absorbé les leucocytes sont disposés régulièrement sur le frottis (voir Fig. 1 et 2).

Tableau II

Examen électrophorétique du sérum de la malade E. B. avant (* Sérum *A*) et après le test d'absorption (* sérum *B*)

Sérum de la patiente E.B.	*	% protéines					
		Albumine	Globulines				
			alpha 1	alpha 2	béta 1	béta 2	gamma
Expérience I	*A*	38	6	11	7	6	32
	B	42,5	6,5	9,5	8	7	26,5
Expérience II	*A*	38	5,5	10,5	7,5	7	31,5
	B	41	6,5	9,5	8,5	9,5	25

L'étude électrophorétique (électrophorèse sur papier «Elphor»), faite avant et après le test d'absorption, a mis en évidence une diminution nette des gamma-globulines et une diminution peu marquée des alpha-2-globulines (voir Tabl. II).

Nous pouvons *conclure* de ces résultats que le *facteur* responsable *du phénomène L.E.* peut être *absorbé par des noyaux cellulaires isolés*. Ceci parle en faveur de l'hypothèse que le facteur L.E. est une substance ayant un caractère d'anticorps antinoyaux, sans que l'on puisse exclure avec certitude la possibilité que ce facteur soit un enzyme perdant son activité après avoir agi sur des noyaux.

P. MIESCHER et M. FAUCONNET

Clinique Médicale Universitaire, Lausanne, le 30 janvier 1954.

Summary

The serum containing the L.E. factor loses its activity after having been put in contact with isolated cell nuclei. The electrophoretic examination shows a clear diminution of the gamma globulin and a slight diminution of the globulin alpha 2. That the factor responsible for the L.E. phenomenon can be absorbed by isolated cell nuclei is an argument in favor of the hypothesis that this factor is a substance having the character of an antinuclear anti-body, without one being able to exclude with certitude the possibility that this factor is an enzyme.

Migration of "Storage PNA" from Cotyledon into Growing Organs of Bean Seed Embryo[1]

OSAWA and OOTA[2] have shown that in every growing organs of germinating bean embryo protein is accumulated with simultaneous increase in pentose nucleic acid (PNA) content, and that the reserve organ, the cotyledon, contains a remarkable amount of PNA ("storage PNA") which diminishes rapidly in the course of germination. It seems reasonable to assume that at least part of the "storage PNA" which disappears is recovered in PNA deposited in the anabolic organs. A problem arises here concerning the migrating form of PNA in the embryonic tissues, i.e. whether "storage PNA" is transported as such from the cotyledon into the growing tissues, or as fragments after being decomposed. In other words, whether the increase in PNA content in the latter means simple accumulation of intact PNA molecules transported as such, or resynthesis of PNA from the decomposed fragments in the tissues in question.

Considering the probable relationship between PNA accumulation and protein synthesis, this problem is thought to be worth while pursuing. We have, therefore, conducted preliminary investigations on the behaviour of "storage PNA" in germinating *Vigna sesquipedalis* seeds, and obtained some results which will be reported below.

Firstly, we studied whether the presence of the anabolic organs attached to the cotyledon is an indispensable condition for the disappearance of "storage PNA". Pre-soaked (for 6 h at 35°C) bean seeds were sterilized with 70% alcohol, decoated, and divided into two cotyledon segments under aseptic conditions: one with seedling portion attached and the other without any other organ. These two lots of material were laid spherical side down on separate moist filter papers. After 48 h of aseptic incubation in the dark at 30°C, when the attached seedlings were about 8 cm long, both lots of cotyledons were analyzed for PNA-phosphorus (PNA–P) content, the seedling portions being discarded. Prior to incubation, an aliquot of isolated cotyledon segments was used for estimation of the initial PNA-P content. General methods of fractionation and analysis used were essentially the same as has been described elsewhere[2]. The results are shown in Table I.

Table I.–Change in PNA-P content of bean cotyledon with and without attached seedling

Material	Cotyledon without seedling		Cotyledon with seedling	
Incubation period (hours) .	0	48	0	48
PNA-P (μg/cotyledon segment) . .	31·9	31·3	31·9	16·8

In cotyledons bearing no seedling portion, PNA–P content is found to be retained almost at the initial level during the incubation period, while in cotyledons with seedling portion attached, it is remarkably diminished. That is to say, even with a good water supply, the cotyledon *per se*, i.e. without the seedling portion attached, hardly shows hydrolytic action on PNA. The following explanation may be tentatively given: some factor accelerating the hydrolysis of PNA may be delivered to the cotyledon from the seedling, and/or rapid establishment of equilibrium in the hydrolytic reaction in the cotyledon may be hindered by the removal of reaction products by the growing tissues attached. A further possibility should not be overlooked here that "storage PNA" in the cotyledon migrates as such, without decomposition, into the seedling tissues.

