The fact that all strains of streptococci from the same source were not able to lyse the clot is interesting. If this test indicates virulence, we must admit that both virulent and avirulent types of beta hemolytic streptococci may coexist in the cow's udder. This point might be proven by further studies since in this work cows considered as mastitic were probably suffering from a chronic type of mastitis. In acute cases, one would expect a much higher percentage of fibrinolytic strains if there is a correlation between fibrinolysis and virulence.

The occurrence of lytic strains in cows which we class as normal does not deny the possibility that the test is of value as an indicator of virulence. Ayers and Mudge[7] isolated *S. mastitidis* from cows they believed to be normal. Plastridge and Anderson[8] do not believe these cows are normal, but that they merely temporarily fail to give the usual non-specific tests. These cows, however, may often harbor streptococci, which, possibly, may be virulent.

There is considerable evidence that *S. mastitidis* causes bovine mastitis; still there is the possibility that it is merely an opportunist. By analogy with properties of human streptococci, we do think there is some possibility that fibrinolysis of bovine plasma, by beta hemolytic streptococci from bovine udders, may be a measure of pathogenicity.

Conclusions: 1. Some beta hemolytic streptococci from milk are capable of causing the lysis of clotted bovine plasma. 2. Organisms from cows that are negative to non-specific tests for mastitis may produce this fibrinolysis.

8585 P

Growth Inhibitor in Liver Tissue.

AUSTIN M. BRUES, ELIZABETH B. JACKSON AND JOSEPH C. AUB.
From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University.

Liver has been known for a long time to contain substances which inhibit growth and migration of cells from explants. Walton[1] observed that extracts of liver had an inhibiting effect on tissue cul-

7 Ayers, S. H., and Mudge, C. S., *J. Infect. Dis.*, 1922, **31**, 40.
8 Plastridge, W. N. and Anderson, E. O., Storrs Agr. Exp. Sta. Bul. 195, 1934.
1 Walton, A. J., *J. Exp. Med.*, 1914, **20**, 554.

tures, in contrast to all other tissue extracts tried. Lynch[2] and Lewis[3] noticed that no cells migrate from cultures of chick embryo liver after the sixteenth day, and it has been suggested that the inhibitor in liver may be responsible for this effect. Despite the great amount of work that has been done on extracts and substances which increase growth of tissues *in vitro* or make possible their prolonged cultivation, relatively little attention has been paid to inhibitors. The following is a report of investigations made in this laboratory on the inhibiting power of liver extract.

We have used, in general, first explants of embryonic and sarcomatous tissues, grown by the usual coverslip or Carrel flask techniques in solid plasma media. In order to get a quantitative measure of inhibition, we have compared the average radial growth in a test solution at a given time with that in a control medium consisting of plasma and Tyrode's solution, using tissues explanted at the same time from the same embryonic organ. Of course, Parker[4] has shown that the growth rates of fibroblasts from different parts of the same organ may vary; we have found, however, that by using series of 5 or more explants a growth index can be obtained which does not vary more than 10 or 15% from series to series, or from day to day in the same series.

When fresh, sterile liver tissue is passed through a fine press and the pulp is shaken with double its volume of Tyrode's solution and centrifuged, a reddish, turbid solution is obtained which, when diluted with equal parts of plasma, inhibits growth of fibroblasts to the extent of 75 to 85% of the growth seen in Tyrode's solution alone: in other words, the average growth radius is 15 to 25% of the average radius of growth in the control. In dilutions of 1:6 or 1:8, inhibition of fibroblasts amounts to 30 to 50% and in higher dilutions there is no significant effect. This inhibition has been demonstrated using saline extracts of rat, mouse, and chicken livers, and is not present in extracts of chick embryo liver before the fifteenth day. Extracts of rapidly growing hyperplastic rat liver inhibit in the same degree as those of normal rat livers. The saline liver extracts with which we have dealt have a chloride content of about 0.5%, a nitrogen content of 4 to 5%, of which nine-tenths is protein nitrogen, and a potassium content of less than 0.2%. There is no inhibition of cultures grown in Tyrode's solu-

[2] Lynch, R. S., *A. J. Anat.*, 1921, **29**, 281.

[3] Lewis, W. H., in E. V. Cowdry's *General Cytology*, Univ. of Chicago Press. Chicago, 1924, p. 401.

