Let us discourage the great practice of passing the buck, avoiding responsibility by passing projects from committee to committee, rather than by getting first-hand information.

The choice before us, experimental design or free research, the project or the man, has many ramifications, but it concerns the mainspring of the entire operation, the future of research in America. Let us support the man.

Reference

1. ARTHUS, M. Philosophy of Scientific Investigation. Preface to *De l' Anaphylaxie à l' Immunité,* Paris, 1921. Translated from the French, with an Introduction by Henry E. Sigerist. Foreword by Warfield T. Longcope. Baltimore, Md.: Johns Hopkins Press (1943).

Inactivation of Vaccinia Virus by a Diffusible Component from Hydrolyzed Hyaluronic Acid

J. F. McCrea[1] and F. Duran-Reynals[2, 3]

Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut

PREVIOUS STUDIES from this laboratory have shown that various preparations of hyaluronic acid inactivated vaccinia virus grown in cell culture, and that this effect was markedly increased when the acid was hydrolyzed by hyaluronidase added to the culture medium (*1*). Experiments have, therefore, been carried out to identify the virus-inactivating component or components. In this preliminary note we describe experiments showing that: (a) the virus-inactivating substance is freely diffusible through a cellophane membrane; and (b) the substance, or one of its components, is in all probability glucuronic acid or a glucuronide.

The procedure in 10 basically similar experiments was as follows: an 0.5% solution of purified umbilical cord hyaluronic acid was prepared in 0.1 *M* acetate buffer pH 6.0 containing 0.1 *M* NaCl and dialyzed overnight at 4° against approximately 10 volumes of the same buffer. A purified preparation of dialyzed testicular hyaluronidase (activity 1000 TRU/mg;[4] final concentration 1 : 1000) was then added to the hyaluronic acid and the mixture incubated for 48 hr at 37° in the presence of toluene. A sample of the hydrolyzate was removed and stored at − 20° C, and the remainder dialyzed in cellophane tubing against distilled water at 4° until, as judged by colorimetric tests (*2, 3*), no further N-acetyl glucosamine or glucuronic acid diffused through the membrane. The diffusate (material passing through cellophane) was concentrated to the original volume by lyophilization. The four materials (i.e., hyaluronic acid, hydrolyzate, dialyzate, and diffusate) were sterilized by heat (10 min at 75° C) or filtration and tested together for viral inactivation against dermo or Levaditi vaccinia virus. Tests were carried out on: (*a*) rabbit skin; (*b*) cell cultures; (*c*) the chlorioallantoic membrane of chick embryos; and (*d*) the hemagglutination reaction. Reducing substances, glucosamine, and glucuronic acid were estimated in each fraction. Approximately 50% of the original N-acetyl glucosamine and glucuronic acid present in the hydrolyzate was recovered in diffusible form.

The most striking results were obtained in rabbit skin, and a summary of a typical experiment is given in Table 1. In this experiment equal volumes of diluted virus and test material were incubated at pH 6.0 for 4 hr at 37°; the same virus suspension was incubated with buffer alone as a control. Groups of 4 rabbits then received 0.5 ml of each mixture intradermally, the development of lesions being recorded after 3, 5, and 7 days. Typical lesions developed after 3 days from control injections and in those where virus had been treated with either hyaluronic acid, hydrolyzate, or dialyzate; in the two latter cases the lesions were fre-

TABLE 1

INACTIVATION OF VACCINIA VIRUS BY DIFFUSATE FROM HYDROLYZED HYALURONIC ACID: INTRADERMAL INOCULATION IN RABBITS

(All materials dissolved in 0.1 *M* acetate buffer, pH 6.0)

Test material	Virus dilution*	Type of lesion†
Hyaluronic acid‡	10^{-2}	+++
Hydrolyzate	10^{-2}	+++++
Dialyzate	10^{-2}	+++++
Diffusate	10^{-2}	—
Buffer control	10^{-2}	++++

* Dermo virus, egg-passage strain.

† ++++ Edematous, necrotic lesion, approximately 3–5 cm in diameter; +++ similar lesion without visible necrosis; — no visible or palpable lesion.

‡ Wyeth Institute of Applied Biochemistry, batch 215–2.

[1] James Hudson Brown Memorial Fellow.

[2] Supported by a Research Grant from the National Institutes of Health, USPHS.

[3] With the technical assistance of Robert A. Roosa and Arthur G. Tyrol, Jr.

[4] Kindly supplied by J. Seifter from the Wyeth Institute of Applied Biochemistry.

quently as large as or larger than the control lesions. However, injections of virus treated with diffusate produced at most a small pink spot on the third day, which, in the great majority of cases, faded completely by the 5th to 7th day. Thus, in a series of 9 experiments with diffusates from various samples of hyaluronic acid, highly significant suppression was obtained in 8 instances and at least a 50% reduction in the ninth test. In other experiments, serial dilutions of diffusate-treated virus were inoculated intradermally in rabbits. Compared with virus incubated in buffer at the same pH, the titer of treated virus was reduced by at least 1 log. Hyaluronic acid alone caused some suppression in an occasional animal.

