6. Scheidegger, J. J., *Intern. Arch. Allergy Appl. Immunol.*, 1955, v7, 103.

7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.

8. Ishizaka, T., Ishizaka, K., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 845.

9. Green, H., Barrow, P., Goldberg, B., *J. Exp. Med.*, 1959, v110, 699.

10. Müller-Eberhard, H. J., Nilsson, U., *ibid.*, 1960, v111, 217.

11. Becker, E. L., *J. Immunol.*, 1959, v82, 43.

12. Harboe, M., Müller-Eberhard, H. J., Fudenberg, H., Polley, M. J., Mollison, P. L., *Immunology*, 1963, v6, 412.

13. Dixon, F. J., *Mechanism of Cell and Tissue Damage Produced by Immune Reactions*, Benno Schwabe & Co., Basel, 1962, p71.

14. Shulman, N. R., *Ann. Intern. Med.*, 1964, v60, 506.

15. Yachnin, S., Laforet, M. T., Gardner, F. H., *Blood* 1961 v17, 83.

16. Springer, G. F., Rapaport, M. J., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 103.

17. Arquilla, E. R., Hamashige, S., Miller, A., *Fed. Proc.*, 1964, v23, 506.

Received September 2, 1964. P.S.E.B.M., 1964, v117.

Interferon in Dermal Crusts of Human Vaccinia Virus Vaccinations Possible Explanation of Relative Benignity of Variolation Smallpox.* (29659)

E. FREDERICK WHEELOCK

Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University, and University Hospitals, Cleveland, Ohio

Interferon, a protein with virus inhibitory properties synthesized in cells in response to viral infection, has been detected in a variety of cell culture and animal systems infected with vaccinia virus(1-4). Further, recent reports have revealed the presence of interferon-like substances in acute pharyngeal washings of patients with influenza(5), in human cerebrospinal fluid(6), in sera of patients with clinical viral infections(7) and in sera and organ extracts following intravenous administration of viruses to a patient with acute myelogenous leukemia(8).

This project on detection of interferon in dermal crusts of vaccinia virus vaccinations of man was conceived in an attempt to provide an explanation of the relative benignity of variolation smallpox.

Variolation, a method of vaccination against smallpox, was utilized in ancient China and is still in use in primitive areas of the world today. This method consists of the intradermal inoculation of crusts from the skin lesions of patients with smallpox. Although variolation usually produces a mild form of smallpox, occasionally severe or even fatal disease occurs.

The mechanism responsible for the mildness of variolation smallpox is unknown. However, the recent discovery of interferon suggested the possibility that this substance might be present, together with variola virus, in the crusts of smallpox skin lesions and thus influence initial virus multiplication at the vaccination site.

Since smallpox dermal crusts were not available for the present investigation, crusts from vaccinia virus vaccinations were examined for interferon. In this communication the detection of interferon in 4 of 5 vaccinia vaccination crusts is reported and the possible role of interferon in limiting the spread of vaccinia virus in vaccinations and in attenuating the clinical course of variolation smallpox is discussed.

Materials and methods. Dermal crusts from vaccinia virus vaccinations were ground to

* This investigation was conducted under the sponsorship of Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported in part by Office of The Surgeon General, Dept. of the Army, and in part by a grant from Robert Hamilton Bishop, Jr. Endowment Fund.

TABLE I. Properties of Substance in Extracts of Vaccinia Virus Vaccination Dermal Crusts Capable of Inhibiting Sindbis Virus CPE.*

Treatment of extracts	Effect on inhibitory property†
Trypsin, 0.1 mg/ml for 1 hr, 37°C	+
Acidification at pH 2, 24 hr	—
Centrifugation 105,000 *g*, 3 hr	—
Desoxyribonuclease 0.5 mg/ml, 1 hr, 37°C	—
Ribonuclease 0.5 mg/ml, 1 hr, 37°C	—

* Cytopathic effects in human fetal lung cells.
† Destroyed (+), Preserved (—).

a fine powder in a mortar and suspended in phosphate buffered saline (PBS) at pH 7.2 at a concentration of 0.5%. The pH was adjusted to 2 for 24 hours at 4°C and then readjusted to 7.2 and the extracts centrifuged at 8000 *g* for 30 minutes at 4°C. The supernates were then assayed for interferon and subjected to further studies as indicated in Table I.

Virus. Sindbis virus (Egypt AR 339 strain) and Simliki Forest virus (obtained from Dr. Delphine Clark) were grown in 12-day-old chick embryos inoculated intravenously.

Interferon assay. Crust extracts were first tested for interferon in tube culture monolayers of established human fetal lung cells and those which significantly protected cultures against Sindbis virus challenge were further studied by the plaque reduction technique.

