THE ACID-SOLUBLE PHOSPHATE DISTRIBUTION IN TERMINAL ILEUM STRIPS OF RAT AS AFFECTED BY FASTING TREATMENTS

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by

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INTRODUCTION

In general, the digestive tract is a tube designed for receiving, digesting and absorbing chemical substances which constitute the ingested food. The digestive tract has to move its contents from an area of one physiological activity to another for the purpose of physical and then enzymatic disintegration, then on to areas of absorption and, finally, to the area of waste elimination. Both enzymatic and motile activity depend upon the physiological status of the intestines and are controlled by numerous mechanisms, either extrinsic or intrinsic in origin.

Included in the extrinsic mechanisms is the cephalic phase as shown by Pavlov to produce gastric secretions by conditioned reflexes. This mechanism is generally thought to be effected through the vagus nerve since sectioning abolishes the reflex, although Uvnas (1) has presented evidence, that, in addition to the vagus, there is a hormonal mechanism involved for producing the digestive or "appetite" juices. Other extrinsic mechanisms include the hormonal activation of enzymatic and motility activities of the intestines and accessory organs. This phase of control as well as a third phase, secretogogues, has been recently reviewed by Grossmann (2) in which he regards a specific hormone governing intestinal motility still in question.

Intrinsic control, dependent upon irritative and pressure

stimuli affecting local areas of the intestine, seems to be an activity reflexing through the Meissner and Auerbach plexi; however, both extrinsic and intrinsic controls seem only to modify existing activity states which have myogenic origin.

In using intestinal segments in the physiology and pharmacology laboratories, it has been the practice of the experimenter to starve the animals prior to sacrificing so that the intestinal segments could be obtained from a more uniform group of animals and also to be fairly free of fecal contents. Picchioni (3) observed increased motility in rat intestinal strips obtained from animals starved for 24 hours and fed for about an hour before sacrificing. Kroeger (4) observed not only activity changes but chemical changes occurring as the result of a brief feeding period prior to sacrificing starved animals.

The latter determined the amounts of acid-soluble phosphate in the intestinal tissue. The importance of the acid-soluble phosphates, which includes the high energy phosphate compounds, as a source of energy for tissue activity has been the subject of many reviews among which are Lipmann (5), Mommaerts (6) and Lundsgaard (7). Both muscular activity and transportation of certain chemical substances across cell boundaries are quite dependent upon energy from phosphate metabolism.

The purpose of this work is to restudy the effects of fasting and feeding treatment upon the phosphate distribution in the terminal ileum of the rat in order to determine whether the muscular or the mucosa structure is most affected by such treatments. In addition, it seemed advisable to re-examine the dependability of this technique for possible use as an end point in further studies to elucidate the nature of the intestinal controls, either hormonal or nervous.

To summarize, the objectives of this study are:

1. To restudy, by the analysis of the acid-soluble phosphate distribution, the effects of fasting treatment upon the terminal ileum of the rat.

2. To determine, by the analysis of the acid-soluble phosphate distribution, whether the mucosa or muscular structure is most effected by such treatments.

3. To determine, by the analysis of the acid-soluble phosphate distribution, the dependability of this technique for use as an end point to determine the nature of intestinal control.

LITERATURE SURVEY

Grossman (2), in a review, states that the small intestine of an animal shows an increase in motility within a few minutes after feeding, and this effect occurs even when food is admitted to the stomach through a gastrotomy. This effect cannot be abolished by sectioning of the vagus, which suggests the presence of a hormone that may have an effect on the activity of the small intestine. But, he states, this hormonal mechanism should not be fully accepted until such a hormone has been clearly demonstrated.

Picchioni (3) reported that greater activity for intestinal strips was obtained if the rats had been fed prior to sacrificing. Kroeger (4) reported that when rats were fasted for 24 and 48 hours prior to sacrificing, it not only caused activity changes but chemical changes as well. He noted significant reductions in the amounts of Creatine phosphate and energy-rich phosphate in the combined portions of muscularis and mucosa of the small intestine, whereas, the same treatment caused an increase in the amounts of Adenosine triphosphate in the same segments. He also showed that in rats starved for 48 hours and then fed for 10 and 15 minutes prior to sacrificing, the amounts of Creatine phosphate, energy-rich phosphate and Adenosine triphosphate were restored to control levels.

The enzymatic splitting of Adenosine triphosphate and Creatine phosphate is supposed to be the chemical reaction most closely coupled to the energy liberation in muscle contractions; thus, the amount of energy liberated in muscle depends upon the amounts of Adenosine triphosphate and Creatine phosphate available and the enzymatic activity

of ATPase as well.

Sarzana (8) found in experiments performed on pigeons' skeletal muscle, that fasting caused a noticeable loss of acid-soluble phosphates. With a 25% decrease of body weight, the phosphoric ester compounds decreased as much as 60% in skeletal muscle and 30% in the heart muscle. Other fractions of the acid-soluble phosphate group were less affected.

EXPERIMENTAL PROCEDURE

I. Generalities

The animals used in this study were Sprague Dawley Albino rats of either sex ranging in weight from 170 to 275 grams. The rats were fed a standard ration of Purina Laboratory Chow, pellet form, except as noted for the experimental groups. Sixty animals were divided into six experimental groups, each consisting of ten rats. The control group was fed ad libidum until time of sacrificing. Group II consisted of ten animals which were starved for 24 hours, then sacrificed. Group III consisted of ten animals starved for 48 hours, then sacrificed. For Group IV, ten animals were fasted for 72 hours prior to sacrificing. Group V, ten animals were starved for 48 hours and at the end of the fasting period they were given a pellet of food to nibble for ten minutes. Group VI consisted of ten animals which were starved for 48 hours and at the end of the fasting period they were given a pellet of food to nibble for fifteen minutes prior to sacrificing.

The following design was used as a systematic way of counterbalancing any differences that could occur due to chance if all animals in each group were sacrificed at one time.

IV-I	IV-II	IV-III	IV-V	IV-VI *
VI-I	VI-II	VI-III	VI-V	VI-IV
V-I	V-II	V-III	V-VI	V-IV
III-I	III-II	III-V	III-VI	III-IV
II-I	II-III	II-V	II-VI	II-IV
I-II	I-III	I-V	I-VI	I-IV

* Roman numerals represent experimental groups.

