amount of the gibberellin having an R_F of 0.8 to 0.9 obtained from the acetone extract was somewhat less than that found in comparable fractions of ethanol and methanol extracts.

The wheat endosperm test was the simplest procedure for detecting gibberellin-like activity and was used routinely during the current investigation. Results obtained through the use of this assay confirmed those obtained by pea-seedling and wheat-leaf assays. Two gibberellins were detected when ethanolic and methanolic extracts were examined for gibberellin-like activity.

A summary of the results obtained by the use of the various extraction, chromatographic, and assay procedures are given in Table 1. The R_F value for the component having the major share of the gibberellin-like activity was similar to that for gibberellic acid, regardless of the chromatographic solvent system employed. The second gibberellin-like fraction was separated by the amyl alcohol–pyridine–water and butanol–acetic acid–water solvent systems but could not be separated by the butanol and ammonium hydroxide solvent. The "second" gibberellin was not extractable with 50-percent acetone.

The wheat gibberellin that resembles gibberellic acid in its chromatographic mobility was found in wheat and malted wheat. The slower-moving gibberellin was found only in malted wheat and in larger amounts in the embryo end of the sprouted seed and in the outer layers of the seed, presumably in the aleurone. Both gibberellins appear to increase in quantity during the first 3 or 4 days of germination.

According to Harris (*7*) one of the principal roles of the embryo during the early stages of germination is secretion of a gibberellin-like hormone into the endosperm and aleurone portions of the seed. The apparently greater amounts of gibberellin, particularly of the new substance, in the embryo and adjacent areas at the start of germination in malted wheat would support Harris's hypothesis.

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Interferon Production in Chickens Injected with *Brucella abortus*

Abstract. *Intravenous injection of large numbers of live, virulent* Brucella abortus *in chickens resulted in the appearance in the serum of a viral inhibitor indistinguishable from interferon. Inhibitor was detected as early as 3 hours after inoculation of brucellae and reached a peak between 6 and 12 hours.*

The antiviral substance, interferon, is produced by vertebrate cells in response to infection with different viruses (*1*). The nature of the essential stimulus that induces cells to make interferon is still unknown. To test the possibility that intracellular infectious agents other than viruses may induce the production of interferon, experiments were designed in which *Brucella abortus* was used instead of virus.

A modification of the procedure for the production of circulating interferon in intact animals by the intravenous injection of viruses was used (*2*). White Leghorn roosters about 1 year old were injected in the cubital vein with 10^{10} live *Brucella abortus* organisms in a 2-ml volume.

The virulent, CO_2-requiring ES strain (*3*) was grown on tryptose agar for 48 hours and suspended to the desired concentration in nonpyrogenic physiological saline solution. Blood samples were obtained from individual chickens by cardiac puncture at different times after inoculation with bacteria, and serums were assayed for inhibitor activity by the plaque-reduction method (*4*) in primary chick embryo cultures. Cultures in 60-mm petri dishes were exposed for 20 hours to 3 ml of twofold serial dilutions of serum and then challenged with 40 to 60 plaque-forming units of vesicular stomatitis virus. The titers of inhibiting substances were calculated from the dilution of the serum which reduced the plaque count to 50 percent of that of the controls; the titers were expressed as inhibitor units per 3 ml of serum.

Figure 1 shows that antiviral activity was detected as early as 3 hours after inoculation of brucellae and rose steadily, reaching a peak at about 12 hours; by 24 hours the inhibitor activity was much reduced, and no activity was detectable 48 hours after inoculation. The properties of this inhibitor were compared to those of an interferon in allantoic fluid from chick embryos infected with the WS strain of type A influenza virus (*4*). The properties of the brucellae-induced inhibitor in chicken serum were as follows. The inhibitor was nondialyzable, stable at *p*H 2.6 for at least 24 hours, and stable to heating at 56°C for 1 hour; it did not sediment at 105,000*g* for 1 hour, and it was inactivated by incubation for 3 hours at 37°C with crystalline trypsin (50 μg/ml). Serum containing inhibitor activity was incubated with 10^5 plaque-forming units of vesicular stomatitis virus for 1 hour at 37°C and then diluted to test for infectivity. No inactivation of virus was detected, indicating that the action of the inhibitor is not on the virus directly but on the cells. These properties of the serum inhibitor are identical to those of interferon produced in chick embryo cells upon stimulation by virus.

