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.

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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.

TABLE I.
Effect of the Oral Administration of Choline or Methionine to Young Rats on a 1% Coramine Diet.†

| No. days | Rat final wt | Liver wt wet | Liver wt dry | Liver wt as % body wt | Liver fat, % wet wt | Liver fat, % dry wt | Liver, % solids | Gain in wt, g/day |
|---|---|---|---|---|---|---|---|---|
| | | | | Control. | | | | |
| 5 | 63.9 | 3.05 | 0.97 | 4.80 ± 0.10* | 4.83 ± 0.21* | 15.11 | 32.23 | +3.47 |
| 9 | 70.0 | 3.36 | 1.06 | 4.78 ± 0.09 | 4.22 ± 0.12 | 13.38 | 31.47 | +3.30 |
| 14 | 99.7 | 4.78 | 1.54 | 4.79 ± 0.09 | 4.01 ± 0.27 | 12.41 | 32.10 | +3.93 |
| 19 | 122.4 | 5.59 | 1.74 | 4.56 ± 0.10 | 3.52 ± 0.21 | 11.19 | 31.46 | +4.03 |
| 24 | 132.4 | 6.10 | 1.96 | 4.60 ± 0.08 | 4.09 ± 0.14 | 12.94 | 31.93 | +3.56 |
| 28 | 152.9 | 6.56 | 2.08 | 4.27 ± 0.09 | 4.27 ± 0.07 | 13.47 | 31.69 | +3.85 |
| | | | | 1% Coramine. | | | | |
| 5 | 52.2 | 4.32 | 1.49 | 8.31 ± 0.12 | 6.72 ± 0.54 | 19.82 | 34.14 | +1.50 |
| 9 | 66.4 | 5.81 | 1.89 | 8.76 ± 0.19 | 7.66 ± 0.42 | 23.33 | 32.53 | +2.56 |
| 14 | 83.8 | 6.87 | 2.38 | 8.25 ± 0.18 | 8.62 ± 0.50 | 25.19 | 34.33 | +2.76 |
| 19 | 97.6 | 7.04 | 2.44 | 7.31 ± 0.25 | 7.88 ± 0.39 | 22.55 | 34.69 | +2.71 |
| 24 | 106.4 | 7.85 | 2.70 | 7.38 ± 0.14 | 8.22 ± 0.71 | 23.29 | 34.58 | +2.70 |
| 28 | 119.3 | 8.35 | 2.93 | 6.98 ± 0.17 | 8.60 ± 0.19 | 24.17 | 35.17 | +2.73 |
| | | | | 1% Coramine + 2.4% Methionine. | | | | |
| 5 | 39.1 | 3.09 | 0.94 | 7.94 ± 0.27 | 4.59 ± 0.19 | 15.06 | 30.45 | —0.40 |
| 9 | 47.8 | 3.81 | 1.21 | 7.95 ± 0.14 | 5.19 ± 0.16 | 16.32 | 31.75 | +0.26 |
| 14 | 63.1 | 5.32 | 1.80 | 8.33 ± 0.28 | 6.89 ± 0.87 | 20.89 | 33.48 | +1.13 |
| 19 | 72.0 | 5.84 | 1.84 | 7.92 ± 0.17 | 5.40 ± 0.31 | 16.63 | 32.41 | +1.41 |
| 24 | 84.7 | 6.22 | 2.04 | 7.44 ± 0.19 | 6.26 ± 0.52 | 18.88 | 32.45 | +1.62 |
| 28 | 80.3 | 5.79 | 1.90 | 7.21 ± 0.10 | 5.49 ± 0.34 | 16.51 | 32.94 | +1.34 |
| | | | | 1% Coramine + 0.5% Choline. | | | | |
| 5 | 40.1 | 3.37 | 1.04 | 8.52 ± 0.21 | 3.98 ± 0.18 | 12.85 | 30.72 | +0.76 |
| 9 | 53.9 | 4.06 | 1.36 | 7.59 ± 0.21 | 6.68 ± 0.42 | 19.76 | 33.16 | +1.95 |
| 14 | 75.3 | 5.53 | 1.81 | 7.37 ± 0.24 | 6.17 ± 0.65 | 18.49 | 32.40 | +2.53 |
| 19 | 82.9 | 5.92 | 1.85 | 7.16 ± 0.16 | 4.74 ± 0.18 | 15.33 | 30.89 | +2.39 |
| 24 | 95.2 | 6.26 | 1.98 | 6.55 ± 0.19 | 4.58 ± 0.23 | 14.27 | 31.62 | +2.42 |
| 28 | 109.0 | 7.32 | 2.41 | 6.66 ± 0.13 | 4.52 ± 0.23 | 13.66 | 32.84 | +2.55 |

