Such changes in pattern of the muscle lactic dehydrogenase may suggest that an *in vivo* diagnostic would be possible by electrophoretic investigations of this enzyme in the blood plasma.

J. Charpentier
R. Goutefongea

Laboratoire de Recherches sur la Viande,
Centre National de Recherches Zootechniques,
Jouy-en-Josas, Seine-et-Oise, France.

[1] Cossard, J., Vet. Med. Doct. Thesis, Alfort, Paris (1957).
[2] Briskey, E. J., and Wismer-Pedersen, J., *J. Food Sci.*, **26**, 297 (1961).
[3] Hughes, B. P., *Nature*, **188**, 1020 (1960).
[4] Dreyfus, J. C., Demos, J., Schapira, F., and Schapira, G., *C.R. Acad. Sci., Paris*, **254**, 4384 (1962).
[5] Hann, A. M. F. H., *Biochim. Biophys. Acta.*, **11**, 258 (1953).
[6] Keeler, R. F., and Young, S., *Biochem. J.*, **81**, 93 (1961).
[7] Schapira, G., and Dreyfus, J. C., *C.R. Soc. Biol.*, **152**, 1705 (1958).
[8] Charpentier, J., and Goutefongea, R., *Ann. Biol. Anim. Bioch. Biophys.*, **3** (4), 381–389 (1963).
[9] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[10] Charpentier, J., and Goutefongea, R., *Eleventh Conf. European Meat Res. Workers*, Budapest (1963).
[11] Allen, J. M., *Ann. New York Acad. Sci.*, **94**, 937 (1961).

Sedimentation Behaviour and Molecular Weight of Choline Acetyltransferase

The human placenta is known to be a rich source of choline acetyltransferase (acetyl CoA: choline-*o*-acetyltransferase *E.C.* 2.3.1.6)[1]. Investigations have shown that this enzyme is very similar, in its kinetic behaviour and specificity, to choline acetyltransferase from other sources. In the foregoing work and in the course of purification of the placental enzyme (Morris, D., unpublished) it was of interest to determine its molecular weight, and to compare its sedimentation characteristics with that of rabbit brain enzyme.

In the investigation reported here, the sedimentation coefficients of placental enzyme and rabbit brain enzyme were compared by two methods, both permitting the examination of small amounts of an assayable component in a mixture. Sedimentation was carried out, either in a continuous sucrose gradient[2] or in the Yphantis-Waugh partition cell[3]. A preliminary estimate of the diffusion coefficient of rabbit brain enzyme, using diffusion in agar gel[4], has permitted the calculation of a provisional molecular weight for this enzyme.

Placental enzyme, from freeze-dried homogenates of several placentæ, and rabbit brain enzyme from an acetone-dried powder, were partially purified by ammonium sulphate fractionation. Three placental and one rabbit brain preparations were subjected to centrifugation in a continuous sucrose gradient at + 3° C, as described by Martin and Ames[2], but using 0·04 M phosphate buffer, *p*H 6·0. The results of 4 such experiments are shown in Table 1.

In one experiment pig heart malate dehydrogenase and yeast alcohol dehydrogenase (Boehringer) were included as marker enzymes in each tube. After centrifugation the tube contents were fractionated and assayed for choline acetyltransferase[5], for alcohol dehydrogenase[2] and for malate dehydrogenase by a modification of the method of Siegel and Bing[6]. In the tubes containing placental enzyme consistent unexplained losses of marker enzyme occurred; the values shown for these enzymes were recorded from the rabbit brain tube only. Having located the positions of maximum enzyme activity, the $S_{20\cdot w}$ (sedimentation coefficient) values shown were computed using tables of sucrose density and viscosity, as described by Martin and Ames[2]. A partial specific volume of 0·725 cm³/g was assumed for each enzyme.

