The Distribution of Guanidines, Phosphagens and N-Amidino Phosphokinases in Echinoids

BY D. E. GRIFFITHS,* J. F. MORRISON AND A. H. ENNOR

Department of Biochemistry, John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T., Australia

(Received 9 October 1956)

The class Echinoidea assumed prominence in the field of comparative biochemistry when it was shown by Needham, Needham, Baldwin & Yudkin (1932) that the jaw muscles of *Strongylocentrotus lividus* contained both phosphocreatine and phosphoarginine. Further work by Baldwin & Needham (1937) showed that extracts of the jaw muscles of two other echinoids, *Paracentrotus lividus* and *Sphaerechinus granularis*, were capable of phosphorylating both creatine and arginine. This result suggested that the muscles of both of these organisms contained phosphocreatine and phosphoarginine, although neither phosphagen was identified. Earlier Meyerhof (1930) had advanced the hypothesis that phosphoarginine was the characteristic phosphagen in the muscles of invertebrates and phosphocreatine was characteristic of the vertebrates. The occurrence of both phosphagens in the echinoids studied provided biochemical support (Needham *et al.* 1932) for the evolutionary theory of Bateson (1886), namely that the echinoderms were the immediate precursors of the primitive chordates. Later work has shown that the premises on which Meyerhof's hypothesis was based are not correct. It has been shown that phosphocreatine is not confined to the vertebrates and those animals intermediate between vertebrates and invertebrates, but that it is also present in sponges (Roche & Robin, 1954) and annelids (Hobson & Rees, 1955). Moreover, other phosphorylated guanidines, e.g. phosphotaurocyamine, phosphoglycocyamine and phospholombricine, also occur in invertebrate muscle (Thoai, Roche, Robin & Thiem, 1953*a*; Thoai & Robin, 1954).

The earlier work on echinoids was concerned mainly with the study of the distribution of free guanidines. Arnold & Luck (1933) showed that arginine was present in the jaw muscles of *Strongylocentrotus franciscanus*, and Holtz & Thielmann (1924) found arginine in *Arbacia pustulosa*. The first positive identification of creatine in echinoids was made by Greenwald (1946), who isolated potassium creatinine picrate from the testes of *Strongylocentrotus* sp. Before this, creatine had been identi-

fied by the non-specific Jaffe reaction (cf. Myers, 1920).

The development of improved techniques for the identification and estimation of guanidine compounds and the availability of two Australian echinoids, *Centrostephanus rodgersii* and *Heliocidaris erythrogramma* Valenciennes, prompted an investigation of the distribution of guanidines and phosphagens in these genera. At the same time a study was made of the distribution of the transphosphorylases involved in the metabolism of these compounds. The results of such a study are described here and indicate that *C. rodgersii* contains arginine, phosphoarginine and arginine phosphokinase only, whereas *H. erythrogramma* contains creatine, phosphocreatine and creatine phosphokinase, as well as arginine, phosphoarginine and arginine phosphokinase.

Abbreviations. The following abbreviations will be used: PA, phosphoarginine; APK, arginine phosphokinase; PC, phosphocreatine; CPK, creatine phosphokinase; ATP, adenosine triphosphate; ADP, adenosine diphosphate; TCA, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid.

MATERIALS

Specimens of *C. rodgersii* and *H. erythrogramma* were collected in the Sydney area during the summer months. The gonads and jaw apparatus (Aristotle's lantern) were excised and frozen immediately by dropping them into liquid N₂. When the tissues had reached the temperature of liquid N₂, they were removed, placed in polythene bags and stored in solid CO₂ for transport to the laboratory. No distinction was made between male and female gonads.

Phosphagens. PA was isolated from the tail muscle of the sea-crayfish (*Jasus verreauxi*) as a barium salt by the method of Ennor, Morrison & Rosenberg (1956) and converted into the sodium salt as described by Morrison, Griffiths & Ennor (1957). Sodium PC was synthesized by the method of Ennor & Stocken (1948). Phosphotaurocyamine was isolated as the barium salt after phosphorylation of taurocyamine by POCl₃ (cf. Ennor & Stocken, 1948). Phosphoglycocyamine was prepared as described by Rosenberg (1955) and isolated as the sodium salt.

