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THE MANAGEMENT OF MECONIUM ASPIRATION SYNDROME*

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SUMMARY: Roberton NRC. (Rosie Maternity Hospital, Cambridge, United Kingdom). The management of meconium aspiration syndrome. Turk J Pediatr 33: 65-78, 1991.

Meconium aspiration is one of the important causes of mortality and morbidity in the neonatal period. Recently, advances for preventing meconium aspiration syndrome by the use of fetal monitoring, mechanical ventilation and supportive treatment have decreased the incidences of morbidity and mortality in this syndrome. In this paper, the management of this syndrome is reviewed and recent advances noted. *Key words: meconium aspiration syndrome, newborn.*

The incidence of meconium aspiration syndrome varies, perhaps as an indication of the standards of intrapartum care. In Europe, the incidence is between 1:1000 - 1:2000 whereas in North America and the Middle East rates of 2-6:1000 have been reported, with a mortality ranging from 5-30% (Table I).

It is a common cause of neonatal mortality and morbidity in otherwise normal term babies and frequently co-exists with hypoxic-ischemic encephalopathy (HIE) babies who have suffered severe birth asphyxia.

TABLE I: Incidence of Meconium Aspiration Syndrome (MAS)

Years	Incidence/1000 Births	Mortality	Country	Reference
1970-74	2.4	28%	U.S.A.	Carson et al[1]
1976-77	0.9	0%	Sweden	Hjalmarson[2]
1976-84	3.0	7.2%	Lebanon	Mounla[3]
1983-84	0.5	0%	U.K.	Field et al[4]
1984-88	3.4	?	Saudi Arabia	Roberton[5]
1985-89	1.8	4.5%	U.K.	Roberton[6]
1973-87	6.5	4.2%	U.S.A.	Wiswell et al[7]

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Etiology

Three things have to happen for a baby to develop meconium aspiration syndrome (MAS): he has to pass meconium, he then has to inhale it, and finally the inhaled material has to damage the lungs.

About 15-20% of babies are born in the presence of meconium stained liquor[8], and MAS is primarily a disease of term and post-term pregnancies[9,10] and only occurs in a very small percentage of preterm pregnancies unless there is concomitant infection with listeria[11]. It is of course, a very poor marker of fetal asphyxia, and is not a feature of small-for-dates pregnancies[12].

Once passed, the meconium can be inhaled either prenatally or postnatally. Meconium inhalation can occur pre-delivery since it is found in the lungs of stillborn babies[13], and in the lungs of babies who die in the early neonatal period, but could not have inhaled meconium intrapartum[14-16]. Hooper, et al[17] have recently shown that prolonged fetal hypoxia can stimulate fetal breathing to the extent that amniotic fluid is inhaled.

Postnatally, meconium inhalation can occur if the baby breathes or gasps with his mouth, pharynx or larynx full of meconium stained liquor. It is, of course, crucial to realize that meconium can be inhaled whether or not the baby is asphyxiated. A normal baby with his mouth and pharynx full of meconium stained liquor may inhale it, and become critically ill even if he did not suffer any problems pre-delivery.

Once inhaled, meconium has at least four deleterious effects on a neonatal lung (Fig. 1).

1. It creates a ball valve mechanism in the airways, whereby air can be sucked past the plug but cannot be exhaled.

2. It acts as a chemical irritant: 24-48 hours after inhalation there is an exudative and inflammatory pneumonitis with alveolar collapse and cellular necrosis.

3. The organic nature of the inhaled material, although initially sterile, may pre-dispose the baby to pulmonary infection, particularly with *E.coli*[18].

4. It displaces surfactant off the alveolar surface[19].

These four factors combine to cause severe airway and alveolar disease, but from studies in animals it may be that severe disease will only occur if there is co-existing asphyxial lung damage[20].

