- Galambos, R. (1961). *Proc. nat. Acad. Sci., Wash.*, **47**, 129.
- Gardner, W. J. (1961). *Perspectives Biol. Med.* **4**, 169.
- Gatt, S. & Racker, E. (1959). *J. biol. Chem.* **234**, 1024.
- Geiger, A. (1957). In *Metabolism of the Nervous System*, p. 245. Ed. by Richter, D. London: Pergamon Press Ltd.
- Geiger, A. (1958). *Physiol. Rev.* **38**, 1.
- Gerschman, R. (1959). 21st int. Congr. *physiol. Sci.: Symp. & spec. Lect.* p. 222.
- Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P. & Fenn, W. O. (1954). *Science*, **119**, 623.
- Gilbert, D. L., Gerschman, R., Ruhm, K. B. & Price, W. E. (1958). *J. gen. Physiol.* **41**, 989.
- Haugaard, N. (1946). *J. biol. Chem.* **164**, 265.
- Haugaard, N. (1955). In *Underwater Physiology Symposium*, p. 8. Washington, D.C.: National Academy of Sciences, National Research Council.
- Haugaard, N., Hess, M. & Itskovitz, H. (1957). *J. biol. Chem.* **227**, 605.
- Hess, B. (1956). *Biochem. Z.* **328**, 110.
- Hotta, S. S. (1962). *J. Neurochem.* **9**, 43.
- Huggett, A. St G. & Nixon, D. A. (1957a). *Biochem. J.* **66**, 12P.
- Huggett, A. St G. & Nixon, D. A. (1957b). *Lancet*, ii, 368.
- Kaufman, S. (1955). In *Methods in Enzymology*, vol. 1, p. 714. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Korkes, S. (1955). In *Methods in Enzymology*, vol. 1, p. 486. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Korkes, S., del Campillo, A. & Ochoa, S. (1952). *J. biol. Chem.* **195**, 541.
- Kunitz, M. & McDonald, M. R. (1946). *J. gen. Physiol.* **29**, 393.
- Lambertsen, C. J., Ewing, J. H., Kough, R. H., Gould, R. & Stroud, M. W., III. (1955). *J. appl. Physiol.* **8**, 255.
- Lambertsen, C. J., Kough, R. H., Cooper, D. Y., Emmel, G. L., Loeschke, H. H. & Schmidt, C. F. (1953). *J. appl. Physiol.* **5**, 803.
- Lipmann, F. & Tuttle, L. C. (1945). *J. biol. Chem.* **159**, 21.
- McIlwain, H. (1953a). *Biochem. J.* **55**, 618.
- McIlwain, H. (1953b). *J. Neurol. Psychiat.* **16**, 257.
- Maddock, S., Hawkins, J. E. & Holmes, E. (1939). *Amer. J. Physiol.* **125**, 551.
- Quastel, J. H. (1961). *The Chemistry of Brain Metabolism in Health and Disease*, p. 5. Springfield, Ill.: Charles C. Thomas.
- Racker, E. (1958). *Ciba Found. Symp.: The Regulation of Cell Metabolism*, p. 205. Boston: Little, Brown and Co.
- Riggs, B. C. (1945). *Amer. J. Physiol.* **145**, 211.
- Ross, G. (1955). *Pflug. Arch. ges. Physiol.* **261**, 334.
- Sacks, W. (1957). *J. appl. Physiol.* **10**, 37.
- Scholander, P. F. (1947). *J. biol. Chem.* **167**, 235.
- Sokoloff, L. (1959). In *The Handbook of Physiology*, sect. 1, vol. 3, p. 1843. Ed. by Field, J. Washington, D.C.: American Physiological Society.
- Sonnenschein, R. R., Stein, S. N. & Perot, P. L., jun. (1953). *Amer. J. Physiol.* **173**, 161.
- Stadie, W. C., Riggs, B. C. & Haugaard, N. (1944). *Amer. J. med. Sci.* **207**, 84.
- Stadie, W. C., Riggs, B. C. & Haugaard, N. (1945a). *J. biol. Chem.* **160**, 191.
- Stadie, W. C., Riggs, B. C. & Haugaard, N. (1945b). *J. biol. Chem.* **160**, 209.
- Sutherland, V. C., Burbridge, T. N. & Elliot, H. W. (1955). *Amer. J. Physiol.* **180**, 195.
- Tschirgi, R. P. (1962). *Fed. Proc.* **21**, 665.
- Van Goor, H. & Jongbloed, J. (1949). *Enzymologia*, **13**, 313.
- Van Harreveld, A. (1962). *Fed. Proc.* **21**, 659.