The procedure followed in this study was essentially the same used by Kroeger (4). The animal was killed by cervical dislocation and the throat was cut to permit bleeding. Immediately the abdominal cavity was exposed and the intestines were packed in crushed ice and the animal was placed in the deep freeze for a period of ten to fifteen minutes. The intestines were removed, placed in a glass plate which had a centimeter scale, and the last 24 centimeters strip was removed and washed in ice cold saline. This intestinal strip was then divided into four equal segments approximately six centimeters in length. The segments were placed on an absorbent towel, frozen with liquid air, then placed in the deep freeze. The intestinal strips were thawed up to -20°C. and the excess mesenteric fragments were cut off with a razor blade. The intestinal strips were split longitudinally along the mesenteric attachment line and spread flat on absorbent toweling with serosa side down and again frozen. The mucosa was scraped off with a razor blade while the segments were frozen. The mucosa section was frozen again with liquid air, placed in a chilled stainless steel cylinder and the tissue was powdered with a chilled piston, according to Le Page's method (9). The frozen plug of firmly packed finely powdered tissue was weighed on a Roller Smith balance, then placed in a centrifuge tube containing 5 ml. of 10% Trichloroacetic acid. The plug was disintegrated by a stirring rod, The stirring rod was removed and washed down quantitatively with 1 ml. of 10% of Trichloroacetic acid. The tube was transferred to an International Centrifuge clinical model, mounted in the deep freeze after all the mucosa segments were disintegrated, and then centrifuged for fifteen minutes. After centrifuging at the temperature of the

deep freeze, (-10 to -5°C.), the supernatant was carefully removed and placed in a 25 ml. volumetric flask and the residue was re-extracted with 5 ml. of ice cold 5% Trichloroacetic acid. After rehomogenizing the packed residue, the tube was again centrifuged for a period of fifteen minutes in the cold and the supernatant added to the first extraction. The combined extracts were brought up to volume with ice cold 5% Trichloroacetic acid, thoroughly mixed and kept in the deep freeze until transfer of aliquots for assay were made.

The same procedure was employed for the muscularis.

II. Phosphate Determination

The technique used in this study for the phosphate determination was that of Ernster, Zetterstron and Lindberg (10). This method consists of the quantitative extraction of phosphomolybdate by a mixture of isobutanol and benzene, 1-1, from an acidified aqueous solution. The phosphate compounds analyzed by this technique are:

1. Inorganic phosphate phosphorus, I.P.-P.
2. Creatine phosphate phosphorus, Cr.P.-P.
3. Phosphate phosphorus hydrolyzed at 100°C. for 35 minutes in 10 N. sulfuric acid.
4. Total acid-soluble phosphate phosphorus, T.A.S.P.-P.

The methods for the determination of each of the foregoing compounds will be described in the order listed.

1. Inorganic phosphate phosphorus.

   Three ml. of aliquot portions from the extract were placed in cold Folin-Wu tubes and 5 ml. of isobutanol-benzene, 1-1 mixture, was added. To this were added 0.5 ml. of 10 N. H_2SO_4

and 0.5 ml. of 10% ammonium molybdate reagent. The tubes were shaken for fifteen seconds, and then 3 ml. of the organic phase were placed in standard cuvette (Coleman B). To this was added 1 ml. of isobutanol-benzene mixture, 1-1, and 1 ml. of acidified ethyl alcohol. The color was developed by the addition of 0.5 ml. of dilute stannous chloride reagent.

2. Creatine phosphate phosphorus determination.

The organic phase remaining in the Folin-Wu tube after the removal of the inorganic phosphate sample was sucked off by means of a pipet and the tube was placed in a water bath at 20°C. for thirty minutes to hydrolyze the Creatine phosphate. The newly formed phosphomolybdic acid was extracted with 5.0 ml. of isobutanol-benzene reagent, 3 ml. of the organic phase was transferred to a standard cuvette and the procedure carried forward as for the inorganic phosphate phosphorus determination.

3. Phosphate phosphorus hydrolyzed at 100°C. for 35 minutes.

Three ml. of the Trichloroacetic acid tissue extract were transferred by pipet to a Folin-Wu tube. After adding 0.5 ml. of 10 N. H_2SO_4, the tube was placed in a steam bath for thirty-five minutes. The tube was then rapidly cooled to room temperature and 0.5 ml. of 10% ammonium molybdate reagent was added. The phosphomolybdic acid was extracted with 5 ml. of isobutanol-benzene and the color sample prepared as previously described.

4. Total acid-soluble phosphate phosphorus.

One ml. of the Trichloroacetic acid tissue extract was placed

in a 20 x 150 ml. Pyrex test tube and 0.15 ml. of 10 N. H_2SO_4 was added. The tube was placed in an oven at 150 to 180°C. for sixty minutes at which time a brown liquid residue remained. The tube was removed and cooled slightly before the addition of 1 to 2 drops of 30% H_2O_2. The tube was replaced in the oven for a period of thirty minutes at the same temperature after which the tube was removed and cooled slightly. Two ml. of distilled water were used to rinse down the side of the tube and the tube was then placed in a steam bath for thirty minutes to hydrolyze the pyrophosphates. Upon completion of this procedure, the tube was cooled in running tap water and 0.5 ml. of 10% ammonium molybdate was added. The phosphomolybdic acid was extracted with 5.0 ml. of isobutanol-benzene and the color samples prepared as previously described.

III. Development of Techniques

1. Calibration of Coleman Universal Spectophotometer, Model 14

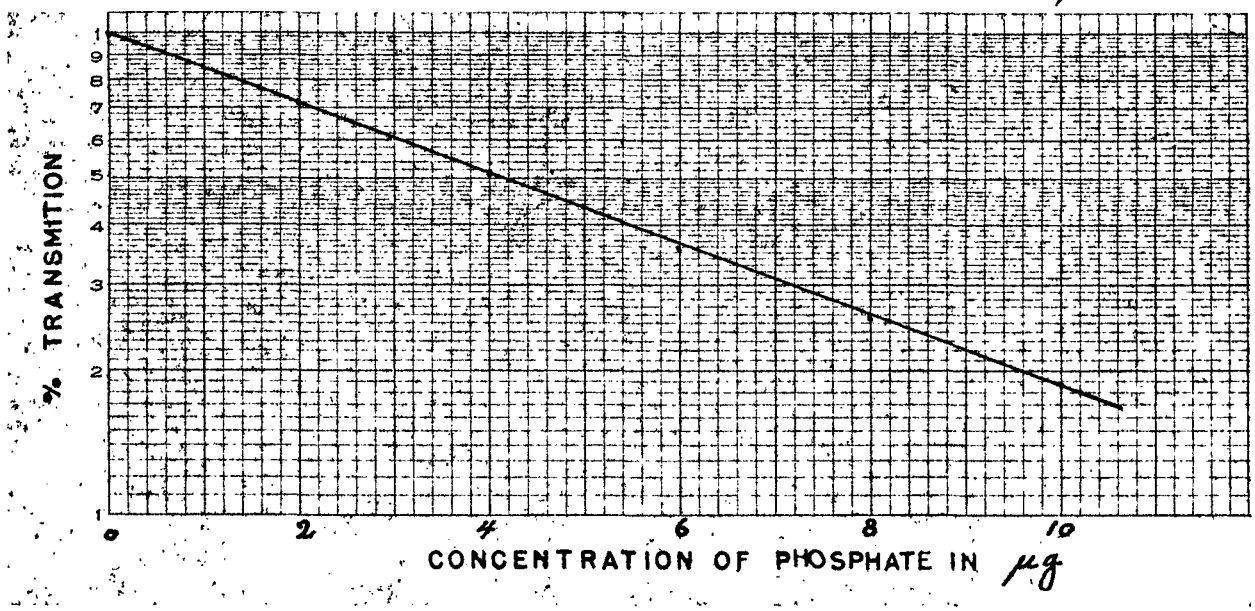
A calibration curve for phosphorus was prepared by extracting 2,4,6,8,10 micrograms of phosphate with Trichloroacetic acid, Figure I, in accordance with the technique employed for the phosphate determination in the tissue extracts. Three ml. of 5% Trichloroacetic acid was used for the preparation of the blank sample. Desiccated monopotassium phosphate was used to prepare the phosphorus standards.

