Blood Constituents. Isomannide injections did not produce methemoglobin in the dog as shown by spectrophotometric analysis. Solutions of isomannide made isotonic with blood were found to rapidly hemolyze red blood cells (rabbit, dog and man). This effect, similar to urea, is not due to damage to the cell and is entirely an osmotic pressure phenomenon and not erythrolytic as shown by the fact that hemolysis is completely prevented by the presence of isotonic salt solution. In certain animals a transient hemoglobinuria is produced by massive injections of isomannide solutions. When this compound is injected dissolved in normal salt solution the production of hemoglobinuria is less frequent and appears only after repeated administration. The authors have never observed hemoglobinuria in animals, or with massive doses in man, when isomannide is given orally. The clearance from the blood of isomannide as shown in Graph 2 demonstrates the rapid diminution of the amount in the dog's blood until a concentration of the order of magnitude of 180 mg % is reached. At this point the diminution

follows a die-away curve with the slow elimination of isomannide and continued diuresis. In the dog, after the intravenous injection of isomannide solution the concentration of the compound in the urine was found as high as 10%. At this time the concentration in the blood was approximately 0.2%, which indicates the capacity of the kidneys of some dogs to concentrate this compound 50 fold. The great solubility of isomannide in water permits ready absorption from the alimentary tract in man, dogs and rats, producing diuresis rather than catharsis as does mannitol.

Summary. Isomannide when administered orally to man or by vein to dogs is excreted unchanged in the urine in large quantities producing an increase in urine volume. Isomannide when ingested in large quantities or administered intravenously produced no toxic symptoms. Like urea, isomannide permeates the red blood cells eliciting the characteristic osmotic phenomena. The diuretic activity of isomannide is likely due to its lack of tubular absorption, thus increasing the osmotic pressure of the fluid upon which the water-absorbing cells are acting and thereby increasing the volume of urine excreted.

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Sulfanilamide and Derivatives in the Treatment of Experimental Tuberculosis of Guinea Pigs.

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Sulfanilamide has been found by Rich and Follis¹ to exert a markedly inhibitory effect upon experimental tuberculosis of guinea pigs when given in 2 daily doses of 0.1 g and 0.5 g divided into 4 doses. Treatment was started 3 days before the subcutaneous injection of human tubercle bacilli. Smithburn,² however, failed to observe any beneficial effects from the daily administration of 0.2 g to guinea pigs by intraperitoneal injection 24 hours before the intracerebral inoculation of virulent human bacilli. Greey, Campbell and Culley³ have reported that sulfanilamide (Prontylin powder) in 3

¹ Rich, A. R., and Follis, B. H., Jr., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77.

² Smithburn, K. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 574.

³ Greey, P. H., Campbell, H. H., and Culley, A. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 22.