Guanidines. Arginine, glycocyamine and creatine were products of British Drug Houses Ltd.; the last two compounds were purified by recrystallization from water.

* Australian National University Research Scholar.

Taurocyamine (*N*-amidinotaurine) was prepared by treatment of taurine with *S*-ethylthiourea as described by Brand & Brand (1942); its purity was checked by elementary analysis.

Arginase. Arginase was prepared from ox liver by the method described by Greenberg (1951). The preparation was taken to stage F and freeze-dried. The enzyme was dissolved in 0.05M *N*-ethylmorpholine buffer (pH 8.4) before use. Such solutions contained sufficient Mn^{2+} ions to activate the enzyme but insufficient to interfere with the colorimetric method for the estimation of guanidines.

Creatine phosphokinase. CPK was prepared as described by Ennor & Rosenberg (1954*a*). The freeze-dried enzyme was dissolved in 0.05M *N*-ethylmorpholine buffer of the appropriate pH before use.

Arginine phosphokinase. APK was prepared by the method of Morrison *et al.* (1957) and stored as a 2.3% (w/v) solution in 50% (v/v) glycerol. Dilutions of the enzyme were made with 0.05M *N*-ethylmorpholine buffer of the appropriate pH.

METHODS

Estimation and detection of guanidines. The guanidine content of tissue extracts was estimated by the colorimetric method of Rosenberg, Ennor & Morrison (1956). Guanidines were demonstrated on paper chromatograms by spraying with a 1:1 mixture of 5*N*-NaOH and the α -naphthol-diacetyl reagent of Rosenberg *et al.* (1956). Monosubstituted guanidines were demonstrated also by the Sakaguchi reaction as described by Acher & Crocker (1952).

Estimation of protein. Protein was estimated colorimetrically by the biuret method of Gornall, Bardawill & David (1949), crystalline bovine-serum albumin being used as the standard.

Study of guanidines

Extraction of guanidines. The method used for the extraction of guanidines from tissues was essentially that of Thoai, Roche, Robin & Thiem (1953*b*). The frozen material was treated in a Waring Blender with 2 vol. of water and the mixture then adjusted to pH 3 by the addition of 10*N*- H_2SO_4 . (Large amounts of CO_2 were evolved on the addition of acid to extracts of the jaw apparatus.) It was then boiled for 2 min., cooled, and the precipitate removed by centrifuging. Samples were withdrawn for the determination of the guanidine content and the remainder was treated with a saturated solution of basic lead acetate until there was no further precipitation, and the excess of lead was removed with H_2S . All the extracts obtained were clear, but were sometimes coloured and in these cases the colour was removed by treatment with a small amount of active charcoal. The extract was then freeze-dried and the residue dissolved in a small volume of water; insoluble material was removed by centrifuging and the supernatant examined enzymically and by paper chromatography.

Paper chromatography. Paper-chromatographic examination of the tissue extracts was carried out by the ascending method at room temperature (20–22°). Sheets of Whatman no. 3 MM paper (12 in. $\times$ 12 in.) were used as a better resolution of guanidines was obtained than with Whatman no. 1 paper.

Many solvent systems were investigated, including those recommended by Block & Zweig (1955) and Roche, Thoai & Hatt (1954). Development of tissue extracts in alkaline solvents led to excessive 'tailing' of the guanidine spots,

and there was not sufficient resolution of guanidines in the acidic solvents used by these authors to enable positive identification. As will be seen below, extracts of the tissue of *H. erythrogramma* contain arginine and creatine, and excellent separation of these guanidines was obtained with *n*-butanol-6*N*-HCl (70:30, v/v; Makisumi, 1952) and phenol-water (80:20, w/v) saturated with SO_2 (Crawford, 1951); see Table 1. The following solvents were used in conjunction with *n*-butanol-HCl and phenol-water- SO_2 even though the separation of guanidines was not as good: *n*-propanol- NH_3 soln. (sp.gr. 0.88)-water (60:30:10; Hanes & Isherwood, 1949); pyridine-*isopentanol*-water (80:40:70; Roche *et al.* 1954); acetone-*n*-propanol- NH_3 soln. (2*N*)-water (50:10:30:10), acetone- NH_3 soln. (2*N*)-water (60:30:10) and *n*-propanol-dioxan- NH_3 soln. (2*N*) (40:10:50).

