2. Awapara, J., and Seale, B., *ibid.*, 1952, v194, 497.
3. LaDue, J. S., Wroblewski, F., and Karmen, A., *Science,* 1954, v120, 497.
4. Wroblewski, F., and LaDue, J. S., *Ann. Int. Med.,* 1955, v32, 345.
5. Siekert, R. S., and Fleisher, G. A., *Proc. Staff Meet. Mayo Clinic,* 1956, v31, 459.
6. Schapira, G., Dreyfus, J. C., Schapira, F., and Kruh, J., *Am. J. Physical Med.,* 1955, v35, 313.
7. Karmen, A., *J. Clin. Invest.,* 1955, v34, 131.
8. Steinberg, D., Baldwin, D., and Ostrow, B. H., *J. Lab. Clin. Med.,* 1956, v48, 144.
9. Dounce, A. L., Barnett, S. R., and Beyer, G. T., *J. Biol. Chem.,* 1950, v185, 769.
10. Sibley, J. A., and Lehninger, A. L., *ibid.,* 1949, v177, 859.
11. Beck, W. S., *ibid.,* 1955, v212, 847.
12. Bessey, O. A., Lowry, O. H., and Brock, M. J., *ibid.,* 1946, v164, 321.
13. Andersch, M. A., and Szczypinski, A. J., *Am. J. Clin. Path.,* 1947, v17, 571.
14. Aronson, S. B., and Volk, B. W., *Arch. Neurol. Psych.,* 1956, v75, 568.
15. Agress, C. M., Jacobs, H. I., Glassner, H. F., Lederer, M. A., Clark, W. G., Wroblewski, F., Karmen, A., and LaDue, J. S., *Circulation,* 1955, v11, 711.
16. Molander, D. W., Wroblewski, F., and LaDue, J. S., *J. Lab. Clin. Med.,* 1955, v46, 831.
17. Siegel, A., and Bing, R. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 604.
18. Sibley, J. A., and Lehninger, A. L., *J. Nat. Cancer Inst.,* 1949, v9, 303.
19. Warburg, O., and Christian, W., *Biochem. Z.,* 1943, v314, 389.
20. LaDue, J. S., and Wroblewski, F., *Circulation,* 1955, v11, 871.

Received January 9, 1957. P.S.E.B.M., 1957, v94.

Pressor and Myotrophic Activities of Incubated Plasma.*† (23007)

G. M. C. MASSON, G. HELMCKE,‡ A. C. CORCORAN AND IRVINE H. PAGE
Research Division, Cleveland Clinic Foundation and Frank E. Bunts Educational Institute, Cleveland, O.

Plasma from children in the hypertensive phase of acute glomerulonephritis was reported to have pressor activity which diminished or disappeared during recovery(1). These samples had been at room temperature for sometime; activity was increased by incubation at 37°C for an additional 12 hours, a procedure which elicited similar, but less pressor activity in samples from normal children. The possibility was raised that the pressor agent might be angiotonin, liberated because the plasma of hypertensive subjects contained increased amounts of renin.

Accordingly, a survey was done in which we tested in Dibenamine-treated, anesthetized rats, or pithed rats, the pressor activity of plasma from 28 patients with established hypertension, 14 patients in malignant phase of essential hypertension, 3 with hypertension proven to be of renal origin, 7 with hypertension and chronic glomerulonephritis and 6 normotensive patients with renal disease of systemic lupus erythematosus as well as 11 normal subjects. For these tests, blood was taken by Dr. Carlos Nijensohn under sterile conditions into heparin-containing tube, immediately centrifuged, plasma separated and divided in 2 fractions. One fraction was incubated for 24 hours at 37°C and the other frozen. Both were assayed at the same time, using angiotonin as the reference standard for pressor activity. Freshly frozen plasma in doses to 0.2 ml gave irregular and small pressor responses similar to those elicited by the same volume of saline. The incubated samples were all pressor, approximately in the same degree and without reference to presence of hypertension. In brief, the survey indicated that pressor activity in question, while apparently not of immediate significance in problem of hypertension, represented at least an interesting property of shed, pre-

* This study was supported in part by grant from Cleveland Area Heart Soc. and was preented at the annual meeting of the Amer. Physiol. Soc., 1956.

