LIX. THE DEAMINASES OF ADENOSINE AND ADENYLIC ACID IN BLOOD AND TISSUES

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In this paper an account is given of the activity of these enzymes as they appear in blood and tissue extracts. Whereas the deaminase of adenosine acts therein as one may expect, that of adenylic acid has certain unusual characteristics. The most striking fact is the great susceptibility of the adenylic deaminase to the inhibiting action of certain anions, though the anions of maleic and citric acids are practically without effect; besides which, there are other inhibiting substances in tissues (probably anions also) but not in voluntary muscle. The result of their action is a protection of the adenylic acid as it forms in tissues generally and a simulation of a proportionality of action to substrate concentration. From such effects also it may be occasionally concluded that adenylic acid deaminase is absent or in very small concentration when in fact very appreciable amounts are present.

From this study, voluntary muscle appears as a very exceptional tissue in so far as the mean *effective* deamination rate of adenylic acid therein would appear to be of the order of 500–1000 times greater than that in other tissues. The adenosine deaminase behaves very differently and its action is practically independent of the buffer used provided that the *p*H be maintained.

The distribution of the enzymes has been studied for 36 tissues of the rabbit with results of interest; it being shown, for example, that the appendix contained the highest concentration of adenosine deaminase in the group studied and presumably in the whole body.

As regards the course which the deamination of adenylic acid takes in tissues, it is shown that not only in voluntary muscle, but also in brain, nerve tissue, the auricle of the heart and blood maintained at normal *p*H the deamination is direct, but that in six other tissues examined and probably in the remaining tissues of the body, the deamination occurs only after a preliminary dephosphorylation.

The statement in the preliminary report in *Nature* [1938] that plasma was found to contain no pure adenylic acid deaminase is now revised, since further experiments have shown that it may contain small variable amounts, but these are of the order of 0·005 % of that present in skeletal muscle. This has appeared from the study of high nucleotide concentrations (buffered at *p*H 7·0) the enzyme being practically inoperative at low concentrations.

Properties of the deaminases in blood and tissues

Methods. Water extracts of tissues ground with pure quartz sand (Merck's) were used throughout unless otherwise stated. The efficiency of extraction with water and saline extracts etc. is commented on below. For the ammonia and other estimations the procedure described in the previous paper [Conway & Cooke, 1939] was used.

Time and enzyme activity

Here we may distinguish at least two effects, firstly that on the enzyme-substrate activity and secondly the ageing or deterioration of the enzyme.

For adenosine deaminase acting for a few hours at room temperature and provided that the substrate concentration does not fall below 5 μg./ml. amino-N, the action shows a linear relation with time. The same applies for the adenylic acid deaminase when the action is negligibly small compared with the substrate concentration.

The activity of the extracts is found to decrease but little over 24 hr. at room temperature, but the deaminase of adenylic acid rapidly deteriorates at 38–40° (unbuffered or buffered at *p*H 7·0) giving only a small fraction of its activity after a few hours. Similar observations at the higher temperatures have not been made with adenosine.

Substrate concentration

Adenosine. The enzyme in laked blood and tissue extracts acts independently of the substrate concentration beyond a certain very small concentration level. From the data in Table I this level is from 2 to 5 μg./ml. amino-N for a 1 in 5 dilution of rabbit blood. The activity is proportional to substrate concentration when this is below *ca.* 2 μg./ml. amino-N or about 0·003 % of adenosine.

Table I

Adenosine concentration in mixture at zero time	Deamination in 1 min.	% of full deamination after long period
Amino-N μg./ml.	NH_3-N μg./ml.	
0·62	0·35	56·4
1·15	0·55	47·6
2·88	0·98	34·1
5·75	1·20	20·9
14·8	1·22	8·4
29·6	1·20	4·1

Blood dilution 1 in 5. Maleic acid buffer (*M*/20) at *p*H 6·8.

Adenylic acid (muscle). The effect of the substrate concentration is here very different. With laked blood and dilutions of 1 in 5 to 1 in 50 and a *p*H of *ca.* 7·0 the typical course of the deamination with increase of substrate is shown in Figs. 1 and 2. In such experiments with either human or rabbit blood the curve shows three sections. In the first comparatively short stage the increase of deamination rate with substrate is much less than in the second, beginning at about 0·1–0·2 %. From this on to 1 % and more the course is strictly linear. At or beyond 1 % it bends to reach saturation rate of the deamination. The same features of the curve may be observed independently of the buffer used, but the actual rate of deamination will be found to vary as one buffer or another is employed. Even without any added buffer (except the adenylate) the upper parts of the curve can be followed at comparatively steady *p*H (using short observation times) and found to correspond with the above description.

As shown below, about 90 % of the deamination is here proceeding by direct deamination of the adenylic acid and not after a preliminary dephosphorylation.

At the saturation level of the adenosine deaminase (about 0·005 %) the NH_3 formation from adenosine is *ca.* 1000 times greater than from adenylic acid, but at *ca.* 100–200 times this concentration the rate approaches that of adenosine and

finally considerably exceeds it. The physiological action will be confined to the lowest concentration levels.

