Effect of immune serum on $C^{14}O_2$ production from glucose-1-C^{14} by polymorphonuclear leucocytes (PMNL). Each vessel contained 2×10^7 cells in calcium-free Krebs-Ringer phosphate and 1 μc of glucose-1-C^{14} in 5.55 μmoles of 1-C^{14} glucose (radioactivity 8.5×10^4 counts/min/1 μmole); 0.1 ml serum or 10.5 mg γ-globulins when indicated. The values are given for 2×10^7 cells and for 20 min of incubation.[a]

	PMNL control	PMNL + normal serum	PMNL + immune serum	PMNL + normal γ-globulins	PMNL + immune γ-globulins
$C^{14}O_2$ from glucose-1-C^{14} (counts/min)	1,690	2,370	15,529	1,740	8,150
Glucose equivalents (nmoles)	19.9	27.9	182.6	20.5	95.8

[a] The experiment reported in this table is a typical one and has been selected from nearly 20 experiments. 6 different immune sera, each of them at several stages of immunization, were tested. The stimulation of $^{14}CO_2$ production induced by immune serum relative to that induced by normal serum ranged from 4 to 12 times, using different sera under the experimental conditions indicated in this table.

not be ruled out. However, it has been found that antiserum to leucocyte granule membranes has a stabilizing action on isolated granules[20].

It is known that leucocytes are able to pinocytose macromolecular substances, including proteins[21,22]. The effect of pinocytosis on the activity of some dehydrogenase in guinea-pig polymorphonuclear leucocytes has been reported[22]. On this line it is possible that the specific binding of immune gamma-globuline to antigenic determinants of the plasma membrane of PMN leucocytes facilitates the pinocytosis uptake of the globuline. In this case, it remains to be ascertained whether the plasma membrane modifications induced by the specific binding of antibodies represent per se the trigger mechanism for the stimulation of respiration of PMN leucocytes or whether an increased pinocytosis must occur as an intermediate step.

Studies are now in progress on the effect of antileucocyte antibodies on the glycolytic activity, on the oxidative enzymes involved in the stimulated oxygen uptake, and on the morphological modifications of the plasma membrane and of the granules[23].

Riassunto. Gli anticorpi antileucociti provocano una marcata stimolazione della respirazione, rotenone, Antimicina A e cianuro insensibile, ed una aumentata attività del ciclo degli esosomonofosfati nei leucociti di cavia.

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The Metabolism of Tri-Alkyl Phosphates[1]

Tri-alkyl phosphates (1) are reactive towards nucleophiles and have been used to alkylate a variety of functional groups[2]. The di-alkyl phosphate entity (usually dimethyl or diethyl) forms an integral part of a number of organophosphorus insecticides and recently trimethylphosphate (TMP, I $R = CH_3$) has been shown to possess a 'functional' sterilizing action in male rodents[3]. The metabolism of organophosphorus insecticides and tri-aryl phosphates is well documented but little is known about the fate of the simple tri-alkyl phosphates, even though they are known to react chemically by an alkylating mechanism.

Chromatograms of rat and mouse urine from either oral or intraperitoneal administration of ^{32}P-TMP (100 mg/kg and 1 g/kg respectively) revealed one radioactive area corresponding to ^{32}P-dimethylphosphate (II, $R = CH_3$). At similar dose levels ^{32}P-triethylphosphate (TEP, I $R = CH_2CH_3$) was excreted by both species as ^{32}P-diethylphosphate (II, $R = CH_2CH_3$). Apart from traces of trimethylphosphate in rat urine within 6 h of i.p. administration, in neither case was the tri-alkyl phosphate excreted unchanged and mouse bladders cannulated 1 h after oral dosing (1 g/kg) contained only the corresponding ^{32}P-metabolite. ^{32}P-TMP and ^{32}P-TEP are rapidly excreted as their metabolites by both rat and mouse with 90% recovery of radioactive material in urine within 16 h and nearly complete recovery over 96 h.

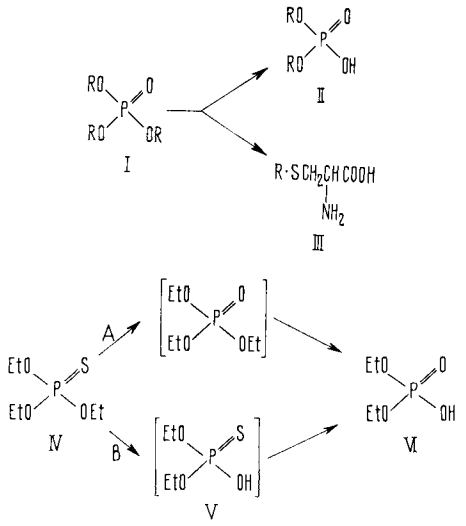
Both ^{32}P-dimethylphosphate[4] and ^{32}P-diethylphosphate are excreted unchanged, there being no mono-alkyl phosphate or phosphoric acid produced in either case. This contrasts with previous work in which di-alkyl phosphates[5], particularly dimethylphosphate[6,7] and diethylphosphate[8], have been reported to be metabolized to the mono-alkyl phosphate and phosphoric acid. However, as these products were obtained from dialkyl-aryl phosphates, they would be produced by de-arylation of initially de-alkylated metabolites. The organophosphorus compounds[9] *trans*-phosdrin[10], *cis*- and *trans*-bomyl[10] and troline[4], for example, are metabolized by de-arylation solely to dimethylphosphate whereas tri-aryl phosphates, such as tri-*o*-cresyl phosphate[11], are degraded through their di- and mono-aryl derivatives to phosphoric acid. The biological mono dealkylation of TMP and TEP, therefore, appears analogous to their chemical reactivity in which further de-alkylation occurs only under extremely severe conditions[12,13].

