their surface side-arms of low electron density and blurred limits (Figure 1). The total length of the shaft has not been determined. However, the cilium in Figure 2 ran a trajectory of more than 2 μm into the neuropile closely attached to a dendrite originating in the same cell. The proximal centriole is located next to the basal body or distal centriole and approximately 90° from it (Figure 1). The Golgi apparatus is also constantly associated with the base of the cilium. Often two or more of these structures can be counted in a single section, suggesting that actually a cluster of them encircles the basal body. A variable number of dense granular masses 70–80 nm in size and irregular in profile are generally present in the area between the Golgi elements and the basal body (Figures 1 and 2). Striated filaments of indefinite longitude are constantly seen in association with the basal body. They are composed of longitudinally arranged subunits (Figure 2), about 7 nm thick, crossed at 50 nm intervals by bars of electron-dense material. Some cross-sections of cilia have been examined showing the 9+0 pattern of microtubules which is usual in neural cilia, and a single cilium of the 9+2 pattern was also observed. Due to the size of the neurons the chances for a section to intersect a cilium are low and it is easy to overlook a cross-section of cilium. Thus it is difficult to assess the percentage of ciliated neurons in the spinal cord.

Discussion. Functional questions related to the neural cilia remain open. One concerns the possible motility of these structures. Cilia exhibiting a 9+0 pattern are usually considered non-motile. However, there is increasing evidence[20–23] that the 9+0 pattern does normally exist in motile forms and this could be the case with the neural cilia. At any rate, the crucial question is what purpose, if any, is subserved by them. The opinions in the literature are divided. Sensory functions of an unspecified nature were attributed to the 9+0 cilia by several authors on the basis of SJÖSTRAND's[24] pioneer description of the highly modified cilium of retinal rods. However, 2 sources of evidence make this generalization untenable. First, the existence of 9+2 cilia in sensory receptors[25–28] indicates that lack of central fibers is not synonymous with sensory function. Second, 9+0 cilia do occur in the most diverse cell types of various organs[5] to which no sensory properties can be reasonably attributed.

The cilia being found in an increasing number of neural structures are always in association with the Golgi apparatus. Thus some metabolic relationship is suggested, perhaps the release under the form of dense granular bodies of substances concentrated by the Golgi cisternae and incorporated by the cilium. It is hoped that future experimental work will be able to answer some of these questions.

Resumen. Un estudio electro-microscópico de la médula espinal del axalote reveló la existencia de neuronas ciliadas, tanto en medulas normales como en segmentos medulares implantados por tiempos variables en la aleta dorsal de animales de la misma especie. La cilia que es única e implantada cerca de una dendrita se extiende en la neuropila por una longitud de varios μm.

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Toxicity of Deoxycholate at pH below 7·3 as a Potential Cancerostatic Property

Deoxycholic acid (DCA) is known to increase the permeability of cell membranes. In our experiments with decorporation of metals from yeast cells using DCA (details will be published elsewhere), we found a striking effect of pH at biological ionic strength and temperature (Figure 1). The decorporation of (labelled) cobalt takes place only below pH 7.3, and the onset of the biological activity is extraordinarily sharp. As any elimination of essential metals lowers the viability of cells, a study was made on the over-all toxic activity of DCA in dependence on pH.

Methods. Commercial baker's yeast (production Kolín) was used; 100 mg (or less) of it was agitated in stoppered tubes with 8–20 ml of a solution containing a constant amount of DCA (production Spofa, CSSR) and of KH_2PO_4; concentration of NaOH and NaCl were variable so that at any pH used the ionic strength remained constant 0.16. The temperature of 37 °C was maintained,

using Wobster ultrathermostat. The incubation period was 1 h; prolongation to 3 h did not alter the results significantly. After centrifugation and washing, the yeast was transferred into a polarographic vessel with a solution of glucose, phosphate, K, Mg, Ca. After aeration, the vessel was stoppered and the decrease of oxygen (linear with time) recorded polarographically. The slope (corresponding to respiration rate) was compared with the slope obtained in the corresponding standard (i.e. yeast incubated without DCA), and so the toxicity evaluated as decrease of respiration rate. To be sure that it is not a selective inhibition of respiration only, we performed some parallel measurements of CO_2-evolution in anaerobic glycolysis; the values of toxicity were identical. The pH was measured using radiometer compensating pH-meter. As no nutrient medium was used in incubation, the pH-values of solutions before and after incubation coincided, within the limits of 0.03 units.

