

- Meyman-Blanchet, T., Zajdela, F. & Chaix, P. (1959). *Biochim. biophys. Acta*, **36**, 596.
- Huggett, A. St G. & Nixon, D. A. (1957). *Biochem. J.* **66**, 12P.
- Linnane, A. W., Vitols, E. & Nowland, P. G. (1962). *J. Cell Biol.* **13**, 345.
- Magasanik, B. (1961). *Cold Spr. Harb. Symp.* **26**, 249.
- Meek, G. A. (1958). *Proc. 4th nat. Conf. Electron Microscopy, Berlin*, **1**, 187.
- Millonig, G. (1961). *J. biochem. biophys. Cytol.* **11**, 736.
- Minnaert, K. (1961). *Biochim. biophys. Acta*, **50**, 23.
- Novikoff, A. B. (1961). In *The Cell*, vol. 2, p. 299. Ed. by Brachet, J. & Mirsky, A. E. New York: Academic Press Inc.
- Palade, G. E. (1953). *J. Histochem. Cytochem.* **1**, 188.
- Pennington, R. J. (1961). *Biochem. J.* **80**, 649.
- Serlin, I. & Cotzias, G. C. (1955). *J. biol. Chem.* **215**, 263.
- Slonimski, P. P. (1953). *La Formation des Enzymes Respiratoires Chez la Levure*. Paris: Masson et Cie.
- Slonimski, P. P. (1955). *Proc. 3rd int. Congr. Biochem., Brussels*, p. 242.
- Strittmatter, C. F. (1957). *J. gen. Microbiol.* **16**, 169.
- Thyagarajan, T. R., Conti, S. F. & Naylor, H. D. (1961). *Exp. Cell Res.* **25**, 216.
- Tustanoff, E. R. & Bartley, W. (1962). *Biochem. J.* **84**, 40P.
- Vitols, E., North, R. J. & Linnane, A. W. (1961). *J. biochem. biophys. Cytol.* **9**, 689.
- Yotsuyanagi, Y. (1962a). *J. Ultrastruct. Res.* **7**, 121.
- Yotsuyanagi, Y. (1962b). *J. Ultrastruct. Res.* **7**, 141.

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## Quantitative Analysis of Phospholipids by Thin-Layer Chromatography

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The quantitative analysis of phospholipid mixtures extracted from different tissues still poses a problem. Several techniques are available (McKibbin, 1957; Lea, Rhodes & Stoll, 1955; Hanahan, Dittmer & Warashina, 1957; Marinetti, Erbland & Kochen, 1957; Schmidt, Fingerman & Thannhauser, 1962; Dawson, 1960; Collins, 1959; Rauser, Bauman, Kritchevsky, Heller & O'Brien, 1961) which have been used by investigators according to their preferences or the character of the problem. Each method has some inherent disadvantages. Several investigators have made use of thin-layer chromatography for quantitative analysis of phospholipids (Wagner, 1961; Jatzkewitz, 1961; Habermann, Bandtlow & Krusche, 1961; Honegger, 1962; Doizaki & Zieve, 1963; Robinson & Phillips, 1963). Recent progress in the qualitative separation of phospholipids by thin-layer chromatography (Skidmore & Entenman, 1962; Skipski, Peterson & Barclay, 1962; Skipski, Peterson, Sanders & Barclay, 1963) has facilitated adaptation of this technique to an improved, efficient and quantitative procedure. The present paper describes the application of thin-layer chromatography to the quantitative analysis of phospholipids in animal tissues.

### MATERIALS AND METHODS

*Standards and tissue phospholipids.* The origins of most of the reference compounds have been described by Skipski *et al.* (1962); additional phospholipids used were phos-

phatidic acids (generously given by Dr E. Baer, University of Toronto, and by Dr L. E. Hokin, University of Wisconsin), sodium salt of monophosphatidylinositol isolated from wheat sprouts (generously given by Dr M. Faure, Pasteur Institute, Paris) and lysophosphatidylcholine (General Biochemicals, Chagrin Falls, Ohio, U.S.A.).

Lipids were extracted from the pooled livers of five male adult albino rats with methanol-chloroform (1:2, v/v) and purified by the method of Folch, Ascoli, Lees, Meath & LeBaron (1951).

