

Pyruvate Dehydrogenase Phosphatase Deficiency: A Cause of Congenital Chronic Lactic Acidosis in Infancy

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Extract

A male child presented on the first day of life with metabolic acidosis with elevated blood lactate (15 mM), pyruvate (0.4 mM), and free fatty acid (1.3 mM) levels and a blood pH of 7.16. The severity of the acidosis was diminished by intravenous administration of glucose in large doses and by bicarbonate. On two occasions, when the acidosis was particularly severe, peritoneal dialysis using an acetate buffer was required. Restriction of the dietary intake of saturated fatty acids or treatment with nicotinic acid also appeared to diminish the severity of acidosis. No improvement was achieved by the administration of thiamine or biotin. Tissues taken at postmortem showed normal activity of gluconeogenic enzymes and pyruvate dehydrogenase. The activity of pyruvate dehydrogenase in tissue homogenates preincubated with ATP was reduced by 60-75% both in liver of the patient and of the controls because of the inactivation of the enzyme by pyruvate dehydrogenase kinase. Addition of Ca^{++} and Mg^{++} to the inactivated enzyme caused a prompt return of the activity to normal in controls but not in the patient. This defect, which was apparent in muscle and liver but not in brain, we attribute to a markedly reduced activity of pyruvate dehydrogenase phosphatase in the patient.

Speculation

Certain cases of chronic congenital lactic acidosis in infancy may be due to pyruvate dehydrogenase phosphatase deficiency.

Chronic congenital lactic acidosis of infancy is a disease characterized by a persistent elevation of blood pyruvate and lactate (9, 15, 16, 21). In three patients with chronic congenital lactic acidosis, markedly reduced activity of pyruvate dehydrogenase (EC. 1.2.4.1) was reported. In two of these, the defect was localized in the pyruvate decarboxylase (EC. 4.1.1.1) component of the enzyme complex (8, 14) and in the third patient in the dihydrolipoyl transacetylase (EC. 2.3.1.12) or the dihydrolipoyl dehydrogenase (EC. 1.6.4.3) component (9).

In this communication, we report a patient with chronic congenital lactic acidosis in whom biochemical investigations on tissue obtained at postmortem revealed a defect in the phosphorylation-dephosphorylation mechanisms controlling the overall activity of the pyruvate dehydrogenase complex in the liver and muscle. The postulated defect is correlated with the clinical manifestations of the disease during the life of the patient.

CASE REPORT

The male patient was born in February 1974 to nonconsanguineous Caucasian parents after an uneventful pregnancy at full term with a birth weight of 3.2 kg. The mother was 27 years old, gravida 3, para 3. A 7-year-old brother was normal. A sister had died at the age of 6 weeks with a chronic metabolic acidosis that had been

noted from the first day of life. At birth in our patient the Apgar score was 8 at 1 min, but acidosis developed during the first day of life and the patient was transferred to The Hospital for Sick Children.

He was a well developed newborn with severe tachypnoea of the Kussmaul type, poor peripheral perfusion, hypotonicity, and diminished consciousness. He did not have evidence of cardiopulmonary disease nor hepatomegaly. His biochemical data on admission are shown in Table 1 and indicated a metabolic acidosis associated with elevated levels of blood lactate (15 mM $\text{N} < 2$ mM), pyruvate (0.4 mM $\text{N} < 0.2$ mM), β -hydroxybutyrate (1.8 mM $\text{N} < 0.5$ mM), acetoacetate (0.36 mM $\text{N} < 0.15$ mM), and alanine (1.0 mM $\text{N} < 0.5$). The plasma free fatty acid level was elevated; the plasma glucose level was normal. Amino acid analysis of plasma and urine showed no abnormalities other than an elevated alanine level. Gas-liquid chromatography showed no abnormal organic acids to be present in the urine.

Therapy was commenced with an intravenous infusion of sodium bicarbonate (4-5 mEq/kg/hr) and glucose (4 mg/kg/min). The clinical and biochemical parameters did not alter appreciably until the 6th day of life when the rate of glucose infusion was increased to 12 mg/kg/min. Within 36 hr the plasma ketones had markedly decreased and the ketonuria had disappeared. The blood lactate had fallen from the previous range of 15-20 mM to 6 mM. The rate of infusion of both glucose and sodium bicarbonate was reduced. Commercial formula feeds (SMA) were commenced. The infant improved clinically and behaved normally.

