establishing the equilibrium level of DCA in tissues will take more days[11,12]. Experiments in animals with different composition and metabolism of BA are to be avoided (e.g. rats inactivate DCA in the liver by hydroxylation to CA[13]).

A still more important problem is to elucidate whether the human body may make use of this selective toxicity in permanently acidified tissues (tumours, inflammations) at natural levels of DCA. The concentration of DCA in blood does not exceed $10^{-5}M$, and, generally, the main part is conjugated[14-17]. However, deconjugation proceeds in the body and DCA is preferred in the transport to the skin, where it appears mainly unconjugated[12], i.e. in the active form. The binding with proteins[18] may help to accumulate DCA in tissues in concentrations sufficient for toxic effects[19]. However, as also other BA combine with proteins, a competition is to be expected (proved in ileal transport[20]), and an excess of other BA (especially in hepatobiliary diseases[15,16]) could inhibit DCA. Thus, DCA-percentage of total BA would be decisive in this kind of supposed natural resistance. In the serum of normals, this percentage varies in the limits of 7–79%[16,17]; an attempt will be made to verify whether there is a significant difference between cancer patients and normal subjects appearing resistant. Further, the incidence of cancer after chronic diseases of liver and bile duct will be rechecked from this point of view. As DCA is a product of intestinal microorganisms, attention should be paid, finally, to factors modifying their activity.

Zusammenfassung. Es wird gezeigt, dass die toxische Wirkung von Desoxycholsäure auf Hefe pH-abhängig ist (pH 7.3). Da das pH der Tumoren niedriger ist, wird die Möglichkeit einer krebshemmenden Wirkung diskutiert.

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[11] K. SJÖVALL and J. SJÖVALL, Clin. chim. Acta *13*, 207 (1966).
[12] L. J. SCHOENFIELD and J. SJÖVALL, Nature *213*, 93 (1967).
[13] S. BERGSTRÖM, in *CIBA Foundation Symposium on the Biosynthesis of Terpenes and Sterols* (Eds. G. E. W. WOLSTENHOLME and M. O'CONNOR; Little, Brown and Co., Boston 1959), p. 185.
[14] J. ROOVERS, E. EVRARD and H. VANDERHAEGE, Clin. chim. Acta *19*, 449 (1968).
[15] I. MAKINSO, S. NAKASAWA and K. MASHIMO, Gastroenterology *56*, 1033 (1969).
[16] D. H. SANDBERG, J. SJÖVALL, K. SJÖVALL and D. H. TURNER, J. Lipid Res. *6*, 182 (1965).
[17] E. MIHAESCO and R. FAUVERT, Rev. fr. Etud. clin. biol. *13*, 200 (1968).
[18] D. RUDMAN and F. E. KENDALL, J. clin. Invest. *36*, 538 (1957).
[19] The toxicity (at lowered pH) seems to appear suddenly, if the ratio DCA substrate exceeds some value (which will probably be a function of the cell type).
[20] K. W. HEATON and L. LACK, Am. J. Physiol. *214*, 585 (1968).

Effect of Actinomycin D on RNA and Protein Synthesis in Regions of Developing Frog Embryos[1]

Endoderm cells of developing amphibian embryos synthesize more DNA-like RNA[2,3] than do the dorsal ectoderm and mesoderm cells yet they become determined and differentiate later[4]. A possible explanation is that much of the endoderm DNA-like RNA (D-RNA) is not translated and this is supported by the lower levels of protein synthesis of the endoderm cells[5]. The reduced capacity of the endoderm cells to translate D-RNA may be due to a failure to transport D-RNA from the nuclei or a reduced capacity to stabilize D-RNA on cytoplasmic ribosomes as polysomal D-RNA, resulting in a shorter half-life of D-RNA. There are many fewer ribosomes in the endoderm regions of developing amphibian eggs and embryos[6]. In anucleolate *Xenopus laevis* embryos this may account for the rudimentary differentiation of the endoderm in the larvae[7]. It is possible that this paucity of ribosomes in the endoderm may account for a reduced level of transport of D-RNA to the cytoplasm, or the failure of much of the endoderm cell D-RNA to be conserved in polysomes in the cytoplasm. It was decided to determine levels of RNA and protein synthesis in dorsal ectoderm-mesoderm and endoderm regions of untreated and actinomycin D-treated gastrula, neurula and tailbud embryos of *Rana pipiens*. If comparison of the levels of reduction of RNA and protein synthesis for the ectoderm-mesoderm and endoderm regions showed that with similar levels of inhibition of RNA synthesis there was greater inhibition of protein synthesis for the endoderm, this would suggest that D-RNA is less stable in the endoderm cells.

