of H_2^3O for CF No. 1 female mice is about 1 mc per g body weight when given in a single injection. The estimated radiation dosage is about 800 rep in the first 3 days and about 1000 rep in the first 12 days after injection. It is concluded that no unexpected factor of biological effectiveness exists for tritium β rays in relation to X or γ rays.

1. Zirkle, R. E., *Am. J. Roentgenol. Radium Ther.*, 1950, v63, 170.

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Cation Exchange Properties of Alginic Acid. (19311)

B. J. LUDWIG, W. T. HOLFELD, AND F. M. BERGER.
From the Wallace Laboratories, Inc., New Brunswick, N. J.

The ability of cation exchange resins to withdraw sodium from the body(1) suggested the investigation of cation adsorbing properties of various naturally occurring substances. This report summarizes the results of *in vitro* experiments conducted to determine the cation adsorbing capacity and ion selectivity of alginic acid. For comparison, similar studies were made with a carboxylic type cation exchange resin Natrinil.*

Alginic acid is obtained from the giant kelp (*Macrocystis pyrifera*). It is a polymeric anhydro-β-D-mannuronic acid chemically related to cellulose and pectic acid. Alginic acid possesses one carboxy group for each anhydromannuronic acid residue, and this free carboxy group is responsible for its ability to form water soluble gels with alkali metal ions. Alginic acid has a theoretical cation combining capacity of 5.7 meq/g.

The sodium and potassium salts of alginic acid are readily soluble in water. For this reason, separation of uncombined cations from the soluble alginate salts cannot be made by the use of the ordinary filtration or centrifuging technics generally employed with cation exchange resins whose alkali metal salts are insoluble. Dialysis of the mixture through a suitable semipermeable membrane such as cellophane provides a satisfactory method of effecting the separation of the diffusible uncombined cations from the adsorbed cations.

* Natrinil is the trade mark of the National Drug Co. brand carboxylic type cationic exchange resin. In all experiments reported here, the acid form of this resin was employed.

Determinations of sodium, potassium and calcium were made using a Perkin-Elmer Model 52-C Flame Photometer. In determining calcium the characteristic calcium oxide band spectrum was employed. In each procedure described, suitable control experiments were conducted to establish the validity of the method under the conditions utilized.

1. *Adsorption of Na^+ from sodium chloride solutions.* The weighed sample of alginic acid was allowed to equilibrate with a sodium chloride solution by shaking at room temperature for 3 hours. The mixture was then dialysed against 3 successive portions of 1 liter of water for a total period of about 40 hours. Control experiments indicated that these dialysis conditions permitted practically quantitative diffusion of the uncombined cations with negligible diffusion of alkali metal alginates. Sodium determinations were made on the supernatant liquid obtained by centrifuging the dialysis bag contents. Curves 3 and 4 of Fig. 1 show the amount of Na^+ adsorbed by 1 g alginic acid or 1 g Natrinil from solutions containing varying amounts of Na^+. The total volume of solution in each instance was 40 ml. Both alginic acid and Natrinil were poor sodium adsorbents in unbuffered sodium chloride solutions, with alginic acid more effective than the synthetic resin. The affinity of each of the adsorbents for sodium was not appreciably influenced by the ion concentration. The sodium adsorption in unbuffered solutions was accompanied by a marked increase in hydrogen ion concentration. Liberation of hydrogen ions by 1 g

