ollowing a 5 mg/kg s.c. dose. Although evidence for codeinone as a metabolite was not obtained in this study, the formation of norcodeine and codeine would implicate its formation as an intermediate. Except for the formation of norcompounds and glucuronide conjugated detoxication products, the metabolic pathways of thebaine (Figure) have some interesting similarities to the biogenetic sequence of opium alkaloids in poppy plant[25-27].

This study demonstrates that rapid metabolism, elimination and lack of persistence of thebaine in rat brain conceivably do not give rise to cellular adaptation in the CNS, consequently thebaine possesses very low potential for tolerance and physical dependence. Repeated administration of thebaine, however, could lead to some accumulation in brain of small quantities of its minor metabolites e.g., norcodeine, normorphine, codeine and morphine, which may be responsible for the low grade dependence recently reported in monkey by the self-administration technique[28].

Zusammenfassung. Rasche Metabolisierung und Ausscheidung sowie Eliminierung von Thebain im Rattenhirn verursacht keine biochemische Änderung der Zellen im Zentralnervensystem, woraus die geringe physiologische Gewöhnung resultieren dürfte.

A. L. MISRA, R. B. PONTANI and S. J. MULE'

New York State Narcotic Addiction Control Commission, Testing and Research Laboratory, Brooklyn (New York 11217, USA), 29 January 1973.

[25] D. H. R. BARTON, Proc. chem. Soc. *293* (1963).
[26] A. R. BATTERSBY, E. BROCHMANN-HANSSEN and J. A. MARTIN, Chem. Commun. *483* (1967).
[27] H. I. PARKER, G. BLASCHKE and H. RAPOPORT, J. Am. chem. Soc. *94*, 1276 (1972).
[28] T. YANAGITA, Symposium on Drugs and Society. Fifth Int. Congress on Pharmacology, San Francisco (1972), p. 133.

Ileal Absorption of Disodium Ethane-1-Hydroxy-1,1-Diphosphonate (EHDP) and Disodium Dichloromethylene Diphosphonate(Cl_2 MDP) in the Chick

The therapeutic potential of the diphosphonate group of compounds dictates that pertinent and relevant information be obtained on their metabolic fate. These compounds have been shown to inhibit the precipitation and dissolution of calcium phosphate in vitro[1-4]. In vivo they were found to prevent soft tissue calcification[1,4] and in certain conditions bone calcification[5-10]. Furthermore they can also diminish bone resorption[1,3,7,9,11]. Because of these properties, a diphosphonate, disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP), has been used successfully in clinical conditions involving either abnormal calcification such as calcinosis universalis[12] and myositis ossificans progressiva[13,14], or increased bone turnover such as Paget's disease[15].

The knowledge of the metabolism of these compounds, and especially their intestinal absorption, therefore became of importance. In a recent multi-species study, MICHAEL et al.[16] noted that EHDP, when fed as part of the diet or given by gavage, was only slightly absorbed in the rat, rabbit and monkey ($< 10\%$). In the dog, EHDP absorption was, on the average, about 20% in young animals and about 14% in old ones, and it was suggested that nearly all of the absorption occurred in the stomach[16]. In man, absorption is in the range of a few percent. The values differ, however, greatly from one individual to another. It is possible that the uneven therapeutic response is due to this variation in absorption. Therefore a better understanding of the latter process is necessary. In the present experiments we have studied the absorption of two diphosphonates by ligated chick ileum.

Experimental. White Leghorn cockerels, in the weight range of 150–238 g, were fasted overnight. In the absorption phase, the chicks were anesthetized with ether, a laparotomy performed, and the ileum exposed. The lumen of the ileum was rinsed with saline, followed by a stream of air to remove excess saline. The length of the ileal segment was from the remnant of the yolk sac to the proximal attachment of the cecal horns. 1 ml of the dosing solution, at pH 7.2, composed of 150 m*M* NaCl, 2 m*M* K_2HPO_4, either 8 m*M* EHDP or 8 m*M* Cl_2MDP, was injected into the lumen between ligations; the concentration of ^{14}C-EHDP and ^{14}C-Cl_2MDP was 0.2 μc/ml[17]. At 15 min post-injection, the chick was killed by pentobarbital (Nembutal®) and the ligated segment removed and its length determined. The luminal contents were allowed to flow into a graduated tube from a cut end and the lumen subsequently rinsed with 30–35 ml of phosphate buffer (150 m*M* NaCl, 2 m*M* K_2HPO_4, pH 6.8), and all tubes were made up to 40 ml. The intestinal tissue was weighed, cut into small pieces and transferred to a homogenizing tube. Phosphate buffer was added in an amount equal to 3 times the weight of the tissue and the tissue thorougly homogenized with a Potter-Elvehjem homogenizer (teflon pestle). 1 ml aliquots of the gut homogenate