2. Hydrolysis of Adenosine triphosphate

Figure 1

Calibration Curve for Phosphorus Determination

Using The Coleman Universal Spectophotometer At 625 mμ

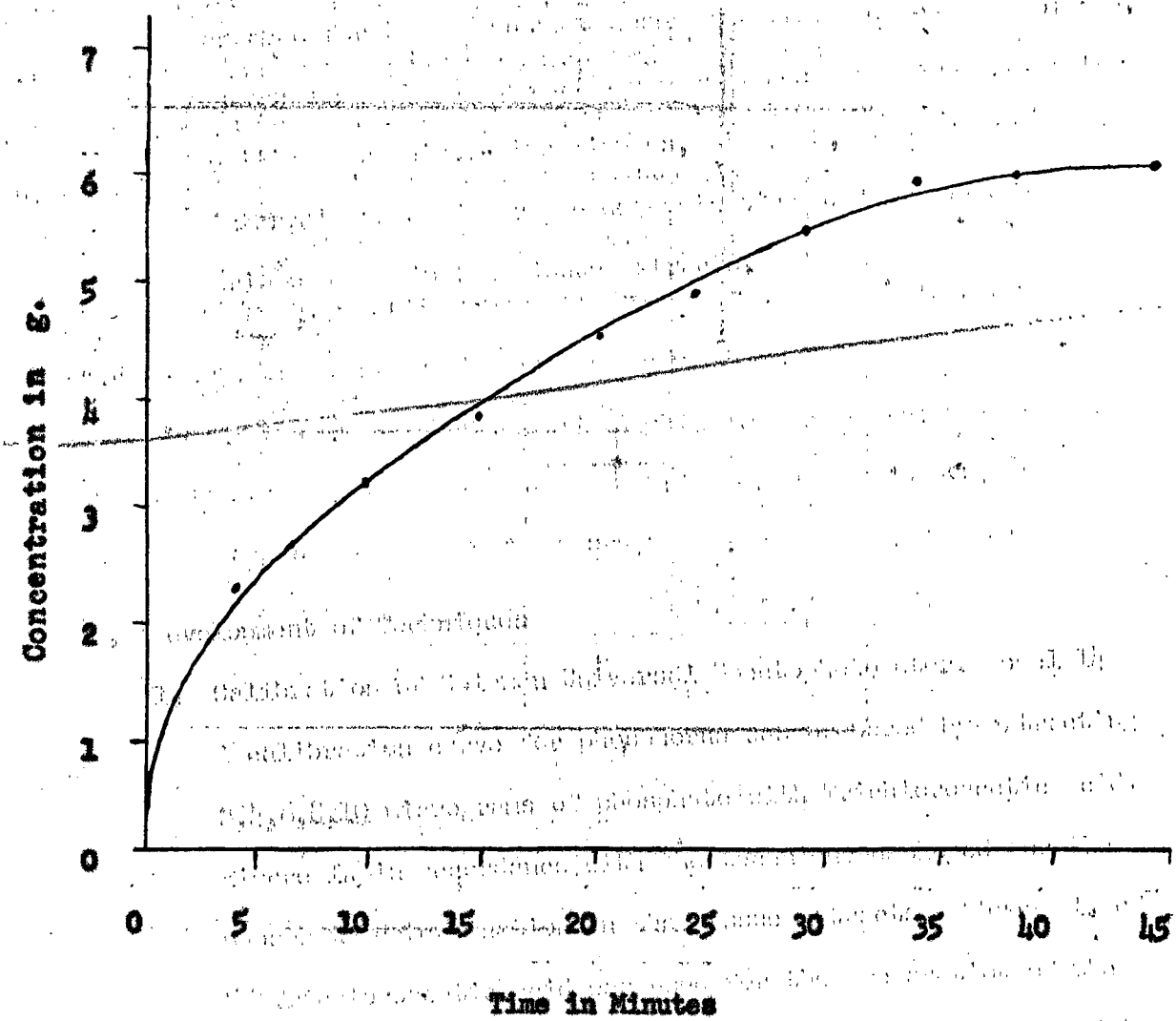


For this determination, 50.7 mg. of Adenosine triphosphate, sodium salt, prepared by Schwarz Laboratories, was dissolved in 100 ml. of 5% trichloroacetic acid, 1 ml. of the solution was diluted to 200 ml. with trichloroacetic acid, and 3 ml. aliquots were placed in Folin-Wu tubes and were hydrolyzed in a water bath. Every five minutes, one tube was removed and assayed for the amount of phosphorus that had been hydrolyzed. It was noted that most of the phosphorus was hydrolyzed after boiling for thirty-five minutes, Figure 2, and this time interval was used for the determination of the Adenosine triphosphate in the tissue extracts.

Figure 2

Hydrolysis of Labile Phosphate from A.T.P.

In 3.0 ml. 5% Trichloroacetic Acid At 100° C.



RESULTS

The abbreviations for the various acid-soluble phosphate compounds used in the subsequent pages are:

I.P.-P. represents the orthophosphate present in the tissues as such.

Cr.P.-P. represents the phosphate hydrolyzed at room temperature in 1 N. H_2SO_4 for 30 minutes and is creatine phosphate phosphorus.

A.T.P.-P. represents the phosphate hydrolyzed at 100°C. for 20 minutes in 1 N. H_2SO_4 and is composed of two-thirds of the phosphate of adenosine triphosphate and one-half of the phosphate of adenosine diphosphate.

P.-P. represents energy rich phosphate and is composed of the sum of the values obtained for Cr.P.-P. and A.T.P.-P.