Biochem. J. (1963) **88**, 45

Labelling of Phospholipids from Inorganic [³²P]Phosphate in Brain Preparations

EFFECT OF ACETYLCHOLINE, CHLORPROMAZINE AND AZACYCLONOL

By W. L. MAGEE, J. F. BERRY, K. P. STRICKLAND AND R. J. ROSSITER

Department of Biochemistry, University of Western Ontario, London, Canada

(Received 22 October 1962)

When slices of brain tissue are allowed to respire in a suitable medium containing inorganic [³²P]-phosphate the phospholipids of the preparation become labelled with the isotope (Fries, Schachner & Chaikoff, 1942; Findlay, Magee & Rossiter, 1954; Strickland, 1954). The labelling of phospholipid in slices of guinea-pig brain is stimulated when acetylcholine together with physostigmine is added to the incubation medium (Hokin & Hokin, 1955a, b). A similar stimulation occurs with the addition of either chlorpromazine [2-chloro-

10-(3-dimethylaminopropyl)phenothiazine hydrochloride] or azacyclonol (diphenyl-4-piperidyl-methanol hydrochloride) (Magee, Berry & Rossiter, 1956). The incorporation of inorganic [³²P]phosphate into the phospholipids of brain slices of cats killed 2 hr. after an intraperitoneal injection of chlorpromazine (25 mg./kg.) also is increased (Strickland & Noble, 1961).

Since chlorpromazine and azacyclonol are representatives of two groups of compounds, each chemically quite distinct, that are known to

possess tranquillizing properties, it was considered of interest to study in more detail the increase in the labelling *in vitro* of phospholipids brought about by the addition of these substances to brain slices.

METHODS

Brain slices. The brains of guinea pigs (approx. 500 g. body wt.) killed by decapitation were quickly removed and placed on a chilled platform. Transverse slices were cut from each cerebral hemisphere free-hand with a safety-razor blade. Each slice was rapidly weighed on a torsion balance and transferred to a Warburg flask containing a measured quantity of Krebs mammalian Ringer solution: glucose (11 mM), NaCl (118 mM), KCl (4.7 mM), CaCl_2 (2.5 mM), MgSO_4 (1.2 mM), KH_2PO_4 (1.2 mM), buffered at pH 7.4 with tris (50 mM). The slices were added to the vessels in a random order. Usually each vessel contained 98–102 mg. of fresh tissue, comprising two slices from each cerebral hemisphere. In certain experiments, slices of liver, kidney and pancreas were prepared in a similar fashion.

Manometry. Appropriate solutions of the substances to be investigated were made up in the medium and, if necessary, the solutions were readjusted to pH 7.4 (glass electrode) with either HCl or NaOH. When all additions had been made, the main compartment of each Warburg flask contained 2.9 ml. of buffered medium, the centre well contained a filter-paper fan moistened with 0.2 ml. of 10% (w/v) KOH for CO_2 absorption, and the side arm contained 0.1 ml. (usually 15 μC) of neutralized carrier-free $\text{H}_3^{32}\text{PO}_4$ (Atomic Energy of Canada Ltd., Ottawa, Ontario).

The vessels were attached to their manometers and gassed for 10 min. with O_2 . The $\text{H}_3^{32}\text{PO}_4$ was tipped into the main compartment and then the vessels were transferred to a water bath maintained at 37.5°. The vessels were shaken to ensure adequate oxygenation of the slices. After 10 min. for temperature equilibration, the consumption of O_2 was measured at intervals for 2 hr. The reaction was stopped by pouring the contents of each flask into 1 ml. of 40% trichloroacetic acid contained in a centrifuge tube maintained at 0° in an ice bath. The tissue was homogenized in a Potter-Elvehjem homogenizer and the resulting suspension was centrifuged. Portions of the supernatant were taken for determinations of phosphocreatine (Rodnight & McIlwain, 1954) and lactic acid (Barker & Summerson, 1941). In some experiments portions of the supernatant were taken for isolation of ATP by the chromatographic procedure of Krebs & Hems (1953). Lipids were extracted, purified and the specific radioactivity of the lipid P was determined as described previously (Findlay *et al.* 1954).

In some experiments lipids from two pooled samples were extracted and subjected to the deacylation and chromatographic procedure of Dawson (1954). The specific radioactivity of each of the separated glycerylphosphoryl derivatives was determined as described by McMurray, Strickland, Berry & Rossiter (1957c). Since the labelled lipids were exposed to 10% trichloroacetic acid before extraction, the deacylated products were derived from both diacyl glycerophosphatides and plasmalogens.

Cell-free preparations. Dispersion (McMurray, Strickland, Berry & Rossiter, 1957b) and mitochondrial (McMurray, Berry & Rossiter, 1957a) preparations obtained from rat brain were incubated with inorganic [^{32}P]phosphate as described above.

