TYROSINASE ACTIVITY IN RELATION TO PHENOLIC TANNING OF THE CUTICLE IN *CARCINUS MAENAS*

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CONTENTS

1. INTRODUCTION

It has been suggested that the mechanism of tanning of the cuticle in Crustacea may be similar to that in insects (Dennell, 1947*b*) but very little is known of the processes resulting in the formation of polyphenols involved in tanning. However, evidence of the existence of the necessary polyphenol-producing mechanism is given by the occurrence of a tyrosine-tyrosinase system in crustacean blood (Pinhey, 1930). A fluctuation in tyrosinase activity noted by Pinhey in *Maia squinado* and *Cancer pagurus* is probably significant in relation to tanning of the cuticle (Dennell, 1947*b*). But the factors responsible for this variability and the nature of the regulation of the formation of polyphenols are unknown. A suggestive line of approach to this problem may be found in the observation of Dennell (1949) that in *Calliphora erythrocephala* a factor holding in check the oxidative activity of tyrosinase may result from the presence of a dehydrogenase system showing a fluctuation correlated with tyrosine oxidation. A balance between tyrosinase activity and a dehydrogenase system both controlled by hormones appears to exist in blowfly larva. The hormonal control of pupation in the blowfly larva first suggested by Fraenkel (1935) and later shown to occur in other Diptera is exercised by Weismann's ring (Hadorn, 1937; Burtt, 1938) which is homologous with the corpora allata and corpora cardiaca of other insects (Burtt, 1937; Day, 1943). It has been pointed out that the hormones secreted by the corpus cardiacum-allatum system show physiological effects similar to those of the sinus gland located in the eyestalks of decapod Crustacea (see Hanstrom, 1949). It is therefore of interest to examine what part, if any, the sinus gland hormone plays in relation to tanning of the cuticle and how far the mechanism of control of tanning in Crustacea corresponds to that in insects. A preliminary account of the findings has been given elsewhere (Krishnan, 1950).

2. MATERIAL AND METHODS

This work has been carried out in *Carcinus maenas* Pennant obtained living from Plymouth and Millport laboratories and on material collected while working

at the Marine Biological Laboratory, Plymouth. For the estimation of tyrosine in the blood, the colorimetric method of Folin and Denis (1912) was used. The fructose determinations were made by the method elaborated by Roe (1934) based on the Selivanoff reaction. An evaluation of the reducing power of blood in different stages of the moult cycle was made by measurement of electrode potentials using a Cambridge *p*H meter as a voltmeter. The cyanide-insensitive respiration of blood and soft parts which showed significant variations in the course of the moult cycle was estimated under various experimental conditions using a Barcroft differential respirometer and the values obtained were taken to indicate the activity of an enzyme system. The rôle of the eyestalk hormone in relation to phenolic tanning was studied by noting the effects of eyestalk extirpation on the enzyme system involved as indicated by the cyanide-insensitive oxygen uptake.

3. Tyrosine and Tyrosinase in the Blood

Tyrosine which appears to be the precursor of polyphenols in insects, is known to occur also in crustacean blood which blackens on exposure, a feature recognised as indicating the oxidation of tyrosine to form melanic pigments (Heim, 1892; Hemmingsen, 1924; Pinhey, 1930). A further similarity to the condition observed in insects is suggested by a fluctuation in the discoloration of the blood in different individuals and in the same individual at different periods (Pinhey, 1930). Such variations in insects have been shown to be significant as indicating a regulation of tyrosinase activity in relation to tanning of the cuticle (Dennell, 1947*a*). The occurrence of similar variations in crustaceans such as *Maia* and *Cancer* might suggest, as has been pointed out by Dennell (1947*b*) a relationship to the elaboration of polyphenols. But it is not known whether there occurs as in insects a rhythm in tyrosinase activity, whether the variations if present are due to quantitative changes in the enzyme or the substrate or both and if when both tyrosine and tyrosinase are present the internal environment has a determining influence on tyrosinase activity.

