TRANSMITTANCE OF TUMOR-INDUCING ABILITY TO AVIRULENT CROWN-GALL AND RELATED BACTERIA[1]

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The relation of bacterial virulence and avirulence to crown-gall tumor induction under otherwise suitable conditions of host susceptibility, temperature, etc., is one of the more interesting aspects of the "crown gall" problem. There is little doubt that elucidation of this problem will aid in understanding the mechanisms whereby virulent bacteria affect host cells. This paper deals with experiments designed to determine whether virulence, as determined by ability to induce tumor formation, can be transmitted to nonvirulent species in the genus *Agrobacterium* and other bacteria.

MATERIAL AND METHODS

The organisms used in this study were: *Agrobacterium tumefaciens*, strain S5-6, which is highly virulent for tomato and *Bryophyllum* but avirulent for bean (Department of Botany, University of Chicago); *A. tumefaciens*, strain NAv-1, which is an avirulent isolate (A. J. Riker, Department of Plant Pathology, University of Wisconsin); *A. tumefaciens*, strain IIBNV6, which is an avirulent isolate (A. C. Braun, Rockefeller Institute for Medical Research, New York); *Agrobacterium radiobacter*, strain RV-3, an avirulent species (A. J. Riker, University of Wisconsin); *Agrobacterium rubi*, which is highly virulent for bean but avirulent for tomato and *Bryophyllum* (L. C. Coleman, Dominion Laboratory, Saanacht, British Columbia); *Rhizobium leguminosarum* (Department of Bacteriology, University of Chicago); and *Escherichia coli*, a wild type (Department of Bacteriology, University of Chicago).

A single synthetic medium was used to culture all organisms (McIntire *et al.*, 1940). Hoaglands A-Z solution at 1 ml per L supplied ionic micronutrients (McIntire *et al.*, 1941); and biotin, pantothenic acid, and nicotinic acid were each added at 0.02 μM per L (Starr, 1946). Stock cultures and subcultures were maintained on potato glucose agar. Tomato (var. Bonny Best), *Bryophyllum*, and broad bean (var. English Windsor) were used as test plants in all experiments. Tomato and bean seeds were grown in garden loam on a greenhouse bench. *Bryophyllum* plants were propagated vegetatively from leaves of a single clone. All plants were inoculated by puncturing the stem with a trident-needle set previously dipped into a liquid culture of the appropriate bacteria. Observations were begun immediately after inoculation and continued for 30 days at which time resulting tumors were graded on an arbitrary scale relative to the size of tumors formed following inoculation with virulent organisms (either S5-6 or *A. rubi*). Most experiments were repeated at least once.

EXPERIMENTAL RESULTS

Transmittance by means of extracts of crown-gall tumor tissues. Sterile extracts of 6 month old bacteria-containing tumor tissue of *Bryophyllum* stem or 1 month old bacteria-containing tumor tissue of tomato stem were prepared by homogenizing the tissues in the cold with equal weights (w/v) of synthetic medium containing 0.01 M citrate. The homogenates were strained through cheesecloth, passed through a medium pore fritted glass filter, and centrifuged at 8,000 × G for 30 min at 4 C. These cell-free extracts were sterilized by filtration through a Selas no. 02 porcelain filter candle. Identical procedures were used to prepare extracts of normal stem tissues of these plants. Aliquot portions (2.5 ml) of the extract were added to 2.5 ml of sterile synthetic medium contained in 50 ml Erlenmeyer flasks. Control flasks, without additions of tissue extract, also were prepared. All flasks were inoculated with 0.1 ml of the appropriate bacteria and

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incubated for 3 days at 26 C. The cultures each were inoculated into stems of young tomato and *Bryophyllum* plants. Subcultures of all treated bacteria and their controls were made onto potato glucose agar and then transferred to liquid media prior to inoculation of tomato plants. Thirty days after inoculation resulting tumors were graded on the basis of their relative size. Isolations of bacteria from these tumors induced by both virulent and altered avirulent bacteria were made on Patel's differential medium (Patel, 1926). The isolated bacteria were transferred to potato glucose agar and reinoculated into tomato plants from synthetic medium.

