acetate solvent[2] combined with the point of elution from the Dowex Cl′ column suggests that it may be guanine diphosphate.

0.01 N HCl/0.1 N NaCl: The sole component of this fraction was ATP which was determined by base analysis and by enzymic assay[6].

Several other unidentified components were located in 0.01 *N* HCl/0.03 *N* NaCl and 0.01 *N* HCl/0.05 *N* NaCl which will be the subject of a future detailed communication.

While the amount of UDPG isolated from mammary gland is of the order of 15 μ moles per 100 g, the amount isolated from the liver of the guinea pig under identical conditions was rarely in excess of 5 μ moles per 100 g. This higher concentration of UDPG in the mammary gland suggests that it may be functional in lactose synthesis where it probably acts as coenzyme of the glucose–galactose transformation[7].

The fact that the UDPG isolated from mammary gland does not pyrophosphorylate to the theoretical amount of UDPG present (based on U.V. absorption) also suggests that UDPGal may be present in the mammary gland.

These observations will be the subject of further study.

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PHOSPHOPROTEIN PHOSPHATASE FROM RAT SPLEEN

by

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We have presented evidence recently for the presence of a specific phosphoprotein phosphatase in ox spleen[1]. Though we were able to separate the enzyme from phosphomonoesterases to a certain extent, the final preparation was slightly active against glycerophosphate. A purified preparation of phosphoprotein phosphatase from rat spleen has been found in the present investigation to be completely free from phosphomonoesterase activity. The action of the enzyme has been tested on several other substrates, including β-casein which has recently been found by PERLMANN[2,3] to be resistant to the action of phosphomonoesterases.

The preparation of the enzyme from rat spleen was effected according to the method previously described by us[1]. The final acetone fractionation step was however omitted. The specific activities of such preparations were of the order of 150 units and represented a 40-fold purification of the enzyme.

For the estimation of enzyme activity 0.2 ml of the purified preparation was mixed with 1 ml of substrate solution, 0.4 ml of 0.01 *M* thioglycollic acid (activator), 1 ml of pH 5.8 acetate buffer and water to a final volume of 4 ml. After incubation at 37° C for 3 hours, the reaction was stopped by the addition of 2 ml of 20% trichloracetic acid and the inorganic phosphorus estimated in the filtrate. The results obtained with the several substrates are summarised in Table I.

It will be evident that while all the phosphoproteins tested are dephosphorylated by this enzyme, no liberation of phosphorus takes place with glycerophosphate. Further, no activity could be demonstrated with the latter substrate by either a change of pH or by the addition of Mg^{+2}. Dephosphorylation of the phosphoproteins was very low in the absence of the activator. In this respect the enzyme resembles the phosphoprotein phosphatase from ox spleen[1].

It is of interest to note that the enzyme attacks all the three casein fractions employed with equal vigour. The pH-activity curves for the three substrates are also quite similar, maximum activity being obtained in each case at pH 5.8. Further, the MICHAELIS constants for their hydrolysis are the same (average value, 0.55 m*M* of protein P) proving thereby that they have the same affinity

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TABLE I

ACTION OF PHOSPHOPROTEIN PHOSPHATASE ON DIFFERENT SUBSTRATES

Substrate	% *Phosphorus split*	
	with activator	*without activator*
"Unfractionated" Casein*	81	18
α-Casein*	78	8
β-Casein*	80	18
Phosvitin**	60	10
Glycerophosphate**	0	1

* Final concentration of 5 mg protein/ml.
** Final concentration of 50 μg of organic P/ml.

for the enzyme. Dephosphorylation of α- and β-casein has also been found to be brought about readily by a phosphoprotein phosphatase preparation from chick embryo[4]. Phosphomonoesterases on the contrary dephosphorylate only α-casein. It is hence justifiable to conclude on these grounds that phosphoprotein phosphatase is an enzyme quite distinct from the phosphomonoesterases. The nature of the phosphorus linkage attacked by this enzyme is not yet clear and remains to be investigated.

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ACCUMULATION D'ACIDE THYMIDYLIQUE CHEZ *E. COLI* B APRÈS IRRADIATION U.V.

par

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De nombreux travaux ont montré que l'irradiation, tant X que U.V., de divers organismes entraîne un ralentissement de la synthèse de l'acide désoxyribonucléique (ADN). Kelner a établi notamment que chez *E. coli* en phase de croissance, la synthèse de l'ADN est bloquée complètement[4]. Ce blocage n'est que temporaire et il s'accompagne d'un enrichissement des bactéries en nucléotides acidosolubles[3]. Dans le présent travail, une accumulation d'acide thymidylique dans les bactéries irradiées est mise en évidence.

Les bactéries sont cultivées dans un milieu synthétique décrit précédemment. Elles sont récoltées au cours de leur croissance logarithmique (densité optique 0.4, mesurée au spectrophotomètre Coleman Univ. M.14 à 700 mμ) et additionnées d'un volume égal de milieu frais. L'irradiation est effectuée dans des boîtes de Pétri ouvertes, disposées sur un agiteur électromagnétique (épaisseur de couche 5 mm). La lampe Mineralight utilisée pour l'irradiation est placée à 30 cm et le flux total est de $9.5 \cdot 10^2$ ergs/mm^2. Environ 1000 ml de suspension bactérienne, irradiée par lots de 100 ml, sont incubés immédiatement après l'irradiation, à 37° et à l'obscurité, pendant des périodes de 30 ou 50 minutes et soumis à une agitation vigoureuse. Pendant ce temps, aucune synthèse de l'ADN n'a lieu dans les lots irradiés. Après l'incubation, les bactéries sont récoltées par centrifugation à 4° C, lavées deux fois à l'aide de NaCl 0.9% et délipidées à l'alcool-éther (3:1). Les culots bactériens sont ensuite