With this object in view the blood of *Carcinus* was examined in different stages of the moult cycle. As the crabs kept in the laboratory tanks moulted only infrequently, the various stages in the moult cycle were collected fresh from their natural environment. To minimise discrepancies arising from the difficulty of fixing the time interval since moulting the averages of two or more evaluations made in similar stages have been taken for purposes of comparison. The phases in the moult cycle recognised in the following study are those described by Baumberger and Olmsted (1928) which are (*a*) the hard crab stage (*b*) the 'pillans' stage representing the condition a few days before ecdysis, (*c*) the moulting stage in which the crab is in the process of emerging from the old shell, (*d*) the soft crab characterised by a soft cuticle, and (*e*) the paper shell stage in which the cuticle is only very slightly hardened. Blood from all stages exposed on filter paper turned black, but marked gradations were shown in the intensity of blackness as may be seen from Table I.

Table I

	Moulting stage.	Soft crab.	Paper shell crab.	Hard crab.	Pillans.
Blood (untreated) ..	+	+	++	++	++++
Blood + KCN ..	—	—	—	—	—
Blood + methyl alcohol	+	+	+++	++++	+++++

If the degree of intensity of blackness is an indication of the extent of enzyme activity as may be inferred from the lack of darkening in the presence of cyanide it may appear that tyrosinase shows fluctuations in activity in different periods of the moult cycle. An additional indication that an enzyme present in the blood is responsible for the blackening of shed blood is provided by the positive reaction with the 'NADI' reagent which has been extensively used to detect tyrosinase and polyphenol oxidases on account of the oxidation it undergoes in their presence.

The feeble darkening of exposed blood of crabs soon after moulting is probably due to a reduction of tyrosinase in the blood. This may be inferred from the suggestion of Pinhey (1930) that tyrosinase is contained in the haematocytes which as observed by Baumberger and Olmsted (1928) are fewer in number at the time of moulting when the blood is much diluted due to a marked absorption of water. If the tyrosinase of the blood is involved in tanning of the cuticle, only very little of it may be expected to be found in the blood at this stage since phenolic hardening is complete immediately after moulting. In the stages following there is a noticeable upward trend in the blackening effect which is most marked in the pillans stage when the new cuticle is being laid down and tanned before its exposure at the time of moulting. Such variations may be attributable to quantitative changes in tyrosinase or to changes in the tyrosine content of the blood. It also appears likely that variations in the inhibition of tyrosinase activity may account for the above observations. Qualitative tests for tyrosine in the blood of *Carcinus maenas* using Morner's reagent and α-nitroso-β-naphthol gave positive reactions indicating the presence of tyrosine (Feigl, 1947) at all stages of the moult cycle. With a view to determine whether the amount of tyrosine varies in different stages, a colorimetric estimation of tyrosine was made using the 'phenol reagent' (Folin and Denis, 1912). It was found that the amount of tyrosine present in the blood was approximately constant, the average value being 0·003%. Pinhey (1930) obtained for *Cancer pagurus* an average value of 0·004%. She found that tyrosine is always present in the blood and is in excess of requirements for oxidase activity, as seen by the fact that when to the blood which has ceased to blacken a small amount of blood which does show blackening is added, discoloration at once appears showing that the blood must have contained the substrate as the 'blackening is very much more intense than that which could be produced from the action of the added enzyme on the small amount of substrate necessarily added with it'. From the observations made on *Carcinus* it may be inferred that tyrosine and tyrosinase are present at all stages of the moult cycle but it is the enzyme which shows a variation in activity.

4. The Redox Potential of the Blood and Tyrosinase Activity

In spite of the presence of both tyrosine and tyrosinase there is no evidence of tyrosinase activity in the blood of intact animals as may be inferred from the absence of a positive ferric chloride test in the blood drawn from the animal in the different stages of the moult cycle. Possibly the elaboration of dihydroxyphenols is confined to a very brief period when they may occur in a concentration too low to be revealed by colour tests. Such a condition is explicable in view of the short duration of the tanning process in this animal. If the above assumption is valid it would appear that the oxidation of tyrosine is held in check by some inhibiting factor probably present in the blood.