Cholinesterase activity. The activity of cholinesterase in slices of guinea-pig brain was measured manometrically at 37.5° (Ammon, 1933). The final concentration of acetylcholine was 10 mM. Under these conditions the production of CO_2 was linear over the entire incubation period of 1 hr. All measurements were made in duplicate and were corrected for non-enzymic hydrolysis of acetylcholine. Esterase activity was expressed as $\mu\text{l. of CO}_2/\text{g. of fresh tissue/hr.}$

Materials. Chlorpromazine (Largactil) and azacyclonol (Frenquel) were generously supplied by Poulenc Ltd., Montreal, Canada, and William S. Merrell Co., Cincinnati, Ohio, U.S.A., respectively.

RESULTS

Brain slices

Acetylcholine. Table 1 shows that 10 mM-acetylcholine, in the presence of 0.15 mM-physostigmine, caused a consistent increase in the labelling of phospholipid, thus confirming the findings of Hokin & Hokin (1955a, b). Furthermore, the increase in the phospholipid labelling was not accompanied by any significant increase in the consumption of oxygen and production of lactic acid by the slices, or in the concentration of phosphocreatine. Although the concentration of ATP showed a small increase, the specific radioactivity of ATP was slightly decreased.

In two experiments in which the labelled phospholipids were separated by the deacylation and chromatographic procedure of Dawson (1954), the greatest stimulation was found in the phosphatidyl-inositol, phosphatidic acid and phosphatidylserine fractions (Table 2). There was some increase in the labelling of lecithin, but no increase in the labelling of phosphatidylethanolamine. In general, the results are in agreement with those of Hokin & Hokin (1955b, 1958).

Chlorpromazine. The addition of chlorpromazine to respiring slices of guinea-pig brain caused an increase in the incorporation of inorganic [^{32}P]phosphate into phospholipid (Table 3). Maximum incorporation was observed when the concentration of chlorpromazine was 0.1 mM. At this concentration there was no increase in the consumption of oxygen, production of lactic acid, concentration of phosphocreatine or in the concentration and specific radioactivity of ATP. When the concentration of chlorpromazine was increased to 1 mM, the labelling of phospholipid was virtually abolished and the respiration was greatly reduced, although aerobic glycolysis was unimpaired. There was a great reduction in both the concentration and specific radioactivity of ATP and in the concentration of phosphocreatine.

The results of 16 further experiments in which the concentration of chlorpromazine was 0.1 mM are shown in Table 1. The mean increase in label-

ling of phospholipid for all experiments was about 50 %. Again there was no significant change in aerobic glycolysis or in the concentration of ATP and phosphocreatine. Confirming the trend observed in Table 3, there was a slight, but statistically significant, decrease in the oxygen consumption and in the specific radioactivity of ATP.

When the labelled lipid was separated into individual phosphatides, there was an increase in the labelling of phosphatidylserine, phosphatidylinositol and phosphatidic acid (Table 2). There was no significant change in the labelling of phosphatidylethanolamine and the labelling of lecithin was decreased.

Azacyclonol. The addition of azacyclonol, a representative of a different group of tranquillizing drugs, to respiring guinea-pig brain slices caused a similar increase in the incorporation of inorganic [³²P]phosphate into phospholipid (Table 3). With azacyclonol, however, the optimum concentration was 1 mM rather than 0.1 mM. At this concentration there was no significant change in respiration and aerobic glycolysis, but a significant decrease in the concentration of ATP and phosphocreatine. With higher concentrations of azacyclonol (5 mM) there was a decrease in oxygen consumption and in the concentration of ATP, with a marked decrease in the concentration of phosphocreatine. The

Table 1. *Effect of 10 mM-acetylcholine (with 0.15 mM-physostigmine) and 0.1 mM-chlorpromazine on the metabolism of slices of guinea-pig brain*

	Acetylcholine			Chlorpromazine		
	Control	With drug	Percentage of control (mean ± s.e.m.)	Control	With drug	Percentage of control (mean ± s.e.m.)
Sp. radioactivity of phospholipid (counts/min./μg. of P)	37.3	54.5	146 ± 5 (8)	43.0	62.8	149 ± 6 (16)
Consumption of oxygen (μmoles/g. wet wt./hr.)	47.8	49.7	103 ± 4 (8)	46.5	43.4	93 ± 2 (12)
Production of lactic acid (μmoles/g. wet wt.)	173	179	106 ± 7 (8)	116	118	103 ± 6 (12)
Concn. of ATP (μmoles/g. wet wt.)	1.03	1.23	120 (2)	1.01	0.93	93 ± 10 (4)
Sp. radioactivity of ATP (counts/min./μg. of P)	3900	3350	89 (2)	4050	3530	87 ± 5 (4)
Concn. of phosphocreatine (μmoles/g. wet wt.)	1.92	1.84	96 ± 3 (8)	1.74	1.68	98 ± 5 (12)

