Richert, D. A. & Westerfeld, W. W. (1957). *Fed. Proc.* **16**, 238.
Romanoff, E. B. & Hunt, C. A. (1954). *Amer. J. Physiol.* **179**, 15.
Schneider, W. C. & Hogeboom, G. H. (1952). *J. biol. Chem.* **195**, 161.
Sonnenschein, N. & Kopac, M. J. (1955). *J. cell. comp. Physiol.* **45**, 361.
Stevens, B. M. & Reid, E. (1956). *Biochem. J.* **64**, 735.
Umbreit, W. W. & Tonhazy, N. E. (1951*a*). *J. biol. Chem.* **191**, 257.
Umbreit, W. W. & Tonhazy, N. E. (1951*b*). *J. biol. Chem.* **191**, 249.
Williams, J. N. Feigelson, P. & Elvehjem, C. A. (1950). *J. biol. Chem.* **185**, 887.

Polyol Dehydrogenase of the Silkworm

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It is becoming increasingly evident that a number of important metabolic events take place in the haemolymph of insects. Examples of the reactions catalysed by enzymes of insect haemolymph are hydrolysis of carbohydrates, fat and proteins, the oxidation of tyrosine and the decomposition of hydrogen peroxide (see review by Buck, 1953). Transaminase has also been detected (Bheemeswar & Sreenivasaya, 1952), as well as a specific phosphatase (Faulkner, 1955).

Recent studies from this laboratory have indicated that the haemolymph of the silkworm, *Bombyx mori* L., contains triphosphopyridine nucleotide-linked dehydrogenases which oxidize L-malate and reduce sugar phosphates (Faulkner, 1956*a*, *b*). It has now been found that hydroxyaldehydes and other carbonyl compounds are reduced by the same enzyme preparation and an account of these studies is given here.

MATERIALS AND METHODS

Dialysed silkworm haemolymph. This was obtained from fifth-instar larvae as described previously (Faulkner, 1956*a*).

Extracts of silkworm tissues. These were obtained by grinding the tissue (0·3–1 g.) with 3 ml. of water at 0° in a motor-driven glass homogenizer. The mixture was centrifuged at 10 000 *g* for 5 min. at 0° and the supernatant was collected. Enzyme activities of the extracts were expressed as units/mg. of protein.

Chemicals. Glycolaldehyde was prepared by the procedure of Powers, Tabakoglu & Sable (1955), glyoxylic acid according to Weinhouse & Friedmann (1951) and D- and L-glyceraldehyde by the method of Perlin & Brice (1956). D-Erythrose and D-threose were gifts from Dr A. S. Perlin, and L-erythrulose from Dr N. Yattrie, both of the Prairie Regional Laboratories, Saskatoon. Other chemicals were purchased as follows: pentoses, sugar acids and uronic acids (Pfanstiehl Chemical Co., Waukegan, Ill., U.S.A.); dihydroxyacetone, DL-glyceraldehyde and L-sorbose (Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.); glucose 6- and ribose 5-phosphates (Schwarz Laboratories Inc., New York, U.S.A.); reduced triphosphopyridine nucleotide (TPNH) and reduced diphosphopyridine nucleotide (DPNH) (Sigma Chemical Co., St Louis, Mo., U.S.A.); D-galactose, D-mannose (Difco Inc., Detroit, Mich., U.S.A.); and methylglyoxal (Bios Chemical Co., New York, U.S.A.); the remaining chemicals were purchased from Fisher Scientific Co., Toronto, Ontario, Canada.

The sugar acids supplied as lactones were converted into the sodium salts by heating with dil. NaOH. Materials used as test substrates were neutralized by addition of HCl or NaOH.

Spectrophotometric determination of dehydrogenase. The standard test system used in the assay of dehydrogenase activity contained the following in a final volume of 2·5 ml.: 2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·5 (18 mM); $MgSO_4$ (4 mM); TPNH (0·1 mM); substrate and enzyme. Cuvettes (1 cm. light path) containing all the components except the substrate were incubated at room temperature (20°) in a Beckman DU spectrophotometer. Optical-density readings were taken at 340 mμ and after a 2 min. equilibration period the substrate (final concn. 2 mM) was added and readings were taken every 30 sec. The blank cell used in the assay did not contain substrate or TPNH. Optical density was plotted against time of incubation and the enzyme activity was calculated from the slope of the initial linear portion of the curve. One unit of enzyme was defined as the amount necessary to reduce the optical density by 0·01/min. Incubations did not normally continue more than 5 min. after the addition of substrate and during this period no appreciable heating of the samples was observed.