The $S_{20\cdot w}$ value of the batch of malate dehydrogenase used was checked at two concentrations using a model *E* Spinco analytical ultracentrifuge. The values obtained were identical (Table 1) and similar to other reported values for mitochondrial malate dehydrogenase[7]. Using

Table 1

Type of centrifugation	Effective duration (h)	Enzyme	Sedimentation distance (cm)	$S_{20,w}$ in Svedberg units
Model *L* Spinco centrifuge Rotor *SW*39 39,000 r.p.m. continuous sucrose gradient	12·8	ChAc., Placenta I ChAc., Placenta II	1·03 1·08	4·67
	12·85	ChAc., Rabbit brain Duplicate	1·18 1·15	5·14
	17·45	ChAc., Rabbit brain	1·59	5·18
		ChAc., Placenta I ChAc., Placenta III	1·37 1·41	4·76 (4·72)
	16·57	ChAc., Rabbit brain	1·48	5·06 (5·02)
		Malate dehydrogenase Alcohol dehydrogenase	1·35 2·29	4·61 8·04
Model *E* Spinco centrifuge 52,640 r.p.m. using wedge cell		Malate dehydrogenase	—	4·58
Model *E* Spinco centrifuge 59,780 r.p.m. using partition cell	0·83 0·8	ChAc., Placenta I ChAc., Rabbit brain	— —	4·52 5·02

ChAc., Choline acetyltransferase.

our value, and assuming that $S_{20\,w}$ is proportional to the distance moved[2], the values of $S_{20,w}$ for the choline acetyltransferases shown in parentheses in Table 1 were calculated. It will be seen that the alternative values for malate dehydrogenase and for the choline acetyltransferases are in very good agreement.

A slightly lower sedimentation coefficient for the placental enzyme was consistently obtained. The results in experiments using a partition cell confirm those described here, and again show a small difference between the two enzymes.

A molecular weight of 67,000 for rabbit brain enzyme was derived from the mean value of $S_{20\,w} = 5{\cdot}13\ S$, and the measured diffusion coefficient, $D_{20\,w} = 6{\cdot}8 \times 10^{-7}$ cm² sec⁻¹.

Using the relationship[8] $\frac{S1}{S2} = \left(\frac{\text{mol. wt. 1}}{\text{mol. wt. 2}}\right)^{2/3}$ and assuming a similar shape and hydration for rabbit brain and placental enzymes, a molecular weight of 59,000 was found for the latter.

G. Bull
A. Feinstein
D. Morris

Agricultural Research Council
Institute of Animal Physiology,
Babraham, Cambridge.

[1] Bull, G., Hebb, C. O., and Ratkovic, D., *Nature*, **190**, 1202 (1961).
[2] Martin, R. G., and Ames, B. N., *J. Biol. Chem.*, **236**, 1372 (1961).
[3] Yphantis, D. A., and Waugh, D. F., *J. Phys. Chem.*, **60**, 630 (1956).
[4] Shantz, E. J., and Lauffer, M. A., *Biochem.*, **1**, 658 (1962).
[5] Hebb, C. O., *Handbook of Experimental Pharmacology*, Supp., edit. by Koelle, G. B., **15** (Springer-Verlag, Berlin, 1963).
[6] Siegel, A., and Bing, R. J., *Proc. Soc. Exp. Biol. and Med.*, **91**, 604 (1956).
[7] Thorne, C. J. R., *Biochim. Biophys. Acta*, **59**, 624 (1962).
[8] Schachman, H. K., *Ultracentrifuge in Biochemistry* (Academic Press, Inc., New York, 1959).

Pteridine Components of Wing Pigmentation in the Butterfly *Colias eurytheme*

Pteridine pigments were first described by Hopkins[1] from the wings of pierid butterflies, and the first chemical characterization of several pteridines from this source was performed by Wieland, Schöpf, Purrman and their associates, as reviewed by Gates[2]. Relatively little attention has been directed to pierid pteridines since, though Weygand and his associates have recently examined some aspects of pteridine biosynthesis in *Pieris brassicae* Linné[3].

Colias eurytheme Boisduval (Lepidoptera: Pieridae) is a convenient subject for investigation of the biological