Antiviral Activity of *Melissa officinalis* (Lemon Balm) Extract.* (29600)

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Melissa officinalis, also known as lemon or bee balm, is a medicinal plant of antiquity. Supposedly it is the "balm" of the Bible (Genesis 37:25, 43:11, King James version) (1), and it was mentioned as a useful medicament by Theophrast, Plinius, and Hippocrates(2). A substantial number of medicinal properties have been attributed to *M. officinalis,* starting with those described by Dioscorides during the first century A.D.(3). Numerous references indicate that, although some therapeutic effects are claimed for alcoholic extracts, the most common preparation, even today, is a hot-water extract which is usually consumed or, more rarely, is applied directly to the affliction. Originally native to Asia Minor and Southern Europe, melissa now is grown widely in Europe as a medicinal plant, as a flavoring, and, by beekeepers, as a rich source of nectar (hence, bee balm). Ethereal oil from melissa has been reported to have antibacterial action *in vitro*(4); aqueous extracts have been reported to have no activity against certain bacteriophage and influenza A virus in embryonated eggs(5). The following report will show, however, that hot-water extracts of melissa do have striking antiviral activity§ against Newcastle, Semliki Forest, vaccinia, and herpes simplex viruses in tissue culture and in embryonated egg systems.

Experimental. Preparation of melissa extract. Melissa can be readily obtained† as "lemon balm herb" in a variety of preparations, including the whole plant, leaves only, chopped, or fine powder. There are no clear data indicating that either the source or the physical form of the dry plant has any effect on the yield of antiviral activity. Usually a mixture of 10 g of plant material and 100 ml of distilled water was boiled for 10 to 20 minutes and then filtered, resulting in a clear dark-brown solution. All preparations were stored at 4°C; when dilutions of the extract were made, distilled water was used.

Antiviral tests in eggs. Embryonated eggs (White Leghorn) 9 to 11 days of age were injected via the allantoic sac with 0.3-ml volumes of virus and of test and control substances. Semliki Forest (mouse brain passage), Newcastle disease (11914, egg passage), vaccinia (WR, mouse brain passage), and herpes simplex (HF, mouse brain passage) viruses were all originally from the American Type Culture Collection (ATCC) and were all used at the appropriate dilution in tryptose phosphate broth. Melissa extracts injected into eggs prior to inoculation with virus protected the eggs from the lethal action of the above 4 viruses (Table I). Even when melissa extract was given 3 to 4 days before Newcastle virus, the protection was substantial. With eggs infected with vaccinia virus it was possible to save eggs from death when melissa extract was administered a few hours after virus. Melissa extract did not protect eggs from the lethal action of ATCC egg-adapted strains of influenza A (PR8) and B (Great Lakes) viruses.

Antiviral tests in tissue culture. In the paper-disc plaque-inhibition test(6), antibiotic-sensitivity discs impregnated with melissa extract induced zones of plaque inhibition in cultures of the 4 sensitive viruses listed above. Monolayers of chick embryo cells in 100-mm plastic petri dishes (Falcon, tissue culture type) were infected with 1 ml of virus suspension and then overlaid with 15 ml of agar medium without neutral red(7). After 4 days of incubation (usually 3 days in the case of Newcastle virus) at 36°C the cells were vitally stained with 0.15% iodonitro-

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§ The antiviral activity described in this paper was first observed while one of the authors (E.C.H.) was employed by the Schering Corp., Bloomfield, N. J.

† S. B. Penick & Co., or Meer Corp., New York City.

TABLE I. Antiviral Effect* of *Melissa officinalis* Extract.