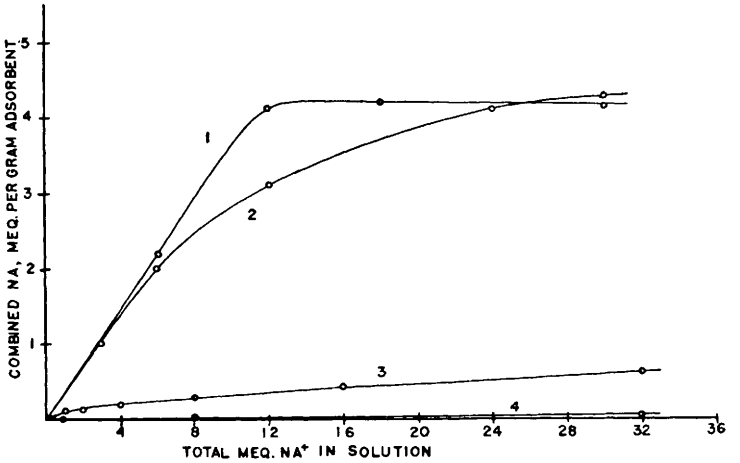

FIG. 1. Adsorption of Na^+ from solutions of varying cation concentrations. Curve 1, alginic acid and Curve 2, Natrinil, in phosphate solution pH 7.6. Curve 3, alginic acid and Curve 4, Natrinil, in unbuffered NaCl solution.

alginic acid in equilibrium with 4 meq Na^+ resulted in a lowering of pH from 6.0 to 2.2. Natrinil produced a corresponding drop in pH to 3.0.

2. *Adsorption of Na^+ from buffered solutions.* The adsorption of Na^+ from solutions containing Na_2HPO_4 and NaH_2PO_4 in a ratio to give an initial pH of 7.6 was determined as described above. Under the conditions employed conversion of alginic acid to its soluble sodium salt took place. Consequently, the extent of sodium adsorption was made by direct analysis of the dialysis bag contents. In experiments using Natrinil the bag contents were ashed and the determination of combined sodium made by analysis of an aqueous solution of the combustion residue. Adjustment of the pH of the medium to approximately that of the small intestine resulted in a marked increase in the adsorption capacity of the adsorbents (Curves 1 and 2, Fig. 1). When equilibrated with solutions containing successively higher concentrations of Na_2HPO_4 and NaH_2PO_4 having a pH of 7.6, alginic acid combined with greater amounts of Na^+ until the maximum of 4.2 meq/g was reached. The inability of this adsorbent to combine with increasing amounts of sodium when equilibrated with amounts of Na^+ above 12 meq/g indicates that the value of 4.2 meq/g is the effective sodium adsorbing capacity of alginic acid under physiological conditions. The cation combining capacity of Natrinil is approximately the same as that of alginic acid under these conditions. However, the synthetic adsorbent appears to require a greater excess of cation to bring about complete saturation.

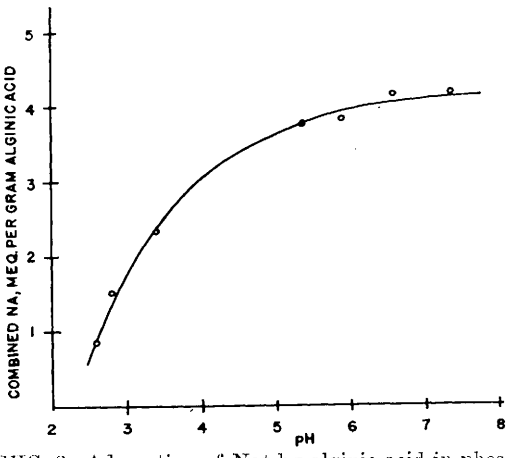

FIG. 2. Adsorption of Na^+ by alginic acid in phosphate buffer solution.

The failure of alginic acid to adsorb its capacity of sodium when equilibrated with less than 12 meq of ion is not due to a mass action type of relationship, but rather to the inability of these low concentrations of sodium phosphate to maintain the pH at a favorable level for efficient adsorption. This is apparent from the data presented in Fig. 2, which shows the sodium adsorbing capacity of alginic acid as a function of pH. In these experiments,

TABLE I. Selectivity of Alginic Acid for Sodium and Potassium at Various Cation Concentrations and Na:K Ratios.