The effect of the substrate concentration in extracts of six different tissues is shown in Fig. 3. The dilution was 1 in 40 with maleic buffering at *p*H 7·0. The

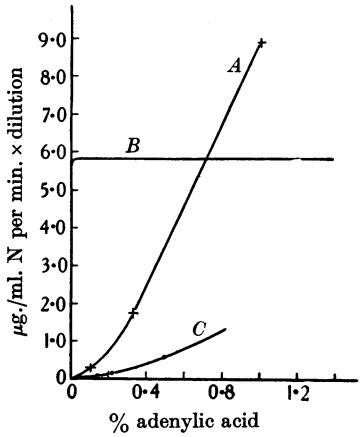

Fig. 1.

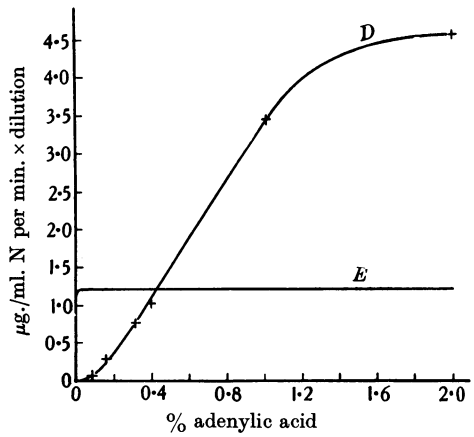

Fig. 2.

Figs. 1 and 2. Deamination rates of m. adenylic acid and of adenosine with respect to substrate in rabbit and human blood. *A*, curve of m. adenylic acid deamination in rabbit blood, with maleic acid buffer at *p*H 7·0. *B*, similar to *A* with phosphate buffer. *C*, curve of adenosine deamination with phosphate buffer, at *p*H 7·0. *D*, curve of m. adenylic acid deamination in human blood as in curve *A*. *E*, curve of adenosine deamination for human blood as in *C*.

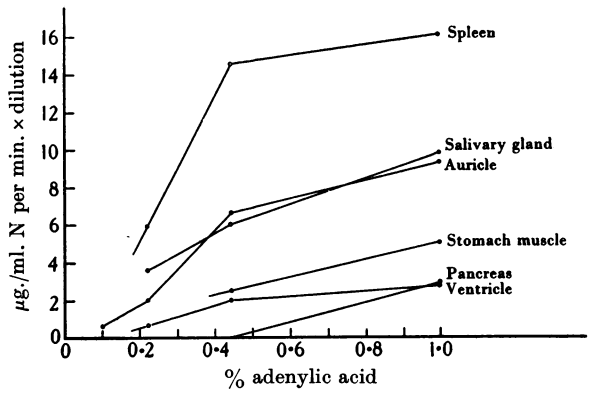

Fig. 3. Deamination of m. adenylic acid in tissue extracts. Maleic acid buffering at *p*H 7·0. Tissue dilution 1 in 50.

general effect is similar to that with blood. Voluntary muscle extract is markedly exceptional to this, the deamination rate increasing by only 10 % over the range 0·08–0·8 % and the mean value being over 70 times greater than the highest recorded in Fig. 3. Of the six tissues, however, only three deaminate the nucleotide in the main directly—these are voluntary muscle, cerebral cortex and the auricle of the heart. The others—as shown below—deaminate it almost if not quite entirely after an initial dephosphorylation, but apart from the extract of voluntary muscle the effect of substrate concentration is similar. From Fig. 3 it will appear

that at possible physiological levels—at and less than 0·1 % of the nucleotide the effective deamination rate in voluntary muscle extract is of the order of 500–1000 or more times as great as in other tissues.

Over the linear region of action of the enzyme with respect to substrate it may be pointed out that a unimolecular reaction relation will be simulated, this relation implying a proportionality of rate to substrate concentration. The underlying cause of the linearity is however quite different. (This does not contradict our previous statement of linearity of action with the time applying when the NH_3 formed is negligibly small compared with the possible total.)

The effect of dilution

With the adenosine deaminase the effect is the expected one. The total deamination rate for 1 ml. blood or 1 g. tissue is independent of the volume in which this is dissolved or dispersed. It is quite otherwise with the adenylic acid deaminase in blood and other tissues. Here the deamination per 1 ml. blood increases greatly with the dilution if the substrate concentration is not very high. This is illustrated in Fig. 4 in which dilution and deamination are expressed logarithmically.

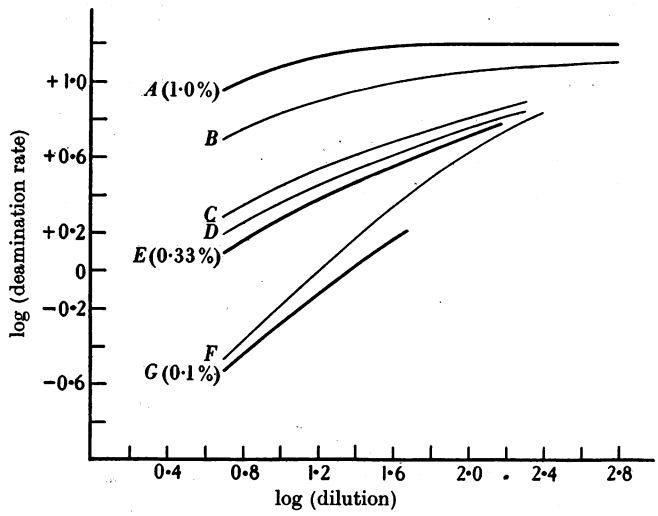

Fig. 4. Effect of dilution on m. adenylic acid deamination in blood. *A*, *E* and *G* are curves for same rabbit blood using 1, 0·33 and 0·1 % nucleotide and CO_2 and bicarbonate buffering at *p*H 7·0. *B*, 0·5 % nucleotide, self-buffering. *C*, 0·5 % nucleotide, CO_2 and bicarbonate buffering. *D*, 0·36 % nucleotide, self-buffering. *F*, 0·40 % nucleotide, maleic buffering. All at *p*H 7·0.