Additions of extracts of normal stem or of crown-gall tissues to media had no effect on the virulence of *Agrobacterium tumefaciens*, strain S5-6 (table 1 and figure 1). Extracts of normal tissues had no influence on the virulence or avirulence of any test organism. Extracts of sterile crown-gall tissue of tobacco had no effect on avirulent bacteria. Sterile extracts of tumorous or normal tissues were without effect on the plants. However, extracts of both *Bryophyllum* and tomato tumor tissue altered strain NAv-1 into a culture highly virulent for both tomato and *Bryophyllum* while moderate virulence was transmitted to strain IIBNV6 by both extracts. Extracts of tomato crown-gall tissue were more effective than those of *Bryophyllum* in transmitting virulence to both *A. radiobacter* and *A. rubi.* Subcultures, prepared from bacteria grown in extract-containing media and subsequently inoculated, induced somewhat larger tumors than did the bacteria inoculated directly into plants from the initial culture. Bacteria isolated from tumors induced by altered bacteria induced larger tumors when reinoculated into tomato plants. These tumors induced by inoculation of subcultures of altered strains and species or by bacteria isolated from tumors were evident macroscopically several days sooner than tumors in-

TABLE 1

Effect of control medium and of media containing extracts of normal and crown-gall tissues of tomato on virulence of virulent and avirulent Agrobacterium

TEST ORGANISM	MEDIUM	INITIAL CULTURES				SUBCULTURES		ISOLATES FROM TOMATO TUMORS	
		In tomato		In *Bryophyllum*		In tomato		In tomato	
		Days*	Tumor†	Days	Tumor	Days	Tumor	Days	Tumor
Agrobacterium tumefaciens, strain S5-6	Control	5	4+	8	4+	6	4+		
	Normal	5	4+	8	4+	6	4+		
	Crown-gall	5	4+	8	4+	6	4+	4	4+
Agrobacterium tumefaciens, strain NAv-1	Control		0		0		0		
	Normal		0		0		0		
	Crown-gall	8	3+	11	3+	6	3+	4	3+
Agrobacterium tumefaciens, strain IIBNV6	Control		0		0		0		
	Normal		0		0		0		
	Crown-gall	8	2+	11	2+	6	3+	4	3+
Agrobacterium radiobacter	Control		0		0		0		
	Normal		0		0		0		
	Crown-gall	11	2+	14	2+	6	2+	4	3+
Agrobacterium rubi	Control		0		0		0		
	Normal		0		0		0		
	Crown-gall	11	2+	14	2+	6	2+	4	3+

* Days from inoculation to first gross swelling.

† Tumors graded 30 days after inoculation relative to size of tumor induced by virulent crown-gall bacteria.

duced by the primary altered culture. Thus, altered avirulent bacteria appear to be able to compete with their unaltered forms *in vitro* or *in vivo*.

Transmittance by means of a bactrin of virulent crown-gall bacteria. A bactrin of virulent crown-gall bacteria was prepared by washing a 24 hr shake culture of strain S5-6 several times with fresh synthetic medium and heating a heavy suspension of these bacteria for 45 min at 60 C. Transfers to fresh media, attempted isolations, and inoculations into plants all demonstrated that no viable cells survived this treatment. Equal volumes of bactrin and fresh medium were mixed and inoculated with 0.1 ml of a culture of the appropriate test organism. Control cultures without bactrin also were prepared. After 3 days' incubation at 26 C the bacteria were inoculated into tomato and *Bryophyllum* plants. Table 2 shows that all tested avirulent bacteria were altered into virulent ones. Strain NAv-1 of *A. tumefaciens* and *A. rubi* were rendered more virulent than were *A. tumefaciens*, strain IIBNV6 or *R. leguminosarum*.

