

orally, alginic acid could combine irreversibly with any ionic calcium present in the stomach contents. If not completely used up in this manner, it could continue to adsorb calcium on passage through the intestine. Available evidence indicates that alginic acid, in a similar manner as the resins, adsorbs cations proportional to their ionic concentration. Since considerably smaller amounts of calcium than of sodium are present in the intestine in a soluble form, a proportionally smaller uptake of calcium would be expected. For this reason, it appears probable that the danger of gradual demineralization of bone with long continued use of alginic acid would not be greater than with the resins.

Preliminary clinical trials carried out by Dr. Mulinos at the New York Medical College and by Drs. Doerner and Feldman at the U. S. Marine Hospital, Staten Island, indicate that alginic acid combines with sodium *in vivo*.

**Summary.** Alginic acid possesses sodium and potassium ion exchange properties which compare favorably with those of a carboxylic type cationic exchange resin. Under *in vitro* conditions alginic acid at pH 7.6 adsorbs 4.2 meq Na<sup>+</sup> per g. The total cation adsorption from solutions containing K<sup>+</sup> and Na<sup>+</sup> is also 4.2 meq/g and the ratio of Na:K combined approximates that present in solution. The adsorption of Ca<sup>++</sup> by alginic acid is irreversible and independent of pH. The hydrophilic character of the sodium and potassium alginates may contribute to the elimination of fluid and cause laxation.

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1. For a review of pertinent publications in this field, see McChesney, E. W., Dock, W. and Tainter, M. L., *Medicine*, 1951, v30, 183, and McChesney, E. W., *J. Lab. Clin. Med.*, 1951, v38, 199.

2. Nilson, H. W. and Lemon, J. M., *U. S. Dept. Int., Fish and Wildlife Service, Res. Rep.*, 1942, No. 4.

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## A Nucleoprotein-Containing Fraction of Intestinal Mucosa. (19312)

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In an attempt to extract from intestinal mucosa that substance, expected to be mucoprotein, which imparts to the tissue its mucous nature, a highly viscous fraction was obtained which appears upon analysis to be approximately 50% nucleoprotein. The remainder of the fraction, not yet separated from the nucleoprotein, is primarily lipid and ash. A description of the preparative method and of the product is given here because of the simplicity of the technic and because of the biochemical demonstration (succeeding paper) of an effect of whole body X-irradiation upon this fraction of intestinal mucosa.

**Preparation of the nucleoprotein fraction.** The nucleoprotein fraction has been prepared

from whole rat intestine and from the intestinal mucosa of rats, rabbits, and sheep. The intestines were cooled on ice as quickly as possible after removal from the animal, then were flushed with cold saline to remove fecal matter. Whole rat intestine was minced in a Latapie mincer. When rat intestinal mucosa was desired, the intact segments of intestine were slit lengthwise, then fed through a device resembling a washing machine wringer; the rollers were made of stainless steel, lightly milled to afford traction. Mucosa was obtained from the cleaned intestine of rabbits or sheep by pressing intact segments with bone spatulas. Whichever the product, whether mucosa or whole intestine mince, it was then blended in the Waring blender with 9 volumes of N/10 NaOH. The blend was filtered through nylon (National Filter Media Co.,

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\* Formerly University of Chicago Toxicity Laboratory. This work was carried out under a research contract with the Atomic Energy Commission.

TABLE I. Chemical Composition of Tactoid from Intestine.

| Sample                                 |    | Rat (pool<br>of several) | Rabbit<br>(1 animal) | Sheep<br>(1 animal) |
|----------------------------------------|----|--------------------------|----------------------|---------------------|
| Total N (mg/ml)                        | O* |                          |                      | 1.10                |
|                                        | T  | 1.71                     | 2.73                 | 2.60                |
|                                        | S  | 1.61                     | 1.76                 |                     |
| Total P (mg/ml)                        | O  |                          |                      | .19                 |
|                                        | T  | .52                      | 1.05                 | 1.18                |
|                                        | S  | .21                      | .26                  | .14                 |
| % dry wt                               | O  |                          |                      |                     |
|                                        | T  | 3.37                     | 3.61                 | 3.96                |
|                                        | S  | 2.09                     | 1.78                 | 1.12                |
| Protein (mg/ml)                        | O  |                          |                      | 4.66                |
|                                        | T  | 1.98                     | 9.66                 | 5.40                |
|                                        | S  | 5.09                     | 7.83                 | .89                 |
| Polysaccharide (mg/ml)                 | O  |                          |                      | .15                 |
|                                        | T  |                          | 1.14                 | 7.40                |
|                                        | S  |                          | .59                  | .25                 |
| Tyrosine (meq $\times 10^{-4}$ per ml) | O  |                          |                      | 43                  |
|                                        | T  | 51.2                     | 72.1                 |                     |
|                                        | S  | 52.2                     | 59.4                 |                     |
| Relative viscosity                     | O  |                          |                      | 2.74                |
|                                        | T  | 200-2000                 | †                    |                     |
|                                        | S  | 1.43                     | 1.47                 |                     |

\* O = original. T = tactoid. S = supernatant.