The ordinates in the graph give the logarithms of the deamination rates (μg./ml./min.) for 1 ml. rabbit blood independently of the volume in which it is contained. The thick lines *A*, *E* and *G* for nucleotide concentrations of 1·0, 0·33 and 0·1 % respectively are for the same rabbit blood with CO_2 dioxide and bicarbonate buffering (*ca.* *p*H 7·0). The other curves are for other blood samples and different conditions of buffering as described under the figure.

It will be seen that the general effect in which dilution causes an ultimate approach to approximately 13·8 μg./ml. NH_3-N/min., is independent of the substrate concentration. With high substrates this is quickly reached, and with low after much dilution. *Dilution at the lower substrate levels can increase the total deamination 60 times or more.*

Maleic acid buffering in blood at comparatively small dilutions appears to have an inhibiting effect on the deamination and this would appear to be associated with some precipitate formation. For tissue buffering in general with the adenylic acid deaminase it is, however, much superior to phosphate, bicarbonate/CO_2 etc. as will appear in the next section.

Specific buffer action

While the ideal procedure would here require the purest enzyme preparations, yet direct examination of very dilute voluntary muscle extracts gives the essential information. When this dilution amounts to 2000 times, the extract is still very active, and provided that the correct conditions of room temperature, buffering and *p*H are present a rate of 0·5 μg. N/min. is obtained which will produce NH_3 in somewhat over $N/500$ strength within 1 hr. In this way we examined five buffers, citric, maleic and phosphoric acids, CO_2/bicarbonate and veronal. The muscle extract dilution in the mixture was 1 in 2000, the buffer strength about $M/20$ (the bicarbonate/CO_2, $M/40$). The *p*H was carefully fixed at 7·4 and the deaminating action not allowed to proceed for longer time than required for formation of $N/5000$ NH_3. The effect on adenosine deaminase of the same buffers was studied in a similar way using laked blood (total dilution of one in eight). Table II shows the results for 0·1 and 1·0 % of the nucleotide and corresponding

Table II

		Adenylic acid deamination in 1 in 2000 voluntary muscle extract μg. N/ml./min. (× dilution)				Adenosine deamination in 1 in 8 diluted blood μg. N/ml./min. (× dilution)		
Buffer mixture at *p*H 7·4	Exp. no.	1 % nucleotide	0·1 % nucleotide	Ratio %	Exp. no.	0·7 % adenosine	0·07 % adenosine	Ratio %
Maleic	1	150	97	65	5	2·54	2·49	98
	2	696	602	86	—	—	—	—
Citric	1	352	289	82	—	—	—	—
Phosphate	1	143	3	2	6	2·71	2·56	94
CO_2/bicarbonate	3	350	3	1	6	2·64	2·82	107
	4	716	37	5	—	—	—	—
	1	· 42 (?)	3	—	—	—	—	—
Veronal	1	327	14	4	6	2·59	2·70	104

The strength of the buffers in the mixtures was from $M/20$ to $M/10$.

concentrations of the nucleoside. It will be seen that a change of adenylic acid concentration from 1·0 to 0·1 % affects only to a small degree the deamination with maleic and citric buffers. With maleic buffer in two experiments the reduction was to 86 and 65 % (with this we may include the effect with an extract dilution of 40, when the reduction was to 90 %). With citric buffer the reduction was to 82 %. With phosphate, bicarbonate and veronal buffers on the other hand, the deamination rate falls almost to zero or less than 3 % in the mean. Apart from its theoretical significance it is of some practical analytical importance to realize that by a suitable choice of buffer the deamination rate of adenylic acid by dilute muscle extract may be increased to over 20–30 times.

If we consider the deamination rate at 1 % nucleotide of the various buffers, citric seems the most efficient with a rate of 352 μg. N/ml./min. In exp. 1 the bicarbonate system gave the very low figure of 42, which may have arisen from

the use of 5 % CO_2/oxygen mixture from a cylinder, alveolar air being used in the other two experiments, though we cannot say why this difference should arise.

With adenosine deamination the results are very different. Here the rate is independent both of the substrate concentration (over the range studied) and of the buffer used.

The dilution was chosen so that the actual deamination rates in the blood-adenosine and the 1 %-adenylic-muscle-extract mixtures were not very different having means of 0·2 and 0·3 μg. N/ml./min. respectively.