*Preparation of plates.* It is advisable to use a thicker layer of silica gel on plates for quantitative analysis of phospholipids than is generally used for qualitative analysis (0.5 mm. on adjustable Desaga applicator). Forty g. of Camag (Muttentz, Switzerland) silica gel, without calcium sulphate binder, was slurried with 90 ml. of 1 mM- $\text{Na}_2\text{CO}_3$  solution and transferred to the applicator. The plates (200 mm.  $\times$  200 mm.) were prepared in the usual manner (Stahl, 1958, 1962). This amount of slurry was sufficient to prepare three or four plates. The plates were allowed to dry at room temperature for 1-2 hr. and could then be stored for several days. Before the experiment, the plates were activated at 110° for 1 hr.

*Application of samples.* Samples (50  $\mu\text{l}$ . or less) were applied to the thin-layer chromatography plate with standardized micropipettes. Where the concentration of the applied material was high (approx. 30-40 mg./ml.) a correction factor was used for the lipid phosphorus remaining on the tip of the pipette after delivery. It was necessary to apply to a plate such a quantity of total lipid that each phospholipid species separated would contain at least 0.4-0.5  $\mu\text{g}$ . of phosphorus. Reference compounds, ranging in amounts from 15 to 50  $\mu\text{g}$ ., were applied together with tissue lipid extracts. For recovery experiments, approx. 100  $\mu\text{g}$ . (3-4  $\mu\text{g}$ . of phosphorus) was applied.

**Chromatography.** Chromatograms were developed with chloroform-methanol-acetic acid-water (25:15:4:2, by vol.). Extreme changes in air humidity may require slight adjustment in the volume of water in the developing solvent. Otherwise, conditions were identical with those described by Skipski *et al.* (1963). The average running time was 2 hr.

**Detection of spots.** The plates were air-dried at room temperature for 20 min. The spots were detected with iodine vapour (Sims & Larose, 1962) and encircled with a fine dissecting needle. Most of the iodine was allowed to evaporate before removal of the spots; a small amount of iodine did not interfere with the phosphorus determinations.

Other detection methods were employed to confirm the identity of the spots: (a) ninhydrin for phospholipids containing free amino groups; (b) the modified Dragendorff reagent (Wagner, Hörhammer & Wolff, 1961) for choline; (c) dinitrophenylhydrazine (Reitsema, 1954) and the Schiff reagent [as modified by Feulgen, Boguth & Andresen (1951) and adapted to paper chromatography by Hack (1953)] for plasmalogens. Esterified fatty acids were detected by hydroxylamine-ferric chloride spray (Whittaker & Wijesundera, 1952). As non-specific tests to reveal the presence of all lipid material, ammonium molybdate-perchloric acid spray (Wagner *et al.* 1961) or 40% (v/v) sulphuric acid spray (Mangold, 1961) was used in addition to iodine vapour.

**Removal of spots.** The areas of silica gel under the origin and above the solvent front were removed with a razor blade. Beginning with the origin of any one running lane, a drop of water was placed on the silica-gel area to be removed for analysis, and the silica gel was transferred to a centrifuge tube by a thin knife spatula. Water caused the silica gel to cohere so that almost the whole spot could be transferred quantitatively. To ensure complete transfer of material, the remaining periphery of the spot was scraped off the plate, and the plate placed vertically and tapped to allow the scrapings to fall on to glazed paper (Glassine). This powder was combined with previously removed material. With the plate held so that the origin was on the bottom, all spots up to the centre of the plate were removed as described. The plate was then turned around and the same procedure was repeated beginning with the solvent front and again working toward the mid-point of the plate. This procedure minimized loss of phospholipid material. The areas in which there were no lipid spots were also removed for analysis and thus, on completion, all of the silica gel was removed from the running lane. Three or four areas (on different levels), where no lipid material was applied, were taken as controls. In the controls, no phosphorus could be detected.

**Elution of phospholipids.** Each sample was eluted from the silica gel by suspending the powder in eluting solvent by gently tapping the tube. The first and second elutions were performed with the developing solvent by using 3 and 2 ml. portions respectively. After centrifuging, the solvent was removed with a capillary pipette. The third elution was made with 2 ml. of methanol, and the fourth with 2 ml. of methanol-acetic acid-water (94:1:5, by vol.). Samples with an expected phosphorus content within the standard-curve range (0.2-5.0  $\mu\text{g.}$ ) were transferred to the digestion tube directly; those with a much greater phosphorus content were transferred to a 10 ml. volumetric flask and an appropriate sample was taken for analysis. All sample

eluates were evaporated under nitrogen to approx. 2 ml., a convenient volume for phosphorus analysis.

