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Neutrality Regulation Mechanisms in Rats Receiving Sulfonic Ion Exchange Resin. (19435)

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Previous studies of the *in vivo* action of cation exchange resins(1,2) have shown that one of their important effects, when fed in either the H– or NH_4– form, is to increase urinary acid excretion (*i.e.*, $BH_2PO_4 + NH_3$). However, if the resin fed contains about 2.4 meq of sodium or potassium per g the urinary acid output remains the same as that of control animals receiving the same diet, without resin. From this it may be reasoned that the net fecal alkali loss which occurs as a result of ingesting the resin, on the specific diet used, must also be 2.4 meq per g. This assumes, of course, that there has been no net change in the alkali reserve of the animals during the experimental period. It constitutes one approach to the problem of determining the total *in vivo* cation uptake of the resin.

Our understanding of the effects of cation exchange resins on neutrality regulation mechanisms is largely based on studies of human subjects. The work of Irwin *et al.*(3) indicates that the ingestion of H-form resins results in a compensated metabolic acidosis. The fact that either H– or NH_4– form resins may cause acidosis is well known, and for the intended purpose, this is to some extent desirable(4). Rats receiving 10% of either carboxylic or sulfonic resin in their diets grow nearly as well as control animals on the same (resin-free) food(5,6), which indicates that they are quite able to deal with this situation over the long term. How they accomplish this is not exactly known, nor has the acidifying action of the resins been sufficiently studied quantitatively. The purpose of the work reported herein has been to study neutrality regulation during a period of resin feeding, and during a subsequent "recovery" period, in order to clarify some of these points more adequately.

Experimental procedures. Twelve female rats served as subjects for these experiments. They were kept in pairs in metabolism cages, with provision for the collection and separation of urine and feces. The former was collected in paraffin-lined beakers over thymol. Deionized water was available at all times. Regular colony diet* was provided *ad lib.* for 5 days before the test procedures began, and during the 5-day recovery period, except that no food was available for the last 16 hours of these periods. The resin period,† which intervened, was also of 5 days' duration; the animals were not fasted at its conclusion, but were immediately transferred to the colony diet. Food consumption records were kept for the resin and recovery periods, and all of the analytical data were reduced to terms of meq per 90 g of colony (or 100 g of resin-containing) diet consumed. For purposes of

* For composition, see reference (7), footnote 4. Analysis indicated that 90 g of this diet contained the following, as meq: Na, 27.4; K, 20.8; Ca, 29; Mg, 11; PO_4, 52; and Cl, 29.

† Diets of pairs 2-6 then contained 10% resin. Ammonium-form (4.6 meq NH_3 per g) and potassium-form (4.1 meq per g) were mixed to give potassium contents described in the Table.

TABLE I. Electrolyte Metabolism and Neutrality Regulation in Rats Receiving Sulfonic Exchange Resin.

Pair No.	mg K/10 g resin	Urinary constituents*						Excess acidt	Fecal constituents*						Net alkali lost‡	Balances		
		pH	H^+	NH_4^+	Na^+	K^+	Inorg. $HPO_4^=$	Cl^-		Na^+	K^+	Ca^{++}	Mg^{++}	$PO_4^=$		Acid bases§	Na^+	K^+
									Resin period									
1	(control)	6.7	1.8	3.5	21.3	14.6	8.9	25.1	—	2	2.5	21.7	7.8	25.2				
2	0‖	5.8	8.4	12.2	14.6	12.1	18.4	24.2	15.3	7.7	4.2	23.5	7.7	12.1	22.1	-6.8	+4.1	+3.7
3	100@	5.9	8.5	8.3	13.4	21.9	19.4	25.9	11.5	9.6	4.6	24.9	7.5	16.5	21.3	+.2	+5.1	+4.5
4	20	6.3	6.2	4.5	14.2	27.7	18.6	25.4	5.4	9.7	7.2	23.6	7.0	14.7	24.0	+1.4	+3.7	+4.3
5	28	6.7	3.8	4.2	14.5	33.8	19.5	26.0	2.7	8.5	7.3	21.9	7.0	14.4	21.5	+9.2	+4.7	+5.9
6	38	7.6	-0.2	1.6	12.2	37.7	15.6	24.9	-3.9	10.4	9.2	22.5	8.0	17.7	24.6	+9.5	+5.2	+8.7
									Recovery period									+11.5
1		6.9	1.7	5.5	23.5	15.1	9.5	25.2	—	3.5	3.0	23.6	9.5	29.6	—	—	+0.4	+2.7
2		7.0	1.7	7.1	25.5	15.7	10.6	27.1	1.6	3.5	2.8	24.6	9.1	27.0	3.0	-1.4	-1.6	+2.3
3		6.7	2.9	5.0	23.6	16.1	8.4	27.1	0.7	3.9	3.0	24.2	7.4	28.3	0.2	+0.5	-0.1	+1.7
4		6.9	1.4	5.0	23.8	16.1	7.9	26.6	-0.8	3.7	3.2	24.1	8.5	29.8	-0.1	+0.9	-0.1	+1.1
5		6.8	2.0	6.6	24.2	17.3	9.5	26.6	1.4	3.8	2.5	22.8	7.6	24.4	2.3	-0.9	-0.6	+1.0
6		7.1	1.3	8.7	24.8	17.2	10.5	28.1	2.8	4.2	4.3	20.3	8.8	25.6	2.0	+0.8	-1.6	-0.7

