$P < 0,01$). L'activité protéolytique, quant à elle, est plus forte lorsque l'éther est utilisé et elle est la plus faible après une anesthésie au nembutal où l'on ne trouve que 7,83 ± 0,58 mg de pepsine (titre 200, Codex) par ml de suc ($P < 0,05$).

En ce qui concerne le FSMG, on obtient des résultats comparatifs analogues selon que l'on rapporte les valeurs du flux seulement au temps, ou au temps et au poids sec de l'organe; il apparaît de toute façon des différences significatives ($P < 0,01$) entre l'anesthésie au nembutal d'une part et celle à l'éther ou à l'uréthane d'autre part; plus précisément le FSMG est statistiquement le plus élevé au cours de l'anesthésie au nembutal, où il atteint 0,185 ml/min et 0,748 ± 0,053 ml/min/g de tissu sec, alors que les valeurs obtenues avec l'éther et l'uréthane sont plus faibles et respectivement de 0,114 et 0,132 ml/mn.

Discussion. Les résultats permettent donc de comparer l'effet de l'éther, du nembutal et de l'uréthane sur quelques paramètres de la fonction gastrique. L'éther qui d'après LEE et THOMPSON[2] ne perturbe pas la sécrétion gastrique, ne semble pas augmenter le FSMG dans nos conditions, bien que chez l'Homme il augmente la circulation sanguine cutanée[18] et accroît le rythme et le débit cardiaque[19]. Par contre, l'uréthane dont l'action sur les sécrétions biliaires et pancréatiques a été étudiée[20] altère de manière significative l'évacuation gastrique[21] mais les effets sur la sécrétion et le FSMG dans nos conditions se rapprochent de ceux obtenus au cours d'une anesthésie à l'éther.

Tableau II. Comparaison inter-groupes (valeur du F) des effets des 3 anesthésiques généraux (éther, nembutal, uréthane) sur le flux sanguin de la muqueuse gastrique (FSMG) chez le Rat

Anesthésiques comparés	Ether et nembutal	Ether et uréthane	Nembutal et uréthane
Acidité libre	3,62	10,56	1,68
	N.S.	$P < 0,01$	N.S.
Activité protéolytique	9,73	0,66	4,96
	$P < 0,01$	N.S.	$P < 0,05$
FSMG (ml/mn)	54,67	3,67	28,67
	$P < 0,01$	N.S.	$P < 0,01$
FSMG (ml/min/g de tissu sec)	18,45	0,28	13,09
	$P < 0,01$	N.S.	$P < 0,01$

Les valeurs numériques représentent la valeur du F calculée selon la méthode de SCHWARTZ[17]; les différences sont considérées significatives pour $P < 0,05$; le terme N.S. signifie $P > 0,05$ (non significatif).

Le nembutal augmente le FSMG, tout au moins par rapport aux deux autres anesthésiques utilisés; cependant il faut souligner le fait que nos conditions expérimentales ne permettent pas de préciser si le nembutal exalte effectivement le FSMG ou si au contraire l'éther et l'uréthane le diminuent, le nembutal ne modifiant alors pas les valeurs que l'on trouverait chez le Rat non anesthésié; l'une de ces deux éventualités ne peut pas être a priori écartée bien qu'ERICSSON[22] ait montré chez le Chien que le nembutal diminue légèrement le débit cardiaque sans modifier le débit sanguin artériel hépatique.

Cependant, compte tenu des résultats obtenus avec l'éther et l'uréthane, il nous semble que le nembutal soit un anesthésique de choix pour l'étude des fonctions gastriques en rapport avec la circulation chez le Rat.

Enfin, il est intéressant de constater qu'au cours de cette expérimentation, nous n'observons pas de corrélations entre le FSMG et l'un des paramètres de la sécrétion gastrique, mais ces corrélations, si elles sont fréquentes[23], ne sont malgré tout pas générales[24].