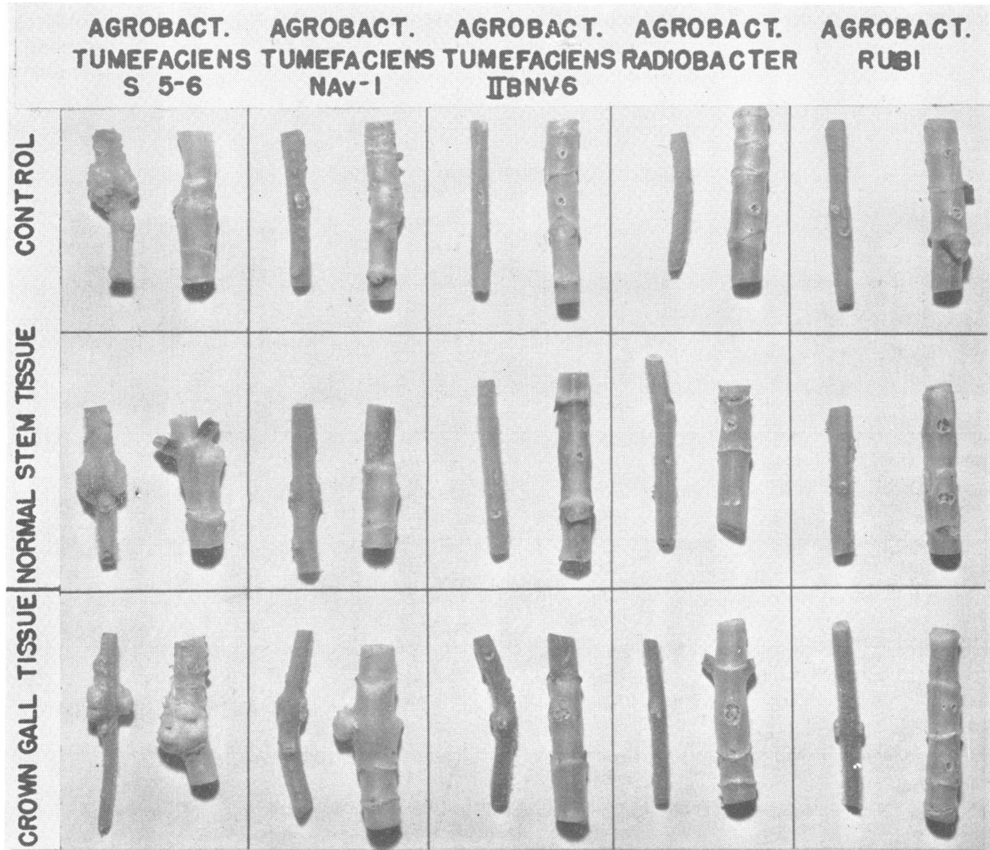

Figure 1. Effect of control medium and of media containing extracts of normal or crown-gall tissues of *Bryophyllum* on the virulence of virulent and avirulent *Agrobacterium* cultures. Tomato plants on left, *Bryophyllum* plants on right. Photographed 30 days after inoculation.

Transmittance by means of a filterable metabolic product of virulent crown-gall bacteria. Following procedures used for the study of filterable transmitting agents (Davis, 1950; Zinder and Lederberg, 1952), a U tube with a pyrex "UF" fritted disc fused into the bottom of the U was employed throughout these experiments. Integrity of the filter disc was demonstrated experimentally several times. Medium in one compartment of U tube was inoculated with a virulent culture, and medium in the other compartment, with an avirulent one. The culture media were forced

TABLE 2

Effect of growing avirulent bacteria in media containing a bactrin of virulent crown-gall bacteria

TEST ORGANISM	MEDIA PLUS BACTRIN		MEDIA	
	Days*	Tumor†	Days	Tumor
Agrobacterium tumefaciens, strain S5-6	7	4+	7	4+
Agrobacterium tumefaciens, strain NAv-1	7	3+		0
Agrobacterium tumefaciens, strain IIBNV6	8	2+		0
Agrobacterium radiobacter	8	2+		0
Agrobacterium rubi	7	3+		0
Rhizobium leguminosarum	10	1+		0

* Days from inoculation to first gross swelling.
† Tumors graded 30 days after inoculation relative to size of tumor induced by virulent crown-gall bacteria.

Table 3 and figure 2 give the results of these experiments. With the exception of *E. coli*, used as a representative of an unrelated gram negative rod, all treated avirulent bacteria were altered into virulent ones. Untreated avirulent control cultures did not induce any tumor formation following inoculation. Of interest is the finding that strain NAv-1 of *A. tumefaciens* can be altered into a culture possessing the virulence of either *A. rubi* or strain S5-6 of *A. tumefaciens* depending on the virulent culture used as the source of the transmitting agent. Further, altered strain NAv-1 can act as a source of the filterable transmitting agent when tested against its avirulent form. This finding, together with the observed virulence of subcultured altered bacteria, conclusively demonstrates that the alterations were permanent. It is of special interest that *A. tumefaciens*, strain S5-6, and *A. rubi* could be made virulent for bean and tomato, respectively, without modification of virulence toward their primary host.

