ACQUIRED IMMUNITY IN PLANTS

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INTRODUCTION

Certain phenomena observed in the development of the embryos of various *Nicotiana* species crosses led to the present investigation. The embryos produced in the crosses *Nicotiana rustica* × *Nicotiana Rusbyi*, *Nicotiana rustica* × *Nicotiana glauca*, *Nicotiana rustica* × *Nicotiana alata*, *Nicotiana rustica* × *Nicotiana Langsdorffii*, *Nicotiana rustica* × *Nicotiana Tabacum*, etc., do not reach the normal size produced by selfing or by mating two plants belonging to the same species. In the first two combinations, the embryos grow from 6 to 10 days, depending upon the environmental conditions; the development then stops and the embryos die within a short time. In the other combinations mentioned the embryos reach a higher stage of development, though they are always behind the normal stage. Development in the majority of these hybrids stops within 15 to 20 days, an exceptional few producing viable seeds. A conspicuous feature in such cases is that a great amount of starch accumulates in the integumental tissue outside of the nucellus which envelops the hybrid embryo and endosperm.

These and some other phenomena, which will be mentioned later, give rise to the question: What is the inhibitor of embryo development?

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Are antibodies produced after interaction and reaction between the embryo and the endosperm (of hybrid nature) on the one hand, and the neighboring tissues of the mother plant on the other hand? In other words, are these reactions similar to the reactions produced by the so-called "*Abwehrfermente*" acting between the mother and the placenta, studied by ABDERHALDEN (1912a, b, c, 1913, etc.) in mammalia and determined by dialysis, ninhydrin reaction and polarization? Or, speaking more generally, is there a lytic reaction, preceded probably by a precipitation *in vivo*? No satisfactory answer can be given to this question until another question is solved, namely: Is the plant organism as well as the animal organism able to produce agents (*Abwehrfermente*, antibodies) which can attack foreign substances? This is the question we hope to answer here.

A brief historical review of the immunological literature treating questions connected with the present work will be given before the experimental work is described.

Historical

When certain foreign substances are parenterally introduced into the animal body, the physiological balance is disturbed. The organism reacts against the invader by the production of agents usually called "antibodies."

The first experimental data demonstrating the production of antibodies in the animal organism appeared near the end of the last century. The earliest observation in what one may call modern immunology, showed that the blood has the power to destroy bacteria. NUTTAL (1888) found that normal defibrinated blood when incubated with tissue containing anthrax bacilli brought about a reduction in the number of bacilli. BUCHNER (1889) pointed out the resemblance of the behavior of the active substance in such reactions to the behavior of enzymes, and called it alexin (protective substance). At about the same time the agglutination phenomenon was discovered by CHARRIN and ROGER (1889) by experimenting with the serum of rabbits rendered immune to *Bacillus pyocyaneus*. These observations were later confirmed by METCHNIKOFF (1891), KRUSE and PANSINI (1892), ISAEFF (1893), ISAEFF and IWANOFF (1894), and many others with different bacteria; but the principal significance of agglutination as a specific immunity phenomenon was studied by GRUBER and DURHAM (1896). At about the same time, agglutinations were found to be characteristic not only of many bacteria but also of red blood corpuscles (BORDET 1896, LANDSTEINER 1899, von DUNGERN

1899), leucocytes (LESCHKE 1913, REESER 1913), spermatozoa (LANDSTEINER 1908), algae (ROSENBLAT-LIECHTENSTEIN 1912, 1913) etc.

The discovery by RUDOLPH KRAUS (1897) that bacterial filtrates produce *precipitates* when mixed with the specific antiserum was a logical outcome of the discovery of agglutinins, and was followed two years later by the observation that this is a general reaction for other than bacterial proteins. TCHISTOWITCH (1889) secured precipitins for eel serum, BORDET (1899) for milk and chicken blood, and later KOWARSKY (1901), JACOBY (1901), MAGNUS and FRIEDENTHAL (1906), MAGNUS (1908), GASIS (1908), and others for plant proteins, while KARASAWA (1910) and GALLI-VALERIO et BORNAND (1912) caused anaphylactic shock with plant antigens.

While immunological investigations have been chiefly directed toward the perfection of methods for application to human therapy and to legal medicine, nevertheless various investigators, including UHLENHUTH (1900, 1901), WASSERMANN (1903), NUTTAL (1902), used the immunological reaction for differentiation of various species of animals. Moreover, the MEZ school, by methodical work for 18 years, has recently drawn a phylogenetical tree of the plants serologically (ZIEGENSPECK 1925a, 1925b, MEZ and ZIEGENSPECK 1926) from about 400 reaction centers. ZADE (1914), also working serologically with certain Leguminosae and Graminae, has even differentiated between varieties.

The work of the MEZ school, often called the "Königsberg school," has recently been reëxamined and questioned at Berlin in the GILG-SCHÜRHOFF laboratory. From the papers appearing thus far, it is obvious that the Berlin serologists do not doubt the specificity of the plant antigens ("Das von mir gewonene Serum war in allen Fällen spezifisch, d.h. es reagierte stets mit dem zum Impfen verwandten Samenauszuge am weitesten"—W. NAY 1927, p. 151). They attack the MEZ method, however, and emphasize the errors which may occur when working with heterogenetic serum which is obtained very frequently.

Both the Berlin and the Königsberg schools used plants for obtaining antigens, while for antibody production the rabbit's body was usually utilized.

It has long been known that many of the warm blooded animals, and even some mollusks, are able to produce antibodies. The question of the production of antibodies in plants, however, has been neglected. The meagre literature treating of this question usually confines discussion to activity of normal precipitins and agglutinins of plant origin on the blood sera and

red blood corpuscles. Thus LANDSTEINER and RAUBITSCHKE (1908) precipitated horse and fowl proteins by extracts of *Phaseolus*, *Pisum*, *Vicia*, and *Lens*. The same extracts agglutinated the red blood corpuscles of horse and fowl. von Eisler and von Portheim (1909) found in *Datura* agglutinins against horse and rabbit red corpuscles and precipitins against horse and rabbit sera. They have shown by these experiments the co-existence of normal agglutinins and precipitins. Precipitin and agglutinin reactions on sera and red blood corpuscles were also produced by ricin, abrin and crotin (WILENKO 1910), by extracts of rice (KANAHARA 1912), of *Zea mays* (LUI and BACELLI 1913), by phaseolin, phaselin, and bean albumin (SCHNEIDER 1912), by extracts of *Cotyledon Scheideckeri* (KRITCHEWSKY 1914) and by extracts of many fruits, roots and leaves (CARBONE and VIGLIANO 1922). Agglutination of bacteria was observed by WAGNER (1915) in the juice of *Solanum tuberosum* and by KOŘINEK (1924) in the juice of *Beta vulgaris*.

Recently J. RODRIGUEZ SARDIÑA (1926, p. 303), investigating the antibody production in plants by injection of bacteria, came to the following negative conclusion.

“Es kann also auf Grund der bis jetzt durchgeführten Untersuchungen gesagt werden, dass sich artspezifische Agglutinine und Precipitine . . . bei *Opuntia*, Kartoffeln, Tomaten, Kurbis und *Vicia Faba* nicht bilden und dass sich dennoch die Pflanzen in dieser Hinsicht anders verhalten als Tiere und Menschen.”

The negative results obtained by SARDIÑA probably are due to the small quantity of the injected antigen. The mechanical constitution of the plant organism differs so materially from that of the animal that it is usually impossible to inject a large quantity of antigen, and even difficult to inject the amount necessary for the induction of antibodies in sufficient quantity for their effects to be observed *in vitro*. When one considers the ratio of the proteins, carbohydrates, water, etc., and the reaction ability of the cells in plants and in animals (MÜLLER 1926), it is obvious that for acquiring immunity in plants one ought to deal in larger amounts of antigen. Since the introduction of sufficient amount of antigen in plants by injection is impossible, one has to apply some other method. We hope we have succeeded by intergrafting certain solanaceous plants. In a graft union the constituents of sap exchanged mutually between scion and stock act as do injected antigens in rabbits.

The technical terminology to be used in the present paper is given in the following paragraph.

Terminology

The word "immunity" includes three different phenomena. A parasite may not attack certain organisms because they may have a covering layer or layers of tissue impenetrable for the parasite in question. In this case we speak of "mechanical immunity." There are, for example, certain citrus-species (STRAPP 1927) which have mechanical immunity against *Pseudomonas citri*. The resistance of an organism against certain parasites may be due to the particular chemical substances which the organism contains, thus making it an unfavorable medium for the parasites. Such an immunity is usually called "physiological immunity." This may be "natural" or "normal" if the organism contains substances without any previous induction, as acid (Comes), alkaloids, etc., which prevent the thriving of the parasites, or "acquired" if the preventive substances are produced only after induction by agents of antigenic nature. An antigen is called any substance which is able to induce the production in an organism of reacting substances against the antigen itself. The preventive substances are called antibodies. In the present work we shall use the term "normal" or "antigenic" extract when an extract has been obtained from a plant in which no production of antibodies has been induced, and the term "immune" extract when the extract has been obtained from a plant in which production of antibodies was induced by grafting.