Table 2. *Effect of 10 mM-acetylcholine (with 0.15 mM-physostigmine), 0.1 mM-chlorpromazine and 1 mM-azacyclonol on the incorporation of inorganic [³²P]phosphate into the phospholipids of slices of guinea-pig brain*

Sp. radioactivity of phospholipid (counts/min./μg. of P)							
Deacylated product	Drug	Acetylcholine		Chlorpromazine		Azacyclonol	
		Mean	Percentage of control	Mean	Percentage of control	Mean	Percentage of control
Total lipid P	—	138	153	129	138	103	180
	+	211		178		185	
Glycerolphosphorylcholine	—	98	136	119	71	152	35
	+	133		84.5		53.5	
Glycerolphosphorylethanolamine	—	12.2	98	14.5	103	13.7	53
	+	12.0		14.9		7.3	
Glycerolphosphorylserine	—	9.8	151	14.1	184	12.8	195
	+	14.8		26.0		25.0	
Glycerolphosphorylinositol	—	1910	163	1990	151	1890	168
	+	3120		3000		3170	
Glycerophosphate	—	3380	152	4210	134	2880	251
	+	5150		5660		7220	

Table 3. *Effect of chlorpromazine, azacyclonol and physostigmine on the metabolism of slices of guinea-pig brain*

Results are means of two experiments expressed as percentages of control values. Control values are means of six or four experiments.

Additions (mm)	Sp. radio-activity of phospholipid	Consumption of O ₂	Production of lactic acid	Concn. of ATP	Sp. radio-activity of ATP	Concn. of phospho-creatine
Chlorpromazine						
0	100	100	100	100	100	100
0.01	123	95	95	100	97	95
0.1	168	93	100	90	83	92
0.3	163	90	107	—	—	63
1.0	7	13	98	29	10	7
Azacyclonol						
0	100	100	100	100	100	100
0.01	100	106	105	87	100	104
0.1	127	102	103	—	—	94
1.0	173	101	92	78	92	67
5.0	26	58	121	62	34	15
Physostigmine						
0	100	100	100	—	—	—
0.1	98	93	111	—	—	—
0.5	113	104	120	—	—	—
1.0	108	97	120	—	—	—
5.0	130	92	135	—	—	—
10.0	121	102	141	—	—	—
Mean control value	38.5 counts/min./μg. of P	46.0 μmoles/g. wet wt./hr.	112 μmoles/g. wet wt.	1.06 μmoles/g. wet wt.	3880 counts/min./μg. of P	1.72 μmoles/g. wet wt.

specific radioactivity of ATP also was considerably reduced, but there was no consistent change in the production of lactic acid.

Examination of the individual phosphatides (Table 2) showed that azacyclonol (1 mm) caused a consistent increase in the labelling of phosphatidylserine, phosphatidylinositol and phosphatidic acid, whereas the incorporation of inorganic [³²P]-phosphate into lecithin and phosphatidylethanolamine was considerably reduced.

Cell-free preparations

It was found that neither chlorpromazine nor azacyclonol, when added to dispersions of rat brain in a wide range of concentrations, caused any increase in the labelling of phospholipid. As reported for the experiments with brain slices, higher concentrations of both chlorpromazine (1 mm) and azacyclonol (5 mm) were inhibitory.

With mitochondrial preparations the results were more equivocal. In each of two experiments there was a small increase in the incorporation of inorganic [³²P]phosphate into phospholipid when the concentration of chlorpromazine was 0.1 mm (Table 4). The same was true for azacyclonol in a concentration of 1 mm. Again, higher concentrations of both substances were inhibitory.

It would thus appear that some degree of structural integrity is necessary for the production of maximum stimulation by chlorpromazine or

Table 4. *Effect of chlorpromazine and azacyclonol on the incorporation of inorganic [³²P]phosphate into the phospholipids of mitochondria from rat brain*

Additions (mm)	Sp. radioactivity of phospholipid (counts/min./μg. of P)		
	Expt. 1	Expt. 2	Mean (% of control)
Chlorpromazine			
0	187	173	100
0.01	209	—	112
0.1	235	200	121
1.0	34.2	24.6	16
Azacyclonol			
0	138	162	100
0.1	141	165	102
1.0	154	174	109
5.0	38.0	18.2	19

azacyclonol, since only a small stimulation was observed in mitochondrial preparations and none in dispersions.

Other tissues

Table 5 shows that, whereas chlorpromazine in a concentration of 0.1 mm increased the incorporation of inorganic [³²P]phosphate into the phospholipids of slices of guinea-pig brain, the same concentration was without effect on liver, kidney or pancreas tissue. Azacyclonol (1.0 mm), on the other hand, caused an increase in the labelling of phospholipids in each of the four tissues studied.