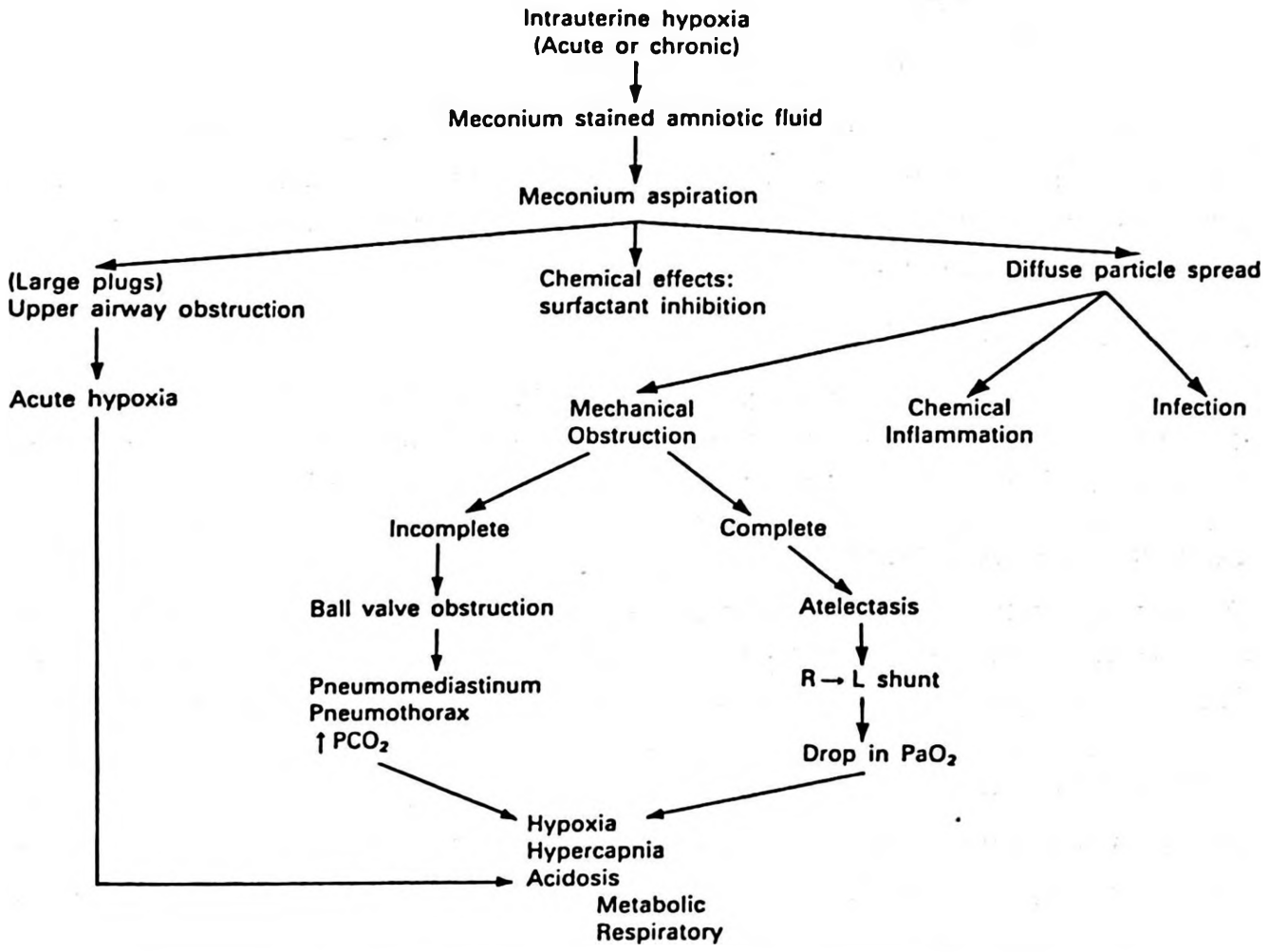

Fig. 1: Schema for the pathophysiology of meconium aspiration pneumonia[48].

SIGNS AND SYMPTOMS

General Appearance

The baby is usually mature or post-mature. The skin, nails and umbilical cord are often stained greenish yellow. The baby is not usually febrile, unless he becomes secondarily infected. Peripheral edema is not a feature, and if present, it suggests either central nervous system (CNS) damage, renal failure, or iatrogenic fluid overload.

Respiratory

The respiratory rate may exceed 120/min with intercostal and subcostal recession, the use of accessory muscles, and flaring of the nostrils. The meconium in the airways causes widespread sticky crepitations, and occasional ronchi, and an overdistended chest with an increased anteroposterior diameter.

Cardiovascular

In uncomplicated MAS the heart rate tends to be rather low, at around 110-125/min, and the blood pressure is maintained within the normal range. Hypotension suggests that there has been asphyxial myocardial damage. If pulmonary hypertension develops, S_2 may remain single, and there may be the murmur of tricuspid incompetence.

Abdominal Examination

The liver and spleen are often palpable due to downward displacement of the diaphragm caused by air trapping. In a severely affected infant, bowel sounds may be absent, and no further meconium will be passed.

Central Nervous System

Depending on the severity of the co-existing neurological insult, the baby may behave normally, or may show any of the neurological features of HIE. Convulsions may occur, and he may be jittery for a period of three-four days.

DIFFERENTIAL DIAGNOSIS

This should rarely be a problem. A term baby who is dyspneic and has meconium stained liquor in his trachea and larynx at delivery has MAS until proved otherwise.

PATHOPHYSIOLOGY

The respiratory failure and hypoxemia in babies with MAS are due to stiff lungs, marked ventilation-perfusion imbalance and pulmonary hypertension precipitating extra-pulmonary right-left shunts.

Bancalari et al[21] (Table II) showed that babies with MAS have a reduced compliance and tidal volume, and an increased airways resistance, but the tachypnea increased the minute volume to twice normal. In the early stages of the disease, when the airways are plugged by meconium, as in the animal model[22],

TABLE II: Respiratory Function in Meconium Aspiration Syndrome (MAS) (Bancalari et al[21])

Investigation	Values in (Mean 1SD)	Normal
Tidal volume (ml)	16.3 (6.7)	24
Minute ventilation (ml/min)	1647 (611)	800
Compliance (ml/cmH_2O)	1.82 (0.76)	4-6
Inspiratory resistance (cmH_2O/L/sec)	100 (67)	15-30

there is a marked ventilation perfusion abnormality which lessens as the lungs recover[23,24]. Later in the disease, shunting increases as the chemical pneumonitis becomes a dominant factor[22]. In those who develop pulmonary hypertension, the pulmonary artery pressure will be higher than the systemic[25] with right-left shunting at the ductus arteriosus[26] and/or the foramen ovale.

INVESTIGATIONS

Blood and biochemistry

The hemoglobin should be checked daily, and coagulation disturbances sought in those who were severely asphyxiated, show a bleeding diathesis or are ventilated. The electrolytes, urea and albumin should be measured daily and abnormalities corrected.

Blood gases

Hypoxia is common, but in cases with mild-moderate disease who are mature, with an efficient respiratory pump, ventilation is not a problem and the $PaCO_2$ may even be low, normal or only slightly raised. A $PaCO_2 > 60$ mm/Hg is unusual, and is seen only in patients with severe lung disease.

Changes in pH initially reflect the metabolic acidemia of intrapartum asphyxia, but if they persist, hypotension and septicemia should be sought.

Electrocardiography and echocardiography

If there has been severe intrapartum asphyxia, there may be electrocardiographic changes suggesting sub-endocardial ischemia and echocardiography will show a reduced cardiac contractility.

Chest X-ray

Many different x-ray patterns may be seen in neonates with MAS. A common early change is widespread patchy infiltration with small pleural effusions in 20-30% of cases. Over-expansion is also common in the early stages, and is best seen on the lateral chest X-ray.

In severe cases, by 72 hours of age, with or without ventilation, the chest x-ray shows diffuse and homogeneous opacification of both lung fields, presumably due to a pneumonitis and interstitial edema secondary to the irritant effects of the inhaled meconium. These changes gradually resolve over the next week, but in severe cases, the X-ray may still be abnormal at 14 days and merge into the changes of bronchopulmonary dysplasia. Air leaks, in particular pneumothorax and pneumomediastinum, are very common in MAS.

Microbiology

All babies suffering from MAS should have full set of swabs taken together with the blood culture as soon after admission to the Neonatal Intensive Care Unit (NICU) as possible. This is the only way to establish the co-existence of sepsis, and since antibiotics will be used in virtually all cases, this provides the essential base-line information upon which further antibiotic therapy can be planned.

TREATMENT

On the basis of the etiology and pathophysiology of MAS outlined above, treatment can be divided into two components. Firstly the impact of meconium in the amniotic fluid must be minimized by meticulous airway toilet in the labor ward; secondly if meconium is inhaled, the baby must be kept alive until the normal lung mechanisms for removing inhaled foreign material have cleared meconium from the airways and alveoli.

Labor ward care

It cannot be emphasized too strongly that the most important part of the treatment of MAS is meticulous removal of meconium from the baby's airway as soon after delivery as possible. Since babies can be born through meconium stained liquor, but not be asphyxiated, and there is no way of identifying which type of meconium staining (thick vs thin: early vs late) is most sinister, this means that someone skilled in laryngoscopy must attend all deliveries at which there is meconium staining of the liquor, even though this may mean attending up to 20 percent of all deliveries.

