VIII. α-GLYCEROPHOSPHORIC ACID AND BRAIN METABOLISM.

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ASHFORD AND HOLMES [1931] found that sodium glycerophosphate increases the oxygen uptake of chopped brain tissue from rabbits, and Davies and Quastel [1932] that it can donate hydrogen to methylene blue in the presence of bullock's brain. In neither of these researches was the isomeride specified. Peters and Sinclair [1933, 1] showed that α-, but not β-, glycerophosphate increases the oxygen uptake of normal pigeon's brain, and [1933, 2] that the effect of pyruvic acid on the extra respiration of the vitamin B_1-deficient brain due to α-glycerophosphate is apparently inconsistent with the scheme of Embden and Meyerhof. The importance of this scheme as a mechanism for glycolysis justified a study of the metabolism of α-glycerophosphate in the brain.

The present work is in two sections, the first dealing with factors that influence the removal of sodium α-glycerophosphate by the brain, the second with the oxidation products of α-glycerophosphate.

THE UTILISATION OF α-GLYCEROPHOSPHATE BY BRAIN TISSUE *IN VITRO*.

The following description applies to all the respiration experiments described.

Normal pigeons, kept on the customary diet of this laboratory, were used throughout. Each bird was guillotined, its cerebrum transferred to a porcelain plate and minced with a bone spatula. Samples of about 150 mg. were placed in duplicate weighed Barcroft bottles of the standard type containing 3·0 ml. of fluid, including Ringer-phosphate solution and additions of substrate. O_2 uptakes at 38 ±0·1° were then determined in the usual way. Shaking was carried out at 60–80 complete revolutions per min., since higher speeds gave bad duplicates by washing tissue on to the sides of the bottle.

At the end of the period of respiration the bottles were taken from the bath and grease was wiped from the necks with a clean duster. When the bottles were cool, the appropriate protein precipitant was added.

The various substrates have been prepared and used as follows.

Lactate. Pure zinc *r*-lactate was decomposed with $NaHCO_3$. The final concentration in the Ringer-phosphate was 0·033 *M*.

Pyruvate. A commercial preparation was repeatedly distilled *in vacuo* until the fraction at 50° was colourless. It was made up to 10 *M* with water and kept at 4°. It was mixed with the Ringer-phosphate in a concentration of 0·019 *M*.

α-Glycerophosphate. Sodium α-glycerophosphate was obtained in solution by treating a sample of Boots's calcium α-glycerophosphate [King and Pyman, 1914] with the theoretical amount of sodium oxalate. The solution was alternately cooled to 0° and heated to 100° until decomposition was complete. The centrifugate always gave negative tests for Ca and oxalate.

Pyrophosphate. A sample of A.R. $Na_4P_2O_7$ was twice recrystallised from water. Just before use it was brought to red heat for 10 min. The required amount was dissolved in a little boiling water, the p_H was adjusted to 7·3 with 10 *N* HCl, and the $Na_4P_2O_7$ was added to the Ringer-phosphate in a final concentration of 0·013 *M*.

Ringer-phosphate solution. NaCl, 0·13 *M*; KCl, 0·0025 *M*; $NaHCO_3$, 0·0014 *M*; KH_2PO_4, 0·1 *M*; enough NaOH (about 0·068 *M*) to bring the p_H to 7·3. Glass-distilled water was used.

After all the additions of substrates had been made, the p_H was adjusted to 7·3 by the addition of NaOH or HCl as needed.

1. *Rate of disappearance of α-glycerophosphate.*

The effects of lactate and pyrophosphate upon the utilisation of α-glycerophosphate were first studied, since lactate is probably a normal metabolite in the pigeon's brain and sodium pyrophosphate profoundly modifies the brain's respiration *in vitro* [Peters and Sinclair, 1933, 2]. The results of eleven pairs of experiments are typified by the two experiments given below.

Oxygen uptake of brain in presence of various substrates.