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), and polyhydric alcohols by the method of West & Rapoport (1949) with the modifications reported previously (Faulkner, 1956*b*).

EXPERIMENTAL AND RESULTS

Reduction of DL-*glyceraldehyde*

In the presence of DL-glyceraldehyde and buffer (pH 7·5) dialysed silkworm haemolymph catalyses the oxidation of TPNH. The reaction does not

occur in the absence of dialysed haemolymph nor when the latter is replaced by heated enzyme, nor does it take place when DPNH is substituted for TPNH. The rate of oxidation of TPNH in the standard test system is proportional to the concentration of enzyme (Fig. 1). These observations indicate that a TPN-linked dehydrogenase which reduces DL-glyceraldehyde is present in the haemolymph of the silkworm. The enzyme, which is active in the range pH 4·0–9·5, has no pronounced maximum between these pH limits.

Although magnesium was included in the standard incubation mixture used to study the reduction of DL-glyceraldehyde, an absolute requirement for the cation could not be shown with either dialysed haemolymph or haemolymph that had been fractionated by treatment with ammonium sulphate and calcium phosphate. Sodium ethylenediaminetetra-acetate (10 mM) had no effect on the rate of reduction of DL-glyceraldehyde in the standard test system with dialysed haemolymph.

It is possible to estimate the amount of DL-glyceraldehyde that can be reduced in 1 hr. by 1 ml. of silkworm haemolymph containing 30 mg. of protein/ml. The specific activity of the glyceraldehyde-reducing enzyme in whole or dialysed haemolymph is about 1·3 units/mg. of protein. If the value of $6{\cdot}22 \times 10^6$ cm.^{2}/mole is taken for the extinction coefficient of TPNH (Horecker & Kornberg, 1948), it may be calculated that 1 ml. of haemolymph could catalyse the reduction of 0·85 mg. of DL-glyceraldehyde/hr. under the standard test conditions.

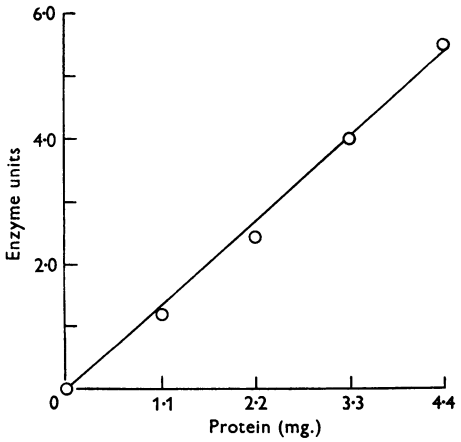

Fig. 1. Relationship between rate of reduction of DL-glyceraldehyde and enzyme concentration. The standard test system was used, with DL-glyceraldehyde (2 mM) as substrate and dialysed haemolymph as enzyme. The total volume incubated was 2·5 ml.

Substrate specificity

A number of sugars and other carbonyl compounds were tested to determine whether they are reduced by a triphosphopyridine nucleotide (TPN)-linked enzyme in silkworm haemolymph. The incubation mixture contained TPNH, Mg^{2+} ions and buffer (pH 7·5) in addition to the enzyme and test substrate. The results are expressed as the rate at which the test substrate was reduced compared with that for D-glyceraldehyde, which was arbitrarily fixed at 100. The concentration of test substrate and of D-glyceraldehyde was 4 mM. Comparative reduction rates with a number of sugars and sugar acids are given in Table 1. It will be seen that glycolaldehyde, D- and L-glyceraldehyde and the tetroses are all readily reduced. In contrast, the pentoses are reduced only slowly and the hexoses not at all. The rate with L-glyceraldehyde is about half that obtained with D-glyceraldehyde. The enzyme has a higher affinity for D- than for L-glyceraldehyde since the Michaelis constant

Table 1. *Rates of reduction of sugars compared with the rate for* D-*glyceraldehyde*

The standard test conditions were used with dialysed haemolymph and with the substrates present at 4 mM. For details see text.

