Relation between the number of congregated portions and the number of notochords in an triangular explant

No. of congregated portions	No. of specimens	Two notochords	One notochord	No differentiation of notochord
two	5	5	0	0
one	13	0	10	3

were formed independently each at either end of the base (Figure 2).

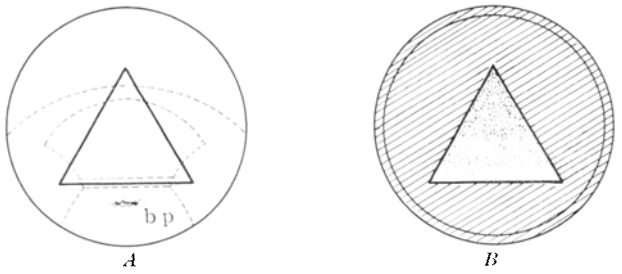

Fig. 1. – Schematic representation of the method of explantation.
A Isolation of a triangle shaped tissue from the uninvaginated dorsal blastoporal area. – *bp* blastopore.
B Culturing of the explant on the collodion membrane.

The histological observations revealed that when a single protrusion was formed, a single rod-like or occasionally a dendritic notochord appeared just at its median, except three cases in which a notochord was not found. On the other hand, when the congregations remained separate at both ends of the base, two notochords always occurred, one in each congregated portion (Table). There was no connection between them.

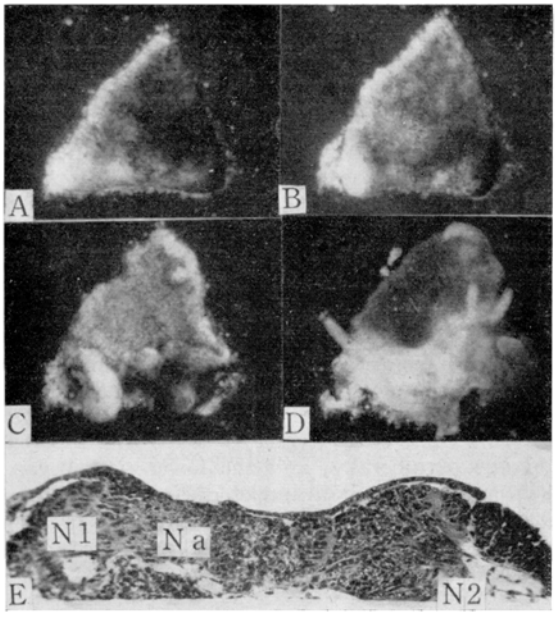

Fig. 2. – Transformation of the shape of an triangular explant in which two independent notochords occur
A one hour after isolation; *B* 24 h after isolation; *C* on the 5th day of cultivation; *D* on the 14th day; *E* notochords in this explant. *N1*, *N2* well differentiated notochord; *Na* scattered, vacuolated cells

Comparing this result with the preceding one, it can be pointed out that in the square explant two notochords are never formed, provided that its lower half is not divided into two parts by a cut, whereas in the triangular explant, two notochords are formed in 28% of cases without cutting. The formation of two notochords in an explant is thus obtained merely by modifying its shape, instead of dividing it into two parts by a cut. Moreover, it must be noticed that even in a triangular explant two notochords appear exclusively when two congregations occur in it. This fact seems to indicate more definite correlation between the congregation of the tissue and the differentiation of the notochord. From this correlation it may be anticipated that the kinetic process of the dorsal marginal zone plays some important role in the determination of the primordia of the axial mesodermal organs.

The details of description and discussion will be published elsewhere.

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Zusammenfassung

Stücke aus der dorsalen Randzone der frühen Gastrula von *Hynobius nebulosus* wurden in Holtfreterscher Lösung auf Kollodiummembranen gezüchtet. In dreieckigen Explantaten entstehen öfters zwei Chordastränge, während in viereckigen Explantaten stets nur ein Chordastrang gebildet wird. Diese Tatsachen weisen auf die Möglichkeit hin, dass Gestaltungsbewegungen des Explantates bei der Determination der Chordaanlage eine wichtige Rolle spielen.

Urinary Excretion of Aluminium after Administration in Chelated Forms

The extent to which aluminium is excreted in urine, following its administration in chelated form, depends upon the chelating agent used, as is the case with other Group III metals. The skeletal deposition of these metals may be due to the stability of their phosphates.

The introduction of chelated aluminium into the body is of interest in connection with research on prophylactic measures against silicosis[1]. Other Group III metals such as yttrium and lanthanum partially deposit in the skeleton and elsewhere after being injected into small mammals as the ethylenediamine tetra-acetic acid (EDTA) chelates. Deposition occurs in spite of the fact that the Y-EDTA and La-EDTA stability constants are far higher than of Ca-EDTA. By trying a number of powerful chelating agents, KROLL *et al.*[2] found some which gave considerably

[1] D. A. SUTTON, Pneumoconiosis Conference, Johannesburg, February 1959.

[2] H. KROLL, S. KORMAN, E. SIEGEL, H. E. HART, B. ROSOFF, H. SPENCER, and D. LASZLO, Nature *180*, 919 (1957).

less skeletal deposition of these metals; and CATSCH and DU KHUONG LÊ[3] discovered certain chelating agents to be more effective than EDTA for removing cerium from the skeleton.