(Expressed as μl. O_2/g. tissue/hr. in the period indicated. Average of duplicates.)

Additions	0–¼ hr.	¼–¾ hr.	¾–1¼ hr.	1¼–1¾ hr.	1¾–2¼ hr.	2¼–2¾ hr.
	Exp. 54. Pyrophosphate not added					
None	910	705	510	600	395	300
α-GP	1430	1095	905	925	615	555
L	2645	1830	1085	990	685	560
L + α-GP	2430	1960	1515	1630	1360	1240
(α-GP) – (Residual)	+520	+390	+395	+325	+220	+255
(L + α-GP) – (L)	−215	+130	+430	+640	+675	+680
	Exp. 72. Pyrophosphate added					
None	1245	1025	670	475	425	250
α-GP	1775	1395	1110	810	660	550
L	2950	2640	2320	1995	1830	1660
L + α-GP	3495	3035	2635	2360	2075	2050
(α-GP) – (Residual)	+530	+370	+440	+335	+235	+300
(L + α-GP) – (L)	+545	+395	+315	+365	+245	+390

When pyrophosphate has been added, the extra oxygen uptake due to α-glycerophosphate is additive to that due to lactate. The four curves are parallel. When pyrophosphate has not been added, the curve for lactate alone starts at a high level and falls off rapidly, but the curve for lactate *plus* α-glycerophosphate starts high and is maintained throughout. There seems to be an interaction between lactate and α-glycerophosphate. An alternative to actual interaction might be that the brain forms pyrophosphate from α-glycerophosphate, and that this pyrophosphate then maintains the extra respiration due to lactate. The effect can be seen in the data of Peters and Sinclair [1933, 1], who however did not remark upon it.

Estimations of the rate of disappearance of α-glycerophosphate were made by a method similar to that described by Meyerhof and Kiessling [1933].

The proteins were precipitated with trichloroacetic acid. Inorganic phosphates were precipitated from the filtrate with baryta and barium chloride at p_H 10. Five volumes of 97% ethyl alcohol were added to the centrifugate and the crude precipitate of Ba α-glycerophosphate was dried *in vacuo*. Aliquot parts were taken for analysis according to Pregl [1930, pp. 180–90].

The sample was weighed into tin cups and treated with HI. The *iso*propyl iodide thus formed was decomposed in alcholic silver nitrate. The silver iodide was filtered off, washed, dried and weighed in the halogen filter stick.

The method was checked against vanillin and pure Ca α-glycerophosphate. Occasional analyses of vanillin were made to insure against faulty reagents.

The maximum error in recovery of known small amounts of Ca α-glycerophosphate added to brain tissue was 5%. In calculating the results no correction

has been made for variations of tissue weight in different bottles since the tissue blank is negligible. There is something estimated as α-glycerophosphate in the brain immediately after death, but in five cases it did not amount to more than 0·023 mg. of Ca α-glycerophosphate per g. of tissue. Single estimations only were made on the precipitates of the impure Ba α-glycerophosphate, because the error of the final analysis was not more than 1 % and the error in recovery could amount to 5 %. The increased accuracy obtained by duplicate estimations was therefore not commensurate with the extra time spent.

The results are collected in Table I.

Table I. *Rate of disappearance of α-glycerophosphate.*

(Calculated as mg. Ca α-glycerophosphate/g. tissue/hr.)