Class	Test substrate	Relative rate of reduction
Diose	Glycolaldehyde	47
Trioses	D-Glyceraldehyde	100
	L-Glyceraldehyde	49
	DL-Glyceraldehyde	100
	Dihydroxyacetone	5
Tetroses	D-Erythrose	131
	D-Threose	104
	L-Erythrulose	19
Pentoses	D-Arabinose	5
	L-Arabinose	12
	D-Ribose	9
	D-Lyxose	2
	D-Xylose	19
Hexoses	D-Glucose	0
	D-Galactose	0
	D-Mannose	0
	D-Fructose	0
	L-Sorbose	0
Sugar acids	Gluconic	0
	Galactonic	0
	Gulonic	0
Uronic acids	Glucuronic	89
	Galacturonic	50
Sugar phosphates	Ribose 5-phosphate	107
	Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate mixture*	0

* Obtained by treatment of hexose diphosphate with aldolase.

for D-glyceraldehyde is 0·066 mM and for L-glyceraldehyde is 2·7 mM in dialysed haemolymph, as determined by the method of Lineweaver & Burk (1934).

The rate of reduction of dihydroxyacetone is only 5 % of that obtained with D-glyceraldehyde, which indicates that the insect enzyme is quite distinct from the glycerol dehydrogenases of *Escherichia coli* (Asnis & Brodie, 1953), *Aerobacter aerogenes* (Burton & Kaplan, 1953; Burton, 1955) and *Pseudomonas salinaria* (Baxter & Gibbons, 1954) with which the reduction of dihydroxyacetone is the faster reaction.

Sugar acids, e.g. gluconic and galactonic acids, are not reduced in this system, but the uronic acids, which retain their carbonyl group at the $C_{(1)}$ position, are readily reduced.

In Table 2 the rates of reduction of some $C_{(1)}$–$C_{(4)}$ carbonyl compounds are compared with that of D-glyceraldehyde. The unsubstituted simple aldehydes and ketones, e.g. formaldehyde and acetone, are not reduced nor are the keto acids, glyoxylic and pyruvic acids. However, the unsaturated aldehyde acrolein, and aldehydes which carry hydroxyl substituents, are reduced.

The dicarbonyl compounds glyoxal, methylglyoxal and dimethylglyoxal are reduced at rates approaching those obtained with an equimolar amount of D-glyceraldehyde. It is interesting that acetylacetone, in which the two carbonyl groups are separated by a $-CH_2$ group, is not reduced.

Identification of end products

Glycerol formation. Sufficient quantities of the product of reduction of DL-glyceraldehyde for analytical purposes were obtained by providing for the continuous formation of TPNH in the incubation mixture. This was achieved by incubating DL-glyceraldehyde in the presence of L-malate and a small quantity of TPN. In this mixture TPN was reduced by the 'malic' enzyme of the haemolymph (Faulkner, 1956*a*) and the following coupled reaction occurred with the 'aldehyde-reducing' enzyme:

$$\text{L-Malate} + \text{TPN}^+ \rightarrow \text{pyruvate} + CO_2 + \text{TPNH}$$

$$\text{TPNH} + \text{DL-glyceraldehyde} \rightarrow \text{'reduced glyceraldehyde'} + \text{TPN}^+$$

Two tubes were each filled with the following mixture: tris buffer, pH 7·5, 20 μmoles; $MgSO_4$, 5 μmoles; L-malate, 30 μmoles; TPN, 0·25 μmole; DL-glyceraldehyde, 10 μmoles; dialysed haemolymph, 0·5 ml. The total volume was 2·05 ml. The enzyme in one of the tubes was inactivated by heating the contents for 5 min. at 100°. Both tubes were then incubated for 3 hr. at 30°. The protein of the second tube was precipitated by heating and both tubes were centrifuged. A volume (1·5 ml.) of each supernatant was treated with 2 ml. of 2:4-dinitrophenylhydrazine (0·1 % in 2N-HCl) and the mixture was incubated at 30° for a further 30 min. It was then centrifuged at high speed and the supernatant dried over silica gel *in vacuo*. The residue was suspended in 0·2 ml. of water and samples (20 μl.) were applied to Whatman no. 1 papers for ascending chromatography. Papers were developed for 18 hr. in the following solvents: (i) *n*-butanol–acetic acid–water (25:6:25, by vol., upper layer; Woiwod, 1949); (ii) *n*-butanol–ethanol–water (4:1:5, by vol., upper phase; Hackman & Trikojus, 1952); and (iii) phenol (80 %, w/v). After development, polyhydric alcohols were detected by spraying the paper with periodate followed by a benzidine reagent (test 1, Cifonelli & Smith, 1954).

