References and Notes

1. L. A. Stauber, *Exptl. Parasitol.* 3, 544 (1954).
2. L. A. Schinazi and G. H. Ball, *ibid.* 5, 541 (1956).
3. W. Grassmann and K. Hannig, *Naturwissenschaften* 37, 496 (1950); Hoppe-Seyler's *Z. physiol. Chem.* 290, 1 (1952); the modification of the original Grassmann-Hannig apparatus used in these studies permits ten strips to be run simultaneously with excellent reproducibility. It was devised by Edwin Weller, to whom grateful acknowledgment is made for helpful advice during the course of the investigation.
4. R. H. Common, W. P. McKinley, W. A. Maw, *Science* 118, 86 (1953).
5. A description of the quantitative details of these changes is in preparation.
6. R. Hegner and R. Hewitt, *Am. J. Hyg.* 27C, 417 (1938).
7. I am indebted to Arthur Hirata for the spectrophotometric data reported here and for his advice concerning the nature of the fast component.

* U.S. Public Health Service Predoctoral Research Fellow, National Heart Institute.

22 January 1957

Concerning the pH Dependence of Enzyme Reactions on Cells, Particulates and in Solution

Considerable attention is being given to the localization of enzymes in and on cells (*1, 2*). Attention has been called to the similarity between the *p*H activity curves of certain enzyme reactions in intact cells and mitochondria (*3*) and in solution. Because of this similarity it has been suggested, for example, with trehalase, lactase, and invertase of yeast, that the enzymes concerned must be peripherally located in the cell. The assumptions are (i) that the internal *p*H of the cell is almost independent of the external *p*H of ambient buffer, (ii) that the permeability of the cell membrane to substrate is independent of *p*H, and (iii) that the extremes of *p*H do not kill the living cells. The assumptions are all valid with yeast (*2, 4*). More recent work with a different approach has verified the conclusion that invertase is located on the surface of yeast cells (*5*).

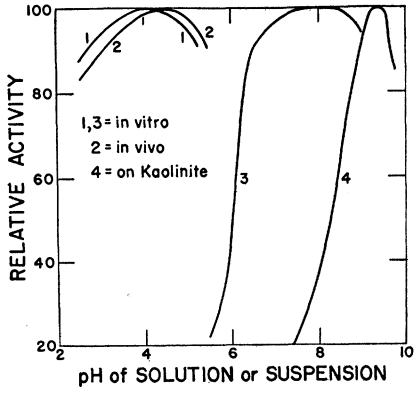

Fig. 1. The effect of *p*H on invertase activity of yeast cells and isolated enzyme (1, 2) and on chymotryptic activity in solution or adsorbed on kaolinite (3, 4).

It is noted here, however, that, if the surface carrying an enzyme is charged, owing to the presence of a polyelectrolyte, one cannot expect that the *p*H for optimum activity of an enzyme will be the same for the enzyme on the surface as compared with the action of the enzyme in solution. A charged surface of a cell or particle will either attract or repel hydrogen ions, depending on the sign of the charge, in an ionic double layer at the surface. An enzyme acting at a surface will thus be exposed to, and in equilibrium with, a hydrogen-ion activity differing from that of the ambient buffer. A comparison of the action of chymotrypsin on a protein in solution and on the surface of kaolinite particles (about 1 μ in size) is a case in point (Fig. 1) (*6*). The *p*H optimum for the enzyme on the surface is at a higher *p*H, and the *p*H of half maximum activity is shifted two units toward higher *p*H, indicating that the hydrogen-ion activity at the surface is about 100 times greater than in solution (*7*). In Fig. 1 data are also plotted from the paper of Wilkes and Palmer (*4*) for the effect of *p*H on invertase activity of yeast cells and of isolated enzyme. Again, the influence of surface is discernible, although less markedly (*8*). As with kaolinite, the data indicate that the surface of yeast has a negative charge density in the *p*H range shown (*9*).

A. D. McLaren

Department of Soils and Plant Nutrition, University of California, Berkeley

References and Notes

1. H. Holter, *Advances in Enzymology* 13, 1 (1952); M. Alexander, *Bacteriol. Revs.* 20, 67 (1956).
2. A. Rothstein, *Protoplasmatologia* 2, E4 (1954).
3. T. P. Singer, E. B. Kearney, N. Zastrow, *Biochim. et Biophys. Acta* 19, 200 (1956).
4. B. G. Wilkes and E. T. Palmer, *J. Gen. Physiol.* 16, 233 (1932).
5. A. J. Demis, A. Rothstein, R. Meier, *Arch. Biochem. and Biophys.* 48, 55 (1954).
6. A. D. McLaren and E. F. Estermann, *ibid.*, in press.
7. According to the modern view, the shape of the curves reflects the ionization of groups in the enzymatic sites [R. A. Alberty, *Advances in Enzymology* 17, 1 (1956)].
8. On the alkaline side of the optima the curves are more nearly coincident; fermentation, however, was a complicating factor with the yeast and the *p*H of the hydrolyzing mixtures dropped toward the optimum (*4*).
9. A. Rothstein and A. D. Hayes, *Arch. Biochem. and Biophys.* 63, 87 (1956).

