Ces produits ont été soumis à l'immunoélectrophorèse selon la méthode de SCHEIDEGGER[4], la révélation étant effectuée par le sérum de cheval anti-sérum humain (ch. 491) de l'Institut Pasteur. La précipitation immunochimique terminée, les plaques sont aussitôt séchées, colorées et mises au contact d'un film radiographique pendant 10 jours. A titre de contrôle, la solution d'albumine marquée a subi le même examen immunoélectrophorétique.

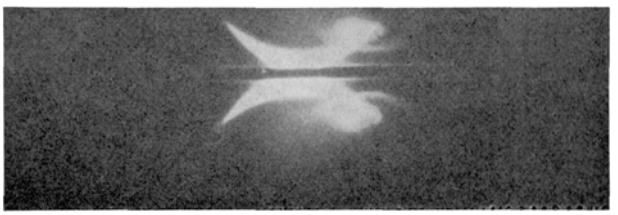


Fig. 2. Autoradiographie d'une immunoélectrophorèse obtenue avec la solution d'albumine radio-active et révélée au moyen de sérum anti-sérum-humain. (On note la présence de deux traits, l'intense correspondant à l'albumine, le second à une protéine contaminante.)

Résultats. A l'examen des diagrammes immunoélectrophorétiques obtenus avec les 3 lots de plaquettes, on n'observe pas de différence appréciable, si ce n'est une légère diminution d'intensité du trait correspondant à l'albumine lorsque les plaquettes lavées 7 fois ont à nouveau subi 7 lavages. Mais, ainsi que le prouve la Figure 1, le trait albumine induit par le lysat de plaquettes incubées dans la solution radio-active impressionne le film radiographique comme le fait la solution d'albumine marquée (Fig. 2). Les liquides des 6e et 7e lavages de ces plaquettes ne contiennent pas d'albumine décelable immunochimiquement. Soumis à l'immunoélectrophorèse, même après concentration, ils ne donnent naissance à aucune ligne de précipitation et sont incapables d'impressionner le film radiographique. Enfin, mis au contact d'immunoélectrophorèses obtenues avec les 2 lots de plaquettes témoins, ce film reste vierge. Le lysat de plaquettes incubées en albumine marquée renferme donc de cette protéine.

Ces résultats permettent d'affirmer que les plaquettes humaines sont capables d'adsorber de façon tenace une protéine en présence de laquelle elles sont incubées, puisque, dans le cas présent, grâce à sa radio-activité, cette protéine est encore décelable après 7 lavages. Ils apportent une nouvelle preuve en faveur de la notion selon laquelle l'atmosphère plasmatique périplaquettaire serait constituée de protéines adsorbées à la surface de thrombocytes.

J. SALMON

Institut de Clinique et de Pathologie médicales, Université de Liège (Belgique), le 27 juillet 1959.

Summary

When brought into contact with radioactive albumin, the blood platelets adsorb this protein firmly, thus proving the existence of intensive surface phenomenon at the level of these elements.

[4] J. J. SCHEIDEGGER, Int. Arch. Allergy 7, 103 (1955).

Transfer of the Antigenicity of Guinea Pig Bone Marrow Implants to the Graft Tissue in Explantation Experiments

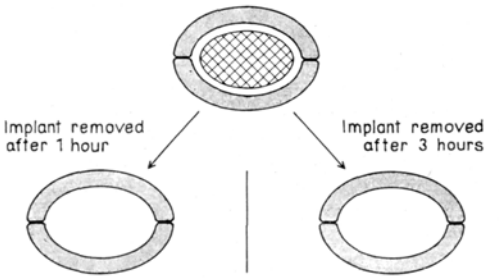
Observations, indicating that differentiating interaction between embryonal tissues is caused by diffusible agents, have been made with regard to primary[1,2] and late[3,4] induction. ROUNDS *et al.*[5] and FLICKINGER *et al.*[6] reported serological evidence for the transfer of the antigenicity between embryonic tissues in chimeric frog-salamander explants, and showed the movement of proteins from the frog embryo chorda mesoderm or larval brain to the salamander gastrula ectoderm. Because the passing of macromolecules in primary induction might be the principal cause of antigenic movement, we found it important to determine whether such a transfer of antigenicity occurs when using an alcohol-treated heterogenous inductor.

