in some instances fell below 70 mg % (more than 3 x the standard deviation below the control mean). Some values continued to remain significantly low during recovery.

Fig. 1 and 2 are representative of 6 animals followed for over a month. Rat 21 was sacrificed on the 43rd day, at which time the liver glutathione concentration was significantly low (85 mg %).

Total glutathione levels were done on 3 rats with blood glutathione concentrations below 10 mg %. Only a small amount of oxidized glutathione was found, an average of 5 mg % for blood and 6 mg % for liver. Therefore, the low reduced glutathione values were not due to an increase in oxidized glutathione.

Initial values on 40 rats for reduced glutathione in liver averaged 184 ± 19 mg %. No change occurred before the 6th day following irradiation. Nine low liver glutathiones (47 to 116 mg %) were found in a total of 23 rats biopsied between 6 to 19 days post-irradiation. Reduced glutathione levels in the blood of these rats were 10 mg % or less.

Inanition was considered as a possible factor in this experiment(10). Forty-eight hours following irradiation a typical series of 10 animals (average weight 183 g) had lost 12 g. However, by the seventh day the average weight had returned to 185 g. Body weights then slowly decreased again. Nine rats with significantly low liver glutathiones (47 to 116 mg %) lost the same amount of weight as 7 rats with no change in liver glutathione values (162 to 222 mg %). At biopsy the stomach was palpated and food particles could be felt. When rats succumbed at biopsy food was always found in the stomach and small intestine. Six rats were starved until their average weight loss was 47 g; yet no decrease was found in liver reduced glutathione values (162 to 196 mg %). Paired feeding experiments were considered useless without determining intestinal absorption(11). However, 9 rats with blood glutathione concentrations from 9 to 20 mg % maintained their average blood glucose level at 120 mg %. Thirteen irradiated animals starved for 24 hours had an average blood glucose level of 76 mg %. Therefore, it was concluded that inanition does not explain the decrease in glutathione levels in liver.

Glutathione may function in the tissues to maintain enzymes in their active SH form. Therefore, it is reasonable to assume that low glutathione levels may interfere with metabolic processes.

Summary. A study has been made of blood and liver glutathione after 500 r total body x-irradiation. The data show first, that irradiation generally causes a decrease in blood glutathione; and second, in some cases, a decrease in liver glutathione.

10. Binet, L., and Weller, G., *Compt. rend.*, 1935, v119, 939.

11. Bennett, L. R., Bennett, V. C., Shaver, A., and Grachus, T., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 439; Lauran, M., and Lartigue, O., *Compt. rend*, 1950, v230, 1426; Buchwald, K. W., *J. Exp. Med.*, 1931, v53, 827.

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Crystal Structures in Dried Smears of Gastric Mucus.* (18915)

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In the course of our studies on acid-free canine mucus, we have examined a large number of specimens microscopically. Air-dried smears were stained with toluidine blue, by a technic previously described(1)†; Wright's stain was also used occasionally.

* This investigation was supported by a research grant from the National Cancer Institute, of the National Institutes of Health, United States Public Health Service.

1. Hess, M., and Hollander, F., *J. Lab. Clin. Med.*, 1944, v29, 321.

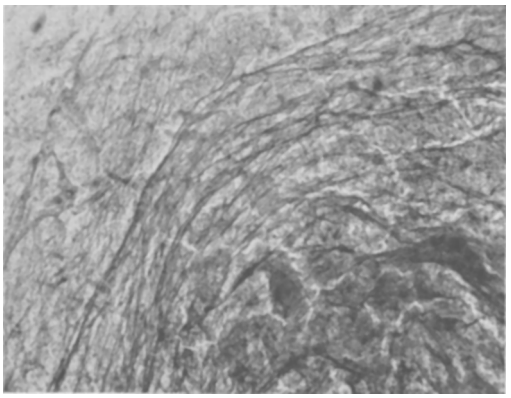

FIG. 1. Smear of transparent gastric mucus (canine) showing long strands of the secretion. Toluidine blue. 38×.

The specimens often contained numerous epithelial and other cells(2), interspersed among small cell-free areas, but many smears were entirely acellular. This was particularly true for "spontaneously" secreted mucus, collected from resting gastric pouches without the application of a mucigogue.‡ The present paper reports the varied microscopic appearance of such acellular mucus, as revealed by careful study of hundreds of specimens.