24 January 1957

Enhancement of Oxidative Esterification of Inorganic Phosphate by Clinical Insulin

In an earlier communication (*1*) it was reported that the addition *in vitro* of Lilly clinical plain insulin increased the oxidative esterification of inorganic phosphate by rabbit tissue (kidney and liver) homogenates and extracts. It was subsequently found that similar enhancement could also be obtained with rat kidney and liver homogenates but not with rat brain homogenates. It was further observed that the Wellcome plain clinical insulin also gave similar results. Crystalline insulin (Lilly or Wellcome), however, had no such effect. This suggested that some component of the solvent used in the manufacture of clinical insulin was possibly responsible for the observed effect. Inquiry showed that the solvent now used in the manufacture of clinical insulin contains appreciable amounts (1 to 2 percent) of glycerol. Glycerol is known to act as a phosphate acceptor during aerobic oxidation in rabbit kidney homogenates (*2*). Thus, it appeared that the enhancement of esterification of inorganic phosphate during oxidation by clinical insulin was possibly caused by the presence of glycerol in the insulin.

Table 1 compares the effects of clinical plain insulin (Lilly and Wellcome) and glycerol on aerobic esterification of inorganic phosphate by rat liver homogenates. The results clearly show that the enhancement of the esterification of inorganic phosphate by clinical insulin is caused by the glycerol present in the insulin solution, which acts as an extra phosphate acceptor in the system (*3*). Rat brain tissue possibly does not con-

Table 1. Effects of the additions of clinical plain insulin and glycerol on the oxidative esterification of inorganic phosphate by rat liver homogenates.*

Experiment No.	Inorganic phosphate esterified (mg)		
	Control	Insulin	Glycerol
1	0.442	0.626	0.682
2	0.498	0.626	0.664
3	0.442	0.591	0.615
4	0.591	0.701	0.720
5	0.536	0.645	0.645
6	0.404	0.572	0.553

* Tissue preparation: Overnight-fasted rats were killed by decapitation, and the liver was quickly removed and chilled on cracked ice for 2 to 3 min. The chilled tissue was homogenized in an all-glass homogenizer with 3 vol of isotonic (0.9 percent) KCl for 5 min at 0°C. The homogenate was centrifuged in the cold for 2 min and the supernatant was used in the experiments. Method: Essentially the same as that used in the earlier communication (*1*). Each manometer vessel contained, in the side arm, 0.2 ml of 5 percent glucose and 0.1 ml of 0.2*M* $MgCl_2$, and, in the main compartment, 0.2 ml of 0.2*M* Na_2HPO_4, *p*H 7.5, 0.1 ml of 0.5*M* NaF, 0.2 ml of 0.01*M* adenosine-5-phosphate, 0.1 ml of 0.00025*M* cytochrome *c*, 0.2 ml of 0.5*M* sodium succinate, and water to make a final volume of 2.5 ml. When insulin or glycerol was added, 0.05 ml of water in the main compartment was replaced by the same volume of a 40 μ/ml clinical plain insulin (Lilly or Wellcome) or a 1.6 percent glycerol solution in distilled water acidified to *p*H 3.3 by HCl. Duration of experiment, 15 min; gas phase, air; temperature 38°C. Wellcome insulin was used in experiments 1, 2, and 3 and Lilly insulin was used in experiments 4, 5, and 6.

tain the enzyme which brings about the phosphorylation of glycerol; hence, clinical insulin had no effect in experiments with rat brain homogenates.

GANGAGOBINDA BHATTACHARYA
Department of Biochemistry, University of Cambridge, England

References and Notes

1. G. Bhattacharya, *Science* **123**, 505 (1956).
2. H. M. Kalckar, *Enzymologia* **2**, 47 (1937).
3. Since this paper was completed, my attention was drawn to abstracts of two papers by A. Ruffo *et al.* [*Chem. Abstr.* **48**, 8274d (1954); *ibid.* **49**, 1212d (1955)] in which the presence of a substance in commercial insulin preparations stimulating oxidative phosphorylation in rat liver homogenates was reported. Furthermore, the active material was also shown to be glycerol.
4. I wish to express my gratitude to F. G. Young for his kind interest in this work and for granting the use of laboratory facilities. My best thanks are due to C. G. Pope of the Wellcome Physiological Research Laboratories for helping me to obtain samples of the Wellcome insulin. Samples of the Lilly insulin were kindly donated by A. J. Kenny of this department. My thanks are also due to the Imperial Chemical Industries Fellowship Fund, University of Cambridge, for the grant of a fellowship.