The incubated mixture was found to contain a substance having the same R_F as glycerol in all three developing solvents. No glycerol was found in the control. It is concluded that glycerol is formed when TPNH reduces DL-glyceraldehyde in the presence of dialysed haemolymph.

Quantitative measurements on the formation of glycerol were made on mixtures of DL-glyceraldehyde, TPN, L-malate and dialysed haemolymph. Samples of the mixtures which had been incubated and deproteinized were analysed by paper electrophoresis at pH 9 in a borate buffer (0·05 M) for 4 hr. under a potential gradient of 10 v/cm. Under these conditions DL-glyceraldehyde and glycerol could be separated and the glycerol was eluted from the paper. The results given in Table 3 show that the amount of glycerol produced is proportional to the time of incubation.

Erythritol formation. The formation of erythritol was demonstrated when L-malate (30 μmoles), TPN (0·25 μmole)

Table 2. *Rates of reduction of carbonyl compounds compared with the rate for* D-*glyceraldehyde*

The standard test conditions were used with dialysed haemolymph and with substrates present at 4 mM.

Class	Name	Relative rate of reduction
Monocarbonyl	Formaldehyde	0
	Acetaldehyde	0
	Acetone	0
	Pyruvic acid	0
	Acrolein	72
	D-Glyceraldehyde	100
	Aldol	37
	Acetoin	29
	Glyoxylic acid	0
Dicarbonyl	Glyoxal	86
	Methylglyoxal	93
	Dimethylglyoxal	98
	Acetylacetone	0

Table 3. *Production of glycerol in dialysed haemolymph*

Incubation medium: tris buffer, pH 7·5, 20 mM; $MgSO_4$, 5 mM; L-malate, 10 mM; TPN, 0·05 mM; DL-glyceraldehyde, 2 mM; dialysed haemolymph, 0·2 ml. Tubes, containing 1 ml., were incubated for the stated time at 30°. Glycerol was separated as described in the text and determined by the method of West & Rapoport (1949).

Incubation time (min.)	Glycerol found (μmoles)
0	0
30	0·34
60	0·74
90	1·07

and D-erythrose (10 μmoles) were incubated in dialysed haemolymph (0·5 ml.), tris buffer, pH 7·5 (20 μmoles) and $MgSO_4$ (5 μmoles) for 3 hr. at 30°. The procedure for working up the incubated mixtures before chromatography was the same as that used for glycerol. Erythritol was resolved by chromatography in the following solvents: (i) *n*-butanol–ethanol–water (4:1:5, by vol., upper phase; Hackman & Trikojus, 1952); (ii) *n*-butanol–pyridine–water (4:1:1, by vol.; Hockenhull, 1953); (iii) phenol (80%, w/v). No erythritol was present in the control mixture which was inactivated at the beginning of the experiment.

Ethylene glycol formation. Glycolaldehyde, L-malate and TPN were incubated with dialysed haemolymph, and the mixture was inactivated and concentrated as described in the section on glycerol formation. Samples of the supernatant were applied to filter paper for development in *n*-butanol–ethanol–water (4:1:5, by vol., upper phase; Hackman & Trikojus, 1952). 'Marker' quantities (0·1 μmole) of ethylene glycol were applied close to the borders of the chromatogram. After development, the strips bearing the 'marker' were cut from the paper and were treated with the periodate–benzidine spray. Portions of the developed chromatogram were then eluted at the level of ethylene glycol as determined on the 'marker' strips and the polyhydric alcohol content of the eluate was measured colorimetrically. In a typical experiment in which glycolaldehyde (10 μmoles), L-malate (30 μmoles) and TPN (0·25 μmole) were incubated with dialysed haemolymph, tris buffer, pH 7·5 (20 μmoles) and $MgSO_4$ (5 μmoles) for 3 hr. at 30°, 0·49 μmole of ethylene glycol was formed, none being detected in a control inactivated at the beginning of the experiment. Under similar incubation conditions DL-glyceraldehyde (10 μmoles) formed 3·42 μmoles of glycerol.

