Summary. Repeated daily oral administrations of tranylcypromine (trans-2-phenylcyclopropylamine) (5 mg/kg, b.i.d.) or of iproniazid (1-isonicotinyl-2-isopropyl hydrazine) (50 and 100 mg/kg, b.i.d.) to male albino rats, Wistar strain, for periods of from 3 to 10 days resulted in cumulative increases in brain serotonin concentration of from 2 to 4 times that produced by a single administration of the drug. The increase in norepinephrine concentration was not appreciably different from that following a single administration of the drug. No significant or consistent effect upon the spontaneous motor activity of the rats was observed.

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Relationship Between Hog Cholera Virus and Virus Diarrhea Virus of Cattle.* (27199)

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Use of a cowpox virus vaccine to protect people against smallpox virus by Jenner(1) was the first example of using one living virus to prevent illness caused by another. Since then many vaccines for man and for animals have been developed, but always by use of homologous virus that has been either inactivated or altered in virulence. Later virologists have considered cowpox virus to be a variant type of smallpox virus; the 2 have been reported to be immunologically related because serum from vaccinated individuals neutralized smallpox virus and serum of alastrim patients neutralized the viruses of vaccinia, of cowpox and of variola(2,3).

The idea of using different viruses as Jenner did has not been generally explored. Recent studies have shown that animals can be protected by viruses immunologically distinct, not by producing measurable neutralizing antibodies, but by inducing neutralizing antibodies to form more quickly (secondary response), thereby giving protection. Because this protection has no direct immunological basis, it is termed resistance. This concept of resistance is presented here.

Materials and methods. Viruses used. Two strains of cattle VD (virus diarrhea) virus were used for inoculation of pigs. One of these, Oregon C24V, cytopathogenic for tissue-cultured bovine kidney cells, was used for neutralization tests. The other, New York 1, that was not cytopathogenic, was maintained in spleen from an infected calf. The fully virulent HC (hog cholera) virus designated strain A, was maintained in spleen from an infected pig. A tissue-cultured derivative that was cytopathogenic was used for neutralization tests.

Animals. Litters of pigs were obtained when they were 8 weeks of age from a disease-free herd maintained by the Institute. Each litter was placed in an isolation unit. One week later one-half of the first litter of 4 pigs was inoculated intramuscularly, each with 1 ml of a 10% spleen emulsion prepared from a calf infected with New York 1 VD virus; the remainder of the litter was left uninoculated as controls. For the second litter of 6 pigs, one-third of the pigs of the litter was given New York 1 VD virus, one-third was

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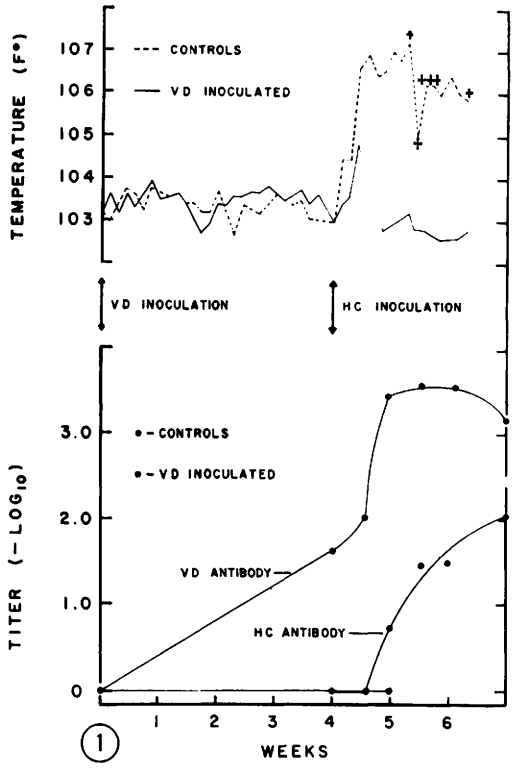

