randomly tritiated 5-fluorotryptophan to *C. purpurea* PRL 1565 was found to be largely in the mycelium and the basic extract which would contain unincorporated labeled 5-fluorotryptophan. In the alkaloid containing extract, the majority of the radioactivity was found to be concentrated in the thin-layer chromatographic spot at R_f 0.55 when developed with ethyl acetate–dimethylformamide–95% ethanol (10:1:1). The low yields of alkaloids produced by the organism prevented conventional fluoride analyses on them. The sensitivity of neutron activation analysis for fluoride was useful in indicating the absence of fluorine in the radioactive, van Urk positive material. The conclusion is that 5-fluorotryptophan was not incorporated into the ergot alkaloids by *C. purpurea* PRL 1565. Two possible explanations could account for the radioactivity of the alkaloid at R_f 0.55: tritium exchange between the tritiated 5-fluorotryptophan and tryptophan or enzymatic reduction of the 5-fluoro group followed by incorporation of the tryptophan. Doubly labeled 5-fluorotryptophan with a better alkaloid producing organism would be required to distinguish between these explanations.

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Gastrointestinal Irritant Effect of Glycerin as Compared with Sorbitol and Propylene Glycol in Rats and Dogs

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Effects of glycerin, sorbitol, and propylene glycol on the gastrointestinal mucosa were compared in rats and dogs. At equivalent, undiluted doses (ml./Kg.) in both species glycerin was more irritating than sorbitol, and propylene glycol was the least irritating of the three compounds studied. The types of irritant effects observed were qualitatively similar, and the degree of severity was dose-dependent in both species. Studies in rats showed that irritation produced by any of the three compounds was reduced by dilution of the dose.

GLYCERIN is a fairly common component of liquid pharmaceutical vehicles. However, there is little definitive information in the literature on its gastrointestinal effects. Johnson *et al.* (1) reported that ingestion of 30 ml. (approximately 0.6 ml./Kg.) of 95% glycerin with orange juice three times daily for 50 days by 14 normal humans produced no consistent changes in body weight or temperature and no overt pharmacological effects. The daily number and consistency of stools was unaffected by the quantities of glycerin fed. Subjectively, four subjects (29%) reported an occasional "sensation of warmth" in the stomach after ingestion, especially in the rare instance when glycerin was taken on an empty stomach. No enterogastric examinations were done, however, to assess gastrointestinal effects directly. These same investigators noted "no ill effects" in 11 fasted rats which received single oral doses of glycerin ranging from 7 to 18 Gm./Kg. (5.6 to 14.3 ml./Kg.). Arnschnik (2) cited "no illness" in dogs after single oral doses of glycerin up to 8 Gm./Kg. (6.3 ml./Kg.). However, at 11 Gm./Kg. orally (8.7 ml./Kg.), the same investigator reported "intestinal disturbances" and vomiting. Kobert (3) and Sollmann (4) stated that repeated ingestion of large doses of glycerin lead to a chronic inflammation of the gastrointestinal tract in man. Deichmann's review of the pharmacology of glycerin (5, 6) cited Kobert's report (3) of the accidental ingestion of 300 Gm. (about 238 ml.) of glycerin by a 2-year-old child who lost consciousness but recovered following stomach lavage. Drill (7) noted that oral administration of 100 to 300 ml. of undiluted glycerin has caused "severe symptoms" in humans, whereas the same amount diluted or mixed with food produces "little or no effect." Sroka (8), in a patient given "more than 100 grams orally" while being treated for kidney stones, observed dizziness, drowsiness, violent headache, bloody diarrhea, and kidney pains (differing from the orginal kidney pains).

The purpose of this study was twofold: (*a*) to compare the gastrointestinal irritation liability of glycerin with that of sorbitol (in aqueous solution) and propylene glycol, two other commonly used constituents of oral liquid pharmaceutical preparations; and (*b*) to determine the effect of water dilution on the gastrointestinal irritant potential of these three compounds.

