IMMUNOLOGIC REACTIONS WITH TOBACCO MOSAIC VIRUS.

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(Received for publication, February 25, 1929.)

Ever since Mayer (1886) demonstrated the infectious nature of tobacco mosaic disease, investigations have been carried out to determine the true nature of the etiologic agent of this malady. Many of the objections to the parasitic theory based on the resistance of the infective agent to chemicals can be met with the argument that the usual methods for the determination of these reactions in the case of known microorganisms are crude when applied to the study of filterable viruses (Ford, 1927). Studies on the purification of virus have been made which may overcome some of the difficulties (McKinney 1927; Vinson and Petre 1929; and Brewer, Kraybill and Gardner 1929). The apparently high resistance to heat exhibited by virus is no greater than that shown by bacterial spores (Duggar and Armstrong 1923). Spores of *B. subtilis* show an increased resistance to heat when tested in vegetable infusions (Williams 1928).

The properties of tobacco virus are frequently likened to those of enzymes. An apparent similarity between them may be due to the fact that they have both been studied in tissue extracts and in an impure state. Falk (1924) maintains that the properties attributed to enzymes are in reality chiefly those of the medium in which they are tested. Allard remarked fourteen years ago that: "It may at least be said that the theory of a parasitic origin for the disease more consistently accounts for all the facts at hand than any enzymic conception yet evolved. It seems not only needless but illogical to abandon a simple, direct explanation for one which leads to complexity of thought and yet fails to correlate all the facts at hand." (Allard 1915).

Various investigators have proposed different hypotheses to explain the origin of tobacco mosaic disease. Some of them express the opinion that the malady is due to purely physiologic response to unfavorable environmental conditions (Egiz 1912; Hunger 1902, 1905 a, b; and Sturgis 1899). Hunger maintains that a phytotoxin is generated spontaneously in the plant which upon transmission to normal plants proves capable of stimulating them also to the production of the phytotoxin. Beijerinck attributes the disease to a "contagium vivum fluidum" (1898), while other workers think the excessive accumulation of oxidizing enzymes is responsible for the morbid condition of the plant (Heintzel 1900, Woods 1899,

1902, and Chapman 1913). Iwanowski, although unable to cultivate a microorganism from affected plants, considers the disease as bacterial in origin (1903). Allard (1916) expresses the opinion that the causal agent is an ultramicroscopic parasite. Several investigators have described microorganisms which they believe to be closely associated with the disease (Palm 1922; Jones 1926 a, b; Link, Jones, and Taliaferro 1926; Jones 1928; and Eckerson 1926). One of these supporters of the parasitic theory of tobacco mosaic disease claims that the foreign cell inclusions, characteristically present in tobacco mosaic tissue are the etiologic factor (Palm 1922).

Olitsky's claim of having cultivated the infective agent of tobacco mosaic disease[1] *in vitro* in the absence of living host cells (1924) is as yet uncorroborated (Mulvania 1925; Purdy 1926; Goldsworthy 1926; and Smith 1928).

Mulvania (1926) injected from 1 to 3 cc. of virus-sap in one ear of a rabbit and 20 minutes later tried to recover the virus by drawing a sample of blood from the uninoculated ear. The virus was not recoverable. He also showed that when normal, fresh, rabbit blood is mixed with virus extract and allowed to stand for 24 hours, the infectivity of the virus is not destroyed but under the same conditions, a filtrate of virus extract is rendered non-infectious. No attempt to produce an antiserum to virus-sap was reported by Mulvania.

Until the recent work of Dvorak (1927), on a virus of potato mosaic disease, no one had undertaken an investigation of the antigenic properties of virus-sap. Dvorak produced antisera in rabbits to sap from healthy potato plants and sap from potato affected with mosaic disease.[2] Precipitin reactions showed that the two antisera had precipitins in common, but that they both exhibited a higher titer for the homologous than for the heterologous antigen. The explanation offered for these results is that the virus had in some way altered the antigenic property of the globulins present in the healthy potato plant.

