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The Antiviral Activity of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride *in Vitro*, *in Ovo*, and in Small Laboratory Animals (33335)

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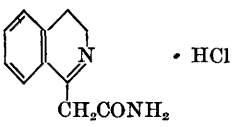
Recent years have witnessed the development of antiviral substances which have been either somewhat group specific in their effect or which have possessed a slightly broader antiviral spectrum. Examples of the former type are the isatin thiosemicarbazones for the pox virus group (1–3), iododeoxyuridine for DNA viruses, especially herpes (4, 5) and amantadine hydrochloride for influenza A (6, 7). Examples of the latter type are 2-dehydroemetine (8) and 1,1,3,3-tetracyanopropene (9) both of which exerted activity *in vivo* against Columbia SK, herpes and Coxsackie virus B1. The present report describes the results of experiments with a new substance, 3,4-dihydro-1-isoquinolineacetamide hydrochloride (10) (DIQA), showing activity *in vivo* against both RNA and DNA viruses but a very narrow spectrum of activity *in vitro*.

Materials and Methods. 3,4-Dihydro-1-isoquinolineacetamide hydrochloride (DIQA) is an odorless, white crystalline solid having the following structural formula:



The substance is highly soluble in water (20% at 20°) and alcohol (10% at 23°). Aqueous solutions are acidic, stable, and unchanged by heat.

In all the present experiments the test compound was dissolved in distilled water and prepared fresh before each experiment.

I. Acute toxicity. The LD_{50} values for mice based on survivors 72 hr after administration of a single dose (mg/kg) were as follows: 375 subcutaneously, 375 intraperitoneally and 750 orally. In addition, the substance exerted an hypnotic effect in all of the animals studied; the HD_{50} in mice was approximately 100 mg/kg intraperitoneally.

II. Preparation of virus pools. a. Columbia SK virus. Swiss albino mice weighing 9–12 g were etherized and infected intracerebrally with a brain suspension of Columbia SK virus. After 48 hr, mice showing signs of paralysis were sacrificed; the brains were excised, minced in saline, homogenized in a high speed blender, and stored frozen as a 20% suspension (11).

b. Herpes virus (Nutley strain). Brain pools were prepared employing the technique described for Columbia SK virus. This strain, isolated from a patient in Nutley, N. J., displays virulence for mice when injected intraperitoneally.

c. Influenza viruses. The PR8 and Asian (Japan 305/57) strains of influenza A virus were propagated in the chorioallantoic cavity of 10-day-old chick embryos. After incubation at 37° for 48 hr, the infected embryos were chilled overnight in the refrigerator followed by aspiration of the chorioallantoic fluid which was then stored frozen until use. Influenza B (DP/66) was propagated at 37° for 72 hr in rhesus monkey kidney cells fed with Eagle's basal medium containing Earle's base and glutamine. Bovine albumin was added to a final concentration of 0.5% to the supernatant of tubes which had shown hemadsorption with guinea pig red blood cells and then stored frozen until use.

d. ECHO 9 virus (Coxsackie virus A 23). Two- to 4-day-old suckling mice were infected subcutaneously with ECHO 9 virus derived from infected monkey kidney cells. When flaccid paralysis of the limbs occurred the mice were killed, the brains were removed and a 10% suspension prepared in saline as described for Columbia SK virus. The supernatant material from this centrifuged suspension was inoculated into rhesus monkey kidney cell cultures containing penicillin (50 units/ml) and streptomycin (50 μg/ml) and fed with medium 199. The tubes were placed in a roller drum and incubated at 37° until typical cytopathic effects were noted. At this time the supernatant fluids were pooled and stored frozen until use.

e. Coxsackie virus B1. Adult Swiss albino mice (Charles River CD-1) were infected intraperitoneally with the GP strain of Coxsackie virus B1. After 72 hr the animals were sacrificed, the livers were excised, minced and homogenized as described for Columbia SK virus and inoculated into rhesus monkey kidney cell cultures as described by Grunberg and Prince (12). The supernatant fluids from tubes showing typical cytopathic effects were pooled and stored frozen until use.

f. Herpes simplex virus (HF Smith and HF 378 strains).[1] The virus was propagated in rabbit kidney cell cultures fed with medium 199. The tubes were incubated at 37° until typical cytopathic effects were noted. The supernatant fluids were then pooled and stored frozen until use.

g. Newcastle disease virus (California strain). The virus was propagated in the chorioallantoic cavity of 10-day-old chick embryos. After incubation at 37° for 48 hr, the infected embryos were chilled overnight in the refrigerator followed by aspiration of the chorioallantoic fluid, which was then stored frozen until use.