With added pyrophosphate			Without added pyrophosphate		
Exp.	α-GP alone	α-GP + lactate	Exp.	α-GP alone	α-GP + lactate
71	17·7	12·8	62	6·3	5·6
72	11·2	9·6	63	3·3	3·6
73	22·2	—	64	2·1	2·1
79	12·1	14·1	65	3·4	8·8
81	19·1	18·2	66	3·9	7·0
84	20·4	25·2	67	3·4	3·2
85	—	14·0	70	4·3	7·0
90	13·4	—	87	5·4	3·1
91	13·9	—	88	7·0	—
Average	16·3	15·7		4·3	5·1

Added $Na_4P_2O_7$ causes a large increase in the rate of α-glycerophosphate disappearance, but the addition of lactate has little effect. In complete combustion 1 mol. of O_2 would remove 0·15 mol. of α-glycerophosphate. Table II is a calculation of the ratio mols. α-glycerophosphate removed/mols. extra O_2 uptake due to added α-glycerophosphate. It shows that only a small part of the α-glycerophosphate could be oxidised completely and, since the ratio varies from 1·1 to 8·8, that the oxidation involved is not a simple one. $Na_4P_2O_7$ doubles the ratio. The effect of pyrophosphate may be connected with the activity of adenyl pyrophosphate as coenzyme to yeast glycerophosphate dehydrogenase [Lehmann, 1934].

2. *The independence of pyruvate and α-glycerophosphate.*

If the Embden-Meyerhof scheme were valid for pigeon's brain tissue, the rate of disappearance of α-glycerophosphate *in vitro* should be increased by added pyruvate. This was tested under anaerobic conditions, with and without added pyrophosphate.

Additions to the Barcroft bottles were made as follows: α-glycerophosphate alone, pyruvate alone and α-glycerophosphate *plus* pyruvate. The α-glycerophosphate in the trichloroacetic extracts was estimated as before and the pyruvate by the bisulphite-iodine titration of Clift and Cook [1932]. For anaerobic experiments, nitrogen was used instead of oxygen. The experiments all ran for 2¼ hours.

Pyruvic acid used in this way does not interfere with the estimation of α-glycerophosphate, but complications arose because aerobically, but not anaerobically, α-glycerophosphate gives rise to a small amount of bisulphite-binding substance, sometimes equivalent to as much as 0·5 mg. pyruvic acid/g. of tissue in 2¼ hours. The assumption was made that the amount of this substance is not affected by the presence of pyruvate, and it was added as a blank. No correction was made for variations in tissue weight.

Table II shows that there is no interaction between pyruvate and α-glycerophosphate, aerobically or anaerobically, with or without added pyrophosphate.

Table II. *Effect of pyruvate on removal of α-glycerophosphate.*

Exp.	Substrates added	α-Glycerophosphate removed As mg. Ca salt/g. tissue/hr. α-GP	α-GP + pyr.	Diff.	Pyruvate removed As mg. acid/g. tissue/hr. Pyr.	α-GP + pyr.	Diff.
	Aerobically						
87	Pyrophosphate not added	5·4	5·95	+0·55	5·27	5·08	−0·19
88	„ „	7·0	7·7	+0·7	2·35	2·08	−0·27
89	„ „	3·0	3·6	+0·6	5·52	4·84	−0·68
90	Pyrophosphate added	13·45	11·6	−1·85	4·16	4·33	+0·17
91	„ „	13·9	13·2	−0·7	4·82	3·10	−1·72
	Anaerobically						
93	Pyrophosphate not added	0·2	0·1	−0·1	1·65	0·79	−0·86
94	„ „	1·9	1·3	−0·6	5·02	4·58	−0·44
96	Pyrophosphate added	2·35	1·6	−0·75	1·83	1·88	+0·05
97	„ „	1·75	1·0	−0·75	2·48	2·13	−0·35

To test the assumption that bisulphite-binding substances are produced aerobically from α-glycerophosphate in equal amounts with or without added pyruvate, estimation of the pyruvate was made in eight experiments by the Neuberg-Case 2:4-dinitrophenylhydrazine method, as modified by Peters and Thompson [1934]. The results (Table III) agree with those in Table II. In this method the bisulphite-binding substance formed from α-glycerophosphate does not appear as a blank.

Table III. *Disappearance of pyruvic acid as estimated by extraction of the 2:4-dinitrophenylhydrazone.*

(As mg. acid/g. tissue/hr.)