One ml of a 10% dilution of crust extract in growth medium was added to one-day-old subcultures of human fetal lung cells in incomplete monolayers grown in screw cap culture tubes. After 24 hours of stationary incubation at 37°C, the medium was aspirated and the cultures washed once with 4 ml of PBS. One milliliter of growth media containing 50,000 $TCID_{50}$ of Sindbis virus was then inoculated into each tube. After 24 hours of incubation at 37°C the cytopathic effects in the extract-treated cultures were compared with virus controls. Significant protection of cultures against virus multiplication was considered to have occurred when there was less than an estimated 25% cytopathic effect (CPE) at a time when control cultures exhibited an estimate of more than 75% CPE.

A modification of the plaque reduction method described by Wagner(9) was employed. Complete monolayers of established human fetal lung cells in 25 ml plastic flasks (Falcon Plastic) were treated for 20 hours with 2 ml of dilutions of crust extracts in growth medium. The cultures were then washed with PBS and challenged with approximately 50 plaque-forming units (PFU) of Sindbis virus.

Interferon titers, expressed as units per 2 ml were determined as reciprocals of the dilution of the extracts required to reduce the plaque counts to 50% of those of virus controls.

Results. The extracts from 4 dermal crusts from vaccinia virus vaccinations were assayed for interferon by the plaque reduction technique as described in materials and methods. Extracts No. 1, 3, 4, and 5 had interferon titers of 20, 42, 40, and 35, respectively. Extract No. 2 contained no interferon. The plaques produced in the extract-treated cultures were reduced in size as well as in number when compared with virus controls, a phenomenon characteristic of interferon action(10).

As controls for the vaccinia virus vaccination dermal crust extracts, the crusts of 5 traumatic skin lesions were obtained. Extracts from these traumatic crusts were made and found to contain no interferon.

The nature of the virus inhibitory property in the crust extracts was studied (Table I). Treatment of extracts with crystalline trypsin, 0.1 mg/ml, at 37°C for 1 hour destroyed the inhibitory property. Acidification at pH 2 and restoration to pH 7.2 did not destroy the ability of the extracts to protect cultures against Sindbis virus challenge, nor did high speed centrifugation remove the inhibitory substance. Strong evidence that the inhibitory property of these extracts is not due to virus is provided by the results of the procedures described in Table I as well as by failure to isolate a virus from any of the extracts in either the chorioallantoic membranes of embryonated chicken eggs or

in "L" cell cultures (derived from mouse fibroblasts), whereas vaccinia virus was readily isolated from the 5 nonacidified crusts in both of these host systems. The virus inhibitor is not a nucleic acid, as indicated by the fact that it was unaffected by treatment with either desoxyribonuclease or ribonuclease. That the inhibitor in the extracts did not affect the virus directly was demonstrated by an experiment in which the extracts were incubated with 10^5 PFU of Sindbis virus for 1 hour with no resultant decrease in virus titer. Hence it is evident that these extracts exerted their protective effect by acting upon the cells rather than upon the virus. The specificity of the virus inhibitor for cells of human origin was shown by the failure of the extracts to protect "L" cell cultures against challenge with Semliki Forest virus. These properties of the virus inhibitor are similar to those previously described for interferon(11).

Discussion and summary. The presence of interferon in dermal cells of vaccinia vaccinations of man is not unexpected since this substance has been detected in a variety of host systems infected with vaccinia virus(1-4). Interferon in vaccination crusts could be either a nonfunctional response to vaccinia virus multiplication or could serve to suppress virus multiplication until immune mechanisms are mobilized. Thus in man, as has been suggested in animal systems(12), interferon may be associated with recovery from infection with vaccinia virus.

The role of interferon in ameliorating the clinical course of smallpox is conjectural. However, several observations tend to support such a relationship. First, it has been found that vaccination with vaccinia virus shortly after exposure to smallpox virus results in a milder subsequent clinical course of smallpox(13). These beneficial effects occur too soon after vaccination to be attributable to antibody production and may be due to interferon. Secondly, although variola virus can be isolated from both the upper respiratory tract and dermal crusts of patients with smallpox, it is believed that the respiratory tract virus produces the characteristic virulent disease, whereas it has been observed that dermal crust virus preparations produce a milder form of smallpox irrespective of route of inoculation(14). These facts can be reconciled if one postulates the presence in dermal crusts of a factor other than virus which has virus inhibitory properties such as interferon, although the possibility does exist that such a factor may also be present in respiratory droplets. That occasional fatal cases of variolation smallpox occur may be due to the absence of interferon in a small percentage of crusts as was found in the present investigation with vaccinia dermal crusts. An alternative explanation for the relatively mild clinical course of variolation smallpox is that the variola virus becomes modified during its dermal passage (15). However, no differences between viruses derived from respiratory and dermal cells have been demonstrated.