T.A.S.P.-P. represents the total acid-soluble phosphate extracted by trichloracetic acid and is composed of I.P.-P., Cr.P.-P., A.T.P.-P. and other phosphate compounds resistant to hydrolysis at 100°C. for 20 minutes in 1 N. H_2SO_4.

The data, mg% of wet weight of tissue, obtained for the various phosphate fractions in both the mucosa and muscularis is shown in Table I. Analysis of variances for ranked data (11) for all acid-soluble phosphate fractions between treatments and segments showed no difference. With the exception of the total acid-soluble phosphate phosphorus, this fraction showed a significant difference for segments at the 5% level of confidence for the muscularis, and at the 1% level of confidence for the mucosa. Whether this was an actual difference or one

SUMMARY of DATA

TREATMENT		T.A.S.P-P		I.P-P		Cr.P-P		A.T.P-P		Δ^{120}-P-P	
		mediam in mg.% of wet weight of tissue									
		†muc.	musc.§	muc.	musc.	muc.	musc.	muc.	musc.	muc.	musc.
GROUP I. control /fed at libidum/	1 Q* above	77.02	69.79	26.20	25.87	22.50	19.80	22.60	16.15	24.13	20.72
	Median	53.80	53.71	19.26	20.68	12.83	13.44	10.90	12.01	16.14	14.23
	1 Q below	37.79	39.22	12.17	15.20	10.03	9.81	4.71	4.67	7.98	8.77
	% of control	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
GROUP II. 24hr. fast.	1 Q above	63.92	51.69	13.87	16.40	15.35	15.14	16.85	8.61	15.04	17.34
	Median	46.96	42.54	12.10	11.86	11.37	12.52	11.85	5.01	13.21	12.89
	1Q below	37.97	35.32	9.16	10.33	8.87	10.92	9.50	2.72	9.74	8.47
	% of control	87%	79%	68%	57%	88%	93%	127%	42%	82%	90%
GROUP III. 48hr. fast.	1 Q above	67.73	46.15	10.67	13.48	11.15	11.63	16.87	8.96	13.64	18.07
	Median	38.70	39.24	8.65	11.09	7.60	10.15	10.92	3.97	10.47	11.24
	1 Q below	32.42	34.44	7.26	9.28	6.19	8.62	4.52	2.68	7.21	10.16
	% of control	72%	73%	45%	53%	59%	75%	100%	33%	65%	79%
GROUP IV. 72hr. fast.	1 Q above	43.96	35.55	10.68	9.96	8.43	8.80	8.34	5.45	12.62	13.98
	Median	34.50	31.59	9.06	8.71	7.07	6.79	5.51	3.66	10.05	10.91
	1 Q below	30.81	28.63	7.78	7.27	6.23	5.35	2.73	2.18	8.74	5.97
	% of control	64%	59%	47%	42%	55%	50%	51%	30%	62%	77%
GROUP V. 48hr. fast. + 10min. feeding	1 Q above	64.88	60.86	20.24	17.20	22.43	12.22	24.24	17.12	17.93	15.94
	Median	55.64	51.92	18.91	16.39	16.48	10.55	14.90	14.21	14.61	13.91
	1 Q below	49.21	45.05	15.55	13.88	12.30	8.23	10.67	8.75	11.23	9.26
	% of control	103%	97%	98%	79%	132%	78%	137%	118%	90%	98%
GROUP VI. 48hr. fast. + 15min. feeding	1 Q above	67.50	64.34	23.86	20.18	19.65	18.40	26.55	19.84	21.46	20.10
	Median	60.75	60.63	22.64	19.86	17.49	17.14	19.24	14.48	16.84	15.81
	1 Q below	55.32	51.31	19.87	18.76	16.19	15.54	14.75	9.96	11.28	7.38
	% of control	113%	112%	117%	96%	136%	127%	176%	121%	104%	111%

* Quartile † Mucosa § Muscularis Δ^{120} Difficult Hydrolyzed P-P.

due to chance, is questionable. Because the different treatments did test significant in all studies, the difference due to segments for the T.A.S.P.-P. was disregarded and the data for all four segments were grouped, thus giving forty assays of a particular phosphate for each treatment in both the mucosa and muscularis.

The fasting treatments tend to decrease the amount of all acid-soluble phosphate fractions, Figure 3-7, both in the muscularis and mucosa of the terminal ileum strips except the A.T.P.-P. in the mucosa. The amount of A.T.P.-P. in the mucosa portion increased after 24 hour fasting approximately by 27%, and then dropped back to control levels at the 48 hour starvation period which was followed by further decrease in the 72 hour starvation period to about 30% of the control values. When the 48 hour fasted animals were fed prior to sacrificing for 10 minutes and 15 minutes, the amount of the acid-soluble phosphate fractions were raised to normal levels, and in most cases, this amount exceeded the control values both in the muscularis and mucosa portions.

The effect of these treatments upon the acid-soluble phosphates in muscularis and mucosa for both the fasting, and the 48 hour fasting with 10 minute and 15 minute feeding periods before sacrificing, is as follows:

I. T.A.S.P.-P.

A. Mucosa: The T.A.S.P.-P. fraction of the acid-soluble phosphates decreased in this portion of the terminal ileum to 87% for 24 hour starvation treatment, and the decrease continued to 72% and 64% of control values for the 48 hour and 72 hour fasting periods respectively. When the 48 hour fasted animals were fed prior to sacrificing for 10 minutes

and 15 minutes, the T.A.S.P.-P. went up to 103% and 113% of the control values, Figure 3.

B. Muscularis: In this portion of the intestinal segments the mg% of the T.A.S.P.-P. was also decreased to 79%, 73% and 59% for the 24, 48 and 72 hour starvation treatments respectively. Feeding prior to sacrificing increased the amount of T.A.S.P.-P. to 97% and 112% of the control levels for the 10 minute and 15 minute feeding periods of the 48 hour fasting animals, Figure 3.

II. I.P.-P.

A. Mucosa: The mg% of I.P.-P. in mucosa decreased to 57% in the 24 hour starvation period and progressively dropped to 53% in the 48 hour period and to 42% of control values in the 72 hour fasting period. When the 48 hour fasted animals were fed 10 minutes and 15 minutes prior to sacrificing, the amount of I.P.-P. increased to 79% and 96% respectively, of the control level.

B. Muscularis: The amount of I.P.-P. in the muscularis portion decreased to 68% in the 24 hour starvation period and dropped to 45% and 47% of control values in the 48 hour and 72 hour fasting treatments. The 48 hour fasted animals when fed for 10 minutes and 15 minutes prior to sacrificing, the amount of I.P.-P. was increased to 79% and 96% mg% toward the control levels, Figure 4.

