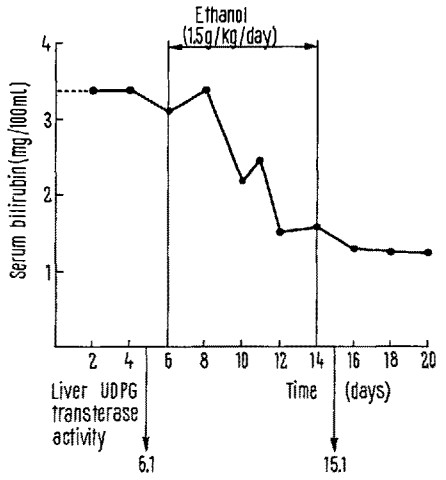
observed in whole homogenates as well as in washed microsomes (Table).

A striking decrease in bilirubinaemia was observed in man during the treatment with ethanol (Figure); bilirubin level was still low 90 days after the end of the treatment. Liver UDPG-transferase activity was 6.1 nmoles of bilirubin conjugated/g of wet liver/min before the treatment, and 15.1 nmoles of bilirubin conjugated/g of wet liver/min at the end of the treatment (Figure).

These data suggest an inductive action of ethanol on liver UDPG-transferase. However, the mechanism of this induction is still uncertain. These observations confirm our preliminary communication[20], and might provide an explanation for the decreased bilirubin levels observed by WALTMAN et al.[11] in new-born babies whose mothers had been given ethanol before delivery.



Behaviour of serum total bilirubin and of liver bilirubin UDPG-transferase in a young man with congenital Gilbert's type jaundice, before and after the i.v. administration of ethanol for 7 days. Conjugated bilirubin always ranged from 0.20 to 0.25 mg/100 ml. Bilirubin UDPG-transferase activity expressed as nmoles of bilirubin conjugated/g of wet liver/min. In our laboratory, normal liver bilirubin UDPG-transferase values range from 14.5 to 44.

Riassunto. La bilirubina UDPG-transferasi epatica si è incrementata nel ratto ed in un paziente affetto da ittero di Gilbert dopo somministrazione di etanolo. Nell'uomo la bilirubinemia si è nettamente ridotta durante il trattamento ed è ancora su livelli normali a tre mesi di distanza dalla fine di questo. Questi dati potrebbero fornire una spiegazione ai bassi livelli bilirubinemici riscontrati in neonati da madre trattata con etanolo poco tempo prima del parto[11].

G. IDÉO, R. DE FRANCHIS,
E. DEL NINNO and N. DIOGUARDI

Istituto di Patologia Medica II dell'Università di Milano, 'Antonio Migliavacca Centre' for Liver Diseases, I-20122 Milano (Italy), 6 July 1970.

[1] O. A. ISERI, C. S. LIEBER and L. S. GOTTLIEB, Am. J. Path. *48*, 535 (1966).
[2] B. P. LANE and C. S. LIEBER, Am. J. Path. *49*, 593 (1966).
[3] E. RUBIN and C. S. LIEBER, Fedn Proc. *26*, 1458 (1967).
[4] E. RUBIN and C. S. LIEBER, New Engl. J. Med. *278*, 869 (1968).
[5] C. S. LIEBER and L. M. DE CARLI, J. clin. Invest. *47*, 62a (1968).
[6] C. S. LIEBER and E. RUBIN, Gastroenterology *54*, 642 (1968).
[7] E. RUBIN, F. HUTTERER and C. S. LIEBER, Science *159*, 1469 (1968).
[8] E. RUBIN and C. S. LIEBER, Science *162*, 690 (1968).
[9] C. S. LIEBER and L. M. DE CARLI, Science *162*, 917 (1968).
[10] C. S. LIEBER and E. RUBIN, New Engl. J. Med. *280*, 705 (1969).
[11] R. WALTMAN, F. BONURA, G. NIGRIN and C. PIPAT, Lancet *ii*, 1265 (1969).
[12] M. BLACK and B. H. BILLING, New Engl. J. Med. *280*, 1266 (1969).
[13] I. M. ARIAS, L. M. GARTNER, M. COHEN, J. BEN EZZER and A. J. LEVI, Am. J. Med. *47*, 395 (1969).
[14] C. F. STRITTMATTER and F. T. HUMBERGER, Biochim. biophys. Acta *180*, 18 (1969).
[15] Y. IMAI, A. ITO and R. SATO, J. Biochem., Tokyo *60*, 417 (1966).
[16] J. R. FOUTS and B. B. BRODIE, J. Pharm. exp. Ther. *119*, 197 (1957).
[17] F. P. VAN ROY and K. P. M. HEIRWEGH, Biochem. J. *107*, 507 (1968).
[18] K. P. M. HEIRWEGH and J. A. T. P. MEUWISSEN, Biochem. J. *110*, 31P (1968).
[19] O. H. LOWRY, N. J. ROSEBROUGH, A. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[20] N. DIOGUARDI, G. IDÉO, E. DEL NINNO and R. DE FRANCHIS, Lancet *i*, 1063 (1970).