A study of the antigenic properties of virus-sap of tobacco mosaic should throw light upon the true nature of the virus. Therefore, the experiments described in this paper, reported previously in preliminary form (Purdy 1928), were undertaken.

Method.

Preparation of Antigens.—As a source of antigens young tobacco plants of a Turkish variety were selected because of a low nicotine-content, which would minimize any toxic effect the alkaloid might have on the rabbits used for immunization. Leaves were collected from healthy tobacco plants and from plants

[1] The host plant in this case was tomato.

[2] Although the filterability of the virus of potato mosaic disease has never been proved, it is nevertheless regarded as belonging to the class of filterable viruses.

that had been infected with mosaic disease for from 10 days to 6 weeks. The particular tobacco virus used was that of Johnson's common field mosaic disease.[3] The diseased and healthy leaves were kept in two separate lots and prepared in vessels, sterilized each time after use to avoid any chance of contaminating the normal sap with virus-sap. Three medium-sized leaves were ground to a pulp in a mortar with 8.5 cc. of saline solution (0.85 per cent). The extract was then centrifugalized for about 5 minutes at a moderate speed and the supernatant fluid was used for injection. At first, the antigens were preserved with 0.5 per cent carbolic acid solution and stored in an ice-chest during the intervals between injections, but later fresh antigen was prepared for each injection to eliminate

TABLE I.

Hyperimmunization of Rabbits.

Rabbit No.	Antigen	No. of injections	Total amount of antigen injected	Period of hyper-immunization	No. of days after last injection, rabbit was bled
1	N*	6	23.0	Feb. 10–Mar. 6	6
2	N	5	16.5	Apr. 10–Apr. 23	10
3	N	6	19.0	Apr. 10–May 11	0†
4	V*	6	21.0	Feb. 10–Mar. 6	6
5	V	5	16.5	Apr. 10–Apr. 23	10
6	V	13	22.8	Apr. 10–June 7	14
7	V	7	9.2	July 25–Aug. 22	0†

* N = Normal sap.
V = Virus-sap.
† Rabbit died before bleeding.

the possibility of the proteins in the extracted sap becoming denatured upon standing.

Immunization of Rabbits.—Each rabbit received from 5 to 13 injections of antigen in the marginal ear-vein, the amount injected at one time varying from 0.5 to 5 cc. Since the rabbits showed toxic reactions when as much as 5 cc. were injected at one time, it was not deemed advisable to use a larger amount of sap extract. The usual interval between injections was 3 to 4 days. Before the fifth and subsequent injections, the rabbit was desensitized intravenously first with 0.5 cc. of antigen, otherwise severe anaphylactic symptoms were evinced. Four rabbits were injected with virus-sap and 3 with normal sap. Table I contains more details of the procedure followed in the injection of individual rabbits.

[3] The original source of the virus was mosaic material supplied by Doctor James Johnson several years ago and subsequently kept free from contamination by other strains of virus at the Boyce Thompson Institute.

Collection of Serum.—In general, samples of serum were drawn from an ear-vein for control purposes, prior to the first injection of the antigen. Thereafter, frequent samples of serum were taken from an ear-vein during the course of injections for use in various tests. From 6 to 14 days after the last injection, the animals were etherized and bled to death aseptically from the carotid artery. The antiserum was allowed to separate from the clot overnight and was pipetted off the following morning, inactivated for one-half hour at 56°C. (water-bath) and stored in sterile tubes in an ice-chest.

Testing of Serum for Antibodies.

Preliminary alexin-fixation experiments were carried out in order to determine whether or not antibodies to virus and normal sap had been produced in the injected rabbits. It was found that in the presence of the homologous antigen, a given amount of serum from the injected animals would fix alexin completely, whereas the same amount of antigen and normal rabbit serum gave no fixation, that is, complete hemolysis occurred on adding sensitized sheep cells. The usual control tubes for antigen, hemolytic system, serum, and cells were included in the tests. All showed that the experiments were properly controlled.