During the stable periods of the infant's life, the basal blood lactate level remained in the range of 4-7 mM ($\text{N} < 2$ mM) and ketonuria was absent. At no time was hypoglycemia apparent. However, intermittent exacerbation of both lactic and ketoacidosis occurred at unpredictable and varying intervals, every 4-12 days, lasting for 3-5 days. When the blood lactate level rose to 9-15 mM, oral bicarbonate therapy (20-25 mEq/kg/24 hr) usually controlled the acidosis. At other times, when the blood lactate level rose to 15-20 mM and the β -hydroxybutyrate to 2.5-3.5 mM, more vigorous therapy was required. It was the episodic nature of this infant's clinical course that made the assessment of the clinical and biochemical effects of therapeutic measures difficult. At the age of 3-4 months, neurologic damage became increasingly evident with intermittent lethargy, irritability, and intermittent generalized seizure activity. The infant did not respond appropriately to stimuli. Death occurred at the age of 6 months.

METHODS

Lactate (2), pyruvate (3), β -hydroxybutyrate (4), acetoacetate (5), citrate (6), and α -ketoglutarate (7) were measured in whole blood samples extracted 1:1 with 0.6 N perchloric acid by enzyme spectrophotometric methods. Free fatty acids were measured in plasma by the method of Laurell and Tibbling (20).

Liver, brain, and muscle tissue was obtained 1 hr after death at

Table 1. Biochemical data on admission

	mEq/ liter		mEq/ liter
Plasma		Blood	
Sodium	134	pH	7.16
Potassium	4.4	pCO ₂ , mm Hg	11
Chloride	110	Urine pH	5.2
Blood		Plasma	
Bicarbonate	3.5	Glucose, mg/100 ml	136
Lactate	15.0	Free fatty acid, mM	1.3
Pyruvate	0.4	Citrate, mM	0.11
β -Hydroxybutyrate	1.8	α -Ketoglutarate, mM	0.025
Acetoacetate	0.36		
Alanine	1.0		

autopsy. The tissues were frozen in liquid nitrogen and stored at -100° until used for determinations. For the enzyme assays to be listed below small pieces of tissue were thawed, homogenized in 10 volumes ice-cold 0.25 M sucrose, 5 mM Tris-HCl buffer, pH 7.4, and centrifuged at $600 \times g$ for 10 min to remove cell debris. Glucose-6-phosphatase was measured by the method of Swanson (30), fructose-1,6-diphosphatase by the method of Pontremoli (24), phosphoenolpyruvate carboxykinase by the method of Roobol and Alleyne (25), and pyruvate carboxylase by the method of Crabtree *et al.* (12).

For the determination of pyruvate dehydrogenase activity the method of Taylor *et al.* was used (31). The assay of whole tissue pyruvate dehydrogenase activity was carried out using 300–400 mg freshly thawed tissue homogenized in 10 volumes ice-cold buffer containing 10 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, 1% fatty acid free bovine serum albumin, pH 7.4. Aliquots of 0.1 ml were then added to the main chamber of 10-ml Erlenmeyer flasks containing 0.8 ml assay buffer (11 mM potassium phosphate, 1.1 mM EDTA, 2.8 mM MgCl₂, 1.6% bovine serum albumin (fatty acid free), 1.2 mM dithiothreitol, 0.3 mM pyruvate labeled with 0.12 μ Ci [$1-^{14}$ C]pyruvate/ml at pH 7.4 and 37°). The flasks were fitted with plastic inserts holding strips of filter paper (1×3 cm) saturated with 0.2 ml Hyamine and closed with a rubber stopper. The reaction was stopped after a given time interval by injecting 0.8 ml 0.08 M citric acid, 0.04 M Na₂HPO₄ buffer (pH 3.0). After 30 min of further incubation the filter strips were removed with forceps and placed in 10 ml toluene-ethanol (9:1, v/v) scintillation fluid and the 14 C activity measured in a Beckman liquid scintillation spectrometer.

Preincubations were carried out by warming aliquots of the homogenate to 37° and adding various effectors (ATP, Ca⁺⁺, and Mg⁺⁺) in concentrations and for the times given in the text. Aliquots of 0.1 ml were then transferred to the assay system above. The time dependence of this assay system is shown in Figure 1 using control tissue from liver and brain with and without preincubation of the homogenate with ATP. Linearity is evident up to a period of 2 min under the conditions used.

