is a striking increase in the amount of P^{32} in the amniotic fluid. In practice this unmistakable change is observed as the difference, for example, between 150 counts/minute for 0.1 ml of normal fluid and 2000 counts/minute for 0.1 ml of infected fluid. In Fig. 3 the counts obtained experimentally have been converted into absolute amounts of P^{32} so that they may be compared with data obtained with other counting methods in other laboratories.

Conclusions. 1. The following observations were made on basic methods of using P^{32} in biological studies: a. P^{32} as received from Oak Ridge is usually sterile but penicillin and streptomycin can be added before direct inoculation into eggs to obviate autoclaving procedures potentially dangerous to laboratory personnel. b. Glassware and needles used for virus work can be decontaminated of P^{32} in 2-4 days with hot sodium metaphosphate (Calgonite®) solutions. c. Simple methods of handling and storing millicurie amounts of P^{32} in biological materials are described. 2. Radioactive phosphorus is of relatively low toxicity for chick embryos; 500-1000 μc per egg is not lethal in 72 hours. 3. P^{32} injected into the yolk sac appears in a 100-fold greater concentration in the allantoic fluid than in the amniotic fluid in 24-96 hours. 4. The partition of P^{32} in the egg fluids is the same for 8- or 9- or 10-day-old embryos with periods of incubation of 24-96 hours following inoculation. 5. Following infection with herpes simplex or vaccinia virus there is a 10-20-fold increase in the amount of P^{32} appearing in the amniotic fluid. 6. Chorioallantoic membrane plaques, whether virus or mechanically induced, concentrate P^{32} in their cells.

1. Libby, R. L., and Madison, C. R., *J. Immunol.*, 1947, v55, 15.

2. Cohen, S. S., *Bact. Rev.*, 1951, v15, 131.

3. Taylor, R. M., and Saenz, A. C., *J. Immunol.*, 1949, v63, 319, 331.

4. Ward, T. G., *Am. J. Hyg.*, 1950, v52, 107.

5. Rafelson, M. E., Winzler, R. J., and Pearson, H. E., *J. Biol. Chem.*, 1949, v181, 583, 595; *Science*, 1950, v112, 231.

6. Graham, A. F., and McClelland, L., *Canad. J. Res. E.*, 1950, v28, 121; Graham, A. F., *loc. cit.*, 1950, 186, 271.

7. Toennies, G., *et al.*, *J. Lab. Clin. Med.*, 1951, v38, 163.

8. Lough, S. A., Isotope Division, U. S. Atomic Energy Commission, personal communication.

9. Boyd, G. A., and Board F. A., *Science*, 1949, v110, 586.

10. Blank, H., McCarthy, P. L., and DeLamater, E. D., *Stain Tech.*, 1951, v26, 193.

11. Nagler, F. P. O., *Australian J. Exp. Biol. Med. Sci.*, 1946, v24, 103.

Received January 29, 1952. P.S.E.B.M., 1952, v79,

Effects of Certain Amino Acids and Related Compounds on Propagation of Mouse Encephalomyelitis Virus.* (19395)

HAROLD E. PEARSON, DOROTHY L. LAGERBORG, AND RICHARD J. WINZLER.

From the Medical Microbiological and Biochemical Departments, University of Southern California School of Medicine and the Los Angeles County Hospital, Los Angeles.

Various factors which affect the propagation of Theiler's GDVII strain of murine encephalomyelitis virus in tissue cultures have been reported(1). The experiments described below were concerned with the effects of certain amino acids, amino acid analogues and other agents on this system; preliminary data were presented elsewhere(2).

Materials and methods. The methods used have been described in detail(1). The virus used was obtained from pooled tissue culture material after 50 to 60 tissue culture passages. It was added to cultures in final dilution of 1:1,000. Cultures were made in 50 ml Erlenmeyer flasks closed with rubber stoppers and containing 3 ml of Simms' X7 solution and

* Aided by a Research Grant from the National Cancer Institute of the National Institutes of Health.