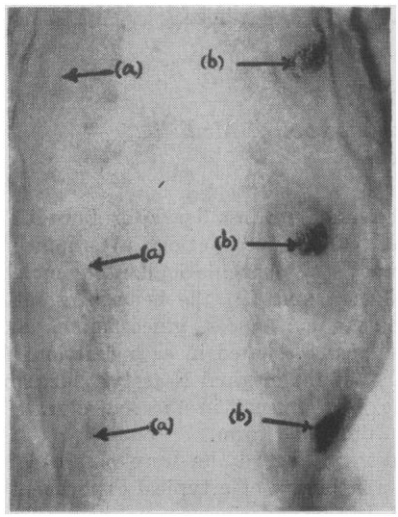

FIG. 1. Effect of incubation with synthetic glucuronic acid on vaccinia virus infection in rabbit skin. Levaditi strain (egg passage) diluted 10^{-3} incubated 4 hr at 37° with an equal volume of: (*a*) 5% glucuronic acid in acetate buffer pH 5.9, and (*b*) 0.5 *M* acetate buffer pH 5.9. Arrows mark injection sites. Note large, necrotic lesions from untreated virus and complete suppression with glucuronic acid-treated virus. Photographed 6 days after inoculation.

It was obvious, therefore, that a virus-inactivating substance or substances, freely diffusible through cellophane, were released when purified hyaluronic acid was hydrolyzed by hyaluronidase. No such substance was obtained on dialyzing unhydrolyzed hyaluronic acid. These observations have been confirmed using: (a) Levaditi virus (egg and testis passage) and dermo virus (egg- and cell-culture passage); (b) hyaluronic acid from several sources including vitreous humor; and (c) testicular and bacterial hyaluronidase. A reduction in titer was also observed when virus was treated similarly and titrated on the chick-embryo chlorioallantois, but in this case, particularly with fully egg-adapted strains, the effect was considerably less marked. Virus maintained in cell culture was inactivated by 2–3 passages in the presence of diffusate. Hemagglutinin titer of either egg-adapted or testis-passaged strains was reduced by 50–70% after 4 hr incubation with diffusate; again the fully egg-adapted strains were less affected.

Since the diffusible products released from hydrolyzed hyaluronic acid are N-acetyl glucosamine and glucuronic acid (either free or as oligosaccharides), we have examined the effect of the synthetic compounds on vaccinia virus.[5] In summary, all the described effects of diffusate could be duplicated by treating vaccinia virus with glucuronic acid under similar conditions (incubation at pH 6 for 4 hr at 37°, final concentration 2%). Again, virus inactivation was best shown in rabbit skin (Fig. 1); in chick-embryos the effect was barely significant. Cell-culture virus became inactive after 1–2 passages in medium containing 0.5% glucuronic acid. Free glucuronic acid and glucuronolactone at pH 5.5–5.9 were approximately equally as active in suppressing lesions in rabbit skin and in inactivating the hemagglutinin. Galacturonic acid showed approximately 50% of the activity of glucuronic acid, but sodium or potassium glucuronate, barium mannuronate, and N-acetyl glucosamine showed little or no activity in any test.

Although we have not yet rigidly excluded the possibility that a dialyzable impurity present in the various preparations of hyaluronic acid and glucuronic acid may be responsible for virus inactivation, the simplest explanation of the above observations would be to assume that vaccinia virus was specifically inactivated by glucuronic acid under the conditions described. That the action was directly concerned with the virus was seen from the observation that at least 4 hr incubation was necessary for complete suppression of infectivity: only partial suppression occurred after incubation for ½–3 hr.

Suppression of the virus in rabbit skin was, however, found to be reversible since the addition of hyaluronidase to an "inactive" mixture of virus and diffusate or glucuronic acid restored the treated virus to the same or a higher degree of activity than that shown by the control virus. This phenomenon explains the fact that, as shown in Table 1, the lesions induced after incubation of the virus with hydrolyzate and dialyzate were actually larger than the control lesions: the hyaluronidase still present in these materials, even after heating, reactivated the virus through spreading in the rabbit skin. A comparable phenomenon has been described when neutralized mixtures of vaccinia and antiserum were injected with hyaluronidase in the skin (*4*). The fact that the same virus-glucuronic acid mixture was significantly suppressed if titrated in rabbit skin, whereas a barely significant reduction in titer occurred in chick embryos, is in agreement with the interpretation that the effect in rabbits was due to reversible inactivation rather than destruction of the virus. Mixtures of vaccinia and antiserum inactive in the rabbit skin also became active when injected into the chick embryo or other tissues (*5, 6*).