RESULTS

BIOCHEMICAL INVESTIGATIONS DURING PATIENT'S LIFE

Based on the repeatedly observed good therapeutic response to intravenous glucose, a low fat, high carbohydrate diet was introduced which supplied protein (2.5 g/kg/24 hr) in skim milk with added glucose to provide a total of 80 cal/kg/24 hr. This was fed at 2-hour intervals for 20 days. During that time the basal lactate levels remained in the range of 7–9 mM. Three moderate exacerbations of the acidosis occurred with lactate levels of the order of 9–12 mM.

Then, while continuing this low fat, carbohydrate diet, nicotinic acid was administered orally in increasing doses up to 300

mg/24 hr over a 10-day period. Blockade of endogenous lipolysis was achieved and was reflected in the fall of the plasma free fatty acid concentration from 0.8 to 0.2 mM. The basal blood lactate levels remained in the range of 4–7 mM. Exacerbations of the acidosis did not occur.

Then nicotinic acid therapy was discontinued and corn oil was added to the diet of skim milk and glucose. Six grams corn oil per day added a further 15 cal/kg/24 hr. On this diet, the basal lactate level was in the range of 7–11 mM. However, several moderate exacerbations of acidosis occurred with blood lactate levels up to 15 mM. At these times, reinstitution of intravenous glucose and bicarbonate resulted in a fall in the degree of acidosis. However, on two occasions, the blood lactate rose to the range of 18–25 mM whereupon peritoneal dialysis was instituted with an acetate buffer solution (Plasmalyte 56). This rapidly controlled the acidosis and the blood lactate levels fell to 10–12 mM over the 48-hr duration of each dialysis. When dialysis was discontinued, glucose and bicarbonate therapy was reintroduced and during the following 4–5 days, the blood lactate levels fell to the range of 3–4 mM.

On three occasions when the modified diet of skim milk, glucose, and corn oil was replaced by SMA oral feeds, a moderate to severe exacerbation of acidosis occurred within 12–24 hr.

Neither thiamine hydrochloride (20 mg im for 5 days) nor biotin (5 mg iv over 12 hr) produced any change in the basal blood lactate concentration.

POSTMORTEM INVESTIGATION

The activities of glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, pyruvate carboxylase, and pyruvate dehydrogenase in the liver homogenate were all within the normal range, whereas the activity of fructose-1,6-diphosphatase was elevated (Table 2).

Since the enzyme activities measured indicated neither a block in gluconeogenesis nor a block in pyruvate oxidation, the possibility of a defect in the control of pyruvate decarboxylation was investigated. Using tissue homogenates of the patient and controls the pyruvate dehydrogenase activity was measured before and after preincubation with ATP, which is known to inactivate pyruvate dehydrogenase by increasing the activity of the controlling enzyme, pyruvate dehydrogenase kinase (13, 14). The results of such an experiment are shown in Figure 2. ATP appeared to have the predictable effect in the case of both the patient and the control. Preincubation of liver homogenate for 2 min with 0.2 mM ATP resulted in marked reduction of pyruvate dehydrogenase activity in both the control and the patient tissue, respectively.

After this, the reactivation of pyruvate dehydrogenase was investigated. It is known that Ca⁺⁺ activates pyruvate dehydrogenase phosphatase, an enzyme which converts inactive phosphopyruvate dehydrogenase to its dephosphorylated active form (13, 33). It can be seen in the right-hand panel of Figure 2 that addition of

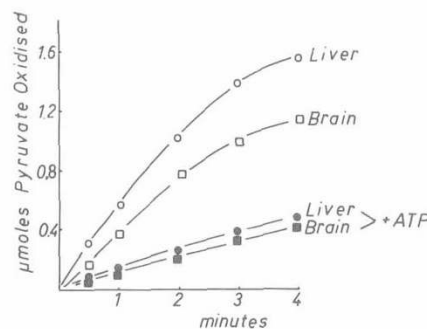


Fig. 1. Time course of micromoles of pyruvate oxidation in liver and brain homogenates. Homogenates of liver and brain (0.1-ml aliquots) from the patient's tissues were incubated for time periods of up to 4 min in a medium containing [$1-^{14}$ C]pyruvate as described by Taylor *et al.* (32), and the 14 CO₂ collected after acidification of the incubation medium to pH 3.0. This experiment was repeated with homogenates which had been preincubated with 0.2 mM ATP for 3 min.

mM Ca^{++} to the control liver homogenate resulted in rapid reactivation of pyruvate dehydrogenase and within 6 min activity was restored almost to that level before inactivation. However, the addition of 2 mM Ca^{++} to the patient's liver homogenate, as shown in the left-hand panel of Figure 1, resulted in only minimal reactivation.

