undifferentiated sarcoma, one osteogenic sarcoma and one plasmocytoma. Metastases occurred in 3 instances. Among the tumors derived by transplantation there were 63 fibrosarcomas, and a few other miscellaneous growths. Variation in differentiation and growth rate was noted in different generations.

Discussion. We have arrived at no conclusion as to the nature of the carcinogenic agent in these experiments. Attention is called to the fact that the procedure described is one of the simplest known for the production of sarcomas. In this connection the production of rat sarcomas by Turner[3] by embedding disks of bakelite (phenol-formaldehyde) is of interest.

In the course of certain surgical procedures, cellophane has been used as a covering or sheath,[4] and left in the human body. We are not acquainted with any reports of the development of sarcoma in man subsequent to its use, but this possibility should not be forgotten.

Conclusions. 1. Sarcomas were induced in albino rats by the insertion of regenerated cellulose film either subcutaneously or by wrapping it around one kidney.

2. These tumors occurred in about 35% of the rats surviving the operation more than 11 months.

3. The tumors were transplantable.

4. This is a simple method for inducing sarcomas experimentally.

5. The foregoing results in rats should be taken into consideration in the surgical use of cellophane in man.

3 Turner, F. C., *J. Nat. Cancer Inst.*, 1941, **2**, 81.

4 Ingraham, F. D., Alexander, E., Jr., and Matson, D. D., *New Eng. J. Med.*, 1947, **236**, 403.

16196

Eugenol as a Stimulus for Gastric Mucous Secretion*†‡

FRANKLIN HOLLANDER AND FRANCES U. LAUBER.

From the Gastroenterology Research Laboratory, The Mount Sinai Hospital, New York City.

In the course of investigations in this laboratory on the characteristics of gastric mucus, various types of stimulation have been studied. These included subcutaneous injection of pilocarpine, gentle mucosal massage, and topical application of a number of aqueous solutions and emulsions including ether (saturated), ethyl alcohol (50%), isotonic NaCl (0.17 N), hypertonic NaCl (0.5 N), clove oil (5%), mustard oil (1%), and distilled water.[1,2,3] Among the several objectives of these investigations was the discovery of a mucus-stimulating agent which induces no parietal secretion and therefore might be adopted as a standard stimulus for further work on the physiology of mucus secretion. Of the nine stimuli already studied, the 5% clove oil-water emulsion was clearly the most suitable for the purpose, as evidenced by the following characteristics of specimens obtained after a single application of this agent: (a) high viscosity, (b) high pH,[4] and (c) a large total volume relative

* A preliminary report of this work was transmitted to the XVII International Physiological Congress (Hollander, F., and Lauber, F. U., *Communications XVII Internat. Physiol. Cong.*, 1947, p. 155).

† This investigation was conducted with the aid of grants from the Altman Foundation, and Wyeth, Inc.

‡ The authors wish to express their thanks to Dr. Sophya Lazard for conducting some of the experiments.

1 Hollander, F., Lauber, F. U., and Stein, J. J., *Am. J. Physiol.*, 1947, **149**, 724.

2 Hollander, F., and Stein, J. J., *Am. J. Physiol.*, 1943, **140**, 136.

3 Hollander, F., Stein, J. J., and Lauber, F. U., *Gastroenterology*, 1946, **6**, 576.

4 Hollander, F., *J. Nat. Cancer Inst.*, 1945, **5**, 367.

TABLE I.
Data for Several Characteristics of Gastric Pouch Mucus.
(Stimuli: aqueous emulsions of clove oil and eugenol.)

			Stimuli			
			Eugenol			
			Low concentration (¼-½%)	Intermediate concentration (1-2%)	High concentration (5%)	Clove oil, (5%)
Volume per exper.		Mean	1.7	4.1	6.4	2.9
		Stand. Dev.	1.89	3.75	3.15	1.19
		No. of exper.	13	21	65	9
pH		Range	4.35-8.60	4.76-8.87	7.53-9.22	7.28-8.47
		Mean	7.69	8.03	8.51	8.10
		Stand. Dev.	1.03	0.57	0.44	0.27
		No. of specimens	42	105	108	27
Consistency	No. (%) of specimens	Viscous	29 (64.4%)	64 (59.8%)	92 (64.8%)	27 (96.4%)
		Fluid	14 (31.1%)	30 (28.0%)	32 (22.5%)	1 (3.6%)
		Mixed	2 (4.4%)	13 (12.1%)	18 (12.7%)	0 (0.0%)
		Total	45	107	142	28
Opacity	No. (%) of specimens	Opaque	26 (59.1%)	54 (56.3%)	104 (81.9%)	26 (96.3%)
		*Non-opaque	18 (40.9%)	42 (43.7%)	23 (18.1%)	1 (3.7%)
		Total	44	96	127	27
Columnar Cells	No. (%) of specimens	Many	28 (68.3%)	76 (72.4%)	35 (83.3%)	19 (95.0%)
		†Few	13 (31.7%)	29 (27.6%)	7 (16.7%)	1 (5.0%)
		Total	41	105	42	20

* Non-opaque = transparent or translucent.
† Few cells = none or a small number of cells per field.

to that secreted spontaneously in the same period of time.