† Dr. Merlin Bumpus graciously performed the physico-chemical studies.

‡ Now resident at Port au Prince, Haiti.

served human blood. Further studies were undertaken which are summarized in this report.

Procedures. Pressor activity was tested by intravenous injection into female rats, 150-200 g body weight. In early experiments these were pithed(2); it was subsequently more satisfactory to prepare them by Amytal anesthesia and subcutaneous injection of 3 mg of pentolinium in solution in 20% polyvinylpyrrolidone. The ganglion-blocker stabilizes blood pressure of the anesthetized rat and also enhances pressor responsiveness(3). Myotrophic properties of incubated plasma were tested in parallel with the frozen fractions, on guinea-pig ileum, rabbit duodenum, rabbit and rat uterus and rabbit aortic strips (4) in comparison with angiotonin and with epinephrine. Most of pressor and myotrophic assays were semi-quantitative, with an accuracy of about 50 to 150% of standard.

Results. (a) Incubation. Samples of both human and dog plasma were incubated for varying periods to 5 days, fractions being removed at intervals for freezing and subsequent assay. The maximum pressor activity was reached between 24 and 48 hours of incubation at 37°C and this remained constant thereafter in both incubated and frozen samples. This maximum was equivalent to 0.8 to 2.0 units(5) of angiotonin/ml; one unit is approximately equal to 1 Indianapolis unit or 1/5 Goldblatt unit. Incubated serum also showed pressor activity, although this demonstration was complicated by serotonin in the control sample.

(b) Site of Sampling. Simultaneous venous and arterial samples from dogs and rats were about equally active, with a suggestion of some greater activity in venous samples. Dr. F. Mason Sones kindly collected samples from children during diagnostic cardiac catheterization; these came from the supra- and infra-renal portions of the inferior vena cava. Dr. Eugene Poutasse graciously obtained samples during renal operations from renal and peripheral veins. These simultaneously collected pairs of samples were inactive before incubation but equally active after incubation, indicating that the ultimate origin of the pressor activity might not be renal.

(c) Myotrophic activity. Incubated samples of plasma caused contraction of rabbit aorta, guinea-pig ileum, rabbit and rat uterus. The direction, latency and duration of these responses more closely resembled those which occurred after application of angiotonin than those due to epinephrine; epinephrine produces relaxation in the rat uterus and contraction in rabbit uterus, while angiotonin contracts both preparations. In the case of rabbit duodenum, epinephrine decreased amplitude of contraction, angiotonin increased tone and incubated plasma provoked an initial decrease in amplitude followed by recovery of contraction and increase in tone. Dr. Leal Prado (then at Mount Sinai Hospital, Cleveland) kindly tested incubated plasma on the frog Laewen-Trendelenburg preparation and found it to be vasoconstrictor.

(d) Nature of agent. The pressor activity was not affected by treatment of rats with Dibenamine or Dibenzyline, by mixing the plasma with sodium thioglycollate or by giving rats bromolysergic diethylamide, confirming the conclusions of Dekanski(6) that it is not an adrenergic amine, not Pitressin, serotonin or, since it is not depressor, histamine.