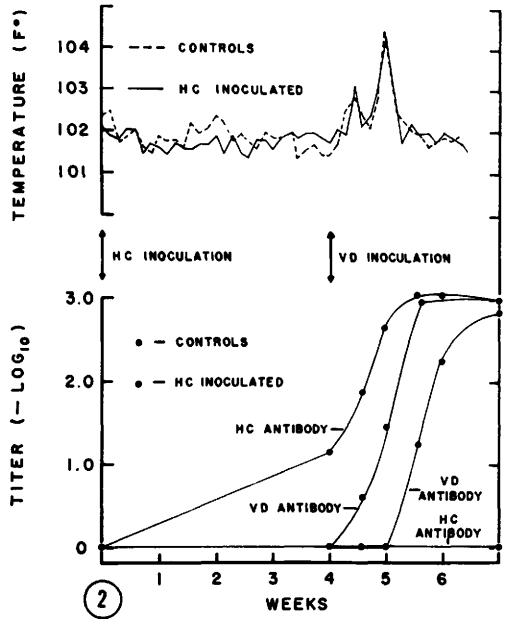

FIG. 1. Response of pigs inoculated with virus diarrhea (VD) virus followed by hog cholera (HC) virus.

FIG. 2. Response of calves inoculated with hog cholera (HC) virus followed by virus diarrhea (VD) virus.

given strain Oregon C24V virus that had been transferred for 9 serial passages and the remainder was left uninoculated. The pigs that were given New York 1 were inoculated as in the first litter and strain Oregon C24V was given intramuscularly, 1 ml of undiluted, infected tissue-cultured fluid to each pig. This was repeated with an additional litter of 7 pigs. After inoculation, temperatures were taken daily for 4 weeks, at which time each pig was inoculated intramuscularly with 1 ml of spleen emulsion prepared from a pig infected with strain A virulent HC virus. Again, temperatures were taken daily and the pigs were observed for signs of illness. Each pig was bled and serum was procured for neutralization tests at time of inoculation with VD virus, or when exposure by contact began, and again at time of inoculation with HC virus and at 4, 7, 10, 14 and 21 days afterwards. Results are shown in Fig. 1.

For reciprocal tests, calves were obtained from a herd of cattle maintained by the Institute whose mothers had no immunity against VD virus. For each test, 2 calves were placed in isolation; 1 was given intravenously 1 ml of a 10% spleen emulsion that contained virulent HC virus while the other served as a control. Temperatures were taken and leukocyte counts were made daily. Then, 28 days after inoculation each calf was given intravenously 1 ml of a 10% spleen emulsion from a calf that had been infected with strain New York 1 virulent VD virus. Temperatures were taken and leukocyte counts were continued. At the time HC virus was inoculated, or exposure by contact began, each calf was bled and serum was obtained. This was repeated at the time VD virus was inoculated and at 4, 7, 10, 14 and 21 days after inoculation. Later, these tests were repeated 3 times. Results are shown in Fig. 2.

Serum neutralizations. Tissue cultures of immature pig kidney cells for HC virus neutralization tests and of embryonic bovine kidney cells for VD virus neutralization tests were prepared. Tenfold dilutions of all serums were made and each dilution was mixed with 100 $TCID_{50}$ of strain A tissue-cultured HC virus. Each of 3 tubes was inoculated with .2 ml of the mixture. In like manner,

TABLE I. Reciprocal Neutralization Tests between Hog Cholera (HC) Virus and Virus Diarrhea (VD) Virus.

Virus used	Serum neutralization test results* VD	HC
VD	10/10	0/10
HC	0/10	10/10

* Denominator indicates No. of serums tested; numerator, those that neutralized 50 to 100 $TCID_{50}$ of virus. Homologous titers for VD ranged from 70 to 3160 and for HC from 100 to 2800.

serum dilutions were mixed with 100 $TCID_{50}$ of Oregon C24V virus and each of 3 tubes of bovine kidney cells was inoculated with .2 ml. Appropriate controls were maintained. Results are shown in Fig. 1 and 2.

Reciprocal neutralization tests were made using 10 sera from cattle that had recovered from an inoculation of VD virus and showed neutralizing antibodies for VD virus, and 10 sera from recovered pigs that had been given modified HC virus and showed neutralizing antibodies for HC virus. These results are presented in Table I.

Results. Sera that contained VD antibodies failed to neutralize HC virus and, in reciprocal tests, sera that contained HC antibodies did not neutralize VD virus (Table I).

