clamitans pituitary extract. Undoubtedly, many factors enter into potential capacity of any ovary to ovulate on being stimulated by pituitary hormones, and without extensive study, a meaningful evaluation is difficult. It is for this reason that individual percentages of ovulation have been omitted from Table I.

It should be noted particularly that *R. pipiens* ovary has now been induced to ovulate *in vitro* during every month of the year. The ovary of a freshly-caught specimen ovulated Aug. 29th, and September ovulation has been induced on several occasions in freshly-collected frogs. Animals kept in forced hibernation in the laboratory may be used routinely from mid-October into early June (no ovulation in Ringer controls) and on 2 occasions ovulation was induced *in vitro* in mid-July from specimens held in cold room since the previous April.

Negative results have been obtained with *R. pipiens* ovary *in vitro* when pituitary extracts from the following animals were added: sexually immature *Ambystoma maculatum* (Nov. 19), 2 sexually mature turtles (*Pseudemys scripta troosti* and *Chrysemys picta*, Mar. 29), and normal and castrated laboratory white rats of both sexes (Dec. and Mar., on several occasions). Human chorionic gonadotropin (Antuitrin-S, Parke-Davis) and combinations of this urinary factor with mammalian FSH (Synapoidin, Schering) failed to induce ovulation either *in vivo* or *in vitro* in repeated assays, nor have they increased significantly the responses obtained with frog pituitary extract. In every instance potential responsiveness of ovarian tissue was verified simultaneously by exposure of other ovarian fragments to *R. pipiens* pituitary extract.

Summary. Ovulation *in vitro* has been induced in a variety of anurans using homoplastic and heteroplastic pituitary extracts, indicating (1) that the reaction as induced in excised ovarian tissue is truly physiological and normal, and (2) that there is a general lack of specificity of pituitary hormones for this response.

1. Wills, I. A., Riley, G. M., Stubbs, E. M., PROC. SOC. EXP. BIOL. AND MED., 1933, v30, 784.

2. Creaser, C. W., Gorbman, A., *Am. J. Physiol.*, 1935, v113, 32.

3. Adams, A. E., Granger, B., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 552.

4. Creaser, C. W., Harkaway, R., *Anat. Rec.*, 1942, v84, 520.

5. Wright, P. A., Hisaw, F. L., *Endocrinology*, 1946, v39, 247.

6. Creaser, C. W., Gorbman, A., *Quart. Rev. Biol.*, 1939, v14, 311.

7. Wright, P. A., *J. Exp. Zool.*, 1945, v100, 565.

8. Samartino, G. T., Rugh, R., *ibid.*, 1945, v98, 153.

Received March 17, 1960. P.S.E.B.M., 1960, v104.

Enzyme Distribution in Human Brain. Lactic and Malic Dehydrogenases.* (25734)

H. RICHARD TYLER (Introduced by George Thorn)
Dept. of Neurology, Harvard Medical School, and Neurological Section, Peter Bent Brigham Hospital, Boston, Mass.

Studies on distribution of enzyme activity in mammalian brain have been carried out by histochemical and microchemical methods. These technics have proved suitable for detailed study of small portions of the nervous system, *i.e.*, Ammon's horn(1) and limited areas of cerebral cortex(2). There have been no systematic studies of normal enzymatic activity on the human central nervous system. The present work was undertaken to define normal enzyme distribution in human gross material and to serve as a baseline for similar studies of various neuropathological entities. Lactic dehydrogenase and malic dehydrogenase activities were chosen because they offered the possibility of comparing information obtained by this method with histochemical

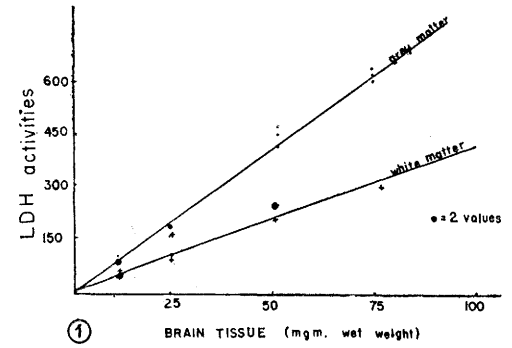
* This investigation was supported by research grant from Nat. Inst. of Neurol. Dis. and Blindness, N.I.H., U.S.P.H.S. Howard Hughes Medical Investigator.

and microchemical data now available. These enzymes are also widespread in their distribution in nervous tissue.

