similar to those obtained in the fractionation of rabbit leukocytes. However, the specific activity of beta glucuronidase is greater in relation to acid phosphatase in human granules than in rabbit granules. The significance of this is difficult to evaluate in view of the known artifacts of these relatively crude separative procedures.

Once granules were obtained, it was possible to study conditions under which the membrane-bound acid hydrolases were released into a non-sedimentable form. It is possible that in pathologic conditions, such as the rheumatic diseases, Chediak-Higashi disease, or juvenile amaurotic idiocy (Spielmeyer-Vogt disease) abnormalities in content or release of enzymes may be found. A specific abnormality in the enzyme content of liver lysosomes has been described by Hers (8) in one form of juvenile glycogen storage disease. It should therefore now be possible to investigate abnormalities of content, or variations in the isozyme pattern, of enzymes contained in human leukocyte lysosomes.

Summary. A method has been described for isolation of human polymorphonuclear leukocyte lysosomes from 25 ml of peripheral venous blood. These granules resembled in content and behavior the granules of rabbit white cells and rat and rabbit liver. The "latent" acid hydrolases could be released from the granules by agents which have been shown to disrupt lysosomes from other tissues: streptolysins, lysolecithin, and etiocholanolone.

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Isosorbide, a New Oral Osmotic Diuretic. (30092)

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The osmotic diuretic properties of hexitols when given intravenously have been known for some time. The diuretic properties of sorbitol were reported by West and Burget (1), by Krantz and Carr(2) and by Leimdorfer(3). A large portion of mannitol given intravenously in man was recovered in the urine by Smith *et al*(4). The use of mannitol as an intravenous osmotic diuretic is mentioned by Krantz and Carr(2). Mazze and Barry(5) have refocused the attention of the clinician in the use of intravenous mannitol as an osmotic diuretic.

Hexitols such as mannitol are not clinically useful osmotic diuretics when given orally. This is because hexitols given orally do not provide the necessary blood level due to (a) slow absorption and (b) exposure to liver oxidation(6,7).

Considering other hexitols and their anhydrides, Smith *et al*(4) observed that the renal clearance in dogs of isomannide and isosorbide (sorbide) was about one-half of that of creatinine, sorbitol, mannitol, dulcitol and sorbitan. However, their report included only renal clearance data on intravenous isosorbide in the dog.

Diuretic activity of isomannide when given orally has been reported(8,9,10). During studies relating to the structure of hexitols

TABLE I. Acute Toxicity of Isosorbide.

Route	Species of animal	Sex	g/kg LD_{50}*	g/kg 0.95 limits	Slope
Oral	Mouse	♂	27.2	22.4–33.1	1.42
"	"	♀	26.8	22.5–31.9	1.32
"	Rat	♂	27.8	23.8–32.5	1.25
"	"	♀	23.9	22.3–25.6	1.10
I.V.	Mouse	♂	14.2	12.2–16.5	1.24
"	"	♀	16.1	14.0–18.5	1.22
"	Rat	♂	16.6	15.6–17.7	1.11
"	"	♀	16.1	15.1–17.1	1.09

* Calculated at 14 days for oral administration and at 7 days for intravenous injection.

and their anhydrides to their acute toxicity and metabolism, it was observed that 1,4:3,6-dianhydro-D-glucitol (commonly referred to as isosorbide) produced diuresis following both oral and intravenous routes of administration. This dihydric alcohol, which is formed by the removal of 2 molecules of water from one molecule of of D-glucitol (commonly referred to as sorbitol), has a very low acute toxicity when given either orally or intravenously to fasted male and female rats and mice. The LD_{50} with the limits and slopes as calculated by the method of Litchfield and Wilcoxon (11) is presented in Table I.

In contrast to hexitols, effective osmotic diuretic doses of isosorbide do not produce diarrhea. The approximate threshold laxative dose for isosorbide given orally to fasted rats is 17.8 g/kg. This is 10 to 20 times greater than the threshold laxative doses for hexitols and hexitans.