**Phosphorus determinations.** A combination of the procedures of Beveridge & Johnson (1949) (digestion and colour development) and Bartlett (1959) and Shin (1962) (measurement of extinction) was used.

## RESULTS

Fig. 1 shows a chromatogram of standard phospholipids that were applied singly and as a mixture. Three reference phospholipids of the highest purity, phosphatidylinositol, phosphatidylcholine and lysophosphatidylcholine, were used for recovery studies. Satisfactory recovery was accomplished for all three phospholipids whether they were applied individually or as a mixture (Table 1).

The chromatographic separation of phospholipids from pooled rat liver is shown in Fig. 2. The individual spots were identified on the basis of the position of standard compounds and by the detection methods listed above. The following lipids were detected: lysophosphatidylcholine, sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, phosphatidylethanol-

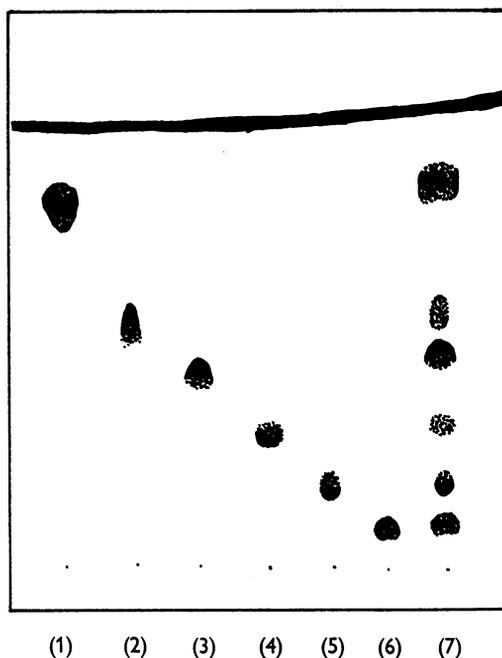


Fig. 1. Thin-layer chromatography of reference phospholipids. Compounds: (1) phosphatidylethanolamine, 50  $\mu\text{g.}$ ; (2) phosphatidylserine, 30  $\mu\text{g.}$ ; (3) phosphatidylinositol, 40  $\mu\text{g.}$ ; (4) phosphatidylcholine, 60  $\mu\text{g.}$ ; (5) sphingomyelin, 50  $\mu\text{g.}$ ; (6) lysophosphatidylcholine, 40  $\mu\text{g.}$ ; (7) mixture of (1)-(6). Separation was performed on a 0.5 mm. silica-gel layer. Detection method: 40% sulphuric acid spray.

Table 1. Recovery of standard phospholipids from silica gel after separation by thin-layer chromatography

Experimental details are in the text. The values for average recovery are given as means  $\pm$  s.d. with the numbers of experiments in parentheses.

Compounds	Applied singly		Applied as mixture	
	Amount applied (range) ( $\mu$ g. of P)	Average recovery of P (%)	Amount applied (range) ( $\mu$ g. of P)	Average recovery of P (%)
Phosphatidylinositol	2.15-3.48	100.5 $\pm$ 4.7 (4)	2.03-2.15	97.9 $\pm$ 3.9 (3)
Phosphatidylcholine	3.45	97.9 $\pm$ 1.2 (6)	3.21-3.46	99.7 $\pm$ 4.8 (3)
Lysophosphatidylcholine	3.34-3.46	99.6 $\pm$ 2.8 (6)	3.43-3.46	102.9 $\pm$ 3.3 (3)

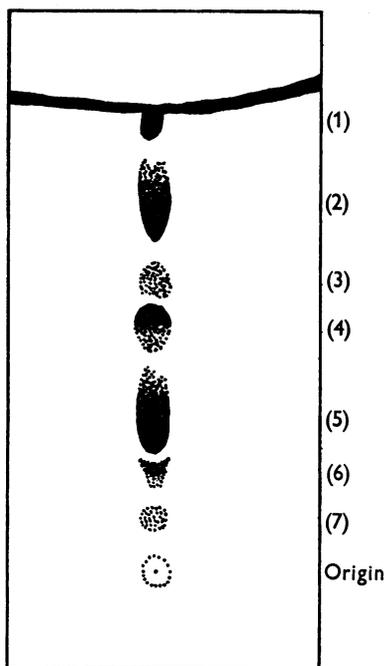


Fig. 2. Thin-layer chromatogram of total lipids extracted from pooled rat liver. A total of 2 mg. of lipid was applied. Each spot was identified as: (1) neutral lipids, cardiolipin and phosphatidic acid (tentative identification of the last two components); (2) phosphatidylethanolamine; (3) phosphatidylserine; (4) phosphatidylinositol; (5) phosphatidylcholine; (6) sphingomyelin; (7) lysophosphatidylcholine. Separation was performed on a 0.5 mm. silica-gel layer. Detection method: 40% sulphuric acid spray.

