ANTIGENS STRUCTURE OF THE PHYTOHEMAGGLUTININ OF PHASEOLUS VULGARIS

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Phytohemagglutinins (PHA) are considered and utilized chiefly as antibodies (1, 5, 12) in the field of experimental and applied medicine.

Dujarrie de la Rivièr and associates are the first to put in the problem as a matter of principle for the antigenic properties in the PHA and carry out experimental studies under this aspect (7, 11).

With renewed interest the question of the antigenic properties of PHA from Phaseolus vulgaris was raised after discovering its mitogenic ability upon leukocytes of the human peripheric blood in vitro (9). *Pearmain* and associates (8, 10) are the first to postulate the hypothesis that this phenomenon represents an immunological reaction of the leukocytes to the special PHA antigenic properties.

We obtained PHA from the seeds of various types Phaseolus vulgaris, among which we chose the one with the most powerfully manifested mitogenetic properties. The PHA thus obtained represents purified globulin fractions, as already established by the same authors in earlier investigations (4, 13).

Proving the antigenic structure of the PHA from Phaseolus vulgaris surges a particular scientific interest in the process of studying the essentials of its mitogenetic capacity. In our present work we give an account of the results obtained from investigations on the antigenic structure of PHA of Phaseolus vulgaris. The experimentation was carried out in accordance with the following plan: 1) determination of the antigenic structure of PHA from Phaseolus vulgaris, type Saxa and their identity with the antigens of PHA from the other sorts of this type; 2) determination of the antigens of PHA from Phaseolus vulgaris, sort Saxa and their related antigens in PHA obtained from the seeds of other sorts of the family Leguminosae — peas, soy-bean and hatchet.

Materials and methods

For revealing the antigenic structure of PHA, we employed immune precipitation sera (IPS), obtained through intravenous immunization of rabbits with PHA. We worked with three IPS types: IPS obtained through threefold injection, every other week, of PHA from Phaseolus vulgaris, sort Saxa, with titer (T) 1000; IPS 6, obtained by means of repeated reimmunizations of the same donor over a period exceeding one year. In the latter instance we resorted to hyperimmunization with a view to obtaining antibodies to the weakest PHA antigens as well. We proceeded from

the results of our studies on the sera antigens in man and some vertebrates, in which we established that the antibodies to far related antigens (which are the weakest) are merely produced during hyperimmunization (2, 3). These findings are in harmony with the principles of the clonal-selectional theory on immunity as postulated by *Burnét* (6). We also employed IPS 7, obtained in the same manner as IPS 6, but by injecting PHA from other sorts Phaseolus vulgaris. IPS 6 had a T — 10000 in relation to PHA of the sort Saxa, T — 100 in relation to PHA obtained from peas and diluted 1 : 100 it gave a doubtfull reaction of precipitation with PHA from soy-beans and a negative one with hatchet PHA. During analogous treatments IPS 7 showed properties similar to those of IPS 6. We employed the method of *Uchterlony* (14), working with 1,5% agar diluted in destilated water. The results of the experiments were accounted for after 10-day stay in room temperature. The fotlowing experiments were thus performed: 1) IPS 1 to 3 gr/% solutions in salt solution of PHA from Phaseolus vulgaris, sort Saxa, sort 4540, 4579 and 4784, which were accordingly studied for electrophoretic fractions and agglutination properties (13) (setting I); 2) IPS 6 to 3 gr % solution of PHA of Phaseolus vulgaris sort Saxa, red cultivated, peas, soy-beans and hatchet (setting II); 3) IPS 6 and IPS 7 to 3 gr% solution of PHA of sort Saxa (setting III).

Results and discussion

The results of the experiments in settings I, II and III are illustrated in figures 1, 2 and 3. In the I set we established five precipitation lines between IPS and PHA of sort Saxa and four stripes with PHA of the remaining sorts of this type, which are identified with four of the Saxa sort antigens. This result indicates that PHA of the sort Saxa possess an antigen by which it is distinguished from the antigens of the PHA of the remaining bean sorts. Thus the differences in the antigenic structures of PHA of various sorts beans is indicative for the presence of genetically determined variations within the limits of this type, present also in their globulin fractions such as the PHA.

In setting II (fig. 2) we established 12 precipitation lines between IPS 6 and PHA of the sort Saxa, some of them particularly delicate, but clearly outlined, 9 with PHA of the sort red cultivated of the same type, 6 with PHA from peas, 4 with PHA of soy-beans and 3 vague and broad precipitation lines with PHA of the hatchet. The precipitation lines established are identified in between them and furthermore display related antigens between the PHA investigated and the PHA of the sort Saxa, in relation to which IPS 6 is obtained.