*All constituents except pH are reported in terms of meq/90 g colony diet ingested. †i.e., increase of titratable acidity + ammonia over that of controls. The former represents alkali required to adjust to pH 7.5. ‡Increase of output of all cations, + decrease of phosphate output, per 10 g resin ingested. §The calculation is: (excess urinary acid output) + (K content of resin) − (Net fecal alkali loss). ‖Usually referred to in previous publications as Win 3000. @Katonium-K, trade-name of Winthrop Stearns, Inc.

convenience, all urine samples were diluted to 350 cc before analysis. The analytical methods used were the same as those previously described(1,8); the data are presented in Table I.

It is evident that a partially compensated metabolic acidosis is the principal effect produced by feeding the ammonium resin: the animals respond by increasing their urinary output of acid phosphate and ammonia. There is, of course, an increase in fecal output of sodium and potassium, but very little net change in calcium and magnesium. That the latter observation is misleading is indicated by the striking decrease in fecal excretion of phosphate. The resin probably affects phosphate metabolism by binding calcium (and perhaps some magnesium), thereby releasing phosphate for absorption. The implication is, therefore, that the amount of calcium and/or magnesium actually bound by the resin is about 1.5 meq per g, not the 0.17 meq which represents the difference in experimental and control fecal outputs of these elements (per g resin ingested). This effect on phosphate metabolism has been observed previously (8,9), but perhaps has not been generally recognized. It still occurs to a slight extent in some pairs during the recovery period, but there is surprisingly little extra fecal sodium and potassium in the recovery period considering that no interval of fasting preceded it, and the digestive tracts were, therefore, not clear of resin when it began.

The decreased absorption of cations, and increased absorption of phosphate are combined to make the factor called "Net alkali loss". This loss is not entirely compensated by the increased urinary output of acid and ammonia, hence the overall effect on acid-base balance is computed as negative. There is no evidence that this effect is significantly compensated, during the recovery period, by increased acid output.

In the pairs receiving potassium resin the acid output is diminished, but by no means in exact proportion to the amount of resin potassium provided. In fact, there is even some excess acid produced in pair 5, which received more than enough potassium to offset the

fecal alkali loss. It is true that a part of this potassium does not actually become available to the animals, but this is taken into account in computing the fecal alkali loss. The latter factor is a measure of the total *in vivo* cation uptake of the resin (other than for hydrogen and ammonium ions), and is quite consistent, averaging 2.4 meq per g for all pairs for the two experimental periods combined. This figure is in good agreement with that previously obtained(1), although arrived at in a somewhat different manner. The data for pairs 3 and 4 confirm the previous observation(2) that a potassium content of 1-2 meq per g resin is probably optimal.

An important feature of the results is the apparent failure of pairs 5 and 6 to compensate, during the recovery period, for the alteration in their alkali reserve which the data indicate occurred during the resin period. The positive balance is represented partly by retained potassium, as is shown by the cation balance data. Since no significant amount of

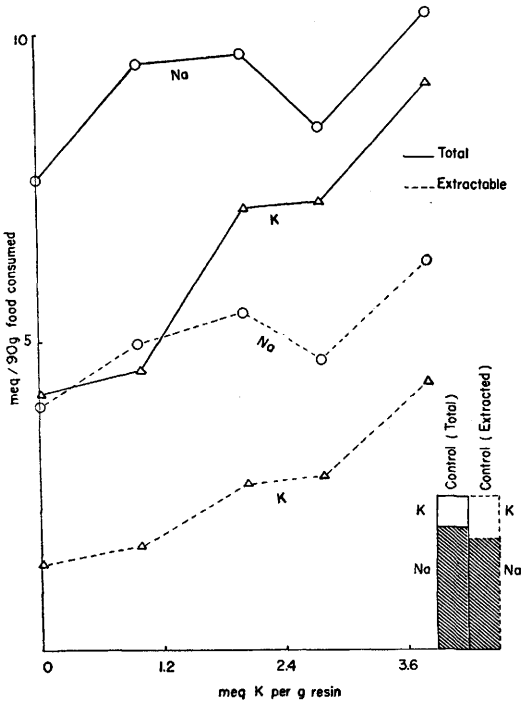

FIG. 1. Total sodium and potassium in feces, as compared to sodium and potassium extractable by water, plotted against potassium content of resin. In control animals virtually all of these elements is extractable.

