XCIX. THE PHOSPHATASE ACTIVITY OF ANIMAL TISSUES.

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(Received March 9th, 1934.)

THE presence of a highly active phosphatase in bone and ossifying cartilage forms the basis of a conception of the process of calcification in these tissues for which much evidence has been accumulated [Robison, 1923]. That calcification does not normally occur in all tissues which contain phosphatase is not necessarily prejudicial to this conception since the animal organism makes wide use of phosphoric esters of varied type and it is not surprising that many of its tissues should contain enzymes by which the synthesis and hydrolysis of these esters can be accomplished. We were, however, interested in the possibility that the presence of phosphatase may be correlated with the calcification which occurs in some of these tissues in certain abnormal bodily states.

The distribution and behaviour of the tissue phosphatases have been the subject of many investigations, which have recently been reviewed by Kay [1932], who discusses the limitations of the experimental methods employed. It has long been recognised that these phosphatases are not all identical, while Akamatsu and his co-workers have shown that more than one type may be present in the same tissue [Akasawa, 1929; Kurata, 1931]. The present experiments were concerned solely with the distribution of the phosphomonoesterase of high optimum p_H which occurs in bone. They were intended to supplement the results already obtained on the relative activities of the tissues of different animal species. It was desired also to eliminate the possibility that a negative or low phosphatase activity reported for any tissue was due simply to lack of magnesium in the tissue or tissue extract. The pronounced effect of magnesium on phosphatase derived from different sources has been described by Erdtman [1927; 1928] and by Jenner and Kay [1931]; the latter concluded that magnesium acts as an accelerator rather than as a coenzyme.

In these experiments we have made a more extended trial of a method which had been found valuable in a study of phosphatase development in rudimentary bones and minute explants of embryonic tissue [Fell and Robison, 1929; 1930]. A number of pieces of each tissue, roughly 1.0–4.0 mg. dry weight, were cut as nearly uniform in size and shape as possible, placed on cover slips and rapidly dried in an evacuated desiccator. The tissue fragments were weighed on a microbalance and placed separately in stoppered tubes of 5 ml. capacity to which 2 ml. distilled water and a small drop of chloroform were added. After standing 24 hours at room temperature, 2 ml. of a 0.2M solution of sodium β -glycerophosphate (p_H 8.9) were added, together with a further drop of chloroform; if necessary the p_H was adjusted to 8.6. From one of the duplicate tests carried out on each tissue examined, a sample was then removed for estimation of inorganic phosphate. This 0-hour value, which rarely exceeded 0.004 mg. P

per ml., represented the sum of the inorganic phosphate present in the glycerophosphate solution *plus* that dissolved out of the tissue and was deducted from the final value obtained. The tubes were placed in an incubator for 48 hours at 25° or 24 hours at 37°; at the end of this time the p_H was checked, and suitable samples were removed for estimation of the phosphate by the Briggs method. When the tests were carried out in presence of magnesium this was added as $MgSO_4$, in the glycerophosphate solution, so that the final concentration of Mg was 0.003M. This concentration was chosen as the most suitable after preliminary tests on a wide range of tissues. It falls within the limits of optimum concentration found by Jenner and Kay [1931]. No added buffers were used in these experiments since their presence is not without effect on phosphatase activity. Sodium glycerophosphate itself has considerable buffering powers at p_H 8.6; and in more than 90 % of the tests the p_H did not fall below 8.5. Any tests in which the final p_H was lower than 8.4 were rejected. The fall in p_H occurred chiefly in the tests with liver, lung, spleen and muscle and was presumably due largely to glycogenolysis. Since the p_H -activity curve of phosphatase is very steep it may be well to state that differences in the final p_H did not account for the observed variation in the activity of duplicate samples.

The majority of the tests were carried out at 25° for 48 hours, the remainder at 37° for 24 hours. The former conditions usually gave somewhat higher values and were considered preferable owing to the greater stability of the enzyme at the lower temperature.

As a result of many hundreds of tests certain comments may be made on this method.

1. It is specially applicable to investigations in which only minute amounts of tissue are available, as in young or small animals, or in which it is desired to study the localisation of the enzyme in particular zones of the tissue.

2. It gives a direct relationship between the activity and the weight of solid matter in the tissue; the dry weight of tissues with variable water content can be determined with greater accuracy than the wet weight and may provide a more suitable basis for comparison.

