The Excretion of Phosphatase in the Faeces

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(Received 2 April 1943)

The mucous membrane of the small intestine is rich in phosphatase which can be liberated into the blood stream or into the intestinal secretion. This accounts for a part of the faecal phosphatase. Estimation of the faecal phosphatase may therefore enable one to study biochemically the activity of the mucous membrane of the small intestine in the intact animal.

The early publications on faecal phosphatase are reviewed by Folley & Kay [1936]. Heymann [1933] found the phosphatase activity to be reduced in rickets, spasmophilia and intestinal infantilism. Armstrong [1936] described the preparation of a strong phosphatase concentrate from dog faeces. Koster's [1938, 1939] most striking finding was the absence of phosphatase from the faeces in some cases of non-tropical sprue. In leukaemia values above the normal range were found. Duckworth & Godden [1941] used faecal phosphatase estimations to measure intestinal secretory activity in rats.

The experiments to be described were performed on the stools of normal adults between 20 and 35 years of age, free from any gross peculiarities of dietetic habit.

EXPERIMENTAL

Estimation of phosphatase

Technique. The stool was weighed, ground with water until homogeneous in a 1 in 400 suspension. Phosphatase was estimated by Bodansky's [1933] method. The increase in 1 hr. in inorganic phosphate, expressed as mg. P/100 g. of untreated or of dried faeces, is referred to as units of phosphatase activity (wet wt.) and (dry wt.) respectively. The former is comparable with the Bodansky unit of phosphatase activity.

Reliability of sampling. Subjects were instructed not to eat foods likely to leave large undigested particles. Grinding of their stools with water gave thorough mixing, and triplicate analyses showed that representative 0.5 g. samples could be obtained.

RESULTS

Solubility of the enzyme

Filtration of the suspensions removed a large and variable proportion of the phosphatase. Thus a stool which contained 2.00×10^3 units (wet wt.) was tested again on four filtrates and the following results obtained: 0.11×10^3 , 0.13×10^3 , 0.13×10^3 , 0.15×10^3 units. Substitution of saline for water did not affect the activity of the suspension.

Activation of the enzyme by Mg

MgCl₂ was added to give concentrations of 0.025 and 0.05 % (approx. 0.005 *M*) which was found by Bowers, Outhouse & Forbes [1940] to be optimal for dog faecal phosphatase. The phosphatase in human faeces is not fully activated with regard to Mg (Table 1), but any difference obtained by activation with Mg is negligible in comparison with the individual variations occurring physiologically.

Table 1. *Activation of faecal phosphatase by MgCl₂*

Stool no.	Conc. added MgCl ₂ in digest			Max. activation % increase
	Nil	0.025 %	0.050 %	
1	3.22	3.58	3.54	11
2	4.28	4.66	4.66	9
3	4.32	4.25	4.62	7
4	15.3	17.3	14.8	13
5	3.52	3.80	3.90	11
6	5.20	—	5.60	8
7	8.6	—	9.6	12
8	9.2	—	10.2	11
9	10.0	—	10.4	4
10	7.34	—	8.00	9

Inhibition of the enzyme by KCN

The results (Table 2) show that this phosphatase is strongly inhibited by 10^{-2} *M* KCN.

Table 2. *Inhibition of faecal phosphatase by KCN*

Stool	Sample	KCN conc. in digest <i>M</i>	Phosphatase activity units wet wt.	Inhibition %
A	1	Nil	2.64×10^3	95
	2	10^{-2}	0.13×10^3	
	3	Nil	2.74×10^3	96
	4	10^{-2}	0.11×10^3	
B	1	Nil	2.96×10^3	94
	2	10^{-2}	0.11×10^3	
	3	Nil	3.42×10^3	97
	4	10^{-2}	0.09×10^3	

Effect of NaF on activity of the enzyme

Experiments similar to those using KCN (Table 3) show that NaF has no effect upon the phosphatase activity of these preparations.

