Fractions of Human Milk and Virus Multiplication[1]

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Human milk contains growth-promoting factors for *Lactobacillus bifidus* var. Penn (*1, 2*). Chemically these factors belong in the group of N-containing polysaccharides (mucopolysaccharides) (*3–5*). In the past, antiviral effect has been ascribed to various mucoproteins and polysaccharides (*6*). The protective effect of different fractions of human milk against several neurotropic viruses has also been reported (*7*). Such observations made it desirable to investigate the possible inhibitory effect of fractions of human milk obtained in the course of the purification of the growth factors for *L. bifidus* var. Penn on virus multiplication.

Preparation of Human Milk Fractions (HMF). The fractions were obtained from 5 different pools of human milk. The pools were skimmed, deproteinized with $ZnSO_4$ and $Ba(OH)_2$ and desalted by means of ion exchange. Subsequently, the materials were adsorbed onto charcoal, washed repeatedly with water, dilute acetic acid, and finally eluted (6 times) with 20% acetic acid and 5% phenol. The details of the procedure have been described elsewhere (*5*). The 6 eluates derived from each of the 5 pools of human milk were lyophilized separately and the dried materials, in appropriate amounts, were dissolved in buffered saline solution for use in the experiments. The pH was adjusted to 7.0–7.2. On testing, only the 3rd, 4th, and 5th eluate showed some degree of inhibition of viral multiplication. Since heating of the HMF solutions to 120° C for 15 min did not destroy the activity, the materials were autoclaved before testing. Up to 30 times the active amount per embryo showed no toxic effect on chick embryos.

The PR8 strain of influenza A and the Enders strain of mumps virus were employed in the experiments. None of the HMF's, in amounts up to 200 mg/ml, inhibited the agglutination of chicken erythrocytes by 4 units of heated influenza A or mumps viruses.

Effect on Virus in vitro. To a solution of HMF (50 mg/ml) was added an equal amount of a 1:10 dilution of active PR8 or mumps virus. Mixtures of equal amounts of saline solution and the virus dilution served as control samples. The mixtures were incubated at temperatures ranging from 4° to 37° C for periods from 2 to 18 hr. After the incubation periods, the infectivity titers for chicken embryos were determined. No significant differences were found between the infectivity titers of the virus samples treated with HMF and the controls.

Effect of Active Influenza Virus and RDE on HMF. HMF solutions (50 mg/ml) were incubated *in vitro* for 18 hr at 37° C with an equal volume of a preparation of active influenza virus containing 10^3 hemagglutinating units/ml or with the receptor-destroying enzyme (RDE) added to a final concentration of 10% (100 units/ml). At the end of the incubation period, the mixtures were heated at 120° C for 15 min. The treatment of the HMF solution with the virus or the RDE did not diminish the inhibitory effect on the propagation of the influenza and the mumps viruses as tested *in vivo*.

Inhibition of Multiplication of Influenza and Mumps Viruses. Influenza: 11-day-old chick embryos were used. The HMF solutions were injected by the allantoic route, followed immediately by a challenging dose of 100 ID_{50} of the influenza virus. An aliquot of the allantoic fluid from each embryo was removed after 24 hr and the samples were pooled. After 48 hr the remainder of the fluids was harvested from each egg and also pooled. In control series, saline solution was substituted for the HMF solutions. The degree of viral propagation was determined by titration of the hemagglutinin in the allantoic fluid pools with 1% chicken erythrocytes employing the pattern test. In several experiments, the infectivity titer of the pools for chicken embryos was also determined.

The HMF's showed considerable difference in their ability to inhibit the multiplication of influenza virus. The range was from 10 to 40 mg in 0.5 ml of saline solution/chick embryo. These amounts inoculated immediately before the challenging dose inhibited the multiplication to the extent that no hemagglutinin was detectable after 24 or 48 hr in the allantoic fluids of the treated eggs. Infectivity titrations in chick embryos of the allantoic fluids collected after 48 hr from the treated eggs showed usually less than 1% of the virus present in the controls. The infectivity titrations were performed in 10-fold steps using 5 eggs for each dilution of the pools.

Inoculation of the HMF solutions up to 24 hr before the virus gave identical results, but when the material was injected 2 hr after the virus, the inhibi-

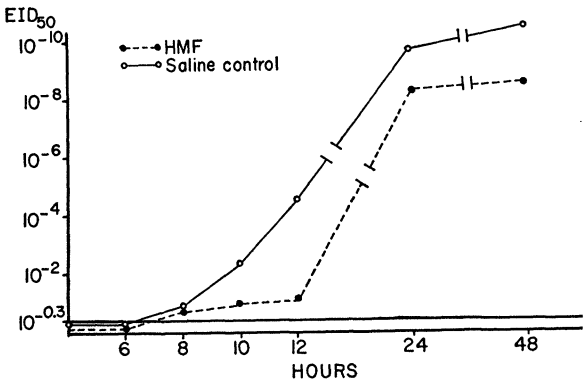

FIG. 1. Influenza virus A (PR8) growth curve in chick embryos in the presence of HMF (0–48 hr) infectivity titration of the allantoic fluids.