Materials and methods. Gastrulae, neurulae and tailbuds (stages 10, 14 and 18 of SHUMWAY[8]) were cut into dorsal ectoderm-mesoderm and endoderm regions and 40–60 explants of each part were cultured in NIU-TWITTY[9] saline alone, or NIU-TWITTY saline containing actinomycin D (60 μg/ml) for 6 h at 20 °C. Either uridine-2-C^{14} (5 μc/ml) or C^{14}-leucine (2 μc/ml) was added to each culture the last 3 h of the 6 h culture period in order to measure RNA and protein synthesis as $\frac{\text{total cpm RNA or total cpm protein}}{\text{total cpm acid soluble pool}} \div \text{total DNA}$[10]. The methods of cutting the embryos, culturing the explants, washing, homogenizing, hydrolysis of RNA and counting the samples have been described[2]. The choice of an actinomycin D concentration of 60 μg/ml was made because it was found that over a 6 h period 30 μg/ml of actinomycin D did not prevent an increase in RNA synthesis as shown by determination of $\frac{\text{total cpm RNA}}{\text{total cpm acid soluble pool}} \div \text{total}$

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[2] R. A. FLICKINGER, M. MIYAGI, C. R. MOSER and E. ROLLINS, Devel. Biol. *15*, 414 (1967).
[3] H. R. WOODLAND and J. B. GURDON, J. Embryol. exp. Morph. *19*, 363 (1968).
[4] B. BALINSKY, Wilhelm Roux Archiv. EntwMech. Org. *143*, 365 (1948).
[5] R. A. FLICKINGER, Expl. Cell Res. *6*, 172 (1954).
[6] J. BRACHET, Progr. Biophys. molec. Biol. *15*, 99 (1965).
[7] H. WALLACE, J. Embryol. exp. Morph. *8*, 405 (1966).
[8] W. SHUMWAY, Anat. Rec. *78*, 139 (1940).
[9] M. C. NIU and V. C. TWITTY, Proc. natn. Acad. Sci. USA *39*, 985 (1953).
[10] Z. DISCHE, in *The Nucleic Acids* (Eds. E. CHARGAFF and J. DAVIDSON; Academic Press, New York 1955), p. 285.

Reduction of RNA and protein synthesis by actinomycin D in dorsal ectoderm-mesoderm and endoderm regions of developing frog embryos

Stage	Total cpm RNA / Total cpm acid soluble pool		÷ total DNA $\times 10^{-3}$	Total cpm protein / Total cpm acid soluble pool		÷ DNA $\times 10^{-2}$	Inhibition of protein synthesis
	Controls	Actinomycin D	Inhibition (%)	Controls	Actinomycin D	Inhibition (%)	Inhibition of RNA synthesis
Gastrulae, stage 10							
Ectoderm-mesoderm	1.4 ± 0.2	0.8 ± 0.2	43	17.5 ± 0.5	10.3 ± 0.8	41	0.95
Endoderm	2.6 ± 0.3	1.7 ± 0.4	35	27.3 ± 1.2	18.6 ± 1.0	32	0.90
Neurulae, stage 14							
Neural plate-dorsal mesoderm	1.8 ± 0.3	0.5 ± 0.1	72	5.7 ± 0.5	3.6 ± 0.4	37	0.51
Endoderm	3.1 ± 0.4	0.7 ± 0.3	77	8.2 ± 1.0	3.9 ± 0.5	52	0.68
Tailbuds, stage 18							
Dorsal axial region	1.9 ± 0.3	0.4 ± 0.1	79	8.3 ± 1.0	5.1 ± 0.4	39	0.49
Endoderm	5.5 ± 1.1	0.9 ± 0.2	84	16.7 ± 1.1	7.9 ± 1.1	53	0.63

Parts of 40–60 cut embryos were cultured in NIU-TWITTY saline alone or with actinomycin D (60 µg/ml) for 6 h at 20 °C. Uridine-2-C^{14} (5 µc/ml) or C^{14}-leucine (2 µc/ml) was added the last 3 h of the incubation. These values are averages of 4 separate experiments.