In order to determine the minimum number of brucellae required to produce detectable inhibitor in the circulation, varying numbers of bacteria were injected intravenously and serums were obtained at 6 to 8 hours after inoculation. In addition, the capacity of heat-killed brucellae to induce inhibitor production was also tested. While 10^{10} live brucellae injected intravenously produced significant amounts of inhibitor in the circulation, 10^8 or fewer bacteria failed to produce a detectable response. Heat-killed brucellae, even when 10^{10} organisms were inoculated, failed to elicit any response. The

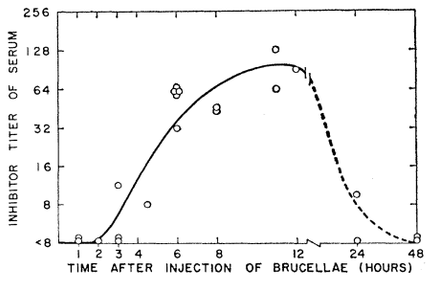

Fig. 1. Inhibitor titers in individual chicken sera collected at different times after intravenous inoculation of 10^{10} brucellae. Titers are expressed as inhibitor units per 3.0 ml of serum.

injection of *Serratia marcescens* (1.5×10^9 viable organisms), *Shigella sonnei* (4.4×10^9), *Bacillus subtilis* (2.8×10^8), and *Staphylococcus albus* (2.2×10^9) did not produce detectable inhibitor. The viable counts varied, although approximately the same mass of the different organisms (adjusted to the turbidity of approximately 5×10^9 brucellae per milliliter) was inoculated. Negative results also were obtained after the inoculation of 5.0 mg of bacterial endotoxin. On the other hand, intravenous injection of 10^9 plaque-forming units of Newcastle disease virus produced a high titer of inhibitor in chicken serum at 6 hours. This result in chickens confirms the observations made by Baron and Buckler in mice (*2*).

An attempt was made to rule out the possibility that breakdown products of brucellae in vivo present in the serums had some direct effect on the cells, thus inhibiting plaque formation. Lysates of 10^{10} live brucellae were prepared by the method Carver and Naficy (*5*) used to make bacterial inhibiting factor, a constituent of certain bacteria which interferes with the replication of group A arboviruses in cell culture. The capacity of vesicular stomatitis virus to produce plaque formation in cell cultures exposed to these lysates for 20 hours did not differ from that in untreated cell cultures. In addition, chick embryo cell cultures exposed to 100 μg of *Escherichia coli* endotoxin (0111:B4) for 20 hours did not show any difference in plaque count from control cultures. These results tend to rule out any carry-over in serums of bacterial products which might have an inhibiting effect on plaque formation. Another inhibitor, termed "serum sparing factor" by Gledhill (*6*), which is produced in mice by endotoxin, also is unlikely to be the inhibitor produced by injection of brucellae into chickens because of the failure of endotoxin to elicit inhibitor production in this species. Consideration must also be given to the possibility (*7*) that foreign RNA or DNA of diverse origins can stimulate interferon production in cell-culture systems. It should be noted that *Escherichia coli* RNA, the only bacterial nucleic acid tested, did not induce interferon production (*7*). In our work, intravenous injection of chickens with 3.0 mg of rabbit liver ribosomes yielded no interferon. The negative results obtained with several bacterial species, other than *Brucella*, also do not support the idea that foreign nucleic acids were responsible for our results.

The data show that a virus inhibitor with properties identical to those described for interferon is found in the blood of chickens after intravenous injection of large numbers of live, virulent brucellae. These organisms were selected because they are intracellular parasites in the hosts they commonly infect and are therefore suited to test the hypothesis that intracellular parasites other than viruses may induce interferon production by cells.

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Immuno-osmophoresis, a Rapid and Sensitive Method for Evaluating Viruses

Abstract. *Characteristic antigen-antibody interactions in agar gel were obtained in 50 to 90 minutes in an electrical field with microgram quantities of three plant viruses, which were either in purified form or in crude plant juice. The method was faster and more senstive than agar-gel diffusion.*

Bon and Swanborn reported (*1*) that a precipitin reaction occurred within half an hour when an extract from the eye lens of cow and its homologous rabbit antiserum were applied simultaneously to separate wells in a thin layer of agar (*p*H 8.4 to 8.5) and exposed to an electrical field running parallel to the line connecting both wells. They suggested that this "very rapid method of immunoelectrophoresis" be named "immuno-osmophoresis," since the antibody movement was due to endosmotic flow. Plant viruses exposed to an electrical field at *p*H 7 are known to move in agar without loss of biological activity (*2*). We report here the immuno-osmophoresis of plant viruses at *p*H values near neutrality and its usefulness in serological studies aimed at quick identification and quantitative evaluation of these antigens of high molecular weight.

Electrophoresis was carried out in 1 percent agar in 0.01*M* tris-succinate buffer, *p*H 6.8 or 7.6, at 4 v/cm, in a cold room at 3°C, without additional cooling. The agar used was Difco Bacto agar, or Difco Noble agar, or agarose. Agarose, the neutral constituent of agar, was isolated from Noble agar according to Hjertén (*3*). The gel layer (16 by 13 by 0.9 cm) was prepared in a translucent plastic box (21 by 13 by 2 cm), and a strip 2.5 by 13 cm was left on both sides of the gel as electrode compartments. Two rows of six holes (0.6 cm in diameter) were arranged symmetrically in the middle of the gel. The distance between the centers of the holes in the direction of the electrical field was 2 cm. Fresh buffer solution was fed continuously into each electrode compartment at one end and the excess solution sucked off at the opposite end to maintain con-