50-100 mg of minced mouse brain from 1-day-old mice. The initial reaction of the medium was adjusted to pH 8-9. Cultures were incubated for 2 days at 35°-36°C. The supernatant fluid obtained after centrifugation of the pooled material from 3 flasks was tested for virus content by determination of the hemagglutination titer for human red blood cells. Serial twofold dilutions were used and the end-point was read from the sediment. The titer of tissue culture supernatant fluid as tested by this method usually was 1280-2560. Virus content was also assayed by intracerebral inoculation of 7 mice each with 0.03 ml of serial 2-fold dilutions. The end-point was the highest dilution fatal for 4 or more mice during the period of observation for 21 days. Control cultures without the compound tested were run routinely. All experiments were repeated one or more times.

Results.† Of the naturally occurring amino acids only lysine, serine and ornithine inhibited virus growth at 1 mg/ml (Table I). Alanine, arginine, aspartic acid, cystine, glycine, histidine, norleucine, phenylalanine, threonine, and tryptophane were inhibitory at 3 mg/ml while leucine, isoleucine, methionine, proline and valine had no inhibitory effect at this level. Tyrosine did not inhibit at 1 mg/ml concentration but could not be tested in greater amount because of insolubility. Synthetic amino acid analogues were highly inhibitory at 1 mg/ml. Thienylalanine, furylalanine, and naphthylalanine reduced the RBC titer by 50% even at a concentration of 0.1 mg/ml. Complete inhibition with this amount was obtained with the ortho-, meta-, or para-fluorophenylalanines. The lowest concentration of lysine found to be inhibitory was 0.5 mg/ml.

Attempts were made to reverse the inhibitory effect of some of the amino acids by addition of one or more amino acids not by themselves inhibitory or by the addition of 1 mg/ml of a combination of each of the amino acids listed in Table I as noninhibitory. The latter combination, however, was inhibitory, so was not tested further. Attempted reversals of lysine inhibition by addition of individual amino acids revealed that only methionine, leucine or tyrosine partially prevented the inhibitory action.

DL-β-2-thienylalanine was tested for protective activity against virus infection in mice. Ten mice weighing 10 to 12 g each received intracerebrally 0.03 ml containing 2 M.L.D. of Theiler's GDVII virus. Twenty-four hours later each was given 25 mg of thienylalanine intra-abdominally; this injection was repeated daily for 4 more days. Ten control mice were

TABLE I. Percentage Reduction in Hemagglutination and Mouse Fatality Titers of Virus in the Presence of Certain Compounds, in Comparison With Control Cultures.

Compound	% reduction in titer	
	Mouse	Hemagglut.
1 mg/ml		
L-Lysine monohydrochloride	—	50–87
DL- " "	—	87
L-α-Aminoadipic acid	75–94	100
D- " "	0	0
α-Ketoadipic acid	75	100
DL-Ornithine monohydrochloride	50–75	—
DL-Serine	50–75	87
DL-β-2 thienylalanine	87–97	100
DL-β-2 furylalanine	97	100
DL-β-1 naphthylalanine	—	80
DL-Fluorophenylalanine†	99	100
DL-Canavanine sulfate	—	100
DL-Allylglycine	0–50	0–50
DL-Methallylglycine	50–87	—
3 mg/ml*		
DL-Alanine	0–50	50–75
L-Arginine monohydrochloride	50–75	100
DL-Aspartic acid	50–75	75–94
L-Cystine	75	—
L-Glutamic acid	50	75
α-Ketoglutaric acid	0	—
Glycine	50–87	87–100
L-Histidine monohydrochloride	50–87	100
DL-Isoleucine	0	0
L-Leucine	0	0
DL-Norleucine	50–75	50–87
DL-Methionine	0	0
DL-Phenylalanine	50–75	50–87
L-Proline	0	0
DL-Threonine	50–75	100
L-Tryptophane	50–87	100
DL- "	0	—
DL-Valine	0	0

* Noninhibitory at 1 mg/ml.
† Ortho-, meta- and para- forms equally active.

