TABLE I.
Skin Lesions and Riboflavin Content of the Liver Associated with Various Amounts of Riboflavin (R) and 2-acetylaminofluorene (AAF) in the Diet.

Group	R, γ/30 g diet	AAF %	Casein, %	Skin lesions	Liver riboflavin, γ/g tissue
1	3	0	18	—	16.0 ± 1*
2	3	.03	18	++++	9.5 ± 1
3	0	0	18	++	12.7 ± 0.9
4	0	.03	18	+++	9.3 ± 2
5	30	.03	18	—	16.0 ± 1.5
6	50	.03	0	+	9.9 ± 1

* Standard error.

ficiency, keeping the liver riboflavin at normal levels. In the absence of protein in the diet, however, the increased riboflavin did not prevent the development of the deficiency.

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Toxicity of Eugenol: Determination of LD50 on Rats.* (17608)

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As a result of the demonstration that eugenol (4-allyl-2-methoxyphenol) is a very effective mucigogue and desquamatory agent in the stomach,(1) this substance has been used for the study of mucus secretion and gastric cytology in man, without gastric resection or gastroenterostomy.(2) Our technic involves administration of an aqueous emulsion of the eugenol by stomach tube, and its reaspiration after about 15 minutes. Prior to such clinical application, however, it was deemed necessary to estimate the limits of safe dosage for this substance. Such a study, conducted on dogs without any gastric operation,(3) revealed a maximum safe dosage of about 0.2 g/kg body weight, when an aqueous emulsion was administered in this same manner without reaspiration. A dose of 0.25 g/kg caused no fatalities, but induced vomiting in 2 of 7 experiments. There was no gross or microscopic evidence of any cumulative effect after repeated administration of this dose—10 times in the course of 3 weeks. At dosage levels around 0.5 g/kg, reactions to the irritant, including vomiting and ataxia, were manifest and in 2 of 6 experiments, the dog died within 24 hours. Hence, a more precise determination of the toxicity, carried out on a large number of animals, was necessary. Such a study on rats is presented herein.

Experimental Procedure. About 200 albino rats, each weighing approximately 200 g, were used in this investigation. The animals, kept in individual wire cages, were fasted for 24 hours before the eugenol was administered and for about 4 hours thereafter. A total of 91 animals were included in the final dosage-mortality analysis. The individual dosage groups were so constituted that each contained equal numbers of males and females. Eugenol (Merck, U.S.P.) was administered undiluted in a single dose, in a manner essentially the same as that reported by Shay(4)

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1. Hollander, F., and Lauber, F. U., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 34.

2. Hollander, F., Hess, M., and Sober, H. A., *J. Nat. Cancer Inst.*, 1947, v7, 365.

3. Lauber, F. U., and Hollander, F., *Gastroenterology*, in press.

4. Shay, H., and Gruenstein, M., *J. Lab. Clin. Med.*, 1946, v31, 1384.

for intra-gastric instillation in rats. If any resistance to passage of the catheter was encountered, the tube was removed immediately and passed a second time. Only 2 of some 200 rats were lost as a result of the intubation, and these 2 deaths occurred within 10 seconds after administration of the material. Since such accidental deaths were easily recognized, they were omitted from the results reported. All animals were observed closely throughout the day of administration of the eugenol, and were checked frequently for at least 10 days thereafter. All but 2 of the deaths occurred during the first 2 days. Those rats in which postmortem changes were considerable, because of death during the night, were discarded; all others were autopsied as soon after death as possible, and tissues from representative animals were taken for microscopic examination. Eugenol was administered in dosages based on body weight. In order to approximate the dosage range suitable for a dosage-mortality study, preliminary runs were made with several groups of 2 rats each. Based on these observations, more extensive runs were made in the range 1.5-2.2 ml/kg (1.6-2.3 g/kg) using 12 animals in each group. Whenever any doubt arose regarding the completeness of administration of the eugenol, the animal was immediately discarded from the group.

Results. Toxic manifestations. The first observable effect after the administration of eugenol was a weakness of the hind legs, which was seen in 100% of the animals. As a rule, this appeared in about 5 minutes, and by 15 minutes, there was complete paralysis of the lower extremities. Simultaneous with, or just after the onset of this hind leg effect, the lower jaw relaxed and remained in an open position for a variable period of time. This was observed in 70 (86%) of the animals. Sixty-four (70%) finally became prostrate and were unable to move, though eye reflexes were still present; 54 of these prostrate animals (60% of the total) went on to complete coma. In no case was an animal observed in which the front legs were affected before prostration or coma set in. Only 5 animals survived the coma; of the remaining 49, at least 10 were observed to arouse one or more times before death. During coma, breathing was irregular, changing from slow, deep, gasping excursions of the chest, to rapid, weak, abdominal respiration. The extremities became cyanotic and cold. Cessation of respiration usually occurred before heart failure. The time of death varied from 1 to 48 hours, 41% of all the deaths occurred within the first 6 hours, 33% in the next 18 hours, and 22% during the second day after administration of the eugenol. One animal died on the third day and one on the sixth. All survivors, whether they became comatose or not, were lethargic and manifested some degree of narcosis—some for as long as 3 days. On the second or third day, the animals usually sat hunched in a corner of the cage. After being prodded, they walked with hunched back and exaggerated high-step of the hind legs. Fifty per cent developed hematuria or urinary incontinence by the third day. By the fifth day, the survivors had all essentially recovered.