Airways suctioning

Although meconium can be inhaled prenatally, most cases of MAS are due to inhalation of meconium when the baby gasps during the last few moments of the second stage of labor, or with the initial few breaths or gasps after his head is delivered. Therefore, as soon as the baby's head is crowned and the mouth and nose visible, both should be efficiently and gently sucked out. Carson et al[1] showed that this would prevent many, but not all babies, from developing MAS, and that in addition, subsequent meticulous airway toilet was necessary. As soon as the pediatrician receives the baby, therefore, he should laryngoscope him, and carefully clear the upper airway of any meconium stained material present. This requires skill and experience in a vigorous howling term neonate. If no meconium is seen down the trachea distal to the vocal cords, there is no need to pass the suction catheter through the cords to suck the baby out[27]. If, however, meconium is seen in the trachea, direct endotracheal suction is indicated[28] and can be repeated several times, since some meconium will stay in the trachea and major bronchial divisions for several minutes after the onset of respiration[29].

Intubation and positive pressure ventilation

The presence of meconium in the airway is the one situation that takes precedence over immediate ventilation in the apneic neonate. Irrespective of the heart rate (even if it is zero) the upper airway must be cleared before the baby is intubated and resuscitated in the usual way. If the baby needs to be intubated for resuscitation, suck out the endotracheal tube before giving Intermittent Positive Pressure Ventilation (IPPV) or use the endotracheal tube as the suction tube[30] and then replace it. After giving the minimum IPPV necessary to cause a satisfactory increase in the heart rate, and an improvement in the baby's color, the endotracheal tube should be sucked out again to ensure that there is no residual meconium.

Bronchial lavage

Instilling water or saline into the lower respiratory tract is damaging, and repeated bronchial lavages can do harm. However if a baby, despite adequate endotracheal suctioning, is thought to have inhaled large amounts of meconium, instill 0.5-1.0 ml of saline down the endotracheal tube, hand ventilate the baby 4-5 times and suck out the tube. If no meconium is obtained, there is no need to persist. Only one or two lavages should be attempted.

Postnatal gastric aspiration

If the baby has inhaled meconium at delivery, it is likely that he has also swallowed some as well. His stomach should, therefore, be emptied as soon after delivery as possible.

Subsequent labor ward care

For the baby who, despite being covered in meconium, has no evidence of intratracheal meconium and who is pink and vigorous with no respiratory distress by 10 minutes of age, it can be assumed that he has not inhaled meconium. Such a baby does not need to be transferred to the neonatal unit, and should be given to his mother for routine postnatal care. Babies should only be transferred to the neonatal unit if they show signs of respiratory disease.

Care in the neonatal unit

If the baby is admitted to the Neonatal Unit (NNU) with respiratory difficulty, it is essential to obtain baseline investigations within the first 30-60 minutes. These must include a set of arterial blood gases, a measurement of blood pressure and a chest X-ray. If these procedures are not carried out in term babies, who may show surprisingly few clinical features of serious cardiorespiratory or acid-base abnormalities, profound acidemia, myocardial damage or serious hypoxia may all be missed.

Oxygen therapy

Despite the damaging effects of meconium in their airways, the relative efficiency of the respiratory system in mature babies enables them normally to control their $PaCO_2$ unless the disease is very severe or respiration is depressed by drugs or asphyxia. Since mature babies are not susceptible to retinopathy of prematurity, hyperoxemia is not a threat, and the prime concern is, therefore, preventing hypoxemia.

Most babies with MAS can, therefore, be managed by the introduction of an adequate concentration of warmed humidified oxygen into a headbox. This can be at a concentration of 80-90% if indicated, and may need to be sustained at this concentration for up to two weeks for those with severe disease. Unlike preterm babies with Respiratory Distress Syndrome (RDS), there is no need to intervene and give Continuous Positive Airway Pressure (CPAP) in the first 36-48 hours, just because the oxygen requirement exceeds 10-60%, or the $PaCO_2$ rises to 45-48.8 mm/Hg or greater.

In many mild cases, oxygen therapy at 40% or less for 24-48 hours is all that is required, and in all cases once the PaO_2 begins to rise, the Fi(fraction of) O_2 can be reduced. Many neonates with mild MAS have a normal PaO_2 in room air by 48 hours of age.

Acid base homeostasis

Carbon dioxide retention is only a problem in severe cases. However, a $PaCO_2$ of 56.3-60 mm/Hg is acceptable, so long as the pH is sustained above 7.25 and other factors in the baby such as his blood pressure, peripheral blood flow, urinary output and clinical condition are acceptable. If the $PaCO_2$ rises above 60 mm/Hg, particularly in the presence of a $PaO_2 < 45$ mm/Hg early in the disease, it is usually an indication for intubation and ventilation.

The first blood gas analysis taken after birth may show a marked metabolic acidemia. So long as the baby is otherwise stable, with a normal PaO_2 and $PaCO_2$ and in particular is passing urine, base deficits of –10 to –15 mmol/L can be left to correct themselves spontaneously and the baby should not be given sodium bicarbonate or THAM. However, once the base deficit has fallen to below –15 mmol/L or the pH below 7.10, it is prudent to correct the acidemia.

If the baby with MAS also has HIE, more rigid control of the blood gases may be called for; hypoxia, hypercapnia and acidemia are all undesirable if there is co-existing brain damage. The conservative management of the blood gases outlined above in babies with uncomplicated MAS may therefore have to be abandoned and the baby intubated and ventilated to teep the $PaCO_2 < 41.3$ mm/Hg.

Continuous positive airway pressure (CPAP)

Although CPAP at pressures of 4-7 cm H_2O increases the PaO_2 in babies with MAS[31], we rarely use it. It is not justified to intubate a baby just to give CPAP. Term neonates do not usually tolerate nasal CPAP and, furthermore, CPAP is likely to increase the risk of pneumothorax in a disease characterized by air trapping.

Intermittent positive pressure ventilation (IPPV)

In the absence of HIE, babies with MAS should only be intubated and ventilated if they collapse and become apneic with, for example, a pneumothorax, or if severe blood gas abnormalities occur: in general this means a $PaCO_2 > 60$-63.8 mm/Hg or a $PaO_2 < 41.3$ mm/Hg in 90% oxygen. Once intubated, these babies virtually always fight the ventilator, and pancuronium should be given.

To prevent air trapping as long an expiratory time as possible should be used, and it is rarely necessary to give more than 2-3 cm of positive end expiratory pressure (PEEP). Rates > 60-80/min should not be used, since particularly in paralyzed babies, this is likely to be associated with an increase in inadvertent PEEP. Initial ventilator settings should, therefore, be:

Rate 60-80/min
Inspiratory time 0.3 sec (i.e., ratio therefore approximately 1:2)
Peak inspiratory pressure 25 cmH_2O
End expiratory pressure 3 cmH_2O
Oxygen concentration 80%

The ventilator settings can then be adjusted in the light of the blood gas analyses.