The findings of these preliminary tests indicated that antibodies to virus and normal sap had been produced in the injected rabbits. Alexin-fixation experiments were then planned for the titration of both antigens with antisera to virus-sap and antisera to normal sap in order to ascertain whether or not the antisera contained alexin-fixing antibodies in common.

Alexin-Fixation Experiments.

The antisera and normal sera were heated for one-half hour at 56°C. (water-bath) just prior to use. Constant amounts (0.05 cc.) of undiluted serum were placed in Wassermann tubes. Fresh guinea-pig serum was then added of a dilution that would contain 2 units of alexin in each 0.1 cc. The antigens were prepared as for injection in the rabbits and were diluted further with saline solution (0.85 per cent). Decreasing amounts of antigen-dilution were pipetted into a series of tubes. The volume of liquid in each tube was then brought up to 0.25 cc. with saline solution. The entire mixture was shaken and placed in a water-bath for one hour at 37°C. At the end of the hour of incubation, sheep cells, previously sensitized with rabbit sensitizer, were added in 0.2 cc. amounts and the tubes were returned to the water-bath for 15 minutes. In some of the experiments, the readings were made immediately after removal from the water-bath; in other experiments the readings were made after the tubes had stood in the ice-chest overnight and the cells had settled out by gravitation. In general, it was found that a satisfactory and quicker method of obtaining the readings was to cool the

tubes under running water immediately after removal from the water-bath, and then centrifugalize them at a low speed for a few moments to throw down any non-hemolyzed cells present. In most of the tests, Madsen's scale was employed for determining the percentage hemolysis occurring in any of the tubes. Using the contents of tubes showing complete hemolysis as the stock solution, dilutions with saline solution were made showing 10 to 90 per cent hemolysis, by increments of 10 per cent.

Three separate experiments were carried out in which 3 different antivirus and 3 different antinormal sera were tested individually for their ability to bind alexin in the presence of virus and normal sap. The results of these experiments showed the smallest amount of antigen used that would react with 0.05 cc. of undiluted antiserum to fix a given amount of alexin. For the sake of discussion, these minimal amounts wherever available have been brought together in Table II. The amount of antigen used is expressed in the equivalent of undiluted sap to facilitate a comparison of the results.

Analysis of the Findings.—In all of 3 experiments (Table II) both antisera fix alexin in the presence of either antigen.

In all 5 titrations (Table II) antivirus sera show a higher titer for the homologous than for the heterologous antigen.

In all 3 experiments (Table II) antivirus sera possess a higher titer for virus than the antinormal sera for the same antigen.

In 3 out of 5 titrations (Table II, Experiments I, II, and IIIa) the antinormal sera exhibit a higher titer for the homologous antigen than for the heterologous antigen. Complete results were not obtained in the other 2 titrations (Table II, Experiment IIIb and c).

In 2 out of 5 titrations (Table II, Experiments I, and IIIa) the antinormal sera have a higher titer for normal sap than the corresponding antivirus sera for the same antigen; in one instance the titer of the antinormal and antivirus sera for normal sap is the same (Table II, Experiment II); in the other 2 titrations, the incomplete results would indicate that the antinormal sera have a lower titer for normal sap than the corresponding antivirus sera (Table II, Experiment IIIb, c).

Discussion.—In interpreting the results of the alexin-fixation experiments summarized in Table II, two possibilities should be taken into consideration—the differences that may exist in both the titer of the

antisera and the concentration of the antigens. These two factors influence the results, but if properly accounted for, they do not invali-

TABLE II.

Minimal Amounts of Antigen Required for Alexin-fixation.