Because the acute LD_{50} studies on isosorbide revealed a low order of toxicity and absence of gross pathology in test animals, additional studies were undertaken to establish diuretic dose-response relationships in the rat.

Methods. Rats* (6-weeks-old weighing from 130-160 g) were used in groups of 5 males and 5 females for each of 9 dosages, calculated on 0.1 log increment. These dosages of isosorbide, given as a 50% W/V aqueous solution, were, respectively, 1.58, 2.00, 2.50, 3.15, 4.0, 5.0, 6.3, 7.9 and 10.0 g per kilo of body weight. In addition, a tenth group served as a control and received no isosorbide. Urinary volumes were measured for individual rats 3 times during a period of 24 hours prior to (—24 to —20 hours, —20 to —16 hours and —16 to 0 hours) and 24 hours after (0 to +4 hours, +4 to +8 hours and +8 to +24 hours) the oral intubation of isosorbide. Food and water were permitted *ad libitum* during both periods. The diuretic response was expressed as a series of ratios calculated from the mean post-drug urine volume obtained from 5 rats for a specified period at each dosage level and the mean pre-drug urinary volume obtained from all 50 rats of the same sex for a comparable period the preceding day.

To determine the dose-response relationships, the data relating the dose and the ratio of urinary output were analyzed in terms of a linear regression. The data were applied to the equation for a straight line ($Y = a + bX$) and the line calculated using the method of least squares, where Y = ratio of urinary volume (post/pre), X = oral dosage of isosorbide in grams per kilogram and b = slope.

Results. The data were found to fit the linear regression equation. The calculated equations of the dose-response curves relating the dose of isosorbide to the ratio of urinary output for the various time intervals are shown in Table II. The linear relationship for 0 to 4 hours is shown graphically for males and females, respectively, in Fig. 1. Diuresis was observed at the lowest dose and was increased 8-9-fold at the highest dose. There was a progressive decrease in the ratio (post/pre) of urinary output, as indicated by the decreased slope of the regression lines with an increase in elapsed

TABLE II. Equations for Dose-Response Curves.

Period	Y* = Males	Y* = Females
0 to 4 hr	1.06 + .79X	.61 + .88X
4 to 8 "	.42 + .62X	.57 + .64X
0 to 24 "	1.29 + .27X	1.23 + .29X

* Where Y = ratio of urinary volume (post/pre), X = oral dosage of isosorbide in g/kg and b = slope.

* From our own Sprague-Dawley Strain Caesarean Derived Colony of Charles River Breeding Laboratories.

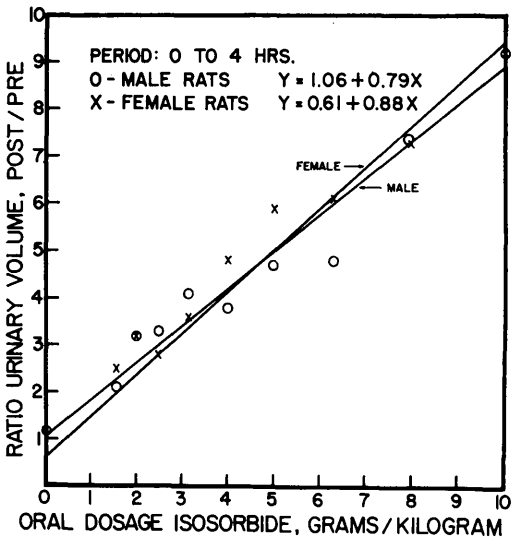

FIG. 1. Relationship between dosage and diuresis among male and female rats, respectively, given isosorbide orally.

time. However, even when considering the 0 to 24-hour period, the ratio (post/pre) of urine output was always greater than one. This indicates the peak diuretic response occurred during the first 4 hours following drug administration.

There was an increase in water intake for the 24-hour period after ingestion of isosorbide as compared to the water intake for the preceding 24 hours which was correlated to the oral dosage of isosorbide and the increased urinary output.