compensation occurs in these pairs (or in pair2) during the recovery period, it is evident that nearly complete compensation has already taken place (as, for example, by alteration of the rate of excretion of alkali bicarbonates(10)). Also, the following observation may be of some interest.

Nature of resin-cation combination. It has usually been assumed that extra cations appearing in the feces as a result of resin ingestion are "bound" by it(1,2,7,8). Physical isolation of the resin from the other fecal matter would show definitely whether this is so. It is also possible(8) that hydration of the resin in the intestine leads to the retention of some electrolytes which are not directly associated with it. In order to study this point, 2% aqueous extracts of the feces of pairs 1-6 for the resin period were made,‡ filtered, and analyzed for sodium and potassium, with the results shown in Fig. 1. It is apparent that the amounts of each of these ions in the extracts is directly related to its total concentration in that sample. About half of the sodium is extracted in each case, but the proportion of potassium extracted increases with the total amount present. In fact, in pair 2 the extractable potassium is even less than the amount found in the controls. This could mean that an exchange reaction is occurring during the extraction, with some potassium passing into the resin. This would occur if the K:Na ratio in the fecal residues exceeded that already existing in the resin. It is not clear, however, why such large proportions of these cations are present in a readily extractable form. The feces of these animals were not of a character which would suggest that any extensive water retention had occurred.§ It also seems unlikely that the extracted ions have been released in exchange for other

‡ The finely pulverized feces were extracted with mechanical shaking for 45 minutes, then filtered. The pH of all extracts was 6.7.

§ If the resin does cause any appreciable water retention, some of the extra cations appearing in the feces may not represent a loss of alkali, since the anions would also be retained in the intestine. This would help to explain the results in pair 2, but not in 5 and 6.

cations present in normal feces, except as noted above.

Summary. Rats receiving sulfonic acid resin respond by increasing their urinary output of acid phosphate and ammonia, but not in amounts sufficient to compensate for their fecal alkali loss. Those receiving moderate amounts of potassium resin show little change in acid-base balance, while those on high potassium resin intakes show a partially compensated alkali retention. The net fecal alkali loss amounts to 2.4 meq per g resin fed, but a large part of this represents increased phosphate absorption, presumably due to the binding of calcium and/or magnesium by the resin. There is suggestive evidence that some of the extra alkali appearing in the feces as a result of resin ingestion is not in direct combination with the resin, and this may have some bearing on the problem of neutrality regulation.

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Protective Effect of Moisture on Denaturation of Human Serum Albumin By Heat (19436)

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It is well known that various proteins and living organisms are more resistant to heat when dry than when in the liquid state. However, certain materials when dried to a critical level (5-10% moisture) are more unstable than the same material in solution(1). While studying this interesting fact it was noted that dialyzed albumin, though denatured by heat when thoroughly dry, was protected when containing a certain amount of moisture.

This observation is published since it may have some bearing on the discordant results obtained in studies on viability of dried bacterial cultures.

Procedure. Twenty-five per cent human serum albumin containing 1/15,000 merthiolate was dialyzed for 24 hours at 4°-6°C against several changes of distilled water. This was made up to 5% solution with distilled water and 4.5 ml quantities distributed into a number of 20 ml ampoules. To one-third of these was added 0.5 ml of water, to another third 0.5 ml of M NaCl and to the last third 0.5 ml of 5 M NaCl. This material was then dried from the frozen state in the ampoules. They were then divided in 6 groups and placed in desiccators over sulfuric acid of the following specific gravities: 1.83, 1.73, 1.61, 1.53, 1.42, and 1.31. They remained for over a week in the desiccators at room temperature under a vacuum of 1-10 mm of Hg. During this time the moisture content of the albumin in each desiccator reached a predetermined level depending upon the concentration of the sulfuric acid(2). One ampoule of each preparation was then removed from the desiccators and the moisture content determined by the U.S.P. method. The remainder of the ampoules were then sealed under air. The sealed ampoules were then autoclaved at 250°C for 2 hours, opened and reconstituted with 5 ml of distilled water. A photoelectric colorimeter (Lumetron) was used to measure the turbidities using a green (530) filter. The results were plotted as percent transmission of a water control.