amine and several lipid components that moved with the solvent front. Phosphatidal compounds moved together with their corresponding phosphatidyl analogues. No hydrolysis of phosphatidal compounds was evident, as indicated by the clear well-defined spots with positive tests for plasmalogens and by the negative tests for aldehydes at the solvent front. The material located at the front was composed of the neutral lipids and a small amount of phospholipids free of amino groups or choline. A very small amount of phosphorus-

Table 2. Quantitative analysis and recovery of rat-liver phospholipids, expressed as percentages of total phosphorus

Details are given in the text. A total of 50.68  $\mu$ g. of P was applied. Results are given as means  $\pm$  s.d. of four replicate experiments on lipids from five pooled livers.

Spot no.	Compounds	Average recovery of P (%)
(1)	Phospholipids at the front (cardiolipin etc.)	5.12 $\pm$ 0.15
(2)	Phosphatidylethanolamine	25.32 $\pm$ 1.40
(3)	Phosphatidylserine	3.02 $\pm$ 0.26
(4)	Phosphatidylinositol	8.81 $\pm$ 0.36
(5)	Phosphatidylcholine	54.96 $\pm$ 1.40
(6)	Sphingomyelin	1.83 $\pm$ 0.07
(7)	Lysophosphatidylcholine	0.87 $\pm$ 0.13
(8)	Non-lipid phosphorus (at origin)	0.23 $\pm$ 0.08
Total recovery		100.84 $\pm$ 3.3

containing material, which was probably non-lipid, remained at the origin.

The relative amounts of liver phospholipids are given in Table 2. The major components were phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. The remaining phospholipids were present in amounts varying from 0.9 to 5.0%. Lysophosphatidylcholine was present consistently in all liver extracts studied with this procedure. Where phospholipids and neutral lipids were separated by silicic acid-column chromatography before separation on thin-layer chromatography, the amount of lysophosphatidylcholine tended to increase with storage of samples.

## DISCUSSION

The presence of both phosphatidyl and phosphatidal derivatives was observed in the spots identified as phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and phosphatidylcholine. Another disadvantage of the present method is that certain nitrogen-free phospholipids (cardiolipin, phosphatidic acid) cannot be differentiated. The value of 5.1% for the total quantity of these

compounds in liver phospholipids agrees with values reported by other investigators (Macfarlane, 1961; Macfarlane, Gray & Wheeldon, 1960; Hübscher & Clark, 1960; Dawson, 1960; Collins & Shotlander, 1961). In general, the results obtained for the composition of pooled rat-liver phospholipids are within the range reported in the literature (Schmidt, Benotti, Hershman & Thannhauser, 1946; Levine & Chargaff, 1952; Kainova, 1960; Creasey, Hankin & Handschumacher, 1961; Biezenski, 1962; Getz, Bartley, Stirpe, Notton & Renshaw, 1962).

Quantitative thin-layer chromatography of phospholipids is superior to quantitative paper chromatography in that a greater amount of starting material can be applied without sacrificing completeness of separation; up to 10 mg. can be used if the thickness of the silica-gel layer on the plate is increased. Further, thin-layer chromatography makes it possible to obtain clear-cut separations of the components without 'tailing', sufficient material may be recovered to perform several chemical tests, radioactivity can be measured and gas-liquid-chromatographic analyses of fatty acids or aldehydes can be performed. Quantitative thin-layer chromatography possesses an advantage over single-column chromatography in that a more complete separation of the majority of individual components can be attained with greater efficiency.

Dawson and other investigators (Dawson, 1960; Dawson, Hemington & Davenport, 1962; Morgan, Hanahan & Ekholm, 1963) have reported procedures in which the deacylated moieties of phospholipids are chromatographed, and from which information on phosphatidyl and phosphatidal analogues can be obtained. However, these procedures give no information on the presence of lyso-compounds.

The principal advantage of the method described above over previously described methods for quantitative thin-layer-chromatographic analyses of phospholipids (Habermann *et al.* 1961; Honegger, 1962; Doizaki & Zieve, 1963; Robinson & Phillips, 1963) is the more efficient separation: phosphatidylinositol and phosphatidylserine were not separated by the previous methods. The elution of samples from silica gel before digestion instead of direct digestion of silica gel with adsorbed samples makes it simpler to analyse samples with a wider range of phosphorus content and also permits other chemical analyses of the eluates.