III. Cr.P.-P.

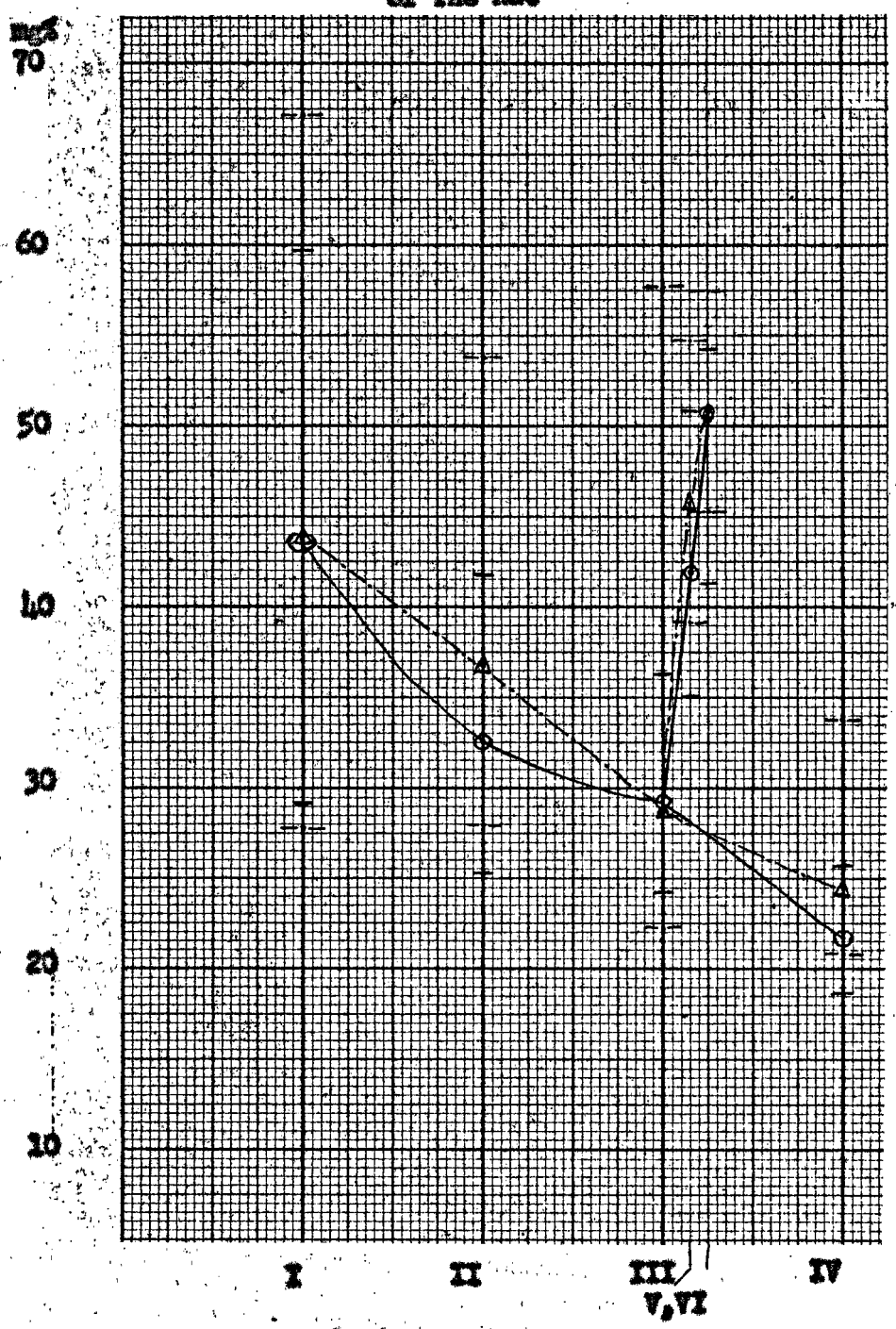
A. Mucosa: The mg% of Cr.P.-P. decreased during the 24 hour fasting treatment to 88% of the control with further decrease to 59% and 55% during the 48 hour and 72 hour fasting treatments. This decrease was followed by an increase in the animals that were starved for 48 hours and

