man that this drug inhibits transiently the accumulation of I^{131}(7). Certainly, cellular respiratory studies are in order, and renal oxygen uptake determinations before and during Phenylbutazone administration would be helpful.

Excretory studies of other renal tubular substances would be enlightening, especially of sodium. From clinical studies, it might be assumed that tubular reabsorption of sodium is increased. If so, this is indeed paradoxical since mercurial diuretics inhibit tubular excretion of PAH and decrease reabsorption of sodium.

Conclusions. 1) Phenylbutazone causes retention of salt and water in approximately 80% of patients receiving this drug with clinical edema becoming manifest in approximately 10%. 2) Phenylbutazone markedly depresses renal tubular excretion of para-aminohippurate. 3) Excretion of para-aminosalicylic acid does not appear to be inhibited by this drug. 4) Further studies are needed to clarify the relation, if any, between decreased excretion of para-aminohippurate and the clinically observed increased reabsorption of sodium.

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1. Wilkinson, E. L., and Brown, H., *Am. J. Med. Sci.,* 1953, v225, 153.

2. Green, J., and Williams, P. O., *Lancet,* March 21, 1953, v1, 575.

3. Goldring, W., and Chasis, H., *Hypertension and Hypertensive Disease,* The Commonwealth Fund, New York, 1944.

4. Marshall, E. K., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 471.

5. Boger, W. P., Beatty, J. O., Pitts, F. W., and Flippin, H. F., *Ann. Int. Med.,* 1950, v33, 18.

6. Kuzell, W. C., Schaffarzick, R. W., Brown, B., and Mankle, E. A., *J.A.M.A.,* 1952, v149, 729.

7. Werner, S. C., *Bull. N. Y. Acad. Med.,* 1953, v29, 523.

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Effect of Ethionine and Other Materials on Semliki Forest Virus Infection in the Mouse.* (20686)

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Semliki forest virus was isolated by Smithburn and Haddow(1) from mosquitoes collected in Uganda. Although the virus has not been recovered from man, antibodies for it have been demonstrated in human serum specimens collected in Uganda and in India (2,3). In mice, the virus produces a fatal disease following inoculation by the intracerebral, subcutaneous, intramuscular or intraperitoneal routes(1). Animals inoculated intracerebrally with the virus usually die within one week and within 2 weeks after intraperitoneal inoculation. Paralysis of one or both posterior extremities usually constitutes the first sign of infection but hyperirritability and convulsions may occur. Animals may recover from paralysis.

Since the peripheral inoculation of mice with Semliki forest virus produces a disease involving the central nervous system, this virus provides a useful means for the investigation of materials which may alter resistance of the mouse to neurotropic viruses. The Semliki forest-mouse model has been used in a study involving a number of amino acids, purines, pyrimidines, and thiosemicarbazones. The most significant results were obtained with DL-ethionine and 5-phenoxythiouracils.

Materials and methods. The strain of Semliki forest virus used in these experiments was obtained in 1951 from Dr. H. M. Powell of Lilly Research Laboratories who, in turn, had received it from Dr. Smithburn. Five intracerebral passages in mice have been

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TABLE I. Effect of Ethionine and Other Materials on the Course of Semliki Forest Virus Infection in the Mouse.

Compound	Treatment			Proportion of inoculated mice surviving infection	
	Dose*	Route	Days	Treated	Untreated
	Virus inoculated intracer.				
DL-e†	.15	Diet	−3 to 6	33/64	33/68
DL-e	20–30	I.P.	−1, 0 only	3/20	2/24
	Virus inoculated intraper.				
D-e	.15	Diet	−3 to 6	20/27	8/24
L-e	.15	"	"	19/30	8/24
DL-e	.15	"	"	205/378	111/413
DL-e	.15	"	"	13/20	8/20
DL-e	.15	"	−3 to 1	11/20	8/20
DL-e	.15	"	0 to 2	7/19	8/20
DL-e	.3	"	"	11/19	8/20
DL-e	20–30	I.P.	−2 only	3/10	3/10
DL-e	20–30	"	−1 "	26/39	13/40
DL-e	20–30	"	0 "	22/39	14/40
DL-e	20–30	"	1 "	7/29	9/29
DL-e	20–30	"	2 "	5/20	7/20
DL-m‡	1.0	Diet	−3 to 6	20/59	16/59
DL-e	.15	"	"	27/56	16/59
DL-m and DL-e	1.0 and .15	"	"	22/60	16/59
Dichlorophenoxy thiouracil	.75	"	"	16/40	8/55
Adenine sulfate	.03	"	"	8/15	8/17
Benzimidazole	.1	"	"	3/16	7/16
Purine derivatives§	.02–.1	"	"	23/115	57/130
Thiosemicarbazones‖	.03–.08; 5	I.P.	"	18/69	17/66
Atabrine hydrochloride	.05	Diet	−2 to 6	7/22	14/26
Antibiotic M5-8450		I.P.	−3 to 6	9/10	4/10

* Materials administered in diet are listed in terms of concentration (%) and those given intraperitoneally (I.P.) in mg/dose.