Total cation present (meq)	Ratio $Na^+:K^+$	Combined cation (meq/g)	Ratio Na:K
12	1:1	4.1	1.1 :1
18	1:1	4.3	1.1 :1
18	5:1	4.1	4.7 :1
18	25:1	4	31.6 :1
24	1:1	4.3	1.15:1
30	1:1	4.3	1.15:1
30	5:1	4.2	4.85:1
60	1:1	4.5	1.25:1
60	5:1	4.2	5 :1
120	1:1	4	1.2 :1

alginic acid was equilibrated with 40 ml of phosphate buffer solution containing 30 meq Na^+. The phosphate mixtures were chosen to give the equilibrium pH values indicated in Fig. 2.

3. *Selectivity of alginic acid for sodium and potassium ions.* The adsorption of Na^+ and K^+ from solutions containing mixed sodium and potassium phosphates at pH 7.6 and at varying ratios of Na:K was determined. The cation-adsorbent ratios found favorable for sodium saturation at this pH were employed, that is 12 to 120 meq cation per g of adsorbent. Determinations of combined sodium and potassium were made by analysis of the dialysed bag contents as described earlier. The data in Table I indicate that the total cation adsorption from a solution containing both K^+ and Na^+ is the same as that obtained when Na^+ is present exclusively. The data also indicate that the "selectivity" of the adsorbent is determined by the cation ratio present in solution. The ratio of Na:K combined in all cases approximates that present in solution throughout the range of cation: adsorbent ratios studied.

4. *Adsorption of Ca^{++} by alginic acid.* Because of the difficulty of finding a suitable buffering agent compatible with calcium ions, calcium adsorption studies were carried out on unbuffered solutions prepared from calcium chloride and calcium hydroxide. One g of alginic acid was shaken with 200 ml of solution containing 10 meq Ca^{++}, the mixture allowed to stand overnight, and the supernatant liquid filtered and analyzed for uncombined calcium. By adjusting the ratio of calcium hydroxide and calcium chloride used it was possible to obtain equilibrium pH values from 3.0 to 11.8. Over this pH range, the calcium adsorption per g of alginic acid was 5.4 $\pm$ 0.3 meq. This value can be considered constant within the experimental error of the analytical procedure used. It is also equal to the alkali titration value of 5.2 meq/g obtained for the alginic acid employed in these studies. Natrinil was found to have a calcium adsorption capacity of the same magnitude, combining 5.5 meq/g at pH 7 and above. However, upon lowering the pH below 6, the calcium combining capacity dropped sharply, so that little if any calcium was removed from solution at pH 3 and below. In this respect the synthetic resin exchange material differs markedly from alginic acid.

Discussion. The studies reported in this paper show that *in vitro* alginic acid is capable of adsorbing sodium and potassium ions as efficiently as the synthetic cation exchange resins. The selectivity for sodium exhibited by the natural material also appears to be similar to that observed with the resins. Alginic acid, although itself insoluble in water, differs from the exchange resins in forming water soluble sodium and potassium salts. Alginic acid also differs from the resins in being broken down to a certain degree in the digestive tract(2).

The free acid form of carboxymethylcellulose was also explored for its cation adsorbing properties. In general it behaved in a similar manner as alginic acid, forming a soluble sodium and potassium salt and an insoluble calcium salt.

The use of alginic acid as a cation exchange material *in vivo* appears worthy of study. This substance, which for satisfactory sodium removal must be taken in daily doses of at least 45 g, is more palatable than the resins. The sodium alginate formed in the intestine is extremely hydrophilic and this property assists in the removal of fluid. The mildly laxative property of the sodium alginate gel also constitutes a desirable property.

The ability of alginic acid to adsorb calcium indiscriminately over a wide range of hydrogen ion concentrations may unfavorably influence cation adsorption *in vivo*. When given

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^+ per g. The total cation adsorption from solutions containing K^+ and Na^+ is also 4.2 meq/g and the ratio of Na:K combined approximates that present in solution. The adsorption of Ca^{++} 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.

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)

ROBERT N. FEINSTEIN AND CARRIE L. BUTLER. (Introduced by J. M. Coon.)

From the United States Air Force Radiation Laboratory and the Department of Biochemistry, University of Chicago.*

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 blendor with 9 volumes of N/10 NaOH. The blend was filtered through nylon (National Filter Media Co.,

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