Table 5. *Effect of 0.1 mM-chlorpromazine and 1 mM-azacyclonol on the incorporation of inorganic [³²P]phosphate into the phospholipids of slices of guinea-pig brain liver, kidney and pancreas*

Results are means of two experiments.

		Sp. radioactivity of phospholipid (counts/min./μg. of P)			
	Drug	Chlorpromazine		Azacyclonol	
		Mean	Percentage of control	Mean	Percentage of control
Brain	—	37.3		52.2	
	+	51.7	139	84.5	162
Liver	—	52.6		53.9	
	+	51.0	97	88.9	165
Kidney	—	194		226	
	+	193	99	336	149
Pancreas	—	60.6		53.8	
	+	58.9	97	107.5	200

Cholinesterase

Since many workers have reported that chlorpromazine is an inhibitor of both plasma (pseudo-) and red-cell (true) cholinesterase (see Erdős, Baart, Shanor & Foldes, 1958, for references), the possibility remains that the addition of chlorpromazine to respiring brain slices may cause an increase in the concentration of acetylcholine in the slice. Thus the observed stimulation of lipid labelling might be a manifestation of the acetylcholine stimulation described by Hokin & Hokin (1955*a*, *b*). The possibility seemed particularly important since Zeleny, Lindaur & Kozák (1957) reported that the administration of chlorpromazine to rats causes an increase in the concentration of acetylcholine in the brain.

Table 6 shows that 0.1 mM-chlorpromazine, the optimum concentration for lipid labelling, did not inhibit the cholinesterase activity of slices of guinea-pig brain. In fact, in concentrations of 0.1 and 0.01 mM, chlorpromazine appeared to cause an increase in the activity of the enzyme. At higher concentrations (1 mM) chlorpromazine was somewhat inhibitory, but it will be recalled that with this concentration of chlorpromazine the labelling of phospholipid was inhibited rather than stimulated (Table 3). These effects of chlorpromazine on the activity of cholinesterase are in agreement with those reported by others for brain preparations (Sinigaglia, 1955; Bernsohn, Namajuska & Cochran, 1955).

Table 6 also demonstrates the classical inhibitory effect of physostigmine on cholinesterase activity. The figures are in agreement with those reported (see Strickland & Thompson, 1955). As can be seen from Table 3, the addition of physostigmine to respiring slices of guinea-pig brain also caused some degree of increase in labelling of phospholipid, but only at high concentrations. In concentrations

Table 6. *Effect of chlorpromazine and physostigmine on the cholinesterase activity of slices of guinea-pig brain*

Additions (mm)	Cholinesterase activity (μl. of CO ₂ /g. of wet tissue/hr.)		Mean inhibition (%)
	Expt. 1	Expt. 2	
None	3630	3430	0
Chlorpromazine			
0.01	—	3630	—6
0.1	4230	3720	—13
1.0	—	2460	28
Physostigmine			
0.001	1380	1630	57
0.01	360	—	80
0.1	40	—	99

as great as 10 mM physostigmine had little effect on the oxygen consumption of the slices, although aerobic glycolysis was stimulated. At a concentration of 0.1 mM, which causes almost a complete inhibition of cholinesterase activity (Table 6), the incorporation of inorganic [³²P]phosphate into phospholipid was not increased (Table 3). The results also show that physostigmine alone, at a concentration of 0.15M (the concentration employed when it was added together with acetylcholine in the experiments reported in Tables 1 and 2), is not likely to have been responsible for the observed increases in phospholipid labelling.

It may thus be seen that chlorpromazine, in a concentration (0.1 mM) that is optimum for stimulation of phospholipid labelling, has a negligible effect on cholinesterase activity whereas physostigmine in the same concentration almost completely inhibits cholinesterase activity but has no effect on phospholipid labelling. Thus the hypothesis that chlorpromazine exerts its effect by inhibiting the activity of cholinesterase, so causing an increase in the concentration of acetylcholine in the slices, is not tenable.

Table 7. *Effect of acetylcholine and chlorpromazine, acetylcholine and azacyclonol and chlorpromazine and azacyclonol on the incorporation of inorganic [^{32} P]-phosphate into the phospholipids of slices of guinea-pig brain*

	Sp. radio- activity of phospholipid (% of control)
Expt. 1	
Chlorpromazine (0.1 mM)	126
Acetylcholine (10 mM) with physostigmine (0.15 mM)	122
Chlorpromazine + acetylcholine and physostigmine	142
Expt. 2	
Azacyclonol (1 mM)	180
Acetylcholine (10 mM) with physostigmine (0.15 mM)	136
Azacyclonol + acetylcholine and physostigmine	207
Expt. 3	
Chlorpromazine (0.1 mM)	122
Azacyclonol (1 mM)	127
Chlorpromazine + azacyclonol	141

Additive effects

The stimulatory effects of acetylcholine (plus physostigmine), chlorpromazine and azacyclonol on the incorporation of inorganic [^{32} P]phosphate into the phospholipids of slices of guinea-pig brain are almost additive when each pair of substances was tested in combination (Table 7). It thus seems likely that the mechanism whereby acetylcholine affects phospholipid labelling is different from that for either chlorpromazine or azacyclonol, and that each of these substances affects lipid labelling by a different mechanism.