22 January 1957

Influence of Prenatal Maternal Anxiety on Emotionality in Young Rats

The purpose of the observations reported in this article (*1*) was to test the hypothesis that emotional trauma undergone by female rats during pregnancy can affect the emotional characteristics of the offspring. By now, a good deal of evidence favoring this possibility has accumulated from diverse sources, including teratology (*2*), pediatrics (*3*), experimental psychology (*4*), and population biology (*5*). While none of the studies done has directly confirmed this hypothesis, many of them indicate that such hormones as cortisone, adrenalin, and adrenocorticotropic hormone, injected into the mother during pregnancy, have drastic effects on the fetus via the maternal-fetal blood exchange. Since strong emotion may release such substances into the mother's blood stream, there are grounds for supposing that it may have an important influence on fetal behavioral development. This experiment was the first in a projected series designed to examine this question in detail.

The rationale of the procedure was to create a situation which would predictably arouse strong anxiety in female rats, and to provide them with a standard means of reducing this anxiety; then to expose them to the anxiety-arousing situation during pregnancy, but block the accustomed means of escaping it. The assumption was that strong, free-floating anxiety would be generated in the pregnant females, and that any endocrine changes resulting would be transmitted through the maternal-fetal blood exchange to the fetus. The experiment was done by training five randomly chosen female hooded rats in a double compartment shuttlebox, first to expect strong shock at the sound of a buzzer, and then to avoid the shock by opening a door between the compartments and running through to the safe side. When the rats had learned this, the five experimentals, together with five control females, were mated to five randomly chosen males in a large cage. As soon as the experimentals were found to be pregnant (by vaginal smears), they were exposed to the buzzer three times every day in the shock side of the shuttlebox, but with the shock turned off and the door to the safe side locked. This procedure was terminated by the birth of a litter. The controls were placed in breeding cages during the same time.

Possible postnatal influences were controlled by cross-fostering in such a way as to yield a design with six cells, each containing ten offspring with two main variables—namely, prenatal and postnatal treatment. The data obtained from tests given to the young were examined by means of analysis of variance. In all tests of significance, three error estimates were used: the within-cell variance, the within-plus-interaction variances, and the within-plus-interaction plus between-postnatal-treatment variances. Thus, as shown in Table 1, all tests of significance reported involve three F values.

The emotional characteristics of the 30 control and 30 experimental offspring were compared by two tests given at 30 to 40 and 130 to 140 days of age. In test A, measures of amount and latency of activity in an open field were taken in three daily sessions of 10 minutes each. In test B, emotionality was measured by latency of leaving the home cage, and latency of reaching food at the end of an alley way leading out from the cage after 24 hours' food deprivation. In the second test, the maximum time allowed an animal to reach food was 30 minutes. In the measures used, low activity and high latency were taken as indices of high emotionality.

The results are summarized in Table 1. On test A, striking differences between experimentals and controls were obtained in amount of activity, both at 30 to 40 days and at 130 to 140 days. On the first testing, a significant interaction was obtained which probably represents genetic variation. On the second measure, experimental animals showed a much higher latency of activity than controls at both ages of testing. In neither of these activity measures were there any significant differences due to postnatal treatment or interaction besides the one mentioned.

In test B, experimental animals were slower to leave the home cage than controls at the first age of testing. There was no significant difference between groups in this measure, however, at 130 to 140 days of age. Similarly, experimentals showed a much higher latency than controls in getting to food at the end of the alley way at the first age of testing. The difference was less at the later age of testing. At both ages, significant interaction variances were found. As before, both may well be due to genetic variation. On neither of the measures used in test B were any significant differences found between methods of postnatal treatment.

It is clear from this analysis that the experimental and control animals differ strikingly on the measures of emotionality used, and that these differences persist to a great extent into adulthood. While there is no question about the reliability of these differences, there is some ambiguity regarding their cause. Thus, we do not know exactly how the stress used had effects. It is possible that

Table 1. Comparison of experimental and control animals on two tests of emotionality.

Item	Test A		Test B	
	Amount of activity (distance)	Latency of activity (seconds)	Latency to leave cage (minutes)	Latency to food (minutes)
	Tests given at age 30 to 40 days			
Experimentals	86.0	146.3	14.9	23.7
Controls	134.5	56.8	5.2	11.8
F values	(15.79, 14.21, 13.57)	(8.51, 7.91, 8.07)	(16.13, 16.46, 15.62)	(31.73, 25.66 25.87)
p	$< .001$	$< .01$	$< .001$	$< .001$
	Tests given at age 130 to 140 days			
Experimentals	114.5	71.5	4.8	11.6
Controls	162.3	26.8	2.1	6.2
F values	(9.77, 9.12 8.76)	(4.95, 4.79, 4.57)	(2.39)	(4.48)
p	$< .01$	$< .05$	$> .05$	$< .05$