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

(2,3) has been demonstrated with progressive age. Increases in the calcium and aspartic acid content of arterial elastin with aging have also been shown(2-6).

The demonstration that collagen from animal skin contains about 0.47% carbohydrate, probably in the form of glucose and galactose (7-9), stimulated us to investigate this carbohydrate fraction in the human aorta. Preliminary studies revealed the presence of a similar carbohydrate fraction in the albuminoid portion of the human aorta. The purpose of this investigation was therefore to determine whether or not this albuminoid carbohydrate varies with age, sex, or various pathological processes.

Materials and methods. The albuminoids were extracted by the method of Highberger (10), which was modified for our purposes. Tryptic digestion was eliminated because of the probable degradation of collagen following such treatment(11,12). It is therefore not possible to determine with certainty which albuminoid fraction contained the carbohydrate measured by our procedure, although theoretically the elastin should not be altered by this method. All samples were obtained from autopsy specimens and were refrigerated for no more than 60 hours before treatment. Only aortic tissue lying proximal to the origin of the coeliac artery was used in these experiments. The aortae were placed in absolute alcohol, shaken, and refrigerated for at least 6 hours. The alcohol was then changed and the procedure repeated for a minimum of 6 hours. Following dehydration, the samples were cut into approximately one-half inch squares, and random samples weighing about 15 g were placed in small gauze bags. From 4 to 8 of the sample bags were extracted with 750 ml of acetone in a large Soxhlet-type extractor for at least 16 hours. Following this the samples were washed with enough absolute alcohol to insure the removal of the remaining acetone, and the washings were discarded. Further extraction was then carried out for from 10 to 12 hours with 750 ml of absolute alcohol. The samples were then washed with distilled water and drained thoroughly. Treatment with 4 changes of half-saturated calcium hydroxide solution followed. This was accomplished by the use of a mechanical shaking machine. At the end of each shaking period (55 minutes) the samples were squeezed so as to express the excess calcium hydroxide before being placed in a fresh solution. The filtrate from the fourth treatment gave no precipitate on acidification with acetic acid, indicating the absence of mucoids. The samples were then washed thoroughly with distilled water, drained thoroughly, and washed with cold 0.007 N acetic acid. Four to 6 acetic acid washings were used, all washings being stirred well and thoroughly drained. After the exhaust liquid was found to be acid to litmus, several distilled water washings were used until the wash gave a negative reaction for calcium when tested with ammonium oxalate. The material was then dehydrated with several changes of absolute alcohol, washed several times with ethyl ether, and air-dried. The samples were then removed from their bags and weighed on an analytical balance. Each sample was then quantitatively transferred to a large Pyrex test tube to which was added 25 ml of 5% sulfuric acid. The tubes were placed in a boiling water bath for one hour, cooled in cold water, and the contents filtered into 100 ml volumetric flasks. The residue and filter paper were washed thoroughly with distilled water, and the flasks diluted with distilled water to the mark. After thorough shaking, 10 ml of the clear brownish solution was pipetted into a test tube containing 10 ml of 10% trichloroacetic acid solution. This procedure was necessary in order to reduce the amount of coloration. The contents of the tubes were shaken thoroughly, allowed to stand at least 10 minutes, and filtered into a dry test tube. One ml of the relatively colorless filtrate was pipetted into a test tube containing 10 ml distilled water, and the solution was shaken thoroughly. A 5 ml aliquot of this solution was then pipetted into an Evelyn macrocolorimeter tube; the tube was placed in a cold water bath. The carbohydrate was estimated by the method of Seifter *et al.*(13) and calculated on a glucose basis.

Duplicate samples from the same aorta were

TABLE I. The Albuminoid Carbohydrate Content of Human Aortae.

	No. of samples	Mean carbohydrate content, mg glucose/g albuminoid	Stand. dev.	t	Probability
Age group (yr)					
0-19	5	10.5	3.3		
20-39	9	10	5.4	.19	> .5
40-59	6	10.5	3.2	.0	1
60-69	11	11	2.2	.36	> .5
70-87	11	11.9	12.8	.24	> .5
	42	Avg 10.9	7.1		
♂	27	10.9	8.3		
♀	14	10.5	4.3	.17	> .5
Normal	8	8.9	5.8		
Pathological*	29	11.6	7.8	.91	> .05
Calcified†	9	9.4	2.5	.24	> .5

* Specimens which showed gross plaque formation, ulceration, or sclerotic changes.
† Gross observations.

analyzed in 4 cases, and a triplicate sample was determined in one case. The average percentage deviation from each mean was approximately 12%. The possibility that different parts of the same aorta are not homogeneous in respect to carbohydrate content may partially account for this figure. A 90% recovery was obtained by the addition of 6.25 mg of glucose to a duplicate sample. A total of 42 separate specimens was used for the analyses.

Results. The 42 samples were subdivided into 5 age groups and the youngest age group used as the base line for statistical analyses which were performed by Mr. G. Mestler of this department. The youngest specimen was obtained by pooling the aortae from two stillborn infants. All other samples were obtained at random from a series of autopsies. The data revealed no significant age changes in albuminoid carbohydrate content (Table I).

Likewise, no sex (Table I) differences could be found from the experimental results. All aortae were examined grossly for pathological processes. Histological examinations of these tissues will be reported in a later paper. The specimens were subdivided, on the basis of gross findings, into a normal group in which no pathological changes were observed, and a pathological group in which plaque formation, ulcerations, or calcifications were found. In comparing these 2 groups (Table I), no significant carbohydrate differences were observed. Those samples which showed definite calcified areas were also compared with the normal group. Again, no statistically significant changes could be seen from the data (Table I).