Previous authors have emphasized the importance of the oxidation-reduction potentials of the medium in determining biological oxidations. Graubard (1933) observed that tyrosinase activity in *Drosophila melanogaster* is dependent on the state of the environment which enables the enzyme present to be involved in the reaction. Similarly Figge (1940) showed that tyrosinase is active only within a certain range of redox potentials and that any shift of potential from the optimum

either to the negative or positive side would inactivate the enzyme. That this holds good not only in vitro but also in vivo is shown by the observations of this author that phenol-indophenol with an E'o value of +0·227 at *p*H 7 produces palor in amphibian larvae by inhibiting tyrosinase in melanophores although it stimulates melanogenesis in the connective tissue cells. The differential reaction of cells to the same substance is explained in the light of the view mentioned above that tyrosinase is active at an optimum potential. It has been pointed out that the melanophores maintain a more positive and connective tissue cells a more negative potential so that with the addition of phenol-indophenol there is a shift of potential away from the optimum in the melanophores and towards the optimum in the connective tissue cells thus accounting for the opposite responses observed. Further Dennell (1947*a*) suggested that the oxidation of tyrosine in the third larval instar of *Sarcophaga* before puparium formation is prevented by the low redox potential of the blood.

In the light of the above observations the redox potential of the blood of *Carcinus* was studied. It is realized that blood may contain a complex mixture of reducing substances whose proportion may affect the redox potentials of the blood. But it is known from the work of Kuwana (1937) that the reducing power of the blood of silkworm shows a stable and unstable part the latter disappearing on exposure concurrently with the darkening of the blood. That this unstable reducing power may be due to a dehydrogenase which is diminished on exposure, has been suggested by Dennell (1949) as a result of observations in the larva of *Calliphora erythrocephala*. In the following study an attempt has been made to estimate the unstable reducing power of the blood (see Dennell, 1947*b*) whose disappearance or diminution on exposure is accompanied by melanosis. For this purpose the electrode potentials of the blood as soon as drawn, in the different stages of the moult cycle was determined using the Cambridge *p*H meter, with a saturated KCl-calomel electrode of potential of +0·250 V at 20°C. (+C 0·76 MV for 1°C.) as a reference electrode.

TABLE II

No.	Moulting stage.	Soft crab.	Paper shell crab.	Hard crab.	Pillans.
1	+0·283 V	+0·320 V	+0·272 V	+0·260 V	+0·355 V
2	+0·295 V	+0·308 V	+0·270 V	+0·250 V	+0·318 V
3	+0·286 V	+0·304 V	+0·280 V	+0·218 V	+0·333 V
4	+0·292 V	+0·290 V	+0·286 V	+0·242 V	+0·320 V
5	..	+0·296 V	+0·288 V	+0·216 V	+0·332 V
6	..	+0·302 V	+0·276 V	+0·242 V	+0·322 V
Average ..	+0·289 V ±0·006	+0·303 V ±0·017	+0·279 V ±0·009	+0·238 V ±0·022	+0·330 V ±0·025

Table II and fig. 1 show the average values of redox potentials of the blood of *Carcinus* in different stages of the moult cycle. It is seen that the values show a rise sometime before moulting and at the time of shedding the exuviam the potential falls below the level before and after the process. The oxidation-reduction characteristics of the blood indicate a correlation with the active and inactive periods of tyrosinase activity of the blood in the course of the moult cycle, the potentials being high at the pillans stage when tyrosinase activity as seen by the blackening of blood is most marked.

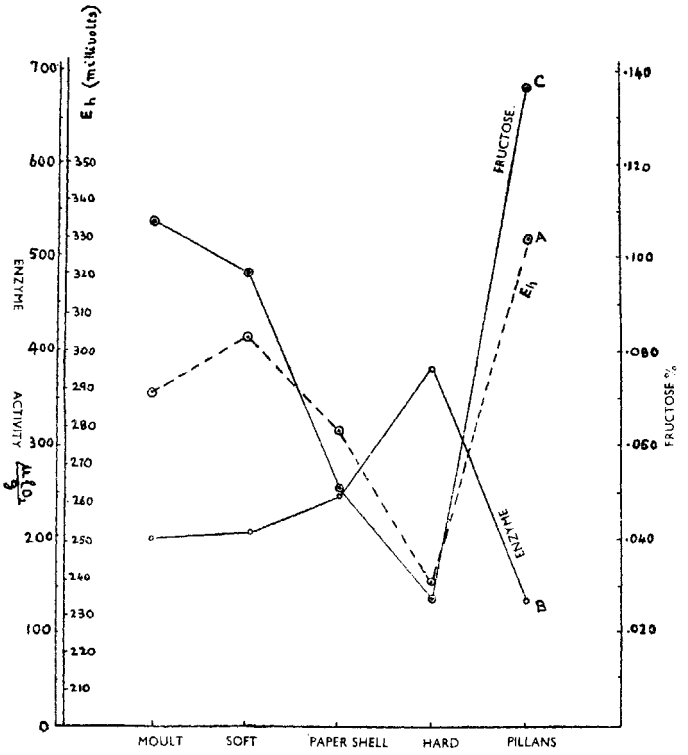

FIG. 1.—Variations in redox potentials of the blood, enzyme activity and fructose values in the course of the moult cycle of *Carcinus maenas*.

