equilibrium is established between dissociated and undissociated molecules is rapid compared with the rate of separation during sedimentation no resolution of the components is observed and the sedimentation constant assumes an intermediate value.

Adair *et al.* (1951), in their studies on the oxalic acid degradation product of elastin, failed to detect the component of molecular weight 6000 in the ultracentrifuge and obtained identical sedimentation constants for material fractionated by porous dialysis and also for the unfractionated material. This would be explained by assuming an equilibrium between the components.

On extrapolation to zero concentration the sedimentation constant is found to be 0·71 Svedberg unit and the diffusion coefficient $11{\cdot}33 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. These values taken with the value for the partial specific volume lead to a molecular weight at infinite dilution of 6870. In view of the errors involved in the estimation of such a low molecular weight by sedimentation velocity and the nature of the extrapolations this may mean identity with the 6000 molecular-weight component described by Adair *et al.* (1951). A reliable value of the maximum molecular weight approached at high concentrations cannot be given since sedimentation and diffusion coefficients depend on concentration in cases where there is no possibility of dissociation occurring. By analogy with the material prepared by the oxalic acid route a value of 84 000 might be expected for the maximum molecular weight.

SUMMARY

1. The preparation of a soluble protein by treating elastin with hot concentrated aqueous urea is described.

2. Sedimentation, diffusion and other physical measurements have been made on this material.

3. The results have been explained in terms of a reversible dissociation favoured by dilution and a value given for the minimum molecular weight.

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Glycerylphosphorylethanolamine in Rat Brain

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The presence of glycerylphosphorylethanolamine (GPE) in aqueous extracts of pig and rabbit liver has been reported by Campbell & Work (1952). More recently, Walker (1952) in carrying out an investigation of the amino compounds in various animal tissues has shown a small amount of glycerylphosphorylethanolamine to be present in ox brain but was unable to detect it in rat brain. Interest in this compound lies chiefly in its possible role as an intermediate in the synthesis of phosphatidylethanolamine, one of the constituents of the kephalin fraction of the phospholipids.

Hahn & Hevesy (1937) demonstrated by means of experiments with radioactive phosphorus (^{32}P) that there is an uptake of phosphorus by the phospholipids of brain tissue, and it has been further shown by Dawson & Richter (1950) that when due allowance is made for the slow rate of the entry of ^{32}P into the brain this uptake is comparable with that of other tissues. Very little, however, is known of the route by which these compounds are synthesized in the tissues. Popják & Muir (1950) investigated possible precursors of the phospholipids using ^{32}P and calculated that α-glycero-

phosphate was a likely intermediate in phospholipid synthesis. Using a partially purified enzyme preparation from rat liver, Kornberg & Pricer (1952*a*, *b*) have shown that L-α-glycerophosphate can serve as a precursor of phospholipids. Kennedy (1953) has also shown that DL-α-glycerophosphate is incorporated into phospholipids by isolated rat-liver mitochondria.

On the other hand, the presence of ethanolamine *O*-phosphoric acid (EPA) in rat brain tissue was demonstrated by Ansell & Dawson (1951), who further showed on the basis of experiments with ^{32}P that it cannot arise solely from the breakdown of phosphatidylethanolamine, and therefore may be a precursor of this phospholipid.

In this paper the presence of glycerylphosphorylethanolamine in rat brain tissue is reported and evidence of its existence in an uncombined form obtained. Experiments with ^{32}P have been carried out in order to determine the relationship of this compound to ethanolamine phosphoric acid on the one hand, and lipid-bound glycerylphosphorylethanolamine on the other. A preliminary account of this work has already been reported (Ansell & Norman, 1953).

EXPERIMENTAL

Chromatography of brain extracts

Young rats (30–100 g.) were decapitated and the heads immediately frozen in liquid oxygen. The brains were dissected out, crushed and weighed. Extracts were then prepared by one of the following procedures.

(i) *Tungstic acid precipitation.* Tungstic acid extracts of brain were prepared as described previously (Ansell & Dawson, 1951), except that, after heating, the mixture was immediately centrifuged. Extracts were desalted in an apparatus similar to that described by Consden, Gordon & Martin (1947). This method was used in all tracer experiments.