Finally, special attention should be given to the experiments reported by Isaacs *et al* (16) who showed that the vaccinia vaccination reaction can be completely suppressed in man by prior subcutaneous administration of interferon. The close antigenic and structural relationships between variola and vaccinia viruses make it probable that virus-interferon relationships are also similar although it has been shown that virulent strains of a given virus type often induce less interferon(17,18). If interferon is present in variola dermal crusts as it is in vaccinia crusts, then the inoculation of interferon together with variola virus in variolation could similarly temporarily suppress virus multiplication, permit host defense mechanisms to mobilize and result in a relatively benign clinical course of smallpox.

The technical assistance of Mrs. Juanita Ruffier is gratefully acknowledged. The author wishes to thank Dr. William Staples for cooperation in obtaining the dermal crusts employed in these experiments.

1. Nagano, Y., Kojima, Y., *Compt. Rend. Soc. Biol.*, 1958, v152, 1627.

2. Glasgow, L. A., Habel, K., *J. Exp. Med.*, 1962, v115, 503.

3. Friedman, R. M., Baron, S., Buckler, C. E., Steinmuller, R. I., *ibid.*, 1962, v116, 347.

4. Glasgow, L. A., Habel, K., *ibid.*, 1963, v117, 149.

5. Gresser, I., Dull, H. B., PROC. SOC. EXP. BIOL. AND MED., 1964, v115, 192.
6. Gresser, I., Naficy, K., *ibid.,* 1964, v117, 285.
7. Wheelock, E. F., Sibley, W. A., *Lancet,* 1964, v2, 382.
8. Wheelock, E. F., Dingle, J. H., *New England J. Med.,* 1964, v271, 645.
9. Wagner, R. R., *Virology,* 1961, v13, 323.
10. Gifford, G. E., Toy, S. T., Lindenmann, J., *ibid.,* 1963, v19, 294.
11. Isaacs, A., *Advances in Virus Research,* Academic Press, New York and London, 1964, v10, 1.
12. Baron, S., *ibid.,* 1964, v10, 39.
13. Dixon, C. W., *Smallpox,* J. & A. Churchill, London, 1962, p184.
14. ———, *ibid.,* 1962, p298.
15. ———, *J. Hyg.,* 1948, v46, 351.
16. Scientific Committee on Interferon, *Lancet,* 1962, v1, 873.
17. Ho, M., Enders, J. F., *Virology,* 1959, v9, 446.
18. Enders, J. F., *Trans. Studies Coll. Physicians,* Phila., Pa., 1960, v28, 68.

Received September 28, 1964. P.S.E.B.M., 1964, v117.

Relative Distribution of Cholesterol in Plasma and Liver Compartments of Chicks Fed Different Fatty Acids. (29660)

G. A. LEVEILLE AND H. E. SAUBERLICH
U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colo.

Numerous studies have been concerned with the influence of dietary lipids on serum cholesterol levels in man and other animals (1-7). Most of these studies have indicated that the hypocholesterolemic effect of certain dietary fats is related to their unsaturated fatty acid content. However, other reports have shown that unsaturation *per se* cannot account for the hypocholesterolemic effect of dietary lipids. Hegsted *et al*(8) have shown serum cholesterol levels to be positively correlated to the intake of unessential polyunsaturated and monounsaturated fatty acids in cholesterol-fed rats. Also, recent reports have shown medium chain triglycerides (a liquid fat composed of C_8 and C_{10} triglycerides having an iodine number of 0.2) to be hypocholesterolemic(9-10).

In a previous report(11), it was shown that dietary fats can influence the distribution of cholesterol between serum and liver. This effect of dietary fat was attributed to its fatty acid composition. The data presented here support this conclusion in chicks fed free fatty acids.

Materials and methods. Male Hy-line White Leghorn chicks were fed a commercial diet for 10 days. Ten chicks were then assigned to each of various experimental groups on the basis of body weight in such a manner as to have similar initial mean weights (105-108 g). The chicks were reared in heated cages having raised wire floors. Food and water were supplied *ad libitum.* Food consumption and body weights were determined at weekly intervals throughout the 3-week experimental period.

The basal diet employed had the following composition in g/100 g of diet: Assay protein C-1,* 29.4; glycine, 0.4; DL-methionine, 0.3; salt mix,† 5.31; vitamin mix,† 4.0; glucose to 100. Fatty acids and cholesterol were added at a level of 10% and 2% of the diet, respectively, as indicated in the Tables. All additions to the basal diet were made at the expense of glucose. The purity of the fatty acids used was determined by gas chromatography. The stearic acid used was essentially pure, the oleic and lineoleic acids were 68 and 77% pure, respectively.

At termination of the experimental period, the chicks were bled by cardiac puncture, then sacrificed by cervical fracture. The livers were excised, blotted to remove excess blood, weighed, frozen and stored at —20°C until analyzed. Plasma cholesterol and lipid phosphorus were determined as previously described(13). Liver fat and cholesterol were determined as described earlier(11).

* Archer-Daniels Corp., Midland, Mich.
† See Leveille *et al.*(12).