Specific inhibitors of adenylic acid deaminase in blood and tissues

The mechanism whereby a linear proportionality of deamination to substrate concentration is simulated is obvious enough from the above study. Very probably the anion of the special buffer is absorbed on to the enzyme system displacing the adenylic acid. Over a certain range a proportionality effect can be simulated on increasing the nucleotide concentration, particularly if this is not adsorbed as readily as the buffer.

The practical independence of substrate concentration seen in the muscle extracts with maleic or citric buffers and in a 1 in 40 as in a 1 in 2000 tissue dilution, does not appear in blood or general tissue extracts in dilutions up to 1 in 50. Here the bicarbonate and free phosphate concentrations are negligible (in a special blood experiment the bicarbonate was totally removed) yet the type of curve shown in Figs. 1 and 2 remains. Some other inhibitors are present which, as may be expected, are progressively weakened in effect by dilution as shown in Fig. 4. These inhibitors appear to be generally distributed in tissues as may be judged from Fig. 3 and may act in a similar way here on dephosphorylation as on deamination processes since vegetable adenylic acid in blood shows a somewhat similar curve on a much lower level; also in most of the tissues in Fig. 3 a dephosphorylation actually precedes the deamination (at least to a very large extent).

By shifting the *p*H of diluted blood towards 6·0–6·5 with citric buffer, the effect of the inhibitor is much weakened, the activity at 0·1 % nucleotide being 62 % of that at 1·0 %. The indication is that the inhibiting substance is an anion of a weak acid with *p*K value in the region of 7·0 and may be possibly a protein anion or anions. In voluntary muscle extracts such inhibitors have no appreciable effect in reducing the activity of the enzyme.

The question arises, however, how far do the normal CO_2 and bicarbonate concentrations in muscle affect the adenylic deaminase. As shown, in very weak dilutions (1/2000), *M*/40 bicarbonate and 38 mm. CO_2, the effect is very great. With stronger extract (1 in 40), 80 mm. CO_2 and *M*/40 bicarbonate the effect is much less, the deamination of 0·1 % nucleotide being 40 % of that of the 1 % solution. It may be presumed that with the very high enzyme concentrations in the tissue itself and a reaction around *p*H 7·0, the effect is not marked.

*The p*H *effect*

Adenylic acid. The effect is shown in Fig. 5 where the activity is given in terms of a 100 value at *p*H 7·0. The *p*H buffers used were maleic acid below *p*H 7·0 and bicarbonate above, the strengths being *ca.* *M*/20; a nucleotide concentration of 0·25 % was used in the mixture and blood dilutions of 1 in 40 (higher nucleotide concentrate would have been somewhat more satisfactory on the alkaline side). The slope of the curve on each side of *p*H 7·0 was determined and the values at 7·0 regarded as 100.

The curve does not show the sharp-peaked optimum close to 6·0 described previously by Schmidt [1928]. It may be noted at the same time that the question of specific buffer inhibition was not studied by this worker.

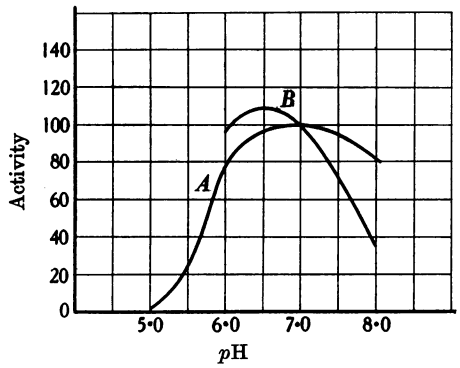

Fig. 5. Effect of *p*H on the deamination rate of m. adenylic acid (curve A) and of adenosine (B) in laked rabbit blood.

Adenosine. The adenosine curve was determined with phosphate buffers and 0·2–0·3 % adenosine. It does not differ appreciably from the results obtained using purified enzyme preparations [Schmidt, 1928].

The path of the deamination of muscle adenylic acid in tissues

The question here is what proportion of the deamination proceeds directly from the nucleotide or after an initial dephosphorylation. It may be considered for adenylic acid added to extracts, or for deaminations proceeding normally from the nucleotide complex. The study of the inorganic phosphate is not of much value for this purpose since phosphate may be transferred to other substances directly or after an initial liberation. We have used two deamination methods, however, whereby the question has been satisfactorily dealt with.

First method. In this method the deamination rate is examined after adding adenylic acid, after adding adenosine alone (enough to saturate the enzyme, which will occur after very small additions) and after adding adenylic acid plus adenosine. The difference between the deamination rates in the second and third mixtures will give the true or direct nucleotide deamination. Such a method will also give a measure of the true adenylic deaminase.

The method is not of immediate value in determining the path of normal deaminations owing to the much greater rate in general of adenosine deamination as compared with that of small added amounts of adenylic acid.

Second method. In this method the deamination rates of similar concentrations of yeast and muscle adenylic acids are studied in the same tissue extract or laked blood mixture.

Assuming that vegetable adenylic acid is not directly deaminated in animal tissues and that the enzymic dephosphorylation rates of both acids are the same (with acidic hydrolysis the yeast adenylic acid goes faster), we obtain the amount of direct deamination of the muscle nucleotide by the difference in deamination rates.