Summary. Effects of ether, nembutal or urethane anaesthesia on gastric secretion and mucosal blood flow (MBF) are studied in the Rat; these 3 anaesthetics alter neither volume nor total acidity of juice, but free acidity and proteolytic activity are more importance after ether anaesthesia; MBF is highest in nembutal anaesthetized rats; therefore nembutal is the best anaesthetic for studies of digestive physiology or pharmacology.

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Formation of 4-Ketocyclophosphamide by the Oxidation of Cyclophosphamide with $KMnO_4$

Investigations into the metabolism of cyclophosphamide (I) have included attempts to mimic the metabolic processes by purely chemical means[1, 2]. In one such study, NORPOTH et al.[3] reported on the production, in the presence of acidic $KMnO_4$, of one of the known in vivo metabolites of cyclophosphamide, namely 2-carboxyethyl N,N-*bis*(2-chloroethyl)phosphorodiamidate (II). The $KMnO_4$-mediated formation, from cyclophosphamide, of two additional in vivo metabolites, 2-[*bis*(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorin-4-one-2-oxide (4-ketocyclophosphamide, III) and 2-[(2-chloroethyl)amino]-tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide (the N-monodechloroethylated analogue, IV) is now reported.

Methods. Radioactively-labelled [^{32}P]cyclophosphamide, of initial specific activity 2.3 mCi/mmole (Radiochemical Centre, Amersham) was appropriately diluted, before use, with non-labelled cyclophosphamide.

For the oxidation reactions, a solution of [^{32}P] cyclophosphamide (5 mg) and $KMnO_4$ (10 mg) in the appro-

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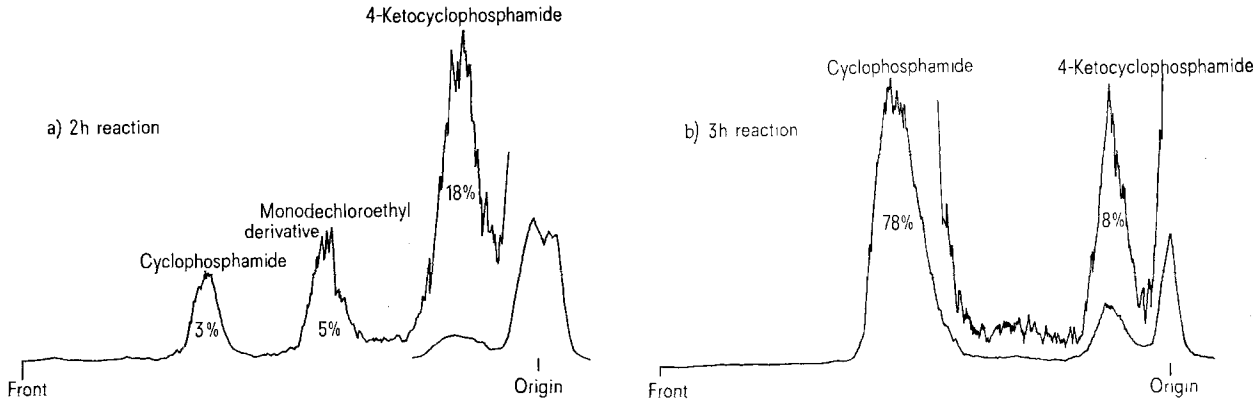


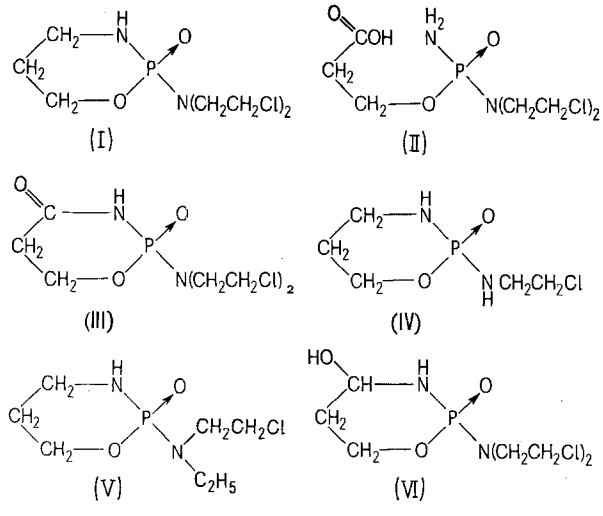
Radiochromatogram scan: TLC on alumina of products from the $KMnO_4$ oxidation of cyclophosphamide a) in water: 3 developments in $CHCl_3$; b) in acetone: 1 development in $CHCl_3$.