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MATERIAL

The following plants were used in the experiments:
Solanum tuberosum L., *Solanum Melongena* Mill., *Solanum Lycopersicum* L., *Solanum Dulcamara* L., *Solanum nigrum* L., *Physalis peruviana* L., *Salpiglossis sinuata* Ruiz et Pav., *Browalia speciosa* H., *Capsicum pyramidale* Mill., *Lycium Barbarum* L., *Datura ferox* Nees, *Datura Wrightii* Nort., *Petunia violacea* Lindl., and certain species of *Nicotiana*, namely: *Nicotiana Tabacum* L., *Nicotiana Rusbyi*, *Nicotiana rustica* L., *Nicotiana glauca* R. Gr., *Nicotiana Langsdorffi* Wm., *Nicotiana alata* Link and Otto,

Nicotiana Sanderæ Hort., *Nicotiana longiflora* Cav., *Nicotiana suaveolens* Lehm., *Nicotiana quadrivalvis* Pursh, F_1 (*Nicotiana rustica* $\times$ *Nicotiana Tabacum*), F_1 (*Nicotiana rustica* var. *humilis* $\times$ *Nicotiana rustica* var. *brasila*), F_1 (*Nicotiana rustica* var. *humilis* $\times$ *Nicotiana rustica* var. *texana*) and F_1 (*Nicotiana Tabacum* $\times$ *Nicotiana Rusbyi*.)

All were grown in the greenhouse and in the garden of the BUSSEY INSTITUTION at HARVARD UNIVERSITY.

METHODS

Grafting

All of the methods of grafting described by WINKLER (1924) were tried; most frequently, however, simple whip grafting, tongue whip grafting, cleft grafting, saddle grafting, and budding were used. The best results were obtained by simple whip grafting (figure 1). The scion and the stock were bound together by common string 0.5–1.5 mm thick.

FIGURE 1.—Scion (A) and stock (B) prepared for grafting.

Better and faster union between scion and stock was obtained when the components were moistened by 1 percent water solution of $MgSO_4$. This increased the percentage of successful unions.

Absorption

In addition to the grafting, we have tried to induce the production of antibodies by the absorption method used by SHEWIRJEFF (1903),

MOKRZECKI (1903) and RUMBOLD (1915), and modified for our purposes. By this method we did not succeed in introducing a great amount of antigenic extract, and we did not obtain any positive results.

Testing the precipitin reaction

Uhlenhuth's ring method (1909) and MEZ's prescription (1924) were applied for testing the precipitin reaction. The plant tissues (leaves and stems) were crushed in a mortar and extracted by distilled water 2 hours. In applying Uhlenhuth's ring method the extracts were made in the ratio of 1 part of plant tissues to 2 parts of distilled water. This extract was used as "normal concentration" and was further diluted for testing the precipitation capacity.

Chloroform was used successfully for the preservation of the extracts. Toluol at the top and chloroform at the bottom were used for keeping extracts a longer time. The test tubes which were used for testing the precipitation capacity of extracts after Uhlenhuth's method were 3–4 mm in diameter and 30–35 mm long. For successful layering of both component extracts, pipettes of about 0.5 mm diameter were prepared. The tubes were boiled 2 hours in 0.5 percent solution of Na_2CO_3 and 4 hours in distilled water changed four times before using. The formation of Uhlenhuth's ring was observed usually for 40 minutes; sometimes it persisted even longer. In applying MEZ's prescriptions the material for both extract components was treated by: (a) tartaric acid-alcohol-ether; (b) ether, (c) chloroform or (d) chloroform-alcohol before the extraction. The extracts were obtained by taking an equal quantity of treated tissues and distilled water. The development of the reactions was observed for 24 hours. The precipitation capacity following the Uhlenhuth's ring method was tested after the treatment of the materials by the above mentioned chemical agents also.

A description of the experiments and the results obtained between the normal extracts precedes the description of the acquired precipitins.

NORMAL PRECIPITINS

It has been known for a long time that the serum of normal unimmunized animals often has the capacity to produce agglutinin and precipitin reactions when not too greatly diluted. Thus ASCOLI (1903) found that ox-serum contains precipitins for the sera of man, dog, pig, goat, rabbit, guinea pig, and fowl; dog-serum for the sera of fowl and guinea pig. GAETHGENS (1910) pointed out that the presence of the normal precip-

itins depends to a certain extent upon the age of the rabbit. The sera of 5-weeks rabbits usually do not contain normal precipitins, while the sera of many adult and old rabbits contain them. NOGUCHI (1903), LOEB (1921), CHARKOVITCH (1921) and others found normal precipitins in the sera of cold-blooded animals. CANTACUZÈNE (1912) succeeded in precipitating the normal sera of horse and rabbit by serum of *Eupagurus prideauxii*. He stated that the normal precipitins of *Eupagurus prideauxii* are very labile and that a temperature of 55°C destroyed them. LANDSTEINER and REICH (1907), HEUER (1922) and others believe that the normal precipitins are not specific and, contrary to CANTACUZÈNE, that they are more resistant than the antibodies that result from immunization. Concerning the rôle which the normal agglutinins play during the acquiring of immunity, BOISSEVAIN (1922) offered the hypothesis that specific immune agglutinins are merely nuclei of antigens charged with normal agglutinins.

It has already been mentioned in the introductory notes that the normal extracts of plant organs contain agents which precipitate the normal sera of many animals and agglutinate their red blood corpuscles. The data of mutual precipitations of the normal extracts of certain solanaceous plants investigated by the author are shown in the following tables: 1, 2, 3, 4, 5, 6, 7, 8, and 9.

The capacity of precipitin reactions was determined by the size and density of the precipitation ring (Uhlenhuth's ring) and recorded as: — (none), ± (trace), +, ++, +++, +++++, the last representing the greatest amount found. The most striking precipitin reactions are given in the tables mentioned.

TABLE 1
Normal precipitin reaction of Solanum nigrum extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Capsicum pyramidale</i>	+	+++	+++++	+++++	+++	+++
<i>Nicotiana glauca</i>	—	+	+	—	—	—
<i>Nicotiana rustica</i>	±	++	++	+	—	—
<i>Physalis peruviana</i> ¹	—	+	++	++	+	±
<i>Datura Wrightii</i>	+++++	+++++	+++	+++	+++	++
<i>Solanum Dulcamara</i>	—	—	—	—	—	—
<i>Salpiglossis sinuata</i>	±	++	+++	++	++	+
<i>Lycium Barbarum</i> ²	—	—	—	—	—	—

¹ After 30 minutes two lytic rings appeared at both sides of the precipitin ring.

² Simple lytic ring.

TABLE 2

Normal precipitin reaction of Physalis peruviana extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Capsicum pyramidale</i> ¹	—	—	—	—	—	—
<i>Lycium Barbarum</i>	—	—	—	—	—	—
<i>Nicotiana rustica</i>	±	+++	+++	++	++	+
<i>Nicotiana glauca</i>	—	++	++	++	+	+
<i>Solanum Dulcamara</i>	+++	++++	++++	++++	+++	+++
<i>Solanum Melongena</i>	—	—	—	—	—	—
<i>Solanum nigrum</i> ²	—	+	++	++	+	±
<i>Solanum tuberosum</i>	—	—	—	—	—	—
<i>Salpiglossis sinuata</i>	—	—	—	—	—	—

¹ Simple lytic ring.² After 30 minutes two lytic rings appeared from both sides of the precipitin ring.

TABLE 3

Normal precipitin reaction of Solanum Dulcamara extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Capsicum pyramidale</i>	++	+++	++++	++++	+++	+++
<i>Nicotiana rustica</i>	—	—	—	—	—	—
<i>Nicotiana glauca</i>	—	—	—	—	—	—
<i>Salpiglossis sinuata</i>	+++	++++	++++	++++	+++	+++
<i>Solanum Melongena</i>	—	—	—	—	—	—
<i>Solanum nigrum</i>	—	—	—	—	—	—
<i>Lycium Barbarum</i>	+	+++	+++	+++	++	++
<i>Datura Wrightii</i>	++++	++++	++++	++++	+++	+++
<i>Physalis peruviana</i>	+++	++++	++++	++++	+++	+++

TABLE 4

Normal precipitin reaction of Salpiglossis sinuata extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Capsicum pyramidale</i> ¹	—	—	—	—	—	—
<i>Nicotiana rustica</i>	+++	++++	++++	+++	+++	+++
<i>Nicotiana glauca</i>	+	++	+++	+++	++	++
<i>Nicotiana Tabacum</i>	+++	++++	++++	++++	+++	+++
<i>Solanum nigrum</i>	±	++	+++	+++	++	++
<i>Solanum Melongena</i>	+	+++	+++	+++	++	++
<i>Solanum Dulcamara</i>	+++	++++	++++	++++	+++	+++
<i>Lycium Barbarum</i> ¹	—	—	—	—	—	—
<i>Datura Wrightii</i> ¹	—	—	—	—	—	—
<i>Solanum Lycopersicum</i>	+	++	+++	+++	++	++
<i>Physalis peruviana</i>	—	—	—	—	—	—

¹ Simple lytic ring.