The guanidines present in tissue extracts were further characterized by enzymic means as the presence of impurities made the preparation of crystalline derivatives difficult. Moreover, the enzymes used (arginase, CPK and APK) show such a high degree of specificity that they provide an excellent means for the characterization of arginine and creatine. Tissue extracts adjusted to pH 8.5 with *N*-NaOH were incubated with arginase in a system which contained 0.1 ml. of extract, 0.1 ml. of *N*-ethylmorpholine buffer (0.5*M*, pH 8.5), 0.1 ml. of arginase (containing 0.1–1.0 mg. of protein) and 0.7 ml. of water. The mixture was incubated at 37° for 1–2 hr., after which 1.0 ml. of an EDTA-NaOH mixture containing 0.4 ml. of 0.2*M* EDTA (adjusted to pH 7.6) and 0.6 ml. of 5*N*-NaOH was added. The presence of arginine was detected by estimation of the total guanidine before and after incubation with arginase. By this means it was also possible to estimate the amount of arginine in the extract as it is almost completely destroyed by arginase. Appropriate control experiments were always carried out to ensure that the extracts contained no substances capable of inhibiting arginase activity and thus producing misleading results.

Arginine and creatine were also characterized by incubation with APK and CPK in a system which contained 0.1 ml. of extract (pH 8.5), 0.1 ml. of ATP (5×10^{-2} *M*), 0.1 ml. of $MgSO_4$ (10^{-2} *M*), 0.1 ml. of *N*-ethylmorpholine buffer (0.5*M*, pH 8.5), 0.2 ml. of APK or CPK (containing 1 mg. of protein) and 0.4 ml. of water. The reaction mixture was incubated at 5° for 1–2 hr. and the guanidine disappearance measured after the addition of 1.0 ml. of the EDTA-NaOH mixture described above.

When chromatographic analysis of the guanidines present in tissue extracts indicated the presence of both creatine and arginine, enzymic identification of the guanidines was

Table 1. R_F values of guanidine compounds

Guanidine	R_F values	
	Butanol-6 <i>N</i> -HCl (70:30, v/v)	Phenol-water (80:20, w/v) saturated with SO_2
Creatine	0.52	0.67
Arginine	0.25	0.39
Glyocyamine	(trails)	0.50
Taurocyamine	0.46	0.73

carried out by colorimetric estimation after each of the following treatments: (1) incubation of samples of the extract with APK and CPK; (2) incubation of a sample of the extract with CPK after destruction of the arginine with arginase; (3) incubation of the separated guanidine with the appropriate *N*-amidino phosphokinase. For this purpose arginine and creatine were separated by passing the extract through a column of Zeo-Karb 216 (Na form) when creatine passed through. The column was washed with water and the adsorbed arginine eluted with 2*N*-NH₃ soln. The eluate was freeze-dried after removal of most of the ammonia by aeration. This method was used only for the extracts from the jaw muscles of *H. erythrogramma* which contained an unknown inhibitor of arginase.