With setting III (fig. 3) 12 precipitation lines are noted between IPS 6 and PHA of the sort Saxa and 9 bands with IPS 7. This outcome serves as control to the II setting and confirms the results obtained with the latter. In this case a difference is observed also in the antigenic structure of PHA of various sorts of a given type. The results of analogous investigations carried out by *Dujarrie de la Rivièr* and assoc. (7, 11) are similar in their essential aspects; they establish antigenic properties in the PHA of Phaseolus vulgaris and those of Pisum sativum (peas) and point out the importance of this fact. However, these authors have worked with gross,

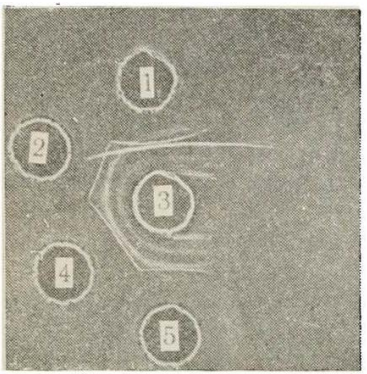

Fig. 1
1 — PHA from Phaseolus vulgaris Saxa
2 — PHA from Phaseolus vulgaris 4540
3 — IPS I
4 — PHA from Phaseolus vulgaris 4579
5 — PHA from Phaseolus vulgaris 4784

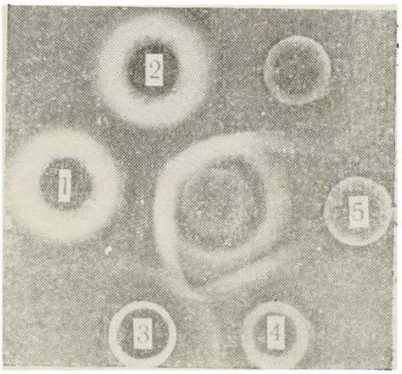

Fig. 2
1 — PHA from Phaseolus vulgaris Saxa
2 — PHA from Phaseolus vulgaris — red cultivated
3 — PHA from soy-beans
4 — PHA from peas
5 — PHA from hatchet
6 — IPS 6

unpurified water-salt extracts, which besides the globulins, contain also albumin and other components. This circumstance obviates the reaching to precise conclusions concerning the PHA structure isolated, being bound to globulin fractions. Nonetheless, they too establish an antigenic unity between Phaseolus vulgaris and Pisum sativum, as well as antigenic differences between PHA of vegetable sorts of the same type. They prove similar differences between the various sorts of peas, whereas our results discover similar differences between the various sorts of the type Phaseolus vulgaris. The results of their investigations point to the fact that the antigenic properties of PHA are related to the globulin fractions of the water-salt extracts employed by them. In our work, in which we employed pure globulin fractions of the seeds of the respective vegetable types and hyper immune precipitation sera in addition, the results obtained provide for a rather clear characteristics of the antigenic structure of the PHA, sort Saxa from Phaseolus vulgaris and the antigenic analogy i. e. biological congenerity of the same and remaining comparatively studied PHA.

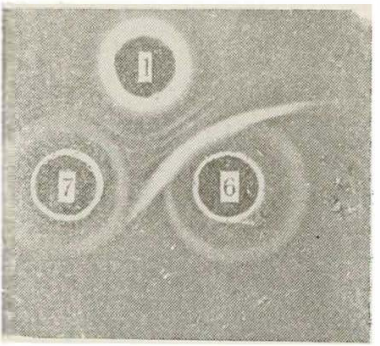

Fig. 3
PHA from Phaseolus vulgaris Saxa; 6—IPS 6; 7—IPS 7

The complex antigenic structure thus established of PHA from Phaseolus vulgaris could explain its wide range of action, manifested in the lymphocytes of human peripheric blood in vitro. We could assume that the high percentage of transformation and division among PHA treated

lymphocytes is due to the common features of certain antigenic components of the PHA and the bacterial or viral antigens, to which the human organism has been a priori sensibilized. In support to this hypothesis are the well established facts that Pirquet (+) individuals exhibit a considerably stronger reaction as compared to Pirquet (—) individuals to the effect of PHA. This question could possibly receive a definitive answer with identification of the antigens of PHA from Phaseolus vulgaris, sort Saxa, proved by us, with the antigens of various bacteria and vira, causing morbid conditions in humans.

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АНТИГЕННАЯ СТРУКТУРА ФИТОГЕМАГГЛЮТИНИНА PHASEOLUS VULGARIS

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РЕЗЮМЕ

Посредством метода двойной диффузии в агаре при использовании гиперимунных преципитационных сывороток установлено, что фитогемагглютинин Phaseolus vulgaris сорта Сакса в форме очищенного глобулина представляет собой мозаику из 12 антигенов. В нем установлены 6 родственных антигена с фитогемагглютининами гороха, 4 с фитогемагглютининами сои и 3 с фитогемагглютининами.

В границах вида Phaseolus vulgaris среди различных его разновидностей (сортов) устанавливают кроме общих антигенов и антигены специфические для каждого из них.

На основании установленной сложной антигенной структуры фитогемагглютинина Phaseolus vulgaris высказывается предположение о вероятной общности между некоторыми из последних и антигенами различных бактерией и вирусов, что может быть причиной сильной митогенетической способности фитогемагглютинина последнего на лейкоциты периферической крови человека.