3. A large number of tissues from the same animal can be conveniently examined.

4. Although in certain tissues reasonably good agreement was obtained between duplicate samples, in others a wide variation was found, indicating uneven distribution of the enzyme. A limited number of tests may therefore fail to give a fair indication of the average phosphatase activity of a tissue.

5. Since duplicate determinations can only be carried out on separate pieces of tissue it is impossible to check the accuracy of individual values.

6. The assumption that no loss of phosphatase activity occurs during drying may not be equally correct for all tissues, and is difficult to verify exactly in the case of tissues with variable phosphatase content. There was, however, no indication of any serious loss of activity.

7. Because of the variation in individual values, the method is not the most suitable for studying the exact effect of p_H and other factors on the phosphatase activity of different tissues.

As these comments indicate, the method possesses definite advantages for certain purposes but does not replace the usual method in which the moist tissues are ground with chloroform water.

The average values of A/W obtained for tissues of animals of different species are shown in Table I, those for tissues of rats of different ages in Table II. The tissues include some for which no data were previously available. In general

Table I. *Phosphatase activity (A/W) of mammalian tissues. (Average values.)*

A = inorganic phosphate (mg. P) liberated from 0.1 M Na β -glycerophosphate in 48 hours at p_H 8.6 and 25°.
 W = weight of tissues (mg.).

Final conc. of MgSO ₄ added ...				None							0.003 M						
Animal	...	...	...	Rat	Rabbit	Guinea- pig	Mouse	Cat	Dog	Rat	Rabbit	Guinea- pig	Mouse	Cat	Dog		
No. of animals examined	...	...	...	3	3	1	2	1	1	3	3	1	2	1	1		
Age of animals (weeks)	...	...	...	4	6-8	4	12	9	5	4	6-8	4	12	9	5		
Tissue																	
Femur epiphysis				0.25	0.22	—	—	—	—	0.60	0.56	—	—	—	—		
Costochondral junction				0.28	0.53	—	—	0.88	0.73	1.10	1.10	—	—	3.18	2.40		
Rib cartilage				<0.01	<0.01	<0.01	<0.02	0	0	0.02	<0.01	—	<0.02	0.01	0.03		
Trachea				0.03	<0.01	<0.01	<0.02	0.03	0.02	0.12	0.02	0.06	0.02	0.14	0.27		
Intestinal mucosa				—	0.43	2.1	0.67	3.0	2.6	—	1.13	2.42	6.77	7.75	11.8		
Kidney cortex				0.38	0.16	0.32	0.66	0.46	0.41	0.55	0.22	0.79	2.70	1.50	1.4		
Bladder				0.07	0.06	0.07	0.13	0.06	0.03	0.16	0.16	0.13	0.50	0.35	0.08		
Aorta				0.09	<0.01	<0.01	<0.01	0	0	0.38	<0.01	<0.01	0?	0?	0		
Dorsal aorta				0.09	<0.01	—	0	—	0	0.38	<0.01	—	0	—	0		
Cardiac muscle				—	0.01	0.03	0.05	0.01	<0.01	—	0.02	0.05	0.09	0.04	0.02		
Skeletal muscle				<0.01	—	0.02	0.03*	0.01	—	—	—	—	0.10*	0.01	0.01		
Lung				0.17	0.07	0.10	0.06	0.11	0.05	0.61	0.20	0.25	0.10	0.21	0.28		
Liver				0.03	0.06	0.02	0.03	0.02	0.06	0.05	0.12	0.04	0.06	0.07	0.26		
Spleen				—	0.07	—	0.03	0.03	0.09	—	0.21	—	0.15	—	0.36		
Pancreas				—	0.03	—	0.03	0.02	0.11	—	0.06	—	0.04	0.03	0.36		
Thyroid				0.16	0.02	0.03	0.12	0.02	0.01	0.41	0.05	0.13	0.30	0.07	0.04		
Adrenal				0.09	0.03	0.33	0.02	0.06	0.03	0.26	0.05	0.73	0.05	0.29	0.11		
Thymus				—	0.06	—	0.03	—	0.11	—	0.07	—	0.10	—	0.48		

* Intercoastal muscle.

