phenomenon. Doses of 0.25 cc of whole blood or greater produce a long lasting tolerance.

1. Billingham, R. E., Brent, L., Medawar, P. B., *Nature,* 1953, v172, 603.
2. ———, *Phil. Trans.,* 1956, vB, 239, 357.
3. Ashley, F. L., Stein, H., Peterson, R., Grazer, F., Longmire, W. P., *Transpl. Bull.,* 1958, v5, 29.
4. Puza, A., Gombos, A., *ibid.,* 1958, v5, 30.
5. Billingham, R. E., Silvers, W. K., *J. Immunol.,* 1960, v85, 14.
6. Stark, R. B., Brownlee, H., Grunwald, R. D., *Ann. N. Y. Acad. Sci.,* 1958, v73, 772.
7. Stark, R. B., Dwyer, E., *Surgery,* 1960, v46, 277.
8. Mariani, T., Martinez, C., Smith, J. M., Good, R. A., *Ann. N. Y. Acad. Sci.,* 1960, v87, 93.
9. Martinez, C., Smith, J. M., Aust, J. B., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 736.
10. Felton, L. D., *J. Immunol.,* 1949, v61, 107.

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Effect of Reserpine on Peptic Ulceration and Gastric Blood Flow in Dogs.* (26508)

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The clinical use of rauwolfia alkaloids has found wide acceptance since their recent introduction to this country mainly for treatment of hypertension and certain anxiety states. However, as with many drugs, these compounds produce undesirable side effects on other physiological systems. Duodenal ulcer, hemorrhage, and perforation have been reported in patients treated with reserpine(1,2). It is not known whether patients treated with rauwolfia drugs develop gastric complications as a result of therapy, because of their disease process and emotional constitution, or a combination of these factors. We attempt in this study to elucidate some of the effects of reserpine on gastric physiology and peptic ulceration in experimental animals.

Peptic ulceration does not occur spontaneously in either dogs or cats. However, ulceration can be produced in laboratory animals by administration of histamine in beeswax(3). This method has proven useful in evaluation of surgical procedures and medications on peptic ulcer diathesis.

The first part of this study demonstrates the effect of reserpine on histamine-induced ulceration in dogs and cats. The second portion shows the effect of reserpine on gastric blood flow.

Method. A. *Ulcer provocation.* Adult mongrel dogs and cats were used in these experiments. The histamine in beeswax mixture was prepared as described by Code and Varco(3). The dogs were kept in separate cages and the cats were kept 4 to a cage. All animals received food and water *ad libitum.* Although the exact amount of food ingested was not measured, the animals receiving reserpine consumed less of the rations offered.

The animals were divided into 6 groups. Group I (9 dogs) received 30 mg of histamine in beeswax I.M. daily. Group II, (8 dogs) received 1 mg of reserpine I.M. in addition to the histamine. Group III, (8 dogs) received only 1 mg reserpine daily. Group IV (8 cats) received 15 mg histamine in beeswax daily. Group V (8 cats) received 0.5 mg of reserpine in addition to the histamine. Group VI (11 cats) received 0.5 mg reserpine only.

Each animal was injected with histamine in beeswax and/or reserpine intramuscularly at the same time each day. The dosage of reserpine used caused miosis, diarrhea, and lethargy in both dogs and cats. At time of death or sacrifice, the stomach and duodenum of all animals were examined grossly for erosion or ulceration. For the purpose of this

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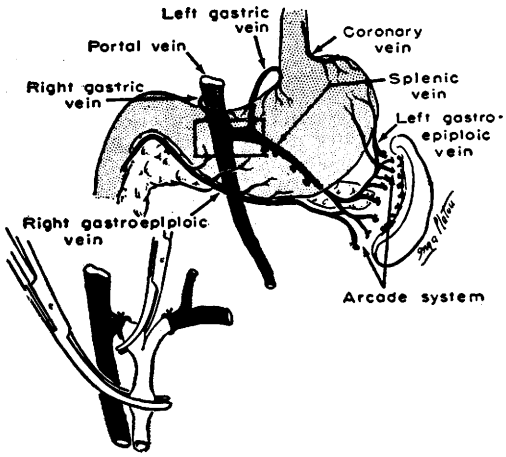

FIG. 1. Diagram of blood flow preparation used.

study "average time of ulceration" is average time of survival of those dogs that died with peptic ulcers. If any doubt existed as to their presence, microscopic sections of questionable areas were prepared and examined.