DISCUSSION

The present experiments confirm the findings of Hokin & Hokin (1955*a, b*) that the addition of 10 mM-acetylcholine, together with 0.15 mM-physostigmine, to respiring slices of guinea-pig brain causes an increase in the incorporation of inorganic [^{32} P]phosphate into phospholipids, and also that the increase in phospholipid labelling is not accompanied by any increase in the consumption of oxygen, production of lactic acid, concentration of phosphocreatine or in the concentration and specific radioactivity of ATP. Hokin & Hokin (1953) showed that the increase in the incorporation of inorganic [^{32} P]phosphate into the phospholipids of pancreas slices, stimulated to secrete by the addition of carbamoylcholine, also was not accompanied by any change in the specific radioactivity of acid-soluble phosphorus compounds.

Apparently acetylcholine stimulates labelling from [^{3} H]inositol (Hokin & Hokin, 1958), but not

from [^{14} C]glycerol (Hokin & Hokin, 1955*a*, 1958). Hokin & Hokin (1959, 1960) suggested that the increase in labelling of phosphatidic acid is related to the transport of cations across biological membranes. Such a suggestion has its attractions. It has been criticized, however, on the grounds that the synthesis of phosphatidic acid is not sufficiently rapid (see, for example, Järnfeldt, 1961).

The experiments with chlorpromazine confirm work previously reported from this Laboratory (Magee *et al.* 1956; Strickland & Noble, 1961). The findings for chlorpromazine and azacyclonol resemble those for acetylcholine in that the increased incorporation of inorganic [^{32} P]phosphate into phospholipids is not accompanied by any increase in oxygen consumption, aerobic glycolysis or in the concentration or specific radioactivity of acid-soluble phosphate compounds. The cholinesterase experiments (Table 6) provide evidence against the hypothesis that chlorpromazine stimulates phospholipid labelling as a consequence of its anticholinesterase properties. In fact, differences in the pattern of the labelling of individual phosphatides, as well as the additive nature of the effects (Table 7), strongly suggest that the stimulation of lipid labelling produced by chlorpromazine is distinct from that caused by acetylcholine. Similarly, the effects of chlorpromazine and azacyclonol may be different in origin. The stimulatory effect of 0.1 mM-chlorpromazine on phospholipid labelling has been observed in this Laboratory in slices of guinea-pig, mouse, rat, rabbit and cat brain, but not in slices of guinea-pig liver, kidney or pancreas (Table 5). In contrast, azacyclonol (1 mM) increased the incorporation of inorganic [^{32} P]phosphate into the lipids of slices of all the tissues investigated (Table 5). Acetylcholine stimulates lipid labelling in brain and pancreas slices, but has no effect on slices of liver or kidney (Hokin & Hokin, 1953).

Although the stimulatory effect of chlorpromazine may be produced when the drug is present in quite low concentrations, such as are possible when the drug is used therapeutically, reports of the effect of tranquillizing drugs on phospholipid labelling in the brains of intact animals are conflicting. Ansell & Dohmen (1956) found that chlorpromazine inhibited the incorporation of inorganic [^{32} P]phosphate into the phospholipids of rat brain, and Ansell & Morgan (1958) reported that azacyclonol in large doses also was inhibitory. On the other hand, Wase, Christensen & Polley (1956) and Wase & Christensen (1960) reported that in some regions of the brain, notably in the hypothalamus, low concentrations of chlorpromazine stimulated the labelling of brain phospholipids. Grossi, Paoletti & Paoletti (1960) found that the administration of chlorpromazine to rats enhanced

the incorporation of radioactivity from [^{14}C]-acetate into the fatty acids of brain phospholipids. In the experiments of Strickland & Noble (1961), in which cats were killed 2 hr. after an intraperitoneal injection of chlorpromazine (25 mg./kg.), an increase in the ^{32}P -labelling of the phospholipids of brain slices was observed.

The mechanism whereby chlorpromazine stimulates phospholipid labelling is not known. Apparently some degree of structural integrity is necessary and the effect is confined to brain tissue, although it may be observed in many areas of the brain (Strickland & Noble, 1961). The stimulation is not confined to labelling from inorganic phosphate. It has been reported with [^{14}C]acetate, [^{14}C]mevalonic acid or [^{14}C]glucose (Grossi *et al.* 1960), and with [^{14}C]glycerol, [^{14}C]glycine or [^{14}C]serine, but not with [^{14}C]choline or [^{14}C]ethanolamine (Pritchard & Rossiter, 1963).