TABLE 3

Effect of a filterable metabolic product of virulent crown-gall bacteria on the virulence of avirulent bacteria

TEST ORGANISM		VIRULENT TEST STRAIN		AVIRULENT TEST STRAIN		SUBCULTURES FROM INITIAL CULTURE OF AVIRULENT STRAIN	
Virulent	Avirulent	Days*	Tumor†	Days	Tumor	Days	Tumor
S5-6	NAv-1	6	4+	7	3+	6	3+
S5-6	IIBNV6	5	4+	7	2+	6	3+
S5-6	*Agrobacterium radiobacter*	6	4+	6	2+	6	3+
S5-6	*Rhizobium leguminosarum*	6	4+	10	1+	8	2+
S5-6	*Escherichia coli*	6	4+		0		0
S5-6	*Agrobacterium rubi*	5	4+	6	2+	5	2+
Agrobacterium rubi‡	S5-6	4	4+	8	2+		
Agrobacterium rubi‡	NAv-1	6	4+	13	3+		
NAv-1§	NAv-1	5	3+	5	3+		

* Days from inoculation to first gross swelling.
† Tumors graded 30 days after inoculation relative to size of tumor induced by virulent crown-gall bacteria.
‡ Broad bean as test plant, all other tests on tomato plants.
§ NAv-1 used as virulent strain was made virulent by treatment with metabolic products of S5-6.

alternately into the opposite compartment with positive oxygen pressure. At least 5 complete fluid cycles were made in a 6 hr incubation period. Virulent and initially avirulent cultures each were inoculated into test plants at the end of the incubation period. Repeated transfers of the subcultures of all tested bacteria were made prior to their inoculation into plants.

To determine whether the filterable transmitting agent was a filter passing form of the crown-gall bacteria, i.e., an "L form" (Klieneberger-Nobel, 1951), the presumably sterile medium which was exchanged 8 to 10 times with medium of a culture of *A. tumefaciens* (S5-6) was inoculated into tomato plants and also subcultured on potato glucose agar and synthetic medium. No

tumor formation was noted in the test plants (figure 2), nor were any visible bacteria isolated after extended incubation of the media. The sterile, exchanged fluid, however, retained the ability to transmit the property of virulence to an avirulent strain (IIBNV6) of *A. tumefaciens* at the end of the cycling period.

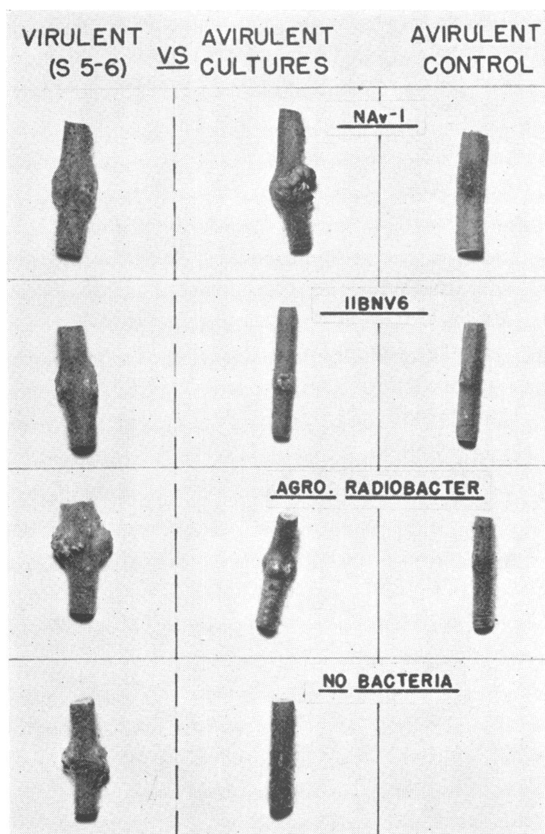

Figure 2. Representative experiments on the effect of a filterable metabolic product of virulent crown-gall bacteria on the virulence of avirulent *Agrobacterium*. Photographed 30 days after inoculation.

The time relations of transmittance of virulence by the filterable agent from strain S5-6 were examined by withdrawing aliquot portions of an exchanged culture of strain IIBNV6 at the end of each complete flushing cycle and inoculating this culture into tomato plants. Table 4 gives the average diameters of tumor formed on 10 plants inoculated with cells taken at each cycle interval. It is seen that the relative effectiveness of transmittance increased with increasing number of cycles.