As regards the first point it has been shown by Schmidt [1928] that the purified enzyme from muscle is extremely specific and does not deaminate the vegetable nucleotide at an appreciable rate, and this we have also found for voluntary

muscle extract at room temperature (vide Table VI). In blood we have found that after elimination of dephosphorylation activity by dialysis the deamination rate of vegetable adenylic acid (in 1 % strength) falls almost to zero when examined over 24 hr., whereas that of the muscle adenylic acid remains practically unchanged. This is seen by comparing the two last columns of Table III.

Table III

	Mixture	Phosphate μg./ml. P after 2 hr.	Phosphate μg./ml. P after 20 hr.	NH_3 μg./ml. N after 2 hr.	NH_3 μg./ml. N after 20 hr
Undialysed	Blood control	34·3	46·0	1·8	5·3
	M. adenylic mixture (0·8 %)	29·9	30·7	124·0	215·0
	V. adenylic mixture (0·8 %)	41·8	64·0	9·3	32·3
Dialysed	Blood control	1·4	1·6	0·7	0·3
	M. adenylic mixture (0·8 %)	1·8	1·8	120·0	192·0
	V. adenylic mixture (0·8 %)	1·4	0·0	4·5	4·7

The blood dilution in mixture was 1 in 4·2, *p*H 8·0–8·3.

Table IV

Tissue	M. adenylic acid deaminated μg. N/ml./min.	V. adenylic acid deaminated μg. N/ml./min.	True nucleotide deaminated. II minus III μg. N/ml./min.
Blood	12·2	0·6	11·6
Auricle	13·3	2·7	10·6
Salivary gland	14·3	8·8	5·5
Jejunum	4·7	2·3	2·4
Stomach muscle	6·1	1·5	4·6

1 in 20 water extract of tissues were used. 1 vol. of extract plus 1 vol. of 2 % adenylic acid at *p*H 7·0. No other buffering used. Room temperature. The deamination rates are reckoned for the original tissue.

Table V

Tissue	Adenosine deaminated 0·16 % μg. N/ml./min.	Adenosine deaminated 0·32 % μg. N/ml./min.	Adenosine plus m. adenylic acid deaminated 0·16 % adenosine + 0·8 % m. adenylic acid in mixture μg. N/ml./min.	True nucleotide deaminated μg. N/ml./min.
Blood	4·15	4·15	17·0	12·9
Auricle	6·3	6·7	17·1	10·6
Salivary gland	15·5	15·8	21·6	6·0
Jejunum	54·8	57·3	60·4	4·4
Stomach muscle	1·74	1·69	5·1	3·4

Similar conditions to those for Table IV, but maleic acid (*M*/20) used for buffering at *p*H 7·0.

As regards the second point—the equality of dephosphorylation of the two nucleotides—perhaps the best proof of this in the tissues generally is a comparison of the results from the two methods. This comparison is given in Tables IV and V for five tissues. The correspondence is quite good, especially when it is considered that analytical inaccuracies will have a big effect in the results from the salivary gland and jejunum extracts. Table III shows how little can be deduced from direct studies of the free phosphate.

With this second method we can examine the effect of adding amounts of each nucleotide to tissue extracts of a similar concentration to the nucleotide in the original tissue and so gain information as to the normal path.

For 11 tissues studied in this way (Table VI) voluntary muscle, the cerebral cortex, conducting nerve, auricle of heart and blood (under normal conditions) show direct deamination as the major path (75–90 %).

In the second group—which is sharply demarcated from the first—the deamination proceeds almost if not quite entirely by the indirect route. This group includes the ventricular muscle of the heart, stomach muscle, salivary gland, kidney, liver and jejunum. At high nucleotide concentrations apparently an appreciable fraction goes directly but as the nucleotide concentration is dropped from 1 to 0·1 % the NH_3 formations from the two nucleotides become the same. It is of interest to note how closely the deamination rates of the two substances approach each other in these different tissues. *From the composition of this second group we may infer that the general visceral deamination of adenine nucleotide is an indirect process.* The auricle of the heart is an interesting exception.

Table VI

I	II	III	IV	V	VI	VII	VIII
Tissue	Exp. no.	*p*H	Nucleotide concentration %	M. adenylic acid deaminated μg. N/ml./min. (× dilution)	V. adenylic acid deaminated μg. N/ml./min. (× dilution)	True nucleotide deaminated at 0·1 % μg. N/ml./min. (× dilution)	Ratio of VI to V %
Voluntary muscle	7	7·0	1·0	987·0	0·05		0·005
	7	7·0	0·1	886·0	0·05	886·0	0·005
Cerebral cortex	4	7·0	1·0	17·1	2·7		15·8
	2	7·0	0·1	3·22	0·55	2·87	10·9
Sciatic nerve	4	7·0	1·0	5·4	0·70		13·0
	2	7·0	0·1	2·59	0·61	1·98	23·6
Auricle	4	7·0	1·0	13·3	2·70		20·3
	2	7·0	0·1	2·93	0·72	2·21	24·8
Blood	3	7·4	1·0	4·25	0·48		11·3
	3	7·4	0·05	0·45	0·04	0·41	8·9
Blood	6	7·0	1·0	12·2	0·60		4·9
	6	7·0	0·1	0·27	—	< 0·27	
Salivary gland	4	7·0	1·0	14·3	8·8		61·6
	2	7·0	0·1	3·18	3·05	0·03	95·8
Kidney	1	7·0	1·0	6·50	3·68		56·8
	1	7·0	0·1	1·67	1·61	0·06	96·6
Liver	1	7·0	1·0	5·76	4·04		70·2
	1	7·0	0·1	1·13	1·07	0·06	94·6
Stomach muscle	4	7·0	1·0	6·10	1·50		24·6
	2	7·0	0·1	0·18	0·19	−0·01	105·5
Jejunum	4	7·0	1·0	4·70	2·30		48·9
	2	7·0	0·1	1·21	1·12	0·09	92·8
Ventricle of heart	1	7·0	1·0	4·72	4·78		101·5
			0·1			(0·00)	
Blood	5	8·3	0·1	0·028	0·027	0·01	96·4