Reversibility

At pH 7·5 the equilibrium of the polyol dehydrogenase in silkworm haemolymph favours the complete reduction of added aldehydes, e.g. glyceraldehyde or glycolaldehyde, and it was not possible to demonstrate the oxidation of glycerol or ethylene glycol by incubating them with TPN and dialysed haemolymph. However, at pH 8·5 and at a high substrate concentration (M), several polyhydric alcohols reduce TPN. Of the compounds tested, 2:3-butanediol was most rapidly oxidized and propylene glycol and glycerol were oxidized at much lower rates. At the same concentration (M) no oxidation of ethylene glycol or sorbitol was observed.

Comparison of the 'malic' enzyme and polyol dehydrogenase

It has been established that silkworm haemolymph contains a TPN-linked malic dehydrogenase (Faulkner, 1956*a*). It was therefore important to determine whether the oxidation of L-malate and polyhydric alcohols is catalysed by the same enzyme. Evidence that two enzymes are involved was obtained when the rates of oxidation of L-malate and propylene glycol separately and in mixtures were compared. In a typical incubation experiment the basic medium contained tris buffer, pH 8·5 (16 mM); $MgSO_4$ (2 mM); TPN (0·2 mM); dialysed haemolymph, 0·5 ml. The total volume was 2·5 ml. The rate of oxidation of L-malate (8 mM) was 0·028 optical density (*E*) unit/min., and with propylene glycol (M) was 0·015 *E* unit/min. and with a mixture of substrates was 0·041 *E* unit/min., which is almost the sum of the individual rates.

Factors affecting the reduction of ribose 5-phosphate and DL-glyceraldehyde in the haemolymph

The incubation conditions for studying the reduction of sugars and carbonyl compounds were the same as those used for reducing sugar phosphates (Faulkner, 1956*b*). Experiments were carried out to determine whether all these reductions are catalysed by the same enzyme. To this end, the rates of reduction of ribose 5-phosphate and DL-glyceraldehyde were compared under a number of experimental conditions, namely in mixtures of ribose 5-phosphate and DL-glyceraldehyde and in different protein fractions of the haemolymph. The effect of inhibitors on the reduction rates was also tested.

In a typical experiment with dialysed haemolymph, a mixture of DL-glyceraldehyde and ribose 5-phosphate (both 2 mM) was reduced at a rate of 0·0235 *E* unit/min., whereas the rate with DL-glyceraldehyde alone was 0·023 *E* unit/min. and with ribose 5-phosphate was 0·024 *E* unit/min. At this concentration both substrates saturated the enzyme. If two enzymes were involved the reduction rate of the mixture should have approached the sum of the rates obtained with the compounds present individually. A similar result was obtained when the rate of reduction of a mixture of galactose 6-phosphate and DL-glyceraldehyde was measured.

Reduction rates of DL-glyceraldehyde and ribose 5-phosphate were compared in whole blood, in dialysed haemolymph and in three protein fractions obtained by treating whole blood with ammonium sulphate. The results of these measurements are given in Table 4 and they indicate that, irrespective of the treatment of the haemolymph, the ratio between the reduction rates of the sugars lies within the limits 0·93–1·16, although there was a fourfold difference in specific activity between two of the samples (I and II).

Further evidence that ribose 5-phosphate and DL-glyceraldehyde are reduced by the same enzyme is provided by the finding that the reduction of both substances is inhibited by ethylene glycol, propylene glycol and glycerol (Table 5). The observed inhibition of the rate of oxidation of TPNH is not due to the production of more TPNH by oxidation of the polyhydric alcohols, since at pH 7·5 no reaction takes place with TPN (see the

Table 4. *Rates of reduction of* DL-*glyceraldehyde and ribose 5-phosphate in protein fractions of the haemolymph*

The standard test conditions were used, with buffer, pH 7·5, and DL-glyceraldehyde and ribose 5-phosphate at 2 mM.

Treatment	Rate of reduction (units/mg. of protein)		Ratio of rates (glyceraldehyde: ribose phosphate)
	Glyceraldehyde	Ribose phosphate	
Fresh haemolymph	1·10	1·01	1·09
Dialysed haemolymph	1·30	1·39	0·93
Fresh haemolymph treated with $(NH_4)_2SO_4$			
I 0–40% of saturation	0·50	0·52	0·96
II 40–65% of saturation	2·06	2·19	0·94
III 65–95% of saturation	2·22	1·91	1·16

Table 5. *Effect of polyhydric alcohols on reduction of* DL-*glyceraldehyde and ribose 5-phosphate*

The standard test system was used with dialysed haemolymph and with DL-glyceraldehyde and ribose 5-phosphate both at 2 mM.