Secondly, the time course of change in contents of total-, acid-soluble-, and PNA–P of both seedling and cotyledons were investigated. Pre-soaked seeds were set out in moist sand placed in porcelain pots, and germinated in the dark at 30°C. The sprouts were harvested at desired stages covering 4 days of cultivation. As the 0-day-old material, pre-soaked seeds were employed. The results obtained are shown in Table II.

[1] We are indebted to Dr. CH. KOYAMA, Chemical Institute of Nagoya University, for the use of a Lauritsen apparatus, and also to Dr. A. SIBATANI, Laboratory of Cytochemistry, Yamaguti Medical School, for his valuable suggestions during this work. P^{32} used in this experiment was provided by Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A.

[2] S. OSAWA and Y. OOTA, Exper. *9*, 96 (1953).

Table II.–Time-course of changes in contents of total-, acid-soluble-, and PNA-P of seedling (S) and cotyledon (C) of germinating bean seed.

Age of embryo (days)	Phosphorus (μg/embryo)								
	Total			Acid-soluble			PNA		
	S	C	Sum	S	C	Sum	S	C	Sum
0	19·1	355·1	374·2	6·3	143·0	149·3	7·8	63·1	70·9
1	69·8	294·6	364·4	36·5	140·3	176·8	14·7	55·8	70·5
1·5	—	—	—	—	—	—	23·0	46·8	69·8
2	179·0	166·3	345·3	120·0	89·0	209·0	39·6	35·4	75·0
3	233·0	114·2	347·2	185·8	56·0	241·8	54·0	23·9	77·9
4	295·0	63·3	358·3	207·0	25·4	232·4	64·5	18·3	82·8

It is noticeable that practically the whole amount of PNA which disappeared from the cotyledon is recovered as increase in PNA content of the seedling as far as the initial stage of germination is concerned. Later, however, as the age of the tissues progresses, the balance is lost, the increase in seedling PNA growing slightly over the decrease in „storage PNA". Presuming that this is not brought about by chance, the occurrence of such "PNA balance" would favour the idea that, at least in the early germination stage, the seedling owes its PNA increase solely to the cotyledon from which this amount of PNA has been transferred as the intact molecule into the seedling, where it subsequently acquires some capacity of *de novo* synthesis of PNA. It may be noted *en passant* that in the seedling the sum: acid-soluble-P plus PNA-P practically equals the total P level at certain stages of germination. This implies that the amount of acid-insoluble-P compounds[1] other than PNA is small or even negligible in the tissue concerned. It is evident that the cotyledon contains a large quantity of acid-insoluble-P compounds other than PNA (estimated as the difference between the total-P and the sum of acid-soluble-, and PNA-P), which diminishes rapidly with the lapse of time, presumably being transported into the growing tissues as acid-soluble compounds.

The last series of experiment deals with the behaviour of radioactive P^{32} previously absorbed in the embryo. If in the seedling tissues PNA is to be resynthesized from lower units than nucleoside level, the resulting PNA would be tagged with active P. If on the other hand "storage PNA" is transported as such, or at least as a molecule larger than nucleotide, radioactivity would not be detected in the seedling PNA, provided that no P-turnover takes place in PNA in embryonic tissues. In this respect, however, we have only contradictory evidences available: it is known that in *Eschericia coli* culture, the turnover of neither phosphorus[2] nor carbon[3] in the PNA molecules occurs in the synthetic process of PNA; but it has been confirmed that renewal of P in PNA takes place, even under stationary conditions, in the cases of keratin synthesis in the plume of pigeon[4], of egg white formation in the hen's oviduct[5], or of enzyme formation in the mouse pancreas[6].

[1] We have found that DNA-P of the seedling portion estimated by the diphenylamine test is less than 3% of PNA-P throughout the germination stage.

[2] F. W. Putnam, Exper. Cell. Res., Suppl. *2*, 346 (1952). – Y. Fujisawa and A. Sibatani (in press).

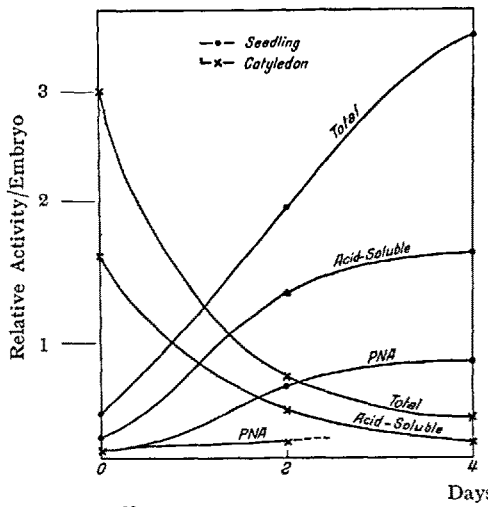
[3] L. A. Manson, Fed. Proc. *12*, 242 (1953).