† Not done (very viscous).

No. 1002) under suction. It was brought to pH 11 with vigorous stirring and placed in the cold room (2-5°C). After a period of one to 4 days without disturbance, a separation takes place into 2 liquid layers. The upper, watery layer increases further in amount as the material remains in the cold. The lower layer is considerably more viscous and forms a cohesive mass which shrinks with the passage of time, expressing more watery fluid, and itself becoming viscous to the point of almost complete gelation. If the pH is not reduced to 11 before placing in the cold room, the two-phase separation will still occur, but it may take several weeks, at the end of which time it will be found that the pH has decreased to and below pH 11, presumably by absorption of CO<sub>2</sub> from the air and by reaction of the alkali with the glass vessel. The cohesive, viscous mass will hereafter be referred to as the "tactoid," a not entirely accurate usage of the expression of Freundlich(1) for the microscopic or submicroscopic particles which when properly oriented, form such a mass. We have also prepared such a tactoid from calf thymus chromosomes or whole thymus, but we have not found it possible to prepare it from alkaline solutions of rabbit kidney, lung,

TABLE II. Phosphorus Fractions of Rat Intestine Tactoid.

| Phosphorus fractions<br>(as % of total P) | Age of tactoid, days |    |    |
|-------------------------------------------|----------------------|----|----|
|                                           | 16                   | 28 | 32 |
| Acid-soluble P                            | 28                   | 24 | 20 |
| Lipid P                                   | 6                    | 3  | 9  |
| Nucleic acid P                            | 60                   | 69 | 69 |
| Phosphoprotein P                          | 6                    | 4  | 2  |

heart, liver or testis.

*Chemical composition of the tactoid.* The chief difficulty in characterizing the tactoid lies in its irregular composition, dependent upon time, temperature, pH, speed and time of centrifuging, if any, and undoubtedly biological factors. As the material stands in the cold room, fluid is continually expressed, and its chemical composition is thus constantly changing. Table I summarizes pertinent information on the chemical composition of alkaline blends of intestine, before and after formation of tactoid.

Phosphorus fractionations have been performed on some tactoids, by the method of Schneider(2). The results are given in Table II and provide strong evidence for the high nucleic acid content of the tactoid. In addition, a portion of sheep tactoid was extracted to remove acid-soluble and fat-soluble

materials, then treated with perchloric acid(3) to dissociate nucleic acids from protein. The soluble portion of the resulting product showed an absorption spectrum very similar to that of the nucleic acids.

The carbohydrate moiety of the nucleic acid was measured by the diphenylamine reaction(4), the orcinol reaction(5), and the carbazole reaction(6). The results indicated clearly the presence of carbohydrate of the desoxypentose type, with only a trace of pentose.

The ash content of these tactoids is quite high, usually about 25% of the dry weight. Besides the alkali metals, the only cation present in appreciable concentration is aluminum. Although it has been definitely established that the aluminum rises directly from the intestine (possibly partly from the diet) and is not a procedural contaminant, its significance in the formation of the tactoid is unknown.

*Summary.* A nucleoprotein-rich fraction has been obtained from alkaline blends of in-

testinal mucosa by placing the blend in the cold with or without adjustment of the pH to 11. After several days (or weeks, if the pH is not previously adjusted), a 2-phase liquid system becomes evident. The heavier, viscous, continually contracting phase, designated here as the tactoid, generally has a composition of approximately 50% nucleoprotein, 25% fat, and 25% ash. The nucleic acid is almost entirely of the desoxypentose type. The ash contains appreciable amounts of aluminum, the significance of which in the formation of the tactoid is unknown.

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### Effect of Whole-Body X-Radiation on Rat Intestine and Intestinal Nucleoprotein (19313)

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In the preceding paper, a method was described for the isolation from intestinal mucosa of a tactoid fraction which has been identified as approximately 50% nucleoprotein. The present paper describes biochemical changes in this fraction after the animals receive a lethal dose of X-rays to the whole body.

*Experimental.* Since, as described in the preceding paper, the process of tactoid formation is a continuing one, for quantitative measurements it is necessary to prepare the intestine solutions, draw aliquots for analysis, etc., at some regular, though arbitrary, time.

To achieve this aim, all intestines of a given experiment were quickly frozen in dry ice and acetone after cleaning. All were then thawed, blended with N/10 NaOH, filtered, and placed in the cold room as nearly simultaneously as possible. When tactoid formation appeared adequate, a control sample and its corresponding sample from X-rayed animals were centrifuged in the same centrifuge load. Animals used were Maguran male rats weighing 220-290 g. X-radiation administered was 800 r to the whole body. Controls and X-rayed animals were fasted 24 or 48 hours after the irradiation and then were sacrificed.

*Results.* Table I indicates that 24 hours after whole body X-radiation, the weight of intestine has decreased significantly, the rela-

\* Formerly University of Chicago Toxicity Laboratory. This work was carried out under a research contract with the Atomic Energy Commission.