Material and methods. Human brains were obtained from Dept. of Pathology, Peter Bent Brigham Hospital. Fresh material obtained within 3 hours of death was used. Strominger and Lowry(3) have shown, in rabbits, little change in lactic dehydrogenase and malic dehydrogenase activities after death in this time interval. The brain was taken directly from cranial cavity, brought in iced container to the laboratory and then placed in the deep freeze for 24 to 36 hours. Previous experiments had shown this increased the yield of enzymatic activity. This has also been noted by Strominger and Lowry(3). The brain was then thawed at 24°C, and samples between 20-90 mg of tissue were dissected with care to insure homogeneity of tissue (*i.e.*, all gray matter, no blood vessels, tissue from various nuclei, etc.) and immediately weighed. The tissue was then finely ground and chopped in 3 ml of metal-free double distilled water, by use of glass stirring rods. This solution was then allowed to stand for 36 to 48 hours at 24°C, centrifuged at 3000 rpm and the supernatant used for further tests. Both lactic dehydrogenase and malic dehydrogenase activities were measured by disappearance of DPNH measured at 340 mμ using a "Coenzometer." A unit of activity of both malic and lactic dehydrogenase was defined as a change of 0.001 optic density unit/minute/cc of solution. The reaction mixture for lactic dehydrogenase consisted of 1.8 ml metal-free doubled distilled H_2O, 1 ml of 0.1 M PO_4 buffer at pH 7.4 (Mallinckrodt A.R.); 0.1 ml of 0.002 DPNH (Sigma) adjusted to pH 7.4, 0.1 ml of 0.001 M pyruvate (Mathieson, Coleman and Bell) and a 0.1 ml solution of enzyme. All reagents were prepared freshly each day. The reaction was initiated by addition of pyruvate solution and readings were taken over a 3 minute period. Control reactions without addition of substrate showed no measurable decrease in DPNH concentration in 3 minutes. The reaction mixture for malic dehydrogenase consisted of 0.3 ml of 0.2 M Sodium Pyrophosphate (J. T. Baker Chemical Co. analyzed reagent) at pH 8.3, 0.2 ml of 0.002 DPNH (Sigma) prepared as above, 2.3 ml of metal-free distilled H_2, 0.1 ml of enzyme and 0.1 ml of 0.0076 M oxaloacetic acid (H. M. Chemical Co., Ltd). The reaction was initiated by addition of oxaloacetic acid and readings taken for 2 minutes. Control reactions without addition of substrate showed no measurable decrease in DPNH concentration in 3 minutes. Enzyme activities were given/mg wet weight of sample studied. Protein content of gray matter was also roughly estimated by E280 absorption of solution on Beckman spectrophotometer. Values of enzyme activity obtained were confirmatory of enzyme activity as calculated on wet weight basis. Presence in white matter extracts of a slight opalescence interfered with E280 measurements and prevented comparison of white and gray matter extracts. Wet weight was used since it appeared to be a more specific, accurate and reproducible baseline and it allowed comparison. Therefore data are reported on this basis. Purified enzyme solutions of lactic dehydrogenase and malic dehydrogenase obtained from Nutritional Biochemicals Corp. were used as standards. All samples for comparison from different areas were run in one batch to prevent undue variation in time permitted for enzyme extraction. All measurements were made in triplicate on individual samples. Multiple samples were taken from each area.

Results. A number of experiments were performed using H_2O, isotonic saline, PO_4 buffer pH 7.4, and veronal buffer pH 8.6 as extracting medium for LDH in both gray and white matter. H_2O consistently proved to be the most efficient extractor of enzyme activity. When ground up brain tissue was placed in the solvent there usually was a slow increase in LDH enzyme activity over 4-8 hours. From 12 hours to 48 hours most preparations showed constant activity which did not vary more than 10-15%. About 20% of preparations showed a peak activity at 24 hours that was within 20% of the activity measured at 12 hours and 72 hours. After 72 hours all preparations showed a slow decline in activity (when kept at room temperature) which resulted in about 50% activity loss in 10 days.