Experiment No.	Undiluted serum (56°C.)	Undiluted antigen	Results	
			Hemolysis	Fixation
	cc.	*cc.*		
I	AV† 4	.006 V	—	Complete
	AV 4	.02 N*	Partial	—
	AN 1	.02 V*	Partial	—
	AN 1	.002 N	—	Complete
II	AV 4	.0015 V	—	Complete
	AV 4	.008 N	—	Complete
	AN 1	.01 V	—	Complete
	AN 1	.008 N	—	Complete
IIIa	AV 4	.001 V	—	Complete
	AV 4	.03 N	—	Complete
	AN 1	.03 V	—	Complete
	AN 1	.02 N	—	Complete
IIIb	AV 6	.0006 V	—	Complete
	AV 6	.1 N	—	Complete
	AN 3	.05 V	—	Complete
	AN 3	.02 N*	75%	—
IIIc	AV 5	.0006 V	—	Complete
	AV 5	.05 N	—	Complete
	AN 2	.1 V	—	Complete
	AN 2	.02 N*	85%	—

* Minimal amount of antigen was not determined.

† AV = Antiserum to virus-sap.
AN = Antiserum to normal sap.
V = Virus-sap.
N = Normal sap.

date the conclusions that may be drawn from these results. The following discussion will demonstrate this point.

Since antivirus serum fixes alexin in the presence of normal sap, it is fair to assume that antigenic substances in the normal sap were also

present in virus-sap used for the production of the antivirus serum. Likewise, since antinormal serum binds alexin in the presence of virus-sap, the reaction may be attributed to antigenic substances in virus-sap that were also present in normal sap. Without further knowledge of the antigenic nature of normal and virus-sap, the results obtained from these experiments may be examined to advantage on the assumption that the two antigens, virus and normal sap, are identical in composition. It is then possible to compare the titer of the antisera and the concentration of the antigens from the results of a single experiment in which antivirus and antinormal sera are titrated with the same preparations of homologous and heterologous antigens. The normal antigen and antinormal serum should be used as the basis for comparing the titer of the antisera and the concentration of the antigens, since the assumption that virus-sap is identical to normal sap in composition remains to be proved.

For example, in Experiment I (Table II) comparing the reaction of the 2 antivirus and antinormal sera to normal sap, the antinormal serum is the higher titered one. By further comparison of the reactions of antinormal serum with virus and normal sap, the former antigen appears to be less than one-tenth the concentration of the latter. By a similar comparison of the titrations in Experiment II (Table II), both antisera have the same titer, but virus-sap appears to be a somewhat weaker antigen than normal sap.

In all 5 sets of titrations, antivirus sera exhibit a higher titer for the homologous than for the heterologous antigen. On the other hand, the incomplete results of Experiment IIIb (Table II) indicate that the antinormal serum may manifest a higher titer for the heterologous than for the homologous antigen, depending in this instance upon the concentration of the antigens. Also, the antinormal sera may show a lower titer for the homologous antigen than that of the corresponding antivirus sera (Table II, Experiment IIIb, c) because of the higher titer of the latter. These results demonstrate the importance of titrating the 2 antisera with both antigens. Without the information thereby gained relative to the concentration of antigen and titer of antiserum, no dependable conclusions can be drawn from the results of a titration.

A careful study of the results obtained from these experiments makes

it evident that differences in the titer of the antisera or concentration of the antigens, identical in composition, are sufficient to explain the reactions of antinormal sera with both antigens, but not to account for the reactions of antivirus sera with virus and normal sap. For instance, in Experiment I (Table II) the antivirus serum is weaker in titer for normal sap than the corresponding antinormal serum, and the normal sap seems to be more highly concentrated than the virus-sap, from a comparison of the reactions of antinormal serum with both antigens. Under these conditions and with antigens of identical composition, the antivirus serum would be expected to show a higher titer for normal than for virus-sap, but in reality the reverse is true and antivirus serum exhibits a higher titer for virus than for normal sap. The only adequate explanation for such a result is that the 2 antigens differ in composition. Virus-sap apparently contains some antigenic substance or substances either not found in normal sap, or only in small amounts. It is possible that some foreign antigenic substance is present in virus-sap not found in normal sap, or that some of the normal antigens present in healthy tobacco sap have been altered by mosaic disease, thereby changing their antigenic properties.