[4] M. Grenson, Biochim. Biophys. Acta *8*, 481 (1952).

[5] M. Grenson, Biochim. Biophys. Acta *9*, 102 (1952).

[6] M. de Deken-Grenson, Biochim. Biophys. Acta *10*, 480 (1953).

Seven hundred seeds were soaked in 100 ml of 0·0025 M KH_2PO_4 solution containing about 1 mc P^{32} for 5 h at 35°C, then thoroughly rinsed with distilled water, and set out in pots filled with sand. Incubation was carried out for 4 days in the dark at 30° C. No harmful effect of the radioactive P added was observed on the growth of embryo. Appropriate numbers of embryo were harvested at 2 day intervals, and after being decoated and well rinsed, they were separated into cotyledon and seedling portions. They were homogenized in cold 0·9% NaCl solution. An aliquot of homogenate and acid-soluble fraction isolated from another aliquot were used for determination of radioactivity contained in total- and acid-soluble-P compounds, respectively.



Time course of P^{32} accumulation in various fractions of bean seed embryo

As to the determination of the activity of PNA, the specific activity (activity per P) of PNA prepared in a purified state from an aliquot of homogenate by the procedure described in Davidson *et al.*[1] was estimated and then multiplied by total PNA-P of the homogenate estimated separately. Prior to the estimation of activity, each sample was digested, and P was isolated as ammonium phosphomolybdate; activity in terms of discharging velocity was assayed by means of a Lauritsen electroscope.

The Figure shows the time course of accumulation of P^{32} in various fractions of embryonic tissues in the cultivation period. The total radioactivity attained at

[1] J. N. Davidson, S. C. Frazer, and W. C. Hutchison, Biochem. J. *49*, 311 (1951).

the end of the experimental period by the embryo, including both the seedling and a pair of cotyledons, is found markedly to exceed the initial total activity. In all probability, this is due to radioactive P from the seed-coat which is discarded in the analytical procedures. The general trend indicated in this figure is that the absolute amount of P^{32} in each fraction of seedling (or cotyledon) increases (or decreases) parallel with the increase (or decrease) in total-P content of the fraction concerned. Thus, in the seedling PNA a remarkable amount of P^{32} is incorporated. This apparently implies, against the indication given above, that there is a *de novo* synthesis of PNA in the growing organs. The situation, however, can not be so simple, for, as is shown in the figure, the cotyledon itself also accumulates P^{32} slightly but definitely in "storage PNA" molecules, and furthermore the P-turnover would be expected to take place in the seedling PNA to a considerable extent. Hence, the apparent P^{32} incorporation into PNA accumulated in the growing tissues does not necessarily exclude the possibility that "storage PNA" molecules are transported as such into the tissues in question. It may be noted here that some authors, basing mainly on their histochemical observations, have suggested macromolecular transfer of PNA in the processes of spermatogenesis[1], or induction of the amphibian neural tissue[2].

Addendum. The authors were not aware when they prepared the present manuscript, that N. FRIES and B. FORSMAN (Physiol. Plantarum *4*, 410 [1951]) had reported that the ultraviolet-absorbing material, exuded from the roots of pea seedlings, consists mainly of mononucleotides and probably also polynucleotides, thus suggesting migration of intact nucleotide molecules through the membrane of, at least, embryonic tissues of plants. In their paper above-mentioned, FRIES and FORSMAN cited LUNDEGÅRDH and STENLID who had first demonstrated the exudation of nucleotides from the seedling roots.

Y. OOTA and S. OSAWA

Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan, November 2, 1953.

Zusammenfassung

Die in den Kotyledonen der Leguminose *Vigna sesquipedalis* als «Reserve-PNA» reichlich gespeicherte Pentosenukleinsäure (PNA) nimmt rasch ab, sobald der Same zu keimen beginnt. In den frühen Stadien der Keimung ist die Abnahme des «Reserve -PNA» scheinbar aufgehoben durch die Zunahme des PNA in den wachsenden Geweben des Keimlings, während später, wenn das Alter der Gewebe sich erhöht, die Gesamtzunahme an PNA in den wachsenden Geweben allmählich die PNA-Abnahme in den Reserveorganen überwiegt. Kotyledonen, die von den Keimlingen abgetrennt und auf feuchtem Filtrierpapier geeignet warm gehalten wurden, zeigen praktisch keine Abnahme im Gehalt an «Reserve-PNA».