In an effort to determine the chemical nature of the filterable transmitting agent, sterile culture fluid exchanged with the medium from a culture of strain S5-6 was divided into several portions. To these were added $MnCl_2$, 0.01 M (final conc), or protamine sulfate, 1 mg per ml. Other portions

TABLE 4

Time relations of transmittance of virulence to an avirulent strain by a filterable metabolic product of virulent crown-gall bacteria

NO. FLUSHING CYCLES	TOTAL CONTACT TIME OF CELLS AND MEDIA	DAYS FROM INOCULATION TO TUMOR APPEARANCE	TUMOR DIAMETER
	min		*cm*
1	17	8	0.61
2	65	6	0.67
3	138	5	0.81
4	262	4	0.96
5	357	4	1.05

TABLE 5

Inactivation of filterable transmitting agent from virulent crown-gall bacteria with heat and nucleic acid precipitants

TEST ORGANISM	GROWTH MEDIUM	NONE		$MnCl_2$ (0.01 M)		PROTAMINE (1 mg/ml)		100° C FOR 10 MIN	
		Days*	Tumor†	Days	Tumor	Days	Tumor	Days	Tumor
IIBNV6	Fresh		0		0		0		0
IIBNV6	S5-6	6	2+		0		0		0
S5-6	Fresh	5	4+	5	4+	5	4+	5	4+

* Days from inoculation to first gross swelling.

† Tumors graded 30 days after inoculation relative to size of tumor induced by virulent crown-gall bacteria.

were heated to 100 C for 10 min or were left untreated. Each portion then was inoculated with 0.1 ml of a culture of avirulent strain IIBNV6 and incubated 3 days at 26 C. Identical procedures were carried out with fresh medium inoculated with either strain S5-6 or avirulent strain IIBNV6. Heating, $MnCl_2$, and protamine each inactivated the filterable agent, but had no effect on virulence of strain S5-6. *A. tumefaciens* (strain IIBNV6) acquired the property of

virulence in untreated culture fluid of strain S5-6 but did not acquire virulence in fresh medium (table 5). Since protamine and $MnCl_2$ have been shown to precipitate nucleic acid without precipitating protein (Kaufman *et al.*, 1951), and since heating at 100 C inactivates nucleic acid, it is probable that the filterable transmitting agent is nucleic acid and not protein.

Transmittance by means of the desoxyribonucleic acid of virulent crown-gall bacteria. Cells of *A. tumefaciens*, strain S5-6, grown in shake cultures for 36 hr in 6 L of synthetic medium were collected in a Sharples centrifuge and frozen at −20 C. Thawed cells were mixed with alumina granules to 30 per cent of the weight of bacteria, the resultant paste moistened with citrate buffer, pH 6.8, to a final conc of 0.005 M and ground 2 to 4 hr in a Fisher mortar grinder at 4 C. Bacterial protoplasm was collected in a Spinco ultracentrifuge and precipitated with 3 volumes of chilled alcohol. Desoxyribonucleic acid was isolated and purified by the method of Chargaff and Zamenhof (1948). The desoxyribonucleic acid was over 90 per cent pure; no ribonucleic acid was detectable.

Cultures of virulent and avirulent bacteria, each containing 1.0 mg desoxyribonucleic acid per 0.7 ml synthetic medium, were incubated 3 days and the bacteria then inoculated into tomato plants. Control cultures of all tested bacteria were set up: (a) without desoxyribonucleic acid, (b) with desoxyribonuclease-treated desoxyribonucleic acid (Tsuboi, 1950), and (c) with the same conc of purified sperm desoxyribonucleic acid. As usual, subcultures of all cultures were made and subsequently inoculated into tomato plants. Desoxyribonucleic acid from the cells of strain S5-6 was without effect on tomato plants.

Table 6 and figure 3 summarize the results of these experiments. With the exception of the initially virulent strain S5-6, no tumor-inducing ability was observed in these bacteria grown: (a) in synthetic medium alone, (b) in media containing the desoxyribonuclease-treated desoxyribonucleic acid, or (c) in media containing sperm desoxyribonucleic acid. All avirulent organisms, however, were altered permanently into virulent, tumor-inducing bacteria when grown in the presence of the desoxyribonucleic acid from virulent crown-gall bacteria.