The rabbit tissues were extracted with water, 1 part by weight to 20 vol. 1 vol. of extract was added to 1 vol. of nucleotide at *p*H 7·0. No other buffering was used except for blood and voluntary muscle, but the NH_3 formation was not allowed to exceed $N/5000$ strength, and no appreciable change of *p*H occurred. In the blood experiments 3, 6 and 5 CO_2/bicarbonate ($M/40$), maleic acid ($M/20$) and bicarbonate ($M/10$) were used, the blood dilution ranging from 1 in 5 to 1 in 8·8. Maleic acid buffering was used with the voluntary muscle extract. Room temperature.

For each experiment the tissues from two rabbits were used immediately after killing. After grinding the extract was usually allowed to stand for a few hours.

The above studies of deamination were carried out without further buffering than that provided by the nucleotide or the tissue extract. At the 1 % levels the nucleotide buffering is very appreciable. With the 0·1 % additions the NH_3 formation was not allowed to exceed about $N/5000$ with the result that no appreciable shift in pH occurred.

Seeing that in these 1 in 40 extracts the tissue Mg concentration has fallen considerably the effect of Mg addition was examined. No appreciable change from the above description was found.

The distribution of the deaminases in tissues

These were measured by the deamination rates of adenosine and adenylic acid under standard conditions, and the unit chosen was the μg. N/ml./min., the amount of enzyme being given per 1 g. or 1 ml. of the original tissue. A pH of 7·0 was selected as most suitable since it corresponds to the optimum formation of NH_3 from adenylic acid in tissues, is most representative of the actual tissue pH and is also close to the adenosine optimum (multiplying the adenosine results by 1·09 will give the value for the optimum pH).

1 in 20 water extracts of the tissues were made—grinding to a fine suspension with quartz sand—and an equal volume of substrate added. For adenylic acid

Table VII

Tissue	Adenosine deaminated	M. adenylic acid deaminated at 1 % (direct and indirect)
Alimentary:		
Appendix	59·7 (2)	8·3 (2)
Jejunum	52·6 (3)	6·6 (4)
Peyer's patches	36·8 (1)	12·1 (1)
Duodenum	29·6 (1)	—
Duodenum mucosa scrapings	23·1 (2)	14·4 (2)
Jejunum mucosa scrapings	21·1 (2)	11·6 (2)
Colon	10·7 (1)	—
Ileum	8·1 (2)	—
Caecum	6·5 (1)	—
Pyloric mucosa	2·8 (2)	2·2 (2)
Stomach muscle	2·0 (3)	5·1 (4)
Glandular:		
Spleen	40·2 (3)	14·8 (2)
Testicles	26·6 (3)	16·5 (2)
Salivary glands	19·7 (5)	9·8 (4)
Suprarenals	15·3 (3)	15·5 (2)
Pancreas	12·6 (3)	3·0 (2)
Thyroid	8·3 (2)	—
Kidney	7·7 (2)	5·6 (2)
Ovary	6·5 (2)	5·6 (1)
Liver	3·7 (3)	2·8 (3)
Pituitary	2·6 (2)	24·6 (2)
Muscular:		
Auricle	6·8 (6)	9·4 (2)
Diaphragm	2·5 (2)	108·0 (2)
Ventricle	2·3 (2)	2·8 (2)
Stomach muscle	2·0 (3)	5·1 (4)
Skeletal muscle	0·9 (4)	1145·0 (6)
Nervous:		
Spinal cord	14·7 (1)	14·8 (1)
Brain (whole)	8·6 (1)	12·2 (2)
Cerebral cortex	5·6 (1)	15·9 (3)
Pituitary	2·6 (2)	24·6 (2)
Sciatic nerve	0·8 (3)	4·8 (3)
Respiratory:		
Lungs	8·0 (3)	6·0 (2)
Circulatory:		
Auricle	6·8 (6)	9·4 (2)
Whole blood	5·9 (5)	14·0 (5)
Artery	4·1 (2)	2·2 (2)
Ventricle	2·3 (2)	2·8 (2)
Plasma	0·04 (2)	0·04 (2)
Miscellaneous:		
Embryonic tissue	10·6 (2)	10·7 (2)
Bone marrow	8·3 (2)	9·7 (2)
Uterus	3·7 (1)	6·5 (1)
Skin	0·0 (1)	0·0 (1)
Bone	0·0 (1)	0·0 (1)

The units in which the deamination is expressed are μg. N/ml./min. for the original tissue.
Tissues ground with quartz in 20 vol. water. To 1 vol. extract 1 vol. of nucleoside plus 0·5 vol. buffer (phosphate $M/20$) or 1 vol. extract plus 1 vol. of 2 % nucleotide. pH 7·0. Room temperature.

this consisted of 2 % nucleotide which had been neutralized to *p*H 7·0 with NaOH and for adenosine 0·5 % adenosine buffered with phosphate at *p*H 7·0 to make *M*/20 in the mixture. No buffer except that of the nucleotide itself in 1 % strength in the mixture was used in studying the distribution of the adenylic deaminase.