When these large babies start to improve on IPPV, weaning can usually be rapid. Once they are in 50-60% oxygen and on pressures < 22/3 cmH_2O, they will usually be off pancuronium and will be breathing well on their own. The rate can then be reduced quickly, and they can be extubated without a period of CPAP.

Pulmonary vasodilators

Babies with MAS do benefit from these drugs[32,33]. In general, we would reserve them from those already on the ventilator, but as an alternative to intubating them and giving IPPV, it is sometimes worth trying the effect of a single i.v. push of tolazoline in a spontaneously breathing baby who just has severe hypoxemia if his blood pressure is stable, and he has a normal pH and $PaCO_2$. Whether ventilated or not, if there is an acceptable response in PaO_2 after a bolus of 2 mg/kg/i.v. of tolazoline, this can be followed by an i.v. infusion of 2 mg/kg/hr.

Physiotherapy

Intuitively this should be good for patients whose airways are plugged with nasty extraneous material. In practice, in the common mild-moderate cases who are not

ventilated and require 60% O_2, the prognosis is excellent whatever therapy is given, and I see little point in disturbing the baby to give chest physiotherapy. In the severely affected neonate who is intubated and paralyzed, 6-8 hourly percusion and endotracheal tube suction should be carried out. If this results in the production of significant amounts of greenish material, the benefits are clear cut, but if it makes the neonate irritable, restless and hypoxic, and little or no meconium is obtained, it is contraindicated.

Steroids

Randomized prospective controlled trials in animals and humans have shown no benefit from these drugs[34,35]; they should not be used.

Antibiotics

In the first few hours it it impossible to be certain whether a baby has just MAS, or MAS complicated by intrapartum infection with, for example, the Group B streptococcus. The presence of organic material in the lung may predispose to pneumonia[18]. Therefore, after taking a full set of cultures, but not doing a lumbar puncture, we put all neonates diagnosed as MAS on broad spectrum antibiotics using penicillin and gentamicin in most cases, though, in preterm babies who may be at risk for listeria, ampicillin would be substituted for penicillin. We would continue the drugs until the baby was in the convalescent stage of his illness and requiring < 30-40% oxygen.

Blood volume and blood pressure

Anemia is not usually a problem in babies with MAS either immediately after delivery or as a result of blood sampling, but if the hemoglobin falls below 13 g/dl, the baby should be transfused[36]. Blood pressure control is essential in all neonates with MAS, aiming to keep the pressure above 50-55/30 mmHg. Hypotension is usually only a problem in those babies who have suffered intrapartum asphyxia with co-existing myocardial injury or after administration of tolazoline. If hypotension does occur, an infusion of albumin or blood (15 ml/kg) should be given. If this is not effective, dopamine should be given starting at 5 μg/kg/min intravenously.

Fluid and electrolyte balance

In uncomplicated MAS this is rarely a problem. Intravenous fluids should be started at 60 ml/kg/day on the first day and increased thereafter as indicated. If the MAS was accompanied by severe birth asphyxia, a much stricter fluid regime giving only 40-45 ml/kg/24 hours is indicated. The initial fluid given should be 10% Dextrose, with electrolytes added after 24-48 hours. Hypoglycemia is always a possibility, and babies with MAS should have their blood glucose checked twice or thrice a day for the first 48 hours.

Nutrition

In the first day or two of the disease enteral feeding is not justified. Mild-moderate cases of MAS have a short duration so that some enteral feeding can usually be started by 2-3 days of age in most babies. For those still on IPPV at this stage, and for the small number of non-ventilated cases who by 5-6 days are not suitable for enteral feeding, intravenous nutrition can be started.

MONITORING

There is a temptation to undermonitor the large full-term baby with MAS, because, despite marked tachypnea and a dreadful chest X-ray he often looks surprisingly pink and vigorous. This temptation should be resisted, not only because of the importance of early detection of blood gas abnormalities, electrolyte disturbance or hypotension before secondary effects develop, but because the baby with MAS has a major risk of sudden deterioration with a tension pneumothorax.

Heart rate and respiration (apnea) must be monitored continuously, and if continuous blood pressure recording is not available, it should be checked by one of the oscillometric devices on admission and 2-4 hourly thereafter.

Since hyperoxemia is not a threat to mature babies, in mild cases requiring 30-40% oxygen by headbox adequate control can be obtained by measuring blood gases on samples drawn 2-3 times a day by direct arterial puncture. Umbilical catheterization is indicated for ease of sampling in infants requiring high FiO_2, IPPV, and in those with neurological problems, in whom frequent blood gas measurements are necessary. Transcutaneous PO_2 and PCO_2 monitors are less accurate in term babies with their thick skin[37]. Pulse oximetry, however, is a very effective way of monitoring oxygenation.

COMPLICATIONS

Pneumothorax, air leaks

These are the most feared and common complications, occurring in 15-20% of unventilated babies[38,39], and in 30-50% of those who require IPPV[40,21]. Pulmonary interstitial emphysema is rare, but pneumomediastinum, pneumopericardium and pneumoperitoneum can all occur.

Pneumothoraces may be both bilateral and under tension, and commonly cause acute deterioration in the baby's condition. It is essential, therefore, that babies with severe MAS are only cared for in units that have the wherewithall to respond instantaneously and effectively when a tension pneumothorax occurs.

Pulmonary hypertension (persistent fetal circulation)

The North American experience would suggest that this is a common complication of MAS and on histological and clinical grounds it would appear to be particularly common in fatal cases[41]. The incidence of this complication does seem to vary[42,43], but this may be merely a matter of definition. In my experience it is rare, and usually responds promptly to tolazoline and conventional ventilation.

Bronchopulmonary dysplasia

Both in the literature[44,45] and in my experience this appears to be a rare complication of MAS, though it may occur in any baby who survives after long-term high pressure IPPV.

Outcome

With good labor ward care, most cases of MAS should be mild; only 10% of cases admitted to the Cambridge Neonatal Unit require IPPV. Even for these cases, survival should be the rule except for those who are virtually impossible to resuscitate in the labor ward, or those who have fetal co-existing HIE. Neurological sequelae in survivors are primarily limited to babies with HIE, but long-term damage to the lungs may occur even in those who are not ventilated[46,47].

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PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS IN A TURKISH FAMILY*

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SUMMARY: Yılmaz E, Özgüç M, Coşkun T, Beksaç S, Çakar N, Ayter Ş, Özalp I. (Departments of Medical Biology, Pediatrics, Obstetrics and Gynecology, and Morphology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Prenatal diagnosis of cystic fibrosis in a Turkish family. Turk J Pediatr 33: 79-84, 1991.