TABLE 6

Effect of growing virulent and avirulent bacteria in media containing the desoxyribonucleic acid of virulent crown-gall bacteria

TEST ORGANISM	CONTROL MEDIA ONLY		DESOXYRIBONUCLEIC ACID FROM S5-6		SUBCULTURES OF DESOXYRIBONUCLEIC ACID TREATED BACTERIA		DESOXYRIBONUCLEASE TREATED S5-6 DESOXYRIBONUCLEIC ACID		COMMERCIAL SPERM DESOXYRIBONUCLEIC ACID	
	Days*	Tumor†	Days	Tumor	Days	Tumor	Days	Tumor	Days	Tumor
S5-6	5	4+	5	4+	5	4+	5	4+	5	4+
NAv-1		0	5	3+	5	3+		0		0
IIBNV6		0	5	2+	5	2+		0		0
Agrobacterium radiobacter		0	5	2+	5	2+		0		0
Agrobacterium rubi		0	5	3+	5	2+		0		0

* Days from inoculation to first gross swelling.

† Tumors graded 30 days after inoculation relative to size of tumor induced by virulent crown-gall bacteria.

Cultural differentiation of A. tumefaciens and A. radiobacter. Several differential media have been used to distinguish *A. tumefaciens* and *A. radiobacter*. These include mannitol-aniline blue agar, mannitol-congo red agar (Hendrickson *et al.*, 1934), and glycerophosphate agar (Sagen *et al.*, 1929). Altered and unaltered strains of *A. tumefaciens* and *A. radiobacter* obtained by several of the above techniques were grown in each of these media. Only the glycerophosphate agar gave results of diagnostic value. Altered and unaltered cultures of *A. radiobacter* gave positive results (white precipitate and brown halo) while cultures of *A. tumefaciens* did not. It appears that acquisition of the property of virulence by

Figure 3. Transmittance of virulence to avirulent *Agrobacterium* by means of polymerized desoxyribonucleic acid isolated from the cells of virulent crown-gall bacteria. Photographed 30 days after inoculation.

A. radiobacter from *A. tumefaciens* does not alter one diagnostic cultural characteristic of the former. Further, *A. tumefaciens* did not acquire this cultural character from the cultural fluids of *A. radiobacter*.

DISCUSSION

The property of virulence, as determined by the capacity to induce crown-gall tumor formation in susceptible plants, can be transmitted easily to avirulent bacteria of three species of *Agrobacterium*, less effectively to a strain of *R. leguminosarum*, and not at all to a strain of *E. coli*. Coleman and Reid (1949), using mainly serological criteria, reported interconversion of *A. radiobacter* and *A. tumefaciens* and Manil (*personal communication*) obtained transfer of root nodule-inducing properties from rhizobia to *A. radiobacter*. With the exception of *E. coli*, the bacteria under consideration are related very closely morphologically (Brown and Leonard, 1932; Hofer, 1941), serologically (Stapp, 1927; Coleman and Reid, 1945), and biochemically (Riker *et al.*, 1930, 1946). Conn (1942) placed them all in the same family (*Rhizobiaceae*). This indicates that very similar genetic complements and close taxonomic interrelationships must be present in organisms which are positively disposed to the induced acquisition of a new character. This would explain the decreased effectiveness of altered rhizobia and the failure of *E. coli* to induce tumor formation following transmittance experiments.

Inoculations of bacteria isolated from the tumors induced by altered bacteria, or of those bacteria subcultured several times prior to their inoculation, induced tumors which were larger and appeared sooner than tumors induced by altered bacteria taken at the end of an initial transmittance experiment. Since virulence can neither be attained nor accentuated by passage through susceptible hosts or by successive *in vitro* transfers (van Lanen *et al.*, 1952), it can be inferred that the number of altered individual bacteria present at the end of a transmittance experiment was initially small but subsequently increased *in vivo* or *in vitro* relative to the number of unaltered bacteria in a total inoculum.

We have demonstrated that the effective transmitting agent for virulence is nucleic acid,

specifically, highly polymerized desoxyribonucleic acid. Two generalized modes of action of this nucleic acid may be postulated. First, the transmitting agent is itself an hereditary unit which enters the unaltered cell and functions as a part of the genetic complement of the altered cell. Second, transmitting agents may act to alter existing hereditary units or to evoke the formation of new hereditary units, these units then forming part of the genetic complement.