The 2 antisera were now tested for the presence of precipitins.

Precipitin Experiments.

Four precipitin tests were set up, using equal parts of undiluted antiserum and antigen-dilution, varying from 1:10 to 1:100,000. The antigens were prepared as for injection in rabbits but it was found necessary to centrifugalize the extracts at high speed for 15 minutes to obtain sufficiently clear antigens. The supernatant fluid was further diluted with saline solution (0.85 per cent). The serum was first pipetted into precipitin tubes and the antigen-dilution was slowly "layered" on top of the serum. After incubation for 1 hour at 37°C. (water-bath), the tubes were examined for the presence of a ring of precipitate, formed at the region of contact between serum and antigen. The contents of the tubes were then thoroughly shaken and placed in an ice-chest overnight. On the following morning a record was made of the precipitate formed in the tubes.

Analysis of the Findings.—No precipitate was formed in a dilution of antigen exceeding 1:100.

A precipitate formed in the case of both antisera in the presence of either antigen.

A very slight precipitate formed in a mixture of normal sap and normal serum when the antigen was undiluted.

The virus-sap produced a heavier, more flocculent precipitate than the normal sap with both antinormal and antivirus serum. A greater amount of precipitate was obtained in a mixture of antivirus serum and virus-sap than in the case of antinormal serum and virus-sap.

Discussion.—The fact that the antigens failed to give a precipitate in a dilution exceeding 1:100, may be due to the fact that the antigen is prepared by the extraction of whole sap high in water-content. The antigens referred to as "undiluted" are in reality considerably diluted.

The heavier precipitate characteristically formed in a mixture of antivirus serum and virus-sap strongly suggests the presence of a specific precipitin for virus-sap in the antivirus serum. In comparing the reactions of the 2 antisera with their homologous and heterologous antigens, the same objections raised to a comparison of the results in the alexin-fixation experiments should be taken into consideration. In these tests, also, the titer of the 2 antisera and the concentration of the antigens may vary. It was concluded, therefore, in the face of these difficulties that the presence of a specific precipitin for virus-sap in antivirus serum could be more readily demonstrated by precipitin-absorption experiments.

Precipitin-Absorption Tests.

After a number of preliminary tests, it was found that the most satisfactory method for the absorption of the precipitins from the serum was as follows:

The antigens were freshly prepared as for the precipitin experiments and used in a dilution of 1 part of extracted sap to 4 parts of saline solution. The antisera were absorbed by adding 1 cc. of antigen-dilution to 2.5 cc. of inactivated serum and incubating for 1 hour at 37°C. (water-bath). The precipitate formed was then removed by centrifugalization and 1 cc. of antigen-dilution was added to the clear supernatant fluid. This process was repeated until none of the tubes showed any precipitate upon removal from the water-bath. The tubes were then set in the ice-chest overnight to allow ample opportunity for a precipitate to form. If the tubes contained no precipitate on the following morning the absorption of the serum was considered complete. The absorbed serum was then tested with both antigens for the presence of precipitins, as described in the precipitin experiments. During the process of absorption, several precipitin tests were carried out with the partially absorbed sera.

Two different antivirus sera and 1 antinormal serum were used in the 3 absorption tests. Two portions each of antivirus and antinormal serum were absorbed separately with both normal and virus-sap. Control tubes of antisera were included, treated in a similar manner to the sera absorbed except that saline was added each time in the place of antigen. Other appropriate control tubes were also used.

Analysis of the Findings.—All of the precipitins to normal sap in both antisera were completely absorbed by either antigen.

After complete absorption of antivirus serum with normal sap, a heavy precipitate was formed upon the subsequent addition of virus-sap.