Prenatal diagnosis of cystic fibrosis (CF) was made in a Turkish family whose first born child was diagnosed at necropsy as having CF. Two consecutive pregnancies followed. The fetus of the second pregnancy was diagnosed as having CF by the microvillar enzyme assay and was aborted. The diagnosis was verified by the DNA polymerase chain reaction analysis using chorionic villi from the abortus. In the third pregnancy, amniocentesis was performed in the 17th week, and KM19 polymorphism linked to CF was used to assess the status of the fetus. Since the fetus was determined to be a carrier, the family was advised to continue with the pregnancy.
Key words: cystic fibrosis, DNA amplification, KM19 polymorphism, prenatal diagnosis.

Cystic fibrosis (CF) is a common recessive genetic disorder affecting approximately 1 in 2500 Caucasians. The disease causes defective regulation of chloride ion transport in exocrine gland epithelia and is linked to a locus on chromosome 7q31 coding for cystic fibrosis transmembrane regulator (CFTR) protein[1].

Clinically, CF is characterized by recurrent respiratory tract infections and malabsorption. Although the survival rate of the disease has improved in recent years, the therapy is still symptomatic, being directed to the good control of pulmonary infections and provision of an optimal nutritional support[2]. Lack of

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radical therapy warrants that families affected by the disease seek prenatal diagnosis.

In the mid 1980s, decreased levels of microvillar enzymes in the amniotic fluid of women carrying fetuses affected by CF were demonstrated, providing a good, but not an absolute method, of prenatal diagnosis due to false positive and false negative results[3]. The discovery of tightly linked informative flanking markers made possible a DNA-based carrier test for the disorder, and prenatal diagnosis of CF was established by chorionic villus sampling in the first trimester for families in which there was at least one affected child.

In this report, we describe the use of the polymerase chain reaction (PCR) to analyse the KM19 polymorphism in a family affected by CF with regard to the prenatal diagnosis of the disease.

Material and Methods

The proband (Fig. 1), a three-month-old male, was the first-born child of nonconsanguineous parents. He suffered from chronic, progressive lung disease

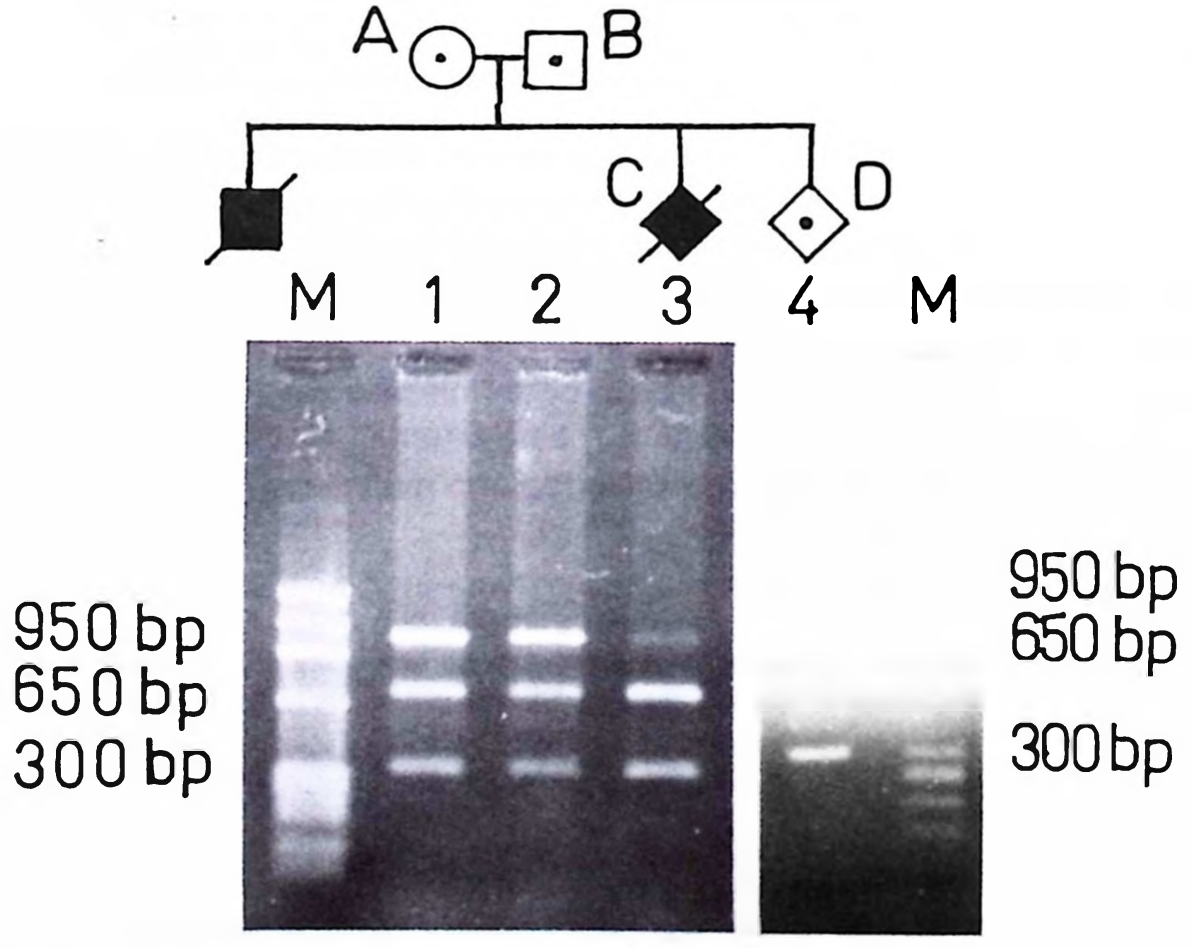

Fig. 1: Results of Pst I restriction digestion of amplified DNA samples. (A1): Mother; obligate carrier, B(2): Father; obligate carrier, D(3): Chorionic villi from abortus D(4): Prenatal diagnosis of fetus, M: øx174 Hae III digest size marker DNA.

and impaired growth. He subsequently died due to severe pulmonary infection. A diagnosis of CF was made at necropsy, and the parents were given thorough genetic counselling.

Prenatal diagnosis was offered to the parents in two subsequent pregnancies. In the second pregnancy, microvillar enzyme analyses of the amniotic fluid sample demonstrated that the fetus was affected by this genetic disorder (The analyses were performed by Prof. DJH Brock, Edinburgh, U.K).

The pregnancy was terminated. DNA was isolated from the chorionic villi of the abortus and analyzed by PCR to confirm the result of the enzyme analysis. In the present pregnancy, amniocentesis was performed during the 17th week of gestation, and DNA was isolated from the amniotic cells.