Table

20 h urinary excretion of aluminium from animals after injection in chelated form

Metal chelate[a]	Percentage[b] of chelate saturated with aluminium	Urinary excretion as percentage of dose	
		c	d
Al-diethylenetriamine pentaacetic acid	96	25	27
Al-ethylenediamine N-(2-hydroxycyclohexyl)-N,N', N'-triacetic acid	94	35	38
Al-ethylenediamine tetraacetic acid	98	40	41
Al-ethylenediamine-N-(2-hydroxyethyl)N,N',N'-triacetic acid	83	80	50
Al-ethylenediamine,-N,N'-bis-(2-hydroxycyclohexyl)-N,N'-diacetic acid	87	80	56
Al-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid . . .	90	95	95
Al-polyphosphate	unknown	42	—

[a] Chelates (containing 1 mg Al) injected intraperitoneally into 450 g male guinea pigs, except Al-tripolyphosphate where 7 mg Al with 190 mg Na-polyphosphate was injected intravenously into a 1500 g female rabbit. Injection solutions were all at pH 7·4.

[b] The percentage saturation of the chelate may influence the fate of the metal (see experiments on yttrium[4]).

[c] Determined by the method of GENTRY and SHERRINGTON[5] on ashed aliquots; results of replicates quoted to the nearest 5%.

[d] Determined by the method used in [a] but on spots cut from paper chromatograms [6] (*Rf* about 0·07) after running aliquots; results are from single runs quoted to the nearest 1%.

Aluminium is now reported to be only partially excreted in the urine after injection into guinea pigs, rabbits and rats as Al-EDTA. Rabbits injected intravenously with large doses (18 mg Al/kg) died after a few days and showed mottling of the livers and to a lesser degree of the kidneys. The livers had undergone fatty change and necrosis in the periphery of the lobules and the kidneys showed tubular necrosis. A rat also died after eight days using the same dose per kg. Aluminium chelates were, however, well tolerated at reasonable dose levels; for example, a 300 g male rat injected intravenously with 3 mg of aluminium (as Al-EDTA) is apparently healthy eight months later, and a pregnant female guinea pig gave birth to a normal litter 52 days after intraperitoneal injection of 2 mg of aluminium as the 1,2-diaminocyclohexane N,N,N',N'-tetraacetic acid chelate. Urinary excretions of aluminium varied with the chelating agent used (Table).

Most of the urinary aluminium was in the sediments, presumably in the form of aluminium phosphate. Aqueous Al-EDTA (25 ml containing 75 mg Al, pH 7·4) was allowed to stand for 86 h at 37°C with powdered defatted ox shin bone (5 g); on treating the sediment with N HCl, 6 mg of aluminium were dissolved off. There was 15 mg of calcium in the supernatant. Al-EDTA reacted even with aqueous sodium orthophosphate at 25°C (pH 7) to give a small precipitate of $AlPO_4$. The reason for the skeletal deposition of Group III metals[2,3] may therefore lie in the stability of their phosphates. The question as to whether calcium chelates are excreted following administration of Group III metal chelates remains, but is not the object of this investigation.

We thank Mrs. O. L. KNIGHT and Mr. M. D. MIDDLETON for assistance with some experiments. Messrs. GEIGY of Basle presented the chelating agents and AMCOH, South Africa, the sodium tripolyphosphate. – This report is published by permission of the South African Council for Scientific and Industrial Research.

D. A. SUTTON and L. W. MARASAS

Pneumoconiosis Research Unit of the Council for Scientific and Industrial Research at the South African Institute for Medical Research, Johannesburg, July 13, 1959.

Zusammenfassung

Der Grad der Aluminiumausscheidung im Urin hängt, nach seiner Verabreichung in Form von Aluminiumchelat, vom verwendeten Chelat ab. Dies ist in Übereinstimmung mit anderen Metallen der Gruppe III. Die Ablagerung dieser Metalle im Skelett beruht wahrscheinlich auf der Stabilität ihrer Phosphate.

Increase of Free 5-Hydroxytryptamine in Blood Plasma by Reserpine and a Benzoquinolizine Derivative

Rauwolfia alkaloids and benzoquinolizine derivatives cause a decrease of the 5-hydroxytryptamine (5 HT) content in various tissues[1]. Thereby the excretion of 5-hydroxyindole acetic acid (5 HIAA), a major metabolic product of 5 HT, is increased in the urine[1]. In isolated blood platelets the 5HT decreases also after addition of reserpine or the benzoquinolizine derivative 2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-benzo[a]quinolizine as methan sulfonate (BQ)[3,4]. It has been suggested that Rauwolfia alkaloids and benzoquinolizine derivatives act by releasing the 5HT from the binding site in the tissue. The released 5HT would then be oxidized by monoamine oxidase (MAO) and excreted as 5HIAA in the urine[5]. There is, however, no proof that 5HT is liberated as such; it might be metabolized, e. g. at the binding sites, and released as 5HIAA. Evidence for the first possibility consists in the observation that incubation of rabbit platelets with reserpine in an atmosphere of nitrogen causes an increase of the amount of 5HT in the plasma[6].

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