priate solvent (water ar acetone, 0.2 ml) was kept at room temperature (20 °C). Aliquots (0.04 ml) were subjected, at suitable time-intervals to thin-layer chromatography TLC) on plates (20 × 5 cm) coated either with alumina (Merck, Aluminium oxid [Type E]) when chloroform was the developing solvent (Solvent A), or with silicic acid (Merck, Kieselgel G) with chloroform-ethanol (19:1) as irrigant (solvent B). After development, the plates were examined for radioactivity using a Berthold LB2723 radiochromatogram scanner. The adsorbants in the areas corresponding to the radioactivity were separately removed, eluted with methanol and subjected to mass spectrometry according to the procedure of Rix et al[4]. Mass spectra were determined with an A.E.I. MS-12 spectrometer, operating at an ion-source temperature of 80–100 °C, and an ionizing voltage of 70 eV.

Results and discussion. The chromatographic procedures used in the present investigation were designed to facilitate the detection ond isolation of oxidation products of cyclophosphamide which are less polar than the 2-carboxyethyl-derivative (II) detected by Norpoth et al.[3] Initially, the oxidation was conducted in aqueous solution and, after 2 h reaction, two mobile radioactive components were observed (TLC, solvent A), additional to cyclophosphamide. Solvent A was preferred for monitoring the oxidations; cyclophosphamide and 4-ketocyclophosphamide have virtually identical Rf values in solvent B. Repeated development was necessary to separate the slowest of the mobile components from abundant immobile products. The slow moring component from had a mass spectrum which corresponded with that reported for 4-ketocyclophosphamide[5] (III) and chromatographic properties (TLC solvents A and B) identical with those of authentic III. The more mobile product (solvent A) gave a mass spectrum which was consistent with a mixture of the monodechloroethylated compound (IV) and a compound not so far reported as a metabolite of cyclophosphamide namely 2-[N-(2-chloroethyl) ethylamino]tetrahydro-2H-1, 3, 2-oxazaphosphorine 2-oxide (V). Thus the peaks[6] appropriate to the compound (IV) were present at *m/e* 198 and 200 (M^+, 2%) and *m/e* 149 [$(M-CH_2Cl)^+$, 100%], whereas the signals at *m/e* 226 and 228 (1 Cl, M^+ for V, 2%) and at *m/e* 211 and 213 [$(M-CH_3)^+$, 14%] were indicative of (V). The identity of IV was further confirmed by comparison (solvents A and B) with authentic material obtained by definitive synthesis. The contribution of the keto-derivative (III) to the total radioactivity after a reaction time of 2 h was estimated as 18% by measurement of the area under the appropriate peak (Figure 1). Similar values were obtained for reaction times of 0.5 h (16%) and 4 h (14%). The values for cyclophosphamide diminished from 24%, at 0.5 h to 1.5% at 4 h.

Oxidation of cyclophosphamide by $KMnO_4$ in acetone also yielded both the keto-derivative (III) and the monodechloroethylated product (IV). The latter compound was not detected after 3 h (Figure 1b), but after 20 h, a radioactive component (3%) was observed at the appropriate Rf value (0.30, solvent A). The mass spectrum of this component contained only the peaks attributable to the compound (IV).

The present studies thus provide a further proof that at least some of the metabolic transformations of cyclophosphamide can be imitated by purely chemical methods. Additionally, the use of radioactively-labelled cyclophosphamide in such reactions affords a method of obtaining the appropriate radioactively-labelled metabolites, useful for further metabolic or chemical studies, without resorting to de novo synthesis. In particular, 4-ketocyclophosphamide is considered to be an in vivo oxidation product of the corresponding 4-hydroxy-



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derivative (VI), a compound believed to be a key intermediate in the transformation of cyclophosphamide into a cytotoxic species[7,8]. Approaches to the synthesis of the 4-hydroxyderivative (VI) have included the attempted reduction of 4-ketocyclophosphamide[9]. The availability of [^{32}P] 4-ketocyclophosphamide, consequent upon the present studies, should facilitate the monitoring of such reactions.