#### SUMMARY

1. A procedure has been described for the quantitative separation of phospholipids by thin-layer chromatography.

2. When standard compounds were applied singly and as mixtures to silica-gel plates, average recoveries were within the range 97.9–102.0%.

3. A well-defined separation of phospholipids of rat liver was achieved on silica-gel plates, with an average recovery of 100.8% of the total phosphorus applied.

4. Analyses of lipids from pooled rat livers gave the following results: phosphatidylcholine, 55.0%; phosphatidylethanolamine, 25.3%; phosphatidylinositol, 8.8%; phosphatidylserine, 3.0%; sphingomyelin, 1.8%; lysophosphatidylcholine, 0.9%; phosphatidic acid and cardiolipin, 5.1%.

5. This procedure, which permits the determination of the main known phospholipids, is simple, rapid, reproducible and reliable.

#### REFERENCES

- Bartlett, G. R. (1959). *J. biol. Chem.* **234**, 466.  
 Beveridge, J. M. R. & Johnson, S. E. (1949). *Canad. J. Res. E*, **27**, 159.  
 Biezenski, J. J. (1962). *J. Lipid Res.* **3**, 120.  
 Collins, F. D. (1959). *Biochem. J.* **72**, 281.  
 Collins, F. D. & Shotlander, V. L. (1961). *Biochem. J.* **79**, 321.  
 Creasey, W. A., Hankin, L. & Handschumacher, R. E. (1961). *J. biol. Chem.* **236**, 2064.  
 Dawson, R. M. C. (1960). *Biochem. J.* **75**, 45.  
 Dawson, R. M. C., Hemington, N. & Davenport, J. B. (1962). *Biochem. J.* **84**, 497.  
 Doizaki, W. M. & Zieve, L. (1963). *Proc. Soc. exp. Biol., N. Y.*, **113**, 91.  
 Feulgen, R., Boguth, W. & Andresen, G. (1951). *Hoppe-Seyl. Z.* **287**, 90.  
 Folch, J., Ascoli, I., Lees, M., Meath, J. A. & LeBaron, N. F. (1951). *J. biol. Chem.* **191**, 833.  
 Getz, G. S., Bartley, W., Stirpe, F., Notton, B. M. & Renshaw, A. (1962). *Biochem. J.* **83**, 181.  
 Habermann, I., Bandtlow, G. & Krusche, B. (1961). *Klin. Wschr.* **39**, 816.  
 Hack, M. H. (1953). *Biochem. J.* **54**, 602.  
 Hanahan, D. J., Dittmer, J. C. & Warashina, E. (1957). *J. biol. Chem.* **228**, 685.  
 Honegger, C. G. (1962). *Helv. chim. acta*, **45**, 2020.  
 Hübscher, G. & Clark, B. (1960). *Biochim. biophys. Acta*, **41**, 45.  
 Jatzkewitz, H. (1961). *Hoppe-Seyl. Z.* **326**, 61.  
 Kainova, A. S. (1960). *Biokhimiya*, **25**, 540.  
 Lea, C. H., Rhodes, D. N. & Stoll, R. D. (1955). *Biochem. J.* **60**, 353.  
 Levine, C. & Chargaff, E. (1952). *Exp. Cell Res.* **3**, 154.  
 Macfarlane, M. G. (1961). *Biochem. J.* **78**, 44.  
 Macfarlane, M. G., Gray, G. M. & Wheeldon, L. W. (1960). *Biochem. J.* **77**, 626.  
 McKibbin, J. M. (1957). *Fed. Proc.* **16**, 835.  
 Mangold, H. K. (1961). *J. Amer. Oil Chem. Soc.* **38**, 708.  
 Marinetti, G. V., Erbland, J. & Kochen, J. (1957). *Fed. Proc.* **16**, 837.  
 Morgan, T. E., Hanahan, D. J. & Ekholm, J. (1963). *Fed. Proc.* **22**, 414.