B. *Blood flow studies.* In the second portion of our experiment, mongrel dogs weighing between 10 and 15 kg were used. The animals were anesthetized with sodium pentobarbital, 30 mg/kg. The carotid artery was cannulated and arterial pressures recorded *via* a Statham transducer on a Sanborn Polyviso recorder. The abdomen was opened and the right gastric, gastroduodenal and left gastric epiploic veins were ligated and divided. Splenectomy and omentectomy were done (Fig. 1). The left gastric vein was cannulated with a polyethylene catheter which was then connected to a Y connector. A polyethylene catheter which was passed into the portal vein *via* the splenic vein was attached to the other limb of the Y connector. In this way, normal gastric flow could be maintained between measurements and gastric venous outflow could be measured by occlusion of the portal vein catheter and collection from the common limb of the Y. The dog was given 2-3 mg heparin/kg. Heparinized saline was used to irrigate the catheters. Following cannulation of the portal and left gastric veins, the dog was allowed to stabilize for 30-45 minutes.

Control values for venous outflow were established by at least 2 measurements at ½ hour intervals prior to administration of the drug. The reserpine was then given *via* the carotid catheter and venous outflow was measured at least 3 times at hourly intervals. There were 3 groups of dogs in this part of the experiment. Group I received no medication; Group II, 1 mg reserpine, Group III, 2.5 mg reserpine.

Results. A. *Ulcer provocation.* It is evident from Table I that reserpine greatly abets histamine ulceration. In fact, reserpine alone appears to be as effective an agent as histamine for production of peptic ulceration and erosions. Reserpine alone caused 50% of dogs to ulcerate in an average time of 6.5 days. The results in cats followed a similar pattern (Table II).

Another interesting finding was that when only histamine was given, ulceration occurred predominantly in the duodenum. However, when reserpine was given alone or with histamine, ulcerations occurred predominantly in the antral and fundic portions of the stomach (Fig. 2 and 3).

All the animals that died with ulceration showed evidence of weight loss, probably due to the decreased intake of food that occurs concomitantly with peptic ulceration. As stated previously, the dogs that received reserpine ate less and this may be a factor in the abetment of histamine induced ulcer.

Baronofsky has shown that nitroglycerine

TABLE I. Dogs.

Group	No. of dogs	Medication	ulceration (%)	Avg time of ulceration, days (± S.E.)*	Duration of survival, days	Type of ulcer
I	9	30 mg histamine in beeswax	44	23.0 ± 9.8	2–40	All duodenal ulcers
II	8	*Idem* + 1.0 mg reserpine	100	2.4 ± .4	1– 4	" gastric "
III	8	1.0 mg reserpine	50	6.5 ± 2.6	4–15	" " "

* p value for difference of all means = <.01.

TABLE II. Cats.

Group	No. of cats	Medication	ulceration (%)	Avg time of ulceration, days (± S.E.)*	Duration of survival, days	Type of ulcer
IV	8	15 mg histamine in beeswax	62.5	9.0 ± .9	3–12	All duodenal ulcers
V	8	*Idem* + .5 mg reserpine	89	2.1 ± .3	1– 5	" gastric "
VI	11	.5 mg reserpine	63	4.6 ± .4	3– 8	2 duodenal, 5 gastric ulcers

* p value for difference of all means = <.01.

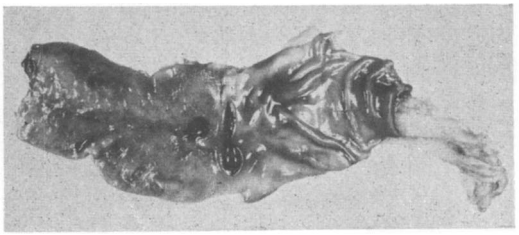

FIG. 2. Ulcer in dog.

and pitressin also abet histamine ulceration (4,5). He suggested that this was due to lowered resistance of the tissues, secondary to ischemia. Recent work has shown that reserpine sensitizes the arterioles in the splanchnic bed to circulating catechol amines(6). The occurrence of gastric rather than duodenal ulceration with reserpine administration stimulated our interest in the effect of reserpine on blood flow in the stomach.

B. *Blood flow studies.* In Group I, the control group, there was very little change in consecutive blood flow measurements (Table III). In Group II there was a slight rise in blood flow, of dubious significance. In Group III, there is a definite decrease in gastric blood flow following administration of 2.5 mg of reserpine. During all these determinations, blood pressure variations did not exceed 15-25 mm Hg.

Discussion. Under these experimental conditions reserpine greatly enhances histamine induced ulceration. Furthermore, reserpine is just as potent as histamine in induction of experimental peptic ulceration. This is in accord with the findings of others that reserpine, like histamine, evokes maximal gastric secretion in patients or Heidenhain pouch dogs(7).

Work done in our laboratory has shown that pretreatment of cats with reserpine renders the cat's esophagus much more susceptible to digestion by perfused human gastric juice(8). Determination of blood flow by the K^{42} method suggests that this increased susceptibility of the esophagus may be due to the decreased blood flow in that organ(9).