Grenell, Mendelson & McElroy (1955) reported that when chlorpromazine was injected into rats there was an increase in the concentration of ATP in the brain, although such an increase was not observed by Weiner & Huls (1961) if the animals were killed by rapid immersion of the whole body in liquid oxygen. It is known that chlorpromazine inhibits adenosine triphosphatases (Abood, 1955; Century & Horwitt, 1956; Bernsohn, Namajuska & Cochrane, 1956; Löw, 1959; Dawkins, Judah & Rees, 1960), so that the drug, acting either *in vivo* or *post mortem*, could be responsible for the increase in the concentration of ATP reported for brain. It seems unlikely, however, that an inhibition of adenosine triphosphatase is responsible for the increase in phospholipid labelling observed in the present experiments since: (a) chlorpromazine caused no increase in the concentration of ATP in brain-slice preparations (Tables 1 and 3); (b) the inhibition of brain adenosine triphosphatase is only slight at a concentration of 0.1 mM (Abood, 1955; Century & Horwitt, 1956; Bernsohn *et al.* 1956); (c) in other experiments fluoride, a potent inhibitor of adenosine triphosphatase, was found to decrease rather than increase the incorporation of inorganic [^{32}P]phosphate into the phospholipids of slices of guinea-pig brain; (d) if the site of action of chlorpromazine were primarily on adenosine triphosphatase, a greater effect would be expected in cell-free preparations, since in these preparations the destruction of ATP by the enzyme is greater. In cell-free preparations, chlorpromazine has little effect (Table 4), whereas fluoride stimulates lipid labelling (McMurray *et al.* 1957a, b).

Chlorpromazine and azacyclonol both stimulated the labelling of phosphatidic acid, phosphatidylinositol and phosphatidylserine (Table 2). Since the labelling of phosphatidic acid was increased, the effect on phosphatidylinositol is consistent with

current views on the formation of this lipid in brain (Thompson, Strickland & Rossiter, 1963). It is more difficult to explain the increased labelling of phosphatidylserine in terms of present suggestions about the formation of this phosphatide in mammalian tissue (Borkenhagen, Kennedy & Fielding, 1961).

In higher concentrations, chlorpromazine (1 mM) and azacyclonol (5 mM) were found to inhibit the consumption of oxygen by brain slices (Table 3), as has been reported frequently. The concentration of phosphocreatine and the concentration and specific radioactivity of ATP also was decreased (Table 3). These changes are most likely the result of the uncoupling of oxidation from phosphorylation, which has been reported for both brain and liver mitochondria (Abood, 1955; Bernsohn *et al.* 1956; Century & Horwitt, 1956; Berger, 1957; Decsi & Méhes, 1957; Dawkins, Judah & Rees, 1959). It seems probable that these decreases in the concentration and specific radioactivity of the acid-soluble phosphates are directly responsible for the reduction in phospholipid labelling observed with the higher concentrations of the drugs.

SUMMARY

1. The addition of chlorpromazine (0.1 mM) or azacyclonol (1 mM) increased the incorporation of inorganic [^{32}P]phosphate into the phospholipids of slices of guinea-pig brain without any increase in the consumption of oxygen, production of lactic acid, concentration and specific radioactivity of ATP or concentration of phosphocreatine.
2. The increase in labelling was confined to phosphatidic acid, phosphatidylinositol and phosphatidylserine.
3. Higher concentrations of chlorpromazine (1 mM) and azacyclonol (5 mM) caused a decrease in the labelling of phospholipids, accompanied by a reduction in the consumption of oxygen, concentration and specific radioactivity of ATP and concentration of phosphocreatine.
4. The results are discussed in relation to the mechanism whereby the addition of these substances causes an increase in the labelling of phospholipids.

This work was aided by a grant from the Medical Research Council of Canada.

REFERENCES

- Abood, L. G. (1955). *Proc. Soc. exp. Biol., N.Y.*, **88**, 688.
Ammon, R. (1933). *Pflüg. Arch. ges. Physiol.* **233**, 486.
Ansell, G. B. & Dohmen, H. (1956). *J. Neurochem.* **1**, 150.
Ansell, G. B. & Morgan, A. (1958). *Biochem. J.* **69**, 30r.
Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.