† We are indebted to Drs. H. Borsook and A. Haagen-Smit for supplies of α-amino and ketoadipic acids, to Dr. V. H. Horowitz for canavanine, to Dr. Karl Dittmer for the allylglycine, methallylglycine and the phenylalanine analogues, and to Dr. Carl Niemann for the ortho-, meta-, and parafluorophenylalanines.

given saline instead of the drug. Nine test mice and 8 untreated controls died in 7-14 days; 1 mouse in the former and 2 in the latter group survived. Preliminary tests showed that the compound was not toxic in the amounts used since animals survived 6 successive daily doses of 25 mg without incident.

Discussion. The reasons for the virus inhibiting action(4-8) of amino acids and amino acid analogues and for differences among them are no more apparent than they are for bacteria and fungi. In the relatively high concentrations used in the present studies only a few naturally occurring amino acids were noninhibitory. Of the naturally occurring amino acids lysine was the most inhibitory. This is particularly interesting since it has been shown that Theiler's virus has a unique effect on protein-bound lysine and histidine, in mouse brain, *in vitro*(3). The amounts of these amino acids and their incorporation of radiocarbon from glucose are markedly reduced, *in vitro,* by virus propagation while other amino acids are not affected by virus growth in this way. Possibly lysine and histidine metabolism are important in the host-virus relationship in this system. Studies of this problem are in progress.

The observation that L-α-aminoadipic and α-ketoadipic acid are as highly inhibitory as lysine is in accord with the data of Borsook, *et al.*(9), and Windsor(10) who showed that these compounds are metabolites of lysine. The inactivity of D-α-aminoadipic acid suggests that the inhibition is due to an interference with specific metabolic processes. There is no explanation at present for the partial reversal of lysine inhibition brought about by methionine and by leucine or tyrosine or for the partial reversal by methionine of the lysine inhibition of $P^{32}O_4$ incorporation into the phospholipid and protein-bound fractions of mouse brain(11). Such reversals indicate a possible metabolic relationship among these amino acids.

Summary. 1. Various amino acids and analogues in concentrations of 1-3 mg/ml inhibit the propagation of Theiler's GDVII strain of mouse encephalomyelitis virus in tissue cultures of one-day-old mouse brain. The most active amino acid was L-lysine which was inhibitory at 0.5 mg/ml. The most effective analogues tested were the ortho-, meta-, or para-fluorophenylalanines, active at 0.1 mg/ml. 2. Partial reversal of lysine inhibition of virus growth was obtained with methionine, leucine, or tyrosine.

1. Pearson, H. E., *J. Immunol.,* 1950, v64, 447.
2. Pearson, H. E., and Winzler, R. J., *Fed. Proc.,* 1949, v8, 409; 1950, v9, 389.
3. Rafelson, M. E., Jr., Winzler, R. J., and Pearson, H. E., *J. Biol. Chem.,* 1951, v193, 205.
4. Spizizen, J., *J. Infect. Dis.,* 1943, v73, 212.
5. Cohen, S. S., and Anderson, T. F., *J. Exp. Med.,* 1946, v84, 525.
6. Spizizen, J., Kenney, J. C., and Hampil, B., *J. Bact.,* 1951, v62, 323.
7. Brown, G. C., and Ackermann, W. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 367.
8. Ackermann, W. W., *J. Exp. Med.,* 1951, v93, 337.
9. Borsook, H., *et al., J. Biol. Chem.,* 1948, v176, 1383, 1395.
10. Windsor, E., *J. Biol. Chem.,* 1951, v192, 607.
11. Rafelson, M. E., Jr., Pearson, H. E., and Winzler, R. J., *Arch. Biochem.,* 1950, v29, 69.

Received January 30, 1952. P.S.E.B.M., 1952, v79.

Carbohydrate Content of the Human Aortic Albuminoid. (19396)

HOWARD MILLER, HAROLD HAFT, AND DOROTHY M. KRAEMER.
(Introduced by J. B. Hamilton.)
From the Department of Anatomy, State University Medical Center, College of Medicine, Brooklyn, N. Y.

The albuminoid (collagen, elastin, and reticulin) of human arteries of the elastic type appears to undergo qualitative as well as quantitative aging changes. Thus, a gradual increase in collagenous substance(1), accompanied by minor changes in elastin content