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Labile Phosphate Changes in Ileum of the Rat. (25048)

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Mass movement of large intestine related to eating is a well-known phenomenon. Grossman(1) states that the small intestine also shows increase in motility within a few minutes after eating and suggested the possibility of hormonal involvement. Picchioni and Edwards(2) noted that isolated intestinal strips had greater activity if animals were fed just prior to sacrifice. Kroeger and Edwards(3) reported when rats were fasted to 48 hours and fed just before sacrificing, this treatment caused not only changes in activity but changes in acid-soluble phosphate fractions related to intermediate metabolism and muscle contraction. Whereas changes in phosphate compounds reported by these authors occurred in combined samples of mucosal and muscularis tissue, our object was to determine the extent to which both tissues separately undergo these changes. The data indicate that each tissue shows significant chemical changes with regard to fasting and feeding treatment but that there is no real difference between the 2 tissues.

Methods. Sixty Sprague-Dawley albino rats of either sex and weighing 170 to 275 g, were divided randomly into 6 equal groups. Rats were fed a standard ration of laboratory pellets† except as noted for different groups. Group I was fed *ad lib* until time of sacrifice. Groups II, III and IV were fasted 24, 48 and 72 hours respectively. Animals in Groups V and VI were fasted 48 hours, then allowed to nibble on food pellets for 10 and 15 minutes prior to sacrifice. All groups were given water *ad lib*. The last 24 cm of ileum was removed, washed with ice-cold saline and divided into 4 segments. These segments were opened longitudinally, laid serosa-side down on absorbent toweling and frozen with liquid nitrogen. After mucosa was cut off with razor blade, both mucosal and muscularis samples were refrozen and pulverized in stainless steel mortar. Extraction and assay methods previously reported(3,4) were used and phosphate fractions determined were: T.A.S.P.-P. representing total acid-soluble phosphates, I.P.-P representing ortho phosphate present in tissue as such, "Cr.P.-P." phosphate hydrolyzed at 25°C for 30 minutes in 1 N.H_2SO_4 and "A.T.P.-P.", the phosphate hydrolyzed at 100°C for 20 minutes in 1 N.H_2SO_4.

Results. Concentrations of various phosphate fractions expressed in mg% phosphorus/wet weight tissue are shown in Fig. 1, 2, 3, and 4, each value representing 40 assays. Analysis of variance and the Mann-Whitney "U" test(5) for ranked data indicates that treatments are significant at least to 0.05 level of confidence. Variation between mucosal and muscularis samples are not significant. All phosphate fractions show a decline in median concentration as period of fasting is extended except A.T.P.P. in mucosal segments. This increases slightly during first 24 hours but is comparable with the muscularis tissue at 72-hour period. This rise was previously noted(3) using combined samples of mucosa and muscularis.

Most concentrations for different fractions decrease gradually, reaching 50 to 60% of control values by 72-hour period. As fasting period is prolonged all phosphate fractions become more uniform in range of concentra-

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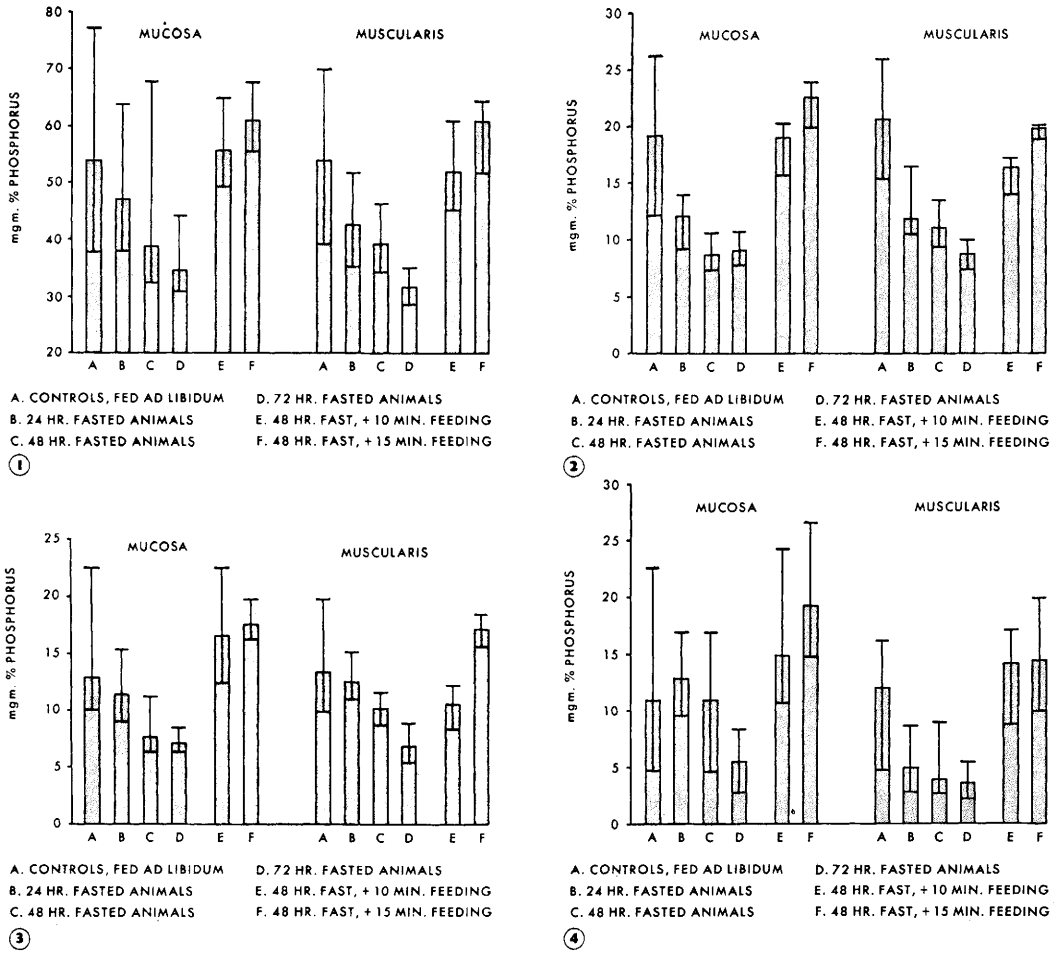