† Ethionine.

‡ Methionine.

§ Purine derivatives: 2,6-diamino, 6-mercapto, 2-mercapto-6-amino, 2,6,8-dithiohydroxy, 2-amino-6,8-dimercapto, 6-hydroxy-8-mercapto, 1,3-dimethyl-2,6-dimercapto, 6-decylamino, 6-methyl, and 2,6-diamino-8-azapurine; 2-thioxanthine, 8-azaxanthine and thioguanine.

‖ Thiosemicarbazones: isatin, cinchoninaldehyde, 5-bromo-2-thenaldehyde, benzaldehyde and *o*-methoxybenzaldehyde.

made in this laboratory. Pooled infected brain tissues from the last 2 transfers served as the inoculum for experiments to be reported. In most experiments, mice were injected intraperitoneally with 0.1 ml of a 10^{-5} dilution of infected brain tissue prepared in Locke's solution containing 5% normal rabbit serum. This inoculum contained approximately 600 intracerebral LD_{50} doses of virus. In a few experiments, mice were inoculated intracerebrally with 0.03 ml of a 10^{-6} dilution of infected tissue. This inoculuum contained approximately 18 intracerebral LD_{50} doses of virus. Inoculated animals were observed for 16-20 days. Materials to be administered in the diet were ground in a mortar and mixed in the basal casein-sucrose diet(4) to give the desired concentrations. Substances to be injected intraperitoneally were dissolved or suspended in sterile distilled water, the pH adjusted to approximately 7.0, and the desired quantity of compound injected in a volume of 0.5-0.8 ml.

Results. When Semliki forest virus was inoculated intracerebrally into mice, DL-ethionine failed to protect animals (Table I). When the virus was injected intraperitoneally, however, treatment with ethionine was observed to reduce the fatality rate. In 31 experiments in which DL-ethionine was fed in the diet in a concentration of 0.15%, 205 of 378 treated mice (54%) survived as compared to 111 of 413 (27%) in the corresponding untreated groups. Both the D- and L-

forms of ethionine protected mice against the virus. When DL-ethionine was administered intraperitoneally in a single dose of 20-30 mg, either on the day preceding or within 1-2 hours after viral inoculation, the survival rate of mice was increased. Protection was not obtained when the compound was injected either 2 days before or one or 2 days after initiation of infection.

Ethionine treated mice which survived infection by Semliki forest virus usually were found to have developed immunity against the virus. All of 15 survivors treated with a single intraperitoneal injection of ethionine within 2 hours after viral inoculation were found to be completely resistant to the same dose of virus given by the same route 2 weeks later. Thirteen of 16 survivors treated with a single intraperitoneal injection of ethionine 24 hours before viral inoculation survived a challenge dose of approximately 18 intracerebral LD_{50} doses of virus given intracerbrally 2 weeks later.

Since ethionine is a structural analogue of methionine, experiments were carried out to determine whether methionine would counteract the protective action of ethionine when both materials were fed in the diet. The combined data from 6 experiments are presented in Table I. In some experiments, the number of surviving mice fed a combination of methionine and ethionine was similar to that of untreated groups while in other experiments methionine appeared to have no effect. A concentration of 2% of methionine in the diet did not produce a result different from that obtained with 1%. Folic acid (10 mg %) and vit. B_{12} (0.005 mg %) in combination with ethionine or with ethionine and methionine or ethionine and homocysteine gave essentially the same results. DL-cystine (2%) was not observed to overcome the protective action of ethionine.

Dichlorophenoxy thiouracil (5-(2′4′-dichlorophenoxy-4-hydroxy-2-mercaptopyrimidine)) when fed in the diet in a concentration of 0.5-0.75% increased the number of survivors (Table I). Similar results were obtained with 4′-bromo, 3′4′-dicholor and 3′4′-dimethyl derivatives of 5-phenoxy thiouracil. In 8 experiments with this group of compounds, 26 of 64 treated mice survived in contrast to 12 of 87 untreated controls.

Antibiotic M5-8450‡ protected mice against Semliki forest virus. Treatment consisted of the intraperitoneal injection of an arbitrary amount of the antibiotic for 3 days before and 6 days after viral inoculation.

The adenine antagonists, benzimidazole and 2,6-diaminopurine, tended to increase susceptibility of mice to Semliki forest virus. Other purine derivatives exhibited a similar effect. In 14 experiments with 13 purine derivatives, 23 of 115 treated animals (20%) survived in contrast to 57 of 130 mice in untreated groups (43%). None of 5 thiosemicarbazones tested altered susceptibility of mice to the virus.