TABLE 5

Normal precipitin reaction of Lycium Barbarum extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Capsicum pyramidale</i>	±	+	++	++	+	+
<i>Nicotiana Tabacum</i>	—	±	±	—	—	—
<i>Nicotiana glauca</i>	—	±	±	±	—	—
<i>Nicotiana rustica</i>	—	—	—	—	—	—
<i>Solanum Melongena</i>	—	±	—	—	—	—
<i>Solanum nigrum</i> ¹	—	—	—	—	—	—
<i>Datura Wrightii</i>	±	++	++	++	+	+
<i>Salpiglossis sinuata</i> ¹	—	—	—	—	—	—
<i>Physalis peruviana</i>	—	—	—	—	—	—
<i>Solanum Dulcamara</i>	+	+++	+++	+++	++	++
<i>Solanum tuberosum</i>	—	—	—	—	—	—
<i>Nicotiana Rusbyi</i>	—	—	—	—	—	—
<i>Datura ferox</i>	—	+	++	++	+	+

¹ Lytic ring.

TABLE 6

Normal precipitin reaction of Solanum Lycopersicum extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Datura Wrightii</i>	++++	++++	++++	++++	+++	+++
<i>Capsicum pyramidale</i>	—	±	+	+	+	±
<i>Salpiglossis sinuata</i>	+	++	+++	+++	++	++
<i>Solanum Melongena</i>	—	—	—	—	—	—
<i>Solanum tuberosum</i> ¹	—	—	—	—	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
<i>Nicotiana rustica</i>	—	—	—	—	—	—
<i>Lycium Barbarum</i>	—	—	—	—	—	—
<i>Datura ferox</i>	±	+	+	+	±	±

¹ Simple lytic ring.

TABLE 7

Normal precipitin reaction of Capsicum pyramidale extract.

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Datura Wrightii</i>	—	±	±	±	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
<i>Nicotiana glauca</i>	—	—	±	—	—	—
<i>Solanum Melongena</i>	—	+	+	+	±	±
<i>Lycium Barbarum</i>	±	+	++	++	+	+
<i>Salpiglossis sinuata</i> ¹	—	—	—	—	—	—
<i>Physalis peruviana</i> ¹	—	—	—	—	—	—
<i>Solanum tuberosum</i>	—	—	—	—	—	—
<i>Solanum nigrum</i>	+	+++	++++	++++	+++	+++

¹ Simple lytic ring.

TABLE 8

Normal precipitin reaction of Nicotiana Rusbyi extract (plant B).

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Nicotiana rustica</i>	—	±	±	—	—	—
<i>Nicotiana paniculata</i>	—	—	—	—	—	—
<i>Nicotiana glauca</i>	—	—	—	—	—	—
<i>Nicotiana Langsdorffii</i>	±	++	+++	+++	++	++
<i>Nicotiana alata</i>	—	+	+	±	±	±
F ₁ (<i>Nicotiana rustica</i> × <i>Nicotiana Tabacum</i>)	—	+	+	+	±	±
<i>Nicotiana Tabacum</i>	—	+	++	+	+	±
<i>Nicotiana suaveolens</i>	±	++	+++	+++	++	++
<i>Petunia violacea</i>	—	++	+++	+++	++	++

The best formation of the reaction rings in the normal precipitations occurs usually from 5 to 15 minutes after the beginning of the reaction. After 15 minutes, in most cases, the ring widens; and then both extracts begin to mix together, the upper layer penetrating into the lower one like small roots. Some particles of the flocculation stick together, forming larger flocules which drop to the bottom; others obviously disappear,—that is, the precipitates dissolve again, the components in the tubes in most cases becoming gradually clearer and clearer after 15 to 20 minutes. In many cases when the precipitation is very weak, the precipitate completely disappears. In some combinations like *Solanum nigrum* + *Lycium Barbarum* (table 1), *Physalis peruviana* + *Capsicum pyramidale* (table 2) *Salpiglossis sinuata* + *Lycium Barbarum*, *Salpiglossis sinuata* + *Datura Wrightii*, *Salpiglossis sinuata* + *Capsicum pyramidale* (table 4) etc., between both layers a lytic (*clear*) ring appears, which is clearer than either extract. When the precipitation ring begins to spread above and below, it sometimes splits into two, with the formation of a lytic ring, appearing at about the same place that first the precipitation ring occurred. This is the phenomenon that gives the appearance of a double precipitation ring. The lytic ring which splits the precipitation ring in two is never as clear as the original component extracts, but clearer of course than the double precipitation ring. When the lytic phenomenon was observed, it usually followed after the precipitation phenomenon.

All reactions of the data reported in tables 1–8 were repeated at least 3 times, and where there was any doubt, 4–5 times. When different plants of the same species were used for the precipitin reaction, the results varied in some combinations within relatively narrow limits.

The greatest variation in the normal precipitin reaction, dependent

upon the individuality of the plants, showed itself in *Datura Wrightii*, *Solanum nigrum*, *Nicotiana Langsdorffii* and *Nicotiana Rusbyi*. Thus the normal precipitin reaction of plant B of *Nicotiana Rusbyi* (table 8) is not identical with the normal precipitin reaction of plant C (table 9).

TABLE 9
Normal precipitin reaction of Nicotiana Rusbyi extract (plant C).

AGAINST NORMAL EXTRACTS OF	THE POTENCY OF THE PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Nicotiana rustica</i>	—	+	+	±	±	—
<i>Nicotiana paniculata</i>	—	—	—	—	—	—
<i>Nicotiana glauca</i>	—	—	—	—	—	—
<i>Nicotiana Langsdorffii</i>	+	+++	+++	+++	++	++
<i>Nicotiana alata</i>	—	+	+	+	±	±
<i>Nicotiana Tabacum</i>	—	±	+	+	+	±
<i>Nicotiana suaveolens</i>	—	++	++	++	++	+
<i>Petunia violacea</i>	±	++	+++	+++	++	++

Some interrelation between the pH of the plant extracts and the normal precipitin reaction was expected. The pH was tested by Clark's (1920) color indicators. The results are presented in table 10. The determinations for the various species were obtained from plants growing under many different environmental conditions. In the table is given the average value of pH.

We can draw the conclusion from the data of table 10 in comparison with those of tables 1 to 9 that no correlation exists between normal precipitin reaction and hydrogen ion concentration.

ACQUIRED PRECIPITINS

By a series of experiments we have tried to answer the question: Is the plant cell (that is, the plant organism) able to acquire the capacity to produce precipitins; and, if so, how far is this ability specific? In order to answer this question, various plants were grafted together, and the physiological and morphological changes in the scions and stocks were studied.

Twenty shoots of *Nicotiana Rusbyi* were grafted on *Nicotiana rustica* the same afternoon. Both the shoots and the stock (*Nicotiana rustica*) plants were chosen as nearly alike as possible in age and size. In the successful grafting an approximately regular circulation of sap from the stock into the scion and *vice versa* begins usually from 3 to 4 days after the grafting was made. On the day when the graftings were made, extracts were obtained and the capacity for a normal precipitin reaction was

TABLE 10

Normal precipitin reaction—maximum titre and average value of pH. (0 = lytic ring, $\pm$ = trace, $++$ = 2, $+++$ = 3, $++++$ = 4, and $-$ = no reaction).