The one disadvantage in the use of clove oil as a standard stimulus arises from its being a mixture of several chemical compounds in variable proportions, rather than a single chemical individual. However, the chief component of this essential oil is eugenol (4-allyl-2-methoxy phenol) which comprises 82-87% of the mixture. Hence, it seemed that this compound may be responsible for a major part of the mucus-stimulating action of the clove oil, and, therefore, better suited for use as a standard mucus stimulus. We have investigated the secretory response of the gastric mucosa to topical application of aqueous emulsions of pure eugenol, and the results are presented in this report.

Procedure. The experimental technique was the same as that used previously for studying the action of clove oil and the other stimuli.[3] The eugenol emulsions were prepared in concentrations ranging from ¼ to 5%. Acacia (5%) was used as an emulsion stabilizer in the earlier experiments; later this was replaced by Tergitol Penetrant-4 (1/40%) with equally good results.§ Control experiments with these substances alone showed that they exert no mucus-stimulating action at these concentrations; the fluid obtained after their application was the same as spontaneously secreted mucus in appearance and rate of formation. As in our previous work, the eugenol stimulus was administered only after the pH of the control (pre-stimulation) specimens had risen above 6.0. In general, collection of the stimulated specimens was continued until the rate of secretion fell to its control value. The pH's were determined electrometrically, with a glass electrode, and were reproducible to ±0.02 of a unit. A total of 99 experiments (*i.e.*, stimulations) was performed with eugenol emulsions, using 9 dogs with Heidenhain pouches.

§ Tergitol Penetrants were kindly supplied by the Carbide and Carbon Chemicals Corp., New York City.

Results. The data on characteristics of mucus specimens obtained after eugenol and 5% clove oil emulsions are presented in Table I. Because of the small frequencies in some of the groups of data, the values for ¼ and ½% eugenol were combined into a single group designated "low concentration," and those for 1 and 2% into an "intermediate concentration" group. The resulting increase in the number of specimens in each group augments the chance of obtaining a significant statistical evaluation of the observations. The frequency of the 5% eugenol data being large, these were retained alone as the "high concentration" group.

Total volume of mucus secreted in response to a single application of stimulus. The mean volume of secretion per experiment increases with the concentration of eugenol in the stimulus, from 1.7 ml for the "low concentration" group to 6.4 ml for the "high concentration." This latter value, for the 5% eugenol concentration, is significantly greater‖ than that for 5% clove oil (2.9 ml per experiment). The datum for 1-2% eugenol ("intermediate" group) is also greater than that for 5% clove oil but the difference is not significant.

pH. As shown in Table I, the mean pH of eugenol-stimulated specimens increases with increasing concentration of the stimulus, the differences between the several groups being significant at or below the 1% level of probability.¶ Corresponding to this, there is an elevation of both the upper and lower limits of the range, but the data become increasingly homogeneous, as evidenced by a decrease in the range itself and in the standard deviation. The mean pH for 5% clove oil is significantly lower** than that of eugenol of the same concentration but is essentially the same as that for 1-2% eugenol. Since the 5% eugenol emulsion gives a higher mean pH and a lower standard deviation than any of the other stimuli which we have previously studied,[5] it is the most effective agent encountered to date for evoking an alkaline response from the stomach.

Consistency, opacity, and columnar cell content. Qualitatively, these physical characteristics of mucus were essentially the same for eugenol as for all the other topical stimuli, except mustard oil emulsion. Some of the specimens were distinctly fluid; others jelly-like or of intermediate viscosity. Some were transparent or translucent; others opaque. Some were cell-free or contained only cellular detritus; others contained many columnar cells—singly, or in ranks and clumps. In microscopic appearance, after being stained with toluidine blue, the eugenol-mucus smears were indistinguishable from those of clove oil-mucus. Numerically, the percentage incidences for the three categories of consistency shown in Table I are essentially the same for all 3 concentrations of eugenol; clove oil, however, gives a markedly higher percentage of viscous specimens. For opacity, the percentages of opaque specimens in the low and intermediate concentration groups are almost identical; whereas the value for 5% eugenol is considerably higher than these, and that for clove oil even greater.†† The values for columnar cell content show this same trend, although the differences are not significant statistically by the χ^2-test ($P>1\%$).