- Berger, M. (1957). *J. Neurochem.* **2**, 30.
- Bernsohn, J., Namajuska, I. & Cochrane, L. S. G. (1955). *Fed. Proc.* **14**, 182.
- Bernsohn, J., Namajuska, I. & Cochrane, L. S. G. (1956). *Proc. Soc. exp. Biol., N.Y.*, **92**, 201.
- Borkenhagen, L. F., Kennedy, E. P. & Fielding, L. (1961). *J. biol. Chem.* **236**, pc28.
- Century, B. & Horwitt, M. K. (1956). *Proc. Soc. exp. Biol., N.Y.*, **91**, 493.
- Dawkins, M. J., Judah, J. D. & Rees, K. R. (1959). *Biochem. J.* **72**, 204.
- Dawkins, M. J., Judah, J. D. & Rees, K. R. (1960). *Biochem. J.* **76**, 200.
- Dawson, R. M. C. (1954). *Biochim. biophys. Acta*, **14**, 374.
- Decsi, L. & Méhes, J. (1957). *Arch. exp. Path. Pharmac.* **230**, 462.
- Erdős, E. G., Baart, N., Shanor, S. P. & Foldes, E. F. (1958). *Arch. int. Pharmacodyn.* **117**, 163.
- Findlay, J. M., Magee, W. L. & Rossiter, R. J. (1954). *Biochem. J.* **58**, 236.
- Fries, B. A., Schachner, H. & Chaikoff, I. L. (1942). *J. biol. Chem.* **144**, 59.
- Grenell, R. G., Mendelson, J. & McElroy, W. D. (1955). *Arch. Neurol. Psychiat., Chicago*, **73**, 347.
- Grossi, E., Paoletti, P. & Paoletti, R. (1960). *J. Neurochem.* **6**, 73.
- Hokin, L. E. & Hokin, M. R. (1953). *J. biol. Chem.* **203**, 967.
- Hokin, L. E. & Hokin, M. R. (1955a). *Biochim. biophys. Acta*, **16**, 229.
- Hokin, L. E. & Hokin, M. R. (1955b). *Biochim. biophys. Acta*, **18**, 102.
- Hokin, L. E. & Hokin, M. R. (1958). *J. biol. Chem.* **233**, 818.
- Hokin, L. E. & Hokin, M. R. (1959). *J. biol. Chem.* **234**, 1387.
- Hokin, L. E. & Hokin, M. R. (1960). *J. gen. Physiol.* **44**, 61.
- Järnfeldt, J. (1961). *Exp. Cell Res.* **25**, 211.
- Krebs, H. A. & Hems, R. (1953). *Biochim. biophys. Acta*, **12**, 172.
- Löw, H. (1959). *Exp. Cell Res.* **16**, 456.
- McMurray, W. C., Berry, J. F. & Rossiter, R. J. (1957a). *Biochem. J.* **66**, 629.
- McMurray, W. C., Strickland, K. P., Berry, J. F. & Rossiter, R. J. (1957b). *Biochem. J.* **66**, 621.
- McMurray, W. C., Strickland, K. P., Berry, J. F. & Rossiter, R. J. (1957c). *Biochem. J.* **66**, 634.
- Magee, W. L., Berry, J. F. & Rossiter, R. J. (1956). *Biochim. biophys. Acta*, **21**, 408.
- Pritchard, E. T. & Rossiter, R. J. (1963). *Canad. J. Biochem. Physiol.* **41**, 341.
- Rodnight, R. & McIlwain, H. (1954). *Biochem. J.* **57**, 649.
- Sinigaglia, M. G. (1955). *Boll. Soc. ital. Biol. sper.* **31**, 1628.
- Strickland, K. P. (1954). *Canad. J. Biochem. Physiol.* **32**, 50.
- Strickland, K. P. & Noble, D. M. (1961). In *Regional Neurochemistry*, p. 489. Ed. by Kety, S. S. & Elkes, J. Oxford: Pergamon Press Ltd.
- Strickland, K. P. & Thompson, R. H. S. (1955). *Biochem. J.* **60**, 466.
- Thompson, W., Strickland, K. P. & Rossiter, R. J. (1963). *Biochem. J.* **87**, 136.
- Wase, A. W. & Christensen, J. (1960). *Arch. gen. Psychiat.* **2**, 171.
- Wase, A. W., Christensen, J. & Polley, E. (1956). *Fed. Proc.* **15**, 496.
- Weiner, N. & Huls, H. N. (1961). *J. Neurochem.* **7**, 180.
- Zeleny, A., Lindaur, V. & Kozák, J. (1957). *Arch. exp. Path. Pharmac.* **231**, 593.

Biochem. J. (1963) **88**, 52

The Intracellular Distribution of Solanesol and Plastoquinone in Green Leaves of the Tobacco Plant

BY J. STEVENSON, F. W. HEMMING AND R. A. MORTON
Department of Biochemistry, University of Liverpool

(Received 12 November 1962)

Plastoquinone (koflerquinone) (I) has been isolated in relatively large quantities from the green leaves of a number of different plants (Crane,

1959a; Crane & Lester, 1958; Kofler, 1946; Kofler *et al.* 1959). Further, the quinone has been shown to be associated with the chloroplasts of green