Polyhydric alcohol (M)	Percentage inhibition	
	DL-Glyceraldehyde reduction	Ribose 5-phosphate reduction
Ethylene glycol	44	58
Propylene glycol	59	56
Glycerol	40	33

Table 6. *Distribution of polyol dehydrogenase in silkworm tissues*

The standard test conditions were used with DL-glyceraldehyde (2 mM) as substrate. Tissues were prepared as described in Methods.

Tissue	Dehydrogenase (units/mg. of protein)
Fifth-instar larval blood	1·30
Fifth-instar larval fat body	1·13
Fifth-instar larval gut	2·21
Pre-pupa, 2 days after spinning	0·22
Pupa, 10 days after spinning	0·27
Adult	0·25

section on reversibility). Glycerol did not inhibit the 'malic' enzyme of the haemolymph, which shows that the alcohol is not a general inhibitor of all TPN-linked dehydrogenases of the haemolymph.

Distribution of polyol dehydrogenase in the silkworm

Polyol dehydrogenase activity was determined in a number of larval tissues as well as in preparations obtained from pupae and adults (Table 6). The enzyme was detected in all the tissues tested. When expressed as units/mg. of protein, the values obtained with pupal and adult preparations were lower than those with larval tissues. With the larval tissues it is interesting that the highest enzyme activity occurs in the gut, which may indicate that much of the fat metabolism in the larva occurs in this tissue.

DISCUSSION

Bacterial extracts that oxidize glycerol to dihydroxyacetone have been obtained from *Acetobacter suboxydans* (Hauge, King & Cheldelin, 1955), *Escherichia coli* (Asnis & Brodie, 1953), *Pseudomonas salinaria* (Baxter & Gibbons, 1954), *Bacillus subtilis* (Wiame, Bourgeois & Lambion, 1954) and *Aerobacter aerogenes* (Burton & Kaplan, 1953). These enzymes require DPN as coenzyme and with each the equilibrium favours the oxidation of glycerol. However, liver extracts from rat or pig possess a DPN-linked glycerol dehydrogenase, the equilibrium of which favours the formation of glycerol from D- or L-glyceraldehyde (Wolf & Leuthardt, 1953; Leuthardt & Wolf, 1954). The extract from liver also reduces acetaldehyde, and Holzer, Schneider & Lange (1955) have suggested that both reductions are catalysed by liver alcohol dehydrogenase.

The enzyme of silkworm haemolymph has its equilibrium in favour of glycerol synthesis and reduces D-glyceraldehyde at least 20 times as rapidly as dihydroxyacetone. It differs from the liver glycerol dehydrogenase in that it requires TPN as coenzyme and does not reduce acetaldehyde; it does, however, reduce a number of substances almost as readily as D-glyceraldehyde: e.g. D-erythrose is reduced to erythritol and glycolaldehyde yields ethylene glycol. The end products formed by the reduction of the other carbonyl compounds have not been identified, but, by analogy, one would expect the carbonyl group of these substances to be reduced to the corresponding carbinol.

The reduction of D-glucuronate and D-galacturonate in silkworm haemolymph is interesting in view of the recent finding that these compounds

are also reduced by enzymes in rat liver (Publitz, Grollman & Lehninger, 1957) and in bacteria (Starr, DeLey & Kilgore, 1957). The reaction is believed to be the first step in the conversion of D-glucuronate into L-ascorbate in animal tissues. The reduction of the uronic acids is interesting because of their structural similarity to the hexose phosphates, which are also reduced in the presence of TPNH and silkworm haemolymph (Faulkner, 1956*b*) at rates which are of the same order as for D-glyceraldehyde.

These specificity studies indicate that sugars having two to four carbon atoms are rapidly reduced and there is a considerable fall off in rate with the pentoses and no reduction of the unsubstituted hexoses. However, if the pentoses and hexoses are substituted in their terminal carbons by an acidic group they are reduced rapidly. From the results given in Table 2 it appears that carbonyl compounds are reduced if the adjacent carbon is unsaturated or is substituted with an hydroxyl group or is part of another carbonyl group.