DNA at hourly intervals. A concentration of 90 µg/ml of actinomycin D gave only 4–5% more inhibition of RNA synthesis than 60 µg/ml, so the latter concentration was used. Since there was no significant increase of inhibition using 90 µg/ml compared to 60 µg/ml, this suggests that actinomycin D is present at a saturation level and that the observed inhibitions are not due to differences in permeability between the ectoderm-mesoderm and endoderm cells. The differential inhibition of protein synthesis in relation to a similar level of inhibition of RNA synthesis also argues against permeability as the cause of the observed results in the two regions.

Results and discussion. The relative percent reductions of RNA and protein synthesis by actinomycin D are given in the Table. At the gastrula stage the reduction in protein synthesis is similar to the reduction of RNA synthesis for the dorsal ectoderm-mesoderm and endoderm regions. By the neurula and tailbud stages the lower ratios of inhibition of protein synthesis/inhibition of RNA synthesis indicate protein synthesis is less affected by actinomycin D than is RNA synthesis. With similar levels of inhibition of RNA synthesis for the ectoderm-mesoderm and endoderm regions of neurulae and tailbuds there is less reduction of protein synthesis for the neurula neural plate-dorsal mesoderm regions and dorsal axial ectoderm-mesoderm of tailbuds than for the endoderm regions (Table).

It might be argued that the estimation of RNA synthesis does not distinguish between incorporation of labeled uridine due to synthesis and that attributable to terminal-labeling of transfer-RNA. However, sucrose density gradient centrifugation experiments with labeled RNA isolated from the ectoderm-mesoderm and endoderm regions of embryos of *Rana pipiens* clearly show the majority of labeled RNA to be in the high molecular weight fraction[11]. Furthermore, in *Xenopus* gastrulae transfer-RNA counts represent only 29% of the combined transfer and high molecular weight RNA counts and only 3.5% of the H^3-uridine is converted to H^3-cytidine[12].

It has been shown that half of the D-RNA synthesized during oogenesis in *Xenopus* is lost by early gastrulation, but most of it is translated before being lost[13]. It is unlikely that this D-RNA contributes to the differential effect of actinomycin D on protein synthesis of the ectoderm-mesoderm and endoderm cells at the neurula and tailbud stages. If maternal D-RNA synthesized during oogenesis were involved, one would expect to see a differential effect of actinomycin D on the protein synthesis of the ectoderm-mesoderm and endoderm cells of early gastrulae.

A plausible explanation for these results is that at the gastrula stage, when levels of RNA and protein synthesis are quite low[5], the RNA synthesis inhibited by actinomycin D is indispensable for protein synthesis. Hence, the inhibition of protein synthesis is proportional to the inhibition of RNA synthesis. This may be because D-RNA synthesis is just beginning[14]. By the neurula and tailbud stages more of the D-RNA has been conserved in the cytoplasm and this accounts for the reduced effect of actinomycin D on protein synthesis. The fact that in relation to the same level of inhibition of RNA synthesis, there is less inhibition of protein synthesis in the neurula neural plate-dorsal mesoderm and the dorsal axial regions of tailbuds than in the belly endoderm cells, suggests a greater conservation of D-RNA in the dorsal ectoderm-mesoderm cells. One possibility is that the presence of fewer ribosomes in the endoderm cells[6] may account for the formation of fewer polysomes and the greater inhibition of protein synthesis in these cells.

Résumé. Aux stades neurula et bourgeon caudal du développement embryonnaire de *Rana pipiens*, traitée à l'actinomycine D, la réduction de la synthèse de protéine est proportionellement moindre que celle du RNA. Aux niveaux semblables de l'inhibition de la synthèse du RNA, la synthèse de protéine a diminué plus fortement dans la région de l'endoderme que dans les cellules de l'ectoderme-mésoderme dorsaux, aux stades embryonnaires mentionnés plus haut. Les données suggèrent que le D-RNA se conserve mieux dans le cytoplasme des cellules de l'ectoderme-mésoderme dorseaux que dans celui des cellules de l'endoderme.

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[11] R. A. FLICKINGER, Expl. Cell Res. *55*, 422 (1969).
[12] R. BACHVAROVA, E. H. DAVIDSON, V. G. ALLFREY and A. E. MIRSKY, Proc. natn. Acad. Sci. USA *55*, 358 (1966).
[13] M. CRIPPA and P. R. GROSS, Proc. natn. Acad. Sci. USA *62*, 120 (1969).
[14] D. D. BROWN and E. LITTNA, J. molec. Biol. *20*, 81 (1966).