(ii) *Trichloroacetic acid precipitation.* To the crushed and frozen brain was added 5 ml. of 12% (w/v) trichloroacetic acid solution, the mixture shaken vigorously and centrifuged immediately. The supernatant was extracted twice with an equal volume of water-saturated ether and the aqueous phase desalted. This method was used only in preliminary experiments.

(iii) *Ethanol precipitation.* This method was based on that of Awapara (1948). The frozen brain was dispersed with a mechanical steel-bladed homogenizer in a volume of ethanol equal to 4 times the water content of the tissue (taken as 80% of the wet weight). After centrifuging, a portion of the supernatant was shaken with four times its volume of $CHCl_3$ and the emulsion centrifuged. The aqueous layer was removed, washed with a little $CHCl_3$ and desalted. This method was used as a mild extraction procedure to confirm the results of experiments with tungstic acid and trichloroacetic acid.

Chromatography. Samples of the desalted extract equivalent to 0·25 g. of brain were subjected to two-dimensional chromatography on Whatman no. 4 paper treated with basic copper carbonate (Crumpler & Dent, 1949). Phenol (A.R.) : water (4:1, w/v) was used as the first solvent and collidine-lutidine (Dent, 1948) as the second. Chromatograms were sprayed with 0·2% ninhydrin in 95% ethanol or (in later experiments) with 0·4% ninhydrin in 90% aqueous *iso*propanol containing 5% (v/v) of collidine (Lewis, 1952).

After 16 hr. at room temperature such chromatograms showed four ninhydrin-reacting compounds. In addition to taurine, EPA and γ-aminobutyric acid, a faint spot appeared in close proximity to the γ-aminobutyric acid and in a position similar to that ascribed to GPE (Campbell & Work, 1952). On some chromatograms these two spots could only be distinguished by the sudden change in the intensity of the ninhydrin colour. In general there was a pink haze on the chromatogram; this may have been due to the copper complexes of α-amino acids of which comparatively large amounts were present.

Confirmation of the presence of glycerylphosphorylethanolamine in rat brain

Although Walker (1952) has reported the presence of free GPE in ox brain he states that it is not found in rat brain and it was therefore thought advisable to confirm the identity of the GPE spot as follows.

The presence of an α-aminocarboxylic acid grouping was excluded by the fact that the compound survived the copper treatment. Phosphorus determinations on the paper containing the spot showed that it contained a significantly greater amount of phosphorus than the surrounding paper.

When a rat was injected intracisternally with ^{32}P and a brain extract was prepared in the usual way, the ninhydrin-reacting area believed to be GPE corresponded exactly with a radioactive spot detected by a radioautograph.

Hydrolysis of a brain extract in 5·5N-HCl for 20 hr. at 100° caused this spot to disappear. In a further experiment the position of GPE was located on a number of heated, unsprayed chromatograms by its fluorescence in ultraviolet light (Woiwod, 1949*a*; Fowden, 1951). These areas were cut out and eluted with water. The eluate was hydrolysed for 20 hr. in 5·5N-HCl at 100° and subjected to two-dimensional chromatography in the presence of basic copper carbonate. No GPE spot was visible but a new faint spot appeared in the position of ethanolamine.

When a solution of GPE, prepared from the ether-soluble phospholipids of brain (see below), was run on a two-dimensional chromatogram with a brain extract the spot due to GPE was indistinguishable from the spot obtained from brain extracts. The same result was obtained with a synthetic sample of L-α-GPE (synthesized by Prof. E. Baer and kindly supplied through Dr R. M. C. Dawson). Similarly, the eluted material from the spot believed to be GPE (located on phenol:collidine chromatograms with ultraviolet light) behaved in the same manner as synthetic GPE on single-dimensional chromatograms using *n*-butanol: acetic acid (Woiwod, 1949*b*), *n*-butanol:ethanol:ammonia (Partridge, 1948) or *n*-butanol:formic acid (Wiggins & Williams, 1952) as developing solvents.

Experiments using radioactive phosphorus (^{32}P)

(*a*) *Experiments* in vivo. Young rats (40–150 g.) were used throughout the investigation. In some experiments 120–300 μc of $KH_2{}^{32}PO_4$ were injected intraperitoneally; in

others, 17–60 μc were injected intracisternally by the method of Jeffers & Griffiths (1942). Although the use of an intracisternal injection has the advantage of producing a high local concentration of ^{32}P within the brain (Bakay, 1951) it was difficult to estimate the exact amount of isotope which had entered the cisterna magna.