* P.E. $= \pm 0.6745 \sqrt{\frac{\Sigma(v)^2}{n\,(n-1)}}$

† Each figure in the table represents the average value obtained from 5 males and 5 females.

which would give some information on the nature of the increase in liver weight and which would help to explain the action of the compound.

*Experimental.* To determine the rate of the liver weight increase 4 groups containing 60 rats each were placed on a diet which consisted of 25% casein, 55% starch, 15% cottonseed oil and 5% salts.[4] Each rat also received a daily dose of one ml of Brewer's yeast extract (equivalent to one g of dried yeast) and one drop of Percomorph oil a week. The rats, weighing 35-45 g, were an inbred mixture of the Sprague-Dawley and Illinois strains. Coramine* was mixed into the diet at a level of 1% for all groups except the controls. One group received an additional supplement of methionine at a level of 2.4% of the diet and one the equivalent of a 0.5% choline supplement in the form of choline chloride. At intervals of 5, 9, 14, 19, 24, and 28 days, 5 male and 5 female rats, each from a different litter, were killed out of each of the 4 groups. The livers were weighed, the fat and water contents were determined by analysis and a specimen was saved for the preparation of histological sections. These were stained by the routine hematoxylin-eosin method. The water content was determined by drying the livers to constant weight in a vacuum desiccator over concentrated sulfuric acid at about 30 mm Hg pressure. The dried livers were then used for the determination of the fat (total lipid) content. They were extracted twice with hot alcohol, four times with an alcohol-ether mixture and ten times with ether. The solvents were evaporated and the fat was determined gravimetrically.

Both choline and methionine were added

[4] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

* The commercial product in 25% solution was used.

TABLE II.
The Influence of the Ingestion of Coramine on the Liver Glycogen of Young Rats.

| Group | No. rats | Liver wt as % body wt | | % liver glycogen | |
|---|---|---|---|---|---|
| | | Range | Avg | Range | Avg |
| Control | 11 | 3.63-5.27 | 4.42 ± 0.09 | 1.40-3.75 | 3.09 ± 0.13 |
| 1% coramine | 12 | 5.56-7.66 | 6.81 ± 0.11 | 1.66-3.28 | 2.39 ± 0.09 |

in amounts calculated to be far in excess of any normal requirements. Methionine in such a massive dose caused a considerable decrease in the growth rate and choline provoked a slight decrease (Table I). In each case the growth rate was in proportion to the food consumed. The comparatively high protein diet supplemented with methionine or choline was chosen in an attempt to provide dietary conditions such as to minimize any liver damage.

The increase in liver weight occurred in surprisingly short time and the livers remained larger than in the controls for the full 28-day period in all of the three experimental groups (Table I). There was a gradual decline in liver wt/body wt ratio in the last 2 weeks in all 3 groups. It is evident that neither choline nor methionine is capable of modifying the increase in the liver wt/body wt ratio caused by coramine. The slight differences noted in the three groups may be explained by the decreased fat content in the groups receiving choline or methionine.

Those rats which received coramine had livers which showed some elevation in the fat content as early as 5 days (Table I) and continued to show roughly a constant level of fat for the full 28-day period. Both choline and methionine reduced the fat content of the liver to a level significantly below that of the coramine rats but slightly above that of the controls. The solid content of the livers was the same in all 4 groups. This becomes evident if one subtracts the excess fat in the coramine series prior to calculation. The absolute increase in liver weight must be due to an increase in both solids and water in the same proportion as that found for the control series.

Since the increase in liver weight was not due to a disproportionate increase in water or fat, experiments were devised to determine whether this might be due to glycogen. Eleven rats, 7 females and 4 males, were placed on the control diet. Twelve rats, 7 females and 5 males, were placed on the 1% coramine diet. At the end of 6 days both groups of rats were killed and the liver glycogen was determined by the method of Good, Kramer and Somogyi.[5] It was decided that a longer period was unnecessary (Table I). The results appear in Table II.