The suggestion that it might be angiotonin was tested in several ways. First, angiotonin was added to plasma and the mixtures incubated; pressor activity of added angiotonin disappeared in 2-3 hours, at which time activity of the pressor substance formed on incubation was not yet detectable. Next, the pressor activity was not enhanced by adding renin-substrate to plasma prior to incubation. Samples of serum of monkeys immune to renin were obtained from Dr. George Wakerlin and incubated, as were also plasma of rats made resistant to renin by repeated daily injections of renin; these samples, presumably containing anti-renin, yielded full pressor activity on incubation. Renal origin of activity was also excluded by demonstrating pressor activity in samples of plasma from 24-hour nephrectomized rats. Lastly, incubated plasma was precipitated with 55% alcohol

at room temperature and the supernatant, after removal of alcohol, was incubated with pepsin, trypsin and chymotrypsin under conditions which rapidly destroyed the activity of control samples of angiotonin; in contrast samples of incubated plasma retained pressor activity.

Further preliminary experiments showed that the activity is not destroyed by standing at room temperature during 9 days or by boiling for 15 minutes, that it is not dialyzable, that it is soluble in 55% alcohol but that the supernatant from plasma rapidly mixed with 4 volumes of alcohol was inactive.

Discussion. The experiments show formation in incubated plasma and serum of an agent which is pressor in intact rats, is vasoconstrictor and directly myotrophic. While these activities correspond to those of angiotonin, this material is not ultimately of renal origin, does not have the properties of angiotonin(7) nor of other myotrophic polypeptides with regard to proteolytic enzymes(8). The experiments do not further elucidate its nature. They suggest that, as a widely distributed property of mammalian blood, it may have ultimate physiological significance and should be of concern in procedures which involve the storage of blood, plasma or serum.

Since our preliminary communication on this substance, 3 articles have appeared which may bear on its nature. Gabr(9) describes isolation from plasma of a long-chain, C18 unsaturated fatty acid, tentatively identified as 3-octodecenoic acid; this substance is possibly a product of phospholipid breakdown and, in low concentrations, causes a slow contraction of isolated jejunum of the guinea pig. More recently, Titus, Weiss and Hadju (10) isolated palmitoyl lysolecithin from plasma and tissues and have characterized its activity as digitalis-like, noting that a large part of the material is present in inactive form. Gayer and Sarre(11) have described a high-molecular weight, acetone- and alcohol-soluble acid which is vasoconstrictor and is present in normal human plasma and, in apparently greater amounts, in plasma of patients with essential hypertension. These observations indicate that there is a group of myotrophic lipid agents with which the above described pressor activity may eventually be identified. The fact that this activity develops during incubation *in vitro* suggests that like palmitoyl lysolecithin, it may exist in active and inactive forms, the latter subject to slow, presumably enzymatic degradation in plasma.

Summary. Incubation of normal human, dog or rat plasma at 37°C for 12 or 24 hours unmasks a pressor and myotrophic activity which is not due to known vasoactive substances.

1. Arneil, G. C., and Dekanski, J. B., *Lancet,* 1954, v2, 1204.

2. Shipley, R. E., and Tilden, J. H., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 453.

3. Page, I. H., and Taylor, R. D., *Circulation,* 1950, v1, 1233.

4. Furchgott, R. F., and Bhadrakom, S., *J. Pharmacol. and Exp. Therap.,* 1953, v108, 129.

5. Green, A. A., and Bumpus, F. M., *J. Biol. Chem.,* 1954, v210, 281.

6. Dekanski, J. B., *Brit. J. Pharmacol. and Chemotherap.,* 1954, v9, 187.

7. Plentl, A. A., and Page, I. H., *J. Biol. Chem.,* 1944, v155, 379.

8. *Polypeptides which stimulate Plain Muscle,* J. H. Gaddum, ed., E. and S. Livingstone Ltd., Edinburgh and London, 1955, p131.

9. Gabr, Y., *Brit. J. Pharmacol. and Chemotherap.,* 1956, v11, 93.

10. Titus, E., Weiss, H., and Hadju, S., *Science,* 1956, v124, 1205.

11. Gayer, J., and Sarre, H., *Klin. Wschr.,* 1956, v34, 334.

Received January 11, 1957. P.S.E.B.M., 1957, v94.