With uniformly-labelled ^{14}C-TMP, 2 further urinary metabolites were detected and identified as *S*-methyl cysteine (III, $R = CH_3$) and its *N*-acetate indicating that TMP, at least as far as its detoxification is concerned, acts in an alkylating capacity. Similarly triethyl-, tri-*n*-propyl, tri-*iso*-propyl- and tri-*n*-butyl-phosphates, apart from being excreted as the di-alkyl phosphate (II), gave rise to the corresponding *S*-alkyl cysteine (III) so that

mono dealkylation (Figure 1) appears to be general in the metabolism of this class of compound.

The loss of the alkyl group in tri-alkyl phosphates is undoubtedly due both to enzymic hydrolysis[14,15] (P-O cleavage) and de-*O*-alkylation (C-O cleavage), the latter being known[16] to involve reduced glutathione as a methyl acceptor. ^{14}C-TMP reacts almost quantitatively with glutathione to produce *S*-methyl glutathione and dimethylphosphate and both can be detected in rat liver homogenates incubated with TMP (1 h at 37 °C in M/15 phosphate buffer). Analogous demethylations occur with methyl-parathion[17], sumithion[17], dimethyl-dichlorovinyl-phosphate[18] and *cis*-phosdrin[10], all requiring glutathione-dependant preparations. As *S*-methyl glutathione is the in vivo precursor of *S*-methyl cysteine[19] it seems that tri-alkyl phosphates in general are detoxified by similar processes to give ultimately the *S*-alkyl cysteine.

Triethylthiophosphate (IV) produced only one phosphorus-containing metabolite, diethylphosphate (VI), which could have arisen from 2 pathways (Figure 2);



Rf values for tri-alkyl phosphate metabolites

S-alkyl cysteine	TLC[a]	Paper[a]
Methyl-	0.38	0.45
Ethyl-	0.44	0.54
n-Propyl-	0.53	0.65
iso-Propyl-	0.54	0.73
n-Butyl-	0.52	0.72
S-methyl glutathione	0.21	0.27
Phosphate		Paper[b]
Dimethyl-		0.46
Diethyl-		0.54
Di-*n*-propyl-		0.74
Di-*iso*-propyl-		0.50
Di-*n*-butyl-		0.79
Diethyl-thio-[22]		0.65

Rf values for metabolites are on Whatman's No. 17 papers, from which the phosphate metabolites were isolated from untreated urine and the cysteine conjugates from acid-hydrolyzed urine, and 250 µ silica gel G plates. Ascending chromatograms were developed in a) *n*-butanol: glacial acetic acid: water 4:2:1, and b) *iso*-propanol: 880 ammonia: water 8:1:1[23], detection being by ninhydrin and molybdate[24] reagents respectively. Di-alkyl phosphates were synthesized by the method of HARLAY[25] and from the tri-alkyl phosphates by controlled hydrolysis. *S*-alkyl cysteines were prepared from cysteine and the appropriate alkyl iodide[26].

route A requiring initial desulphurization, and route B in which alkylation or hydrolysis has primarily occurred. As diethylthiophosphate (V) is excreted unchanged and was not a urinary metabolite, only route A can be operating. This differs to the metabolism of hexamethylthiophosphoramide which is detoxified by 2 similar pathways independently, both by desulphurization and loss of a methyl group[20].

Trimethylphosphate is the only member of this series possessing anti-fertility activity in male rodents[21] and not exhibiting any of the anticholinesterase activity normally associated with organophosphorus compounds. As this sterilizing activity may be associated with in vivo alkylation, the incorporation of ^{14}C-TMP into cellular material is at present being investigated.

Résumé. Les tri-alkyl phosphates (I) sont metabolisées par mono-dealkylation produisant des di-alkyl phosphates (II) et les *S*-alkyl cysteines (III) relatives chez le rat et la souri.

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[1] This work was supported by grants from the Ford Foundation and the Wellcome Trust.

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[9] Abbreviations: Phosdrin, 0,0-dimethyl-*O*-(1-carbomethoxy-1-propen-2-yl) phosphate. Bomyl, 0,0-dimethyl-*O*-(1,3-dicarbomethoxy-1-propen-2-yl) phosphate. Troline, 0,0-dimethyl-*O*-(2,4,5-trichlorophenyl) phosphate. Sumithion, 0,0-dimethyl-*O*-(4-nitro-*m*-tolyl) thiophosphate. Methyl parathion, 0,0-dimethyl-*O*-(4-nitrophenyl) thiophosphate.
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