Figure 3

Total Acid-Soluble Phosphate Phosphorus

Determined In The Mucosa And The Muscularis Of The Terminal Ileum Strips

Of The Rat



⊙ Median for muscularis

— Quartiles for muscularis

△ Median for mucosa

–·– Quartiles for mucosa

I Control

II 24 hr. fast

III 48 hr. fast

IV 72 hr. fast

V 48 hr. fast + 10 min. feeding

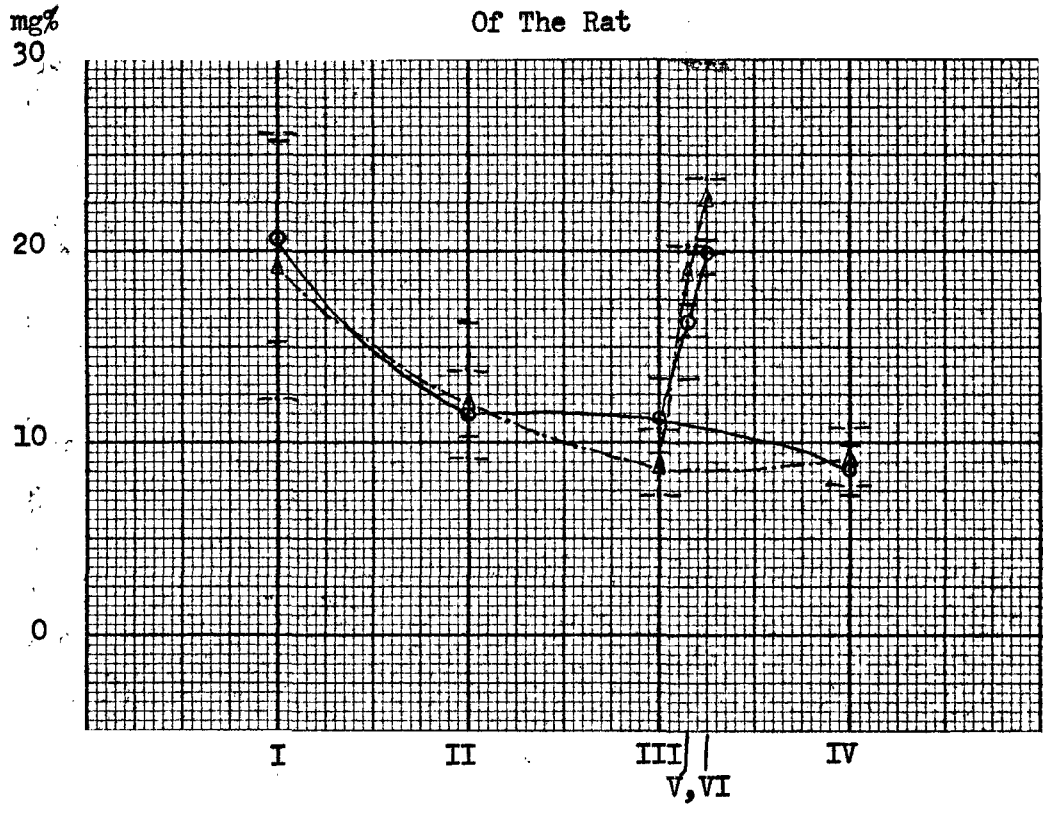
VI 48 hr. fast + 15 min. feeding

Figure 4

Inorganic Phosphate Phosphorus

Determined In The Mucosa And The Muscularis Of The Terminal Ileum Strips

Of The Rat



⊙ Median for muscularis

— Quartiles for muscularis

△ Median for mucosa

-·- Quartiles for mucosa

I	Control	III	48 hr. fast	V	48 hr. fast + 10 min. feeding
II	24 hr. fast	IV	72 hr. fast	VI	48 hr. fast + 15 min. feeding

then fed 10 minutes and 15 minutes before sacrificing of 132% and 136% respectively.

B. Muscularis: Also in this portion of the terminal ileum, the amount of Cr.P.-P. decreases during the starvation treatments to 93%, 75% and 50% during the 24, 48 and 72 hour fasting periods with definite increase to 78% and 127% of the control values in the 48 hour starved animals that were fed at 10 minutes and 15 minutes before sacrificing, Figure 5.

IV. A.T.P.-P.

A. Mucosa: The A.T.P.-P. in the mucosa did not follow the rest of the acid-soluble phosphate fraction, and actually it showed an increase during fasting to 127% at the 24 hour fasting which was decreased to 100% in the 48 hour fasting and was further decreased to 51% in the 72 hour fasting. This was also restored to and above control levels after 10 minute and 15 minute feeding before sacrificing in the 48 hour starved animals.

B. Muscularis: The A.T.P.-P. in the muscularis portion followed the rest of the acid-soluble phosphate fragments and decreased to 42%, 33% and 30% during the 24, 48 and 72 hour starvation treatments, with an increase to 118% and 121% in the animals fed prior to sacrificing, Figure 6.

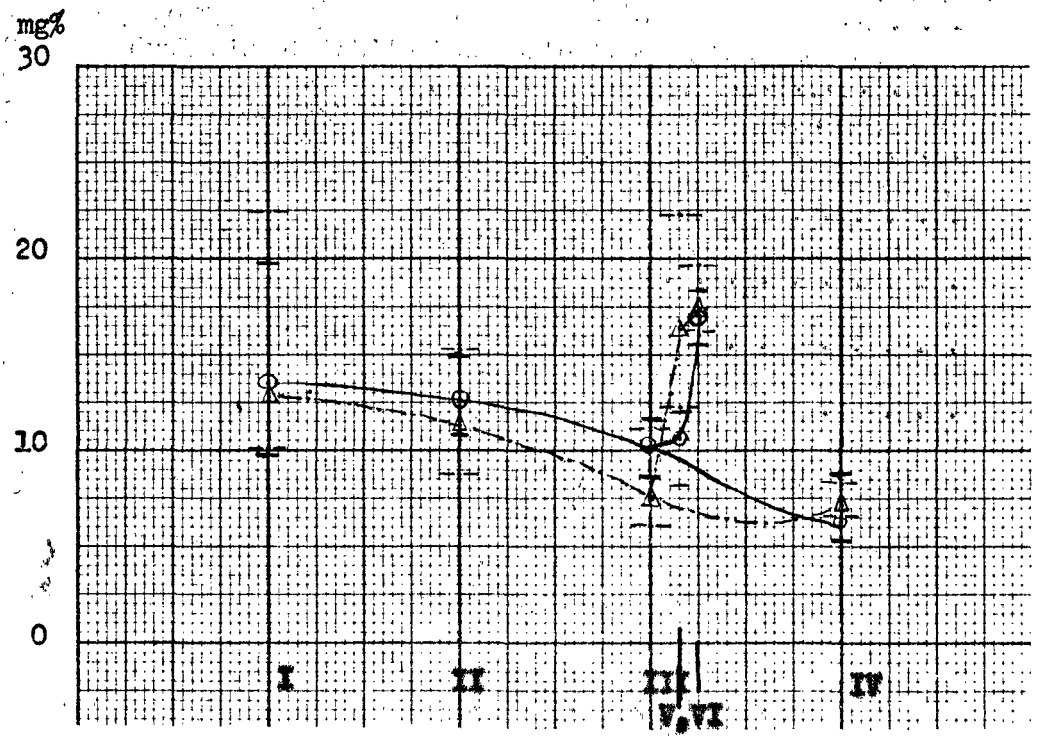
V. Difficulty Hydrolyzed Phosphate Phosphorus.

A. Mucosa: Difficulty Hydrolyzed Phosphate Phosphorus showed a decrease during fasting to 82%, 65% and 62% for the 24, 48 and 72 hour starvation treatments, followed by an increase after feeding the 48 hour starved animals for 10 minutes and 15 minutes before sacrificing, to 90% and 104% respectively.

Figure 5

Creatine Phosphate Phosphorus

Determined In The Mucosa And The Muscularis Of The Terminal Ileum Stripe Of The Rat