METHODS

Female Charles River rats, weighing 150 to 210 Gm., and adult mongrel dogs of either sex, weighing 8.9 to 16.0 Kg., were used. The rats were housed five per cage, and the dogs caged individually, in rooms maintained at 74° F. The compounds studied (glycerin, sorbitol, and propylene glycol) were administered by stomach tube, three times

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TABLE I—GASTROINTESTINAL IRRITATING EFFECTS OF GLYCERIN, SORBITOL, AND PROPYLENE GLYCOL AFTER ORAL ADMINISTRATION t.i.d. FOR 8 DOSES TO RATS

Compd.	Dose	Concn., %	Dose, Vol., ml./Kg.	No. Animals Showing Effect/No. Tested: No Irritation	Slight Hyperemia	Severe Hyperemia	Petechial Hemorrhage	Erosions
Glycerin	0.75 ml./Kg.	100 v/v	0.75	6/10	3/10	0/10	1/10	
	1.50	100	1.50	3/10	2/10	3/10	1/10	1/10
	3.00	100	3.00	0/10	3/10	1/10	4/10	2/10
	3.00	80	3.75	2/10	0/10	2/10	5/10	1/10
	3.00	60	5.00	3/10	5/10	1/10	1/10	
	3.00	40	7.50	4/10	4/10	1/10	1/10	
	3.00	20	15.00	9/10	1/10			
Sorbitol	0.675 Gm./Kg.	90 w/v[a]	0.75	8/10	2/10			
	1.35	90	1.50	7/10	0/10	1/10	1/10	1/10
	2.70	90	3.00	5/10	0/10	2/10	3/10	
	3.00	45	6.67	8/10	2/10			
	3.00	25	12.00	9/10	1/10			
Propylene glycol	0.75 ml./Kg.	100 v/v	0.75	9/10	1/10			
	1.50	100	1.50	9/10	0/10	1/10		
	3.00	100	3.00	8/10	2/10			
	3.00	75	4.00	10/10				
	3.00	50	6.00	10/10				
Distilled water–controls	3.00 ml./Kg.	100	3.00	20/20				

[a] Sorbitol, 90% w/v in distilled water is sorbitol solution U.S.P.

daily (at 10 a.m., 1 p.m., and 4 p.m.), for 3 days. Food and water were withheld during the dosing period (from 8:30 a.m. to 4:30 p.m.) on each day but were available *ad libitum* at all other times.[1] All animals were observed for overt effects following each dose. The animals were sacrificed about 1 hr. after the second dose on the third day (after having received 8 doses). Rats were killed by a blow on the head; dogs received a lethal intravenous dose of a veterinary euthanasia agent.[2] Following sacrifice, the stomach and attached portion of the duodenum (about one-third the entire duodenal segment) of each animal were removed carefully and rinsed in tepid water. The stomach was cut along its greater curvature from cardiac sphincter through the pylorus, and the duodenum was cut longitudinally by extending the incision. The mucosal surface was examined grossly and irritated areas were observed under a binocular microscope.

The degree of irritation was graded according to the following descriptions, arranged progressively from least to most severe: *normal*, appearance of mucosa same as in water-treated controls; *slight hyperemia*, slight reddening of mucosa; *severe hyperemia*, deep reddening of mucosa; *petechial hemorrhage*, hyperemia and pinpoint submucosal hemorrhages; *erosion*, mucosal erosion with associated severe hyperemia and hemorrhage.

RESULTS AND DISCUSSION

The results of this study in rats (Table I) and dogs (Table II) clearly indicate that at equivalent undiluted oral doses glycerin was more irritating to the gastrointestinal tract than either sorbitol or propylene glycol. Chronic dosing (t.i.d. for 8 doses) in both species with glycerin at doses up to 3.00 ml./Kg. produced hyperemia, petechial hemorrhages, and erosions. These effects may be due to the hygroscopicity of the compound which, in high concentration, is somewhat dehydrating and irritating to exposed tissue (9). In contrast, undiluted sorbitol solution U.S.P. was far less irritating than equivalent doses of glycerin, and propylene glycol was relatively innocuous. Gastrointestinal irritation following oral administration of undiluted glycerin has been cited in the literature. The doses used, however, were in the toxic range. Deichmann (6) reported an acute irritation of the entire gastrointestinal tract in rats receiving lethal oral doses of undiluted glycerin. The previously cited "severe symptoms" in humans (3, 7, 8) were produced by doses in the lethal range since Dreisbach (10) estimated the fatal oral undiluted dose in humans to be 100 Gm. of glycerin (79.4 ml.). The highest dose used in the present study, 3.00 ml./Kg. of undiluted glycerin, was sublethal, and was about one-seventh of the reported oral LD_{50} in rats (11).