	Substrates	Pyruvate alone	Pyruvate + α-glycero-phosphate	Difference
	Aerobically			
1	Pyrophosphate not added	5·08	1·72	−3·36
2	„ „	3·82	3·56	−0·26
3	„ „	7·19	2·16	−5·03
4	Pyrophosphate added	5·63	6·28	+0·65
5	„ „	6·11	5·94	−0·17
6	„ „	3·90	4·36	+0·47
	Anaerobically			
7	Pyrophosphate not added	3·25	3·08	−0·17
8	Pyrophosphate added	3·08	1·95	−1·13

It can be seen from these figures that the disappearance of α-glycerophosphate is largely dependent upon the presence of oxygen.

Only two anaerobic experiments were made by the second method, which is much more specific than the first, but the complete agreement between the two sets of experiments strengthens the conclusion that the Embden-Meyerhof scheme does not hold for pigeon's brain tissue with or without added pyrophosphate, aerobically or anaerobically. If anything, the two compounds exercise a sparing action on each other.

The α-glycerophosphate and pyruvate were added in concentrations which had submaximum effects on the respiration. Any interaction would have

appeared in the analyses. The Ringer-phosphate solution was $N/10$ with respect to potassium, a concentration at which the effect on brain glycolysis [Ashford and Dixon, 1935] is maximum. However, as no experiments were made with potassium-free Ringer solution, the effect of potassium on the results is unknown.

No attempt has been made to show that α-glycerophosphoric acid is a normal metabolite in the pigeon's brain, although a very small amount of something estimated as α-glycerophosphate is present in the tissue immediately after death. The work does indicate that the Embden-Meyerhof scheme cannot hold for the normal pigeon's brain. The dual mechanism for brain glycolysis suggested by Ashford and Holmes [1929] and Ashford [1933] implies that the Embden-Meyerhof scheme could hold only for a part of the lactic acid production. Ashford [1933], in two experiments with slices of rabbit's brain, found that the lactic acid produced from the combination of pyruvate and α-glycerophosphate was less than that from pyruvate alone.

Peters and Thompson [1934] favoured the view that the disappearance of pyruvic acid in the pigeon's brain is dependent upon some other change. Peters *et al.* [1935] found that the actions of fluoride and iodoacetate on vitamin B_1-deficient pigeon's brain tissue are consistent with the Embden-Meyerhof scheme so far as pyruvic acid is concerned. In the present experiments α-glycerophosphoric acid did not behave in brain as it does in muscle. In experiments with avitaminous brain by Johnson and Peters [unpublished], vitamin B_1 had no influence upon the disappearance of α-glycerophosphoric acid, though it is known to affect pyruvate. The evidence therefore suggests that in the pigeon's brain there is a glycolytic cycle, but that α-glycerophosphoric acid is not a part of it.

The oxidation products of α-glycerophosphate.

Brain tissue, in contrast to muscle, seems to utilise α-glycerophosphate in appreciable quantity only in the presence of oxygen. It has been suggested that glycerophosphate dehydrogenase produces glyceraldehyde-3-phosphoric acid [Harrison, 1935; Fischer and Baier, 1932], but it is possible that other carbonyl compounds are formed as well. It seemed practicable to detect them by their reducing properties, and a study was accordingly made of the filtrates from brain tissue which had respired in the presence of α-glycerophosphate.

Trichloroacetic acid was unsuitable as a protein precipitant because, when boiled in alkaline solution, it decomposed and formed reducing substances. Samples of Merck, Kahlbaum and B.D.H. trichloroacetic acid all did this. After much experimentation with different substances, zinc, which has frequently been used for this purpose [see especially Somogyi, 1929; 1930], was found to be suitable. In these estimations 0·4 M $ZnSO_4, 7H_2O$ in $2N$ H_2SO_4 was used. It had many advantages: precipitation was carried out in the cold; the blank remained very small even when filtrates were boiled for 5 hours; and glycogen, hexosediphosphate, Robison's hexosemonophosphate and all the common mono- and di-saccharides were soluble in it.

