

centrifugation, thrice triturated with ethanol to remove excess phenol and dried with ether. The highest binding power resulted in the fraction precipitated at 20–30% ethanol concentration (10.869 $\mu\text{g}/\text{mg}$). This fraction was redissolved in phenol-water solution and the ethanol fractional precipitation repeated: the activity resulted at the same ethanol levels, but only a slight increase in binding power was observed (11.961 $\mu\text{g}/\text{mg}$). This fraction was then dissolved in water (2% w/v), at 0°, Barium acetate (3% w/v) was added to assist flocculation, and the precipitates formed by fractional addition of ethanol collected by centrifugation, redissolved in water and treated with Na_2SO_4 ¹: a precipitate was obtained, discarded by centrifugation, and the supernatant was dialysed 48 h against distilled water at 0–4° and dried in vacuo from the frozen state. The highest binding power resulted in the fraction precipitated at the 50–60% ethanol level. Electrophoretic examination shows that the material is not grossly inhomogeneous (only traces of a component with a slow and another with a high mobility seem to be present as impurities).

The collected physical and chemical data on this almost pure preparation of vitamin B_{12} -binding factor are: total nitrogen 8.2%, uronic acids nearly absent, hexosamines 17.1%, reducing sugars 18.3%, non-glucosamine polysaccharides 21.6%, binding power on vitamin B_{12} 22.123 $\mu\text{g}/\text{mg}$. Electrophoretic mobility (ascending) (Fig. 2) is about $-2.3 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \text{ V}^{-1}$.

With inhibition agglutination tests (anti-A and anti-H sera), only a very slight activity at the concentration of 1 mg/ml is present.

A. CRESSERI

Biological Laboratory, Istituto Carlo Erba per Ricerche Terapeutiche, Milano, October 1, 1954.

Riassunto

Vengono riferiti i risultati dell'esame con elettroforesi continua su carta di una preparazione dalla mucosa gastrica di maiale dotata di potere di legame sulla vitamina B_{12} e l'isolamento da essa con metodi di estrazione e di precipitazione frazionata del fattore responsabile di tale legame.

¹ H. SMITH, R. C. GALLOP and J. L. STANLEY, *Biochem. J.* 52, 15 (1952).

Nuclear Uptake of Methionine- S^{35} in the Newt Embryo

The incorporation of glycine- 2-C^{14} predominantly into the nuclei of the dorso-axial structures and induced neural plate of the amphibian embryo has been described in a previous paper, and interpreted as resulting from a higher nuclear metabolism of those regions of the embryo¹. It should be of interest to experiment with another amino-acid, such as methionine, which might be expected to enter into rather more localized metabolic processes than glycine.

In a new experimental series, embryos of *Triturus alpestris* were kept at room temperature in solutions of DL-methionine- S^{35} , with original activities of 37.2 or 40.9 $\mu\text{C}/\text{ml}$. The incubations were all finished before the tracer had decayed for a month. The solutions also con-

tained sodium sulphadiazine (May & Baker) to preserve asepsis. As the ratio of methionine to sulphadiazine was approximately 1.4:1, any antagonism between them, as

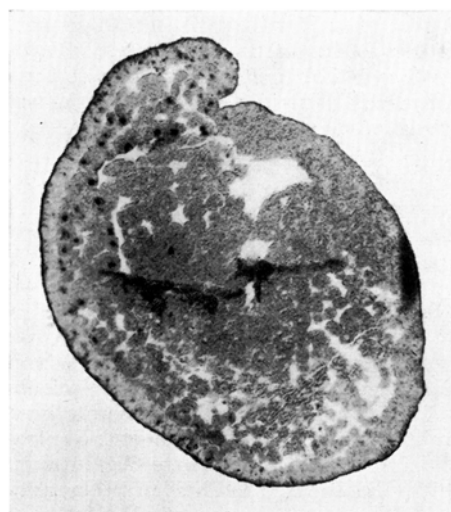


Fig. 1.—Autoradiograph of a mid-gastrula ($\times 40$).

described elsewhere¹, would probably have been very slight. Early blastulae or older embryos were slit open to facilitate the penetration of the tracer, and neural-fold stages onwards were transversally transected. After culture in the tracer solution, the embryos were rinsed three times in a similar solution made with non-radioactive methionine. The technique for preparation of the autoradiographs has been given before², but in the present experiments the exposure of the film was sometimes prolonged up to two months. When the last autoradiographs were developed the tracer had decayed for 3.5 months.

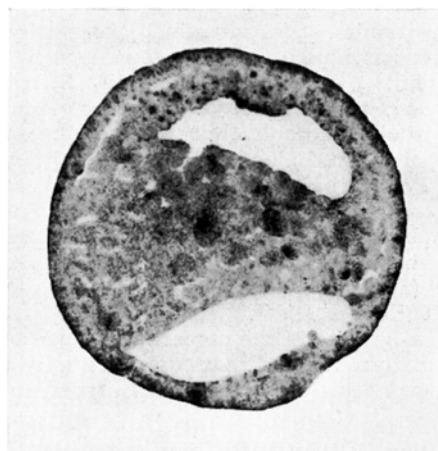


Fig. 2.—Autoradiograph of a neural-plate embryo ($\times 40$).

Embryos kept for 24 h in the tracer solution, from cleavage until the first appearance of the blastopore, show a marked nuclear activity in the animal half, which can be sometimes noticed particularly in the nuclei of the

¹ J. S. HARRIS and H. I. KOHN, *J. Pharm. exp. Therap.* 73, 383 (1941).

² J. L. SIRLIN and C. H. WADDINGTON, *Nature* 174, 309 (1954).