(*b*) *Separation of the glycerylphosphorylethanolamine phosphorus and the ethanolamine phosphoric acid phosphorus of brain extracts.* Tungstic acid extracts of the frozen brains were desalted for 10–20 min. as this process removed most of the highly radioactive inorganic and acid-labile phosphorus compounds of the brain acid-soluble phosphorus. After chromatography the GPE spot was cut out and an equivalent area, measured by weight, around the spot taken as a control. The EPA spot was large and diffuse; hence only a portion of this spot was taken. In this instance it was not possible to take a surrounding area as a control but, as the amount of phosphorus and radioactivity to be estimated was high, it was considered sufficiently accurate to use an equivalent area of paper some distance away. In general chromatograms were run in duplicate.

All spots and control areas were oxidized as previously described (Ansell & Dawson, 1951), and diluted with distilled water for radioactivity and phosphorus determinations.

(*c*) *Separation of acid-liberated glycerylphosphorylethanolamine phosphorus.* The tungstic acid precipitate of brain was washed twice with 5 ml. of water containing 0·2 ml. of tungstic acid solution. It was then mixed with 5 ml. of 12% (w/v) trichloroacetic acid solution (so that the final concentration of trichloroacetic acid was 10%) and allowed to stand overnight (16 hr.) at 4°. After centrifuging, the supernatant was washed twice with an equal volume of ether and a volume equivalent to approximately 250 mg. of tissue was subjected to two-dimensional chromatography in the usual way. The GPE spot obtained in this way was cut out for phosphorus and radioactivity determinations.

(*d*) *Separation of phosphatidylethanolamine phosphorus.* The brain residue obtained in (*c*) was washed thoroughly with trichloroacetic acid solution and water and finally dried with acetone; it was then mixed with a little sand and allowed to stand in a vacuum desiccator overnight.

The powdered, dried brain residue was extracted for 45 min. with 30 ml. of a boiling 1:1 (v/v) $CHCl_3$:ethanol mixture, the extract taken to dryness under reduced pressure and this lipid residue extracted with 15 ml. of warm ether. After allowing the ether solution to stand at 4° for at least 30 min., it was centrifuged to remove ether-insoluble material. The ethereal solution was taken to dryness under reduced pressure. The residue, containing the ether-soluble phospholipids, was hydrolysed with 2 ml. of 0·08 M-$HgCl_2$ solution on a boiling-water bath for 3 hr. (Norman & Dawson, 1953), to liberate GPE from phosphatidylethanolamine. The GPE was isolated by single-dimensional paper chromatography using phenol as the solvent and the ninhydrin-reacting spot oxidized and diluted in the usual way.

(*e*) *Inorganic plus acid-labile phosphorus.* A sample (0·5 ml.) of the tungstic acid brain extract was used for determination of the specific activity of inorganic + acid-labile phosphorus as previously described (Ansell & Dawson, 1951), except that the radioactivity determination was carried out on the aqueous solution before treatment with molybdate.

(*f*) *Radioactivity and phosphorus determinations.* These were carried out as previously described (Ansell & Dawson, 1951). The radioactivity determinations were subject to a variation of $\pm 2\%$ and phosphorus determinations to a variation of $\pm 2{\cdot}5\%$.

(*g*) *Experiments* in vitro. Minced rat brain was suspended in Krebs bicarbonate-Ringer solution (200 mg. wet weight brain tissue/ml.). This suspension (2 ml.) was incubated with 15 μc. of $KH_2{}^{32}PO_4$ in Warburg vessels for 2 hr. (see Table 3). After incubation the contents of each vessel were treated with 0·22 ml. of tungstic acid and heated for 2 min. at 100°. In order to obtain a sufficient volume of extract the contents of two similarly treated vessels were combined.