III. *Infection and treatment of animals.* For all virus infections, the animals were observed daily for signs of illness and death for a period of 21 days. Where greater than 50% survival was noted, the 50% curative dose (CD_{50}) was calculated according to the method of Reed and Muench (13); when CD_{50} values could not be obtained, the activity of the drug was described in terms of the percentage surviving and the mean survival time. In the nonlethal influenza infection of ferrets, temperature response was employed as the criterion for infection and illness.

a. Columbia SK, Herpes (Nutley) and

[1] The HF Smith strain was obtained through the courtesy of Dr. James Smith, Syracuse University while the HF 378 strain was supplied through the courtesy of Dr. M. R. Hilleman, Merck Institute for Therapeutic Research.

Coxsackie virus B1. Swiss albino mice weighing 9–12 g (18–20 g in the case of Coxsackie virus B1) were infected intraperitoneally with 0.1 ml of a dilution of the appropriate virus pool containing approximately 10 LD_{50}. The drug was administered in 0.5- or 1.0-ml amounts by the intraperitoneal route at the following times relative to infection: 24 hr before, immediately, and 24 hr after. All control animals were dead usually by the eighth day after infection.

b. ECHO 9 virus. Two- to 4-day-old suckling albino mice were infected subcutaneously with 0.1 ml of a dilution of the virus pool containing approximately 10 LD_{50}. The antiviral agent was administered in 0.1-ml amounts at the following times relative to infection: 24 hr before, immediately after, and then once daily for 5 consecutive days following infection. Control animals were dead usually by the tenth day after infection.

c. Influenza viruses. Swiss albino mice weighing 9–12 g were lightly anesthetized with ether and then infected intranasally with approximately 0.05 ml of an appropriate dilution of chorioallantoic or tissue culture fluid as described by Prince and Grunberg (14). The infected mice were treated intraperitoneally with 0.5-ml amounts of the antiviral agent at the following times relative to infection: immediately before and 1, 5, 24, 30, 48, and 72 hr after. Control mice had succumbed usually by the tenth day after infection.

Ferrets, weighing approximately 500 g, were isolated for 10 days. Daily determinations were made of the rectal temperature to accustom the animals to handling and to obtain a baseline of normal values (101–103°F). The animals were infected with the PR8 strain of influenza A virus under light ether anesthesia by instillation into the nostrils of 1.0 ml of undiluted chorioallantoic fluid or 1.0 ml of the supernatant of a 5% ferret lung suspension. The effect of repeated subcutaneous administration of the antiviral agent on the course of experimental ferret influenza was observed using monophasic temperature rise (104°F or above) as the criterion for infection (14).

d. Newcastle disease virus. One- to 2-week-old white leghorn chicks obtained from nonvaccinated flocks (SPAFAS) and weighing approximately 100 g were infected intraperitoneally or subcutaneously with 0.5 ml of a dilution of chorioallantoic fluid containing approximately 10 LD_{50} of virus. The drug was administered intraperitoneally or subcutaneously seven times as described for the influenza infection of mice, or intraperitoneally three times as described for the Columbia SK infection of mice. Control animals were all dead usually by the tenth day after infection.

e. Herpes simplex virus (HF Smith and HF 378 strains). Rabbits weighing 1–2 kg were anesthetized with sodium pentobarbital and the cornea was scarified in crosshatch with a scalpel 3 times in each direction. Two drops of a 1–10 dilution of virus harvested from rabbit kidney cell cultures were instilled onto the cornea. Treatment was started 1 hr after infection by administration of 2 drops/eye of the test solution. Treatment was continued 3 times a day for 5 days and then 2 times a day for 2 days. The rabbits were observed up to a total of 14 days. Prevention of keratitis was used as the criterion for antiviral activity.