Table 3. *Effect of NaF upon faecal phosphatase activity*

Stool	Sample	NaF conc. in digest <i>M</i>	Phosphatase activity units wet wt.	Activa- tion %
A	1	Nil	4.08×10^3	1
	2	10^{-2}	4.13×10^3	
	3	Nil	4.20×10^3	0
	4	10^{-2}	4.20×10^3	
B	1	Nil	3.76×10^3	3
	2	10^{-2}	3.89×10^3	
	3	Nil	3.75×10^3	7
	4	10^{-2}	4.02×10^3	

The phosphatase activity of normal stools

Stools from 50 normal adults were analysed for phosphatase activity and water content. The results are given in Table 4.

Table 4. *Phosphatase activity of normal stools*

	Min.	Max.	Mean of 50
Units wet wt. $\times 10^{-3}$	0.8	36.7	6.0
Units dry wt. $\times 10^{-3}$	1.9	103.0	23.0
Dry matter in stool (%)	15.0	47.0	30.0

DISCUSSION

Cloetens [1939 *a, b*; 1940] has presented evidence that the alkaline phosphatase of animal tissues consists of two types, 'alkaline phosphatase I' and 'alkaline phosphatase II'. This classification is based upon activation or inhibition by Mg ions, CN and F. The present results indicate that faecal phosphatase belongs to the subdivision type II of Cloetens, of the group A I of Folley & Kay [1936]. Cloetens found that nearly all the phosphatase of the small intestine of guinea-pigs was of this type.

The most striking feature of the analyses of normal stools is their extreme range of variation; but even the lowest value (0.8×10^3 units wet wt.) is 200 times the maximum value for normal serum. The values were very unevenly distributed: 36 of

them (dry wt. basis) fell below the mean and only 14 above. The maximum value observed by Koster [1939] if converted into comparable units (dry wt.) is 94×10^3 , which is close to the present maximum value of 103×10^3 . If the mean phosphatase activity of the pooled faeces from six normal rats studied by Duckworth & Godden [1941] is converted into comparable units, a value of 1.8×10^3 (wet wt.) is obtained, which lies within the range of 0.8×10^3 to 36.7×10^3 units (wet wt.) given above for normal human stools.

There is probably a negative correlation between the percentage of dry matter in normal stools and their phosphatase activity. Twenty-four stools containing less than 30 % of dry matter had an average of 33×10^3 units (dry wt.) and 26 stools containing 30 % or more dry matter had 14×10^3 . The speed of transit of the stool through the intestine probably influences dry-matter content and phosphatase activity in opposite senses. Koster [1939] states that $MgSO_4$ taken by mouth, or a diet rich in green vegetables, increases the phosphatase activity of the stools. This effect he attributes to activation of the enzyme by Mg ions. Both procedures are, however, likely to increase the speed of transit of the intestinal contents and diminish the percentage of dry matter in the stools.

SUMMARY

1. The phosphatase activity of normal human faeces has been shown to be slightly increased *in vitro* by $0.53 \times 10^{-2} M$ $MgCl_2$, uninfluenced by $10^{-2} M$ NaF, and very strongly inhibited by $10^{-2} M$ KCN.

2. The range of variation of the phosphatase activity of 50 normal stools was from 1.9×10^3 to 103×10^3 units (dry wt.) with a mean value of 23.0×10^3 units.

3. There is probably a negative correlation between the phosphatase activity/unit wt. dry matter and the percentage dry matter in normal stools.

REFERENCES

- Armstrong, A. R. [1936]. *Biochem. J.* **29**, 2020.
 Bodansky, A. [1933]. *J. biol. Chem.* **101**, 93.
 Bowers, R. V., Outhouse, E. L. & Forbes, J. C. [1940]. *J. biol. Chem.* **132**, 675.
 Cloetens, R. [1939*a*]. *Enzymologia*, **6**, 46.
 — [1939*b*]. *Naturwissenschaften*, **27**, 806.
 — [1940]. *Naturwissenschaften*, **28**, 252.
 Duckworth, J. & Godden, W. J. [1941]. *Biochem. J.* **35**, 16.
 Folley, S. J. & Kay, H. D. [1936]. *Ergebn. Enzymforsch.* **5**, 159.
 Heymann, W. [1933]. *Z. Kinderheilk.* **55**, 92.
 Koster, L. [1938]. *Ned. Tijdschr. Geneesk.* **82**, 5856.
 — [1939]. *Acta med. scand.* **101**, 482.