aufwiesen, teils ohne, teils mit Bleiabdeckung der Blattrosette mit 2000 r, 4000 r, 8000 r und 16000 r bestrahlt (50 kVs, 1000 mA, 0,9 mm Al-Filter, 1000 r/min Dosisleistung in einem Focus-Objekt-Abstand von 59 cm).

Die Samen sämtlicher Schoten wurden, an der Mutterpflanze ausgereift, nach Dosen getrennt, gesammelt.

Neben einer grossen Zahl dikotyler Keimlinge kamen bei allen Bestrahlungsserien synkotyle, anisokotyle, trikotyle und vereinzelt tetrakotyle Sämlinge und Mehrlinge vor (Tab. I).

Die Auslösung von Keimblatt-Anomalien durch Röntgenstrahlen unter Anwendung der beschriebenen Methodik ist danach als sichergestellt zu betrachten. Ein nicht zu unterscheidender Effekt bei Embryonen von Pflanzen, deren Blattrosetten bleigeschützt bzw. ungeschützt waren, dürfte auf eine ausschliesslich lokale Beeinflussung hinweisen; eine regulatorische Funktion der unbestrahlten Bezirke scheint nicht stattzuhaben. Über die Dosisabhängigkeit der Aberrationsrate kann vorläufig keine Aussage gemacht werden. Eine solche könnte erst nach Einschaltung von Zwischendosen für den Bereich unter 8000 r erfolgen. Eine strenge Koordination gewisser Aberrationstypen zu bestimmten *Röntgendosen* besteht nicht. Für die Gruppe der auch genetisch besonders interessierenden Trikotylen, die spontan nur zu 0,2% (unter 18997 Kontrollpflanzen wurden 39 trikotyle gefunden) vorkommen, konnte die Feststellung gemacht werden, dass die Regelmässig-Trikotylen (t_r) – gleich starke Ausbildung aller 3 Kotyledonen – bei 2000 r dominieren, bei 4000 r nur noch selten gebildet werden und bei 8000 r ganz verschwinden, während die Unregelmässig-Trikotylen (t_u) mit Erhöhung der Dosis zunehmen. Es liegt nahe zu vermuten, dass der experimentell hervorgerufene Typus «trikotyl», der völlig symmetrisch gebaut ist und auch als häufigste Spontanabweichung vorkommt, seine Entstehung chemischen Umstimmungen in den bestrahlten Embryozellen verdankt, die bei niederen Dosen allein oder zumindest vorwiegend wirksam sind, während mit Zunahme der Ionisationsakte Zellschäden auf direktem biophysikalischem Weg entstehen, die sich dem chemischen Effekt überlagern und zu unregelmässigem Wachstum führen.

Um zu prüfen, ob Beziehungen zwischen dem beeinflussten *Embryostadium* und der Ausbildung des Keimblatt-Typus bestehen, sind Versuche an *Eranthis* im Gange[1]. Wurden die Samen der Schoten verschiedener Etagen des Blütenstandes von *Arabidopsis*-Pflanzen getrennt auf die Abnormitäten ihrer Keimlinge geprüft, so konnte an dem allerdings erst kleinen Untersuchungsmaterial eine Relation zwischen bestrahlter Entwicklungsstufe und Aberrationstypus nicht gefunden werden. Wie Tabelle II zeigt, lieferten diese Versuche, in denen die einzelnen Schoten, ausgehend von einer Markierungsschote, nach ihrer Stellung im Blütenstand geordnet wurden, die Bestätigung für unsere Annahme, dass die Embryonen nur in einem frühen Entwicklungsstadium hinsichtlich ihrer Keimblattausbildung zu beeinflussen sind.

Eine ausführliche Schilderung der Versuche wird, sobald die Selbstungsnachkommenschaften der aberranten Typen ausgewertet sind, in einer Fachzeitschrift erfolgen.

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Summary

A previous experiment with *Eranthis* showed that ROENTGEN irradiation of "undivided" embryos led to a change in the number of cotyledons. In the present publication, the induction of anomalies of the cotyledon was shown after irradiation of early embryo stages on the mother plant in Arabidopsis thaliana, an example of a plant with "complete" embryos.

Which aberration type (synkotyl, anisokotyl, trikotyl, tetrakotyl or multiple seedlings) will arise, seems to be determined neither by the ROENTGEN dose nor by the stage of development on irradiation. The appearance of predominately regular trikotyl types after application of 2000r, and their gradual disappearance with increasing doses in favour of the irregular trikotyl types, suggests that in the lower doses the main influences are upon the purely chemical reactions in the embryos, which are overlaid by cell damages occurring in a directly biophysical way with increase of the physical primary acts and lead to irregular growth.