	AVERAGE VALUE OF pH	<i>Capiscum pyramidale</i>	<i>Datura ferox</i>	<i>Datura Wrightii</i>	<i>Lycium Barbarum</i>	<i>Nicotiana glauca</i>	<i>Nicotiana Langsdorffii</i>	<i>Nicotiana paniculata</i>	<i>Nicotiana Rusbyi</i>	<i>Nicotiana rustica</i>	<i>F₁ (N. rustica N. Tabacum)</i>	<i>Nicotiana Tabacum</i>	<i>Nicotiana suaveolens</i>	<i>Petunia violacea</i>	<i>Physalis peruviana</i>	<i>Salpiglossis sinuata</i>	<i>Solanum Dulcamara</i>	<i>Solanum Lycopersicum</i>	<i>Solanum Melongena</i>	<i>Solanum nigrum</i>	<i>Solanum tuberosum</i>
<i>Capiscum pyramidale</i>	5.8	-		$\pm$	2	$\pm$	2	+	$\pm$	+	0	+	0	-	0	0	4	+	2	4	-
<i>Datura ferox</i>	5.2	$\pm$	-		2							2									
<i>Datura Wrightii</i>	5.0			2																	
<i>Lycium Barbarum</i>	7.2	2	2																		
<i>Nicotiana glauca</i>	5.6	+																			
<i>Nicotiana Langsdorffii</i>	5.6	$\pm$																			
<i>Nicotiana paniculata</i>	5.4	2																			
<i>Nicotiana Rusbyi</i>	5.5	+																			
<i>Nicotiana rustica</i>	5.6	$\pm$																			
<i>F₁ (N. rustica N. Tabacum)</i>	5.2	+																			
<i>Nicotiana Tabacum</i>	5.4	0	2																		
<i>Nicotiana suaveolens</i>	5.6	+																			
<i>Petunia violacea</i>	5.3	0																			
<i>Physalis peruviana</i>	5.1	-																			
<i>Salpiglossis sinuata</i>	7.6	0				2															
<i>Solanum Dulcamara</i>	7.7	0		0		3															
<i>Solanum Lycopersicum</i>	6.0	4	+	4	3							4									
<i>Solanum Melongena</i>	5.4	+		4																	
<i>Solanum nigrum</i>	5.7	2		4																	
<i>Solanum tuberosum</i>	6.4	4		4	0	+															
	6.4	-		4			2														

tested. On the 5th, 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, 50th, 60th, 80th, and 100th days after the grafting, the acquired (immune) precipitin reaction and the normal precipitin reaction (if any) were determined. The difference between the precipitation capacity of the first and of the second (normal) test represents the acquired precipitation potency in the grafted union. In other words, the normal extract from *Nicotiana Rusbyi* and the normal extract from *Nicotiana rustica* were used as a control of immune (acquired) precipitins.

In table 11 is given for illustration a copy of the original notes from which the curves of figure 2 were drawn

FIGURE 2.—Precipitin reaction capacity of shoots of *Nicotiana Rusbyi* plants A, B and C growing as scions on *Nicotiana rustica* stocks.

TABLE 11

Scion—*Nicotiana Rusbyi* (plant B)
Stock—*Nicotiana rustica* (N 28)
Grafting made on March 26, 1927
Test of the precipitin reaction—April 25, 1927
Ratio: 1 part of tissue extracted by 2 parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF NICOTIANA RUSTICA
Immune extract of <i>Nicotiana Rusbyi</i> against <i>Nicotiana rustica</i>	+++
Normal extract of <i>Nicotiana Rusbyi</i>	±

The results obtained in this way were summarized and plotted in figure 2. The curves show the precipitin reaction capacity of the shoots of *Nicotiana Rusbyi* plants A, B, and C growing as scions on *Nicotiana rustica*. When the grafts were made, the extract of plant A manifested no normal precipitin reaction “(−)”, the extract of plant B a “trace”, “(±),” and the extract of plant C showed “+” normal precipitin reaction

with *Nicotiana rustica* normal extract. At 10 days after grafting, the grafted shoots of plant A manifested a trace of precipitin reaction; and this reaction increased in intensity with the time of growth on *Nicotiana rustica* until the 30th day (++++). At later dates, the level of the precipitin reaction remained constant. The increase of the precipitin capacity of B scions began at the same time (10 days). It reached the highest potency, however, 40 days after grafting. The normal precipitin reaction "(+)" manifested at first by the extract of the shoots of plant C, decreased during the first 10 days. Later, it began to increase, and reached the highest point 35 days after grafting. The control tests which were always made when the immune precipitin capacity was tested, showed very slight variations from the control made at the time of grafting (compare the ordinate for 0 days, figure 2).

The curves on figure 2 shows very conspicuously the individual reaction of the several plants. The most striking immunological difference is between plant A and plant C.

A similar series of studies of the immune precipitin reactions was carried out in the subsequent combinations:

1. *Solanum tuberosum* grafted on *Nicotiana rustica*.
2. *Lycium Barbarum* grafted on *Solanum Lycopersicum*.
3. *Nicotiana Sanderae* grafted on *Datura ferox*.
4. *Nicotiana glauca* grafted on *Capsicum pyramidale*.

The highest precipitation capacity in the scion extracts was found to be 35–40–45 days after grafting. In tables 12, 13, 14, and 15 are given the highest immune potency of the four graft combinations mentioned above.

TABLE 12

Scion—*Solanum tuberosum*
 Stock—*Nicotiana rustica*
 Grafting made on March 23, 1927
 Test of the precipitin reaction—May 2, 1927
 Ratio: 1 part of tissue extracted by 2 parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF NICOTIANA RUSTICA
Immune extract of <i>Solanum tuberosum</i> against <i>Nicotiana rustica</i>	+++
Normal extract of <i>Solanum tuberosum</i>	±

TABLE 13

Scion—*Lycium Barbarum*
 Stock—*Solanum Lycopersicum*
 Grafting made on March 25, 1927

Test of the precipitin reaction—April 30, 1927

Ratio: 1 part of tissue extracted by two parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF SOLANUM LYCOPERSICUM
Immune extract of <i>Lycium Barbarum</i> against <i>Solanum Lycopersicum</i>	++
Normal extract of <i>Lycium Barbarum</i>	—

TABLE 14

Scion—*Nicotiana Sanderae*

Stock—*Datura ferox*

Grafting made on April 15, 1927

Test of the precipitin reaction—May 29, 1927

Ratio: 1 part of tissue extracted by two parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF DATURA FEROX
Immune extract of <i>Nicotiana Sanderae</i> against <i>Datura ferox</i>	++
Normal extract of <i>Nicotiana Sanderae</i>	±

TABLE 15

Scion—*Nicotiana glauca*

Stock—*Capsicum pyramidale*

Grafting made on February 10, 1927

Test of the precipitin reaction—March 17, 1927

Ratio: 1 part of tissue extracted by two parts distilled water.

	PRECIPITIN REACTION WITH NORMAL EXTRACT OF CAPSICUM PYRAMIDALE
Immune extract of <i>Nicotiana glauca</i> against <i>Capsicum pyramidale</i>	++
Normal extract of <i>Nicotiana glauca</i>	±

Very slight acquirement of precipitins was found in the following graft unions:

1. *Nicotiana Rusbyi* scion on *Nicotiana glauca*.
2. *Solanum Lycopersicum* scion on *Datura ferox*.
3. *Nicotiana Langsdorffii* scion on *Nicotiana rustica*.
4. F₁ (*Nicotiana Tabacum* × *Nicotiana Rusbyi*) scion on *Nicotiana Sanderae*
5. *Lycium Barbarum* scion on *Datura ferox*.
6. *Datura Wrightii* scion on *Nicotiana Tabacum*.
7. *Lycium Barbarum* scion on *Nicotiana Tabacum*.

No acquirement of precipitins was found in the following graft unions:

1. *Nicotiana Tabacum* scion on *Nicotiana rustica*.

2. *Nicotiana rustica* scion on *Nicotiana quadrivalvis*.
3. *Nicotiana Tabacum* scion on *Solanum nigrum*.
4. *Salpiglossis sinuata* scion on *Datura ferox*.
5. *Nicotiana glauca* scion on *Nicotiana rustica*.
6. *Solanum Melongena* scion on *Solanum nigrum*.
7. *Datura Wrightii* scion on *Solanum nigrum*.
8. *Nicotiana Tabacum* scion on *Solanum Melongena*.
9. *Solanum nigrum* scion on *Nicotiana Sanderae*.
10. *Nicotiana Tabacum* scion on *Solanum tuberosum*.
11. *Nicotiana Tabacum* scion on *Nicotiana Langsdorffii*.

A decrease of the normal precipitins was observed in the following unions:

1. *Solanum tuberosum* grafted on *Datura Wrightii*.
2. *Solanum Melongena* grafted on *Datura Wrightii*.
3. *Solanum Lycopersicum* grafted on *Datura Wrightii*.
4. *Nicotiana Tabacum* grafted on *Datura Wrightii*.

The gradual decrease of the normal precipitin reaction in *Solanum tuberosum* (T), *Solanum Melongena* (M), and *Solanum Lycopersicum* (L) when grafted on *Datura Wrightii*, are presented in figure 3.

FIGURE 3.—Gradual decrease of the normal precipitins in shoots of *Solanum tuberosum* (T), *Solanum Melongena* (M) and *Solanum Lycopersicum* (L) grafted on *Datura Wrightii*.

A lytic ring was obtained from the union: *Solanum tuberosum* grafted on *Nicotiana alata* 20 days after the grafting.

A general question of importance to serology is the precipitin capacity of gradually diluted serum. It also has significance in plant immunology, because the precipitin capacity can be measured in this way. The highest limits of dilution of immune extracts giving precipitin reaction are shown

on table 16 and 17, and in figure 4. The graft union is *Nicotiana Rusbyi* scion on *Nicotiana rustica*.