Tests with other materials. Little or no influence on the course of Semliki forest viral infection in mice was observed as the result of feeding the following materials in the concentrations listed: Acetyl (0.5%), alanyl (0.15%), benzoyl (0.2%), propionyl (0.5%), sulfone (0.5%), and sulfoxide (0.5%) derivatives of methionine; *alpha* amino butyric acid (1%), *alpha* hydroxy *gamma* methyl mercapto butyric acid (0.2%), 5-methyl tryptophane (0.09%), N-2-carboxy-ethyl-tyrosine (0.15%), *p*-chloro (0.5%), and *p*-bromophenylalanine (0.5%), *beta* totylalanine (0.3%), *beta*-3-thienylalanine (0.1%), phenylserine (0.5%), aureomycin (0.1%), and chloromycetin (0.25%).

Discussion. Ethionine has been reported to inhibit multiplication of mouse encephalomyelitis, influenza (type A) and poliomyelitis (type 2) viruses *in vitro* (5-7). Methionine counteracted the inhibition of growth of influenza and poliomyelitis viruses produced by ethionine (6-7). Data have been presented which demonstrate that ethionine increases the survival rate of mice inoculated intraperitoneally but not of animals inoculated intracerebrally with Semliki forest virus. The compound is effective either when fed in the diet or injected by the peritoneal route. Treatment of mice with ethionine is most effective when the compound is given within

‡ Obtained through the courtesy of Dr. H. M. Powell, Lilly Research Laboratories, Indianapolis, Ind.

24 hours preceding the inoculation of virus. Ethionine treated mice which survive Semliki forest viral infection usually are immune to the virus. Although supplementing an ethionine-containing diet with methionine appeared to reduce the protective capacity of ethionine in some experiments, the results obtained were variable and are not interpreted as a true reversal.

Dichlorophenoxy thiouracil likewise was observed to increase the survival rate of mice inoculated with Semliki forest virus. Isatin thiosemicarbazone, however, was ineffective. Both of these compounds protect mice against vaccinia virus(4,8). Antibiotic M5-8450 and the closely related or identical compound, Helenine, protect mice not only against Semliki forest virus(9,10), but also against MM and Columbia SK strains of encephalomyelitis virus(9,11), and the MEF1 strain of poliomyelitis virus(12). 2,6-Diaminopurine, which reduces the fatality rate of mice inoculated with Russian Far East encephalitis virus(13), increases the susceptibility of animals to Semliki forest virus. Although atabrine increases resistance of mice to equine encephalomyelitis virus(14), it exerts little or no influence on Semliki forest viral infection. With the exception of dichlorophenoxy thiouracil(4), isatin thiosemicarbazone(8) and atabrine(14), none of the materials mentioned has been demonstrated to protect mice when the virus is inoculated by the cerebral route.

Summary. 1. Feeding mice D-, L- or DL-ethionine in a casein-sucrose diet increases the survival rate of mice inoculated intraperitoneally with Semliki forest virus. A single, large dose of DL-ethionine injected intraperitoneally within 24 hours preceding viral inoculation by the same route also increases the survival rate. Ethionine does not increase the resistance of mice to Semliki forest virus when the virus is inoculated intracerebrally. Ethionine-treated mice which survive infection usually are immune to the virus. 2. Methionine does not counteract the protective action of ethionine against Semliki forest viral infection in mice. 3. Dichlorophenoxy thiouracil and closely related materials increase the survival rate of mice inoculated intraperitoneally with Semliki forest virus. 4. Benzimidazole, 2,6-diaminopurine and other purine derivatives tend to decrease the resistance of mice to Semliki forest virus.

1. Smithburn, K. C., and Haddow, A. J., *J. Immunol.*, 1944, v49, 141.

2. Smithburn, K. C., Mahaffy, A. F., and Haddow, A. J., *ibid.*, 1944, v49, 159.

3. Smithburn, K. C., *Fed. Proc.*, 1953, v12, 460.

4. Thompson, R. L., Price, M. L., Minton, S. A., Jr., Falco, E. A., and Hitchings, G. H., *J. Immunol.*, 1951, v67, 483.

5. Pearson, H. E., and Winzler, R. J., *Fed. Proc.*, 1949, v8, 409.

6. Ackermann, W. W., *J. Exp. Med.*, 1951, v93, 337.

7. Brown, G. C., and Ackermann, W. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 367.

8. Thompson, R. L., Minton, S. A., Jr., Officer, J. E., and Hitchings, G. H., *J. Immunol.*, 1953, v70, 229.

9. Powell, H. M., Culbertson, C. G., McGuire, J. M., Hoehn, M. M., and Baker, L. A., *Antibiotics and Chemotherap.*, 1952, v2, 432.

10. Shope, R. E., *J. Exp. Med.*, 1953, v97, 627.

11. ———, *ibid.*, 1953, v97, 601.

12. Powell, H. M., and Culbertson, C. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 161.

13. Moore, A. E., and Friend, C., *ibid.*, 1951, v78, 153.

14. Hurst, E. W., Melvin, P., and Peters, J. M. *Brit. J. Pharm. and Chemotherap.*, 1952, v7, 455.

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