It has been suggested that a virus is the transmitting agent. If true, either of the above postulated modes of action is possible. The virus hypothesis has been discussed by Ephrussi-Taylor (1951) who concluded that if transforming agent is a virus, it is a most unique one. Klein and Klein (*unpublished data*) unsuccessfully tried to establish the presence of a lysogenic virus acting as the factor for crown-gall virulence. Zinder and Lederberg (1952) have shown that genetic exchange in species in the genus *Salmonella* is mediated by a filterable agent and have suggested that this agent is associated with bacteriophage. Our results obtained with the same technique differ in several important respects from those of Zinder and Lederberg. Contrary to their findings, the transmitted agent did not have to be evoked in the donor organism by abnormal conditions, transmittance was not unilateral, and positive tests for nucleic acid were obtained. These findings tend to eliminate a virus as the transmitting agent for virulence.

Assuming that transmittance involves the addition of an organized, functional entity to a bacterium deficient for that character (Ephrussi-Taylor, 1951; Zinder and Lederberg, 1952), our results suggest that virulence is genetically controlled. If true, the ability of avirulent forms of *A. tumefaciens*, *A. radiobacter*, and *R. leguminosarum* to acquire virulence is conditioned by the absence of the necessary genetic factors in the avirulent cell. The abilities of *A. rubi* and *A. tumefaciens* to acquire the host range of the other without loss of activity toward their primary hosts imply that specific host virulence is controlled by multiple genetic factors. Factors controlling one host range do not prevent the acquisition of other factors influencing virulence toward a different host. It can be assumed that acquisition of virulence occurs independently of other genetic factors concerned with growth and other characteristics. The retention of a specific cultural characteristic of *A. radiobacter* by its altered virulent form bears out this assumption.

The above hypothesis of the acquisition of virulence can, just as satisfactorily, be modified to fit into the second generalized mode of action of transmitting agents. If we assume that transmitting agents alter or evoke the formation of genetic units, they act as entities which induce directed mutations of preexisting hereditary units or direct the formation of new units. No experimental evidence is available to distinguish among these possibilities.

Within the past year, two reviews appeared on bacterial transformation reactions. Austrian (1952) defined transformation as an hereditary alteration in a susceptible cell resulting from its acquisition, by other than sexual means, of a genetically active unit directing the change. Ephrussi-Taylor (1951) stated that this unit must be a nucleic acid. Both agree that the change must be demonstrable in the progeny of the altered cells. Using the above definition, acquisition of the property of specific tumor-inducing ability by avirulent bacteria may be considered a transformation reaction.

Finally, it may be asked what relation these results have to the alteration of normal plant cells into primary crown-gall tumor cells. Two major hypotheses can account for the role of transmitting or transforming agents in the acquisition by the bacteria of the property of virulence. First, the agent is itself the "tumor-inducing principle" (Braun, 1947) and after duplication in the affected bacterium can be transferred to cells of the host. As indicated above, the possibility of a virus being the transforming agent is not too attractive. Braun (1952) discussed the possibility that a virus is the "tumor-inducing principle" and provisionally rejected it. The second hypothesis suggests that "tumor-inducing principle" is a metabolic product of virulent crown-gall bacteria. As such, its production is controlled by one or more genetic factors which can be transmitted to or evoked in deficient forms by appropriate methods. Our results suggest that altered bacteria acquire the ability to produce the "principle" and, as a result, are capable, in cooperation with other bacterial products (Klein and Link, 1952) and those etiological factors and agents evoked by the host plant, of inducing crown-gall tumor formation.

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SUMMARY

Extracts of bacteria-containing crown-gall tumor tissue, a bactrin of virulent crown-gall bacteria, nucleic acid produced by crown-gall bacteria, and desoxyribonucleic acid isolated from crown-gall bacteria are each capable of transmitting the property of specific host virulence to avirulent strains of *Agrobacterium tumefaciens*, and to *A. rubi*, *A. radiobacter*, and *Rhizobium leguminosarum*. The property of virulence, once acquired, appears to be genetically fixed. The results are discussed in their relation to transformation reactions and to the induction of crown-gall tumors in plants.

REFERENCES

AUSTRIAN, R. 1952 Bacterial transformation reactions. Bact. Revs., **16**, 31–49.

BRAUN, A. C. 1947 Thermal studies on the factors responsible for tumor initiation in crown gall. Am. J. Botany, **35**, 511–519.

BRAUN, A. C. 1952 The crown-gall disease. Ann. N. Y. Acad. Sci., **54**, 1153–1161.