IV. Antiviral tests employing the chick embryo. Ten-day-old chick embryos were treated with the maximum tolerated dose of 2.5 mg of the drug/egg administered in a 1.0 ml amount into the chorioallantoic cavity. One hr later six embryos each were infected intraallantoically with 0.1 ml of an appropriate dilution of chorioallantoic fluid containing approximately 10 EID_{50} of influenza A (PR8) or influenza A (Japan 305/57) viruses. The infected and treated groups as well as untreated controls were incubated for 48 hr at 37° after which time the embryos were examined for viability; in addition, hemagglutination titers were determined employing a 0.5% suspension of washed chick red blood cells mixed with dilutions of the harvested chorioallantoic fluid and incubated for 2 hr at room temperature.

V. In vitro *tests for antiviral activity. a. Contact experiments for direct virus inactivation. In vitro* contact experiments were performed with the Columbia SK and herpes

(Nutley) viruses in the following manner: a virus suspension (1:100 saline dilution of infected mouse brains) was incubated for 1 hr at room temperature in the presence of 2000 μg/ml of the antiviral agent in saline. Control suspensions were similarly incubated in the presence of saline alone. At the end of the incubation period the drug-treated and the control virus suspensions were diluted in saline by 10-fold increments to a dilution of 10^{-7}. Swiss albino mice weighing 9–12 g were then infected intraperitoneally with 0.1-ml amounts of each dilution from the treated and control virus suspensions. The animals were observed for a total of 21 days. The comparative virulence of the drug-treated and the saline-treated virus suspensions as a measure of virus inactivation was then determined by calculation of the LD_{50} values.

b. Tissue culture experiments. As a general procedure all cultures were incubated in the presence of 100–200 μg of the substance/ml 24 hr before infection with various dilutions of the virus. The substance was left in contact with the infected cells throughout the entire incubation period, which varied from 2 to 10 days, depending upon the virus employed. The $TCID_{50}$ value obtained from treated cells was compared with that obtained from untreated cells and a difference of 2 logs or more was considered to represent an antiviral effect.

In experiments with ECHO 9, Coxsackie virus B1 and poliovirus type I (Mahoney strain), tubes of rhesus monkey kidney cells maintained with medium 199 were observed daily for cytopathic effects; rabbit kidney cells maintained with medium 199 were employed in similar experiments with the HF Smith strain of herpes simplex virus.

For experiments with the PR8 and Japan 305/57 strains of influenza A virus and the DP/66 strain of influenza B virus, tubes of rhesus monkey kidney cells, previously grown in the presence of SV5 antiserum, were maintained with Eagle's basal medium containing 0.11% $NaHCO_3$ (final pH = 6.8) and supplemented with L-glutamine to a final concentration of 0.002 mmoles/ml. Influenza-infected cultures were incubated for 3 days at 35° after which time the supernatant fluid was removed. The presence or absence of virus was determined by the hemadsorption technique employing 0.2 ml of a 0.4% suspension of washed guinea pig erythrocytes incubated with the infected monkey kidney cells for 20 min at 4°.

Tissue culture experiments with measles virus were conducted in African green monkey kidney cell cultures maintained with Eagle's basal medium supplemented with L-glutamine and calf serum to a final concentration of 2%. The cells were incubated for 8 days after which time the presence or absence of virus was determined by the hemadsorption technique employing 0.2 ml of a 0.5% suspension of washed rhesus monkey red blood cells incubated with the infected cell sheet for 1 hr at room temperature.

For vaccinia virus, in addition to comparative $TCID_{50}$ as judged by cytopathic effect, activity was also determined by the plaque-reduction method of Nishmi and Keller (15) modified by the use of Eagle's basal medium containing horse serum to a final concentration of 10%, rhesus monkey kidney cells instead of HeLa cells, and stained for 1 hr at 37°. Cells were infected and adsorption allowed to take place for 4 hr at 37°, after which time the inoculum was washed off and the tubes were refed followed by reincubation for 40–48 hr at 37°. The tubes were then emptied, stained with neutral red, and read against an illuminated white background for presence or absence of plaques. A reduction in plaque count of 50% or more as compared to untreated controls was considered to represent an antiviral effect. The substance was in contact with the cells 24 hr prior to infection but was removed during the period of infection and adsorption. The drug was added once again to the cells after adsorption and was present throughout the remainder of the incubation period.