In Table 3 results of similar studies are shown with homogenates of liver and also with muscle and brain homogenates of both the patient and controls.

In order to determine whether this defect was localized in liver, homogenates of liver, muscle, and brain were incubated under five sets of conditions and the activity of pyruvate dehydrogenase was measured (Table 3). ATP reduced the activity of pyruvate dehydrogenase in all three tissues in both the patient and in the controls. The degree of inactivation was somewhat variable, especially in muscle. The extent of inactivation found in the tissues of the patient was within the range of controls in the liver, although slightly lower in muscle and brain. When Ca^{++} (10 mM) and

Mg^{++} (10 mM) were added to reactivate the pyruvate dehydrogenase, activity in the liver of controls was restored in 5 min from 34% of the original activity to 83%. However, with the patient's liver homogenate the degree of reactivation was from 39.9% to 44.1%, indicating a markedly reduced ability of the patient to reactivate the enzyme. A similar effect was seen in muscle. Although reactivation in this case was variable in controls, it was minimal in the tissues of the patient. An alternative method to adding Ca^{++} and Mg^{++} of stimulating conversion of inactive to the active form of this enzyme is by the removal of ATP through the hexokinase reaction. Addition of glucose and hexokinase after inactivation with ATP gave consistent reactivation in controls, but only a very small reactivation in the patient. Addition of Ca^{++} and Mg^{++} to homogenates in the absence of ATP had little effect on the activity of pyruvate dehydrogenase in any of the tissues. In brain, however, although one only control specimen was available, inactivation and reactivation occurred both in patient and control to a similar extent.

Table 2. Activities of key gluconeogenic enzymes and pyruvate dehydrogenase in liver of patient compared with controls

	$\mu\text{mol/min/g liver}$		
	Patient	Controls ¹	Range
Glucose-6-phosphatase	1.70	1.77 ± 0.16	(1.36–2.14)
Fructose-1,6-diphosphatase	3.2	1.72 ± 0.42	(0.91–3.21)
Phosphoenolpyruvate carboxykinase	0.78	0.97 ± 0.08	(0.79–1.32)
Pyruvate carboxylase	0.61	0.66 ± 0.02	(0.61–0.71)
Pyruvate dehydrogenase	0.80	0.59 ± 0.07	(0.45–0.76)

¹ Values for controls are given as the mean \pm SEM. Control values are from two biopsy specimens taken from patients undergoing liver surgery and from four specimens taken at 1–2 hr postmortem.

Table 3. Pyruvate dehydrogenase activity in liver, brain, and muscle of patient compared with controls¹

Preincubation	Pyruvate dehydrogenase activity, nmol/min/g liver				
	(a) No additions	(b) ATP	(c) ATP + Ca^{++} + Mg^{++}	(d) ATP + hexokinase glucose	(e) Ca^{++} + Mg^{++}
Muscle					
Control 1	1,041	197	455	914	1,045
Control 2	1,167	476	1,025	1,171	1,340
Control 3	1,298	547	872	1,243	1,421
Patient					
Expt. i	1,120	606	620	656	1,610
Expt. ii	1,336	694	592	721	1,330
Brain					
Control	430	186	362		484
Patient	605	388	505		606
Liver					
Control 1	790	215	690		715
Control 2	755	167	592		790
Control 3	715	205	645		800
Control 4	630	306	519		705
Control 5	731	362	569		698
Patient					
Expt. i	805	325	374		801
Expt. ii	781	341	356		671
Expt. iii	846	315	341		631

¹ Pyruvate dehydrogenase activity was determined in tissue homogenates as described in Reference 31 after the following preincubations: (a) no additions; (b) plus 0.2 mM ATP for 3 min; (c) plus 0.2 mM ATP for 3 min followed by 10 mM Ca^{++} and 10 mM Mg^{++} ; (d) plus 0.2 mM ATP for 3 min followed by 5 mM glucose and 2 U hexokinase for 3 min; (e) plus 10 mM Mg^{++} and 10 mM Ca^{++} for 3 min.