Table II. *Phosphatase activity (A/W) of tissues of rats of different ages. (Average values.)*

A = inorganic phosphate (mg. P) liberated from 0.1 M Na β -glycerophosphate in 24 hours at p_H 8.6 and 37°.

W = weight of dried tissue (mg.).

Final conc. of MgSO ₄ added...			None			0.003 M		
Age of rat ...	...	...	1 day	4 weeks	6 weeks	1 day	4 weeks	6 weeks
No. of rats examined	...	...	2	3	2	2	3	2
Tissue								
Femur epiphysis			0.23	0.21	0.18	1.23	0.48	0.57
" junction			—	0.48	0.33	—	1.04	0.79
" shaft			0.36	0.07	0.07	1.45	0.27	0.16
Rib bone			—	—	0.14	—	0.31	0.30
Costochondral junction			0.20	0.17	0.18	0.77	0.40	0.52
Tooth point			{ 0.36	0.09	0.06	{ 1.04	0.25	0.13
" root				0.41	0.25		1.18	0.59
Membrane bone			0.40	0.08	0.10	1.26	0.57	0.23
Rib cartilage			<0.01	<0.01	<0.01	0.02	0.01	0.01
Trachea			0.03	0.03	0.04	0.25	0.16	0.24
Intestinal mucosa			0.67	—	0.80	2.94	2.06	2.42
Kidney cortex			{ 0.16	—	0.34	{ 0.37	0.38	0.68
" medulla				—	0.04		0.11	0.15
Bladder			0.06	0.08	0.05	0.20	0.19	0.27
Aorta			0.08	0.02	0.14	0.11	0.45	0.86
Cardiac muscle			—	—	0.06	—	0.10	0.10
Skeletal muscle			<0.01	<0.01	<0.01	0.08	0.01	0.02
Lung			0.29	0.12	0.10	0.42	0.24	1.08
Liver			0.02	—	0.02	0.05	0.04	0.03
Spleen			0.05	—	0.02	0.20	0.07	0.09
Brain			0.06	—	0.04	0.10	0.06	0.07
Thyroid			0.05	0.04	0.04	0.38	0.21	0.42
Adrenal			0.04	0.03	0.06	0.28	0.20	0.23
Thymus			0.02	—	0.02	0.07	0.06	0.08

the relative activities are in agreement with those obtained by other methods. In nearly all cases the activity was decidedly increased in presence of magnesium. High values are shown for intestinal mucosa, kidney cortex, bones and teeth; with regard to the last two, it should be remembered that a large proportion (50–66 %) of the dry tissue fragment consisted of inorganic material, so that the A/W of the organic portion was actually much higher than the value shown. Rib cartilage and skeletal muscle were almost inactive in all species examined, even in the presence of magnesium, but cardiac muscle had a slight but definite activity. Most of the remaining tissues gave intermediate values, rather variable from one species to another. The definite activity of the bladder in all species may be noted [*cf.* Huggins, 1931] and also that of the trachea in the rat, cat and dog. Calcification of the tracheal cartilage occurs not infrequently *in vivo*, especially in old age; and the presence of phosphatase may seem significant when it is recalled that in the rudimentary bone the appearance and increasing activity of this enzyme runs parallel with the hypertrophy of the cartilage cells.

The examination of the aorta was carried out with special interest, in view of the frequency with which this tissue becomes calcified in certain abnormal states. Among the species examined, the aorta of the rat alone showed definite phosphatase activity; and in this respect the contrast between the rabbit and the rat is remarkable. Calcification of the aorta has been frequently observed *in vivo* in both rat and rabbit, and calcification has also been induced *in vitro*

in the excised aortae of both animals [Rosenheim and Robison, 1934]. While therefore the phosphatase present in the rat aorta may affect in some degree the incidence or extent of such calcification, it would seem that the enzyme cannot be the crucial factor in this process.

SUMMARY.

A method of estimation of the phosphatase activity of tissue fragments is described in which the activity is related to the dry weight of the tissue. The application of this method is discussed.

The values obtained by this method for the phosphatase activity of various tissues in presence and absence of magnesium are given for the rat, rabbit, guinea-pig, mouse, cat and dog. The results generally confirm those previously obtained.

The bladder shows definite phosphatase activity in all species examined; the trachea also showed some activity in most species.

The aorta of the rat was exceptional in possessing a definite phosphatase activity, while in the other species this tissue was practically devoid of the enzyme.

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