[1] H. FLEISCH, R. G. G. RUSSELL, S. BISAZ, P. A. CASEY and R. C. MÜHLBAUER, Calc. Tiss. Res. *2*, Suppl. 10 (1968).
[2] M. D. FRANCIS, Calc. Tiss. Res. *3*, 151 (1969).
[3] R. G. G. RUSSELL, R. C. MÜHLBAUER, S. BISAZ, D. A. WILLIAMS, and H. FLEISCH, Calc. Tiss. Res. *6*, 183 (1970).
[4] H. FLEISCH, R. G. G. RUSSELL, S. BISAZ, R. C. MÜHLBAUER and D. A. WILLIAMS, Eur. J. clin. Invest. *1*, 12 (1970).
[5] J. JOWSEY, K. E. HOLLEY and J. W. LINMAN, J. Lab. clin. Med. *76*, 126 (1970).
[6] W. R. KING, M. D. FRANCIS and W. R. MICHAEL, Clin. Orthop. *78*, 251 (1971).
[7] A. B. GASSER, D. B. MORGAN, H. A. FLEISCH and L. J. RICHELLE, Clin. Sci. *43*, 31 (1972).
[8] R. G. G. RUSSELL, A.-M. KISLIG, P. A. CASEY, H. FLEISCH, J. THORNTON, R. SCHENK and D. A. WILLIAMS, Calc. Tiss. Res. *11*, 179 (1973).
[9] R. SCHENK, R. G. G. RUSSELL, H. FLEISCH, and D. A. WILLIAMS, Calc. Tiss. Res. *11*, 196 (1973).
[10] W. R. MICHAEL, W. R. KING and M. D. FRANCIS, Clin. Orthop. *78*, 271 (1971).
[11] R. C. MÜHLBAUER, R. G. G. RUSSELL, D. A. WILLIAMS and H. FLEISCH, Eur. J. clin. Invest. *1*, 336 (1971).
[12] R. L. CRAM, R. BARMADA, W. B. GEHO and R. D. RAY, New Engl. J. Med. *285*, 1012 (1971).
[13] C. A. L. BASSETT, A. DONATH, F. MACAGNO, R. PREISIG, H. FLEISCH and M. D. FRANCIS, Lancet *2*, 845 (1969).
[14] R. G. G. RUSSELL, R. SMITH, M. C. BISHOP, D. A. PRICE and C. M. SQUIRE, Lancet *1*, 10 (1972).
[15] R. SMITH, M. BISHOP and R. G. G. RUSSELL, Lancet *1*, 945 (1971).
[16] W. R. MICHAEL, W. R. KING and J. M. WAKIM, Toxic. appl. Pharmac. *21*, 301 (1972).
[17] Both ^{14}C-EHDP (disodium ethane-1-hydroxy-1,1-dophosphonate and ^{14}C-Cl_2MDP (disodium dichloromethylene diphosphonate) were provided by the Procter and Gamble Company, Cincinnati, Ohio, USA. Specific activity: ^{14}C-EHDP 1.97 mCi/mmole, ^{14}C-Cl_2MDP 0.55 mCi/mmole.

Table I. Ileal absorption of disodium ethane-1-hydroxy-1,1-diphosphonate (^{14}C-EHDP) and disodium dichloromethylene diphosphonate (^{14}C-Cl_2MDP) in the chick

	^{14}C-EHDP	^{14}C-Cl_2MDP
No. of chicks	6	6
Body weight (g)	170 ± 15.0[a]	192 ± 7.5
Length of ileal segment (cm)	19.3 ± 0.9[a]	22.7 ± 1.4
Absorption (% dose)	28.1 ± 1.4[b]	43.3 ± 2.3
In gut tissue (% dose)	15.4 ± 1.9[a]	11.8 ± 2.9
Into body (% dose)	12.7 ± 2.1[b]	31.5 ± 2.5

Values are means ± SE
[a] Difference between EHDP and Cl_2MDP groups not significant ($p > 0.05$). [b] Difference between EHDP and Cl_2MDP groups significant at $p < 0.001$.