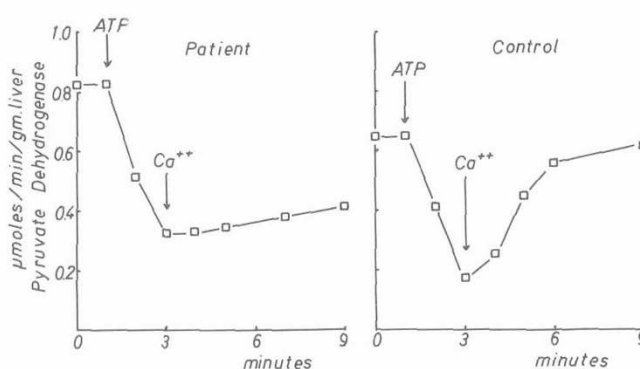


Fig. 2. Activity of pyruvate dehydrogenase in homogenates of the liver of the patient and a control on incubation with ATP and Ca^{++} . See text for details of experiment.

DISCUSSION

CLINICAL AND CHEMICAL STUDIES DURING LIFE

The clinical history, the course of the disease, and the laboratory data obtained during life in this patient provide some very pertinent clues when one considers the etiology of chronic congenital lactic acidosis. That the disease is familial is shown in this case by the fact that a female sib of the patient died at 6 weeks of age with a severe lactic acidosis. Both lactate and pyruvate were elevated, which indicated that pyruvate was not being metabolized and although the lactate to pyruvate ratio was abnormally high, there was no apparent hypoxia. The absence of hypoglycemia at any point during the course of the patient's disease suggested that the lactic acidosis was not due to a primary block in the gluconeogenic pathway such as is seen in pyruvate carboxylase deficiency (10), fructose-1,6-diphosphatase deficiency (1), or type I glycogen storage disease (glucose-6-phosphatase deficiency) (26). The onset from birth, the severity of the acidosis, and the absence of a raised plasma α -ketoglutarate or citrate level made the possibility of Leigh's disease unlikely (17, 36). The absence of abnormal urinary organic acids ruled out the possibility that the lactic acidosis was secondary to a block in propionate or methylmalonate metabolism.

The finding of elevated plasma free fatty acid levels in this patient raised the possibility of a defect in fat metabolism, but this was deemed unlikely, at least in liver, because of the presence of ketone bodies in the plasma which indicated that fatty acids were being oxidized. Nevertheless, it was observed that a high dietary fat intake, especially of saturated fatty acids, made the acidosis worse, whereas a high carbohydrate intake seemed to correct the acidosis. Unsaturated fats appeared to be tolerated better than saturated fats. Reduction of circulating free fatty acid levels by nicotinic acid therapy appeared to have a beneficial effect though the lactic acidosis was still evident. Summarizing this information, we felt that although a plentiful dietary supply of saturated fatty acids had an adverse effect on the acidosis, a primary defect in the catabolism of fat was unlikely to be the cause of the lactic acidosis.

POSTMORTEM STUDIES

The finding of normal activities of the key gluconeogenic enzymes in the liver of the patient confirmed our suspicion that a primary block did not exist in the gluconeogenic pathway. The initial finding of adequate pyruvate dehydrogenase activity was at first disconcerting since a distinct possibility existed of a defect in this enzyme, such as in the case described by Blass *et al.* (8). However, since pyruvate dehydrogenase is a multienzyme complex, the overall activity of which is closely controlled by a phosphorylation-dephosphorylation mechanism (22, 23), we felt that a defect in this control system might be a cause of chronic lactic acidosis.