Extractions were run at 4°, 24°, 37°. Op-



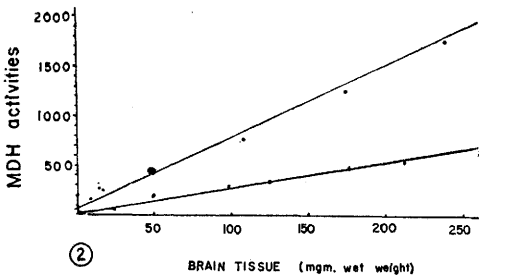


FIG. 1. Relationship of LDH activity to brain extracts.

FIG. 2. Relationship of MDH activity to brain extracts. (Upper curve represents gray matter, lower curve white matter.)

timal extractions occurred at 24° for both white and gray matter. Finely chopped and homogenized material gave roughly the same LDH values on 2 samples. 5.5 ± 1.8 units per mg wet weight for finely chopped brain; 6.0 ± 1.3 units/mg wet weight for homogenized (12 determinations of each). It was therefore elected to use chopped material for the assays reported.

In the range of 0-100 mg of brain tissue/3 cc of solution, there is a linear extraction of enzyme activity. Above 200 mg of tissue there is some decrease in efficiency of extractability of enzyme activity. Both gray matter (cortex) and white matter (centrum ovale) were used in demonstration of linear extraction with increasing amounts of tissue (Fig. 1).

Malic dehydrogenase was extracted with H_2O at room temperature. Analysis of a sample 12 times gave a value of 9.2 ± 2.43 for white matter and 20.4 ± 5.19 for gray matter. In the range of 0-250 mg of both white and gray matter there is a linear extraction of enzyme activity. The range above 250 mg of tissue/3 cc solution was not tested (Fig. 2). When in contact with the extracting solvent malic dehydrogenase activity rapidly increased for the first 6-8 hrs and then stabilized. In one typical experiment there was no significant change from 8 to 82 hrs, then up to 10% rise was gradually noted through 196 hrs.

All data reported here were obtained on material extracted by water at 24°. Since values were reasonably constant from 24-48 hours, no attempt was made exactly to standardize time of activity determinations, but all were performed between 36-48 hours. The most striking result was the clear presence of more enzyme activity in all gray matter as compared to all white matter (Fig. 3 and 4 and Table I for both enzymes). Average value for 43 assays of gray matter for lactic dehydrogenase was 6.1 ± 1.1 units/mg wet weight and for white matter was 3.5 ± 0.6 units (27 assays). A similar finding was noted for malic dehydrogenase with 21.2 ± 4.6 units noted for 39 assays of gray matter and 11.7 ± 2.3 units (21 assays) noted in white matter. In both cases more variation was noted in gray matter samples than in white matter. This suggested the possibility that certain areas of brain might be even more highly concentrated than others. Table II shows a breakdown of the source of gray matter.

Lactic dehydrogenase. The level of enzyme activity extracted from inner part of globus pallidus is more than 2 standard deviations below mean value of all gray matter assays. This probably reflects the great amount of white fibers passing through this nucleus. The caudate and outer part of the globus pallidus show significantly higher extractable enzyme activities than the rest of the gray matter, 8.5 and 9.2 units/mg wet weight respectively. In white matter (Table III), there is less variation. The white tracts of the cerebral hemispheres seem to be reasonably similar in activity. Optic tracts and the septic pellucidum, fornix and cerebellar white matter all tend to run higher than hemispheral values. The olfactory bulb and tract consistently had the lowest lactic dehydrogenase values. Fig. 3 is a graphic representation of lactic dehydrogenase distribution in a typical experiment.