Observations. Smears free of whole cells often contain particles suggestive of cellular debris, with or without a reticulated substratum. Sometimes the smears reveal only dark wisps or long strands of mucus more viscous than the secretion in adjacent areas (Fig. 1). At other times they contain minute vacuoles which may be bubbles of air incorporated by shaking prior to smearing, or as has been reported elsewhere(3), possibly bubbles of CO_2 released spontaneously by the mucus. Frequently, however, the smears contain fern-like crystal structures of variable size (Fig. 2) and these sometimes occur as rectangular outgrowths from cubic nuclei (Fig. 3) or in stellate patterns about a central point of origin. Another type of structure observed in these smears resembles fronds of seaweed; these also may be isolated (Fig. 4) or may appear as symmetrical outgrowths from a central nucleus (Fig. 5). Examination of this nucleus at high power suggests that it is compounded of the same globular elements

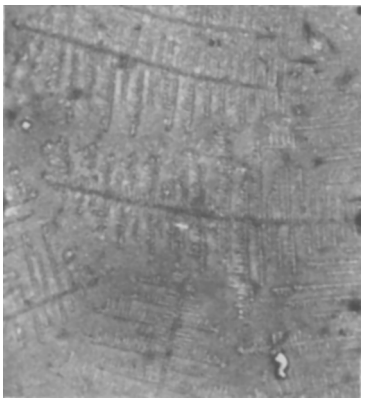

FIG. 2. Smear of transparent gastric mucus (canine), showing rectangular fern-like crystals. Toluidine blue. 38×.

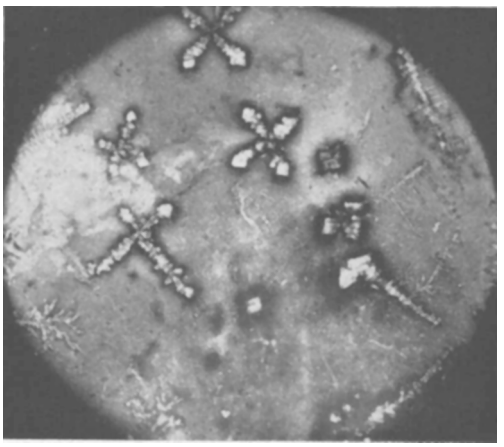

FIG. 3. Smear of transparent gastric mucus (canine), showing relatively large cubic (?) crystals with occasional fern-like structures growing out of them. Toluidine blue. 38×.

† This staining technique was designed for the preparation of smears in which the metachromatic staining is permanent. Attention is therefore called to the fact that the metachromasia of the specimens discussed in this report is generally as pronounced at this time as when the smears were originally prepared, 8 or more years ago. For a modification of this technique applicable to tissue sections, see Hess and Hollander, *J. Lab. Clin. Med.*, 1947, v32, 905.

2. Hollander, F., Stein, J., and Lauber, F. U., *Gastroenterology*, 1946, v6, 576.

‡ A method for stimulating the secretion of pure, acellular mucus in relatively large quantities has recently been developed by Janowitz, Hollander, and Jackson, PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 578.

3. Hollander, F. and Lauber, F. U., *Fed. Proc.*, 1948, v7, 56.

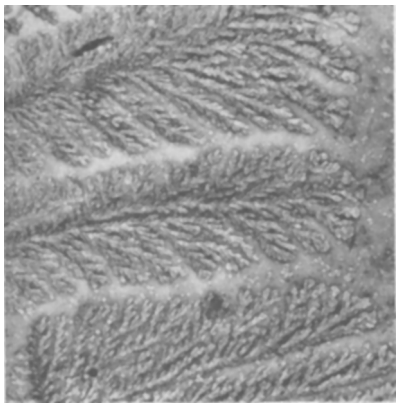

FIG. 4. Smear of transparent gastric mucus (canine), with seaweed-like structures. Toluidine blue. 38×.

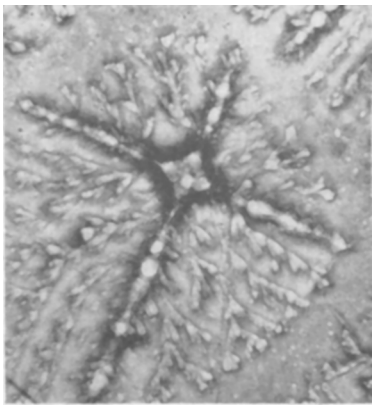

FIG. 5. Smear of transparent gastric mucus (canine), showing seaweed-like structures growing out symmetrically from a central nucleus. Toluidine blue. 38×.

as are visible in the outgrowths from its corners, rather than a cube as in Fig. 3.

These crystals have never been seen to take Wright's stain or toluidine blue. With the latter there is usually a concentration of metachromatic material at their margins, corresponding to the dark lines of variable thickness in some of the photomicrographs. Sometimes, careful examination reveals a thin metachromatic network over their surfaces as well, but the crystals themselves are unstained.