For isolation of the GPE in the extracts an amount of extract equivalent to 250 mg. of brain was subjected to two-dimensional chromatography as previously described. A further sample of the extract was then treated with saturated $Ca(OH)_2$ solution until the pH was 8·5. After allowing the calcium phosphate to precipitate at 4° for 15 min., it was removed by centrifugation and the solution treated with Na_2CO_3 solution until no further $CaCO_3$ was precipitated. The supernatant from this was then desalted and an amount equivalent to 50 mg. of brain subjected to two-dimensional chromatography for the isolation of EPA. The calcium precipitation removed most of the inorganic phosphate of high specific activity which might have contaminated the EPA spot because of the proximity of this substance to the origin on the chromatograms.

The brain precipitate was treated as in the *in vivo* experiments to determine the specific activity of the phosphatidylethanolamine.

RESULTS

Evidence for free glycerylphosphorylethanolamine in rat brain. The spot due to glycerylphosphorylethanolamine was seen on chromatograms of brain extracts which were prepared in three different ways from brain tissue frozen in liquid oxygen. Both the tungstic acid and especially the ethanolic precipitation of the proteins are procedures unlikely to cause hydrolysis of glycerylphosphorylethanolamine-containing phospholipids and it seems most likely therefore that a small amount of free glycerylphosphorylethanolamine is present in rat brain tissue. The amount of rat brain extract examined by Walker (1952) would have been too small to demonstrate its presence; the fact that he observed it in comparatively high concentration in cow brain is not completely conclusive because of the inevitable delay in extracting such slaughterhouse material. Three methods of extraction were investigated because it was found that mild treatment with acid, i.e. 10% trichloroacetic acid solution at 4° for 16 hr., released glycerylphosphorylethanolamine from brain tissue. It was also released under these conditions from DL-α-dimyristylkephalin, the amount corresponding to a 1% breakdown of the kephalin.

The strongest evidence for the existence of free glycerylphosphorylethanolamine in rat brain, however, comes from experiments with isotopes. As can be seen in Table 1, the specific activity of the

glycerylphosphorylethanolamine in brain extracts after short exchange periods was always greater than that of the phosphatidylethanolamine and, as will be seen from later discussion, also greater than the plasmalogen (acetalphospholipid). Furthermore, after a 24 hr. exchange, the specific activities of the glycerylphosphorylethanolamine and ethanolamine phosphoric acid in the extracts were identical. This indicates that the glycerylphosphorylethanolamine found in brain extracts is a genuine component of the acid-soluble phosphorus fraction because all acid-soluble phosphorus compounds of nervous tissue are in isotopic equilibrium within 24 hr. (Samuels, Boyarsky, Gerard, Libet & Brust, 1951).

The concentration of free glycerylphosphorylethanolamine in rat brain is very low, the mean value obtained from the figures from twelve young rats being 2·3 mg./100 g. This value must, however, be regarded as a minimum because recovery of the synthetic substance from two-dimensional chromatograms was never greater than 80 %. Nevertheless, it represents a very small fraction of the acid-soluble phosphorus and free amino nitrogen in rat brain extracts.

The uptake of ^{32}P *into the glycerylphosphorylethanolamine of rat brain* in vivo. In experiments on the uptake of ^{32}P into the glycerylphosphorylethanolamine and other phosphorus-containing fractions of brain, intracisternal and intraperitoneal injections of the isotope were used. Although the glycerylphosphorylethanolamine isolated from the brains of animals injected intracisternally had a high rate of counting after short exchange periods, this mode of injection often proved difficult and sometimes failed completely. However, the results were useful in confirming some of those obtained after intraperitoneal injection in which the counting rate of the glycerylphosphorylethanolamine was often very low.

In Table 1 the specific activities of the ethanolamine phosphoric acid, free glycerylphosphorylethanolamine, acid-liberated glycerylphosphorylethanolamine and phosphatidylethanolamine at given times after either intracisternal or intraperitoneal injection of ^{32}P are shown. It may be seen that the specific activity of the free glycerylphosphorylethanolamine 105 min. after intraperitoneal injection was only 10 % of that of the ethanolamine phosphoric acid; after 349 min. this figure had risen to 20 % and after 24 hr. the two compounds were equally radioactive. It can also be seen that the specific activity of the free glycerylphosphorylethanolamine was always higher than that of the phosphatidylethanolamine and hence, after short exchange periods, is intermediate between that of the ethanolamine phosphoric acid and phosphatidylethanolamine.