The enzyme in haemolymph also differs in its properties from the DPN-linked polyol dehydrogenases of rat liver, *Aerobacter suboxydans*, *Candida utilis* and *Pseudomonas* species, the substrate specificities of which have been extensively studied by McCorkindale & Edson (1954), by Arcus & Edson (1956) and by Shaw (1956). These enzymes oxidize hexitols and ribitols readily and attack erythritol and glycerol slowly or not at all.

The evidence presented here indicates that sugar phosphates and the simpler carbonyl compounds are reduced by the same enzyme in the haemolymph, but the results have to be interpreted with caution until the fraction containing the reducing activity has been purified further.

The actual role that the polyol dehydrogenase of *Bombyx mori* tissues plays in the physiology of the insect will remain in doubt until more is learned of the chemical composition of the haemolymph and other tissues. The present studies have indicated the class of compound that may be reduced in the insect, but the 'natural' substrates are not yet known. It is significant that Wyatt & Kalf (1958) reported finding glycerol in the haemolymph of the giant silkworm (*Platysamia cecropia* L.), and the possibility should now be considered that glycerol is formed *in situ* by reduction of glyceraldehyde in the haemolymph. However, these studies emphasize the fact that active dehydrogenases are present in the haemolymph and add weight to the suggestion that the 'malic' enzyme and other dehydrogenases of the haemolymph make an important contribution to the maintenance of the redox potential (Dennell, 1949; Faulkner, 1956*a*).

SUMMARY

1. Dialysed haemolymph of the silkworm, *Bombyx mori* L., contains a triphosphopyridine nucleotide-linked polyol dehydrogenase that reduces glycolaldehyde to ethylene glycol, DL-glyceraldehyde to glycerol and D-erythrose to erythritol. Among the other hydroxyaldehydes and carbonyl compounds reduced by the enzyme preparation are D-threose, glucuronic and galacturonic acids, ribose 5-phosphate, glyoxal, methylglyoxal and dimethylglyoxal. Hexoses and pentoses are not reduced and dihydroxyacetone is reduced at 5% of the rate for D-glyceraldehyde.

2. The equilibrium of the reaction favours the reduction of the carbonyl compounds at pH 7·5; but the oxidation of butane-2:3-diol, propylene glycol and glycerol in the presence of triphosphopyridine nucleotide can be demonstrated at high substrate concentrations (M) at pH 8·5.

3. The enzyme oxidizing the glycols is distinct from the 'malic' enzyme of the haemolymph, but the reduction of glyceraldehyde and ribose 5-phosphate appears to be catalysed by the same protein fraction.

4. Polyol dehydrogenase has been detected in extracts of the fat body and the gut of larvae, and in extracts of whole pupae and adults.

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REFERENCES

Arcus, A. C. & Edson, N. L. (1956). *Biochem. J.* **64**, 385.
Asnis, R. E. & Brodie, A. F. (1953). *J. biol. Chem.* **203**, 153.
Baxter, R. M. & Gibbons, N. E. (1954). *Canad. J. Biochem. Physiol.* **32**, 206.
Bheemeswar, B. & Sreenivasaya, M. (1952). *Curr. Sci.* **21**, 253.
Buck, J. B. (1953). *Insect Physiology*, pp. 147, 191. Ed. by Roeder, K. D. New York: John Wiley and Sons, Inc.
Burton, R. M. (1955). *Methods in Enzymology*, vol. 1, p. 397. New York: Academic Press.
Burton, R. M. & Kaplan, N. O. (1953). *J. Amer. chem. Soc.* **75**, 1005.
Cifonelli, J. A. & Smith, F. (1954). *Analyt. Chem.* **26**, 1132.
Dennell, R. (1949). *Proc. Roy. Soc.* B, **136**, 94.
Faulkner, P. (1955). *Biochem. J.* **60**, 590.
Faulkner, P. (1956*a*). *Biochem. J.* **64**, 430.
Faulkner, P. (1956*b*). *Biochem. J.* **64**, 436.
Hackman, R. H. & Trikojus, V. M. (1952). *Biochem. J.* **51**, 653.
Hauge, J. G., King, T. E. & Cheldelin, V. H. (1955). *J. biol. Chem.* **214**, 1.
Hockenhull, D. J. D. (1953). *Nature, Lond.*, **171**, 982.