TABLE 16

Precipitin reaction between the immune extract of Nicotiana Rusbyi grafted on Nicotiana rustica and antigenic extract of Nicotiana rustica. (Diluent—distilled water)

IMMUNE EXTRACT DILUTED	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
1:0	++++	++++	++++	++++	++++	++++
1:1	+++	++++	++++	++++	++++	++++
1:2	+++	++++	++++	++++	++++	++++
1:4	+++	+++	+++	++++	++++	++++
1:8	+++	+++	+++	+++	++++	++++
1:12	++	+++	+++	+++	+++	+++
1:16	++	++	+++	+++	+++	+++
1:20	+	++	++	++	++	++
1:24	+	+	++	++	++	++
1:30	±	+	+	++	++	++
1:36	±	±	+	+	+	+
1:50	—	±	+	+	+	+
1:75	—	—	±	+	+	+
1:100	—	—	—	±	±	±
1:200	—	—	—	—	—	—

TABLE 17

Precipitin reaction between immune extract of Nicotiana Rusbyi grafted on Nicotiana rustica and antigenic extract of Nicotiana rustica. (Diluent—distilled water)

ANTIGENIC EXTRACT DILUTED	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
1:0	++++	++++	++++	++++	++++	++++
1:8	+++	++++	++++	++++	++++	++++
1:16	++	+++	+++	++++	++++	++++
1:32	+	+	++	+++	+++	+++
1:64	±	±	+	++	++	++
1:128	—	—	±	+	+	+
1:256	—	—	—	—	—	—

Similar results were obtained from *Nicotiana glauca* grafted on *Cap-sicum pyramidale* and from *Solanum tuberosum* grafted on F_1 (*Nicotiana rustica* var. *humilis* × *Nicotiana rustica* var. *brasilia*).

From a comparison of table 16 with table 17, it appears that the precipitin reaction drops down more quickly when the immune extract is diluted.

The limit of dilution of the immune extract shown in table 16 and in figure 4 is 1:100—not nearly as high a dilution as can be reached with

rabbit serum. But we cannot make a comparison between the rabbit serum and the plant extract. The animal cell (that is organism) is more sensitive and reactable (MÜLLER 1926) and disposes of more antibody formative materials than the plant cell. Moreover, the basic amount of extract (normal dilution) we usually used represents an extraction of 1 part plant organs (leaves and stems) to 2 parts of distilled water. That is relatively a very high dilution.

FIGURE 4.—Precipitation capacity of *Nicotiana glauca* immune extracts against *Nicotiana glauca* normal extracts. Curve P—reaction of scions extracted immediately after cutting. Curve Q—reaction of scions extracted 4 days after cutting. Curve R—reaction of shoots extracted 2 days after-cutting.

The Uhlenhuth's ring observed between an immune and an antigenic extract keeps its form a longer time (table 16 and 17) than in a normal precipitin reaction, and the immune-antigenic extract components mix more slowly.

The immune extracts used in obtaining the data for tables 16 and 17 and curve R of figure 4 were produced from shoots growing as grafts 40 days and kept in a glass of water 2 days after cutting.

Curve R (figure 4) is drawn from the data of 3 tests, and includes the

results from table 16. Curve Q (figure 4) shows the precipitin capacity of two shoots grown as grafts 40 days, and kept 4 days in a glass of water after cutting. Curve P represents the precipitin potency of 4 scions of *Nicotiana Rusbyi* grown 40 days in graft union with *Nicotiana rustica* stocks, as were those of curves R and Q, but extracted immediately after cutting.

Comparing the curves in figure 4, one may conclude that the shoots extracted two days after cutting (curve R) have a higher precipitin potency than the shoots of the other two cases. Probably a part of the precipitin in the shoots extracted immediately after cutting (curve P) is neutralized by the antigenic sap coming up from the stock. The drop of the curve Q probably is due to the failure of antigenic agents over a relatively longer time.

When shoots of different ages were compared, the greatest amount of precipitins was found in the leaves and stems of about 20 to 70 days old. Both younger and older tissues contain less precipitins.

The results obtained from the material treated by tartaric acid-alcohol-ether, ether, chloroform and chloroform-alcohol before the extraction compared with the material not treated are as follows:

TABLE 18¹

a. Material treated by tartaric-acid-alcohol-ether 2 hrs.

Immune extract of <i>Nicotiana Sanderae</i>	+ Antigenic extract of <i>Datura ferox</i>	= +
Normal extract of <i>Nicotiana Sanderae</i>	+ Normal (antigenic) extract of <i>Datura ferox</i>	= -

b. Non-treated material.

Immune extract of <i>Nicotiana Sanderae</i>	+ Antigenic extract of <i>Datura ferox</i>	= + +
Normal extract of <i>Nicotiana Sanderae</i>	+ Normal extract of <i>Datura ferox</i>	= ±

c. Material treated by ether three days.

Immune extract of F ₁ (<i>Nicotiana Tabacum</i> × <i>Nicotiana Rusbyi</i>)	+ Antigenic extract of <i>Nicotiana Sanderae</i>	= ±
Normal extract of F ₁ (<i>Nicotiana Tabacum</i> × <i>Nicotiana Rusbyi</i>)	+ Normal extract of <i>Nicotiana Sanderae</i>	= -

d. Non-treated material.

Immune extract of F ₁ (<i>Nicotiana Tabacum</i> × <i>Nicotiana Rusbyi</i>)	+ Antigenic extract of <i>Nicotiana Sanderae</i>	= + + +
Normal extract of F ₁ (<i>Nicotiana Tabacum</i> × <i>Nicotiana Rusbyi</i>)	+ Normal extract of <i>Nicotiana Sanderae</i>	= +

e. Material treated by ether 2 hours.

Immune extract of <i>Nicotiana Rusbyi</i>	+ Antigenic extract of <i>Nicotiana rustica</i>	= + to + +
Normal extract of <i>Nicotiana Rusbyi</i>	+ Normal extract of <i>Nicotiana rustica</i>	= -

f. Non-treated material.

Immune extract of <i>Nicotiana Rusbyi</i>	+ Antigenic extract of <i>Nicotiana rustica</i>	=++++
Normal extract of <i>Nicotiana Rusbyi</i>	+ Normal extract of <i>Nicotiana rustica</i>	=+

g. Material treated 2 hours with chloroform-alcohol.

Immune extract of <i>Nicotiana Rusbyi</i>	+ Antigenic extract of <i>Nicotiana rustica</i>	=+++
Normal extract of <i>Nicotiana Rusbyi</i>	+ Normal extract of <i>Nicotiana rustica</i>	=+

h. Material treated 2 hours with chloroform.

Immune extract of <i>Nicotiana Rusbyi</i>	+ Antigenic extract of <i>Nicotiana rustica</i>	=+++
Normal extract of <i>Nicotiana Rusbyi</i>	+ Normal extract of <i>Nicotiana rustica</i>	=+

i. Material treated 2 hours with chloroform.

Immune extract of <i>Nicotiana Sanderae</i>	+ Antigenic extract of <i>Datura ferox</i>	=++
Normal extract of <i>Nicotiana Sanderae</i>	+ Normal extract of <i>Datura ferox</i>	=+

¹ In all series of experiments in table 18, 1 part of tissue was extracted by 1 part distilled water

Parallel experiments for observing the precipitation capacity for 24 hours (MEZ) with the components of a, c, e, g, and h in ratio 1:1 were also carried out. The results did not differ from those obtained in 40 minutes.

The most marked decrease of the precipitin capacity was found in the material treated with ether before the water extraction was made.

The normal extract of *Nicotiana Rusbyi* in one case manifested the same precipitin potency (+++) as the immune extract when tested against the antigenic extract of *Nicotiana rustica* in undiluted form. After dilution of the extracts, however, the precipitin potency of the normal extract decreased faster than that of the immune extract; and the Uhlenhuth ring kept its form and the precipitate its density longer in the case of the immune extract than in the normal extract.

Optimum growing temperatures (25°–30°C) and intense light increased the precipitin content.

The data reported in the preceding pages give us an answer to our first question. *Plants,—that is, the cells in the plants,—are able to produce antibodies.* This statement will be confirmed later by other observations of a different nature.

Experiments were made in which it was proposed to test the specific reactivity of the immune extracts. The data obtained are given in tables 19, 20, 21, and 22.

TABLE 19

Acquired precipitin reactions of *Nicotiana Rusbyi* immune extract (*Nicotiana Rusbyi* grafted on *Nicotiana rustica*).

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	20 min.	40 min.
<i>Nicotiana rustica</i>	++++	++++	++++	++++	++++	++++
<i>Nicotiana paniculata</i>	++	+++	+++	+++	+++	+++
<i>Nicotiana glauca</i>	+	++	+++	+++	+++	+++
<i>F₁ (Nicotiana rustica × Nicotiana Tabacum)</i>	±	+	++	++	++	++
<i>Nicotiana Tabacum</i>	—	±	+	+	+	+
<i>Nicotiana alata</i>	++	+++	++++	++++	++++	+++
<i>Nicotiana Langsdorffii</i>	+++	++++	++++	++++	++++	++++

TABLE 20

Acquired precipitin reactions of *Nicotiana Rusbyi* immune extract of table 19 diluted in distilled water 1:4.