⊙ Median for muscularis

— Quartiles for muscularis

△ Median for mucosa

–·– Quartiles for mucosa

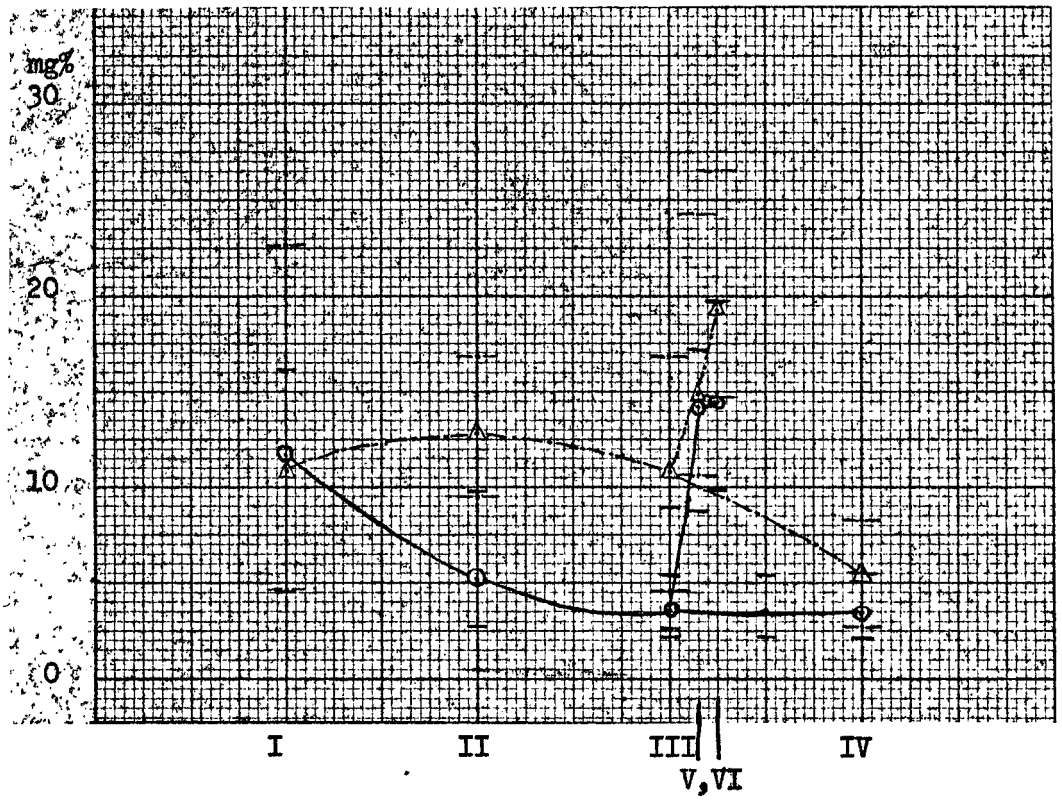
I Control III 48 hr. fast V 48 hr. fast + 10 min. feeding

II 24 hr. fast IV 72 hr. fast VI 48 hr. fast + 15 min. feeding

Figure 6

Adenosine Triphosphate Phosphorus

As Calculated By Subtracting The Sum Of Cr.P.-P. And I.P.P. From Δ^{100} P.-P.



⊙ Median for muscularis

— Quartiles for muscularis

△ Median for mucosa

–·– Quartiles for mucosa

I	Control	III	48 hr. fast	V	48 hr. fast + 10 min. feeding
II	24 hr. fast	IV	72 hr. fast	VI	48 hr. fast + 15 min. feeding

B. Muscularis: Difficulty Hydrolyzed Phosphate Phosphorus decreased during starvation period to 90%, 79% and 77% for the 24, 48 and 72 hour fasted animals, and increased to 98% and 111% after 10 minute and 15 minute feeding of the 48 hour starved animals before sacrificing, Figure 7.

To test the significance of the results obtained in this study, the following statistical measures were employed:

I. Evaluation of Chi-square for testing the significance between treatments.

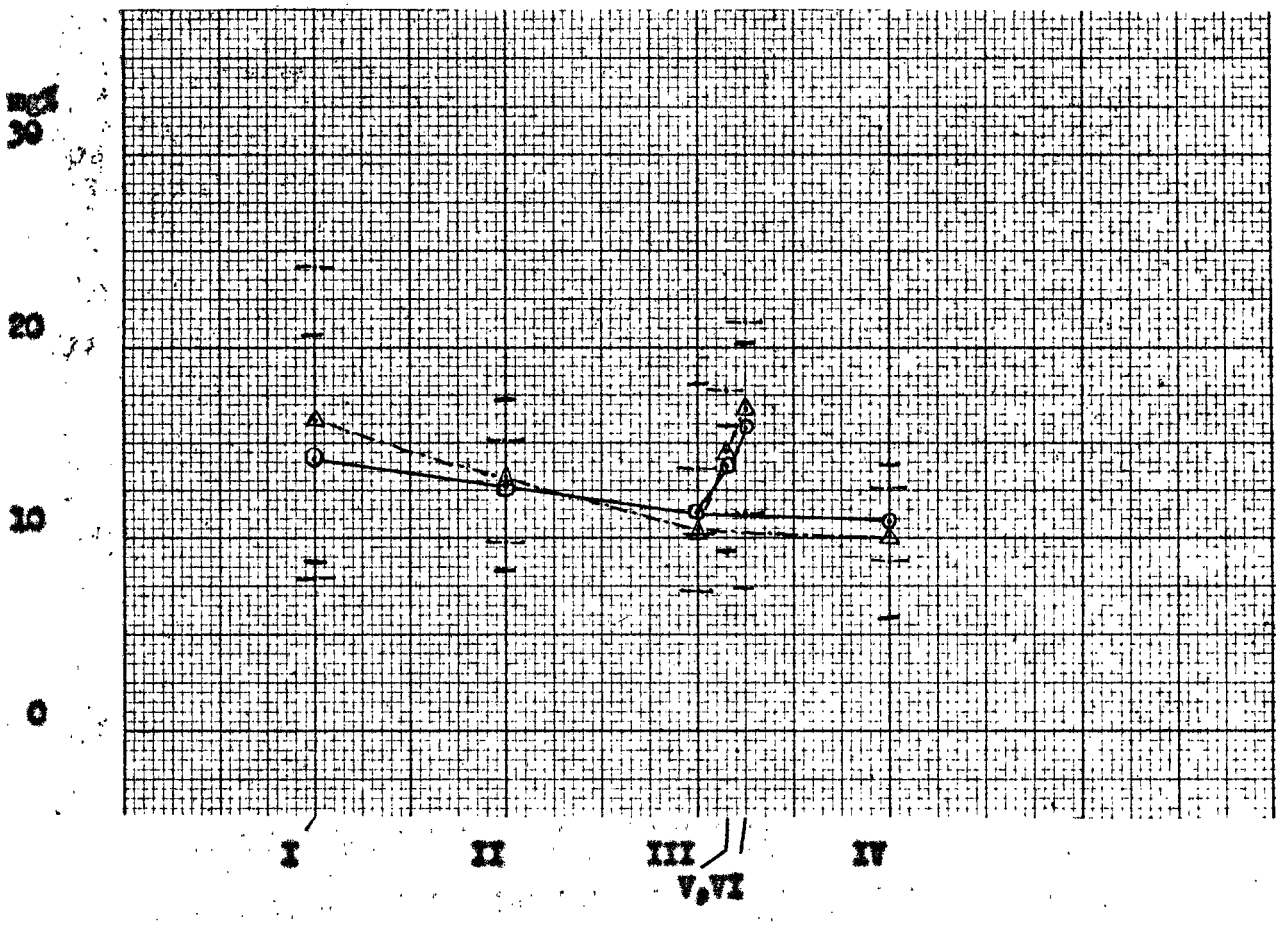
II. Analysis of variances for ranked data(11) for testing the significance between treatments and segments.

III. The Mann-Witney "U" test (11) was used to determine if a significant difference existed between the phosphates of the muscularis and mucosa portion of the intestinal strips under different experimental conditions. This test indicated that the difference found for A.T.P.-P. in the mucosa portion is confident in the 2% level, Figure 6. In addition, there is a significantly greater increase in the amount of difficulty hydrolyzed phosphate in the mucosa following 10 minute and 15 minute feeding treatments after the 48 hour starvation period, Figure 7.