[4] Parker, R. C., *J. Exp. Med.*, 1933, **58**, 401.

tion modified to contain this amount of potassium as KCl. The pH of the extracts is from 7.3 to 7.5.

Shaking the saline extract with ether for a sufficiently long time to precipitate most of the turbid material appears to destroy the inhibitor in large part, although there is slight inhibition in the ether-soluble fraction, corresponding in all probability to the mild inhibition which Carrel[5] has found in the lipoid fraction of serum. Precipitation of the turbid material by acidifying to a pH of 5.0 to 6.0 leaves a supernatant fluid which lacks any marked inhibitory action; in some instances, when the precipitate can be made to go back into solution, it is as inhibitory as the original extract. Oxygenation of the solution and evaporation of it to dryness, do not destroy its inhibitory power, and the inhibitor is stable at 50°C. for 30 minutes and is partly destroyed at 75°C. for the same length of time. Heating to 100° destroys it completely.

Ultrafiltration of the saline extract through a collodion sac yields a non-diffusible residue which is highly inhibitory, while the filtrate, tested on subdivided cultures which have acquired a constant growth rate, stimulates considerably better growth than is produced with Tyrode's solution alone (due, probably, to the presence in it of nutrient substances).

Addition of alcohol to the saline extracts up to 75% by volume, which precipitates most of the proteins, leaves a supernatant fluid which contains the inhibitor, although some of its potency is lost in the process. Seventy per cent alcohol extracts of liver pulp, when evaporated and taken up in Tyrode's solution, are inhibitory, although not in quite as high dilution as are saline extracts of liver. Such alcoholic extracts have been made from chicken, rat, lamb, calf, and steer livers, and when made isotonic and adjusted to a pH of 7.4, have always been found to be inhibitory. When obtained in this form, the inhibitor is found to diffuse through a collodion sac. The inhibitor, in both saline and alcoholic extracts, is removed by Lloyd's reagent and is not removed by shaking with large quantities of blood charcoal. In alcoholic extracts, it is partly precipitated by 40 to 60% acetone and partly soluble in 60 to 80% acetone. The inhibitory action of acetone precipitates is lost, however, when they are purified by reprecipitation from 55% alcohol.

The inhibitor is most effective on fibroblasts, and has been found to have an equal effect on various sorts of embryonic fibroblasts and on the malignant fibroblasts of mouse sarcoma 180. It is effec-

[5] Baker, Lillian E., and Carrel, Alexis, *J. Exp. Med.*, 1925, **42**, 143.

tive on homologous and heterologous tissues alike. It appears to have some inhibiting effect on the migration of monocytes, and has little effect on epithelium. The life-span of inhibited cultures does not seem to be altered and rare mitoses may be seen, but the radius of growth is greatly diminished and the cells (especially in cultures of sarcoma) are smaller.

Further investigations now in progress are planned to determine the exact nature of the growth-inhibiting property of liver tissue.

8586 C

Growth of Human Nervous System. III. Relations between Cerebral Surface, Volume and Length.

RICHARD E. SCAMMON AND MEREDITH B. HESDORFFER.

From the Graduate Faculty and the Institute of Child Welfare, University of Minnesota.

The human cerebrum, excluding its ventricular system, is a solid: and it is possible by the study of its measurements to determine to what degree, if any, the growth of this structure follows the usual geometric laws of the interrelation of dimensions of solids with simple and regular form.

If these dimensions approach the geometric rules of dimensionality, it is to be expected that the following relations may be approximated:

$$CV\ (=)\ k_1CL^a,\ CS\ (=)\ k_2CL^b,\ CS\ (=)\ k_3CV^c,$$

and also the converse expressions. In the above general formulae CV is cerebral volume, CL cerebral length, and CS cerebral surface. The k's are constants to be empirically determined, and the other lower case letters represent powers also to be empirically determined. For objects of simple and regular geometric form it is to be expected that *a* will approach 3; *b*, 2; and *c*, 2/3.

We have tested these assumptions by fitting curves for several of these dimensions as determined from a series of 20 cerebri, ranging from about 5 cc. to over 1,000 cc. in volume and from slightly less than 4 fetal or lunar months to about 50 years in age.[1] All curves were fitted by the method of average logarithms.[2]

[1] For the data see Hesdorffer, M. B., and Scammon, R. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 415.

[2] See Lipka, J., Graphical and Mechanical Computation, New York, 1918, p. 128 *et seq.*