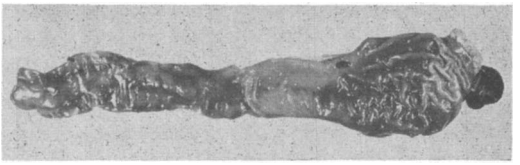

FIG. 3. Ulcer in cat.

Reserpine then could be detrimental to the stomach in 2 ways. In moderate doses it causes augmented acid secretion. In larger doses it causes a decrease in gastric blood flow with resultant ischemia and increased susceptibility to ulceration. There would appear to be, therefore, good evidence to suggest avoidance of rauwolfia derivatives in patients with the peptic ulcer diathesis.

Summary. Reserpine, in large doses, abets histamine induced ulceration in dogs. It also appears to be as ulcerogenic as histamine. Decreased blood flow occurs in the stomach

TABLE III. Effect of Reserpine on Gastric Blood Flow.

Medication	No. of dogs	Mean change in flow (ml) ± stand. error 1 hr	2 hr	3 hr	4 hr
None	6	−3.3 ± 2.1	−2.7 ± 1.4	−.2 ± 1.1	2.5 ± 2.7
1.0 mg reserpine	4	3.3 ± 1.9	4.3 ± 2.2	5.8 ± 4.5	4.0 ± 2.6
2.5 mg "	5	−19 ± 3.7	−20 ± 3.0	−21 ± 3.6	−19 ± 2.3

when 2.5 mg of reserpine is given intravenously to dogs. This may be an additional mechanism for the increased incidence of ulceration.

1. Denny, J. L., Frasher, W. G., Hoytt, D. D., *Ann. J. Med. Sci.*, 1955, v230, 169.
2. Hallister, L. E., Krieger, G. E., Krinzel, A., Roberts, R. H., *Ann. N. Y. Acad. Sci.*, 1955, v61, 92.
3. Hay, L. J., Varco, R. L., Code, C. F., Wangensteen, O. H., *S. G. O.*, 1942, v75, 170.
4. Baronofsky, I. D., Wangensteen, O. H., *Bull. Am. Coll. Surg.*, 1945.
5. ———, PROC. SOC. EXP. BIOL. AND MED., 1964, v62, 127.
6. Burns, J. H., Rand, M. I., *Brit. J. Anesthesia*, 1958.
7. Rider, J. A., Swader, J., Gibbs, J. C., Deroin, J., Agcaoili, L., Moeller, H. C., *Clin. Res. Proc.*, 1957, v5, 39.
8. Leonard, A. S., Griffen, W. O., Jr., Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1960. v103, 190.
9. Leonard, A. S., Griffen, W. O., Jr., Nicoloff, D. M., Doberneck, R. C., Wangensteen, O. H., *ibid.*, 1961, in press.

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Use of Fungizone® in Control of Fungi and Yeasts in Tissue Culture. (26509)

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Fungal and yeast contamination is a troublesome problem to those working in tissue culture laboratories. Mycostatin (Squibb Nystatin), an antifungal antibiotic derived from *Streptomyces noursei* was found by McLimans *et al.*(1) to have little or no effect on growth of mammalian cells and to suppress growth of fungi and yeasts effectively. As it is relatively insoluble in aqueous media, it has been difficult to use in certain tissue culture systems, especially where the cells are being grown in monolayers. Fungizone®*, the sodium deoxycholate complex of amphotericin B(2) an antifungal agent produced by *Streptomyces nodosus*(3), forms a colloidal dispersion in aqueous media and is effective in suppressing growth of many yeasts and fungi(4). Hemphill *et al.*(5) found that Fungizone was effective in controlling growth of certain yeasts and fungi in tissue cultures used for propagation of viruses, and observed no deleterious effects of Fungizone on viral multiplication in the infected cells or of cell multiplication of the uninfected cells. We have studied the effect of Fungizone on growth of a number of established cell lines and of chick fibroblasts in monolayer culture, and have confirmed that Fungizone when used in certain fungistatic concentrations, does not noticeably affect cell multiplication. We have also studied the stability of Fungizone in a number of tissue culture media and determined rate of inactivation at 37°C in these menstra.

Methods. Four media were used in these experiments including Eagle's medium(6) with 10% (v/v) calf serum, Ziegler's modification (7) of Eagle's medium containing 10% (v/v) calf serum, Waymouth's chemically defined medium MB 752/1(8) supplemented with 10% (v/v) calf serum and 0.03% carboxymethylcellulose and Waymouth's medium supplemented with 0.5% carboxymethylcellulose. Among the cell lines used in these studies were Earle's L_{929} strain of mouse fibroblasts, the HeLa (Gey) cell line, a cell line derived from bovine pituitaries, and 2 cell lines derived from samples of peritoneal fluid from mice infected with Ehrlich ascites tumors. Chick fibroblasts were prepared by

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