Preparation of genomic DNA[4]*:* The DNA was extracted from isolated lymphocytes, amniotic fluid cells or chorionic villi and cleared of maternal decidua by proteinase K (200 µg/ml) and sodium dodecyl sulphate (0.2 %) treatment for a 16 hour period at 37 °C, followed by phenol-chloroform isoamyl alcohol (25:24:1) extraction and precipitation with ethanol. DNA was redissolved in a TE buffer (10 mM Tris-HCl, 0.1 mM ethylenediamine tetra acetic acid, EDTA pH 7.0) and kept frozen until analysis.

DNA amplification: KM19 oligonucleotide primer sequences were (50 pmol/L) 5' (AAGGTACATGTTAATTTT) 3' (forward) and 5' (GCTGCATCATATAGTTGCC) 3' (reverse). The reaction mixture consisted of genomic DNA (1 µg), 1xTaq polymerase buffer (25 mM Tris-HCl pH 9.5, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mg/ml bovine serum albumin, 300 µg/ml activated calf thymus DNA), 0.2 mM of each of the deoxyribonucleotides (dATP, dGTP, dCTP, dTTP) and the thermostable DNA polymerase (Amersham Int.), (2.25 U per reaction) in a total volume of 0.1 ml. The initial template denaturation step was performed by adding 100 ml mineral oil to the reaction mixture present in 0.5 ml microcentrifuge tubes and heating it for ten minutes at 92 °C. The temperature cycling conditions for KM19 oligonucleotide primers were: 1 minute at 92 °C (denaturation), 1.2 minutes at 55 °C (annealing) and 2 minutes at 72 °C (extension) for a total of 30 cycles with a final extension step of 10 minutes instead of two minutes to fully extend any remaining single-stranded DNA[5]. The procedure was performed using a household manual system consisting of water baths that could be adjusted to the above temperatures.

PCR product analysis: PCR products were checked by 1.5 % agarose gel electrophoresis and the amplified fragments were visualized with ethidium bromide under ultraviolet light. Thirty microliters of the amplified product were digested by PstI restriction enzyme (25 U of the enzyme incubated at 37 °C for a period of 16 hours). Restriction fragments were observed using ethidium bromide stained 1.5% agarose gel[6].

Results

PCR products resulting from amplification when digested with PstI enzyme yielded electrophoretically separable fragments of different lengths which represented different alleles. (Fig. 1 a - d).

A: Obligate carrier mother; displayed three bands of 950 base pairs (bp), 650 bp and 300 bp each.

B: Obligate carrier father; displayed three bands of 950 bp, 650 bp and 300 bp each.

C: Chorionic villus material from the second pregnancy in which the fetus was found to be affected by microvillar enzyme analyses displayed only two bands of 650 bp and 300 bp each.

D: Amniocytes from present pregnancy displayed three bands of 950 bp, 650 bp and 300 bp each.

Discussion

As in any other recessively inherited autosomal disease, the risk of occurrence of CF in affected families is one in four. If the high risk of occurrence of CF in affected families and the limited impact of the present supportive approaches on the long-term prognosis of the disease is considered, there is a need for the use of methods for in-utero diagnosis of the disease, particularly in Turkey, where the frequency of the disease seems to be high[7].

Although rare (5%), diagnostic errors in the prenatal diagnosis of CF may occur when the microvillar enzyme assay of amniotic fluid is used. Recent developments in the field of DNA technology make it possible to diagnose CF, and many other genetic disorders more accurately. During the search for the CF gene KM19, XV2C and C57, probes have been identified that define the locus D7S23 which lies close to the CF locus[8].

A linkage disequilibrium between particular D7S23 haplotypes and CF has been shown[9]. Therefore, these markers can be used in clinical applications should prenatal testing and carrier detection be offered to families where there is an index CF case. Besides conventional Southern blotting, for haplotyping, sequences flanking these polymorphic regions can be used as oligonucleotide primers to amplify the region and check for polymorphism by use of restriction enzymes (KM19/Pst I, XV2C/Taq I, CS7/Hha I)[9,10]. In this case, the use of PCR technology for DNA amplification obviates the use of radioactivity for probe labeling and shortens the diagnostic procedure considerably. However, since this is not a direct determination of the mutation causing CF, the markers should be tested initially in the parental DNA samples. To make a decision concerning the fetus in regard to prenatal diagnosis, there should be data about the parents regarding the

markers and the linkage phase of the disease should be confirmed by the results of the index case.

In our study, we used KM19 polymorphism in the in-utero evaluation of the present pregnancy in this family. The longer DNA fragment demonstrated by agarose gel electrophoresis after Pst I digestion of the amplified sample was termed "code 1" and the shorter DNA fragment, "code 2", corresponding to the absence (1), or presence (2) of the restriction site. There are two copies of chromosome 7, thus there are three possible allelic combinations:

1/1 Normal: homozygous for the absence of the restriction site.

1/2 Carrier: one chromosome bears the restriction site, the other does not.

2/2 Affected: the restriction site is present on both alleles (homozygote for the disease).

A normal individual (1/1) carries only the 950 bp fragment whereas the individual affected by CF (2/2) carries 650 bp and 300 bp fragments. Any individual who is a carrier of the disease (1/2) exhibits three bands 950 bp, 650 bp and 300 bp.

Analysis of the DNA sample isolated from the chorionic villi of the abortus of the second pregnancy revealed two bands of 650 bp and 300 bp. This pattern was dissimilar to the pattern seen in the obligate carrier parents, who had 950 bp, 650 bp and 300 bp bands. Therefore, the diagnosis that the fetus was affected, was confirmed. The last pregnancy was also evaluated in the same manner, and the fetus was found to be heterozygote (1/2) for CF since an electrophoretic pattern similar to that of the parents was found. The mother was advised to continue with the present pregnancy.

A reliable prenatal diagnosis is now available for CF by using PCR technology if there is data with regard to the marker polymorphism of the parents of the index case. The Δ F508 mutation, which is linked to about 70 percent of the CF alleles of Northern Europeans, is observed in only 30 percent of the alleles of Turks Therefore, this mutation frequency can not be used as a direct test for CF in Turkey. The same situation also applies to other genetic diseases such as phenylketonuria where the alleles in the Turkish and European populations are linked to different haplotypes[11]. Studies are continuing in our laboratory to determine the major mutation in association with CF alleles in the Turkish population. Depending on these results, a direct determination of the mutation will be applied both for prenatal diagnosis and carrier detection.

Note Added in Proof:

Subsequent to the prenatal diagnosis of the index case as a CF carrier by KM-19 polymorphism, the status of the newborn was determined. KM-19 polymorphism was checked by PCR amplification of blood leukocyte DNA obtained from the newborn, and the diagnosis of CF carrier was confirmed.