The unexpected behaviour of adenylic deaminase in tissues as regards the points already considered led us to examine the efficiency of extraction of the enzyme under various conditions. In our experiments the efficiency of extraction is somewhat variable even under strictly standardized conditions, though this may be attributable to varying distribution throughout the tissue. Using pure water, 0·9 % NaCl and 0·9 % NaCl plus 0·05 % KCl we obtained a ratio of 1·0 : 1·2 : 1·6 in the amounts of enzyme action observed in a few experiments. These extractions were made from the same muscle finely cut and mixed, some of which was weighed out and ground with quartz and the fluid to make 1 in 20 extracts. The inclusion of Mg and Ca with the 0·9 % NaCl caused a decrease, which also resulted from the inclusion of some bicarbonate in the extract medium. From this it will appear that saline would have been a more efficient extractor than water and there is also a case for supposing K addition to act better than Na alone, though to decide this many more experiments would be necessary. Since for reasons already given the amount of the deaminase can be given only in a comparative way, the water extract was considered sufficiently serviceable.

Table VII gives a summary of the experiments performed on 36 tissues of the rabbit, the results being considered in the subsequent discussion. The adenylic deamination given in Table VII indicates the sum of both direct and indirect formations at 1 % concentration, from which, as already considered, we must distinguish the pure nucleotide deamination and the effective deamination rate in tissues.

Discussion

The distribution of adenylic acid and adenosine deaminases in the tissues of the rabbit. The distribution of the adenosine deaminase may be roughly divided into a digestive, a glandular, a nervous and a muscular type and in that order with respect to enzyme strength. The jejunum and duodenum were found to contain 53 and 30 units respectively *while the appendix showed the highest of any tissue examined,* namely 60 units. In each case all the intestinal coats were taken together, but scrapings from the mucosa of the duodenum and jejunum gave also no increase over these figures, but rather a decrease.

An explanation of the high appendix concentration compared with that in the ileum, caecum and colon may be given by considering that in the rabbit—and possibly in other herbivora—the caecum serves as a second stomach in which the cellulose walls of cells are digested by special bacteria, further nucleic acid material being liberated. The appendix may then be represented as playing the same role as the duodenum and jejunum to the main organ with respect to the further treatment of nucleic acid.

In the glandular distribution the spleen comes highest, but this tissue is exceptional with regard to the adenylpyrophosphate liberated therein from the red corpuscles. The special glandular activity is shown rather by the testicles, salivary glands, suprarenal and the pancreas, which gave from 27 to 13 units. Of the eleven types of glandular tissue examined the liver gave the second lowest with 4 units and the pituitary lowest with 3 (the pituitary contained relatively a very high proportion of adenylic acid deaminase, namely 25 units).

The nervous distribution of adenosine deaminase is somewhat similar to the glandular, being perhaps a little lower. The difference here is in the compara-

tively higher amount of adenylic deaminase, the ratio being about 3 to 1 in the cerebral cortex and 6 to 1 in the conducting nerve (the latter containing only 0·8 units of adenosine deaminase).

Muscle tissue in general contained the lowest amounts, with the auricle of the heart highest in the group, having 6·8 units, and voluntary muscle lowest with 0·9. The muscle of the stomach wall had 2·0 units and the ventricle of the heart 2·3.

Concerning the distribution of true adenylic acid deaminase, voluntary muscle is very exceptional with an average of 1145 units. A long way after this we have nervous tissue, e.g. cerebral cortex, with 15·8 units (vide Table VI) but only an effective deamination rate of less than 2·9. Conducting nerve also has a high ratio of adenylic acid deaminase to that of adenosine (6 : 1). Blood was found to contain 14 units and the auricle of the heart 9–11. Apart from these tissues the amount of true adenylic acid deaminase found elsewhere was low and its effective action practically zero. Contrary to our first observations plasma was found to contain a very small and variable amount of the true nucleotide deaminase, but at any possible normal concentrations its activity is only a few % of the adenosine deaminase therein (0·05 unit).

The significance of the adenylic acid and adenosine deamination

That NH_3 formation is coincident with activity in tissues is a point that, as is well known, has been emphasized by Embden and others. Adenylic acid was shown by Embden to be the precursor of this NH_3 in voluntary muscle. Later it was found by Lohmann [1929] that adenylic acid was itself a stage in the breakdown of adenylpyrophosphate, the role of which in phosphate transference and the glycolytic cycle was soon recognized. It was subsequently considered—in particular by Parnas—that the deamination of the adenylic acid formed on dephosphorylation and such as escaped resynthesis was merely a detoxication process without any special relation to muscular activity. Certain findings in the present paper point directly against this view and may be summarized as follows.