Zusammenfassung. Oxydation des (^{32}P) Cyclophosphamides durch $KMnO_4$ in Wasser oder Aceton führt zu 4-Ketocyclophosphamid und einem N-dechloroäthylierten Derivat des Cyclophosphamides, beides bekannte Metaboliten der Droge. Die Auftrennung der Produkte gelingt durch Dünnschichtchromatographie und die Richtigkeit der Strukturen wurde durch Massenspectrometrie nachgewiesen.

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Cyclic 3',5'-Adenosine Monophosphate in *Tetrahymena pyriformis*

Adenyl cyclase activity[1] and cyclic nucleotide phosphodiesterase[2] activity have been reported in the ciliate *Tetrahymena pyriformis*. It has been reported that theophylline increases glycogen level in *Tetrahymena*[2]. Since methylxanthines are believed to affect the glycogen metabolism via cyclic AMP system[3], it was of interest to study whether cyclic AMP[4] is present in *Tetrahymena*.

Tetrahymena pyriformis, GL strain, is used in our studies. The cells are grown, harvested and washed as described earlier[5]. Finally, the cells are homogenized by freeze thawing 3 times in 1 ml 0.05 *M* sodium acetate buffer pH 6.2 plus 0.5 ml theophylline (30 mg/ml). The homogenate is centrifuged at 3000 rpm for 20 min and the supernatant taken for assay.

Radioimmunoassy method is used for the determination of cyclic AMP. The details of the method are well documented[6]. The radioimmunoassay kit is purchased from Schwarz/Mann. Protein is determined by the method of LOWRY et. al.[7] The Table includes the results. The amount of cyclic AMP present is expressed as picomoles per mg protein. As can be seen, cyclic AMP is present in cells grown in both media.

The intracellular concentration of cyclic AMP is extremely low, of the order of 10^{-6} *M* or lower, in most tissues. Cyclic AMP-mediated events are triggered by relatively small changes in the intracellular concentration of cyclic AMP. Hence, extremely sensitive methods are necessary to measure the intracellular levels of cyclic AMP. Among the various methods available, the sensitivity of the radioimmunoassay technique for cyclic AMP is reported to surpass that of the other methods[6]. In our experiments described here, we could detect the extremely low level of the cyclic AMP in *Tetrahymena*, using the radioimmunoassay technique.

The significance of the presence of cyclic AMP in *Tetrahymena* can only be speculated at the moment. The effect of theophylline on glycogen metabolism in *Tetrahymena*, may now be examined by studying its effect on phosphodiesterase and the intracellular level of cyclic AMP.

It is reported that *Tetrahymena* contains catecholamines and serotonin and the growth of *Tetrahymena* is inhibited by a variety of adrenergic and/or serotonergic drugs[2]. These data have earlier led to the hypothesis that *Tetrahymena* contains a primitive metabolic control system with several features in common with the intercellular metabolic systems found in metazoa[2]. This similarity can now be extended further with our detection of cyclic AMP in *Tetrahymena* and the reported adenyl cyclase and phosphodiesterase activities. Work is now in progress to measure the effect of several drugs on the intracellular level of cyclic AMP in *Tetrahymena*.

Determination of cyclic AMP in *Tetrahymena pyriformis*, GL.

Medium	Conditions	Amount of C-AMP (*p*moles/mg protein)[a]
With glucose	25 °C + shaking	2.70 ± 0.38
Without glucose	25 °C no shaking	1.07 ± 0.38

[a] The assay is carried out with different aliquots of the homogenate (25 to 100 µl) in duplicate.

Zusammenfassung. In *Tetrahymena pyriformis*, GL., wurde zyklisches AMP nachgewiesen.

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