Acknowledgement

We wish to thank Prof. Safiye Göğüş for diagnosing at autopsy cystic fibrosis in the index case, thus giving us the opportunity of carrying out DNA analysis in this family.

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INTESTINAL EFFECTS OF IRON DEFICIENCY ANEMIA IN CHILDREN*

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SUMMARY: Ercan O, Ulukutlu L, Özbay G, Arda O. (Departments of Pediatrics, Pathological Anatomy, and Histology and Embryology, Istanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Intestinal effects of iron deficiency anemia in children). Turk J Pediatr 33: 85-98, 1991.

A group of 11 children with iron deficiency anemia were studied with respect to intestinal structure and function. In six cases there were histological abnormalities of intestinal mucosa in varying degrees consisting of villous damage, increased activity in the crypts, increased lymphoplasmocytic infiltration and changes in the surface epithelium. Ultrastructurally, microvilli lesions, mitochondrial changes and an increase in lysosomes were observed. Relative malabsorption of iron and d-xylose malabsorption were present in a minority of patients. Functional and structural changes were correlated. Our results suggest that these changes are due to impairment of cell metabolism. *Key words: iron deficiency anemia, intestinal mucosa, mitochondria, d-xylose malabsorption.*

Many compounds in the body known to perform a metabolic or enzymatic function, contain iron[1]. Iron deficiency that has progressed beyond the depletion of iron stores affects tissue iron compounds and these effects have distinctive intracellular and organ distributions that are determined by a variety of factors[2]. It has been demonstrated that in the growing rat the tissues that are most resistant to iron deficiency are those which are fully grown and those which are subjected to a continual obligatory work load, especially of a nature that is essential for survival, like the heart muscle[1]. On the other hand, epithelial cells are constantly renewed, and iron enters these cells when they are produced[3]. Thus, in iron deficiency, one might expect abnormalities of the intestinal mucosa, which are an example of epithelial tissues with a rapid turnover. It is known that in children there is impairment in the absorptive function of the intestinal mucosa associated with iron deficiency[1]. Certain investigators have described morphological changes occurring in the intestinal mucosa[4,5], while others, have found the intestinal

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mucosa morphologically normal[6], and still others have described these morphological changes as insignificant[7].

The present study was undertaken to examine the intestinal mucosa morphologically and functionally in iron deficiency anemia. In previous studies, iron-dependent cytochrome oxidase activity in experimental animals and in children was determined so as to define the precise mechanism by which iron deficiency results in defects in intestinal function[8,9]. However, our aim was to examine the ultrastructure of the intestinal mucosa which had not been previously investigated in iron deficiency.

Material and Methods

The study group consisted of 11 patients hospitalized in the Pediatrics Department of the Istanbul University Cerrahpaşa Faculty of Medicine, between 1983-1985 with the diagnosis of iron deficiency anemia. None of the subjects had a history of infections or chronic disease. There were four females and seven males who ranged in age from one to 12 10/12 years (mean age: 5 9/12 ± 4 8/12 years). One child (Case 1), who was a twin, had a diet which consisted only of cow's milk. Another child (Case 3) had positive guaiac stool tests on four occasions and was operated on later because of a fibromyoma of the jejunum. The other subjects did not receive a balanced diet. There was a history of pica in three patients: Cases 4 and 9 ate earth, and Case 5 ate earth, sand and chalk.

Laboratory studies included hemoglobin, hematocrit, reticulocyte, leukocyte and platelet counts, examination of serum iron, total iron-binding capacity (TIBC), transferrin, osmotic fragility, Hb F, Hb A_2, erythrocyte sedimentation rate, total serum protein, albumin, globulin, liver function tests, and examination of the feces for parasites. Blood smear erythrocyte morphology was evaluated and a differential leukocyte count was performed. Bone marrow aspirates were examined. Mean corpuscular hemoglobin concentration and the serum transferrin saturation level were calculated. Intestinal biopsies were taken from all patients by means of a pediatric Watson biopsy capsule. Light microscopy revealed changes in the villi, crypt zone, surface epithelium, and lamina propria which were graded according to the following criteria: (Grade I) slight shortening and broadening of the villi, slight thickening of the basal layer, increased mitotic activity in the crypt zone, slight compaction of surface enterocytes, and a slight increase in the cellularity of the lamina propria; (Grade II) obvious shortening and thickening of the villi, thickened basal layer and crypt hyperplasia, squat, disordered and more compact enterocytes, an increase in the number of intra-epithelial lymphocytes and the cellularity of the lamia propria is greater; (Grade III) excessive shortening of the villi, mucosal flattening, obvious compaction and disorder of surface enterocytes, obvious thickening of the basal layer,

crypt hyperplasia, an increase in the number of intra-epithelial lymphocytes, and an abundance of cells in the lamina propria.

A part of the specimen was examined in six patients using the Carl Zeiss (EM 10) electron microscope and electron micrographs were obtained. In one patient, the ultrastructure was re-examined.

An oral test dose of 0.5 g/kg d-xylose was used in five patients. At 30-60 minutes after the administration of this test, d-xylose levels of 30-50 mg/dl are normally reached[10]. Since the d-xylose levels in the other five patients were low, 5 grams of oral d-xylose was given to these subjects by the method of Rolles et al[11]. According to this method, a blood d-xylose level exceeding 20 mg/dl (35 mg/dl on average) is usually reached at 60 minutes.

Iron absorption was investigated by an oral iron absorption test. After an overnight fast, blood was collected for a fasting serum iron and ferrous fumarate (65 mg per tablet) was given. Patients weighing 12.5 kg or less received one tablet of ferrous fumarate, patients weighing 12.5-20 kg received two tablets, and patients weighing over 20 kg received three tablets. Blood was drawn at one and three hours following the oral iron test dose. The resulting absorption curves were described as being "elevated" or "flat", depending on whether the maximum rise over baseline values in the serum iron concentrations at either one or three hours was greater or less than 100 μg/dl, respectively.

The Mann-Whitney *U* test and linear correlation were used for statistical analysis.

Results

Some of the hematological and biochemical data are summarized in Table I. Two children had elevated reticulocyte counts: one (Case 8) had a transferrin saturation level of 2.0 percent. TIBC was not determined in the other child (Case 2) but Hb A_2 and Hb F values were normal. The chief findings on the blood smear were hypochromia, microcytosis, anisocytosis and poikilocytosis. Neither patient had parasites.