[1] Supported by a grant of Wyeth Laboratories, Philadelphia.

tory effect was diminished considerably. No hemagglutinin was detectable in the 24-hr harvest but after 48 hr the virus titers were similar to those of the controls. The effect was also less pronounced when the challenge dose of virus was increased to 10,000 EID_{50} and injected immediately after the HMF solution.

The influence of the HMF on the 48-hr growth curve of the PR8 virus is shown in Fig. 1. In this experiment, one series of 40 embryos was inoculated with a solution of 20 mg of HMF in 0.5 ml of saline by the allantoic route, and a 2nd series of 40 embryos with 0.5 ml of saline by the same route. Immediately thereafter both series were challenged intra-allantoically with 100 EID_{50} of PR8 virus. The allantoic fluids of groups of 5 eggs from each series were harvested at the indicated time intervals and pooled, and the infectivity titers for chick embryos of the pools were determined. In this experiment, HMF of low potency had to be used because none of the more potent fractions were available in the quantity needed for growth curves, yet significant differences in the treated and control series could be demonstrated up to the 48th hr of incubation (Fig. 1).

Mumps: Eight-day-old chick embryos were injected with 20 mg of HMF in 0.5 ml of saline solution intra-allantoically and subsequently infected with 10,000 EID_{50} of mumps virus by the same route. The allantoic fluids were harvested after 6 days and pooled. Viral hemagglutinin could not be demonstrated in the pools. The infectivity titrations of the 6-day pools of allantoic fluid showed 0.01% of the virus present in the control samples.

The inhibitor for influenza and mumps virus multiplication found in human breast milk is not identical with the mucoprotein inhibitor of hemagglutination since (a) the HMF does not inhibit hemagglutination; (b) its inhibitory effect is not destroyed by RDE or by incubation with active virus; and (c) the HMF's have been deproteinized.

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Manuscript received November 20, 1952.

Comments and Communications

2,4-D Herbicides Pose Threat to Cotton and Other Susceptible Crops

QUITE reminiscent of the cattle-sheep wars of the Old West is the present controversy between the cotton growers and rice growers in the South. The focal point of this argument is the vast fertile area known as The Delta in the state of Mississippi. However, the controversy is going on in many other areas, wherever 2,4-D herbicides are being used near susceptible crops such as cotton, grapes, beans, and several others.

The problem has become acute in the Mississippi Delta because of the recent introduction of rice as a crop. The growing of rice in this section is economically feasible. But one of the great problems is that of controlling coffee-weed, and this is where the 2,4-D herbicide enters the picture. Rice, of course, is a member of the grass family and as such is not sensitive to 2,4-D, but the coffee-weed is killed by it. The result is that 2,4-D in its various forms is sprayed on the rice paddies to control this weed. The herbicide can be applied by means of ground equipment, but it can be applied much cheaper as a spray from airplanes. This is where the trouble starts that has resulted in damage suits totaling several hundreds of thousands of dollars.

What makes the problem of scientific interest is the fact that cotton, especially, is almost fantastically sensitive to 2,4-D and related compounds. It has been stated facetiously that if one walks through a field of cotton with a label from a 2,4-D container in one's pocket the cotton will be damaged. Yet it is possible that if 2,4-D had been spilled on the label some damage to nearby cotton might occur.

To illustrate a similar condition, a case may be cited that occurred several years ago. On one large plantation a dry spray rig was driven down a plantation road between fields of cotton. This spray rig had not been used to spray 2,4-D since the year before. The amazing fact is that moderate but typical 2,4-D damage showed up on one side of this road for several hundred feet. Presumably this was on the side toward which the wind was blowing at the time the equipment was moved.

The above is an extreme case, to be sure, but many other instances could be cited almost as spectacular. During the summer of 1953 typical 2,4-D damage to cotton appeared along several highways in a number of southern states. As nearly as can be ascertained, this was caused by leaking containers of the herbicide that were being hauled along highways in trucks.

Most of the damage to susceptible crops appears to result from spray application either from the ground or from the air. The use of dusts containing 2,4-D or other hormone-type herbicides was outlawed by the Civil Aeronautics Authority several years ago because of the extreme hazard from drift. The spraying of 2,4-D from airplanes has often resulted in great loss. In one instance 4000 acres of vineyards were damaged by drift from air application of herbicide over grain fields 4–15 miles away. Case after case might be quoted where cotton was damaged both from the ground and from aerial application. It is not at all unusual for