[1] B. HACCIUS, unveröffentlicht.

Inhibition of Thiamine Synthesis by Vitamin B_{12} in Wild Strains of *Escherichia coli*

Inter-relationship has been shown to exist between vitamin B_{12} and amino acids[1], methionine[2], glycine[3] and certain vitamins such as cholines folic[4], acid[5], and ascorbic acid. BURKHOLDER's[6] study on the absorption of vitamin B_{12} by the cells of *Escherichia coli* led JÄNNES[7] to investigate the effect of this vitamin on the synthesis of other vitamins of the B complex by the micro-organism. He observed that vitamin B_{12} had no effect on the production of nicotinic acid, folic acid and biotin, but synthesis of pantothenic acid, was reduced by about 20% in presence of 0·03 γ B_{12}/ml. With 0·06 γ B_{12}/ml the synthesis was completely inhibited in certain cases. In view of this fact it was thought of interest to investigate the effect of vitamin B_{12} on the biosynthesis of thiamine by *E. coli*[8]. The results obtained are presented in this communication.

Since thiamine synthesis in older cultures was rather low, two freshly isolated cultures of *E. coli*, maintained on nutrient agar slants, pH 7, were used for the present study. The glucose salt medium of DAVIS and MINGIOLI[9] modified by the addition of 0·05% sodium chloride as suggested by JOHANSSON[10] was used. One hundred ml of the medium was taken in 250 ml ERLENMEYER flasks and sterilized by autoclaving at 15 lbs. for 15 min. Sterile solution of vitamin B_{12} in appropriate dilutions was added aseptically to the medium. A washed cell suspension of *E. coli* was made from 48 h

[1] L. W. CHARKEY, H. S. WILGUS, A. R. PATTON, and F. X. GASSNER, Proc. Soc. Exp. Biol. Med. *73*, 21 (1950).

[2] A. E. SCHAEFER, W. D. SALMON, and D. R. STRENGTH, Proc. Soc. Exp. Biol. Med. *71*, 193 (1949). – G. M. BRIGGS, E. G. HILL, and M. J. GILES, Poultry Sci. *29*, 723 (1950).

[3] H. MENGE and G. F. COMBS, Proc. Soc. Exp. Biol. Med. *75*, 139 (1950).

[4] A. E. SCHAEFER, W. D. SALMON, and D. R. STRENGTH, Proc. Soc. Exp. Biol. Med. *71*, 202 (1949). – M. B. GILLIS and L. C. NORRIS, Poultry Sci. *28*, 749 (1949).

[5] L. S. DIETRICH, C. A. NICHOL, W. J. MONSON, and C. A. ELVEHJEM, J. Biol. Chem. *181*, 915 (1949). – A. E. SCHAEFER, W. D. SALMON, D. R. STRENGTH, and D. H. COPELAND, J. Nutr. *40*, 95 (1950).

[6] P. BURKHOLDER, Science *114*, 478 (1951).

[7] J. JÄNNES, Experientia *10*, H. 1, 31 (1954).

[8] D. W. WOOLLEY and A. G. WHITE, J. Exptl. Medicine. *78*, 495 (1943). – W. H. PETERSON and M. S. PETERSON, Bact. Rev. *9*, 49 (1945).

[9] B. D. DAVIS and E. S. MINGIOLI, J. Bact. *60*, 17 (1950).

[10] K. R. JOHANSSON, Proc. Soc. Exp. Biol. Med. *83*, 448 (1953).

Inhibition of Thiamine Synthesis by Vitamin B_{12} in Cultures of *E. coli*

Concentration of B_{12} added γ ml	24 h			48 h		
	Growth % Transmission	B_1 content γ/ml	Reduction in synthesis %	Growth % Transmission	B_1 content γ/ml	Reduction in synthesis %
0	64	0·0176	—	55	0·0320	—
0·01	64	0·0136	22·8	57	0·0256	20·0
0·1	65	0·0120	31·8	55	0·0235	26·6
0·5	65	0·0096	45·5	56	0·0200	37·5
1·0	63	0·0080	54·6	57	0·0144	55·0

old culture, growing on agar slants and adjusted to approximately 60% transmission in a Lumetron photoelectric colorimeter with red-filter (650 mμ). One drop of this suspension was added to each of the flasks. The flasks were then incubated at 30°C. Twelve ml of the culture was taken out aseptically from each flask after 24 and 48 h and the growth was measured in terms of percentage transmission in the Lumetron colorimeter. The culture was then centrifuged at 4000 rpm. for 20 min and the supernatant was assayed for thiamine by thiochrome method[1], measuring the fluorescence in a Lumetron Fluorimeter 402 E using the filter accessories for thiamine. Five ml samples in duplicate were taken for each estimation and the results are presented in the Table.