Abscissae, Stages in moult cycle; Ordinates: (i) Redox potentials of the blood in millivolts; (ii) Enzyme activity expressed as O_2 uptake $\left\{\frac{\mu_1 \cdot O_2}{g.\ \text{tissue}}\right\}$; (iii) Fructose as gm. % of tissue. Curve A—Redox potentials. Curve B—Enzyme. Curve C—Fructose content.

5. CYANIDE-INSENSITIVE RESPIRATION AND REDOX POTENTIALS

Discussing the causes of the reducing power of insect blood Dennell (1947*a*) observed that the mere presence of reducing substances such as glucose, uric acid or ascorbic acid cannot by themselves account for the reducing power, which as noted by Kuwana (1937) shows a stable and unstable fraction, the latter disappearing on exposure. From the observation that this unstable reducing power may be removed by treatment with narcotics such as urethane, chloroform (Graubard, 1933; Dennell, 1947*a*) it is inferred that the agency responsible may be of the nature of dehydrogenases which are known to be susceptible to the action of narcotics. Further in *Calliphora erythrocephala* a study of the cyanide-insensitive respiration revealed variations apparently correlated with tyrosinase activity and it has been suggested that the cyanide-insensitive respiration may reflect the activity of a dehydrogenase system (Dennell, 1949). With this possibility in view the blood of *Carcinus* was treated with thymol, urethane and other narcotics but these substances did not produce any appreciable effect on tyrosinase activity. However, it has been observed that the blood of hard crabs darkens more intensely in the presence of methyl alcohol indicating an increase in tyrosinase activity. It has been suggested that the above reaction may be due to the removal of an inhibiting enzyme probably

susceptible to methyl alcohol (see Dennell, 1947*a*; Fraenkel and Rudall, 1947). The action of methyl alcohol was found to have varying effects in the different stages of the moult cycle. Blood from soft crabs did not blacken as markedly in the presence of methyl alcohol as that of crabs in the middle stages of the intermoult period, suggesting a fluctuation in the activity of the enzyme concerned. A correlation between such a fluctuation of enzyme activity and the oxygen uptake of blood and soft parts of the crab in the presence of cyanide suggested that the enzyme-system indicated thereby may be involved in tyrosine oxidation. To determine whether this is indeed correlated with tyrosine oxidation a detailed study of the cyanide-insensitive respiration in different periods of the moult cycle was made.

Enzyme activity though indicated when blood only was used, the values obtained were low compared to those recorded with extracts of muscles. In the following experiments muscle extracts were used. The tissues were ground well with sand in distilled water and the ground tissue after several washings was suspended in 5 ml. of *M*/15 K_2HPO_4 and shaken for about forty minutes. After centrifugation the supernatant fluid was used. 2·5 ml. of the extract and 2·5 ml. of ·1% KCN were taken together in the right hand flask of the respirometer and an equal quantity of phosphate buffer and KCN added to the left hand flask. Under these conditions the extract did not show any oxygen uptake. However, the addition of either glucose or fructose to the extract caused an immediate oxygen uptake indicating the presence of an enzyme system capable of catalysing the oxidation of these substrates. It is to be noted that the addition of fructose caused a greater uptake than the addition of glucose.. The enzymic nature of the reaction was indicated by the absence of an oxygen uptake when the extract was previously boiled. With fructose as the added substrate marked fluctuations in the oxygen uptake were noted in the different stages of the moult cycle. Therefore a detailed study of the cyanide-insensitive oxygen uptake in the course of the moult cycle was made. The oxygen uptakes were referred to the dry weight of the tissues after extraction and the values expressed as $\frac{\mu_1 . O_2}{g.\ \text{tissue}}$ during thirty minutes. The experimental temperature was maintained at 20°C. In referring the oxygen uptakes to the dry weight of tissues, it is realized that marked changes in the composition of tissues in the course of the moult cycle (Hoet and Kerridge, 1926) render the values as obtained above not strictly comparable. But such an anamoly could not be overcome by using wet weights as the water content of the crabs is subject to very wide fluctuations (Maluf, 1939). However, since the object of these estimations is to note the fluctuations in enzyme activity in relation to moulting rather than the determination of absolute values, a certain exaggeration of the values in the early stages after moulting may not vitiate the overall trend of events. The values obtained in the course of the moult cycle are given in Table III.