Holzer, H., Schneider, S. & Lange, K. (1955). *Angew. Chem.* **67**, 276.
Horecker, B. L. & Kornberg, A. (1948). *J. biol. Chem.* **175**, 385.
Leuthardt, F. & Wolf, H. P. (1954). *Helv. chim. acta*, **37**, 1732.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
McCorkindale, J. & Edson, N. L. (1954). *Biochem. J.* **57**, 518.
Perlin, A. S. & Brice, C. (1956). *Canad. J. Chem.* **34**, 85.
Powers, H. H., Tabakoglu, G. & Sable, H. Z. (1955). *Biochemical Preparations*, vol. 4, p. 56. New York: John Wiley and Sons, Inc.
Publitz, C., Grollman, A. P. & Lehninger, A. L. (1957). *Fed. Proc.* **16**, 382.
Shaw, D. R. D. (1956). *Biochem. J.* **64**, 394.
Starr, M. P., DeLey, J. & Kilgore, W. W. (1957). *Science*, **125**, 929.
Weinhouse, S. & Friedmann, B. (1951). *J. biol. Chem.* **191**, 707.
West, C. D. & Rapoport, S. (1949). *Proc. Soc. exp. Biol., N.Y.*, **70**, 141.
Wiame, J. M., Bourgeois, S. & Lambion, R. (1954). *Nature, Lond.*, **174**, 37.
Woiwod, A. J. (1949). *Biochem. J.* **45**, 412.
Wolf, H. P. & Leuthardt, F. (1953). *Helv. chim. acta*, **36**, 1463.
Wyatt, G. R. & Kalf, G. F. (1958). *Proc. 10th int. Congr. Ent., Montreal* (in the Press).

Phospholipids

5. THE EFFECT OF COD-LIVER OIL IN THE DIET ON THE COMPOSITION OF HEN'S EGG PHOSPHOLIPIDS*

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The degree of unsaturation of the lipids of the hen's egg is known to be influenced by the composition of the fats present in the diet (Henriques & Hansen, 1903; McCollum, Halkin & Drescher, 1912; Terroine & Belin, 1927). Cruickshank (1934) found that the effect of a change in the dietary fat reached its maximum after about 16 days, reflecting the time taken for the laying down of the yolk in the oviduct. Although the iodine value of the yolk lipids could be increased greatly by feeding highly unsaturated oils such as linseed or hempseed, relatively saturated fats such as mutton fat or palm oil had only a slight depressing effect, and foreign saturated acids such as lauric and myristic were not transmitted to the yolk.

Reiser (1951) added 2% of cod-liver oil to a fat-free feed and found evidence of pentaene and hexaene structures in the fatty acids of the yolk lipids by spectrophotometric analysis. On a rigorously fat-free diet hexaethenoid acids were found to disappear completely from the egg lipids, and the proportions of the other polyethenoid acids diminished; however, the total amount of unsaturated acids was not affected (Reiser, Gibson, Carr & Lamp, 1951). These workers examined either the total lipids of the yolk, or the glycerides and phospholipids as separated by precipitation with acetone.

* Part 4: Rhodes & Lea (1957).

It has recently been shown (Hanahan, 1954; Rhodes & Lea, 1956*a*) that the fatty acid in the α' position of the phosphatidylcholine (lecithin) and of the phosphatidylethanolamine of normal eggs is apparently always unsaturated, whereas that in the β position is almost entirely saturated. Moreover, the unsaturated acids of the phosphatidylethanolamine and of a small fraction of the phosphatidylcholine possessed unusually high average unsaturation for an animal lipid of non-marine origin (Rhodes & Lea, 1956*a*). The hens had been receiving a normal diet which, however, contained some fish meal.

The object of the present work was to ascertain whether the highly selective deposition of unsaturated and saturated acids in the α' and β positions of the yolk phospholipids would persist when a high proportion of fish oil, rich in polyethenoid acids, was included in the diet.

EXPERIMENTAL

Materials

Basic ration. This was a commercial laying meal containing 5% of white-fish meal.

Defatted meal. The meal (1 kg.) was refluxed with diethyl ether (2 l.) for 2 hr., filtered off and the residue re-extracted with fresh solvent. The final residue was dried and supplemented with 5 mg. of β-carotene and 22 mg. of α-tocopherol/kg.