¹ J. L. SIRLIN and C. H. WADDINGTON, *Nature* 174, 309 (1954).

dorsal lip of the blastopore; some cells in the floor of the blastocoele have also a considerable activity in their cytoplasm. In embryos incubated with tracer for 25 h,



Fig. 3.—Autoradiograph of a neurula ($\times 50$).

from the late cleavage until mid-blastopore stages, there is a very high nuclear charge in the already invaginated mesoderm, and a lesser, although definite, nuclear charge in the presumptive mesoderm and neural plate; the activity in nuclei of other tissues is low (Fig. 1). Early blastulae incubated for 40 h until the neural-plate stage show high nuclear activity in the mesodermal roof of the archenteron, and also a less high, though clear, activity in the neural-plate nuclei. As a consequence of the long incubation, the cytoplasm has a considerable radioactive content, but it is still not nearly as dark as the nuclei (Fig. 2). Neural-fold embryos incubated for 28 h until

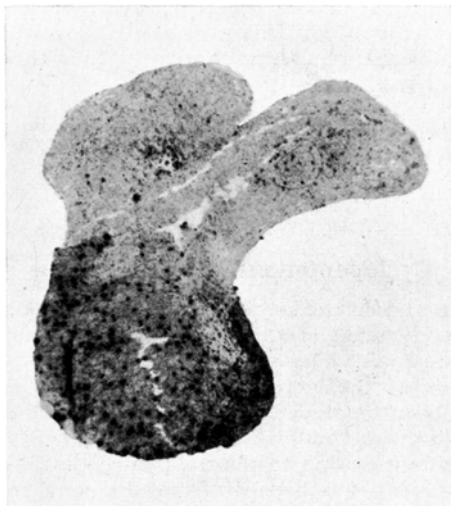


Fig. 4.—Autoradiograph of an exogastrula ($\times 30$).

the neural-tube stage show great activity in the inter-spaces between notochord, somites and neural tube. This may be partly because the flux of the tracer is easier along these spaces, but it must be pointed out that all soluble materials would have been removed during the preparation of the autoradiographs, and the high activity must indicate that the S^{35} is incorporated into the material of the intercellular jelly. Some mesoderm and endoderm nuclei in these embryos also show great

charge; although not clearly seen in the figure there appears to be an increased uptake in the mesoderm and epidermis, a fact which parallels the phenomena described for glycine- $1-C^{14}$ ¹ (Fig. 3).

An *axolotl* exogastrula, kept in the tracer solution for 95 h since the blastula stage, demonstrates clearly that the ectodermal nuclei have a greater uptake than the endodermal nuclei; some active nuclei in the core of the evaginated endoderm are probably somitic (Fig. 4). This is evidence that the nuclear behaviour is tissue-specific, and is almost independent of the diffusion path of the tracer.

These experiments, together with those described earlier, suggest a common pattern in the utilization of DL-methionine- S^{35} and glycine- $2-C^{14}$ by the nuclei in the dorso-axial structures, which are mainly responsible for the laying down of the embryonic organization. To what extent, in this process, the fate of the S^{35} and C^{14} atoms depends on their being part of the amino-acid molecule is at present under investigation.

The author wishes to thank Professor C. H. WADDINGTON for encouragement during the course of the work. This investigation was carried out during the tenure of a British Council scholarship for which grateful acknowledgement is made. The work also received financial support from the Agricultural Research Council.

J. L. SIRLIN

Institute of Animal Genetics, Edinburgh, October 20, 1954.

Zusammenfassung

In der vorliegenden Arbeit wird die Aufnahme von in DL-methionin enthaltenem S^{35} durch die Zellkerne des Mesoderms und der Neuralplatte des Amphibien-Embryos beschrieben. Es wird auf die Ähnlichkeit hingewiesen, die mit der Aufnahme von in Glykokoll enthaltenem C^{14} besteht.

¹ C. H. WADDINGTON and J. L. SIRLIN, J. Embryol. exp. Morph. (in press).

Eine Spinne mit dem Körperbau von *Zilla-x-notata*, aber mit anderem Netzbauverhalten

Bei pharmakologischen Experimenten mit *Zilla-x-notata* Cl. in Bern in den Jahren 1949–1954 (WITT¹) fiel es mir auf, dass die Radienzahl der Netze nicht mit der in anderen Arbeiten angegebenen übereinstimmte. So gibt KÖNIG² für seine *Zilla-litterata* = *Zilla-x-notata*-Netze eine mittlere Radienzahl von 25 mit Extremwerten von 21 und 31 bei 261 vermessenen Netzen an. Ich selber fand in Tübingen bei 48 während der gleichen Monate untersuchten Netze eine mittlere Radienzahl von 24,4 mit Extremwerten von 18 und 32, stimmte also bei den unter Laboratoriumsbedingungen in Tübingen gebauten Netzen mit den Zahlen von KÖNIG aus Freiburg i.Br. überein. In 104 in Bern im Laufe von 4 Jahren wieder in den gleichen Sommermonaten vermessenen Netzen zählte ich durchschnittlich 42,5 Radien mit Extremwerten von 20 und 60, ein Unterschied gegenüber Tübingen, der mit dem T-Test von Student mit $T = 4,7175$ bei $P 0,01 = 3,361$ statistisch stark gesichert werden konnte. Abbildung 1 zeigt ein Netz aus Bern.

¹ P. N. WITT, Helv. physiol. Acta 7, C 65 (1949); Exper. 7, 310 (1951); Behaviour 4, 172 (1952).

² M. KÖNIG, Z. Tierpsychol. 8, 463 (1951).