It should be pointed out that in the above experiments when tissue extracts were used without addition of fructose there was no oxygen uptake. Possibly the naturally occurring substrate of the enzyme was lost in the process of extraction described above or more likely was soon metabolized in this preparation and the added fructose served as a substitute. It was therefore of interest to find whether fructose normally occurs in the tissues and the body fluid and if so whether it shows quantitative changes which may be related to enzyme activity noted above. A quantitative estimation of fructose in the tissues and blood was made by the method of Roe (1934). While the fructose content of the blood was low, with extracts of muscles quite appreciable quantities were indicated. For estimating fructose in the muscles the aqueous extracts of tissues were treated in the same way as for blood and the values obtained were referred to the dry weight of the tissue used for extraction and expressed as gm. % of tissue. Care was taken to see that the stages in which the fructose estimations were made were identical with those used

TABLE III

Enzyme Activity expressed as $\frac{\mu_1 \cdot O_2}{g. \text{ tissue}}$ *(with added fructose).*

No.	Moulting stage.	Soft crab.	Paper shell.	Hard crab.	Pillans.
1	159	171	200	413	111
2	216	181	236	318	120
3	196	188	187	427	148
4	214	193	289	385	113
5	..	188	222	315	127
6	..	278	282	450	204
Average ..	196	200	236	385	137

for enzyme estimation. The variations in the fructose values in the course of the moult cycle are shown in Table IV and fig. 1.

TABLE IV

Fructose in gm. %.

No.	Moulting stage.	Soft crab.	Paper shell crab.	Hard crab.	Pillans.
1	0·117	0·088	0·051	0·026	0·167
2	0·094	0·089	0·053	0·036	0·113
3	0·083	0·109	0·053	0·029	0·170
4	0·132	0·112	0·057	0·024	0·173
5	..	0·085	0·055	0·019	0·095
6	..	0·091	0·043	0·021	0·094
Average ..	0·107	0·096	0·052	0·026	0·135

A correspondence is apparent between the values for fructose and those for enzyme activity, the latter varying inversely with the former (fig. 1). It is noteworthy that the lowest values for enzyme activity as determined by the above experiments and the highest electrode potentials occur at the pillans stage when the crab is preparing for moult. This is also the stage at which tyrosinase activity as judged by the darkening of the exposed blood is most evident. It may be suggested that in *Carcinus*, during the intermoult period chiefly represented by the hard crab stage, tyrosinase activity is held in check by the low redox potential which does not permit of the oxidation of tyrosine. Just before moulting at the pillans stage, the potential rises and the consequent oxidation of tyrosine due to tyrosinase activity leads to the production of phenols responsible for the tanning of the new cuticle which will be exposed at the time of moulting.

6. HORMONAL CONTROL OF TYROSINASE ACTIVITY

Although moulting in decapod Crustacea is a comparatively brief process, preparatory changes antecedent to this, extend over several days and involve physiological changes affecting many aspects of the metabolism of the animal.

For several days previous to moulting calcium is withdrawn from the exoskeleton and stored in the hepatopancreas in crabs (Paul and Sharpe, 1916; Robertson, 1937) or in gastroliths in the crayfish (Kyer, 1942; Scudamore, 1947). Changes in the glycogen content of the hepatopancreas have been noted in relation to moulting in *Callinectes sapidus* (Baumberger and Dill, 1928) and in *Carcinus maenas* (Schonborn, 1910). Similarly the blood glucose in *Maia squinado* shows a rise preceding moulting and a fall succeeding it (Drillhon, 1933). Changes in water content and oxidative metabolism have been shown to commence several days before the onset of moulting (Scudamore, 1947). It is now well established that moulting in decapod Crustacea is hormonally regulated by the endocrine activity of the neurosecretory organs located in the eyestalks (Brown, 1944; Scudamore, 1947). Further considerable evidence exists to show that many features associated with moulting such as changes in water content, oxygen consumption, resorption of materials from the cuticle, are also under hormonal control (Kyer, 1942; Scudamore, 1947). In the light of the above observations it is of interest to discover whether the tyrosinase of the blood, the activity of which seems related to ecdysis and hardening of the new cuticle, may also be regulated by the hormones of the eyestalk.