BROWN, NELLIE A., AND LEONARD, L. T. 1932 Is *Bacterium tumefaciens* a mutant or one of the pleomorphic forms of *Bacillus radiobacter*? Phytopathology, **22**, 5.

CHARGAFF, E., AND ZAMENHOF, S. 1948 The isolation of highly polymerized desoxyribonucleic acid from yeast cells. J. Biol. Chem., **173**, 327–340.

COLEMAN, MADELINE F., AND REID, J. J. 1945 A serological study of strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the "M" and "S" phases. J. Bact., **49**, 187–192.

COLEMAN, MADELINE F., AND REID, J. J. 1949 The conversion of strains of *Alcaligenes radiobacter* and *Phytomonas tumefaciens* in the "S" phase to the "M" phase of the heterologous species. Phytopathology, **39**, 182–190.

CONN, H. J. 1942 Validity of the genus *Alcaligenes*. J. Bact., **44**, 354–360.

DAVIS, B. D. 1950 Non-filterability of the agents of genetic recombination in *Escherichia coli*. J. Bact., **60**, 507–508.

EPHRUSSI-TAYLOR, HARRIET 1951 Genetic aspects of transformations in pneumococci. Cold Spring Harbor Symposia Quant. Biol., **16**, 445–456.

HENDRICKSON, A. A., BALDWIN, I. L., AND RIKER, A. J. 1934 Studies on certain physiological characters of *Phytomonas tumefaciens*, *Phytomonas rhizogenes*, and *Bacillus radiobacter*. II. J. Bact., **28**, 597–618.

HOFER, A. W. 1941 Characterization of *Bacterium radiobacter*. J. Bact., **41**, 193–224.

KAUFMAN, S., KORKES, S., AND DEL CAMPILLO, ALICE 1951 Biosynthesis of dicarboxylic acids by carbon dioxide fixation. V. Further study of the "malic" enzyme of *Lactobacillus arabinosus*. J. Biol. Chem., **192**, 301–312.

KLEIN, R. M., AND LINK, G. K. K. 1952 Auxin as a promoting agent in the transformation of normal to crown-gall tumor cells. Proc. Natl. Acad. Sci. U. S., **38**, 1066–1072.

KLIENEBERGER-NOBEL, EMMA 1951 Filterable forms of bacteria. Bact. Revs., **15**, 77–103.

MCINTIRE, F. C., PETERSON, W. H., AND RIKER, A. J. 1940 Factors affecting the carbon metabolism of the crown-gall organism. J. Agr. Research, **61**, 313–329.

MCINTIRE, F. C. RIKER, A. J., AND PETERSON, W. H. 1941 The role of certain vitamins and metallic elements in the nutrition of the crown-gall organism. J. Bact., **42**, 1–13.

PATEL, M. K. 1926 An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. Phytopathology, **16**, 577–581.

RIKER, A. J., SPOERL, E., AND GUTSCHE, ALICE E. 1946 Some comparisons of bacterial plant galls and of their causal agents. Botan. Revs., **12**, 57–82.

RIKER, A. J., BANFIELD, W. M., WRIGHT, W. H., KEITT, G. W., AND SAGEN, H. E. 1930 Studies on infectious hairy root of nursery apple trees. J. Agr. Research, **41**, 507–540.

SAGEN, H. E., WRIGHT, W. H., AND RIKER, A. J. 1929 The cultural differentiation of *Bacillus radiobacter* and closely related organisms. J. Bact., **17**, 22–23.

STAPP, C. 1927 Der bakterielle pflanzenkrebs und seine beziehungen zum tierschen und menlichen krebs. Ber. deut. botan. Ges., **45**, 480–504.

STARR, M. P. 1946 The nutrition of phytopathogenic bacteria. II. The genus *Agrobacterium*. J. Bact., **52**, 187–194.

TSUBOI, K. K. 1950 Mouse liver nucleic acids. II. Ultra-violet absorption studies. Biochim. et Biophys. Acta, **6**, 202–209

VAN LANEN, J. M., BALDWIN, I. L., AND RIKER, A. J. 1952 Attenuation of crown gall bacteria by cultivation in media containing glycine. J. Bact., **63**, 715–721.

ZINDER, N. D., AND LEDERBERG, J. 1952 Genetic exchange in *Salmonella*. J. Bact., **64**, 679–699.