The respiration period was usually 2 hours. The contents of duplicate Barcroft bottles were poured into centrifuge-tubes containing 2 ml. of the zinc solution and the bottles were washed out three times with 2 ml. portions of distilled water. After standing half an hour with occasional mixing, the precipitates were centrifuged and the centrifugates were filtered into 100 ml. volumetric flasks through Whatman 44 papers previously washed three times with boiling distilled water. (It was found that even the best papers contain water-soluble reducing substances in appreciable amounts.) The precipitates were ground with 0·5 ml. of the zinc solution, the centrifuge-tubes were washed down with 3 ml. of water, the centrifugates filtered into the appropriate 100 ml. flask, and the process was repeated once. The filter-papers were washed once with 5 ml.

of distilled water. When the solutions had been made up to 100 ml., duplicate samples of 10 or 20 ml. were pipetted into boiling-tubes fitted with Kjeldahl bulbs to prevent reoxidation. From this point estimations of the reducing substance were made by the method of Hagedorn and Jensen [1923].

In estimating pure glucose, it was found that reduction of the ferricyanide was complete only at p_H 10 or above. The filtrates were therefore adjusted to p_H 11 with NaOH just before reduction. Recovery of glucose was consistently 97%.

Table IV shows that added lactate is not converted into reducing substances by normal brain tissue under these conditions:

Table IV. *Reducing substances produced by normal brain.*

(Expressed as mg. glucose/g. tissue.)

Exp.	Lactate not added	Lactate added	Difference
	Pyrophosphate not added		
7	2·3	2·35	+0·05
15	1·7	1·7	0·0
30	7·4	7·35	−0·05
33	8·45	8·4	−0·05
34	10·9	10·85	−0·05
35	6·75	7·1	+0·35
36	8·3	7·85	−0·45
37	10·65	11·4	+0·75
40	3·7	3·75	+0·05
43	4·2	4·3	+0·1
Average	6·40	6·50	+0·05
	Pyrophosphate added		
41	4·1	4·0	−0·1
44	4·5	4·5	0·0
46	4·2	4·05	−0·15
47	5·45	5·7	+0·25
Average	4·55	4·55	0·00

Added $Na_4P_2O_7$ does not cause the formation of reducing substance from lactate. No attempt has been made to identify the reducing substance actually estimated, although 10% of the value is due to substance which reduces at 20°.

To make sure that non-reducing carbohydrates were absent, hydrolysis curves were constructed for the two types of filtrate. The filtrates were made up to 100 ml. at a concentration of $2N$ H_2SO_4. Samples were pipetted into boiling-tubes and heated at 100° for varying lengths of time. No great increase in reducing power was seen in five experiments, of which the following is one:

Reducing substances present after hydrolysis.

(Expressed as mg. glucose/g. tissue.)

Time in hours ...	0	¼	½	1	1½	2	3
No addition	2·0	2·05	2·3	2·6	2·85	2·8	2·8
Lactate added	2·2	2·3	2·35	2·6	2·8	2·8	2·85

Reducing substances or potential reducing substances are therefore not formed oxidatively from lactate by normal pigeon's brain tissue *in vitro*. This is in agreement with work by Ashford and Holmes [1931], who found no evidence of synthesis of glycogen or free carbohydrate by chopped rabbit's brain tissue in the presence of lactate.

It was then possible to discover if α-glycerophosphate is changed oxidatively into reducing substances by normal brain tissue. Exactly the same technique

was used as before, since α-glycerophosphate itself has no reducing properties, and lactate is not changed into reducing substances. The results are given in Table V.

Table V. *Production of reducing substances from α-glycerophosphate.*

(Expressed as mg. glucose/g. tissue.)