It is clear from the table that the addition of vitamin B_{12} inhibits the biosynthesis of thiamine by *E. coli*. The more the vitamin added the less is the synthesis and excretion of thiamine in the medium. At 24 h the percentage decrease in the synthesis was 31·8 at 0·1 γ B_{12} level and 54·6 at 1·0 γ level. There does not appear to be any appreciable difference in the inhibition of synthesis at 24 and 48 h, in spite of the fact that slight variations have been obtained between the two periods. It may also be pointed out here that the growth of *E. coli* was not affected by the presence of vitamin B_{12} in the medium.

This phenomenon of the inhibition of thiamine synthesis is interesting, but no explanation seems to be possible at this stage. It may be that vitamin B_{12} blocks in some way the biosynthesis of thiamine by this organism or that the requirement of thiamine is dispensed with in the presence of vitamin B_{12} in the medium. It may also be possible that vitamin B_{12} accelerates the utilization of thiamine by the cells or that the retention of thiamine may be increased by the presence of vitamin B_{12} in the medium. Further work, especially on the thiamine content of the cells, now in progress, may elucidate the role played by vitamin B_{12} in the thiamine metabolism, as far as this organism is concerned.

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Central Drug Research Institute Lucknow, India, July 15, 1954.

Zusammenfassung

Zusatz von Vitamin B_{12} in Kulturnährböden hemmt die Thiamin-Biosynthese von frisch isolierten *E.-coli*-Stämmen. Diese Hemmung steht im Verhältnis zur zugesetzten Menge von Vitamin B_{12}.

[1] The Association of Vitamin Chemists, Inc. "Methods of Vitamin Assay", Inter Science Publishers, New York, N. Y. 1947, p. 73.

DISPUTANDUM

To the Question of Somatic Reduction Divisions in Sea Urchin Micromeres

Some time ago Lindahl[1] has drawn attention to the resemblance of sea urchin micromeres to polar bodies and suggested that they might be the result of somatic reduction divisions. He consequently claimed that sections of *Paracentrotus lividus* and *Strongylocentrotus droebachiensis* embryos revealed the presence of haploid chromosome sets in the micromeres.

On the other hand, a photometric study of the desoxyribonucleic acid content of embryonic nuclei of *Lytechinus variegatus* by McMaster[2] gave no indication of haploidy in micromeres or their descendants.

The present authors had an opportunity to investigate this matter during a visit of the senior author at the University of California, in March 1953. Chromosome counts were made on 32-cell embryos of *Strongylocentrotus purpuratus* obtained through the courtesy of Professor D. Mazia. The material was fixed in 5% acetic acid, stained *in toto* with aceto-orcein-fast-green[3] or by the Feulgen method and mounted whole without dehydration. In many cases most of the cells of one embryo were found to be in various stages of division but reductional groupings or haploid chromosome sets were never seen. Macromeres, mesomeres and micromeres in different regions of one embryo were found to possess each approximately 38–40 chromosomes at metaphase.

The senior author subsequently had the opportunity to reexamine cleavage stages of the Japanese sea urchin *Strongylocentrotus intermedius* by the same methods. The diploid chromosome number of this species is 50[4]. Careful observations of micromere divisions from metaphase to anaphase in 16-cell to 32-cell stages were made. No reductional grouping or haploid chromosome number occurred in any of the cells that were studied. A detailed account of these observations will be published elsewhere by the senior author.

Without adding substantial new evidence, Lindahl[5] more recently published a somewhat fuller account of his views and expressed the opinion that somatic reduction of chromosomes in micromeres is a consequence of secondary pairing of chromosomes. In the present authors' opinion such secondary pairing is not a natural feature of sea urchin cleavage, but the result of acciden-

[1] P. E. Lindahl, Nature *171*, 437 (1953).
[2] R. McMaster, Anat. Rec. *113* (4), 36 (1952).
[3] N. B. Kurnick and H. Ris, Stain Tech. *23*, 17 (1948).
[4] S. Makino and H. Niiyama, J. Fac. Hokkaido Univ. Ser. VI, Zool. *9*, 225 (1947).
[5] P. E. Lindahl, Exp. Cell Res. *5*, 416 (1953).