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Nicotiana rustica</i>	+++	++++	++++	++++	++++	++++
<i>Nicotiana paniculata</i>	±	+	++	++	++	++
<i>Nicotiana glauca</i>	—	±	+	+	+	+
<i>F₁ (Nicotiana rustica × Nicotiana Tabacum)</i>	—	—	—	—	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
<i>Nicotiana alata</i>	+	++	+++	+++	++	++
<i>Nicotiana Langsdorffii</i>	++	+++	+++	+++	+++	++

TABLE 21

Acquired precipitin reactions of *Nicotiana Rusbyi* immune extract of table 19 diluted in distilled water 1:8.

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Nicotiana rustica</i>	++	+++	+++	+++	+++	+++
<i>Nicotiana paniculata</i>	±	+	+	+	+	+
<i>Nicotiana glauca</i>	—	—	±	±	±	±
<i>F₁ (Nicotiana rustica × Nicotiana Tabacum)</i>	—	—	—	—	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
<i>Nicotiana alata</i>	±	+	+	+	+	±
<i>Nicotiana Langsdorffii</i>	±	+	++	++	++	+

TABLE 22

Acquired precipitin reactions of Nicotiana Rusbyi immune extract of table 19 diluted in distilled water 1:16.

AGAINST NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	20 min.	40 min.
<i>Nicotiana rustica</i>	+	++	++	++	++	++
<i>Nicotiana paniculata</i>	—	—	—	—	—	—
<i>Nicotiana glauca</i>	—	—	—	—	—	—
<i>F</i> ₁ (<i>Nicotiana rustica</i> × <i>Nicotiana Tabacum</i>)	—	—	—	—	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
<i>Nicotiana alata</i>	—	—	±	±	—	—
<i>Nicotiana Langsdorffii</i>	—	±	+	+	±	—

Nicotiana Rusbyi extract immunized against *Nicotiana rustica* by grafting always gave the heaviest precipitate when tested against its antigenic extract, namely the normal extract of *Nicotiana rustica*. Then follow in order the extracts of *Nicotiana paniculata*, *Nicotiana glauca*, *F*₁ (*Nicotiana rustica* × *Nicotiana Tabacum*) and *Nicotiana Tabacum*. Three species *Nicotiana rustica*, *Nicotiana paniculata*, and *Nicotiana glauca* belong to the subgenus or section *Rustica*; the species *Nicotiana Tabacum* and *Nicotiana Rusbyi* belong to the section *Tabacum* (Comes). The hybrid *Nicotiana rustica* × *Nicotiana paniculata* is partially fertile, (EAST 1921), while the hybrid *Nicotiana rustica* × *Nicotiana glauca* produces embryos which are viable only 10–15 days. The genetic compatibility between *Nicotiana rustica* and *Nicotiana paniculata* and between *Nicotiana rustica* and *Nicotiana glauca* thus corresponds with the precipitin reaction obtained. *Nicotiana rustica* × *Nicotiana Tabacum* also produces a partially fertile *F*₁ generation; (in back crosses but since *Nicotiana Tabacum* is closely related to *Nicotiana Rusbyi*, the lower precipitin reaction is to be expected. The *F*₁ hybrid *Nicotiana rustica* × *Nicotiana Tabacum* usually gives a precipitin reaction between those of *Nicotiana rustica* and *Nicotiana Tabacum*. The powerful precipitation with *Nicotiana Langsdorffii* extract and with *Nicotiana alata* extract is due chiefly to the normal precipitins (compare with tables 8 and 9); nevertheless, heterogenetic (non-specific) precipitins are also present.

In one case a high precipitin reaction was obtained when *Nicotiana Rusbyi* immune (against *Nicotiana rustica*) extract was tested against *Nicotiana rustica* and against *Nicotiana Tabacum* normal extracts (ex-

tracts undiluted—reaction++). For the elimination of the normal precipitins an experiment was performed as follows:

A. 2cc. *Nicotiana Tabacum* normal extract was added to 2cc. *Nicotiana Rusbyi* normal extract (Tube A).

B. 2cc. *Nicotiana rustica* normal extract was added to 2cc. *Nicotiana Rusbyi* normal extract (Tube B).

After 40 minutes A and B components were filtered separately. The filtrates gave the following results with *Nicotiana Rusbyi* immune extract:

1. A-filtrate+*Nicotiana Rusbyi* immune extract = —

2. B-filtrate+*Nicotiana Rusbyi* immune extract = ++

The reaction potency in the second combination (++) remained about the same even when 3cc. instead of 2cc. of *Nicotiana Rusbyi* normal extract were added to B.

This experiment shows that the precipitation between *Nicotiana Tabacum* normal extract and *Nicotiana Rusbyi* extract (immune against *Nicotiana rustica*) is simply a normal precipitation and not a reaction indicating acquired immunity.

A reaction similar to that shown in table 21 was obtained by an immune extract from a tuber formed in the air on a scion of *Solanum tuberosum* grafted on *Nicotiana rustica* [F_1 (*Nicotiana rustica* var. *humilis* × *Nicotiana rustica* var. *texana*)]. The results are shown in table 23.

TABLE 23

Acquired precipitin reactions of *Solanum tuberosum* (tuber) extract (*Solanum tuberosum* grafted on *Nicotiana rustica* 45 days).

NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.
<i>Nicotiana rustica</i>	+	++	+++	+++	+++	+++
<i>Nicotiana paniculata</i>	—	±	+	+	+	+
<i>Nicotiana glauca</i>	—	—	—	—	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
F_1 (<i>Nicotiana rustica</i> × <i>Nicotiana Tabacum</i>)	—	—	—	—	—	—
<i>Nicotiana Rusbyi</i>	—	—	—	—	—	—
<i>Nicotiana alata</i>	±	+	++	++	++	+
<i>Nicotiana Langsdorffii</i>	+	+++	+++	+++	++	++

In table 24 the normal precipitin reactions of normal extract of *Solanum* (tuber) are given for comparison.

TABLE 24

Normal precipitin reactions of Solanum tuberosum (tuber) normal extract.

NORMAL EXTRACT OF	PRECIPITIN REACTION AFTER					
	1 min.	5 min.	10 min.	20 min.	20 min.	40 min.
<i>Nicotiana rustica</i>	—	—	—	—	—	—
<i>Nicotiana paniculata</i>	—	—	—	—	—	—
<i>Nicotiana glauca</i>	—	—	—	—	—	—
<i>Nicotiana Tabacum</i>	—	—	—	—	—	—
<i>F</i> ₁ (<i>Nicotiana rustica</i> × <i>Nicotiana Tabacum</i>)	—	—	—	—	—	—
<i>Nicotiana Rusbyi</i>	—	—	—	—	—	—
<i>Nicotiana alata</i>	±	+	++	++	+	+
<i>Nicotiana Langsdorffii</i>	+	++	++	++	++	+

It is obvious that a great deal of the precipitation with *Nicotiana alata* and *Nicotiana Langsdorffii* (table 23), if not all, is due to normal precipitins; while the reaction with *Nicotiana rustica* and *Nicotiana paniculata* is evidently acquired.

Another demonstration of specific precipitin reaction is presented graphically in figure 5.

FIGURE 5.—Scheme for showing acquired precipitin reaction after grafting.

THE BEHAVIOR OF THE PLANT PRECIPITIN AND ANTIGEN TO SOME PHYSICAL AND CHEMICAL AGENTS

The behavior of antigen and precipitin to various agents in animals has been studied by many investigators. Although the data exhibits many

discrepancies, they appear to support the statement that high temperature diminishes the precipitin potency both of the immune serums and of the antigens.

In order to determine the influence of high temperatures on the immune and the antigenic extracts, the following experiments were carried out:

Immune and antigenic extracts in separate glass tubes were kept in water baths at 25°C, 60°C, 80°C, and 100°C respectively, for 30 minutes; after which, the precipitin potencies of the extracts were tested. Curves

FIGURE 6.—Decrease of precipitation capacity of the immune extract in 30 minutes at 60°C, 80°C and 100°C temperature.

from the data obtained from the immune extract activity of *Nicotiana Rusbyi* against *Nicotiana rustica* antigen are plotted in figure 6. The curves of normal antigenic *Nicotiana rustica* extracts are plotted in figure 7. The precipitin reaction decreases with the increase of temperature. When the immune extract is treated in the same way, its precipitin reaction with the antigenic extract decreases more strikingly than when the antigenic extracts are so treated.

It was found that filter paper absorbs the precipitins and the antigens, thus causing the reaction potency to diminish.