- Rausser, G., Bauman, A. J., Kritchevsky, G., Heller, D. & O'Brien, J. (1961). *J. Amer. Oil Chem. Soc.* **38**, 544.
- Reitsema, R. H. (1954). *Analyt. Chem.* **26**, 960.
- Robinson, N. & Phillips, B. M. (1963). *Clin. chim. Acta*, **8**, 385.
- Schmidt, G., Benotti, J., Hershman, B. & Thannhauser, S. J. (1946). *J. biol. Chem.* **166**, 505.
- Schmidt, G., Fingerman, L. H. & Thannhauser, S. J. (1962). In *Cerebral Sphingolipodoses*, p. 197. Ed. by Aronson, S. M. & Volk, B. W. New York and London: Academic Press Inc.
- Shin, Y. S. (1962). *Analyt. Chem.* **34**, 1164.
- Sims, R. P. A. & Larose, J. A. G. (1962). *J. Amer. Oil Chem. Soc.* **39**, 232.
- Skidmore, W. D. & Entenman, C. (1962). *J. Lipid Res.* **3**, 471.
- Skipski, V. P., Peterson, R. F. & Barclay, M. (1962). *J. Lipid Res.* **3**, 467.
- Skipski, V. P., Peterson, R. F., Sanders, J. & Barclay, M. (1963). *J. Lipid Res.* **4**, 227.
- Stahl, E. (1958). *Chemikerztg.* **82**, 323.
- Stahl, E. (1962). In *Dünnschicht-Chromatographie*, p. 5. Ed. by Stahl, E. Berlin, Göttingen and Heidelberg: Springer-Verlag.
- Wagner, H. (1961). *Fette, Seifen, Anstrichmittel*, **63**, 1119.
- Wagner, H., Hörhammer, L. & Wolff, H. (1961). *Biochem. Z.* **334**, 175.
- Whittaker, V. P. & Wijesundera, S. (1952). *Biochem. J.* **51**, 348.

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## Induction of Alkaline Phosphatase in a Subcellular Preparation from *Escherichia coli*

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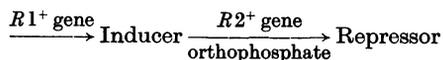
(Received 25 June 1963)

The synthesis of alkaline phosphatase in the cells of *Escherichia coli* has been shown to be under the control of three genes (Garen & Echols, 1962*a, b*). Two of these genes (designated *R1* and *R2*) control the quantitative aspects of enzyme synthesis, and the third (*P*) controls the structure of the enzyme. With the wild-type bacteria (Hfr strain K10 of *E. coli* K12) the three genes are present ( $R1^+R2^+P^+$ ), and regulate the synthesis of alkaline phosphatase according to the content of orthophosphate in the medium (Torriani, 1960). With a high concentration of orthophosphate in the medium, wild-type bacteria have only a low content in alkaline phosphatase. When, however, the phosphate becomes limiting, a high content of alkaline phosphatase is produced. A mutation of either the *R1* or the *R2* gene produces strains of bacteria that have been termed 'constitutive' mutants because the strain is no longer repressible by high concentrations of orthophosphate.

From other genetic studies Echols, Garen, Garen & Torriani (1961) have shown that, for both *R1* and *R2* genes, repressibility is dominant over constitutivity in heterozygous diploids that carry a constitutive gene either *cis* or *trans* to the active structural gene for alkaline phosphatase. The two genes then appear to control the formation of a repressor of alkaline phosphatase synthesis.

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Further, it has been suggested, from a study of the synthetic capacity of  $R1^-$  mutants and a partial diploid  $F'$  strain carrying  $R1^+P^-$  on the episome and  $R1^-R2^+P^+$  on the chromosome (Echols, 1961), that the relation between the genes in control of alkaline phosphatase is of the form:



It was decided to examine the induction of alkaline phosphatase *in vitro* by using a subcellular membrane fraction prepared from the wild-type strain of *E. coli* after the bacteria had been grown in the presence of orthophosphate. The present paper describes the conditions and factors effecting the induction of alkaline phosphatase in an *E. coli* subcellular preparation ( $P_1$ ) prepared in a manner similar to that described by Nisman, Fukuhara, Demailly & Genin (1962).

### MATERIALS AND METHODS

*Materials.* Ribonucleoside triphosphates were obtained from Pabst Laboratories (Milwaukee, Wis., U.S.A.),  $NAD^+$ ,  $NADH$ ,  $NADP^+$ ,  $NADPH$  from Boehringer und Soehne G.m.b.H. (Mannheim, Germany), amino acids (L-form) from California Biochemical Corp. (Los Angeles, U.S.A.), and diethylaminoethylcellulose (DEAE-cellulose) from Eastman Organic Chemicals (U.S.A.). Phosphoenolpyruvate, pyruvate kinase, bovine serum albumin (crystalline), *p*-nitrophenyl phosphate, alkaline phosphatase,