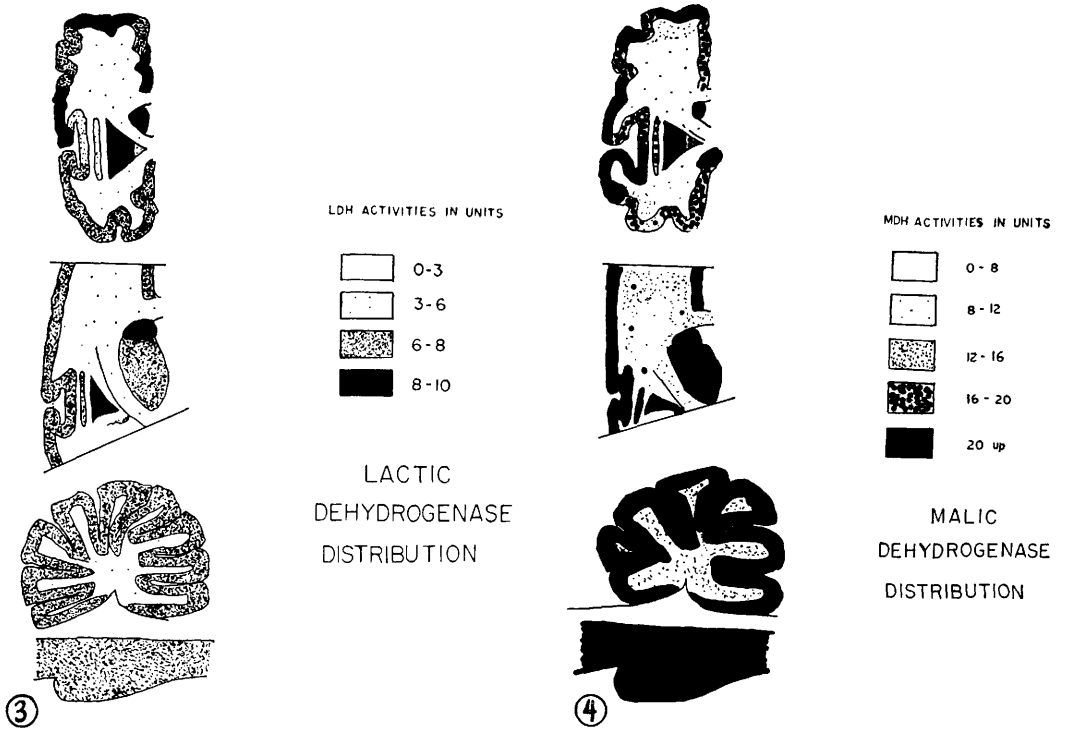


FIG. 3. LDH distribution in brain.
FIG. 4. MDH distribution in brain.

Samples were taken from representative areas as noted and their activity is pictured on the graph. For purpose of illustration activity ranges were used rather than individual values. It can be clearly seen that gray matter values were consistently higher than white matter values. Variations between adjacent activity ranges are probably not significant. Caudate and globus pallidus were consistently among the highest activities in all 3 brains examined.

Malic dehydrogenase. Malic dehydrogenase has a much more uniform distribution in gray matter (Fig. 4) and variation between various anatomical sites is not as striking as in lactic dehydrogenase. Again enzyme activities are plotted as "activity ranges" for purposes of illustration. Changes in adjacent activity ranges are probably not significant. Range in gray matter was 16-23 units/mg wet weight, whereas range in white matter was 10-15 units/mg wet weight. The differences noted with lactic dehydrogenase, *i.e.*, excess of enzyme activity in the optic tracts, septum pellucidum and fornix, and decreased amounts in olfactory bulb and tract are no longer apparent (Table III).

Discussion. Distribution of 2 enzyme activities, extracted under similar conditions from human material, showed certain phenomena in general, as well as individual variations. Both tend to occur predominantly in

TABLE I. Enzyme Distribution in Brain.

	Lactic dehydrogenase activity*		Malic dehydrogenase activity*	
Exp.	Gray matter	White matter	Gray matter	White matter
I	8.1 ± 1.7 (12)	3.8 ± .5 (10)	22.8 ± 6.4 (17)	12.0 ± 2.4 (10)
II	5.3 ± 1.0 (8)	3.5 ± .7 (6)		
III	5.3 ± .9 (23)	3.2 ± .7 (11)	20.0 ± 3.2 (22)	11.4 ± 2.3 (11)
Total	6.1 ± 1.1 (43)	3.5 ± .6 (27)	21.2 ± 4.6 (34)	11.7 ± 2.3 (21)

* MDH and LDH units/mg wet wt. Each exp. was done on different human brain.