Virus	Virus dose†	Virus time‡	Melissa dose (mg/egg)§ 9.6	4.8	2.4	1.2	0.0
Newcastle	10	+ 3	—	8/8 (1)	12/12 (2)	10/12 (2)	0/10 (2)
	100	— 2	0/16 (2)	—	—	—	0/8 (1)
		+ 3	—	66/72(15)	51/71(15)	21/73(15)	0/61(15)
		+24	17/17 (2)	—	—	—	1/17 (2)
		+48	9/10 (1)	—	—	—	0/10 (1)
		+72	5/7 (1)	—	—	—	0/10 (1)
	1000	+ 3	15/17 (3)	11/17 (3)	3/5 (1)	—	0/17 (3)
Herpes simplex	30	+ 3	—	4/5 (1)	8/10 (2)	6/10 (2)	0/13 (2)
	300	+ 3	—	5/10 (2)	4/10 (2)	2/8 (2)	0/13 (2)
	3000	+ 3	—	1/5 (1)	0/5 (1)	0/5 (1)	0/8 (1)
Vaccinia	10	—14	0/9 (1)	—	—	—	0/10 (1)
		— 2	7/9 (1)	—	—	—	0/10 (1)
		+ 2	6/9 (1)	—	—	—	0/10 (1)
	100	+ 2	7/8 (1)	5/9 (1)	—	—	1/9 (1)
Semliki Forest	10	+ 2	6/10 (1)	6/10 (1)	5/10 (1)	—	0/10 (1)
	100	+ 2	5/10 (1)	2/10 (1)	2/10 (1)	—	0/10 (1)
	1000	+ 2	0/10 (1)	0/10 (1)	—	—	0/10 (1)

* Expressed as No. of survivors/No. of eggs inoculated. Figures in parentheses indicate No. of determinations. Data are accumulated from experiments with a number of batches of melissa.

† Expressed as No. of 50% lethal doses.

‡ Expressed as hr before (—) or after (+) injection of melissa extract.

§ Estimated weight determined from dry weights of a number of melissa batches.

tetrazolium chloride(6) contained in 7 ml of a second 1.5% agar overlay. The tetrazolium salt was reduced by the live cells to the brilliant red-purple formazan, thus making plaques and plaque-free zones readily observable.

When ¼-inch antibiotic-sensitivity discs were impregnated with 0.02 ml of melissa extract, plaque-free zones 20 to 30 mm in size were produced in cultures containing 2 to 7 $\times$ 10^3 herpes simplex virus plaques. Similar discs produced plaque-free zones measuring 12 to 16 mm in cultures containing 100 to 200 Semliki Forest virus plaques while 14 to 21-mm zones were produced in cultures with 8 $\times$ 10^2 to 8 $\times$ 10^3 Newcastle disease virus plaques. With vaccinia virus, ½-inch antibiotic-sensitivity discs were used containing 0.08 ml of melissa extract and 40 to 50 mm zones were produced in cultures containing 600 plaques. Within certain limits, this same method can be used for assaying melissa antiviral activity; thus far, however, assays in eggs seem to be more reproducible. It is thought that the plaque-inhibition test lends itself better to testing therapeutic drugs like 5-iododeoxyuridine(8) which produce very large plaque-free zones. Melissa extract, however, seems to act in a protective manner and is at some disadvantage in this type of test since it is applied after virus infection, a situation in which its activity is less than optimal.

So far, melissa extract has not suppressed Newcastle virus infections in monkey kidney cells in liquid culture, nor has it had any effect on plaque formation of vaccinia virus in monkey kidney cells. This suggests that the antiviral activity of melissa extract may be specific for chicken cells.

Effect of gelatin on melissa extract. Melissa contains about 5% tannins(9) and it was thought that the antiviral activity might reside in this fraction. Indeed, when an equal volume of a 1 or 10% gelatin (Nutritional Biochemicals) solution was added to melissa extracts, a heavy precipitate developed which is typical of tannins and of a few nontannin plant polyphenols. On testing the supernate in eggs, no antiviral activity was detected. The precipitate was washed with distilled water and then suspended in a solution of

TABLE II. Inhibition of Melissa Antiviral Effect by Gelatin.

Virus and dose	Time of inj prior to virus inj (hr) 10% Gelatin (.3 ml)	Melissa (9.4 mg)	Water (.3 ml)	Survivors/ total eggs*
Newcastle (100 × LD_{50})	19 to 24	3 to 7	—	3/16 (2)
	24	—	7	0/9 (1)
	3 to 7	19 to 24	—	4/20 (2)
	—	19 to 24	3 to 7	17/19 (2)
	—	3 to 7	19 to 24	17/19 (2)
	—	—	19	0/10 (1)
	—	—	—	0/14 (2)
Herpes simplex (100 × LD_{50})	24	7	—	2/9 (1)
	7	24	—	0/9 (1)
	—	24	7	6/10 (1)
	—	7	24	6/9 (1)
	—	—	—	0/5 (1)

* Numbers in parentheses indicate No. of experiments.