Pyrophosphate not added				Pyrophosphate added			
Exp.	Lactate	Lactate +α-GP	Difference	Exp.	Lactate	Lactate +α-GP	Difference
54	3·5	4·3	+0·8	44	4·5	5·1	+0·6
55	4·9	6·9	+2·0	45	3·4	4·2	+0·8
56	4·2	5·3	+1·1	46	4·0	6·2	+2·2
57	4·0	5·0	+1·0	47	5·7	6·9	+1·2
58	6·8	14·6	+7·8	49	3·6	5·3	+1·7
				50	4·6	5·6	+1·0
Average	4·7	7·2	+2·5		4·3	5·6	+1·3

Fairly large amounts of reducing substance are formed from the added α-glycerophosphate, and pyrophosphate has little effect on this formation. Hydrolysis did not increase the reducing power of the filtrates, and the substance cannot be a polysaccharide or other substance which increases in reducing power on hydrolysis.

Peters [personal communication] noticed that an orange-red precipitate formed slowly when trichloroacetic acid filtrates of brain tissue which had respired with α-glycerophosphate stood for 1 or 2 days with 2:4-dinitrophenylhydrazine. This observation has been confirmed and the precipitate has been partially fractionated.

Aerobic and anaerobic experiments were made with and without added lactate, pyrophosphate and α-glycerophosphate. The red precipitate appeared in large quantities only when α-glycerophosphate had been used and the experiment was aerobic. Lactate and pyrophosphate seemed to have no effect on its appearance. It was therefore formed from an oxidation product of α-glycerophosphate. In order to obtain enough to analyse, large scale experiments were made.

The brains of three normal pigeons were removed, minced and shaken for 3 hours in an atmosphere of oxygen at 38° with 100 ml. of Ringer-phosphate containing α-glycerophosphate in the usual concentration. 20 ml. of 25% trichloroacetic acid were added and the precipitate was centrifuged. 25 ml. of 0·6% 2:4-dinitrophenylhydrazine in 2*N* HCl were added to the centrifugate and the mixture was kept at 38° for 36 hours. To remove unchanged hydrazine, the orange-red precipitate was centrifuged down and washed with boiling *N* HCl until the washings were colourless. The precipitate was transferred to a 25 ml. centrifuge tube and was washed with 3 ml. portions of boiling 75% alcohol *N* with respect to HCl until only a faint orange colour came out in the washings, 250 ml. of which, containing one fraction of the precipitate, were collected.

The residue was dissolved in pyridine and reprecipitated by the addition of 10 volumes of absolute alcohol. This was repeated three times. The product was washed five times with cold absolute alcohol and was dried *in vacuo* over $CaCl_2$. The yield was 3·1 mg.

The melting-point was 299° (corr.), the melting-point of methylglyoxal-2:4-dinitrophenylbishydrazone [Barrenscheen and Dreguss, 1931]. It gave a violet colour with alcoholic potash. (Found: N, 26·01%; calc. N, 25·92%.)

Another preparation melted at 298° (corr.) and had N, 25·78%.

The product was therefore methylglyoxal-2:4-dinitrophenylbishydrazone.

The washings collected in the first fractionation were adjusted to p_H 7 with 10*N* NaOH and were evaporated to dryness *in vacuo* at 40°. To remove inorganic salts and unchanged hydrazine, the residue was washed with boiling *N* HCl until the washings were colourless. The precipitate was dissolved in boiling absolute alcohol and an insoluble residue was centrifuged. Five volumes of cold *N* HCl were added to the centrifugate and the precipitate was recrystallised four times from hot absolute alcohol by the addition of cold *N* HCl. Its melting-point remained constant at 267° (corr.) after recrystallisation from absolute alcohol. It was dried at 120° *in vacuo*.

The crystals had a striking ruby colour. They were insoluble in water but dissolved readily in ethyl acetate and less readily in absolute alcohol. They contained no P or Cl. They gave a violet colour with alcoholic potash and were not acidic in character. (Found: C, 46·27; H, 7·91; N, 15·01%.)