The influence of formaldehyde, toluol, chloroform, H-ion, OH-ion, and certain metal-ions, as Pb-, Fe-, and Hg- on the antigen has been studied by OBERMEYER and PICK (1906) and by PICK (1912). These authors stated that all such agents make a change in the protein molecule similar to that produced by high temperature. LANDSTEINER and LAMPL (1917) did not confirm PICK's conclusion concerning the activity of formaldehyde on the antigen, but BAIVY (1926) found that. . . "par l'action du

FIGURE 7.—Decrease of precipitation capacity of the antigenic extract in 30 minutes at 60°C, 80°C and 100°C temperature.

formol la précipitin perd immédiatement et complètement son activité; la proportion suffisant de formol étant, dans certains cas, de 1 per 1000." ABDERHALDEN (1922), moreover, found a decrease of the "plasma" and "sera" reaction capacity when treated by phenol, totuol and chloroform in so-called Abderhalden's reaction.

The reaction capacity of the immune extract of *Nicotiana Rusbyi*, treated by various chemical agents, against the *Nicotiana rustica* extract is shown in table 25.

TABLE 25

Reaction of treated immune extract of Nicotiana Rusbyi against normal Nicotiana rustica extract.

Immune extract	+ Antigenic extract	=++++
Immune extract saturated with $MgSO_4$ and filtered	+ Antigenic extract	=--
Immune extract saturated with Na_2SO_4 and filtered	+ Antigenic extract	=--
Immune extract saturated with $(NH_4)_2SO_4$ and filtered	+ Antigenic extract	=++++
Immune extract saturated with $Li_2Cr_2O_7$ and filtered	+ Antigenic extract	=--
(Immune extract+formaldehyde (40 per cent) in ratio 20:1) after 4 hours	+ Antigenic extract	=+++
(Immune extract+ chloroform in ratio 1:1) after 2 days	+ Antigenic extract	=+++
(Immune extract+toluol in ratio 1:1) after 2 days	+ Antigenic extract	=+++
(1 cc. Immune extract+1 drop 10 per cent CH_3COOH)	+ Antigenic extract	=++++
(1 cc. Immune extract+1 drop 10 per cent KOH)	+ Antigenic extract	=++++

In 1922, MASON pointed out that precipitin reactions are obtainable within a pH range of 4.5 to 9.5, and the precipitation is no heavier at the iso-electric point either of the serum or of the antigen; if the pH is outside of this range, no precipitate forms, and an existing precipitate will be re-dissolved by such H-ion or OH-ion concentrations.

In the present study, the hydrogen ion concentration has no marked influence on the precipitin reaction. The data for this conclusion are given in table 26.

TABLE 26

Immune extract diluted 1:10 (pH=5.6)	+ Antigenic extract (pH=5.4)	=+to++
(Immune extract diluted 1:10+HCl) with pH=1.8	+ Antigenic extract (pH=5.4)	=++
(Immune extract diluted 1:10+NaOH) with pH=9.8	+ Antigenic extract (pH=5.4)	=++
Immune extract diluted 1:10 (pH=5.6)	+ (Antigenic extract+HCl) with pH=3.0	=+
Immune extract diluted 1:10 (pH=5.6)	+ (Antigenic extract+NaOH) with pH=8.4	=+

The hydrogen ion concentration in the scion is usually altered to a condition nearly intermediate between that of scion plant and stock plant (table 27).

TABLE 27

pH determined by Clark's color indicators (compare table 10).

EXTRACT OF	pH
<i>Lycium Barbarum</i>	7.2
<i>Lycium Barbarum</i> scion on <i>Solanum Lycopersicum</i>	6.8
<i>Solanum Lycopersicum</i>	5.4
<i>Nicotiana Tabacum</i>	5.6
<i>Lycium Barbarum</i> scion on <i>Nicotiana Tabacum</i>	5.8
<i>Nicotiana Tabacum</i> scion on <i>Solanum Melongena</i>	5.6–5.8
<i>Solanum Melongena</i>	5.6–5.8
<i>Solanum Melongena</i> scion on <i>Datura Wrightii</i>	5.6
<i>Datura Wrightii</i>	5.0
<i>Solanum tuberosum</i> scion on <i>Datura Wrightii</i>	5.8–6.0
<i>Solanum tuberosum</i>	6.4
<i>Solanum Lycopersicum</i> scion on <i>Datura Wrightii</i>	5.2–5.4
<i>Solanum tuberosum</i> scion on <i>Nicotiana rustica</i>	6.0–6.2
<i>Solanum tuberosum</i> scion on <i>Nicotiana Tabacum</i>	6.0–6.2
<i>Nicotiana rustica</i>	5.2
<i>Nicotiana rustica</i> scion on <i>Nicotiana quadrivalvis</i>	4.8
<i>Nicotiana quadrivalvis</i>	4.4–4.6
<i>Nicotiana Rusbyi</i> scion on <i>Nicotiana rustica</i>	5.4–5.6
<i>Nicotiana Rusbyi</i>	5.6
<i>Nicotiana Rusbyi</i> scion on <i>Nicotiana glauca</i>	5.6
<i>Nicotiana glauca</i>	5.6
<i>Nicotiana glauca</i> scion on <i>Capsicum pyramidale</i>	5.6
<i>Capsicum pyramidale</i>	5.8
<i>Datura Wrightii</i> scion on <i>Nicotiana Tabacum</i>	5.2
<i>Nicotiana Tabacum</i> scion on <i>Datura Wrightii</i>	5.4
<i>Solanum nigrum</i> scion on <i>Nicotiana Tabacum</i>	5.6–5.8
<i>Solanum nigrum</i>	6.4
<i>Nicotiana Tabacum</i> scion on <i>Solanum nigrum</i>	5.6–5.8
<i>Solanum Melongena</i> scion on <i>Solanum nigrum</i>	5.8
<i>Datura ferox</i> scion on <i>Solanum nigrum</i>	5.2–5.4
<i>Datura ferox</i>	5.2
<i>Lycium Barbarum</i> scion on <i>Datura ferox</i>	5.6

A few years ago it was thought that all of the different antigens (sera, red blood corpuscles, egg albumine, milk, etc.) belonged to one chemical group of substances, namely to the proteins. "No antigen effect without protein" was a thesis generally accepted. Recently, the view that the proteins are the only antigens has been definitely changed. New evidence has indicated an antigenic nature for certain non-proteid substances, namely lipoids, bacterial distillates, carbohydrates, and alkaloids.

In 1909, PICK and SCHWARZ suggested on the antigenic property of the lipoids. MEYER (1910–1912) pointed out that for the antigenic activity, it is necessary to have other additional agents (probably proteins) whose

rôle is "Ictus immunisatorius" (EHRlich und MORGENROTH's term). His statement was received with skepticism. Later (1912-1914) he obtained antisera by lipoid-antigens which reacted with lipoid antigens but not with those of proteins.

In 1911 FORSSMANN has found that emulsion of guinea pig kidney, injected into rabbits, induces antibody formation against sheep blood cells. It was established that this so-called heterogenetic antigen is not a protein but a lipid, soluble in hot alcohol.

GAY and ROBERTSON (1912) denied the antigenic effect both of protamines and also of protamines in combination with caseinogen. GAMALEIA (1927) claimed that distillate products of the group of protamin substances and alkaloids can also cause immunity. YERMOLJEWA (1927) made similar statements. She obtained immune sera which gave the positive "complement deviation reaction" and the "Pfeiffer-phenomenon" with distillates of bacteria. Finally we must mention HEILDERBERGER, GOEBEL and AVERY recent investigations (1926). They have isolated I, II and III Type substances of *Pneumococcus*. Substance Type III is a "nitrogen-free polysaccharide" and "in as high a dilution as 1:6,000,000 it still reacts with Type III anti-pneumococcus serum."

Nevertheless, many serologists up to the present time are very skeptical about the antigenic effect of the substances outside of the protein group.

About the nature of the antigens and antibodies in the plants one can say very little. It is impossible to draw definite conclusions from the observed interaction between scion and stock or from the behavior of the antigenic and immune extracts to various physical and chemical agents reported above.

OBSERVED PHENOMENA EXPLICABLE BY ANTIGEN-ANTIBODY

REACTION IN VIVO

In almost all intergeneric graft unions it was necessary for a satisfactory growth of the scion to maintain an approximate equality between the amount of green mass (leaves and stems) of the scion and the stock in the unions. As soon as the scion predominates, it either kills the stock or injures it so that no more growth is manifested. This occurred most frequently when the stock predominated. This phenomenon was not observed very frequently, however, in the graft unions between species.