Table 1. *Specific activities of the inorganic and acid-labile phosphate, ethanolamine phosphoric acid (EPA), glycerylphosphorylethanolamine (GPE), acid-liberated GPE and phosphatidylethanolamine in rat brain after injection of* ^{32}P

Wt. of rat (g.)	Dose ^{32}P (μc)	Exchange period (min.)	Specific activity (counts/min./mg. P)				Ratio of specific activities		
			Inorganic + acid-labile P	EPA	GPE	Acid-liberated GPE	Phosphatidylethanolamine	GPE/EPA	GPE/phosphatidyl-ethanolamine	Acid-liberated GPE/phosphatidyl-ethanolamine
					Intracisternal					
112	60	66	—	Approx. 500 000	11 500	4 530	4 440	0·02	2·5	1·02
80	55	230	—	607 000	154 000	21 050	21 650	0·25	7·1	0·97
150	55	319	—	578 000	90 000	18 950	22 550	0·16	4·0	0·84
					Intraperitoneal					
40	7·5/g.	105	86 200	25 700	<2 400	—	771	<0·09	<3·1	—
39	7·2/g.	240	87 400	51 550	8 000	—	2 740	0·16	2·9	—
45	7·35/g.	349	69 200	56 450	13 000	—	—	0·23	—	—
70	1·7/g.	1420	—	18 500	19 370	<3 600	2 870	1·05	6·8	<1·25
70	1·7/g.	1455	—	20 400	19 800	2 730	2 795	1·03	7·1	0·98
76	5·0/g.	1440	36 800	38 600	33 300	—	—	0·86	—	—

On the other hand, the specific activity of the glycerylphosphorylethanolamine liberated by acid (approximately 10 mg./100 g. brain) was invariably almost identical with that of the phosphatidyl ethanolamine. This indicates either that part of this phospholipid is hydrolysed by trichloroacetic acid, as was found for synthetic DL-α-dimyristylkephalin, or that another fraction with the same turnover rate is acid-labile.

In order to investigate the nature of the acid-liberated glycerylphosphorylethanolamine the specific activity of the glycerylphosphorylethanolamine liberated from the acetalphospholipid fraction of rat brain was determined. From half the brain the total lipid was extracted, saponified and allowed to stand in 10% trichloroacetic acid solution for 2 hr. at room temperature, according to the method of Schmidt, Benotti, Hershmann & Thannhauser (1946). From the acid extract glycerylphosphorylethanolamine was separated by chromatography and the specific activity determined in the usual way; this must have derived from an alkali-stable, acid-labile, glycerylphosphorylethanolamine-containing phospholipid, e.g. acetalphospholipid. The other half of the brain was treated with tungstic acid and then allowed to stand at 4° with 10% trichloroacetic acid solution in the usual way for the determination of the specific activity of the acid-liberated glycerylphosphorylethanolamine. The specific activity of the phosphatidylethanolamine was determined, using the trichloroacetic acid-precipitated brain as previously described.

In Table 2 the specific activities of the acid-liberated glycerylphosphorylethanolamine, phosphatidylethanolamine and alkali-stable, glycerylphosphorylethanolamine-containing phospholipid are shown. It can be seen that the specific activities of all three are very similar.

The amount of glycerylphosphorylethanolamine liberated from the alkali-stable phospholipid was approximately 120 mg./100 g. brain (a minimum value), a considerably greater amount than that liberated from whole brain tissue.

The uptake of ^{32}P *into the glycerylphosphorylethanolamine of rat brain* in vitro. When minced rat brain was incubated for 1–2 hr. in glucose-bicarbonate Ringer solution containing $KH_2{}^{32}PO_4$, there was a small but definite uptake into the glycerylphosphorylethanolamine isolated on chromatograms (Table 3). Its specific activity was very much lower than that of the ethanolamine phosphoric acid but the synthesis of both was increased when the incubations were carried out in the presence of ethanolamine. The radioactivity detected in the phosphatidylethanolamine was not significant, which confirms earlier observations by Dawson (1953).