Pathology. In 38 (95%) of the 40 animals which were autopsied, eugenol odor was detected in either the stomach, the duodenum, or both. The blood vessels in the peritoneum and mesentery were engorged, as were those in the diaphragm, ribs, and genital organs. The peritoneal wall was glistening and diffusely inflamed, varying from pink to bright red, and fluid was present in the peritoneal cavity. There was diffuse congestion of the kidneys, and the medulla was often pink and poorly delineated. The liver also was usually congested and darker than normal. The glandular stomach contained mucus, clotted blood, and occasionally some areas of mucosal erosion; the fore-stomach appeared normal. Blood clots were often found in the lumen of the small intestine. The lungs were usually mottled and redder than normal. In many animals opened promptly after death, the auricles were still beating; in all instances, the ventricles were engorged and the vessels were injected. The general impression was that of a diffuse inflammation with congestion in the viscera.

The microscopic studies yielded evidence of pathologic change, especially following the use

TABLE I.
Dosage-Mortality Data for Eugenol.

Group No.	Eugenol dose, ml/kg	No. of rats per group	No. of deaths following administration (by days) 1*	2	3	4-10	Total	%
T-13	1.5	12	2 (2)	2	—	—	4	33
20	1.6	12	6 (3)	1	—	—	7	58
14	1.75	11	0 (0)	2	—	1†	3	27
16	1.75	11	1 (0)	1	1	—	3	27
17	1.9	12	3 (1)	1	—	—	4	33
15	2.0	11	7 (5)	1	—	—	8	73
22	2.1	11	8 (6)	1	—	—	9	82
21	2.2	11	9 (3)	2	—	—	11	100
	Total	91	36 (20)	11	1	1	49	
			74%	22%	2%	2%	54%‡	

* Values in parentheses give number of deaths in first 6 hrs.
† This death occurred on the sixth day.
‡ This represents the proportion of all deaths among all the animals treated, whereas the other percentages on this line give the proportions of all the deaths which occurred in the groups indicated.

of the highest dosages (2.1 and 2.2 ml/kg). Damage appeared to be greatest in the lungs, liver, kidney, stomach and duodenum, with relatively minor or no changes elsewhere. The lungs were emphysematous, with distended bronchi, and intense congestion was evident in the larger blood vessels as well as in the alveolar septa. In the liver, extensive congestion of the central veins and sinusoids was observed. At the higher dosages, the kidneys showed evidence of mild tubular dilatation, with the tubular epithelium swollen and distended with blood. The effects of eugenol on the nervous system in general were not studied histologically, but the possibility of their occurrence must be entertained in view of the gross observation of narcosis and malfunction of limbs and jaw following administration of the irritant. The wall of the forestomach was normal, showing no apparent reaction to the eugenol. The glandular stomach mucosa sometimes showed evidence of subacute inflammatory reactions with extensive leucocytic infiltration. In general, the columnar cells were distended with mucus, and desquamated masses of surface epithelium were noted in the lumen of the viscus. In the mucosa of animals given high dosages, areas of desquamation were distinctly visible. Parietal cells were well preserved, but the peptic cells were degenerate in appearance. Duodenitis was sometimes seen. There was evidence of excessive mucus production not only in the stomach, but also in the duodenum and other areas of the small intestine.

LD50. The mortality data are summarized in Table I. Using the statistical technique described by Bliss(5,6) the LD50 for eugenol in albino rats under the conditions herein described was found to be 1.8 ml/kg (1.93 g).

Antagonistic agents. Several stimulants were tested for possible therapeutic action in animals poisoned with eugenol: Picrotoxin (1 mg/kg), coramine (5 mg/kg), strychnine (0.75 mg/kg), caffeine (10 mg/kg), and metrazol (7.5 mg/kg). Each of these was tested on a group of about 10 rats that had received eugenol in single doses of 2.0 ml/kg. The drugs were injected intraperitoneally when the animals were already in coma or, if no coma occurred, when they were in deep narcosis. In some animals, repeated injections of the same dosage were made. No statistically significant reduction in mortality was observed in any of these experiments.

Summary. The LD50 for pure eugenol, administered to rats by stomach tube, and without subsequent reaspiration, was estimated to be 1.8 ml (1.93 g/kg). Any use of this datum as a guide to safe dosage in man,

5. Bliss, C. I., *Ann. Appl. Biol.*, 1935, v22, 134.
6. Bliss, C. I., *Quart. J. Pharm. Pharmacol.*, 1938, v11, 192.

however, may take cognizance of the fact that the conditions of the rodent studies are considerably more severe than any which might be encountered in clinical studies. In the first place, the rat experiments employed eugenol *per se,* whereas work with dog and man is done with aqueous emulsions of the irritant. Furthermore, the rat retains all of the eugenol administered, because of its inability to vomit, whereas dog and man can reject part of the irritant in this manner if the dose is excessive, and this affords an additional factor of safety.

The salient features of the toxic manifestations of eugenol in the rat were as follows: Paralysis occurred initially in the hind legs and the lower jaw. The forelimbs were unaffected unless general prostration or coma ensued. In those animals in which the acute symptoms subsided, the animals remained lethargic, showed signs of urinary incontinence and frequently hematuria, and gave evidence of impaired function of the hind legs for several days. Gross and microscopic observations of the tissues suggested that profound changes in fluid distribution had occurred in response to acute irritation of the gastrointestinal tract. The net impression of the effect of the eugenol, from the microscopic evidence, was essentially that of circulatory collapse with resultant congestion.

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