The Columbia SK and herpes (Nutley) strains employed produced neither a cytopathic effect nor hemagglutination. Accordingly, cultures were incubated "blind" for 8 days in the presence of 100 μg/ml of the drug added to the tubes 24 hr before infection with various dilutions of the virus. After this time, the presence of virus was determined

TABLE I. Effect of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride (DIQA) on Neurotropic Virus Infections of Mice.

Virus	Route of infection	Treatment schedule[a]	CD_{50} (mg/kg)[b]
Columbia SK	i.p.	i.p. × 3	102
Herpes (Nutley)	i.p.	i.p. × 3	108
ECHO 9	s.c.	s.c. × 7	77
Coxsackie virus B1	i.p.	i.p. × 3	inactive at 200 mg/kg

[a] Intraperitoneally 24 hr before, immediately after, and 24 hr after infection; subcutaneously 24 hr before, immediately after, and once daily for 5 days after infection.

[b] Based on survivors at 21 days after infection, survivors accumulated from 7 experiments, 10 animals/dose.

by intraperitoneal infection of weanling mice with 0.1 ml of undiluted tissue culture fluid (medium 199). Death of the animals was taken to indicate presence of virus in the cell cultures. A difference of 2 logs or more between the $TCID_{50}$ of the treated and untreated cells was taken to represent an antiviral effect.

Results and Discussion. I. In vivo *experiments. a. Columbia SK, herpes (Nutley), ECHO 9 and Coxsackie virus B1.* The results of experiments in mice with neurotropic infections produced by the above viruses are given in Table I. The data in Table I show that DIQA exerted an appreciable antiviral effect against the Columbia SK herpes, and ECHO 9 infections of mice but was without effect when tested against Coxsackie virus B1. It should be pointed out that protection of mice from death in the Columbia SK and herpes infections could be accomplished only if an intraperitoneal treatment schedule was employed in which a dose was administered immediately after infection. Repeated dosing by the subcutaneous or oral routes or dosing by the intraperitoneal route with omission of treatment immediately following infection were without effect in suppressing the lethal effects of these infections. Because of the small size of the suckling mice used in the ECHO 9 experiments, only subcutaneous drug administration was employed.

Although subcutaneous administration of the drug to mice infected intraperitoneally with Columbia SK virus did not protect against death, additional experiments were performed to determine whether or not repeated treatments by the subcutaneous route could decrease the amount of virus appearing in the brain after 72 hr, as compared to untreated controls. Accordingly, 10 mice weighing 9–12 g were infected with 10 LD_{50} of virus intraperitoneally and treated subcutaneously with 100 mg of the agent/kg at the following times relative to infection: 30, 24, and 2 hr before followed by 2, 24, 30, and 48 hr after. At 72 hr the mice were killed, the brains were removed, triturated in a mortar and pestle and diluted by 10-fold increments in saline. Comparative virulence titrations of the pooled brain suspensions from the treated as well as from untreated mice were performed by intraperitoneal injection of 0.1 ml of the various dilutions into weanling mice.

The results of the experiments revealed that repeated subcutaneous administration of the agent, although not effective in suppressing the lethal effects of Columbia SK virus, quantitatively decreased the amount of virus appearing in the brain after 72 hr as compared to the controls (LD_{50} of mouse brain suspension from treated mice $= 10^{-6.4}$ vs LD_{50} of mouse brain suspension from control mice $= 10^{-8.4}$). Thus the substance produced a sufficient effect to reduce the yield of virus in the brains of the treated mice by a factor of 100.

b. Influenza viruses. The effects of the drug against the PR8 and Japan 305/57 strains of influenza A virus in mice are summarized in Table II.

The data in Table II show that DIQA produced a definite increase in the number of survivors at 10 and 21 days when compared to the untreated controls. For the PR8 strain, statistical analysis showed that for both the 10- and 21-day periods the survival rates were significantly ($p < .001$) higher in the treated group. For the Japan 305/57 strain, the 10- and 21-day survival rates were also significantly higher in the treated group ($p < .01$ and $p < .05$, respectively). The effects on survival, based on a maximum of 21 days,

TABLE II. Effect of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride (DIQA) on Intranasal Influenza A Infections of Mice.