Table 2. *Specific activities of acid-liberated glycerylphosphorylethanolamine (GPE), phosphatidylethanolamine and alkali-stable, GPE-containing phospholipid of rat brain after the intraperitoneal injection of* ^{32}P

Wt. of rat (g.)	Dose ^{32}P (μc/g.)	Exchange period (min.)	Specific activity (counts/min./mg. P)			Ratio of specific activities	
			Acid-liberated GPE	Phosphatidylethanolamine	Alkali-stable, GPE-containing phospholipid	Acid-liberated GPE/phosphatidylethanolamine	Alkali-stable, GPE-containing phospholipid/phosphatidylethanolamine
64	4·7	119	540	628	622	0·86	0·99
58	5·1	298	1150	1102	1453	1·04	1·32
60	5·0	1484	7160	6180	7460	1·16	1·21

Table 3. *Synthesis of radioactive glycerylphosphorylethanolamine (GPE) by minced rat brain*

(Each Warburg vessel contained 400 mg. minced rat brain, 3·6 ml. Krebs bicarbonate-Ringer solution, 15 μc. $KH_2{}^{32}PO_4$ (3 μg. P) in 0·2 ml., 0·2 ml. of 5·4% (w/v) glucose. Temp. 37°. Gas phase, 95% O_2/5% CO_2.)

	Time of incubation (min.)	Additional substrate	Specific activity (counts/min./mg. P)			
			Inorganic + acid-labile P	EPA	GPE	Phosphatidylethanolamine
Expt. 1	0	—	7640×10^3	0	0	<207
	120	—	6620×10^3	71 000	573	<600
	120	Ethanolamine, 0·04 M	6720×10^3	175 000	2 100	0
Expt. 2	0	—	—	0	0	<226
	60	—	—	—	2 200	<228
	60	Ethanolamine, 0·04 M	—	—	13 500	<455
	120	—	—	—	13 500	<183
	120	Ethanolamine, 0·04 M	—	—	52 200	<226

DISCUSSION

From evidence presented in this paper it appears certain that glycerylphosphorylethanolamine is present in the acid-soluble phosphorus fraction of rat-brain tissue. Because of our incomplete knowledge of the lability of kephalin fractions the fact that it is present in brain extracts prepared from frozen brains under mild conditions is not in itself conclusive. It was demonstrated, for example, that glycerylphosphorylethanolamine is liberated from brain tissue under acid conditions at a low temperature. However, isotopic experiments, both *in vivo* and *in vitro*, demonstrated that its specific activity was not consistent with its being derived from any glycerylphosphorylethanolamine-containing phospholipid, unless it is assumed that the turnover of such phospholipids is extremely heterogeneous.

When ^{32}P was injected intraperitoneally and intracisternally into young rats the specific activity of the glycerylphosphorylethanolamine in the brain extracts after short periods was always greater than that of the phosphatidylethanolamine and therefore glycerylphosphorylethanolamine must be considered as a possible phospholipid precursor. Previously, Ansell & Dawson (1951) suggested that ethanolamine phosphoric acid could not be excluded as a precursor of phosphatidylethanolamine on the basis of a comparison of the specific activities of the ethanolamine phosphoric acid and ether-soluble phospholipids. The direct comparison of the specific activity of ethanolamine phosphoric acid with that of phosphatidylethanolamine reinforces this conclusion. On the other hand, Popják & Muir (1950) are of the opinion that the *in vivo* synthesis of phospholipids, by which they mean phosphatidylethanolamine and phosphatidylcholine, proceeds by way of a common precursor, namely α-glycerophosphate. The results of Kornberg & Pricer (1952*a*) suggest, however, that L-α-glycerophosphate is not an exclusive *in vitro* precursor of phospholipids because they have shown that phosphorylcholine can be incorporated as a unit. In view of these results it would appear that there are alternative pathways for phospholipid synthesis in tissues. It seems unlikely, however, that glycerylphosphorylethanolamine lies on the major synthetic pathway of phosphatidylethanolamine because its specific activity would have to be very much greater in relation to the phosphatidylethanolamine than the experimental finding to compensate for the relatively small amount present in rat brain.