Methods. — The presumptive epidermis of the common newt (*Triturus vulgaris*) was used in sandwich experiments with the bone marrow of guinea pig as inductor. The bone marrow was fixed in 70% alcohol 2–4 h at 4°C. Each series consisted of 21–27 explants. After 1 or 3 h, the explants were opened and the inductor tissue was removed. The explants were thoroughly washed and homogenized in a conical glass grinder in 0·5 cm^3 suitably buffered saline. The prepared suspension was centrifuged at 3000 *g* and 25000 *g* and the clear supernatant was used for titrations in ring precipitation tests. Antiserum prepared against fresh guinea pig bone marrow homogenate was used throughout the experiments. Several series of intravenous and intramuscular injections with Freund complete adjuvants were administered for immunisation. Ring precipitation tests were carried out, using titrations of antigen solutions, and the results were read after half an hour at room temperature. A few experiments were also made with anti-guinea pig bone marrow serum coupled to Lissamine Rhodamine (RB-200)[7]. After two absorptions with charcoal and one absorption with acetone-dried rabbit liver, the cells of explants were stained in small tubes, after having been separated from each other by suction through a narrow Pasteur pipette. After careful washings, the cells were put on a glass plate and result was read using a fluorescence-microscope. The usual indirect control was made, and also staining with a normal rabbit serum pool coupled to RB-200.

Results. — As seen in Fig. 1, normal rabbit serum pool tends to precipitate an antigen solution made from ectodermal cells of newt gastrula. Anti-guinea pig bone marrow serum, again, seems to precipitate the antigen solution of explants from which the inductor has been removed after 1 h. In experiment I the antigen titer is on the level of the controls, but in experiment II the corresponding titer is higher. When the inductor was removed after 3 h, it was possible to show a higher degree of guinea pig bone marrow antigenicity in solution made from explants especially in experiment II. In both experiments, the transferred antigenicity seemed to in-

[1] M. C. NIU and V. C. TWITTY, Proc. nat. Acad. Sci., Wash. *39*, 985 (1953).
[2] M. C. NIU, Proc. nat. Acad. Sci., Wash. *44*, 1264 (1958).
[3] C. GROBSTEIN, Exp. Cell Res. *13*, 575 (1957).
[4] C. GROBSTEIN and J. DALTON, J. exp. Zool. *135*, 479 (1957).
[5] D. E. ROUNDS and R. A. FLICKINGER, J. exp. Zool. *137*, 479 (1958).
[6] R. A. FLICKINGER, E. HATTON, and R. E. ROUNDS, Exp. Cell Res. *17*, 30 (1959).
[7] C. S. CHADWICK, M. G. MCENTEGART, and R. C. NAIRN, Lancet *1*, 412 (1958).

crease when the inductor was allowed to remain for 3 h instead of 1 h.



Two fold titers of antigen solution

EXP. I control	21 explants 1/2	1/2	22 explants 1/1	1/64
EXP. II control	27 explants 1/2	1/64	22 explants 1/1	1/512

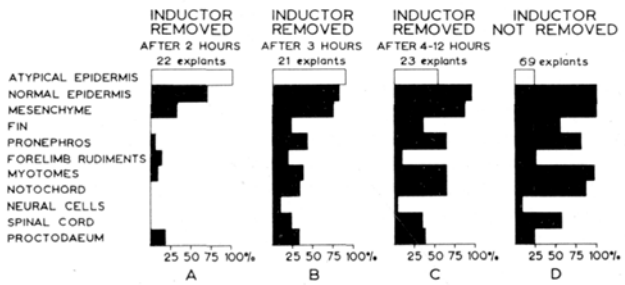


Diagram showing the frequency of the various differentiations induced in explants of *Triturus*, when alcohol-treated bone-marrow of the guinea-pig was used as inductor. (Toivonen-58)

Control titers of the rabbit anti-guinea pig bone marrow serum. Ring precipitation tests.