Discussion.—During the process of precipitin-absorption, the antinormal serum was usually absorbed completely by the first addition of antigen while the largest amount of antigen used in these tests was required by the antivirus serum for complete absorption with virus-sap. For uniformity of method, the same quantity of antigen was used in every case for the absorption of the 4 different lots of serum in a single experiment. Consequently, it was necessary to set up complete precipitin tests at various stages during the process of absorption, otherwise the results would be incomplete, for the final precipitin tests showed that some of the absorbed sera were so highly diluted that no reaction occurred in the corresponding control tubes of unabsorbed serum.

The point of most interest in these experiments has been fully demonstrated by this method of precipitin-absorption, namely, that after complete absorption of an antivirus serum with normal sap, the absorbed serum will still give a heavy precipitate with virus-sap. As stated in the discussion of the results of the alexin-fixation experiments, the specific reaction may be due either to a foreign antigenic substance present in the virus-sap, or to the formation during the course of mosaic disease of an altered plant protein.

As a logical sequence to the precipitin-absorption tests, it was decided to repeat the experiments and instead of using tobacco sap for the final precipitin tests to substitute sap from healthy and mosaic diseased hosts of the virus other than tobacco.

Precipitin Tests with Tobacco Virus from Other Hosts.—Tomato, pepper, and petunia plants, common hosts of tobacco mosaic virus, were inoculated with the

TABLE III.

Precipitin-absorption Tests with Virus from Various Hosts of Tobacco Mosaic Virus.

Tube No.	Serum	Antigen used for absorption	Undiluted absorbed serum	Antigen dilution 1:10	Results	
					After incubation for 1 hr. at 37°C. (water bath)	After standing in ice-chest overnight*
			cc.	*cc.*		
1	AV†	N tobacco	.2	.2N tobacco	No ring	0
2	"	" "	.2	.2V tobacco	Ring	+++
3	"	" "	.2	.2N tomato	No ring	0
4	"	" "	.2	.2V tomato	Ring	+++
5	"	" "	.2	.2N pepper	No ring	0
6	"	" "	.2	.2V pepper	Heavy ring	++++
7	"	" "	.2	.2N petunia	No ring	0
8	"	" "	.2	.2V petunia	Very faint ring	++
9	"	" "	.2	.2 saline	No ring	0
10	"	" "	.2 saline	.2N tobacco	" "	0
11	"	" "	" "	.2V tobacco	" "	0
12	"	" "	" "	.2N tomato	" "	0
13	"	" "	" "	.2V tomato	" "	0
14	"	" "	" "	.2N pepper	" "	0
15	"	" "	" "	.2V pepper	" "	0
16	"	" "	" "	.2N petunia	" "	0
17	"	" "	" "	.2V petunia	" "	0
18	"	Saline	Unabsorbed serum .2	.2N tobacco	No ring	++
19	"	"	"	.2N tomato	Ring	+
20	"	"	"	.2N pepper	No ring	+
21	"	"	"	.2N petunia	" "	±
22	"	"	"	.2V tobacco	" "	++
23	"	"	"	.2V tomato	Heavy ring	+++
24	"	"	"	.2V pepper	Ring	++++
25	"	"	"	.2V petunia	Ring	++
26	"	"	"	.2 saline	No ring	0

* The following symbols are used:

0 = no precipitate.
± = very slight precipitate.
+ = slight precipitate.
++ = moderate precipitate.
+++ = heavy precipitate.
++++ = very heavy precipitate.

† AV = antiserum to virus-sap.
AN = antiserum to normal sap.
V = virus-sap.
N = normal sap.

strain of virus used throughout these experiments. The identical procedure used above in the precipitin-absorption tests was followed. Four sets of experiments were carried out. The results obtained from one of these tests are recorded in Table III. A parallel series of tubes with the corresponding unabsorbed serum was included in these experiments.