Actinomycin D Inhibition of Intestinal Transport of L-Histidine and of Vitamin D Action

Renal aminoaciduria and reduced intestinal histidine transport were observed in rabbits with experimental vitamin D deficiency rickets[1], while administration of high doses of vitamin D to intact animals resulted in increased intestinal absorption of histidine[1]. Since absorption may involve a protein carrier in the intestinal mucosa, the present study was carried out to determine the effect of actinomycin D, an inhibitor of protein synthesis[2], on histidine absorption.

White rabbits weighing 700–1000 g, fasted for 12 h, were used. Under light thiobarbital anesthesia the small intestine was rapidly excised, washed in normal saline and everted, using the technique of WILSON and WISEMAN[3]. 3 or 4 sacs, each of about 3.5 cm long, were obtained from each animal. These sacs were each filled with 1.5 ml of L-histidine solution and placed in a flask containing 25 ml of the same solution. The solution used was Krebs-Henseleit bicarbonate saline, containing 0.3% (w/v) glucose in which L-histidine (Wako Pure chemical Industory; chemically pure grade) was dissolved. At the end of incubation the sacs were removed from the flask and the volume of the sac content was measured. Samples of the initial amino acid solution and of the sac fluid were analyzed with an automatic amino acid analyzer (Model KLA3, Hitachi Co.). The weight of each sac was determined after drying for 2 h at 110 °C. The rate of accumulation of histidine in serosal fluid during the incubation was calculated in mmoles/g dry wt. of sac per 90 min.

[1] M. SUGAI and I. MATSUDA, Biochim. biophys. Acta *170*, 474 (1968).
[2] E. REICH, Science *143*, 684 (1964).
[3] T. H. WILSON and G. WISEMAN, J. Physiol., Lond. *124*, 414 (1954).

Effect of vitamin D_3 and actinomycin D on the rate of L-histidine transport by intestine

	Km mmolar	V max mmolcs/g/90 min	
Control	16.4	100.0	(6)
Vitamin D[a]	16.4	200.0	(6)
Actinomycin D[b]	35.7	58.9	(5)
Actinomycin D and vitamin D[c]	16.4	111.1	(6)

[a] Vitamin D 18 h before experiment. [b] Actinomycin 20 h before experiment. [c] Vitamin D 18 h and actinomycin 20 h before experiment. Numbers in parentheses represent the number of animals used.

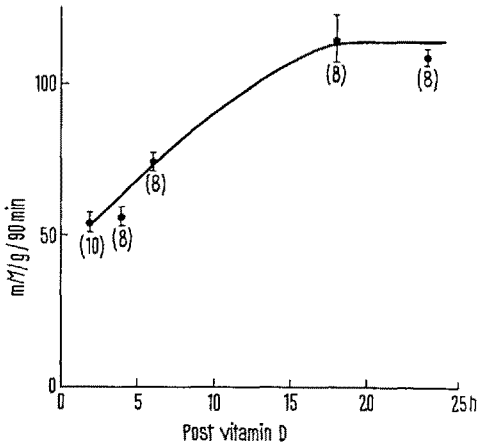


Fig. 1. The time course effect of vitamin D on the intestinal transport of histidine at 20 mM concentration. Each result is the mean $\pm$ S.D