Wenn Samen zuerst mit einer Phosphatlösung behandelt werden, die P^{32} enthält und dann zum Keimen gebracht werden, so findet man die Radioaktivität nicht nur im akkumulierten PNA der wachsenden Gewebe, sondern auch in dem im Abbau begriffenen PNA der Kotyledonen. Auf diesen Tatsachen fussend, wird die Möglichkeit einer makromolekularen Wanderung des «Reserve-PNA» von den Kotyledonen in den Keimling kurz diskutiert.

[1] G. MONTALENTI, G. VITAGLIANO, and M. DE NICOLA, Heredity *4*, 75 (1950). – G. MONTEFOSCHI, Caryologia *4*, 25 (1951). – B. BATTAGLIA and M. SARÀ, Sci. Genet. *4*, 36 (1951). – H. J. DALED, Arch. Anat. Micr. et Morph. expér. *40*, 183 (1951).

[2] J. BRACHET, Symposia Soc. Exp. Biol. *6*, 173 (1952).

Die Speicherung von Blutproteinen in den Histiozyten nach vorhergehender Histamineinwirkung

Unlängst haben wir über eine mikrotechnische Methode berichtet, die eine Sichtbarmachung der Speicherung von Proteinen in den Retikuloendothelzellen ermöglicht[1]. Besonders bedeutungsvoll erschien uns die Beobachtung, dass die Zellen des retikuloendothelialen Systems nicht nur artfremde, sondern auch arteigene Serumproteine zu speichern vermögen, und wir folgerten daraus, dass die fortwährende Einverleibung und der Abbau von Blutproteinen eine wichtige physiologische Funktion dieser Zellen darstelle. Unsere neueren Untersuchungen haben unsere früheren Ergebnisse bestätigt und wesentlich weiterentwickelt.

Bei den neuen Versuchen wurde die in unserer ersten Mitteilung angegebene Technik der Eiweissfärbung insofern modifiziert, dass die vom subkutanen Bindegewebe verfertigten Häutchenpräparate in der Goldchloridlösung (2–4 %) 30 min fixiert, in reinem Methanol wenigstens 15 min lang gewaschen und dann auf die schon mitgeteilte Weise gefärbt wurden.

Es wurde in dieser Versuchsreihe jungen Ratten arteigenes und artfremdes Serum *subkutan* eingespritzt, um das Eiweiss in unmittelbaren Kontakt mit den Histiozyten zu bringen. 70–80 g schwere Ratten erhielten verdünntes Rattenserum unter die Rückenhaut gespritzt. Es konnte bei den nach 6 h getöteten Tieren in den Histiozyten des subkutanen Bindegewebes eine äusserst starke, grosstropfige Eiweißspeicherung nachgewiesen werden. Jene Tiere, die Pferde- oder Kaninchenserum injiziert bekamen, lieferten ganz ähnliche Bilder; ein auffallender quantitativer Unterschied war nicht zu beobachten. Bei unbehandelten jungen Ratten waren in den Histiozyten keine Eiweissgranula nachweisbar.

Diese Versuche haben ergeben, dass *die Histiozyten arteigenes und artfremdes Serumeiweiss mit der gleichen Bereitwilligkeit speichern* und dass auch im Abbau der verschiedenen Eiweissarten kein wesentlicher Unterschied besteht, da das aufgenommene Eiweiss in allen Fällen nach 48 h fast völlig aus den Zellen verschwindet. Die Speicherbarkeit der Proteine ist also nicht von der Artspezifität des Eiweisses abhängig, sondern es sind hierfür seine allgemeinen physikochemischen Eigenschaften, sein makromolekularer Charakter ausschlaggebend.

Diese experimentellen Ergebnisse legten den Gedanken nahe, durch Anwendung von Histamin als permeabilitätssteigerndem Agens *die Akkumulation der eigenen Blutproteine des Versuchstieres in seinen Histiozyten* hervorzurufen zu versuchen. Unseren Versuchen zufolge hatte sich *insbesondere nach lokaler Anwendung des Histamins* unseren Erwartungen gemäss tatsächlich *Eiweiss in grossen Mengen in den subkutanen Histiozyten angereichert.*

Die enthaarte Rückenhaut junger Ratten wurde in halbstündigen Intervallen viermal nacheinander je 5 min lang mit einer Histaminlösung (2 % Histaminchlorhydrat in 40 %igem Alkohol) leicht eingerieben und 2 h nach der letzten Behandlung das Tier getötet. Bei diesen Tieren fanden wir in der Subkutis in den die Kapillaren begleitenden (Abb. 1) sowie in den im Bindegewebe verstreuten Histiozyten (Abb. 2) eine reiche grosstropfige Eiweißspeicherung. In den Kontrolltieren dagegen, deren Haut auf ganz analoge Weise nur mit

[1] N. JANCSÓ und A. JANCSÓ-GÁBOR, Exper. *8*, 465 (1952).