Comment. Failure to react with toluidine blue suggests that these crystals are not gastric mucins. Pepsinogen also is ruled out by reason of its possessing a different crystalline appearance, as reported by Northrop *et al.* (4). Pepsin could not be present because the mucus specimens had not been acidified before smearing. Some inorganic salt such as NaCl is likewise a possibility. Henning and Norpoth(5) offered such an explanation for the "lattice structures" (Gitterbildungen) observed microscopically in unstained dried drops of filtered human gastric juice. These structures, which included both simple cubic crystals and delicate fern-like structures, resembling the crystals reported herewith for relatively pure pouch mucus, were believed to be crystals of NaCl, modified by the presence of foreign substances from saliva or regurgitated duodenal fluid. However, these investigators offered no evidence to support their contention, nor have we been able as yet to demonstrate that the crystals seen in non-acid mucus are NaCl. On the contrary, it is now generally recognized that such rectangular crystal forms are non-specific(6), and a similar skeletal growth with successive branching has been grown from pure NH_4Cl by Bunn(7). It may be suspected that these crystals are artefacts, deriving from the stains used in preparing the specimens, but this can not be the case because we have watched the patterns in Fig. 2 and 5 growing under the microscope in a slowly drying *unstained* preparation. It is evident, therefore, that characterization of the mucus crystals as NaCl is not warranted without direct chemical evidence. Further attempts to identify these structures will be of interest, but they must be made with the aid of suitable microchemical methods.

Conclusion. Several characteristic crystal patterns have been observed microscopically in dry smears of pure gastric mucus from

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5. Henning, N., and Norpoth, L., *Z. f. klin. Med.*, 1933, v126, 1.

6. Chamot, E. M., and Clyde, W. M., Handbook of Chemical Microscopy, John Wiley & Sons, Inc, New York, 2nd Ed., 1938, v1, pp. 333 and 335.

7. Bunn, C. W., Chemical Crystallography, The Clarendon Press, Oxford, 1945. (Fig. 8, Plate I).

canine Heidenhain pouches. Although their chemical composition has not been established, there is reason to believe that they are neither pepsinogen, pepsin, nor one of the mucins.

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Effects of Cortisone and 11-Desoxycortisone on Pain Thresholds in Man.* (18916)

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While testing 11-desoxycortisone† in allergic disorders, it was noted that the tooth pain thresholds of 5 patients decreased with this steroid medication. Thus the patients, over a period of hours, were able to recognize a smaller stimulus. Since these patients were not trained, the decreases in pain thresholds could be, and tentatively were, interpreted as a normal learning curve. Because cortisone and also ACTH therapy occasionally produce euphoria and other psychic changes(1,2), it is remotely possible that the mechanism of the analgesic effect of cortisone is similar to that of the opiates.

Method. To test these hypotheses, trained subjects were used to assay the effects on pain thresholds of 11-desoxycortisone and cortisone. As a control for this study a placebo (cholesterol) was administered. Absolute blind test conditions were used in that the vials of suspended steroid were labeled A, B, and C and the identity of the injected suspension was unknown both to the experimenter and the subjects.

Four trained male subjects were used for these tests. At the beginning of each experiment 2 or more control pain thresholds were determined with both the tooth and warm wire algesimeters. The saline suspension of steroid (25 mg) was administered by intramuscular injection and the pain thresholds were then measured at hourly intervals for 6 hours. The experimental period extended over 7 hours and the subjects were permitted to resume their usual activities between pain threshold determinations. Each of the 4 subjects received 11-desoxycortisone, cortisone, and the placebo (cholesterol) at weekly intervals in an arbitrary but varied sequence. Two types of algesimeters were used: the warm wire algesimeter of Lee, Williams, and Pfeiffer(3), and the tooth algesimeter of Goetzl, Burrill, and Ivy(4). The warm wire algesimeter consists of a nichrome resistance wire (crimped to provide a tip for application of the stimulus) in series with a milliammeter, a rheostat, and a source of electrical current. The current was varied by means of the rheostat. The instrument was calibrated in increments of 10 milliamperes with a range of 400-1000 milliamperes. The conduction heat stimulus was applied to the skin of the volar surface of the wrist. The tooth algesimeter provided induced alternating current as a secondary coil was moved toward a rotating primary coil. The calibration was in increments of 0.2 volt with a range of 0 to 4.2 volts The induced electrical stimulus was applied to the filling of a tooth. Two pain thresholds were determined with the algesimeters. Using the warm wire algesimeter on the skin of the volar surface of the wrist, the wrist pain threshold was

* This investigation was supported in part by a research grant from the National Heart Institute, of the National Institutes of Health, Public Health Service.

† Compound S of Reichstein.

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4. Goetzl, F. R., Burrill, D. Y., and Ivy, A. C., *Quart. Bull. Northwestern Univ. Med. School*, 1943, v17, 280.