Strain	Dose (mg/kg; i.p. × 7[a])	Treated survivors at days 10	Treated survivors at days 21	MST[b]	Controls survivors at days 10	Controls survivors at days 21	MST[b]	T/C[c]
PR8	100	72/182[d] (40)	46/182 (25)	12.8	24/165 (14)	12/165 (7)	8.3	1.5
Japan 305/57	100	13/23 (56)	11/23 (47)	13.2	2/19 (10)	2/19 (10)	8.8	1.5

[a] Immediately before infection and then 1, 5, 24, 30, 48, and 72 hr after infection.
[b] Mean survival time calculated in days.
[c] MST of treated animals/MST of control animals.
[d] Number of survivors/no. in group; percentage given in parentheses.

were also reflected in a longer mean survival time for the treated animals as compared to the controls. Accordingly, the T/C ratio for the drug was 1.5 in the PR8 and Japan 305/57 infections, representing a 50% increase in survival time. DIQA was inactive in the influenza B infection of mice when administrered intraperitoneally at a dose of 100 mg/kg.

The effect of DIQA on the course of experimental influenza in ferrets as judged by temperature response is shown in Table III.

TABLE III. Effect of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride (DIQA) on the Intranasal Influenza A (PR8) Infection of Ferrets.

Experiment	Inoculum	No. of animals with temperature rise[a]/no. of animals infected: 104°F or higher	105°F or higher
1. Treated[b]	Lung	1/6 (16)[d]	0/6 (0)
Controls		5/6 (83)	2/6 (33)
2. Treated	Chick CAF[c]	6/11 (55)	3/11 (27)
Controls		9/10 (90)	4/10 (40)
3. Treated	Chick CAF	6/10 (60)	0/10 (0)
Controls		9/10 (90)	5/10 (50)
Total			
Treated		13/27 (47)	3/27 (11)
Controls		23/26 (88)	11/26 (42)

[a] Occurred 24–72 hr after infection in all cases.
[b] Subcutaneously (100 mg/kg) at the following times relative to infection: 24 and 2 hr before, 2, 24, and 48 hr after.
[c] CAF = chorioallantoic fluid.
[d] Percentage given in parentheses.

When each experiment was statistically analyzed individually, the control groups showed no significant differences among the three experiments at either 104 or 105°F. The treated groups showed some, but still not a significant difference among the three experiments at both temperature levels. Since the ferret experiments thus appeared to be homogeneous and because there was rather too little data in any one experiment to obtain treatment significance, it was felt appropriate to pool the experiments in order to make the test of treatment. This was done (see totals, Table III) and the 104°F temperatue rise was significantly ($p < .01$) less frequent in the treated group than in the control group. Moreover, the 105°F temperature rise was significantly ($p < .05$) less frequent in the treated group than in the control group.

Thus the data indicate a consistent antiviral effect due to DIQA as measured by a decrease in the number of acute febrile episodes as compared to the untreated controls. Lethargy, sneezing, and other clinical signs were not used as indicators of illness since they were not consistently a part of the experimental disease. The ability of the drug to decrease the number of febrile episodes was not an antipyretic side effect since it was possible to show that the antiviral agent had no effect in ferrets on the pyrogenicity of *Escherichia coli* lipopolysaccharide.

c. Newcastle disease virus. In the intraperi-

TABLE IV. Effect of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride (DIQA) on the Development of Herpes Keratitis of the Rabbit Eye.

Herpes strain	Concentration of DIQA (mg/ml)[a]	No. eyes negative/no. eyes infected: Treated	Controls	$p <$
HF Smith	200	25/42 (60)[b]	3/36 (8)	0.001
	100	5/16 (31)	3/36 (8)	0.10
HF 378	200	16/24 (67)	0/24 (0)	0.001
	100	8/24 (33)	0/24 (0)	0.01

[a] Beginning 1 hr after infection, 3 times daily for 5 days and 2 times daily for 2 days; each treatment 2 drops/eye.