To elucidate the problem whether or not the hormones of the eyestalk control the process of tanning, normal animals have been compared with those in which the eyestalks have been removed, with reference to the cyanide-insensitive respiration, fructose content and tyrosinase activity. For these experiments hard crabs of the same carapace breadth and of similar stage in relation to the moult cycle were chosen to ensure similarity of members of a batch used for experiments. Animals in very early stages of the hard crab phase as indicated by the nature and appearance of the shell were selected. The suitability of such a stage for purposes of these experiments lies in the fact that the hard crab stage represents the longest phase in the moult cycle when it may be presumed that some uniformity of general metabolic features may be established so enabling comparative experimental observations over several days to be made.

For each set of experiments twenty-four male crabs were divided into two groups of twelve each. In one group both eyestalks were extirpated. Cautery was not applied as haemorrhage was little and the wound was soon closed by a blood clot. The operated crabs were segregated in individual jars. The remaining twelve animals were used as controls and similarly segregated in individual jars. Each set of experiments was continued for nearly three weeks. During this period a test animal was killed every third day and enzyme activity as indicated by the cyanide-insensitive oxygen uptake with added fructose was estimated. The method followed was the same as in previous experiments. At the same time the fructose values of the muscles were also determined. Tyrosinase activity was estimated by

TABLE V

Days after eyestalk extirpation.	Normal animals.		Operated animals.	
	$\frac{\mu_1 . O_2}{g.\ \text{tissue.}}$	Fructose gm. %	$\frac{\mu_1 . O_2}{g.\ \text{tissue.}}$	Frustose gm. %.
3 days	318	0·045	118	0·136
6 days	242	0·055	120	0·087
9 days	113	0·173	155	0·102
12 days	127	0·095	180	0·115
15 days	192	0·117	171	0·114
18 days	188	0·160	219	0·107

the observation of the extent of darkening of exposed blood. The values obtained were compared with those of the control animals in the corresponding periods of the experiment. Table V gives the average values of enzyme activity and fructose in control and operated animals.

It was seen that three days after the operation, the eyestalkless animals showed a marked reduction of enzyme activity compared with the control animals (fig. 2). In the later stages of the experiment an increase in enzyme values of the operated animals was noted. From the absence of a uniform decrease of enzyme activity following the eyestalk removal, it would appear that the relation between them may not be a simple and direct one. Experimental animals killed about fifteen days after the eyestalk extirpation showed evidence of an incipient moult but died before completing it. At the corresponding period none of the control animals showed signs of an impending moult. It seemed clear that the eyestalk extirpation in the test animals had hastened the onset of moulting and the reduction of enzyme activity noted above is consistent with this feature. In view of the trends in enzyme