It is possible that glucuronic acid interacts with vaccinia virus in a way similar to its conjugating action in detoxication mechanisms (*7*). If this is so, the

[5] We are indepted to E. R. Weidlein, Jr., and Corn Products Refining Company for gifts of pure synthetic glucuronic acid, glucuronolactone, and glucuronates.

acid or analogous compounds may play a part in defense mechanisms when the ground substance is attacked during infection. The experiments reported are being extended to other viruses and to bacteria.

References

1. DURAN-REYNALS, F., and DURAN-REYNALS, M. L. *Science*, **115**, 40 (1952).
2. MORGAN, W. T. J., and ELSON, L. A. *Biochem J. (London)*, **28**, 988 (1934).
3. DISCHE, Z. *J. Biol. Chem.*, **167**, 189 (1947).
4. VIEUXCHANGE, J. *Compt. rend. soc. biol.*, **128**, 901 (1938).
5. ANDREWES, C. H. *J. Pathol. Bacteriol.*, **31**, 671 (1928).
6. MYERS, R. J., and CHAPMAN, M. J. *Am. J. Hyg.*, **25**, 16 (1937).
7. ARTZ, N. E., and OSMAN, E. M. *Biochemistry of Glucuronic Acid*. New York: Academic Press (1950).

News and Notes

The Cooperative Research Foundation

MANY programs have been developed in the past decade in the hope of increasing international understanding and cooperation in many different fields of common interest. Among these is an interesting experiment undertaken by a San Francisco group in 1950 which led to the establishment of the Cooperative Research Foundation, or CORE as it is commonly called. CORE is a true cooperative whose membership at present includes administrators, professors, scientists, and engineers from universities, research institutes, government agencies, and industry, both in the U. S. and abroad. The organization, with research offices in San Mateo and Washington, D. C., provides services through which scientists can exchange information, knowledge, and skills, through consultation and education, and can use available trained personnel and facilities to the greatest advantage in solving mutual scientific and technical problems. Members offer consultation, time, and facilities to other members, to government agencies, to universities, to research institutes and to industry.

During the past two years, CORE has been particularly active in building a cooperative program with the Conselho Nacional de Pesquisas of Brazil, an organization similar to the National Science Foundation in the United States. CORE's South American membership has grown rapidly through the leadership of such members as Admiral Alvaro Alberto, President of the Conselho, Dr. Cortes Pla, Head of the Science and Technology Section, Pan American Union, and Prof. Olympio da Fonseca, Director of the Instituto Oswaldo Cruz, in Rio de Janeiro. In the United States new members having interests similar to those of the Latin American members joined CORE to cooperate in the programs of the Foundation. To illustrate the type of direct working relationship developing between CORE members, a cooperative project in oil shale technology was initiated by one of CORE's board of directors, Prof. B. E. Lauer, Head of the Department of Chemical Engineering at the University of Colorado. For several years Prof. Lauer's department has been doing research on oil shale retorts which utilize fluidized solids techniques. Since Brazil is interested in making use of its extensive deposits of oil shale, CORE members in Brazilian universities became interested in the work at Boulder, and through travel grants awarded by the Conselho have spent short periods with Prof. Lauer in Colorado. Reports on the Colorado experiments have been sent to CORE members in several Brazilian research centers. The ground has thus been broken, through CORE's efforts, for a direct working level exchange of knowledge and mutual cooperation in oil shale technology.

Since its inception, CORE members have assisted the Conselho in placing the recipients of Brazilian fellowships and scholarships for graduate study in universities. In July of this year, with the cooperation of the California Academy of Sciences, CORE will open a San Francisco Liaison Office for the Conselho in the Academy buildings at Golden Gate Park. A committee of CORE members from the San Francisco Bay region has been formed to integrate the operation of this office with the scientific activities of the area. This committee includes Mr. Belford Brown, Vice President, The San Francisco Bank; Dean Morris Steward, Graduate School, University of California; Mr. George Tenney, President, McGraw-Hill Company of California; Dr. Robert Miller, Director of the California Academy of Sciences; Mr. R. L. Champion, U. S. Liaison Officer, Conselho Nacional de Pesquisas; Mr. John Alison, President, The Transit Van Corporation, and the writer. The committee plans to assist in obtaining fellowships for graduate study for Brazilian scientists who wish to attend universities in the area, to obtain subsistence grants and travel fellowships.

Later this year, with the cooperation of the National Academy of Sciences, the program of the office will be broadened to include a number of projects involving scientists from many different countries. By the end of the year a San Francisco International Relations Center for Scientists and Engineers will be in operation. The main functions of the center will be: (1) to facilitate contacts between the visiting scientists and appropriate scientific personnel in the area; (2) to serve as a liaison office between the National Research Council and the local scientific organizations and societies, and as a general coordinating agency for international scientific activities in the area; (3) to offer such other assistance to visiting scientists as other institutions of the area may not be equipped to give.