Results. The toxicity of DCA for yeast at 37 °C and ionic strength 0.16 is zero at physiological pH (7.36) and above, but rises discontinuously by lowering the pH of solution below 7.3, the 'transition pH' being 7.30–7.33 (Figure 2). Using different samples of yeast, the transition pH was found to be the same. Preliminary experiments with erythrocytes and He-La cells indicated no dependence of the start of toxicity on substrate used. Moreover, we found that even surface tension of pure solutions of DCA shows a break at pH 7.3, not shifting with DCA concentration. (Details will be presented later.) Hence, the transformation of DCA proceeds without participation of organic material.

Additional experiments proved that cholic acid (CA) is entirely non-toxic in the pH-range 6.6–7.5. Glycodeoxycholic acid (prepared according to CORTESE[1]) was found to have a lower activity in the standard 1-h experiments, but the full toxicity of DCA developed after 5 h of incubation. We concluded that the proteolytic enzymes of yeast cells split the peptide bond in a slow process that was responsible for this delay.

Discussion. The sudden break in biological and physical properties of DCA at the transition pH (valid only at the physiological ionic strength and temperature) is principally different from the usual dependence of many biological processes on pH. In the latter case, the curve is continuous and can be correlated with dissociation of reacting species. In our case, some abrupt qualitative change in the structure of lower aggregates of DCA is to be anticipated, resembling recrystallization. Recent studies[2,3] indicated both hydrophobic and hydrophilic binding in the association process of bile acids (BA). It might be accepted that under 'critical constellation' of counterions, and especially of H^+-ions (DCA is a weak acid), the energy of hydrogen bond may prevail even in dimers. Hence, both 3α- and 12α-hydroxyls become hidden inside the aggregates, which become more lipophilic, and consequently more active biologically. In CA, the 7α-hydroxyl group probably cannot, for steric reasons, be hidden perfectly.

Physiological significance. A substance, harmless under physiological conditions, but toxic at slightly lowered pH, has been wanted[4] as cancerostatic for a therapy based on permanent acidity of all kinds of malignant growth; pH of the interstitial fluid in tumours is 7.1–6.4[5–7]. As DCA is known to interact with membrane lipids of probably all cells, its toxic form should be universally toxic, and hence be the required substance. Moreover, due to higher tendency to polymerization at lower pH[8], it can be expected to accumulate more in the permanently acid tumours than in muscles acidified only transitorily.

Therapeutic experiments with DCA (in man, harmless in peroral doses of 1.2 g daily[9]), or better with glycodeoxycholic acid (perhaps less irritating[10]), should not be made without analyses of BA in the blood. Even after attainment of the normal composition of serum BA,

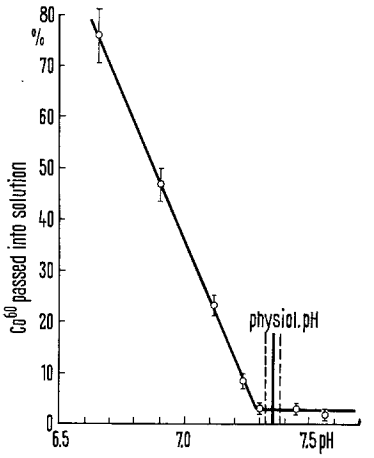

Fig. 1. Decorporation of Co^{60} from yeast by deoxycholic acid at different pH and constant ionic strength (0.16). 20 mg precontaminated yeast, 10 μmol DCA in 5 ml. 1 h incubation at 37 °C.

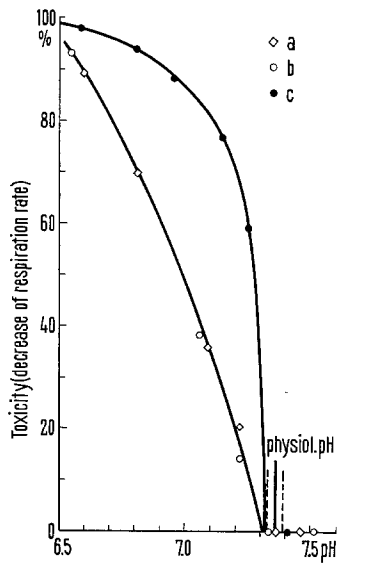

Fig. 2. Toxicity of DCA in yeast dependent on pH, at ionic strength 0.16. 1 h incubation at 37 °C. a) 100 mg yeast, 8 μmol DCA in 8 ml. b) 100 mg yeast, 20 μmol DCA in 20 ml. c) 100 mg yeast, 40 μmol DCA in 20 ml.

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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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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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