Study of the phosphagens

Isolation of phosphagens. The jaw apparatus was extracted with 2 vol. of ice-cold 9% (w/v) TCA by treatment in a Waring Blender for 2 min. The mixture was filtered on a Büchner funnel (Whatman no. 531 paper) and the filter cake re-extracted with 1 vol. of ice-cold 5% (w/v) TCA. After filtration the filtrates were combined, adjusted to pH 9 by the addition of 10*N*-NaOH and the resultant precipitate was removed by centrifuging. These operations were carried out in the cold room at 5°. The pH of the extract was then adjusted to 8.2 by the addition of 5*N*-HCl, excess of barium acetate (M) was added and the pH readjusted to 8.2. The insoluble barium salts were removed by centrifuging. To the supernatant was added 4 vol. of ethanol, the pH was adjusted to 8.2 and the precipitate was allowed to settle overnight. The precipitate was removed by centrifuging, washed with ethanol and ether and dried *in vacuo*.

The gonads were extracted as described above, but 1 vol. of ethanol was added to the TCA extract before neutralization in order to precipitate the glycogen. Further fractionation was carried out as described by LePage (1951).

The water-soluble, ethanol-insoluble barium salts containing the phosphagen were suspended in a small volume of water and sufficient 5*N*-HCl to effect solution was added. The solution was immediately adjusted to pH 9 by the addition of 5*N*-NaOH. The inorganic phosphate so precipitated was removed by centrifuging and discarded; 4 vol. of ethanol was added to the supernatant and the barium salt of the phosphagen collected by centrifuging, washed with ethanol and ether and dried *in vacuo*.

Preparation of the sodium salts of the phosphagens. Conversion of the barium salts into sodium salts by the addition of Na₂SO₄ led to a marked loss of phosphagen when PC was present, so that an ion-exchange column was used from which 85% recoveries were obtained. Solutions of the barium salts were passed through a column of Zeo-Karb 225 (Na form). The effluent was freeze-dried and the residue dissolved in a small volume of water. In no case did the material so prepared contain guanidine other than that bound to P.

Paper chromatography. Paper chromatography of the tissue phosphagens in solvent systems used by other authors, e.g. propanol-NH₃ soln.-water (Hobson & Rees, 1955), pyridine-isopentanol-water (Roche *et al.* 1954), proved unsatisfactory owing to the marked 'tailing' of the compounds. Moreover, there was such poor separation of marker phosphagens, PA, PC, phosphotaurocyamine and phosphoglycocyamine, that these solvents could not be relied on for use in positive identification of unknown phos-

phagens. Attempts were made to find solvents which would give good separation of the various phosphagens, but they were unsuccessful. The phosphagens were therefore identified by chromatography of the free bases after acid hydrolysis. The barium salts of the phosphagens were dissolved in *N*-HCl and allowed to hydrolyse by standing at room temperature for 24 hr. Alternatively the solutions of the sodium salts of the phosphagens were spotted on the paper and hydrolysed by allowing the paper to remain for 1 hr. in a jar filled with HCl vapour before development with solvent. Both procedures were effective in hydrolysing the phosphagens of echinoid tissues. The conditions for chromatography were the same as those described previously.

Enzymic characterization of phosphagens. Solutions of the sodium salts of the phosphagens were incubated separately with APK and CPK (1 mg. of protein/ml.) for 1 hr. at 5° under the conditions described for the determination of APK activity (Morrison *et al.* 1957). The release of arginine or creatine by the respective phosphokinases (which contained no phosphatase or phosphoamidase activity) in the presence of ADP was taken as evidence for the presence of the relevant phosphagen.

Study of the N-amidino phosphokinases

The jaw apparatus only of both echinoids was examined for the presence of *N*-amidino phosphokinases. The frozen jaw apparatus was passed twice through a mincer and extracted with 2 vol. of water by stirring mechanically for 30 min. The mixture was centrifuged for 15 min., the residue re-extracted with 1 vol. of water for 30 min., centrifuged and the supernatants were combined. The extract was then fractionated with solid (NH₄)₂SO₄. All operations were carried out at 5°. APK and CPK activities were determined under the same conditions as described by Morrison *et al.* (1957) for the determination of APK activity. In each case, the appropriate controls for phosphoamidase activity were set up.

RESULTS

The results of the investigation into the nature of the guanidines present in extracts of the jaw muscles and gonads of *C. rodgersii* and *H. erythrogramma* are shown in Table 2.