Antigens

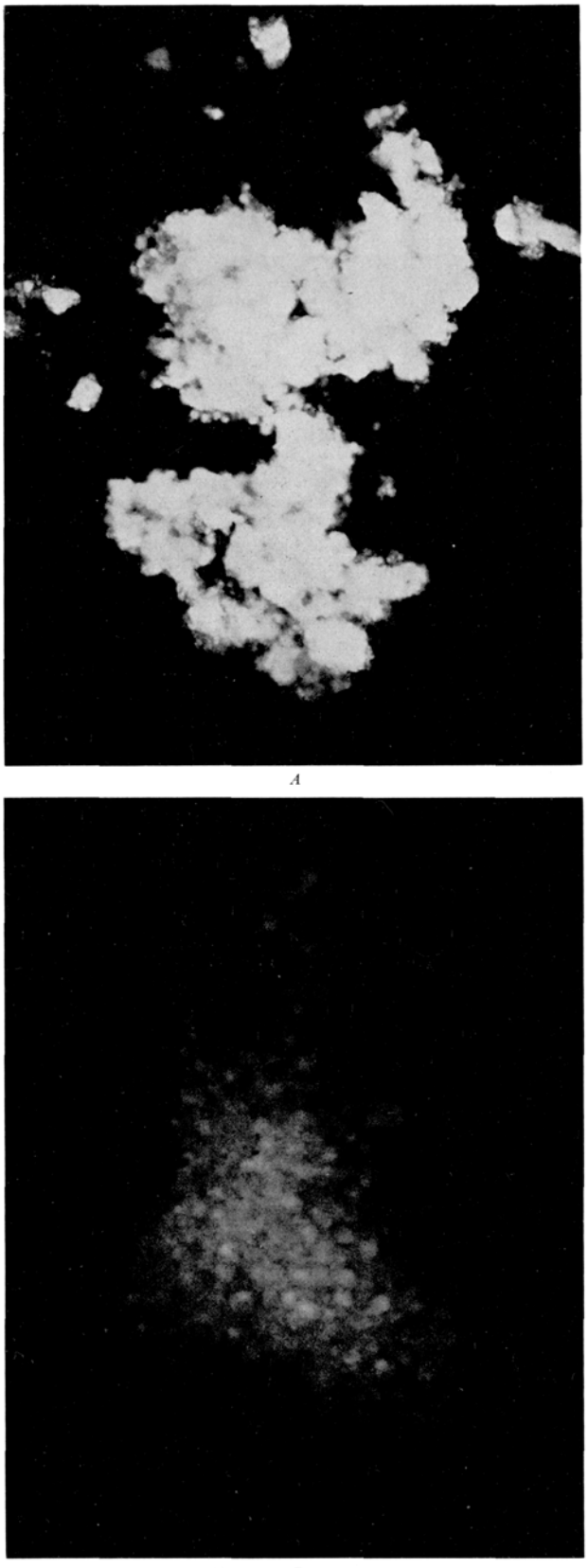
	Homogenate of fresh guinea pig bone marrow 5,5 mg/ml*	Homogenate of guinea pig bone marrow treated in 70% ethanol half an hour 3,5 mg/ml*
Antiserum	1/5000	1/1000
Normal rabbit serum pool	—	—

*the initial concentration of antigen solution

Fig. 1. – Two experimental series of transfer of antigenicity from implant to totipotent ectoderm. Two flod titers of antigenic solutions have been made by ring precipitation techniques using normal rabbit serum pool as control. A control titer of anti-guinea pig bone marrow serum is determined with fresh and fixed bone marrow material. Toivonen's[8] experiments show the movement of inducing effect as a function of time

The staining experiments with fluorescent antibodies showed that the bone marrow antigenicity passed into the reaction material (Fig. 2). In the control slides, some yellow autofluorescence was seen but the typical reddish colour seen in experimental cases was absent. It was not possible to localize transferred antigens because sectioning of dispersed cells was not performed. Some trials have been made by embedding the material in paraffin, but dehydration with alcohol or butanol seems to destroy the transferred antigens.

Discussion. – It is obvious that the above-mentioned data do not suggest any quantitative approach to the problem of transferred antigens, particularly as no attempt was made to measure the protein content in the scanty antigen material. However, it has been demonstrated that there was an obvious increase of bone mar-



A

B

Fig. 2. – The epidermal cells were stained with fluorescent (RB-200) anti-guinea pig bone marrow serum (*A*) or fluorescent normal rabbit serum pool (*B*) after 3 h explantation. The definitely lower intensity of fluorescence observed in Figure *B* resembles that observed in indirect controls. The photographic process was the same in both cases

row proteins in *Triturus* tissue during the cultivation from 1–3 h. The titer is seemingly affected by newt proteins in solution, which might explain the high titers observed in experimental series compared to the titers observed by adjusting the antiserum. It has been noted before that to obtain some mesodermal induction, a bone marrow implant must act on ectoderm for at least 3 h[8]. Thereafter the degree of induction shows only slight increase. Even if our results do not justify correlation of the movement of antigens to the primary induction phenomenon, they offer two important suggestions. First, it seems probable that the handling of inductor tissue with 70% alcohol, even when denaturing the greater part of soluble proteins, leaves certain antigenic material active and this may pass into the reaction material during the time primary induction has been assumed to occur. Secondly, the same kind of movement of antigens as observed by FLICKINGER[6] can be demonstrated when using 'chimeric explants' of fixed adult heterogenous tissues and newt totipotent ectoderm. The residual inductor material after removal of implant has been considered minimal[8]. This suggestion is supported by the fact that, in a number of slides stained with fluorescent antibodies, the fluorescence was observed to be localized to the ectodermal cells and not to extracellular material.

The results, which have been confirmed using fluorescent antibodies in preliminary experiments, open up possibilities for closer analysis of transferred material during primary induction; adult antigens provide a good tool for preparing efficient antibodies and it has been noted previously that the effect of mesodermal inductor might be inhibited by antibodies against inducing tissues[9].