Another preparation was made in a different way. Cold *N* HCl was added to the washings from the first fractionation as above. The precipitate was extracted with boiling 75% alcohol *N* with respect to HCl until only a faint orange colour came out in the washings. The mixture was precipitated again by cold *N* HCl, and the process was repeated until boiling 75% alcohol *N* in HCl dissolved all the precipitate. The final precipitate was then extracted with boiling 30% alcohol *N* with respect to HCl, and the residue was recrystallised from absolute alcohol by the addition of cold *N* HCl, as above. The properties of this substance were the same as before, but the melting-point remained constant at 262° after recrystallisation. (Found for two preparations by this method: C, 45·58, 43·93; H, 5·93, 6·03; N, 15·98, 16·90%.)

0·143 mg. substance was mixed with 3·523 mg. camphor. $\Delta = 5{\cdot}0°$; mol. wt. 308.

It has been very difficult to prepare enough of the ruby-coloured compound for analysis, and the melting-points show that at least two of the samples were impure. The analyses agree well enough to allow a calculation of the empirical formula. The assumption has been made that the compound is a 2:4-dinitrophenylhydrazone and contains four nitrogen atoms.

Sample	Mol. wt.	% N	% C	% H	% O	M.P. (corrected)
I	—	15·01	46·27	7·93	30·81	267°
II	—	15·98	45·58	5·93	32·51	262°
III	308 ± 30	16·90	43·93	6·03	33·14	269°
Required for:						
$C_6H_{16}O_2.C_6H_4O_4N_4$	316	17·72	45·57	6·33	30·38	—
$C_6H_{16}O_3.C_6H_4O_4N_4$	332	16·87	43·37	6·02	33·73	—
$C_7H_{16}O_3.C_6H_4O_4N_4$	344	16·28	45·35	5·81	32·56	—

Preparation III was probably the purest, and the analysis agrees well with the formula $C_6H_{16}O_3.C_6H_4O_4N_4$. There is little evidence to show its structure. It gives a violet colour with alcoholic KOH like many monose 2:4-dinitrophenylhydrazones, is not acidic since it is insoluble in half-saturated Na_2CO_3 and is completely insoluble in water. The melting-points of 2:4-dinitrophenylhydrazones follow no general rule, so that conclusions cannot be drawn from its very high melting-point. The most likely compound to form such a hydrazone would be a carbonyl compound of high molecular weight.

The compound appears only when the tissue has been incubated aerobically with α-glycerophosphate, but there is nothing yet to show whether it is formed directly from α-glycerophosphate or is produced from oxidation products of α-glycerophosphate by the action of the reagents used in its isolation. The methylglyoxal is certainly formed in this fashion, since there is only a slight turbidity in the mixture of trichloroacetic acid extract with 2:4-dinitrophenylhydrazine, even after 2 hours at 38°. Furthermore, after 12 hours, if the precipitate is centrifuged, methylglyoxal-2:4-dinitrophenylbishydrazone will continue to precipitate for another 12 hours. The compound forming the ruby-coloured hydrazone may very well be a condensation product of some precursor,

and analogous to the α-keto-γ-valerolactone-γ-carboxylic acid into which pyruvic acid polymerises [Wolff, 1899].

The ruby-coloured compound and methylglyoxal osazone account for only a small part of the α-glycerophosphate which actually disappears. The maximum yields, 4·0 mg. and 12·1 mg., respectively, were obtained in an experiment using 300 mg. of α-glycerophosphate and 3·7 g. of brain tissue, the incubation period being 3 hours. Since calculations show that 150 mg. of the α-glycerophosphate would have disappeared during the time, only 3% of the α-glycerophosphate could be accounted for in this way unless the compounds actually isolated are indicative of true intermediates in the oxidation of α-glycerophosphate.