(1) An average of about 40 times more true adenylic acid deaminase exists in voluntary muscle than in any other tissue examined, but the effective deamination rate is greater by about 500–1000 times or more, owing to the absence therefrom—or at least the ineffective action—of specific inhibitors of the enzyme.

(2) Though quite considerable amounts of the deaminase exist in the red corpuscle and other tissues, the action of the enzyme is reduced to a few % or less of its full action by special inhibition (in addition to that produced by the CO_2 and bicarbonate system). In this way adenylic acid can be said to be conserved and presumably as a physiological process. Yet adenylic acid is freely permeable across the membrane of the red corpuscle and will therefore escape into the plasma. We have demonstrated this permeability to adenylic acid added both to oxalated and to heparinized blood and examined immediately after shedding, and also a similar permeability for voluntary muscle.

(3) An adenine nucleotide apparently already exists in plasma and its pharmacological action cannot be considered as markedly less than that of muscle adenylic acid [Bennet & Drury, 1931].

(4) The deamination of adenine nucleotide in the duodenum or jejunum is not direct but occurs after dephosphorylation. Here the total effective deamination of the nucleotide for normal concentrations is only a minute fraction of that in skeletal muscle—probably considerably less than 1 %—yet we may suppose that across the intestinal wall there pass amounts of nucleotide at least as great

as the adenylic acid set free momentarily and escaping re-esterification in voluntary muscle.

A point which has appeared conclusive to Lohmann [1935] against the special role of adenylic deaminase is the absence—as he found—of this enzyme in the muscle of the crab—including the claw muscle. We have investigated this point in turn and found for the claw muscles from four crabs a mean value of 40 units of the deaminase. While this amounts only to about 4 % of that in the rabbit's leg muscle, it is still very appreciable and higher than that found in any of the other tissues of the rabbit. Considering the many factors discussed above which inhibit the action of this enzyme it is not surprising that its absence is occasionally and erroneously concluded.

Direct nucleotide deamination can also occur normally in nerve tissue, the auricle of the heart and in red corpuscles though these have no obvious relationship. It may be noted that the conservation of adenylic acid in general tissues by the action of the specific inhibitors and CO_2 (or bicarbonate) combined with its permeability (as we may deduce from muscle and red corpuscles) may lead to the activity of this substance as a local vasodilator.

Concerning the deamination of adenosine, this may be firstly considered as merely a process in the metabolism of surplus nucleic acid, and in the intestinal wall all the normal deamination of adenine nucleotide derives from this substance. Besides this, the widespread occurrence of adenosine deaminase in tissues and in blood may be regarded from two aspects. Adenosine may be looked upon as an accidental formation from free adenylic acid, set free in turn from A.T.P. in the glycolytic process, and that when formed it is detoxicated by deamination. Adenosine is apparently the most active substance pharmacologically of its class, and that it should be rapidly deaminated in the intestinal wall may be linked with the detoxication view. We may consider too that adenosine deamination in the general tissues is a physiological mechanism for obtaining a ready supply of NH_3—which they cannot obtain directly from blood —and that the role of this NH_3 may be of a similar kind, though much more slowly formed, than the NH_3 in voluntary muscle. What this role may be is so far obscure, but various possibilities can be suggested such as permeability changes caused thereby, requirement for local synthesis of amino compounds, local neutralizations etc.

Summary

1. The relation of adenylic acid deaminase to substrate concentration is affected by specific buffer inhibition and also by special inhibitors in tissues. These appear to act by displacing the adenylic acid from an adsorbing surface. Among the buffers so acting are CO_2 and bicarbonate, phosphate and veronal. Maleic and citric acids do not act in this way. The effect of the inhibition is to simulate a linear relation of action to substrate up to very high concentrations (about 1 % or more). The special tissue inhibitors are either absent from or ineffective in voluntary muscle. Dilution of blood—by lessening the concentration of the inhibitors can very greatly increase the deamination of adenylic acid per ml. blood.

Similar inhibitions of the action of adenosine deaminase have not been found.

2. Methods for determining the path of the deamination of adenine nucleotide are given. It is shown that (apart from voluntary muscle) nerve tissue, the auricular muscle of the heart and red corpuscles deaminate adenylic acid normally in a direct way, but the kidney, liver, intestine, smooth muscle, ventricular muscle of heart and blood after loss of CO_2 deaminate it only after an initial dephosphorylation.

3. The distribution of adenosine and adenylic acid deaminases has been studied for 36 tissues of the rabbit. The amount of adenylic acid deaminase in voluntary muscle has a mean value 40 times greater than that in any other tissue examined, but its effective action on possible normal concentrations is greater by about 500–1000 times or more. Through the inhibition of the adenylic acid deaminase in blood, although there is more of this than the corresponding adenosine enzyme, the action on minute amounts is less than 1 % of that on the nucleoside. Even those tissues, apart from voluntary muscle, which normally deaminate the nucleotide directly are less effective deaminators of small amounts of adenylic acid than of adenosine.

The highest concentration of adenosine deaminase was found in the vermiform appendix and somewhat lesser amounts in the duodenum and jejunum.

4. Contrary to Lohmann's finding [1935], adenylic acid deaminase in very appreciable amounts was found in the claw muscles of the crab.

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