[b] Percentage given in parentheses.

toneal infection, the agent was without effect when 100 mg/kg was administered intraperitoneally three times to chicks; the rate and extent of death was similar to those observed for untreated controls. Similarly the substance was inactive (50 mg/kg) when administered intraperitoneally seven times to chicks. In the subcutaneous infection, the substance was inactive when administered subcutaneously seven times at a dose of 200 mg/kg.

d. Herpes simplex (HF strains). The results of experiments with herpes keratitis are summarized in Table IV. These data show clearly that DIQA effectively suppressed the development of keratitis as produced by the 2 strains of herpes simplex virus tested.

II. Experiments with the chick embryo. The substance at a level of 2.5 mg/egg was without antiviral activity against the PR8 and Japan 305/57 strains of influenza A virus as judged by viability of the embryos and hemagglutination titers of the chorioallantoic fluid.

For the PR8 strain, 1 out of 12 embryos survived the infection. The geometric mean hemagglutination titer for the 12 eggs was 1:1024. In the controls, 3 out of 11 survived the infection with a geometric mean hemagglutination titer of 1:938.

For the Japan 305/57 strain, 1 out of 12 embryos survived. The geometric mean hemagglutination titer for the 12 eggs was 1:832. In the controls, 2 out of 11 survived the infection with a geometric mean hemagglutination titer of 1:896.

III. In vitro *experiments. a. Contact studies with Columbia SK and herpes (Nutley) virus.* The results of contact experiments in which suspensions of the above viruses were incubated in saline for 1 hr at room temperature in the presence of 2000 μg/ml of the antiviral agent are summarized in Table V.

The data in Table V show that the compound was not able to exert a direct antiviral effect *in vitro* on the viral particles per se. These data may also indicate that the previously described activity of this substance against these same viruses *in vivo* cannot be explained on the basis of localized inactivation within the peritoneal cavity of the mouse.

b. Tissue culture experiments. The results of tissue culture experiments are summarized in Table VI. The data in Table VI reveal that the compound is an antiviral agent with limited activity in cell cultures, being without effect against the six viruses that it was active against *in vivo*. Conversely, the activity noted in tissue culture against Coxsackie virus B1 was not observed in mice. This lack of correlation is in contrast to other known antiviral agents with the exception of another isoquinoline derivative, 2-dehydroemetine, which is inactive in tissue culture but active *in vivo* against Columbia SK virus (8).

It should be noted that in the case of

TABLE V. *In Vitro* Effect of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride (DIQA) on the Infectivity of Columbia SK and Herpes Virus Suspension.

Virus	LD_{50}[a] of virus suspension after incubation for 1 hr at room temperature in the presence of: Saline + DIQA[b]	Saline alone	Δlog
Columbia SK	$10^{-6.4}$	$10^{-6.7}$	0.3
Herpes (Nutley)	$10^{-6.8}$	$10^{-6.5}$	0.7

[a] Determined by titration of incubation mixtures intraperitoneally into 9–12 g mice.

[b] Concentration = 2000 μg/ml.

TABLE VI. Activity of 3,4-Dihydro-1-isoquinolineacetamide Hydrochloride (DIQA) in Rhesus Monkey Kidney Cell Cultures.

Virus	Concentration (μg/ml)	$TCID_{50}$ Treated	$TCID_{50}$ Controls	Δlog	Parameter measured	Tissue culture activity	*In vivo* activity
Coxsackie virus B1	200	$10^{-1.2}$	$10^{-3.8}$	2.6	—[a]	+[e]	0
	100	$10^{-2.8}$	$10^{-3.8}$	1.0	—[a]	0	
ECHO 9 (Coxsackie virus A23)	200	$10^{-4.8}$	$10^{-5.6}$	0.8	—[a]	0	+
Poliovirus type I (Mahoney)	200	$10^{-2.6}$	$10^{-2.5}$	0.1	—[a]	0	0[f]
Columbia SK	100	$10^{-3.3}$	$10^{-4.7}$	1.4	—[b]	0	+
Influenza A (PR8)	200	$10^{-3.7}$	$10^{-4.3}$	0.6	—[c]	0	+
Influenza A (Japan 305/57)	200	$10^{-4.4}$	$10^{-4.4}$	0.0	—[c]	0	+
Measles (Edmonston)	200	$10^{-1.2}$	$10^{-1.4}$	0.2	—[c]	0	—[g]
Herpes (Nutley)	100	$10^{-4.5}$	$10^{-5.8}$	1.3	—[b]	0	+
Herpes (HF Smith)[h]	200	$10^{-5.4}$	$10^{-5.6}$	0.2	—[a]	0	+
Vaccinia	200	$10^{-2.7}$	$10^{-4.3}$	1.6	—[a]	0	ND
		(Av pfu per tube) Treated	Controls	Reduction (%)			
Vaccinia	200	3.1	11.9	75	—[d]	+	ND
	100	11.6	11.9	2.5	—[d]	0	ND

