ether layer, nitrogen was bubbled through the reaction mixture until no further odor of ether was given off. The yield of malonyl coenzyme A so prepared was 78% as measured by CoA disappearance by the nitroprusside test. All of the thiocresol produced was removed by the extraction as shown by a negative nitroprusside test on a similarly treated blank solution containing 5 μmoles of thiocresol. The hydroxamic acid derivative of the malonyl coenzyme A was chromatographed according to the method of STADTMAN AND BARKER[5] and found to be identical to a similarly prepared malonyl monohydroxamate from analytically pure mono-*p*-thiocresyl malonate.

Further work is being done using other aromatic thiols which might give better yields of intermediate thioester or of malonyl coenzyme A.

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A new phosphoinositide containing four phosphates per inositol

In a previous study it was found that if the total lipid extract from brain cytoplasmic particulate fractions which had been incubated with ^{32}P under conditions of oxidative phosphorylation was chromatographed according to a method which separates the usual phosphatides[1], considerable amounts of radioactivity streaked[2]. This streaky material could, however, be resolved into discrete spots, termed A ($R_F = 0.06$), B ($R_F = 0.30$), and C ($R_F = 0.51$), if the lipid extract was chromatographed in phenol–ammonia on silicic acid-impregnated paper[2]. This communication deals mainly with the isolation and characterization of the water-soluble products of mild alkaline hydrolysis of the material in Spot B.

Spots B and C were not present in amounts sufficient to give a phosphorus stain on paper chromatograms, even when the chromatograms were maximally loaded with respect to total lipid present in the extracts. The spots were therefore routinely assayed by their radioactivity. It was found that Spots B and C were formed on incubation of brain-cortex slices in the bicarbonate saline of KREBS AND HENSELEIT[3] containing glucose and $^{32}P_i$.

10 kg of beef brain were homogenized with 0.3 N $HClO_4$, and the residue was serially extracted with a total of 40 l of ethanol – chloroform (1:1). After washing with 0.1 N HCl, the chloroform extracts were concentrated and the phospholipids were precipitated with acetone. The total yield of lipid at this stage was 258 g. The

phospholipids were deacylated by a modified method of DAWSON[4] and then brought to about pH 3.5 with Dowex-50 (H^+). In order to prepare radioactive marker, brain-cortex slices were incubated with $^{32}P_i$, and a radioactive band corresponding to Spot B was cut out from the phenol–ammonia chromatogram and was deacylated by the method of DAWSON[4]. The deacylated radioactive material was added to the large-scale

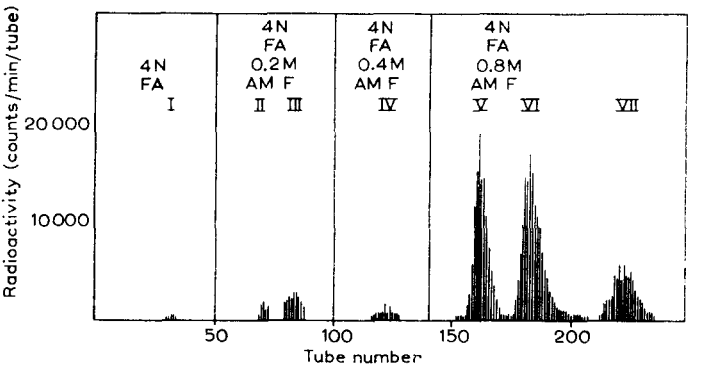

Fig. 1. Chromatography on Dowex-1 (formate) of the products of mild alkaline hydrolysis of radioactive "Spot B". FA, formic acid; AM F, ammonium formate.

non-radioactive preparation. Dowex-1 (formate) was added to the hydrolysate until all of the radioactivity was adsorbed. The resin was poured onto a column and washed with water followed by 2.9 N formic acid. 0.8 N ammonium formate in 4 N formic acid was then passed through the column, and the eluate was collected. Ammonium formate was removed from the eluate by successively treating with Dowex-50 (H^+) and lyophilizing. The lyophilized material was dissolved in water, applied to a fresh Dowex-1 (formate) column, and gradient elution was started[5]. The mixing vessel contained 250 ml of water; 10-ml fractions were collected. Aliquots from each tube were counted and assayed for total phosphorus content. Fig. 1 shows the elution pattern obtained. The three peaks of ^{32}P radioactivity showed a distribution of phosphorus as determined chemically which coincided exactly with the distribution

TABLE I

ANALYTICAL DATA ON NEW PHOSPHOINOSITIDE

For inositol assays the samples were first hydrolyzed in sealed tubes in 6 N HCl for 48 h in the autoclave and then dried in the oven overnight at 100°. Glycerol was determined by the method of HANAHAN AND OLLEY[6]; α-substituted glycerol was determined by the same method, only omitting the acid hydrolysis step.

Peak	*Ratios*			
	Glycerol: inositol	*α-substituted glycerol: inositol*	*Phosphorus: inositol*	
			Microbiological[6]	*Periodate*[7]
V	n.s.*	—	2.96 ± 0.10**	—
VI	0.75	0.91	3.90 ± 0.18	3.80
VII	n.s.	—	4.08 ± 0.24	3.74

* No significant amounts detected.
** Standard error of mean.

of radioactivity in each peak; this indicated that the radioactivity and the phosphorus determined chemically were in the same compound. The yield of cyclohexylamine salts of Peaks V, VI, and VII were 130 mg, 228 mg, and 19.5 mg, respectively. Analyses of the major radioactive peaks are shown in Table I. Peak V contained no significant glycerol but contained phosphate and inositol which gave a ratio which agrees closely with 3:1. Peak VI contained phosphate, α-substituted glycerol, and inositol which agreed closely with a ratio of 4:1:1. Peak VII contained no significant glycerol but contained phosphate and inositol which agreed closely with a ratio of 4:1. These data indicate that Spot B contains a tetraphosphoinositide. Elementary analysis of the barium salt of Peak VI gave: C, 18.2; H, 2.75; and P, 20.6 % (corrected to the barium-free anion). This shows fairly good agreement with the theoretical percentage composition for glycerol phosphoryl inositol triphosphate, (Calc.: C, 19.1; H, 2.65; and P, 21.8 %). Our preliminary interpretation of the results is that Peak VI is glyceryl phosphoryl inositol triphosphate, and that Peak V and Peak VII are probably further degradation products, being, respectively, inositol triphosphate and inositol tetraphosphate. Since these substances were obtained by deacylation of the intact lipid the data suggest that the mother substances may be phosphatidyl inositol triphosphate, which is the next higher homologue of the series, phosphatidyl inositol, phosphatidyl inositol monophosphate[9–11], and phosphatidyl inositol diphosphate[10,11]. It is likely to differ from the phosphoinositide isolated from brain by KLENK AND HENDRICKS[12], since they obtained complex products containing hexosamine and hexoses (probably in glycosidic linkage with inositol), ethanolamine, and phosphate, on hydrolysis in 0.1 N $Ba(OH)_2$.

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