Analysis of the Findings.—When antiserum to virus-sap of tobacco was completely absorbed with normal tobacco sap (Table III):

(*a*) No precipitate was formed upon adding normal sap of tobacco, tomato, pepper, or petunia plants.

(*b*) A heavy precipitate was formed upon the addition of virus-sap of tobacco, tomato, pepper, and petunia plants.

Unabsorbed antiserum to virus-sap of tobacco produced some precipitate when mixed with the normal sap of tobacco, tomato, pepper, and petunia plants.

Discussion.—Since the 4 different host plants, tobacco, tomato, pepper, and petunia used in these experiments are all members of the same family, the *Solanaceae*, the occurrence of precipitins in antiserum to virus-sap of tobacco common to the 4 plants is to be expected. The presence of antigenic substances in tobacco common to all of these different plants still leaves the possibility unaltered that the antigenic substance in virus-sap of tobacco, tomato, pepper, and petunia specific for antiserum to tobacco virus-sap, may be plant protein altered by disease. If the specific antigenic substance is foreign virus protein, for example, its antigenic property is apparently not altered by multiplication in different hosts.

In the hope of determining the presence of an antibody, specific for virus itself, a series of experiments were undertaken to test the power of inactivation of the antiserum to virus-sap on virus.

Inactivation Experiments with Virus-Sap and Antiserum.

Known amounts of antiserum were pipetted into tubes and varying amounts of freshly extracted virus-sap, diluted with saline solution (0.85 per cent), were added to the serum. The volume of liquid in each tube was made constant by the addition of saline solution. In 4 out of the 6 experiments carried out, the mixture of serum and virus-sap was held at room temperature for several hours; in the other 2 cases the mixture was kept for 1 hour at 37°C. (water-bath). Two different antisera to virus-sap, 2 to normal sap, and several samples of normal rabbit and guinea-pig serum were tested.

After the serum had remained in contact with the virus-sap for a given period, the contents of each tube were shaken thoroughly, poured into a dish and used for inoculation into young Turkish tobacco plants.

The inoculation of the plants was accomplished by scarifying 3 leaves on each plant with a sterile needle, dropping on the inoculum with a capillary pipette, and rubbing it in with a sterile cork. Every precaution was taken throughout the

TABLE IV.

Summary of Results of Inactivation of Virus-sap with Antisera and Normal Serum.

Serum used for inactivation of virus	No. of individual mixtures of serum and virus-sap tested	No. of plants inoculated	No. of plants		Per cent of healthy plants
			Diseased	Healthy	
Active AV*.....	5	23	3†	20	86
(56°C.) AV.....	8	34	8‡	26	76
(56°C.) AV + alexin.....	7	32	3§	29	90
Total.....	20	89	14	75	88
Active AN.....	2	8	7	1	12
(56°C.) AN.....	1	6	3	3	50
(56°C.) AN + alexin.....	1	6	4	2	33
Total.....	4	20	14	6	30
Active normal.....	3	13	13	0	0
(56°C.) normal.....	1	6	6	0	0
(56°C.) normal + alexin.....	2	10	8	2	20
Total.....	6	29	27	2	6
Saline.....	6	26	26	0	0

* AV = antiserum to virus-sap.

AN. = antiserum to normal sap.

† In 3 of 5 tests, complete inactivation occurred.

‡ In only 3 of 8 tests, complete inactivation occurred.

§ In 6 of 7 tests, complete inactivation took place. In the seventh instance, the corresponding amount of active serum also failed to produce complete inactivation.

entire procedure to avoid accidental infection of the plants. Records of the occurrence of mosaic were made as the disease developed, for a period of 4 weeks, when the plants were discarded.

Control tests were included in which the virus-sap was placed in contact with antiserum to normal sap, normal rabbit and guinea-pig serum, and saline solution or tap water.

In a series of experiments, the antisera to virus-sap and normal sap were drawn from the marginal ear-vein of the rabbit and allowed to stand on the clot overnight. The sera were tested on the following day in the active state, and after inactivation for one-half hour at 56°C., both with and without the addition of small amounts of fresh, normal, rabbit or guinea-pig serum (alexin).