Figure 7

Difficulty Hydrolysed Phosphate Phosphorus

As Calculated By Subtracting The Amounts Of $\Delta^{'oo}$ From T.A.S.P.-P.



⊙ Median for muscularis

— Quartiles for muscularis

△ Median for mucosa

–·– Quartiles for mucosa

I	Control	III	48 hr. fast	V	48 hr. fast + 10 min. feeding
II	24 hr. fast	IV	72 hr. fast	VI	48 hr. fast + 15 min. feeding

DISCUSSION

Previous workers have reported that fasting treatments will decrease the amounts of the acid-soluble phosphates; however, the amount of these compounds will return toward control values following a brief feeding period prior to sacrificing. The control values obtained in this study are within the general range of concentration obtained by Kroeger (4), as well as by previous workers, see Table II. However, the total acid-soluble phosphate phosphorus is much lower as well as the inorganic phosphate than that reported in the starved animals. This may be partially explained because the wet weight of tissue used for comparative purposes is quite variable. Better techniques for comparative purposes are the dry weight method, or the more accurate method using the nitrogen content of the tissue residue.

No histological studies were made to assure the purity of the muscularis and mucosa samples used for assay. This, also, may have been a source of error; however, it was assumed that percentage-wise, the values obtained represent the muscularis and mucosa layers respectively. It is to be noted that the mucosa muscularis present in the mucosa represents a source of error. It is realized that enzymatic hydrolysis of the phosphate fraction, or even separation and analysis of the individual compounds, offers a more accurate technique of assay. Acid hydrolysis of the acid-soluble phosphates has been criticized by many, Rowles & Stocken (12) and Ennor & Stocken (13), but the object of this study is to observe relative changes, not absolute variations, in the amount of the phosphate compounds.

From the data obtained, it appears that there is a continuous decrease in the acid-soluble phosphates during fasting conditions with the exception of Adenosine triphosphate phosphorus, which agrees with the results reported by earlier investigators, Kroeger (4), Sarzana (8). The mucosa seems to be the least stable of the two tissues because the depletion occurs largely in the mucosa portion in the 24 and 48 hour fasting periods, although total depletion at the 72 hour period is about the same for the two tissues. Adenosine triphosphate phosphorus in the mucosa was found to increase during the 24 and 48 hour fasting periods. This increase together with the decrease in Creatine phosphate phosphorus might indicate a transfer of the energy-rich phosphate bonds from a storage unit to an active donor; however, with increased starvation time, as in the 72 hour fasting period, even the donor phosphate, Adenosine triphosphate phosphorus, has been greatly depleted, as is the case with the other fractions.

In the second part of this study where the 48 hour starved animals were fed for ten and fifteen minutes prior to sacrificing, the acid-soluble phosphate fractions increased and most of them exceeded the control levels after the fifteen minute feeding period. The fractions which showed the greatest increase were Creatine phosphate phosphorus and Adenosine triphosphate phosphorus, and this occured in the mucosa samples. The Creatine phosphate phosphorus in the mucosa after ten minute feeding increased to 132% of control values, whereas, Adenosine triphosphate phosphorus in ten minute feeding increased to 137% of normal values. This increase of both Creatine phosphate phosphorus and Adenosine triphosphate phosphorus in both muscularis and mucosa substantiates the

Table II

Values of the Acid-Soluble Phosphate Compounds Reported for Smooth Muscle in Various Animals

Animal	Type Muscle	mg % Phosphorus (Wet Weight)			
		T.A.S.P.	I.P.	Cr.-P.	A.T.P.
Dog	Longitudinal Intestinal		23.2-26.0	3.2-7.4	10.9-12.2
Rats	Uterine	43.1	22.7	0.7	2.3

T.A.S.P. represents Total Acid-Soluble Phospate
I.P. represents Inorganic Phosphate
Cr.P. represents Creatine Phosphate
A.T.P. represents Adenosine triphosphate

hypothesis that here is a mechanism, nervous or hormonal, responsible for preparing the intestines for receiving and handling of food material, especially absorption, and also, that the amounts of energy-rich compounds are more dependent upon activity states than a purely nutritional depletion and restoration.

This method of assay for acid-soluble phosphates may prove to be of value in discriminating whether a hormonal and/or nervous mechanism is responsible for the activation of the intestinal tract. Other workers have used intestinal motility as an end point in the above mentioned study, but intestinal motility is not always apparent and readily noticeable. Chemical data, such as presented here, might offer a solution to the problem; however, it must be realized that, as yet, no direct correlation between the amounts of the phosphate compounds and intestinal activity has been established.

SUMMARY

1. Studies were made in terminal ileum of rat to re-examine the effects of presacrificing, fasting and feeding treatments on the distribution of acid-soluble phosphate fractions.

2. Studies were made to determine by the analysis of the acid-soluble phosphate fractions, whether the changes occurring under different fasting and feeding treatments are primarily in the mucosa or muscularis portion of the intestines.

3. Studies were made to determine by the analysis of the acid-soluble phosphate distribution in terminal ileum segments, the dependability of this technique for use as an end point to determining the nature of intestinal control, hormonal and/or nervous.

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THE ACID-SOLUBLE PHOSPHATE DISTRIBUTION IN TERMINAL ILEUM STRIPS OF RAT AS AFFECTED BY FASTING TREATMENTS

An Abstract of a Thesis

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the Faculty of the Graduate School

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Master of Science

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by

Dimitris P. Papandrianos

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Abstract

It was the purpose of this study to re-examine the effects of fasting and feeding treatments upon the phosphate distribution in the terminal ileum of the rat in order to determine whether the muscular or mucosa structure is most affected by such treatments. In addition, it seemed advisable to re-examine the dependability of this technique for possible use as an end point in further studies to elucidate the nature of the intestinal controls, nervous and/or hormonal.

The experimental procedure followed in this study was essentially the same used by Kroeger.

From the data obtained, it appears that there is a continuous decrease in the acid-soluble phosphates during fasting conditions. The mucosa portion of the intestines seems to be the most labile of the two tissues. Adenosine triphosphate phosphorus in the mucosa was found to increase during the 24 and 48 hour fasting periods. When the 48 hour starved animals were fed for ten and fifteen minutes prior to sacrificing, the acid-soluble phosphate fraction increased toward normal levels. The decrease in the amounts of energy-rich phosphate seemed to be more dependent upon activity states than nutritional, depletion and restoration.

Chemical data, such as presented here, may prove of value in discriminating whether a hormonal and/or nervous mechanism is responsible for the activation of the intestinal tract.