It has been shown by Schmidt *et al.* (1946) that acetalphospholipids are completely broken down in 10% trichloroacetic acid in 2 hr. at room temperature. It has further been demonstrated by Thannhauser, Boncoddo & Schmidt (1951) that the ethanolamine-containing acetalphospholipid yields glycerylphosphorylethanolamine on mild hydrolysis with acid. It would be expected therefore that the acid-liberated glycerylphosphorylethanolamine in the present work was derived from such an acetalphospholipid. This acid-liberated glycerylphosphorylethanolamine (10 mg./100 g. brain) corresponds to 20 mg. acetalphospholipid/100 g. brain (derived from palmitaldehyde) but the amount of acetalphospholipid present in rat-brain tissue calculated from the aldehyde value of Anchel & Waelsch (1944) is 600 mg./100 g.

In an attempt to reconcile these results, experiments were performed in which the glycerylphosphorylethanolamine liberated by acid from the alkali-stable phospholipids was determined. The value obtained corresponded approximately to 240 mg. acetalphospholipid/100 g. brain; this is minimal in view of the incomplete recovery of glycerylphosphorylethanolamine from chromatograms. However, this value for the acetalphospholipid content of rat brain is still considerably lower than that obtained by Anchel & Waelsch (1944) and this may be accounted for by the existence in brain of acetalphospholipids that do not contain ethanolamine (Klenk & Böhm, 1951; Lovern, 1952). The large amount of glycerylphosphorylethanolamine liberated by acid from the extracted acetalphospholipids as compared with that liberated from whole brain tissue would indicate that the lability of acetalphospholipids to acid is very much less in the latter case. It is possible, therefore, that brain acetalphospholipid exists in a combined form within the tissue.*

The fact that synthetic DL-α-dimyristylkephalin is hydrolysed by 10% trichloroacetic acid at 4° suggests that the acid-liberated glycerylphosphorylethanolamine derived partly from ethanolamine-containing acetalphospholipid and partly from phosphatidylethanolamine. If this is so then the two phospholipids must have similar rates of synthesis. This conclusion is supported by the fact that the ethanolamine-containing acetalphospholipid was shown to have a similar specific activity to both the acid-liberated glycerylphosphorylethanolamine and phosphatidylethanolamine.

SUMMARY

1. The presence of a low concentration of free glycerylphosphorylethanolamine (2·3 mg./100 g. brain) in rat-brain extracts has been demonstrated; this concentration is minimal.

* Since this paper was accepted for publication, a report which is in agreement with the above suggestion has come to our notice (Schmidt *et al.* (1953). *Fed. Proc.* 12, 265) of the comparative stability of the plasmalogens (acetalphospholipids) in an unsaponified, lipid-extracted brain.

2. After injection of ^{32}P into young rats the specific activity of the free glycerylphosphorylethanolamine after short periods was always greater than that of the phosphatidylethanolamine. This suggests that glycerylphosphorylethanolamine is not an *in vivo* breakdown product of phosphatidylethanolamine, although other considerations indicate that it does not lie on the main synthetic pathway.

3. The specific activity of a combined form of glycerylphosphorylethanolamine labile to 10% trichloroacetic acid at 4° was always identical with that of phosphatidylethanolamine. The specific activity of the ethanolamine-containing acetal-phospholipid was also similar.

4. The synthesis of radioactive glycerylphosphorylethanolamine by minced rat brain has been accomplished. No *in vitro* synthesis of phosphatidylethanolamine could be demonstrated.

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The Bases of the Nucleic Acids of some Bacterial and Animal Viruses: the Occurrence of 5-Hydroxymethylcytosine

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Recent studies on the multiplication of viruses have directed attention increasingly toward their nucleic acids. Hershey & Chase (1952) have shown that most, if not all, of the sulphur-containing protein of coliphage T2, which appears to be present in the outer shell of the virus, does not enter the infected cell. However, deoxyribonucleic acid (DNA), apparently organized within the virus, is in some way transferred to the host cell, and appears, therefore, to participate more intimately in the transmission of genetic properties. On infection of *Escherichia coli* with bacteriophage T2, T4 or T6, there is immediate cessation of synthesis of ribonucleic acid (RNA) and net synthesis of DNA is detectable in about 10 min. (Cohen, 1947, 1951). A similar apparent redirection of DNA synthesis during virus multiplication is characteristic of certain induced lysogenic systems, but in this case synthesis of RNA