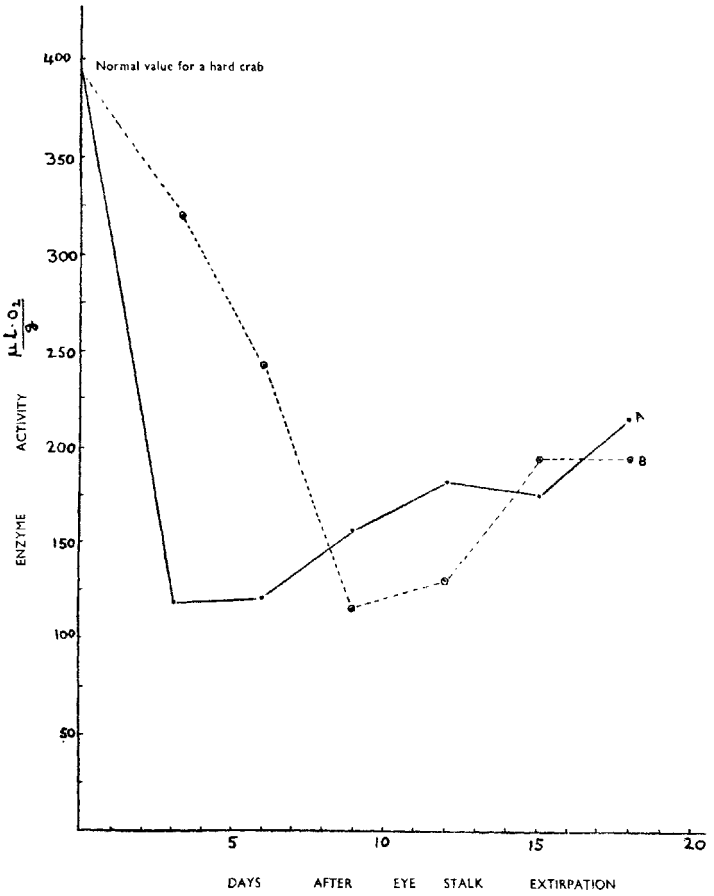

FIG 2.—Changes in enzyme activity as a result of eyestalk removal. Abscissae, days after eyestalk extirpation; ordinates, enzyme activity expressed as O_2 uptake $\left\{\frac{\mu_1 \cdot O_2}{g.\ \text{tissue}}\right\}$; Curve A changes in experimental animals; Curve B controls in corresponding periods as in the experimental animals.

activity in relation to moult cycle already noted and the effect produced on it by the eyestalk extirpation it may be reasonable to infer that the enzyme activity noted above constitutes only a part of a system controlled by the hormone of the eyestalk. This hormone in normal animals has been shown to have an inhibiting effect on moulting and its elimination seems not only to hasten moulting but also to set in train all the events associated with moulting in advance of their normal occurrence (Scudamore, 1947). It may be inferred from fig. 2 on which a parallel may be seen between Curve B, showing the variations in enzyme activity in the control animals and the Curve A, representing the values in experimental animals, that in the latter the fall and subsequent rise of enzyme activity is advanced in point of time.

In another set of similar experiments paper shell crabs were used instead of hard crabs and over a period of fifteen days the cyanide-insensitive respiration and fructose values were determined. Differing from the effect of eyestalk extirpation in hard crabs there was an irregular rise of oxygen uptake in the experimental animals. The effect of the removal of eyestalks on the enzyme activity at this stage was different from that at a later stage.

Additional experiments to throw light on the relation between eyestalk extirpation and enzyme activity in question have been performed in which the operated animals without eyestalks were at different periods injected with saline extracts of eyestalks of animals in stages corresponding to those of the experimental animals. The amount of extract injected was equivalent to one eyestalk per animal (*see* Brown and Scudamore, 1940). The enzyme activity as indicated by the cyanide-insensitive oxygen uptake was estimated one hour after injection. But in the eyestalkless crabs subsequently injected, the enzyme activity gave anomalous values. In the above experiments it has been noted that a rise in enzyme activity in the test animals appeared to affect tyrosine oxidation, the blood on exposure blackening less intensely than before eyestalk injection.

7. Discussion

With the recording of tyrosinase activity in the blood of *Maia squinado* and *Cancer pagurus* (Pinhey, 1930) and the suggestion that it may be related to the tanning of the cuticle in decapod Crustacea (Dennell, 1947*b*) it may be reasonable to expect a mechanism of tanning similar to that in insects. The observations recorded in the foregoing study of *Carcinus maenas* support such a view. Not only the oxidation products of tyrosine appear to be utilized for tanning of the cuticle as may be inferred from the occurrence of maximum tyrosinase activity at the pillans stage when the new cuticle is laid and tanned, but also the mechanism by which tyrosine oxidation is held in check till the appropriate period, appears to show a close parallel to that in insects. Dennell (1947*a*, 1949) from a study of cyclorraphous Diptera suggested that the activity of an enzyme system having an influence on the redox potentials of the blood so controls the internal environment affecting tyrosine oxidation, that the polyphenols are formed only at the time of tanning of the cuticle. In *Carcinus maenas* a striking correlation seen between the fluctuations in the cyanide-insensitive oxygen uptake in different stages of the moult cycle and tyrosinase activity may suggest as in insects, a causal relation between the two processes. However, the precise nature of the enzyme or enzymes involved and the mechanism of enzyme action as well as the rôle of fructose in the intermediary metabolism are not known and constitute a problem in enzyme chemistry.