It may be seen that arginine only is present in the jaw muscles and gonads of *C. rodgersii* and that both arginine and creatine are present in similar tissues of *H. erythrogramma*. Also the guanidine content of the gonads of both echinoids is much greater than that of the jaw muscles, which is the reverse of the findings in vertebrate tissue (cf. Ennor & Rosenberg, 1952). The proportion of the total guanidine in *H. erythrogramma* which is represented by arginine is much less in the jaw muscles than in the gonads.

A study of the phosphagens present (Table 3) shows that only PA is present in the jaw muscles and gonads of *C. rodgersii*, whereas these tissues in *H. erythrogramma* contain both PA and PC. No detailed investigations were carried out to determine the relative amounts of each of the phosphorylated guanidines in the tissues of *H. erythrogramma*, but qualitative analyses indicated that

the ratio PA:PC was about the same as that of the free guanidines.

Examination of aqueous extracts of the jaw muscles of *C. rodgersii* showed that only APK was present. Fractionation of the extract showed that the major portion of the enzyme was precipitated between the addition of 42 and 56 g. of $(\text{NH}_4)_2\text{SO}_4$ /100 ml. No CPK activity was detected in the original extract or in any of the fractions obtained as a result of $(\text{NH}_4)_2\text{SO}_4$ fractionation. The APK isolated did not phosphorylate creatine, glyco-cyamidine or taurocyamine. The enzyme is similar to that isolated from crayfish muscle (Morrison *et al.*

1957) in that it is activated by Mg^{2+} and Mn^{2+} ions, but not by Ca^{2+} ions.

Aqueous extracts of the jaw muscles of *H. erythrogramma* contained both APK and CPK, which could be separated by $(\text{NH}_4)_2\text{SO}_4$ fractionation. Table 4 shows that CPK is precipitated between the addition of 28 and 35 g. of $(\text{NH}_4)_2\text{SO}_4$ /100 ml., whereas APK is precipitated between the addition of 42 and 63 g. of $(\text{NH}_4)_2\text{SO}_4$ /100 ml. It is also clear in *H. erythrogramma* that the total units of activity of APK and CPK are approximately equal. The APK isolated was activated by Mg^{2+} and Mn^{2+} ions, but not by Ca^{2+} ions, and in this respect is

Table 2. *Distribution of guanidines in the jaw muscles and gonads of C. rodgersii and H. erythrogramma*

Methods used for the identification of guanidines have been described in the text.

Echinoid	Tissue	Guanidine content (mg./100 g.)		Phosphoryl- ation by CPK (%)	Phosphoryl- ation by APK (%)
		Creatine	Arginine		
<i>Centrostephanus rodgersii</i>	Jaw muscles	0	82.5	—	60
	Gonads	0	250	0	80
<i>Heliocidaris erythrogramma</i>	Jaw muscles	27	10	24*	36*
	Gonads	77	124	30†	—

* Tests carried out on arginine and creatine separated by passing through a column of Zeo-Karb 216 (Na form).

† Test carried out on creatine remaining in extract after destruction of arginine with arginase.

Table 3. *Distribution of phosphagens in the jaw muscles and gonads of C. rodgersii and H. erythrogramma*

Methods used for the identification of phosphagens have been described in the text. Abbreviations: PA, phospho-arginine; PC, phosphocreatinine; APK, arginine phosphokinase; CPK, creatine phosphokinase.