It is evident that the glycogen content of the livers taken from the coramine treated rats, although somewhat lower, was not seriously different from that of the control rats. Glycogen storage was not responsible for the liver enlargement. The increase in liver weight in the glycogen series was less marked than in the series reported in Table I. This discrepancy may possibly be explained by the fact that this experiment was conducted during the hottest time of the year. The general phenomenon of significant liver enlargement, however, is still apparent.

Microscopic examination showed that the livers from rats receiving coramine had intracellular fat globules. Close correlation existed between the histological picture and the gravimetric analysis for fat. A prominent feature of the liver parenchyma was the large number of mitoses and binucleate cells. There was a slight increase of interlobular connective tissue in the livers of the experimental animals. Aside from the significant decrease in fat in the livers of rats receiving coramine plus choline or methionine no other effects due to these lipotropic agents were observed. Livers from rats after the 28-day experimental period were little different from those of the 5-day groups.

*Discussion.* In spite of the fact that cora-

[5] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

mine seems to promote a very rapid "regeneration" of liver tissue, the liver shows relatively little damage even after 28 days on a coramine diet. It is quite possible that necrosis or cirrhosis or both might have been produced by continuing the experiment for several months. Blumberg and Grady[6] found that over 300 days were required to produce fibrosis in rats with fatty livers.

It is surprising that the full effects of coramine on the liver wt/body wt ratio are manifest in 5 days or less and that no obvious progressive change occurs from then up to the 28th day. The growth rate was depressed, but not seriously, and all of the 180 experimental animals appeared to be in good condition at the time of autopsy.

Essentially none of the results presented, either chemical or histological, indicate any serious abnormality in the liver parenchyma of the coramine treated rats. A hypothesis as to the nature of the action of coramine on the liver cannot, however, be advanced. The rapidity and degree of enlargement of the liver superficially resembles the effect produced in organs by their respective trophic hormones. Whether this resemblance is more than superficial remains to be determined.

*Summary.* (1) Coramine produces a great increase in the liver wt/body wt ratio in growing rats. (2) This increase which is manifest within 5 days or less and which is still apparent for at least 28 days cannot be prevented by the ingestion of choline or methionine. (3) The percentage of water and glycogen in these livers is essentially the same as in the controls. Coramine causes some elevation in the fat content which may be prevented by the administration of choline or methionine. (4) On microscopic examination, aside from the fat globules observed in the livers of rats receiving coramine alone, no marked pathological changes were evident. Binucleate cells and mitoses were present in large numbers.

The authors wish to express sincere thanks to Dr. T. Hernandez for carrying out the microscopic examination of the histological sections.

[6] Blumberg, H., and Grady, H. G., *Arch. Path.*, 1942, **34**, 1035.

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## Growth of Animal Tissue Cells in Artificial Media.*

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Multiplication and growth of tissue cells *in vitro* depend on the presence of two kinds of substances in the culture media. Substances of a protein nature present in extracts from embryonic tissues were shown by Carrel[1] to act as growth promoting factors. These high molecular weight substances are present only to a very limited extent in blood plasma or serum. Attempts at the isolation of these very labile substances, grouped by us under the name "embryonin",[2] have shown that they can be purified by the extraction methods outlined by Hammarsten[3] for the isolation of a nucleo-protein. Other methods of purification have so far failed. These results have been confirmed by Davidson and Waymouth.[4]

When the culture media (plasma, serum, embryo extract) are dialysed against Ringer-glucose solution, and Tyrode's solution is added (furnishing inorganic phosphate and bicarbonate), the tissue cells disintegrate

* Aided by grants from Rask-Orsteds Fond and from Kong Christian den Tiendes Fond.

[1] Carrel, A., *J. Exp. Med.*, 1913, **17**, 14.

[2] Fischer, A., and Astrup, T., *Pflügers Arch.*, 1943, **247**, 34.

[3] Hammarsten, E., *Z. physiol. Chem.*, 1920, **109**, 141.

[4] Davidson, J. N., and Waymouth, Ch., *Biochem. J.*, 1945, **39**, 188.