Discussion. The results of this investigation thus revealed that the albuminoid carbohydrate content of human aortae does not significantly change with age, sex, or pathology. They did, however, clearly demonstrate the presence of a constant carbohydrate fraction in the albuminoid portion of the human aorta. The exact significance of this carbohydrate fraction cannot be presently evaluated. An attempt to isolate and determine the nature of this carbohydrate fraction is being undertaken.

Summary. 1. The presence of a constantly occurring carbohydrate fraction in the human aortic albuminoid has been demonstrated. 2. This carbohydrate fraction does not appear to be altered by age, sex, or pathological changes in the aorta. 3. The possible significance of this carbohydrate has been discussed.

1. Troitzka-Andreva, A. M., *Arkh. biol. Nauk.*, 1930, v30, 519.

2. Lansing, A. I., Rosenthal, T. B., and Alex, M., *J. Gerontol.*, 1950, v5, 211.

3. ———, *J. Gerontol.*, 1950, v5, 112.

4. Blumenthal, H. T., Lansing, A. I., and Gray,

S. H., *Am. J. Path.*, 1950, v26, 989.

5. Lansing, A. I., Blumenthal, H. T., and Gray, S. H., *J. Gerontol.*, 1948, v3, 87.

6. Lansing, A. I., Alex, M., and Rosenthal, T. B., *J. Gerontol.*, 1950, v5, 314.

7. Beek, J., *J. Am. Leather Chem. Assn.*, 1941, v36, 697.

8. ———, *J. Research NBS*, 1941, v27, 507.

9. Grassmann, W., and Schleich, H., *Biochem. Z.*, 1935, v277, 320.

10. Highberger, J. H., *J. Am. Leather Chem. Assn.*, 1936, v31, 93.

11. Cassel, J. M., and Kanagy, J. R., *J. Am. Leather Chem. Assn.*, 1949, v44, 425.

12. ———, *J. Research NBS*, 1949, v42, 557.

13. Seifter, S., Dayton, S., Novic, B., and Muntwyler, E., *Arch. Biochem.*, 1950, v25, 191.

Received February 1, 1952. P.S.E.B.M., 1952, v79.

The Measurement of Systemic Blood Flow.* (19397)

STANLEY J. SARNOFF, ERIK BERGLUND, AND PHILIP E. WAITHE.

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Various technics for the measurement of cardiac output or large portions thereof have been proposed by Gregg and Shipley(1), Wegria *et al.*(2,6), Seely and Gregg(3), Eckstein *et al.*(4), and Bohr and Henriques (5). The method to be described below measures systemic blood flow, *i.e.* cardiac output minus coronary flow. It is relatively simple to perform and has the virtue of being accomplished by cannulation only of intrathoracic vessels.

Recent work has shown the adaptability of the Potter turbinometer to the problems of measuring total aortic flow(7). This flowmeter records pulsating flow as accurately as steady flow, and thus the necessity for using a buffer bottle or pressure reservoir in the system has been eliminated along with the undesirable shift of blood from dog to bottle and vice versa that occurs with variations in arterial pressure. Further, the sensing element is a tube 2.5 inches x 0.5 inch and significantly decreases the amount of blood required to fill the system when compared to a system using a rotameter with a buffer bottle.

Method. Mongrel dogs of both sexes were anesthetized with morphine sulfate, 4 mg per kg intramuscularly, and chloralose, 48 mg per kg, and urethane, 480 mg per kg intravenously. Dogs in excess of 14 kg were used. Smaller dogs may be used if the cannulae are made of a size smaller than those listed in Fig. 1. After a tracheotomy cannula is inserted, positive pressure breathing is maintained with a modified Starling pump. With the dog on its right side, the left ribs 3, 4 and 5 are then partially removed. The first 3 sets of intercostal vessels are ligated, but the aorta is not dissected free in order to eliminate the ooze that might otherwise occur after heparinization. The brachycephalic artery is then cleared from its point of origin to its bifurca-

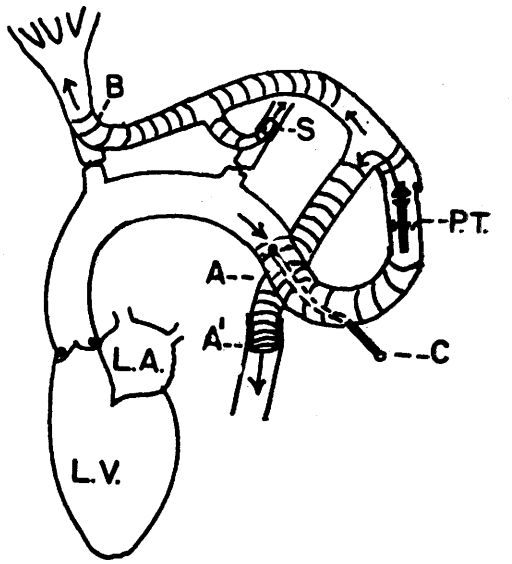

FIG. 1. Schema of aortic flow technic. See text. Desirable minimal internal diameters of cannulae are 10 mm for A, 9 mm for A', 8 mm for B, and 3 mm for S for use in dogs from 15 to 20 kg.

* Aided by a grant from the National Heart Institute, U. S. Public Health Service.