Echinoid	Tissue	Phosphagens present	Guanidine liberated by	
			APK	CPK
<i>Centrostephanus rodgersii</i>	Jaw muscles	PA	+	0
	Gonads	PA	+	0
<i>Heliocidaris erythrogramma</i>	Jaw muscles	PA and PC	+	+
	Gonads	PA and PC	+	+

Table 4. *Arginine phosphokinase (APK) and creatine phosphokinase (CPK) activity of fractions isolated by ammonium sulphate fractionation of extracts (100 ml.) of the jaw apparatus of C. rodgersii and H. erythrogramma*

A unit of enzyme activity is defined as the amount of enzyme which releases 1 μ mole of creatine or arginine in 1 min. at pH 7.2 and 5°. Specific activity (S.A.) is defined as the number of units of enzyme activity/mg. of protein. Fraction 1 was obtained by the addition of 21 g. of $(\text{NH}_4)_2\text{SO}_4$ to the extract. Fractions 2–5 inclusive were obtained by the stepwise addition of 7 g. of $(\text{NH}_4)_2\text{SO}_4$ to the supernatant from the preceding fraction. Fraction 6 was obtained by the addition of 14 g. of $(\text{NH}_4)_2\text{SO}_4$ to fraction 5 (or fraction 4 in *C. rodgersii*).

		<i>H. erythrogramma</i>			
Fraction	<i>C. rodgersii</i> APK 10 ² S.A.	APK		CPK	
		10 ² S.A.	Total units	10 ² S.A.	Total units
Original	230	12	56.3	8.3	39.0
1	68	1.7	3.2	3.1	5.6
2	49	1.4	0.97	11.4	7.9
3	26	1.2	0.68	41	23.0
4	244	10.4	6.45	5	3.1
5	—	74	11.8	0.6	0.1
6	825	81	10.5	0	0

similar to APK isolated from crayfish muscle and from *C. rodgersii*. The CPK, on the other hand, was activated by all three cations and is thus similar to CPK isolated from rabbit muscle (Rosenberg & Ennor, 1955; Kuby, Noda & Lardy, 1954). A comparison of the activation of the two enzymes by Mg^{2+} , Mn^{2+} and Ca^{2+} ions is shown in Fig. 1.

DISCUSSION

The present work has clearly established that the echinoid *Centrostephanus rodgersii* contains only one phosphagen (PA) in the jaw muscles and thus provides the second example of a mono-phosphagen type in this group. The first example was described by Baldwin & Yudkin (1950), who found only PA in *Arbacia punctulata*. The general assumption (cf. Baldwin & Needham, 1937; Wald, 1952; Florkin, 1949) that both PA and PC are found in echinoids is thus disproved and the results emphasize the necessity for examining a number of species within a class before concluding that a particular phosphagen(s) is characteristic of that class.

The occurrence of both PA and PC in certain echinoids and only PA in others suggests that some members of this group may contain only PC, but no such echinoid has been described. The investigations up to the present have covered an insufficient number of echinoids to demonstrate with certainty any pattern so far as the occurrence of phosphagens is concerned. Nevertheless, it is of interest to note that those echinoids whose jaw muscles have been

shown to contain both PA and PC [*Paracentrotus lividus*, *Sphaerechinus granularis* (Baldwin & Needham, 1937); *Strongylocentrotus lividus*, *Echinus esculentus* (Baldwin & Yudkin, 1950; *Helicodidaris erythrogramma* (present work)] all belong to the order Camarodonta (Hyman, 1955a). Conversely, members of other orders, e.g. Aulodonta (*Centrostephanus rodgersii*) and Stirodonta (*Arbacia punctulata*), contain only PA. No members of the other orders have been investigated.

The only free guanidines which were detected in the present work were arginine and creatine, although it is possible that others were present at concentrations less than 1% of the total guanidine. Thus it might have been expected that *H. erythrogramma*, which contains PC, would also contain trace amounts of glycoeyamine in view of the results of Roche, Thoai, Robin, Garcia & Hatt (1952) and Thoai & Robin (1951), which indicated the presence of glycoeyamine and the enzyme methylating glycoeyamine in *Paracentrotus lividus* and *Sphaerechinus granularis*.