The consistently higher position of clove oil over eugenol, in regard to all three of these physical characteristics, is probably the result of the action of one or more compounds in the essential oil other than eugenol. In addition to the latter, clove oil contains vanillin, methyl alcohol, furfural, caryophyllene, acetyl

‖ This difference is significant at the 1% level of probability; $t = 3.3$, $n = 72$, $P = 0.18\%$.

¶ The difference between the mean-pH's for the low and intermediate concentrations gave the following statistics in the t-test: $t = 2.6$, $n = 145$, $P = 1\%$. For the intermediate and high concentration groups, $t = 6.9$, $n = 211$, $P < 0.01\%$.

** $t = 4.8$, $n = 133$, $P < 0.01\%$.

[5] Hollander, F., Lauber, F. U., and Stein, J. J., in preparation.

†† These differences in relative frequencies are statistically significant at the 1% level of probability, as shown by the χ^2-test. For the first of these—with categories of opaque and non-opaque specimens, and "high" and "intermediate" concentrations of eugenol—$\chi^2 = 17.4$, $n = 1$, $P < 0.01\%$. For the second—with categories of opaque and non-opaque specimens, and 5% clove oil and "intermediate" concentrations of eugenol—$\chi^2 = 14.8$, $n = 1$, $P = 0.01\%$.

eugenol, and eugenol acetyl salicylate,[6] and it is possible that some of these substances are particularly potent as desquamating agents, even in small quantities.

Summary. The characteristics of gastric mucous secretion, stimulated by topical application of aqueous eugenol emulsion in several concentrations, have been investigated on 9 Heidenhain pouch dogs. A 5% emulsion of clove oil, which had previously been found to be superior to all other mucus stimuli, was used as a basis of reference for determining the secretory value of eugenol. It was found that 5% eugenol yields larger volumes of secretion, with a higher pH, than the clove oil. The percentages of specimens possessing high viscosity, opacity, and columnar cell content are lower for the eugenol than for the clove oil. Since we have already cited reasons[3] for believing that *pure* gastric mucus is transparent, cell-free, and of variable consistency, it may be that the secretion yielded by eugenol differs less from pure mucus than does the fluid obtained with clove oil.

Five per cent eugenol emulsion is the most effective stimulus to mucus secretion which we have found to date—especially since it yields larger volumes of mucus which tend to have a higher pH than those induced by any of the other stimuli. The latter characteristic indicates also that eugenol has virtually no stimulating effect on the parietal cells. Furthermore, this substance is a pure compound, the major component of clove oil, whereas the latter is a mixture of at least 7 different chemical substances. Since the minor components of the essential oil may also exert some physiological effect on the mucosa, their absence from the pure eugenol enhances its value as a standard. Hence, we propose to adopt an aqueous emulsion of eugenol as a standard stimulating agent for further work on the physiology of gastric mucous secretion.

[6] Wood, H. C., Jr., and Osol, A., *The Dispensatory of the United States of America*, 23° ed., Philadelphia, 1943.

16197

The Influence of Coramine on the Liver of the Young Rat.

FRED G. BRAZDA AND ROLAND A. COULSON.
(With the technical assistance of Lucy C. Gremillion.)
From the Department of Biochemistry, Louisiana State University School of Medicine, New Orleans, La.

Coramine (nikethamide) is a compound which provokes a number of different pharmacological actions. It is capable of curing black tongue in dogs by virtue of its close structural relationship to nicotinamide;[1] it is often employed as a medullary stimulant; and it has a pronounced ability to increase the liver weight of young rats.[2] Coulson and Brazda[2,3] have presented evidence which indicates that the unsubstituted nitrogen of the heterocylic ring and the di-ethyl substitution of the amido nitrogen are both in part responsible for the liver enlargement which follows the administration of the compound.

In preliminary experiments the absolute liver weight increase caused by coramine by the end of a 28-day period could not be prevented by the inclusion of 1.2% methionine or 0.5% choline in the diet.[2] This weight increase suggested that the liver cells were being injured by the coramine and that this was followed by the very rapid regeneration which is seen in many types of liver injury. It was deemed desirable to design experiments

[1] Smith, D. T., Margolis, G., and Margolis, L. H., *J. Pharm.*, 1940, **68**, 458.

[2] Coulson, R. A., and Brazda, F. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 1.

[3] Brazda, F. G., and Coulson, R. A., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 19.