FIG. 1. Influence of fasting and feeding treatments on T.A.S.P.-P. concentrations in ileal segments of rat intestine. Concentrations shown as median values with quartile limits.

FIG. 2. Influence of fasting and feeding treatments on I.P.-P. concentrations in ileal segments of rat intestine. Concentrations shown as median values with quartile limits.

FIG. 3. Influence of fasting and feeding treatments on Cr.P.-P. concentrations in ileal segments of rat intestine. Concentrations shown as median values with quartile limits.

FIG. 4. Influence of fasting and feeding treatments on A.T.P.-P. concentrations in ileal segments of the rat intestine. Concentrations shown as median values with quartile limits.

tion. A.T.P.-P. values in the muscularis fall off rapidly during first 24 hours but decrease more gradually during next 2 days of fast.

When animals are allowed to nibble briefly for 10 and 15 minutes, there is a rapid return of all phosphate fractions towards normal and in many cases their values exceed controls. These altered values show the same uniformity as seen with fasted animals. Examination of alimentary tract showed no evidence of passage of food particles beyond the stomach and a few animals with marked responses had food particles only in their mouths.

Samples of tissue from all experimental groups were analyzed for both water and Kjeldahl nitrogen. These results indicated that changes in phosphate are not a concentration or dilution effect by water or other substances.

Discussion. Most values obtained in this study compare favorably with phosphate values reported for smooth muscle(6,7,8), however, the T.A.S.P.-P. content is considerably below those previously reported by Kroeger and Edwards(3). The finding that values for I.P.-P. and T.A.S.P.-P. decrease with fast-

ing are also in disagreement. No satisfactory explanation can be offered except those of strain difference and climatic conditions.

Adding the values for I.P.-P., Cr.P.-P. and A.T.P.-P. leaves rather small quantities of difficultly hydrolyzable phosphate. Values for this latter fraction do not show the decline exhibited by other fractions but remain quite constant. The trauma to which these tissues are exposed during separation is appreciable, although ortho phosphate content is not excessive.

Wide variation of concentration values for various fractions in the control group is perhaps best accounted for by animals' random eating patterns. Some animals may have just eaten, others not for several hours. As animals are subjected to more uniform treatment, range of concentration decreases. The apparent increase of A.T.P.-P. in the mucosa after 24-hour fast is perhaps accounted for by this mechanism, however this may be an example of transfer of energy-rich phosphate bonds from a storage unit to an active donor. With increased starvation time, even the donor becomes greatly depleted. The brief feeding of 48-hour fasted animals, causing shifts of phosphate values towards control amounts, suggests a mechanism responsible for preparing intestines for receiving and handling of food material. Incomplete data shows a similar picture for 24- and 72-hour fasted animals. Rapidity of these alterations indicates that amounts of energy-rich phosphate compounds are more dependent on activity states than purely nutritional depletion and restoration. A study of this "chemical reflex" may prove of value in determining whether hormonal and/or nervous mechanism is responsible for activation of intestinal tract. Investigation is now underway to determine sensory mechanisms for this reflex.

Summary. 1. Both muscularis and mucosa of rat show significant changes in amounts of energy-rich phosphate compounds when animal is fasted or fasted and briefly fed. 2. With increasing periods of starvation to 72 hours, the easily hydrolyzable phosphate compounds, Cr.P.-P. and A.T.P.P., diminish in both tissues in a predictable manner. 3. The mucosa appears to be more labile of the 2 tissues, although the muscularis shifts in the same direction and to nearly the same magnitude. 4. The assay of either Cr.P.-P. or A.T.P.P. on whole intestinal segments will provide sufficient data as would the independent assay of each, in the major tissues comprising the intestine, to follow the nature of the mechanism involved.

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Fluorometholone, A Preferentially Anti-Inflammatory Corticoid. (25049)

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Biological activities of hundreds of analogs and derivatives of hydrocortisone synthesized in the past decade have begun to establish certain patterns. Potencies far beyond that of hydrocortisone have become commonplace(1-5) and separation of "glucocorticoid" from "mineralocorticoid" properties has been achieved with several compounds. Many compounds which are potent in bioassays measuring changes in carbohydrate metabolism are also potent in bioassays measuring suppression of experimental inflammation, involution of lymphoid tissue, production of eosinopenia, and suppression of ACTH secretion. This