Phosphorylation (and inactivation) of pyruvate dehydrogenase is catalyzed by a specific kinase, which requires magnesium ions and ATP, and which may be subject to inhibition by high concentrations of pyruvate or ADP (Fig. 3). Conversion of pyruvate dehydrogenase phosphate back to the active pyruvate dehydrogenase is catalyzed by a specific phosphatase requiring the divalent metal ions, Mg^{++} and Ca^{++} (13, 33). This regulatory system has been demonstrated to be active in muscle, brain, liver,

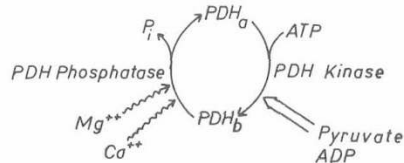


Fig. 3. Diagram to show activators and inhibitors of pyruvate dehydrogenase interconversion. Squiggly arrows indicate activation, thick arrows denote inhibition.

kidney, and adipose tissue (13, 22, 32, 33, 35), but a theory or concept as to how the system operates to modulate pyruvate dehydrogenase activity *in vivo* has only recently been proposed (33, 34).

In order to explain the change from the active to the inactive form of pyruvate dehydrogenase as a result of the transition from fed to a fasted state, it has been proposed that on fasting, free fatty acid levels increase, thus leading to increased tissue levels of long chain acyl-CoA thioesters, which inhibit the mitochondrial adenine nucleotide translocase system (23, 28) and the citrate-transporting system (11, 18), whereupon the intramitochondrial concentrations of both citrate and ATP rise. This has two effects: first both these compounds effectively chelate Ca^{++} and Mg^{++} and prevent them from activating the phosphatase. Second, the elevated level of ATP ensures that the kinase brings about inactivation of the pyruvate dehydrogenase complex. When fatty acyl-CoA levels fall, the intramitochondrial ATP and citrate level fall, Ca^{++} and Mg^{++} are released, and the phosphatase restores the inactive pyruvate dehydrogenase to the active form again.

We have demonstrated in the patient that pyruvate dehydrogenase phosphatase is defective in muscle and liver, having an estimated activity between 0 and 10% of normal. The fact that some activity of the phosphatase exists explains why the pyruvate dehydrogenase isolated from postmortem tissues appeared to be almost fully activated. Since postmortem ATP levels in all tissues fall rapidly to extremely low levels, inactivity of pyruvate dehydrogenase kinase would result. The low activity of the phosphatase in this patient would then be able to dephosphorylate the enzyme and restore full activity. However, "*in vivo*", one would expect the predominance of the kinase and the deficiency of the phosphatase to keep the pyruvate dehydrogenase complex in the inactive form. Resulting from this inability to fully oxidize the available pyruvate would be an elevation of pyruvate and lactic levels resulting in chronic lactic acidosis. Any circumstance leading to elevation of tissue fatty acyl-CoA esters would make the pyruvate dehydrogenase enzyme complex more inactive, a situation that concurs with our observations that the lactic acidosis was exacerbated by dietary fatty acids and relieved by reduction of circulating fatty acids and use of nicotinic acid therapy.

There are two important clinical points arising from consideration of the biochemical aspects of lactic acidosis. It has been reported in two cases of congenital lactic acidosis that transfusion of patient with citrated blood caused a severe bout of acidosis (19, 27). Consideration of the effect of citrate on pyruvate dehydrogenase phosphatase activity dictates that any activity in this enzyme would be drastically reduced by removing the activators Ca^{++} and Mg^{++} . If this enzyme is already defective in activity, citrate would have the effect of putting the pyruvate dehydrogenase complex completely in the inactive form. Also, acetate has been shown *in vitro* to activate the enzyme by inhibiting the kinase (31), which might explain why peritoneal dialysis against acetate buffer in the patient was successful in alleviating the acidosis.

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40. Requests for reprints should be addressed to: B. H. Robinson, M.D., Research Institute, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, M5G 1X8 (Canada).
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Corrigendum

The Abstracts of the 13th and 14th Annual Meetings of the European Society for Paediatric Endocrinology were published in the August, 1974 issue of this Journal.

Because of a printing error, the headings of the two groups of abstracts were transposed which resulted in the abstracts of the 14th Annual Meeting in Berlin (pages 666-679) appearing under the heading of the 13th Annual Meeting in Paris and, conversely, the abstracts of the 13th Annual Meeting in Paris (pages 680-692) appearing under the heading of the 14th Annual Meeting in Berlin.

Additionally, the heading "Read by Title" mistakenly preceded Abstract 45 on page 673 instead of in its correct position preceding Abstract 45 on page 687.

The Publisher sincerely regrets these errors.