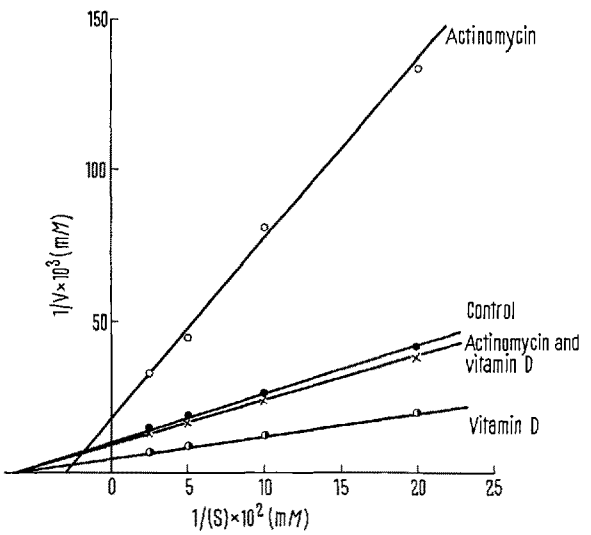


Fig. 2. Transport of histidine by ileal sacs of normal, vitamin D-treated, actinomycin-treated and actinomycin and vitamin D-treated rabbits (LINEWEAVER-BURK plots). Each point is the mean of the result of 5 or 6 samples.

In the first experiment, the animals were sacrificed at intervals of 2, 4, 6, 18 and 24 h after the i.m. injection of 20,000 IU Vitamin D_3 and studies of intestinal histidine transport at 20 mM concentration were made. The results indicate that treatment produced a significant increase in the rate of histidine transport, reaching a peak 18 h after the injection (Figure 1).

In the second experiment animals of one group were injected s.c. with actinomycin D dissolved in 0.85% NaCl solution in the amount of 0.6 µg per g body weight. The control group received the same volume of 0.85% NaCl. 2 h after the actinomycin injection, half of the rabbits of each group were given 20,000 IU of vitamin D_3 in solution while the other half were given the solvent alone. The animals were fasted and 18 h later intestinal preparations were made.

The results were plotted by the LINEWEAVER-BURK[4] method (Figure 2). The Km (kinetics constant) and the Vmax (apparent limiting velocity) of absorption in each group are listed in the Table. The value of Km and Vmax of the control and Vitamin D treated animals are slightly different from those in a previous communication[1], because the number of animals was increased in the present studies. The Vmax of animals that received vitamin D was markedly elevated, while the Km was identical to that of the control, suggesting that the vitamin D stimulated the intestinal transport system of histidine.

In contrast, animals treated with actinomycin alone showed an apparent decrease of Vmax and an increase of Km. When actinomycin was given 2 h before the vitamin D injection, the Vmax was significantly decreased as compared with vitamin D-treated animals. The data indicate that actinomycin has an inhibiting effect on the intestinal histidine transport and blocks the response of the transport to vitamin D. It has been reported that administration of vitamin D resulted in an increase of the rate of RNA labelling with ^{3}H-uridine in chicken intestinal mucosa[5].

The accepted concept of the mode of action of actinomycin D is that it blocks DNA directed synthesis of mRNA and in this way inhibits protein synthesis[2]. When comparison was made between the groups receiving actinomycin alone and both actinomycin and vitamin D, it is shown that vitamin D does increase the Vmax and prevent the increase of the Km.

Since the amount of actinomycin used in the present study is assumed to be sufficient to block mRNA synthesis in the intestine[5], the data may indicate that the rate of histidine transport is stimulated by vitamin D not only through the induction of RNA and protein synthesis, but also through some other mechanism. However, such an interpretation must be viewed with caution, because actinomycin has an injurious effect upon cell synthesis.

Zusammenfassung. Eine grosse Gabe Vitamin D bewirkte bei Kaninchen eine Erhöhung der Histidinabsorption durch den Darm. Vorbehandlung mit Actinomycin D hemmte den Histidintransport und verhinderte die Stimulation desselben durch Vitamin D.

M. SUGAI and I. MATSUDA

Hokkaido University School of Medicine, Department of Pediatrics, Sapporo (Japan), 6 July 1970.

[4] H. LINEWEAVER and D. BURK, J. Am. chem. Soc. *56*, 658 (1934).
[5] A. W. NORMAN, Biochem. Biophys. Res. Comm. *23*, 335 (1966).