0.05% trypsin (Difco) and 0.05% EDTA (Versene, Fisher) in a magnesium and calcium-free Earle's balanced salt solution. After several hours at 37°C, the precipitate was digested; the trypsin action was destroyed by heating at 56°C for 1 hour and this digest, when tested in eggs, showed considerable protective activity against Newcastle virus. Gelatin alone treated in a similar manner had no activity.

When 0.3 ml of 10% gelatin was injected into eggs prior to or after injection of melissa extract, the protective effect was all but eliminated (Table II). On the other hand, the medium used in the agar-overlay tissue culture tests contained 0.5% gelatin which by no means eliminated the antiviral effect. Even when gelatin was removed from the medium, no marked increase in size of the plaque-free zones occurred, although a slight increase in zone size did at times seem to occur. Presently, there is no explanation why gelatin has such a dramatic effect on antiviral activity of melissa extract in eggs but not in plaque plates. It is thought that the active agent(s) of melissa does not necessarily react with all proteins since mixture of melissa extract with bovine serum in no way affected the antiviral activity as measured in virus-infected eggs. Melissa extract did not form precipitates when mixed with either bovine serum or bovine serum albumin. It also has been found recently that, when the active agent is freed from gelatin by trypsin-EDTA digestion, it no longer will precipitate gelatin. In this case, the sites of gelatin-attachment may still be occupied by peptides, but this does not seem to inhibit the antiviral activity.

Additional properties of melissa extract. Melissa extracts retain substantial antiviral activity after having been autoclaved for 15 minutes. The active principle seems to be quite soluble in water but not in common organic solvents. Lead acetate will precipitate the active portion, suggesting that it is a plant polyphenol(10). The active material can be freed from lead by addition of AG-50W-X resin (Calbiochem) in the hydrogen form.

Melissa extract was not virucidal for Semliki Forest, Newcastle, vaccinia, and herpes simplex viruses. These viruses were added directly to undiluted extracts of melissa, left at room temperature for 1 hour, and then diluted, in 10-fold increments, in tryptose phosphate broth. Plaque counts in chick cell monolayers agreed closely with those from virus controls containing no melissa. When mixed with influenza A or B virus, melissa extract had little effect on the lethality of this virus for eggs.

Some eggs are killed by injection of 0.3 ml of undiluted melissa extract into the allantoic sac. It was thought, however, that the true toxicity of the antiviral agent could only be resolved once pure material was available. Already there is some indication that the gelatin precipitation technic frees melissa extract of some toxicity. Experiments in mice

have been postponed until it has been determined whether melissa extract has any action on cells other than those of the chicken. Apparently this crude material is not very toxic for mice since they tolerate 10 mg injected subcutaneously daily for 5 days. This again has little relationship to the toxicity that might be seen in a purified preparation. Experiments in chickens, however, must now be considered.

Preliminary experiments indicate that melissa extract suppresses the production of infectious Newcastle virus. It is suggested that, since the active agent has an affinity for at least one protein, it may also have a similar affinity for a few others, such as those at the cell surface. Also, melissa extract is protective in its action and this protection can be reversed by gelatin, which suggests that its antiviral effect involves the cell surface, perhaps blocking the entrance or exit of certain viruses. There is no evidence that this reaction involves the myxovirus receptors since neither influenza A or B can be prevented from killing eggs. Although there is suggestive evidence that the antiviral substance in melissa extract is a tannin or tannin-like polyphenol, its activity is unrelated to that of tea tannins(11) or tannic acid(12) which are virucidal and act primarily on influenza A virus.

Summary. Hot-water extracts of the plant *Melissa officinalis* (lemon balm herb), when injected into embryonated eggs, protect them against the lethal action of Semliki Forest, Newcastle, vaccinia, and herpes simplex viruses. Plaques produced by these viruses in chick embryo cell monolayers can be suppressed by applying melissa extract-impregnated antibiotic-sensitivity discs to the agar overlay surface. Injection of 10% gelatin into eggs before or after injection of melissa extract largely eliminates the antiviral effect. Melissa extracts precipitate gelatin and the antiviral activity can be recovered from the precipitate. It is suggested that the active moiety is a tannin or tannin-like polyphenol that perhaps acts at the cell surface.

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