Table II. Comparison of absolute transfer of ^{14}C-EHDP and ^{14}C-Cl_2MDP across chick ileum in situ

	Absorption (μmoles/cm/h)	Into body
EHDP (8 m*M*)	0.46	0.21
Cl_2MDP (8 m*M*)	0.61	0.44

were added to a small screw-cap bottle, dried at 65 °C, and solubilized with Soluene 100 (Packard®) at 65 °C. A 0.1 ml aliquot of the solubilized material was added to 10 ml of liquid scintillation solution made from 600 ml toluene and 400 ml ethylene-glycomonoethylether in which were dissolved 80 g Naphtalene (Merck® Nr. 6200) and 7.0 g Butyl-PBD (Ciba-Geigy®). The radioactivity was measured in a Packard Tricarb scintillation spectrometer. The luminal solution (0.2 ml aliquot from 40 ml) was counted in the same fashion. Quench corrections were made by the use of internal standards.

Results and discussion. The values, given in Table I, indicate that both diphosphonates are absorbed from chick ileum. Within the 15 minute absorption period, about 28% and 43% of the injected EHDP and Cl_2MDP, respectively, left the intestinal lumen. About 15% and 12% of EHDP and Cl_2MDP, respectively, remained in the intestinal tissue, whereas 14% and 32% of these compounds were tranferred to the body, i.e., left the intestinal region. Cl_2MDP was absorbed and was transferred into body to a significantly greater degree than EHDP ($p < 0.001$).

In Table II, the amount of the diphosphonates that was transferred out of the lumen or entered the plasma per unit time and per unit length as calculated from the specific activity of the labelled dosing solution and the amount of radioactive label that was transferred are given.

As before, the rate of translocation of Cl_2MDP was somewhat greater than that for EHDP. It is interesting to compare the tranfer rate of diphosphonates to that of inorganic phosphate (P_i). In a different but comparable study in chicks[18], the percentage of the dose of P_i absorbed from ileal segment into the body was found to be 27.6% and 24.5% for luminal concentrations of 5 m*M* and 20 m*M* respectively. Assuming a linear relation between these 2 concentrations, the interpolated percentage for 8 m*M* would be 27%, and the transfer rate of P_i into the body would be 0.55 μmoles/cm/h. This value for P_i transfer is greater than that for EHDP, but not too dissimilar from the value for Cl_2MDP.

These results indicate that the diphosphonates can cross the intestinal epithelium, and that the intestinal tract might represent a significant route of entrance of these compounds into the body. However, it should be recalled that absorption was occurring from a washed intestinal loop containing little or no residual ingesta. The previous suggestion that diphosphonates are little absorbed from the gastrointestinal tract of mammals was based on feeding experiments or the administration of the compounds per os to the fasted animal[16]. It was proposed by MICHAEL et al.[16] that this low absorption rate might be due to binding of the diphosphonates to some endogenously secreted or dietary substances. Also the study of MICHAEL et al.[16] was done with mammals only, whereas the current results were obtained in birds and therefore might reflect species variability in the absorption of EHDP and Cl_2MDP. The present experimental protocol does provide a defined system for the systematic assessment of the effect of various factors on diphosphonate translocation across the intestine.

Résumé. Une proportion non négligeable de disodium ethane-1-hydroxy-1, 1-diphosphonate (EHDP) et de disodium dichloromethylenediphosphonate (Cl_2MDP) peut être absorbée au niveau de l'ileum de poulet. L'absorption du Cl_2MDP est significativement supérieure à celle de l'EHDP.

R. H. WASSERMAN[19], J.-P. BONJOUR[20] and H. FLEISCH[20,21]

Department of Physical Biology, New York Veterinary College, Cornell University, Ithaca (N.Y., USA); and Department of Pathophysiology, University of Berne, Hügelweg 2, CH-3012 Bern (Switzerland), 24 April 1973.

[18] R. H. WASSERMANN and A. N. TAYLOR, J. Nutr. *103*, 586 (1973).
[19] Department of Physical Biology, New York State Veterinary College, Cornell University, Ithaca (N.Y., USA).
[20] Department of Pathophysiology, University of Berne (Switzerland)
[21] Acknowledgements. We thank Miss LINDA SENIOR for her skilful technical assistance. This work has been supported by the Procter and Gambel Company, by the US National Institute of Health (Grant No. AM 07256) and by the Swiss National Research Fund (Grant No. 3.326.70 Sr). R. H. W. was supported, in part, by the John Simon Guggenheim Foundation.

Correlation of Physicochemical Parameters and Biological Activity in Steroids. 9α-Substituted Cortisol Derivatives[1]

The nature of the relationship between chemical constitution and biological activity in steroids is of both practical and theoretical importance. Recently, we demonstrated that a given molecular modification in a

[1] This investigation was supported in part by Public Health Service Research Grants No. AM-14824 (to M.E.W.) and No. GM 07492 (to C.H.) from the National Institutes of Arthritis and Metabolic Diseases, and General Medical Sciences, U.S. Public Health Service.