Two other fractions, which have not been identified, were found in small quantities in the working up of the 2:4-dinitrophenylhydrazones. One is soluble in water, is yellow, gives a red colour with alcoholic potash and melts at 128° (corr.). It is like glyceraldehyde-2:4-dinitrophenylhydrazone. The other accompanies the ruby-coloured compound. It is insoluble in water, soluble in 30% alcohol *N* with respect to HCl, melts at 178° (corr.), and gives a violet colour with alcoholic KOH.

There is as yet no proof that the brain can oxidatively synthesise long carbon chains from short, although a high Meyerhof quotient has frequently been obtained with brain tissue [Warburg *et al.*, 1924]. Ashford and Holmes [1929] and Holmes and Ashford [1930] could show no synthesis of glycogen or other carbohydrates from lactic acid. The present work confirms this for the pigeon's brain so far as lactic acid is concerned. If, however, the 6-carbon atom compound described above were really synthesised by the brain from α-glycerophosphate, and even if α-glycerophosphate were an abnormal metabolite, such a formation would demonstrate oxidative synthesis under special circumstances.

Summary.

The metabolism of α-glycerophosphoric acid in the brain of normal pigeons has been investigated *in vitro* by means of a mince incubated with various substrates in Ringer solution buffered with potassium phosphate.

1. The rate of aerobic removal of sodium α-glycerophosphate is unaffected by added sodium lactate, but is increased by added sodium pyrophosphate.

2. Added sodium pyruvate has no effect upon the aerobic or anaerobic removal of α-glycerophosphate, with or without added pyrophosphate. Therefore the Embden-Meyerhof scheme does not hold for the pigeon's brain.

3. There is aerobic production of reducing substances from α-glycerophosphate.

4. From solutions in which brain tissue has respired with α-glycerophosphate the 2:4-dinitrophenylbishydrazone of methylglyoxal and the 2:4-dinitrophenylhydrazone of a compound of probably six carbon atoms can be prepared.

5. The possibility is discussed that the brain may synthesise oxidatively a compound of six carbon atoms from α-glycerophosphate.

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REFERENCES.

Ashford (1933). *Biochem. J.* **27**, 903.
—— and Dixon (1935). *Biochem. J.* **29**, 157.
—— and Holmes (1929). *Biochem. J.* **23**, 748.
—— —— (1931). *Biochem. J.* **25**, 2028.
Barrenscheen and Dreguss (1931). *Biochem. Z.* **233**, 305.
Clift and Cook (1932). *Biochem. J.* **26**, 1788.
Davies and Quastel (1932). *Biochem. J.* **26**, 1672.
Fischer and Baier (1932). *Ber. deutsch. chem. Ges.* **65**, 337.
Hagedorn and Jensen (1923). *Biochem. Z.* **135**, 46.
Harrison (1935). *Ergebn. Enzymforsch.* **4**, 297.
Holmes and Ashford (1930). *Biochem. J.* **24**, 1119.
King and Pyman (1914). *J. Chem. Soc.* 1238.
Lehmann (1934). *Biochem. Z.* **272**, 95.
Meyerhof and Kiessling (1933). *Biochem. Z.* **264**, 40.
Peters, Rydin and Thompson (1935). *Biochem. J.* **29**, 63.
—— and Sinclair (1933, 1). *Biochem. J.* **27**, 1677.
—— —— (1933, 2). *Biochem. J.* **27**, 1910.
—— and Thompson (1934). *Biochem. J.* **28**, 916.
Pregl (1930). Quantitative organic microanalysis, 2nd English Ed. (London.)
Somogyi (1929). *J. Biol. Chem.* **83**, 157.
—— (1930). *J. Biol. Chem.* **86**, 655.
Warburg, Posener and Negelein (1924). *Biochem. Z.* **152**, 309.
Wolff (1899). *Liebig's Ann.* **305**, 154.