The grafts of *Nicotiana glauca* on *Capsicum pyramidale* (see table 15) grew relatively well 4-5 weeks after grafting and sometimes gave shoots 25 cm long, of which few produced even flowers. After 4-5 weeks, the

growth of the scion shoots stopped and the scions began to die. On *Capsicum pyramidale* stock on which 5 scions of *Nicotiana glauca* had died, further attempts were made to graft new scions of the same species as before, but none of these scions grew. Since the inhibition of growth of the scion shoots approximately 4-5 weeks after the first grafting, coincides with the period of greatest antibody production, the failure of subsequent grafts on that stock which had already killed their scions is undoubtedly due to the antibodies induced in the stock by the first series of graftings.

FIGURE 8.—*Nicotiana Tabacum* var. *macrophylla*. G-shoot grafted on *Datura Wrightii*. N-non grafted shoot.

When *Nicotiana Tabacum* var. *macrophylla* is grafted on *Datura Wrightii*, the scions grow relatively normally, especially if equilibrium between the mass of the scion and the stock is retained. The flowers, however, could not develop normal corollas, and the top of the calyx leaves was usually more or less destroyed (figure 8). In periods of intense sunshine and unsatisfactory water supply, all corollas, as well as the greater part of the calyx leaves are shrunk and destroyed. In cloudy weather, when there was an adequate water supply for 10 or 12 days, the scions developed almost normal, but not completely normal, flowers.

The highest degree of disturbance in corolla and calyx is accompanied by a disturbance in the cell division of the pollen mother cells. The non-grafted *Nicotiana Tabacum macrophylla* plants growing around the grafted

FIGURE 9.—N—normal chloroplasts of *Nicotiana glauca* grafted on *Capsicum pyramidale* from a distance of 2 cm from the callus. A—agglutinated chloroplasts of the same graft union in the tissue near to the callus.

ones in the same environmental conditions always showed normal cell division in the pollen mother cells and normally developed pollen grains;

FIGURE 10.—*Solanum tuberosum* grafted on *Nicotiana rustica*.

while the shoots grafted on *Datura Wrightii* showed about 25–30 percent of abortive pollen grains, the corolla being entirely destroyed. The appear-

ance of abortive pollen grains is due to the abnormality in the reduction cell division in the pollen mother cells.

Abnormal heterotypical and homeotypical cell division of the pollen mother cells with an abortion of about 50 percent of pollen grains was observed also in the scion when *Nicotiana Langsdorffii* was grafted on *Solanum nigrum*. Regular spindles were seldom observed in the heterotypical division of *Nicotiana Langsdorffii* scion. The chromosomes were irregularly spread in the pollen mother cells as in species hybrids. Irregularity in second division was observed also. In the plant from which originated

FIGURE 11.—*Solanum tuberosum* grafted on *Nicotiana Sanderae*.
T—tuber, C—callus, N—*Nicotiana Sanderae* stock.

the scions, growing in the same environmental conditions, was observed normal cell division and no abortive pollen grains. The destruction of corolla and of calyx leaves and the disturbance in the pollen mother cell division are undoubtedly due to the stock activity.

In some graft unions the mutual reactivity of scion and stock is indicated by an agglutination of the plastids on both sides of the callus. In figure 9A are shown agglutinated chloroplasts of *Nicotiana glauca* grafted on *Capsicum pyramidale*. Figure 9N shows normal chloroplasts of the same scion at a greater distance from the callus. Agglutination of chloroplasts was also observed in the unions *Solanum tuberosum* grafted on *Nicotiana*

Sanderae and *Solanum nigrum* grafted on *Nicotiana Sanderae*. The effect of agglutinin upon some of the cell elements is evident in a small area around the callus. The agglutinated zone spreads very slowly. In some cases the agglutination process results in death of the tissues.

The behavior of the starch in the graft unions is of particular interest. A very striking phenomenon was observed in the union *Solanum tuberosum* grafted on *Nicotiana rustica*. In this union, the scion of *Solanum tuberosum* formed aerial tubers (figure 10) near to the callus. The starch components produced in the scion were accumulated and dehydrated in the aerial tubers in form of starch grains. Although the branches of tobacco stock required further nutrition such as was accumulated in aerial tubers (the stock had branches with capsules in which very weak and small seeds were formed) they did not utilize the materials formed in the potato. When the top of the scion was cut just above the aerial tubers, the nutrients accumulated in the scion were not utilized by tobacco; instead, new shoots arose at the top of the tubers (figure 11). There are two possible explanations for the accumulation of starch above the callus. Either (1) the enzymes (the mobilizers of the starch) are inactivated by antibodies produced in the stock against the specific substances of the scion, so that the dehydration process is accelerated, or (2) the starch components have a specific antigenic stereochemical nature and are attacked directly by the antibodies coming from the stock, thus causing the dehydration. It is not impossible that both processes are involved.

REICHERT (1913), concerning the specificity of starch, says: "In the hemoglobin research it was shown that the hemoglobin molecule is modified in specific relationship to genus, species, etc., which may be taken to mean that the form of protoplasm that is expressed by the term erythrocyte is correspondingly stereochemically modified; with starch it has been found that the molecule is likewise changed in specific relationship to genera, species, etc., which accordingly may also be taken to mean that during synthesis the products of activity are altered in their molecular peculiarities in specific relationship to the stereochemic modifications of the forms of protoplasm which produce them."

Starch can be hydrolyzed and its derivatives again synthesized into starch grains. In these processes is there a despecification and later a specification, or are the hydrolyzed components of each particular starch specific? Further investigations are needed to answer this question.

In the unions *Nicotiana glauca* grafted on *Capsicum pyramidale*, *Nicotiana Tabacum* grafted on *Datura Wrightii*, *Nicotiana Tabacum* grafted on

Solanum Melongena, *Solanum Lycopersicum* grafted on *Nicotiana glauca*, *Nicotiana Tabacum* grafted on *Solanum Lycopersicum*, and *Solanum nigrum* grafted on *Nicotiana Sanderæ*, an abundant accumulation of starch grains was also observed above the callus. Figure 12 represents the callus union *Solanum Lycopersicum* (L) grafted on *Nicotiana glauca* (C). In some species graft union—as *Nicotiana glauca* grafted on *Nicotiana rustica*, *Nicotiana Tabacum* grafted on *Nicotiana rustica*, *Nicotiana Rusbyi* grafted

FIGURE 12.—Longitudinal section through the callus of the graft union—*Solanum Lycopersicum* grafted on *Nicotiana glauca*, stained with iodine. C—callus. *Solanum Lycopersicum* (scion) tissue contains abundance of starch, *Nicotiana glauca* tissue (stock) contains no starch near to the callus.

on *Nicotiana rustica*, etc., the stocks sometimes lost all leaves and the unions (that is, the scions) grew over 3–4 months without any great evidence of injury. These observations show that a part of the nutrients produced in the scions can be utilized by the stocks, apparently after a change when in passing the callus.

The physiological phenomena, expressed in morphological formations in the callus after an interaction between scion and stock, are in the same

way morphologically represented in the species embryos after an interaction between the mother plant and the embryo of hybrid nature. This parallelism leads to the conclusion that the antibodies produced in the mother plant are responsible for the characteristic phenomena observed in the species embryo development.

This conclusion is supported also by the precipitin reaction obtained between the extract of placenta of *Nicotiana rustica* plants on which have grown seeds of hybrid nature—*Nicotiana rustica* × *Nicotiana Langsdorffii*—and the normal extract of *Nicotiana Langsdorffii*. In 9 such tests the precipitin reaction “trace” was obtained four times, while in 9 other tests no precipitin reaction was observed between *Nicotiana Langsdorffii* normal extract and an extract of *Nicotiana rustica* placenta after selfing.

SUMMARY

1. Normal precipitins and lysins were found between many plant extracts of the Solanaceae.

2. In grafting certain species and genera of the Solanaceae, mutual induction of antibodies in scion and in stock was found.

3. The acquired immunity in such plants was tested by precipitin reactions.

4. The induced antibodies were specific in certain species.

5. Heterogenetic (non-specific) precipitins were also found.

6. The reactivity of the plants in acquiring immunity is, to a certain extent, an individual character.

7. The tested plants acquired the highest precipitin potency about between 30 and 45 days.

8. In some graft unions no precipitins were found.

9. In certain graft unions, which showed relatively high normal precipitins, a decrease of precipitins after grafting was observed.

10. No correlation between hydrogen ion concentration and precipitin reaction was found.

11. The effects of various physical and chemical agents on the antigenic and the immune extracts were observed.

12. High temperature inactivates both extracts.

13. Intensive light and optimum growth temperature increase the production of antibodies.

14. An agglutination of plastids near the callus was observed in certain graft unions.

15. In some intergeneric graft unions irregular cell divisions in the pollen mother cells were found. Abortive pollen grains were also observed.

16. Physiological interaction between scions and stocks accompanied by different morphological phenomena in the scions and stocks were observed, which are explicable by postulating antibody production.

17. Starch accumulated above the callus fails to pass down into the stock.

18. A parallelism was drawn between the interaction of scion and stock and that between mother plant and embryos of hybrid nature.

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