[a] Cytopathic effect.
[b] Lethality of tissue culture supernatant for baby mice.
[c] Hemadsorption.
[d] Plaque counts.
[e] + = active; 0 = inactive; ND = not determined.
[f] To be published—observation in rhesus monkey.
[g] Infection of monkey could not be accomplished with the strain employed.
[h] Rabbit kidney cell culture used in place of rhesus kidney cell cultures.

vaccinia virus, activity *in vitro* could be detected if the sensitive plaque-reduction method was employed.

The *in vivo* antiviral properties of DIQA against viruses from such diverse groups as the picornaviruses, myxoviruses, and herpesviruses make it a substance of unusual interest from both the clinical and biological points of view. Although the mechanism of action of DIQA has not been defined, the present data namely, the necessity of simulataneous treatment to detect activity *in vivo* and the lack of direct inactivation *in vitro* of viruses against which it is active *in vivo*, might possibly suggest that DIQA prevents the virus from attaching to or penetrating the host cell.

Summary. 3,4-Dihydro-1-isoquinolineacetamide hydrochloride (DIQA) is a novel antiviral agent active *in vivo* against Columbia SK virus, herpesvirus, ECHO 9 virus (Coxsackie virus A23) and the PR8 and Japan 305/57 strains of influenza A virus. The substance was without effect *in vivo* against Coxsackie virus B1, Newcastle disease virus and influenza B virus. The compound was inactive when tested in tissue cultures against the viruses for which it had an antiviral effect *in vivo*. It was also inactive in tissue cultures against poliovirus type I and measles virus. Activity *in vitro* could be detected against Coxsackie virus B1 and vaccinia virus but, in the latter case, only if a plaque-reduction method was employed. The antiviral agent did not display anti-influenza activity *in ovo*.

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Energy Sources in Hibernator Heart: Pyridine Nucleotide Metabolism* (33336)

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Previous experiments from this laboratory on the metabolism of hibernator hearts have shown that the pyridine nucleotide metabolism of fluoride-inhibited heart homogenates from bats (*Myotis sp.*) differ markedly from nonhibernator rat heart homogenates (1). Fluoride-inhibited heart homogenates from bats were able to consume oxygen using glucose-6-phosphate as substrate with either nicotinamide adenine dinucleotide (NAD+) or nicotinamide adenine dinucleotide phosphate (NADP+). Rat heart homogenates under the same conditions consume oxygen only with NAD+.

However, the source of energy available to an animal during hibernation and the source of readily available energy for arousal remain obscure (2). Blood glucose levels are lowered and free fatty acids increase during hibernation (3), but the enzymatic means by which hibernator tissue can accomplish an apparent shift away from a glucose-based metabolism to a lipid metabolism in the hibernating state remains largely unknown except in brown fat (4). The present studies offer further evidence that the metabolism of the pyridine nucleotides in tissues from hibernators is altered with the onset of hibernation.

Methods and Materials. Ground squirrels *(Citellus tredecemlineatus)* captured in the vicinity of Chicago, Illinois, were maintained in separate cages throughout the winter months at 4–5° with a 10–14 day-night light cycle. They were fed mouse breeder chow and given free access to water. The animals hibernated readily under this regimen, arousing periodically and reentering hibernation. The average bout of hibernation lasted 12–14 days but some animals hibernated for as long as 10 weeks. Animals were considered for experiments as hibernators after 5 days of continuous hibernation. Aroused, cold-exposed animals were used only if they remained awake for a minimum of 5 days. A total of 54 animals was used in these experiments.

Animals were killed by cervical dislocation.

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