Although Baldwin & Needham (1937) showed that extracts of the muscles of *P. lividus* and *S. granularis* were able to phosphorylate both creatine and arginine, their results did not indicate whether this phosphorylation was carried out by one or two enzymes. The present work clearly establishes the presence of two enzymes of approximately equal activities in *H. erythrogramma*. Moreover, the general properties of the APK and CPK present are similar to those of APK isolated from crayfish muscle (Morrison *et al.* 1957) and CPK isolated from rabbit muscle (Rosenberg & Ennor, 1955) and from *Pyura stolonifera* (Griffiths, Morrison & Ennor, unpublished observations). The echinoids possess seven different groups of muscle in their jaw apparatus (Hyman, 1955b), and it has been shown by Needham *et al.* (1932) that both the protractor and retractor muscles of *Strongylocentrotus lividus* contain PA and PC. It would seem therefore that in this species and in *H. erythrogramma* both phosphagens are present in the same muscles, and it follows that the free guanidines and the transphosphorylation enzymes are also present.

There would seem little point, in view of the present state of knowledge, in speculating on the possible evolutionary reasons for the appearance of the two phosphagens in the jaw muscles of *H. erythrogramma*. It is difficult to picture the functional interrelationship which might exist between the two phosphagens, for although the role of PA in invertebrate muscle appears to be similar to that of PC in vertebrate muscle it is hard to conceive of the two phosphagens both serving as 'reservoirs' for the adenylic acid system in the same muscle. However, in the absence of evidence to the contrary, it must be assumed that such is the role of

Fig. 1. Activation by bivalent metals of arginine phosphokinase (APK) and creatine phosphokinase (CPK) fractions from the jaw muscles of *H. erythrogramma*. Fractions were isolated and activity determinations carried out in the forward reaction as described in the text. □, CPK fraction; ▨, APK fraction.

the two phosphagens, although the possibility (Ennor & Rosenberg, 1952) that one or other of these phosphagens 'may contribute to endergonic reactions directly and without the mediation of the latter (adenylic acid) system' must be borne in mind. It is not clear how the function of each phosphagen may be determined. Ennor & Rosenberg (1954*b*) have shown that the incorporation of ^{32}P into PC in rabbit muscle is extremely rapid, and it is likely that this would also occur with PA and PC in echinoid muscle; it would thus be difficult to detect any differential incorporation of ^{32}P into the two phosphagens, so that the application of ^{32}P to the problem would seem of little value.

SUMMARY

1. Two echinoids, *Centrostephanus rodgersii* and *Heliocidaris erythrogramma* Valenciennes, have been examined for the presence of free guanidine, their phosphorylated derivatives and their respective *N*-amidino phosphokinases.

2. *C. rodgersii* has been shown to contain arginine, phosphoarginine and arginine phosphokinase. *H. erythrogramma*, on the other hand, contained both creatine and arginine together with their phosphorylated derivatives and *N*-amidino phosphokinases.

3. The creatine and arginine phosphokinases in *H. erythrogramma* are readily separable and are similar in properties to those enzymes isolated from vertebrate and invertebrate sources.

4. Methods for the chromatographic and enzymic characterization of creatine and arginine are described.

We are indebted to Dr J. W. Evans, Director of the Australian Museum, Sydney, for the use of Museum facilities, and to Mrs J. W. Evans, Miss Elizabeth Pope and Miss P. McDonald of the Australian Museum for assistance in locating and collecting specimens. Our thanks are also due to Miss Pope for the identification of the specimens and to Mr D. Maguire and Miss B. Giddey for technical assistance.