From the observations made on the effect of eyestalk extirpation in relation to the cyanide-insensitive oxygen uptake, it may appear that the hormone of the eyestalk has a controlling influence on the enzymes regulating tyrosinase activity in that a removal of the source of this hormone in the hard crab stage brings about a

reduction in enzyme activity which seems to characterise animals preparing for moult. This is explicable in view of the fact that the eyestalk hormone is known to have an inhibiting effect on moulting and its removal accelerates the onset of moulting. The relationship between the enzyme activity noted above and the hormone of the eyestalk suggests a striking parallel to that between certain metabolic processes accompanying moulting and the sinus gland hormone noted by Scudamore (1947). It has been pointed out that, in the crayfish, the formation of gastroliths, increase in oxygen consumption and in water content, commence some days before the moult and that these changes could be induced to take place much earlier than in normal animals by bilateral eyestalk extirpation or sinus gland removal. The suggestion made by the above author that these metabolic changes may not be separately controlled by hormones but are associated to form a single functional system controlled by the eyestalk hormone, supports the inference that could be made from the results obtained for the regulation of tyrosinase activity in *Carcinus maenas*. It appears reasonable to suggest that tyrosine oxidation as a precursor to the tanning of the newly formed cuticle at the time of moulting may be only a part of a series of metabolic processes associated with moulting and that any change in the timing of the moulting process may bring about corresponding changes in the processes linked up with moulting.

In this connection it is of interest to compare the hormonal regulation of moulting in Crustacea and insects. It has been shown that in insects a dualistic control is exercised by two hormones, a juvenile hormone responsible for the development of nymphal characteristics and a moulting hormone inducing adult traits, the effect produced at any stage being the result of the two hormones exerting their influence in varying degrees (Wigglesworth, 1940). In Crustacea it is known that a moult-inhibiting hormone is produced by the sinus gland or as has been recently observed secreted by the neurosecretory centres in the eyestalk and stored in sinus gland (Bliss and Welsh, 1952). The physiological effects of this hormone suggest a similarity to the juvenile hormone of insects, produced by the corpus allatum or its homologue the Weismann's ring of Diptera. But it is not known whether in Crustacea there is evidence of a moult-inducing hormone comparable to that secreted by the intercerebralis-cardiacum system of insects (*see* Scharrer and Scharrer, 1944). In this connection the observations of Scudamore (1947) are suggestive. He noted that injections of extracts of brain or thoracic ganglia in crayfish result in an increased oxygen consumption and stimulate gastrolith formation, which characterise animals preparing for moult. If this is an indication of a moult-inducing hormone in Crustacea, a very striking parallel would seem to exist with the condition described in insects in regard to a dualistic hormonal control of moulting.

8. Summary

1. Tyrosine and tyrosinase occur in the blood of *Carcinus maenas*. Tyrosinase activity shows variations in different stages of the moult cycle, while tyrosine content is more or less unchanged.

2. The fluctuations in tyrosinase activity appear to be related to the changing redox potentials of the blood estimated electrometrically. The potentials show a fall in the stages following moulting and a rise before the onset of moulting. The rise of potential coincides with the period of most intense tyrosinase activity.

3. It is suggested that low redox potential of the blood may be due among other factors to the activity of an enzyme system, indicated by the cyanide-insensitive oxygen uptake in different periods of the moult cycle, showing a depression in the period before moult and a rise after the moult.

4. The fructose content of the muscles shows variations in the course of the moult cycle and it appears suggestive that such variations may be related to the enzyme activity as estimated by the cyanide-insensitive oxygen uptake.

5. From the observations made it is suggested that the tyrosinase activity is kept in check for a greater part of the intermoult period by the low redox potential of the blood arising from the activity of an enzyme system, the depression of which sometime before moulting raises the

redox potential so as to make possible tyrosinase activity and the elaboration of polyphenols involved in tanning.

6. The control of moulting by a hormone elaborated in the neurosecretory organs of the eyestalk has been studied in relation to the enzyme system noted above. Experimental evidence is adduced that the removal of the eyestalks accelerates the normal course of rise and fall in the activity of the enzyme in relation to moulting. It is suggested that the eyestalk hormone has a controlling influence on the enzyme system in question as a part of the physiological system related to moulting.

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