After treatment of the labeled glycogen with β amylase, the radioactivity was recovered in the liberated maltose.

While G-6-P increased the activity of yeast glycogen synthetase, the stimulation obtained was usually less than 2-fold (see Table II) as compared with 15- to 20-fold in the case of the animal enzymes[1,2].

A full report of these findings will be published elsewhere.

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The identification of lysolecithin in lipid extracts of brain

The presence of lysolecithin in lipid extracts of a number of mammalian tissues has recently been reported[1–5]. Its presence in extracts of brain has not, as far as we are aware, been described, although PIGHINI[6] in 1932 had obtained an alcohol-soluble, ether-insoluble substance from human brain which, both from its solubility properties and from certain of its biological actions, appeared to resemble a lysophosphatide. It was decided therefore to re-investigate PIGHINI's work, using more modern chromatographic methods for the separation of lysolecithin. Initial experiments showed that lipid extracts of normal human brain, prepared by PIGHINI's method, contained a haemolytic substance having the same R_F value as lysolecithin. We therefore next decided to extract the brain lipids by the method of FOLCH *et al.*[7] and to characterise this haemolytic substance more precisely.

Human brains were obtained post-mortem as soon as possible after death from cases in which there had been no clinical evidence of nervous disorder. Meninges were removed and the surface of the brain washed free from blood in gently running tap-water. Cerebral cortical grey matter was then dissected off and extracted. In addition, a number of scattered plaques were dissected from the unfixed brain of a case of multiple sclerosis.

One specimen of a normal human frontal pole was obtained post-operatively, immediately placed in a vacuum flask at 0°, brought to the laboratory and treated as above. Whole rat brains were also used, and extracted immediately after killing by decapitation.

The choline-containing phosphatide fractions were separated from the total lipid extracts[7] on Al_2O_3 columns and then applied to silicic acid-impregnated papers[8]. Chromatograms were developed with $CHCl_3$–CH_3OH (4:1, v/v). Egg lysolecithin[9] was used as a marker.

Selected lanes were cut from each paper, stained with phosphomolybdic acid, washed and reduced with $SnCl_2$[10]. Areas corresponding to the lysolecithin spots were cut out from unstained lanes and eluted with CH_3OH–$CHCl_3$ (5:1, v/v). The eluates were evaporated in a rotary evaporator at 40° and dissolved for analysis in a known volume of $CHCl_3$.

Total P and P in the alkali-labile phospholipids (following hydrolysis by the method of SCHMIDT *et al.*[11]) was determined as described previously[12]. Fatty acids were determined titrimetrically after hydrolysis of lipid samples for 2 h in 2 N NaOH, followed by acidification and extraction of the fatty acids with ether. Choline was estimated by the method of WEBSTER[13], and haemolytic activity tested by the procedure of COLLIER[14].

Paper chromatograms of the choline phosphatides obtained from human brain have regularly shown the presence of 3 spots staining with phosphomolybdic acid, the slowest of which has an R_F resembling that of lysolecithin. Elution of this slowest spot from unstained papers, followed by re-chromatography on paper, showed the presence of a single spot with R_F corresponding to that of lysolecithin (Fig. 1).

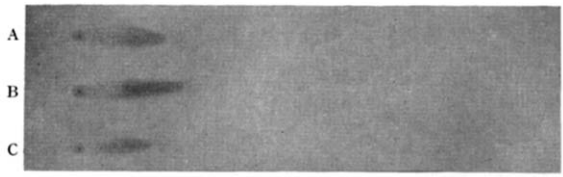

Fig. 1. Chromatogram of human brain lysolecithin. A = Brain lysolecithin. B = Brain lysolecithin + added egg lysolecithin. C = Egg lysolecithin.

After elution from unstained papers this substance was in each experiment found to be haemolytic and to give total P:alkali-labile lipid P:choline:fatty acid ratios close to 1:1:1:1 (Table I). These data indicate to us therefore that total lipid extracts of brain contain appreciable amounts of lysolecithin. Confirmatory evidence of a preliminary nature has been obtained by infrared spectroscopy kindly carried out by Dr. W. L. G. GENT. It is possible of course that the lysolecithin in these extracts may have been formed in the brain post-mortem, or during the chemical manipulations involved in its isolation. It has, however, been isolated from the post-operative specimen of human brain in amounts comparable to those present in the post-mortem material, and is also present in rat brain extracted within 3 min of killing. It has also been shown that storage of the total lipid extract at —10° for 48 h causes no apparent increase in the size of the lysolecithin spot; furthermore, when lecithin was taken through the entire procedure of extraction and chromato-

TABLE I

ANALYTICAL DATA ON THE ELUATES

Tissue	μmoles/ml eluate solution				Molar ratios (A:B:C:D)
	A Total P	B Alkali-labile lipid P	C Choline	D Fatty acids	
Rat brain	0.31	0.29	0.30	—	1 : 0.93 : 0.98 : —
Rat brain	4.57	4.43	4.57	4.56	1 : 0.97 : 1.00 : 1.00
Rat brain	7.06	7.48	7.01	6.65	1 : 1.06 : 0.99 : 0.94
Post-operative human brain	0.47	0.41	0.48	—	1 : 0.87 : 1.02 : —
Post-mortem human brain	7.10	6.80	6.00	6.40	1 : 0.96 : 0.85 : 0.90
Post-mortem human brain	1.44	1.44	1.51	1.52	1 : 1.00 : 1.05 : 1.06
Post-mortem human brain	1.60	1.52	1.62	1.70	1 : 0.95 : 1.01 : 1.06
Post-mortem human brain	0.86	0.90	0.88	0.93	1 : 1.05 : 1.02 : 1.08
Post-mortem human brain	1.51	1.54	1.57	1.61	1 : 1.02 : 1.04 : 1.07
Plaque tissue from brain of case of multiple sclerosis	0.89	0.92	0.90	—	1 : 1.03 : 1.01 : —

graphic separation used in the brain experiments no evidence of lysolecithin formation was observed.

Although our methods are not yet entirely suitable for quantitative work (and for this reason the data in Table I are expressed as μmole/ml eluate solution), the amounts of lysolecithin present would appear to correspond to 1–4 μmoles/g fresh tissue. Further work is now in progress to put these observations on a quantitative basis.

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