The results of 6 separate experiments are summarized in Table IV.

Analysis of the Findings.—An examination of Table IV will show that:

Of 89 plants inoculated with virus-sap mixed *in vitro* with antiserum to virus-sap, 75 remained healthy.

The 26 corresponding control plants inoculated with the same amount of virus-sap in saline solution or tap water developed 100 per cent mosaic disease.

Of 20 plants inoculated with virus-sap mixed *in vitro* with antiserum to normal tobacco sap, only 6 remained healthy.

Twenty-seven of 29 plants inoculated with virus-sap mixed *in vitro* with normal rabbit or guinea-pig serum developed mosaic disease.

Discussion.—In respect to the power of inactivation of virus-sap exhibited by the antiserum to virus-sap, in comparison to that shown by antiserum to normal sap, and normal rabbit or guinea-pig serum, the results are so significant as to require no comment.

Several hundred plants were inoculated in these experiments, but since the concentration of virus varies with every preparation of virus-sap, the amount of antiserum required for complete inactivation cannot be determined in advance. The results of experiments in which a considerable excess of virus-sap was used are not reported in this paper. There is also some evidence to show that an excess of antiserum to virus-sap may inhibit an inactivation of the virus.

The results would seem to indicate further that antiserum to virus-sap, when heated for one-half hour at 56°C. (water-bath) loses some of its power to inactivate virus-sap (Table IV). The original strength of the antiserum can apparently be fully restored by the addition of rabbit or guinea-pig alexin. This behavior of the antiserum to virus-sap suggests that a lytic antibody for virus may be present in the antiserum. The importance of such a finding necessitates further efforts to corroborate these results.

Preliminary experiments indicate that the antiserum to virus-sap

of tobacco is also capable of inactivating virus-sap of tomato, pepper, and petunia plants.

SUMMARY.

1. Antisera were produced, separately, in rabbits to normal sap from healthy Turkish tobacco plants and to irus-sap from tobacco plants, affected with mosaic disease.

2. The immunologic reactions of the antisera were studied by means of:

(*a*) Alexin-fixation tests.

(*b*) Precipitation experiments, including: Precipitin-absorption tests with the same tobacco virus multiplied in tobacco, tomato, pepper, and petunia plants.

(*c*) Experiments with the inactivation properties of both antisera and normal serum on virus-sap.

3. From the results obtained from the above experiments, the following conclusions were drawn:

(*a*) Normal-tobacco-sap and virus-tobacco-sap possess antigenic substances in common.

(*b*) Normal sap and virus-sap of tomato, pepper, and petunia plants contain antigenic substances in common with normal sap of tobacco.

(*c*) Virus-saps of tomato, pepper, and petunia plants, have antigenic substances in common with virus-sap of tobacco, that are either not present in the normal tobacco sap or present only in small amounts.

(*d*) The two antisera possess alexin-fixing antibodies and precipitins in common.

(*e*) All of the precipitins to normal tobacco sap may be removed from either antiserum by absorption with virus-sap of tobacco.

(*f*) Specific precipitins for virus-sap of tobacco, tomato, pepper, and petunia are present in the antiserum to tobacco virus-sap and cannot be removed by complete absorption with normal sap of tobacco.

(*g*) Antiserum to virus-sap of tobacco, when used in an appropriate amount, has the power of completely inactivating virus-sap.

A corresponding quantity of antiserum to normal tobacco sap, or normal rabbit or guinea-pig serum, does not exhibit the same preventive action on virus-sap.

(*h*) There is some evidence that a specific antibody to virus-sap, lytic in nature, is present in the homologous antiserum.

In closing, the writer gratefully acknowledges the valuable criticism and suggestions offered by Doctor F. P. Gay and Doctor L. O. Kunkel in the course of these experiments.

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