REFERENCES

- Acher, R. & Crocker, C. (1952). *Biochim. biophys. Acta*, **9**, 704.
- Arnold, A. & Luck, J. M. (1933). *J. biol. Chem.* **99**, 677.
- Baldwin, E. & Needham, D. M. (1937). *Proc. Roy. Soc. B*, **122**, 197.
- Baldwin, E. & Yudkin, W. H. (1950). *Proc. Roy. Soc. B*, **136**, 614.
- Bateson, W. (1886). *Quart. J. micr. Sci.* **26**, 511, 535.
- Block, R. J. & Zweig, G. (1955). *Paper Chromatography and Paper Electrophoresis*, p. 93. New York: Academic Press Inc.
- Brand, E. & Brand, F. C. (1942). *Org. Synth.* **22**, 59.
- Crawford, T. B. B. (1951). *Biochem. J.* **48**, 203.
- Ennor, A. H., Morrison, J. F. & Rosenberg, H. (1956). *Biochem. J.* **62**, 358.
- Ennor, A. H. & Rosenberg, H. (1952). *Biochem. J.* **51**, 606.
- Ennor, A. H. & Rosenberg, H. (1954*a*). *Biochem. J.* **57**, 203.
- Ennor, A. H. & Rosenberg, H. (1954*b*). *Biochem. J.* **56**, 308.
- Ennor, A. H. & Stocken, L. A. (1948). *Biochem. J.* **43**, 190.
- Florkin, M. (1949). *Biochemical Evolution*, p. 20. New York: Academic Press Inc.
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
- Greenberg, D. M. (1951). *The Enzymes*, vol. 1, p. 893. New York: Academic Press Inc.
- Greenwald, I. (1946). *J. biol. Chem.* **162**, 239.
- Hanes, C. S. & Isherwood, F. A. (1949). *Nature, Lond.*, **164**, 1107.
- Hobson, G. E. & Rees, K. R. (1955). *Biochem. J.* **61**, 549.
- Holtz, F. & Thielmann, F. (1924). *Z. Biol.* **81**, 296.
- Hyman, L. H. (1955*a*). *The Invertebrates*, vol. 4, p. 461. New York: McGraw-Hill Inc.
- Hyman, L. H. (1955*b*). *The Invertebrates*, vol. 4, p. 509. New York: McGraw-Hill Inc.
- Kuby, S. A., Noda, L. & Lardy, H. A. (1954). *J. biol. Chem.* **210**, 65.
- LePage, G. A. (1951). *Manometric Techniques*, p. 186. Minneapolis: Burgess Publ. Co.
- Makisumi, S. (1952). *J. chem. Soc. Japan (Pure Chem. Sect.)*, **73**, 737. [Quoted in *Chem. Abstr.* (1953), **47**, 5848.]
- Meyerhof, O. (1930). *Die Chemische Vorgänge in Muskel*. Berlin: Springer.
- Morrison, J. F., Griffiths, D. E. & Ennor, A. H. (1957). *Biochem. J.* **65**, 143.
- Myers, R. G. (1920). *J. biol. Chem.* **41**, 119.
- Needham, D. M., Needham, J., Baldwin, E. & Yudkin, J. (1932). *Proc. Roy. Soc. B*, **110**, 260.
- Roche, J. & Robin, Y. (1954). *C.R. Soc. Biol., Paris*, **148**, 1541.
- Roche, J., Thoai, N. v. & Hatt, J. L. (1954). *Biochim. biophys. Acta*, **14**, 71.
- Roche, J., Thoai, N. v., Robin, Y., Garcia, I. & Hatt, J. L. (1952). *C.R. Soc. Biol., Paris*, **146**, 1899.
- Rosenberg, H. (1955). *The Synthesis and Breakdown of Phosphocreatine in Animal Tissue*. Ph.D. Thesis: Australian National University.
- Rosenberg, H. & Ennor, A. H. (1955). *Biochim. biophys. Acta*, **17**, 261.
- Rosenberg, H., Ennor, A. H. & Morrison, J. F. (1956). *Biochem. J.* **63**, 153.
- Thoai, N. v. & Robin, Y. (1951). *C.R. Soc. Biol., Paris*, **145**, 1674.
- Thoai, N. v. & Robin, Y. (1954). *Biochim. biophys. Acta*, **14**, 76.
- Thoai, N. v., Roche, J., Robin, Y. & Thiem, N. v. (1953*a*). *C.R. Soc. Biol., Paris*, **147**, 1241.
- Thoai, N. v., Roche, J., Robin, Y. & Thiem, N. v. (1953*b*). *Biochim. biophys. Acta*, **11**, 593.
- Wald, G. (1952). *Trends in Physiology and Biochemistry*, p. 337. New York: Academic Press Inc.