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State Serum Institute, Helsinki, Department of Pathology, Section II and Zoological Laboratory, University of Helsinki (Finland), September 9, 1959.

Zusammenfassung

Der Übertritt von Antigenmaterial aus heterogenem Induktor in Ektoderm wurde mit der Ringpräzipitation untersucht. Nach Entfernung des Implantates wurde als Antigen ein Ektodermextrakt verwendet und als Induktor alkoholbehandeltes Knochenmark von Meerschweinchen. Die ins Ektoderm übergetretene Antigenmenge war bei 3 h alten Kulturen grösser als bei 1 h alten.

Addendum: Since this preliminary note was sent to the editor, a paper has appeared[10] showing the transfer of guinea pig antigenicity from the liver cells (treated with alcohol) to the totipotent ectoderm. The fluorescent antibody technique was used and the explants were prepared from *Triturus alpestris* ectoderm.

[8] S. TOIVONEN, J. Embryol. exp. Morph. *6*, 479 (1958).

[9] T. VAINIO, Exp. Cell Res. *15*, 184 (1958).

[10] R. M. CLAYTON and A. ROMANOVSKY, Exp. Cell. Res. *18*, 410 (1959).

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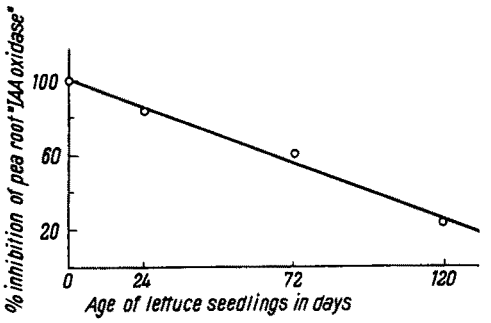
A Thermostable Substance from Lettuce Seeds and Seedlings Inhibiting β-Indolyl-acetic Acid Oxidation

During the course of an investigation into the growth substances present in lettuce seeds and seedlings (variety Grand Rapids), the IAA[1]-oxidizing activity of homogenates of these tissues was tested. The presence of 'IAA oxidase', i.e. the complex of enzymes responsible for the oxidation of the auxin[2], could not be demonstrated either in ungerminated seeds or in seedlings up to the age of 20 days. A thermostable inhibitor (or inhibitors) of IAA oxidation was, however, detected in homogenates of both



The relationship between the age of lettuce seedlings extracted and the degree of inhibition of 'IAA oxidase' produced by the extracts (Lettuce seedlings were germinated in complete darkness).

seeds and seedlings. This inhibitor was tested on an IAA-oxidizing system prepared from roots of 8-day old etiolated Alaska pea seedlings. 100 g (fresh weight) of root tissue was ground in 100 ml 0·15 *M* phosphate buffer, pH 6·0. Cell debris was removed by squeezing through cheese cloth and centrifuging at 700 × g for 5 min. The supernatant served as the source of the IAA oxidase. Lettuce homogenates were prepared in a similar way, by grinding 1 g of seeds or seedlings in 10 ml of phosphate buffer pH 6·0. Three ml of the IAA oxidase suspension were mixed with 1–3 ml of lettuce homogenate, 1 ml 10^{-4} *M* $MgCl_2$, and 0·5 ml 10^{-3} *M* IAA. The reaction mixture was then made up to 10 ml with phosphate buffer and allowed to incubate for 1 h at 26°C in the dark. Residual IAA was estimated by the method of TANG and BONNER[3] after the reaction mixture had been cleared with 30% trichloracetic acid (TCA). Preliminary experiments[4] showed that addition of TCA to the reaction mixture increased the intensity of the colour developed with the reagent. Calibration curves containing the appropriate amount of TCA were therefore prepared.

The effect of dialysis on the degree of inhibition produced by homogenates of lettuce on pea root 'IAA oxidase'

Age of seedlings in days	% inhibition of pea root 'IAA oxidase'		
	Before dialysis	After dialysis	
		24 h of dialysis	84 h of dialysis
0	100	60	—
24	84	52	50
35	68	46	—

It will be seen in the Figure that IAA oxidation was entirely prevented in the presence of 1 ml of a homogenate of ungerminated lettuce seeds. As germination

[1] IAA = β-Indolyl-acetic acid.

[2] W. D. BONNER, JR., Ann. Plant. Physiol. *8*, 427 (1957).

[3] J. W. TANG and J. BONNER, Arch. Biochem. *13*, 11 (1947).

[4] S. BLUMENTHAL-GOLDSCHMIDT, Ph. D. Thesis, Hebrew University, Jerusalem.