No signs of illness were noted in any pig inoculated with VD virus whether of spleen or of tissue-cultured origin. Littermates exposed by contact also showed no signs of illness. All pigs showed a temperature elevation 2 to 4 days after inoculation with HC virus. In the pigs that had not been inoculated previously with VD virus, the temperatures remained elevated and the pigs died from 8 to 15 days after inoculation. In contrast, the pigs initially given VD virus showed a temperature elevation of lesser degree that lasted for only 1 to 2 days, after which the temperature returned to the normal range, the pigs continued to eat and showed no other signs of illness. These pigs developed an average antibody titer of 27 against VD virus 28 days after inoculation but showed no titer against HC virus. The littermates exposed by contact showed no titer to either virus and showed no illness. After inoculation with HC virus, the pigs that had been given VD virus initially showed the presence of HC antibodies 7 days after inoculation, and 21 days afterwards their average titer was 515. The titer against VD virus had increased from 27 to 729 and paralleled the development of titer against HC virus. In the control pigs no antibodies developed against HC virus before the animals died from the inoculation with virulent hog cholera virus. Mean temperature responses and antibody production can be seen in Fig. 1.

In the reciprocal experiments, following inoculation with HC virus no temperature elevations were noted in any of the calves, and their leukocyte count remained in the normal range. After inoculation of VD virus, all calves developed typical diphasic temperature elevations and leukopenias. A low neutralizing antibody titer for HC virus developed in inoculated calves but not in those exposed by contact. After inoculation with VD virus, calves that had been given HC virus initially showed VD antibodies 4 days afterwards and on the 21st day the average titer was 988. The HC antibody titer also rose in the calves and paralleled development of VD antibody. Calves that had not been inoculated with HC virus showed VD antibodies 11 days after inoculation with VD virus and an average VD antibody titer of 720 twenty-one days afterwards (Fig. 2).

Discussion. From this evidence, the conclusion can be made that VD virus protects pigs against lethal doses of hog cholera virus. Failure of the 2 viruses to share a common neutralizing antibody indicates that an additional response must be in operation. Indeed, tests in pigs previously inoculated with VD virus show the appearance of HC antibodies within 7 days, whereas, after vaccination with HC virus only, antibodies are not found until 11 days afterwards. VD virus seems to create in the pig a state of secondary response to hog cholera virus which otherwise would be fatal. In this way it resembles superficially the effect induced by a single inoculation of homologous inactivated virus. HC virus apparently failed to provide complete protection in calves subsequently given VD virus because the same signs of illness occurred that were shown by control calves al-

though a definite secondary response effect was noted.

Textbooks usually do not differentiate completely between resistance and immunity; such indefinite factors as nutrition, heredity and environment as nonspecific factors of resistance are usually mentioned. While nonspecific factors may be important, now the inference can be drawn that resistance to a particular organism may be due to previous infection by an immunologically distinct organism that is related to the extent of inducing a state of accelerated antibody response. This relationship could be indicated by complement-fixation or perhaps by gel-diffusion, as was found for adenovirus-infectious canine hepatitis virus(4) and for mucosal disease and swine fever viruses(5). Heterotypic responses also have been noted within the human adenovirus group following vaccination (6,7) and complement-fixing antibodies for poliovirus have been observed in individuals following ECHO 9 infection(8). While serological studies have not been made, cross-protection among certain of the group A arboviruses has been found(9-12). Much of the variation in clinical response to infectious agents may result from some previous infection that has induced resistance and, by accelerating antibody production, has reduced severity of clinical illness. If this is true, then this resistance concept introduces an entirely new means of controlling diseases.

Summary. Pigs inoculated with VD (virus diarrhea) virus survived inoculations of virulent HC (hog cholera) virus that killed littermates that had not received VD virus. Survival was signalized by accelerated antibody production both to HC and VD viruses. In reciprocal tests, HC virus produced secondary response in calves. Reciprocal neutralization tests between VD virus and HC virus showed they did not share a common antibody. Protection appeared related to accelerated antibody production that resembled the secondary response induced by inactivated viral antigen.

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Effect of Bacterial Endotoxin in Germ-free Mice. (27200)

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There has developed an increasing awareness that the characteristic reactivity of mammals to endotoxin may be a consequence of the continued presence of Gram-negative bacteria in the intestinal tract from shortly after birth. Animals reared in an environment without contact with living bacteria provide a means for determining whether the intes-

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