TABLE II. Enzyme Distribution in Various Areas of Gray Matter.

	Units/mg wet wt	
	LDH	MDH
Frontal cortex	7.5	22.1
Temporal "	6.1	19.4
Parietal "	6.2	20.3
Occipital "	7.1	19.8
Putamin	6.8	21.2
Caudate	8.5	23.1
Globus pallidum, inner part	3.2	20.3
" " , outer "	9.2	22.0
Claustrum	4.6	20.0
Thalamus	5.9	20.7
Brain stem reticulum	6.8	21.4
Amygdala nucleus	4.5	11.8
Cerebellar cortex	6.2	21.1

gray matter. In individual nuclei lactic dehydrogenase varied independently of malic dehydrogenase, however. It has been suggested that these enzymes are more concentrated in nerve cells and/or synaptic areas(2).

Robbins *et al.*(4) found more malic dehydrogenase and lactic dehydrogenase in cortex than in white matter of rabbit and monkey brain and noted that the malic dehydrogenase/lactic dehydrogenase ratio is slightly higher for cortex than for white matter. We could confirm these observations. They also noted areas where the correlation of enzyme concentration was not parallel. Lactic dehydrogenase showed more variation than malic dehydrogenase in central white matter and optic nerves. Strominger and Lowry(3) previously have noted increase of lactic dehydrogenase in optic nerve and tracts by their microtechnics, and their coworkers have commented on the marked differences noted in histologically different areas of brain, and they have spoken of enzymatically different types of tracts.

TABLE III. Enzyme Distribution in Various Areas of White Matter.

	Units/mg wet wt	
	LDH	MDH
Frontal	3.6	12
Temporal	3.6	12
Occipital	3.5	13
Corpus callosum	3.6	11
Cerebellar white	4.4	15
Optic tracts	4.5	10
Septum pellucidum and fornix system	4.2	10
Olfactory bulb and tract	2.9	13

The higher concentration of lactic dehydrogenase in the caudate nuclei and other central gray areas is of interest in that it correlates with the observations of Himwich and Fazekas(5) that the caudate nucleus has one of the greatest oxygen consumptions found in brain tissue.

We were unable to find significant differences between the various areas of cortex, a finding noted by Bennett *et al.*(6) to be true of cholinesterase. He noted that cholinesterase activity differed in motor and visual somesthetic cortices, but that this was not true of lactic dehydrogenase. Our observations confirm his observations on lactic dehydrogenase and show similar diffuse distribution for malic dehydrogenase.

Summary. Distributions of enzymatic activities of lactic dehydrogenase and malic dehydrogenase have been studied in human brain. Their activities are increased in gray matter as compared to white matter. There are areas where concentrations of these enzymes seem consistently elevated, with higher concentration of lactic dehydrogenase in caudate nuclei, as compared to other gray matter, and optic nerve as compared to other white matter. In some areas lactic dehydrogenase activity shows no correlation with malic dehydrogenase activity. The significance of these variations is not as yet apparent.

1. Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L., Farr, A. L., Albios, R. W., *J. Biol. Chem.*, 1954, v207, 39.

2. Pope, A., Hess, H. H., *Metabolism of the Nervous System,* Pergamon Press, New York, 1957, p72-86.

3. Strominger, J. L., Lowry, O. H., *J. Biol. Chem.*, 1955, v213, 635.

4. Robbins, E., Roberts, N. R., Eydt, K. M., Lowry, O. H., Smith, D. E., *ibid.*, 1956, v218, 897.

5. Himwich, H. E., Fazekas, J. F., *Am. J. Physiol.*, 1941, v132, 454.

6. Bennett, E. L., Krech, D., Rosenzweig, M. R., Karlsson, H., Dye, N., Ohlander, A., *J. Neurochem.*, 1958, v3, 153.

Received January 4, 1960. P.S.E.B.M., 1960, v104.