Thus, the irritating properties of undiluted glycerin have been observed in two species at doses below that required to produce overt toxicity.

According to Drill (7), the gastrointestinal irritating potential of glycerin in humans was reduced by dilution of the dose. Johnson, Carlson, and Johnson (1) reported a similar observation in rats and dogs. Results of the present study in rats confirm these earlier investigations. Rather severe irritation was produced by 3.00 ml./Kg. of undiluted glycerin; but when the same dose was given as 80%, 60%, 40%, or 20% dilutions in distilled water, the irritation was reduced as the concentration was decreased. Similar results were obtained with both sorbitol and propylene glycol, although the initial irritation produced by either compound undiluted was less than that produced by glycerin. It appeared that irritation was reduced to minimal

[1] Rats received Purina rat chow. Dogs received 1 lb. of Red Heart dog food daily.

[2] Marketed as Euthanasia Solution by Arnold Laboratories, New Castle, Ind. Each milliliter of the solution contains 100 mg. each of sodium pentobarbital, sodium secobarbital, and mephenesin.

TABLE II—GASTROINTESTINAL IRRITATING EFFECTS OF GLYCERIN, SORBITOL, AND PROPYLENE GLYCOL AFTER ORAL ADMINISTRATION t.i.d. FOR 8 DOSES TO DOGS

Compd.	Dose, ml./Kg.	Dog No.	Description of Stomach and Duodenum
Glycerin	0.75	1	Appeared "normal."
	1.50	2	All mucosa severely hyperemic with widespread petechial hemorrhages.
	3.00	3	All stomach mucosa slightly to severely hyperemic with several localized areas of petechial hemorrhages; duodenal mucosa mostly "normal" with scattered hyperemic areas.
		4	Several localized severely hyperemic areas containing several small erosions; duodenum appeared "normal."
Sorbitol[a] soln.	0.75	5	Appeared "normal."
	1.50	6	Most of stomach slightly to severely hyperemic; duodenum appeared "normal."
	3.00	7	Appeared "normal."
Propylene glycol	0.75	8	Appeared "normal."
	1.50	9	All stomach mucosa slightly hyperemic; duodenum "normal" except for several small, localized slightly hyperemic areas.
	3.00	10	Appeared "normal."
Distilled water—controls	3.00	11	Appeared "normal."

[a] 0.75 ml./Kg. of sorbitol solution U.S.P. = 0.675 Gm./Kg., as sorbitol. 1.50 ml./Kg. of sorbitol solution U.S.P. = 1.35 Gm./Kg., as sorbitol. 3.00 ml./Kg. of sorbitol solution U.S.P. = 2.70 Gm./Kg., as sorbitol.

levels: (*a*) when 3.00 ml./Kg. of glycerin was administered in a concentration of under 40% v/v; and (*b*) when 3.00 Gm./Kg. of sorbitol was administered at concentrations of 45% w/v, or less. Propylene glycol, even undiluted, produced only minimal irritation.

These studies in rats and dogs have shown that repeated oral administrations of high concentrations of glycerin are irritating to the gastrointestinal mucosa. This effect might be a factor for consideration in the development of vehicles for liquid pharmaceutical preparations intended for repeated administration to humans. The results suggest that additional investigations in man would be desirable to establish the possible clinical significance of our observations in animals.

SUMMARY

In this study, undiluted glycerin, administered orally to rats and dogs, produced gastrointestinal irritation to a much greater degree than did either sorbitol or propylene glycol. The degree of severity of the irritant effect of each compound was dose-dependent in both species and was reduced in rats by dilution of the dose.

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