The diuretic activity of isosorbide is related to its metabolic fate. Tracer studies using randomly carbon-14 labeled isosorbide showed that following oral administration to male and female rats, an average of 95% to 97% of the dose administered appeared in the urine, about 2.5% in the feces and contents of the gastrointestinal tract, with approximately 0.5% remaining in the carcass and less than 0.2% being oxidized. Of the radioactivity which appeared in the urine, 96% migrated on paper at the same rate as isosorbide-C^{14} and the rest was indicated to be an ester of isosorbide.

Thirteen week sub-acute toxicity studies were carried out in 4 groups of 48 rats (24 males and 24 females). Three groups were fed diets containing 1, 5 and 10% isosorbide in the basic Purina Laboratory Chow. The fourth group served as a control and received only the basic diet. This study showed isosorbide to be non-toxic on the basis of appearance, uniform survival, normal growth, the ratio of 6 different tissue weights to that of the body weight, urine output and urine analysis, and food and water consumption. Water consumption at the 10% level was related to diuresis. Gross and histologic examination of 21 different tissues, including the kidneys, revealed no pathological alterations when compared to the controls.

Studies in mammalian species including both pharmacologic and toxicologic evaluations have been completed. Clinical studies on isosorbide in volunteers have corroborated the initial studies in animals and will be reported elsewhere.

Summary. Isosorbide has been shown to be an effective diuretic when given orally to rats. The urinary output is related to dosage with an 8- or 9-fold increase for the period of 4 hours following a dose of 10 g/kg of body weight. Based on carbon-14 studies, more than 95% of the administered dose is readily excreted largely unchanged in the urine. Because of its effectiveness and very low order of acute and sub-acute toxicity, isosorbide is considered as an orally effective osmotic diuretic.

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Brain Sphingolipids in Experimental "Allergic" Encephalomyelitis.* (30093)

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Previous work of Edgar(1) showed that the ratios of sphingomyelin to lipid-galactose of brain specimens from 2 cases of multiple sclerosis were considerably lower than the respective ratios found in 7 brains from cases without neurological disease. Since this relatively small amount of material hardly allowed definite conclusions it was thought to be of interest to study these sphingolipid constituents in experimental "allergic" encephalomyelitis (E.A.E.), even more so as they have been implicated recently to be possibly involved in the auto-immunization mechanism underlying that disease(2,3). It thus was decided to examine the ratios of sphingomyelin to lipid-galactose in brains and spinal cords of normal rabbits and rabbits with E.A.E.

Material and methods. Disease-free female albino rabbits (Huntingdon Farm, Philadelphia) of about 2.5 to 3 kg weight were used. E.A.E. was produced by intradermally injecting into the foot pad of each paw 0.2 ml aliquots of an emulsion prepared from 10 g fresh, normal spinal cord of rabbits, 750 mg dry *Mycobacterium butyricum* (from American Type Culture Collection grown in our laboratory), 5 ml Freund's adjuvant, 1 ml Arlacel (Falba), and 5 ml Bayol F. Approximately 2 to 6 weeks following the injection 60 to 80% of the animals developed severe paresis starting at the hind legs. The paretic rabbits and controls were sacrificed by intravenous injection of air. The brains and spinal cords then were removed, freed of blood and meninges, weighed, and kept frozen until lipid extractions could be carried out shortly afterwards. Some brains and spinal cords were placed into formalin for neuropathological examination.

Chemical procedures. The lipids of the brains or spinal cords were extracted with chloroform-methanol 2:1 (v/v) and then washed according to Folch *et al*(4). Thus purified lipid extracts were stored under nitrogen in the cold room until the analysis could be performed. The sphingomyelin analysis utilized the resistance of the component to mild alkaline hydrolysis(5,6). The results presented here were based particularly on the determination of the differences between the values of the total choline and the choline released from the "alkali labile" phospholipids by the hydrolysis. Sphingomyelin was calculated by multiplication of that difference, representing the value of sphingomyelin-choline, by a factor of 6.4. The choline determinations were carried out essentially by the procedure of Smits(7). The accuracy of the sphingomyelin determinations was checked by analysis of pure sphingomyelins prepared in this laboratory(8). A series of earlier ex-

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