Intestinal biopsy findings (Tables II and III) : A satisfactory biopsy was obtained in all patients. In two biopsy specimens, the only pathological finding was edema of the lamina propria (Cases 7 and 11). Six of the specimens showed the following histological abnormalities under light microscopy: shortening and broadening of the villi, activity in the crypts, lymphoplasmocytic infiltration, eosinophils and edema in the lamina propria were increased and changes in the surface epithelium consisting of a decrease in height, disorder and an increase in the intraepithelial lymphocytes. Three biopsy specimens showed Grade I changes, two showed Grade II changes (Fig. 1) and one showed Grade III changes (Fig. 2).

These findings are documented in Table II. Under the electron microscope the main pathological findings of the surface epithelium were disorder and rarefaction of the microvilli, changes in the mitochondria and an increase in lysosomes. A microvillus lesion, along with a loss of surface coating fuzz (Fig. 3), were observed

TABLE I: Hematological and Biochemical Data in Patients With Iron Deficiency

Case No.	Age (Yrs)	Sex	Hb g/dl	Hct 1/1	Ret $x10^9/1$	Leukoc $x10^9/1$	Thr $x10^9/1$	Serum İron µg/dl	TIBC µg/dl	Transferrin saturation %	Transferrin mg/dl
1	1	M	7.2	0.22	110	10.3	210	41.0	503	8.1	865
2	1 6/12	N	5.6	0.17	410	4.8	170	23.3			700
3	7 7/12	F	4.8	0.16	140	4.6	200	6.8	431	1.6	
4	11 4/12	H	9.0	0.27	50	3.2	190	3.0	635.8	0.5	
5	4	M	5.7	0.20	130	3.2	140	26.0	305	8.5	530
6	1 3/12	M	8.8	0.26	60	8.2	380	7.0	426	2.0	405
7	1 3/12	M	9.0	0.26	270	5.1	280	15.3	669	2.2	515
8	10 2/12	F	6.4	0.20	590	4.9	240	25.0	328	7.62	335
9	10	F	8.0	0.22	140	2.8	220	18.0	630	2.0	350
10	12 10/12	M	6.0	0.18	130	3.0	200	25.0	275	9.0	450
11	2	M	7.0	0.21	140	3.4	200	25.0	381	6.56	415
Mean	5 9/12		7.0	0.21	197	4.8	220.9	19.58	458.38	4.8	507.2
SD	4 8/12		1.5	0.04	165	2.2	64	11.0	145	3.4	174.0

Ret : reticulocyte
Leukoc: leukocyte
Thr : Thrombocyte
TIBC : Total iron - binding capacity

TABLE II: Intestinal Biopsy Findings in Patients with İron Deficiency

	VILLI				SURFACE EPITHELIUM			LAMINA PROPRIA		
Case No.	Height	Broadening	Grade of Villous Change	Increase Activity in Crypts	Height	Disorder	Intraepithelial Lymphocytes	Edema	Lymphoplasmocytic Infiltration	Eosinophil Number
1	↓	+	I	+	N	+	↑	++	↑	↑
2	N	+	I	+	N	+	↑	+	↑↑	N
3	N	–	0	–	N	–	N	–	N	N
4	↓	+++	II	++	↓	–	↑	+	↑↑	N
5	N	+	I	++	N	+	N	++	↑↑	N
6	N	N	0	–	N	–	N	++	N	N
7	N	N	0	–	N	–	N	++	N	N
8	↓	++	II	++	↓↓	++	↑↑	–	↑↑	N
9	Flat mucosa		III	+++	↓↓↓	–	↑↑		↑↑↑	↑
10	N	–	0	–	N	–	N	–	N	N
11	N	–	0	–	N	–	N	+	N	N

Fig. 1 (Case 4): Grade II villous damage: villi are broader and shorter, crypt zone hyperplastic. Lymphoplasmocytic infiltration increased in the lamina propria (H+Ex80).

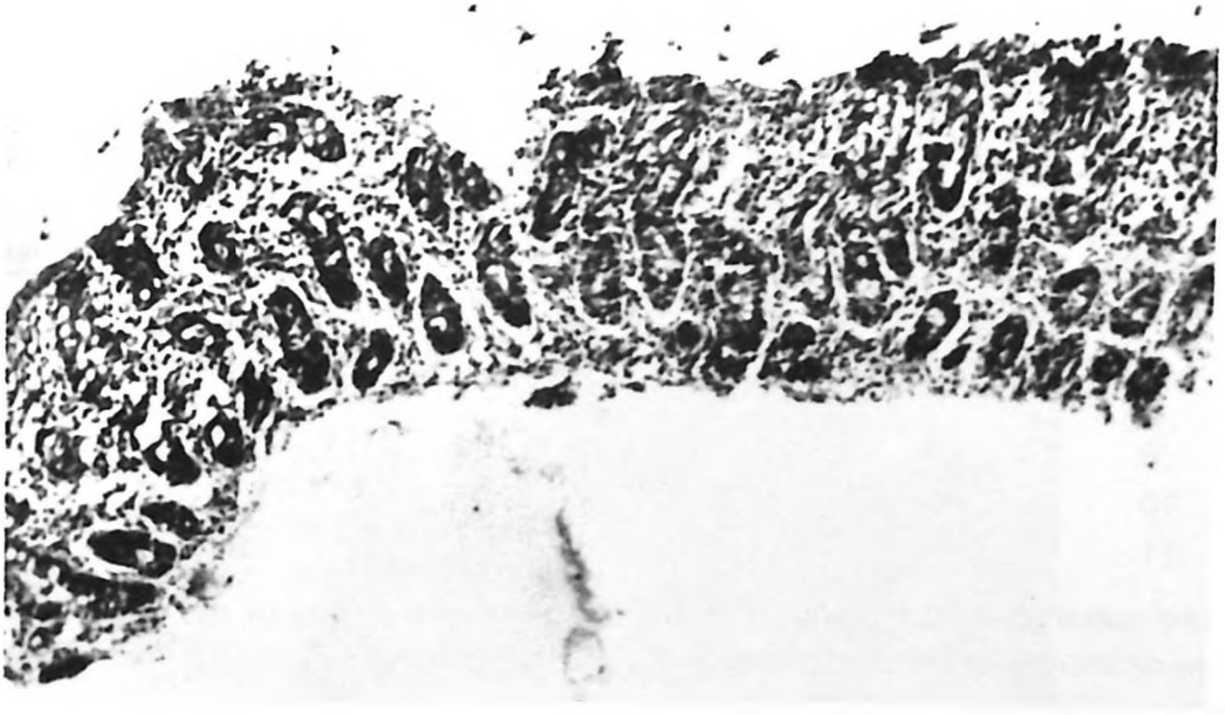

Fig. 2 (Case 9): Grade III villous damage: absence of villous structure, flat intestinal mucosa, surface epithelium irregular and cubic, and mucosa consisting only of crypt zone (H+Ex80).

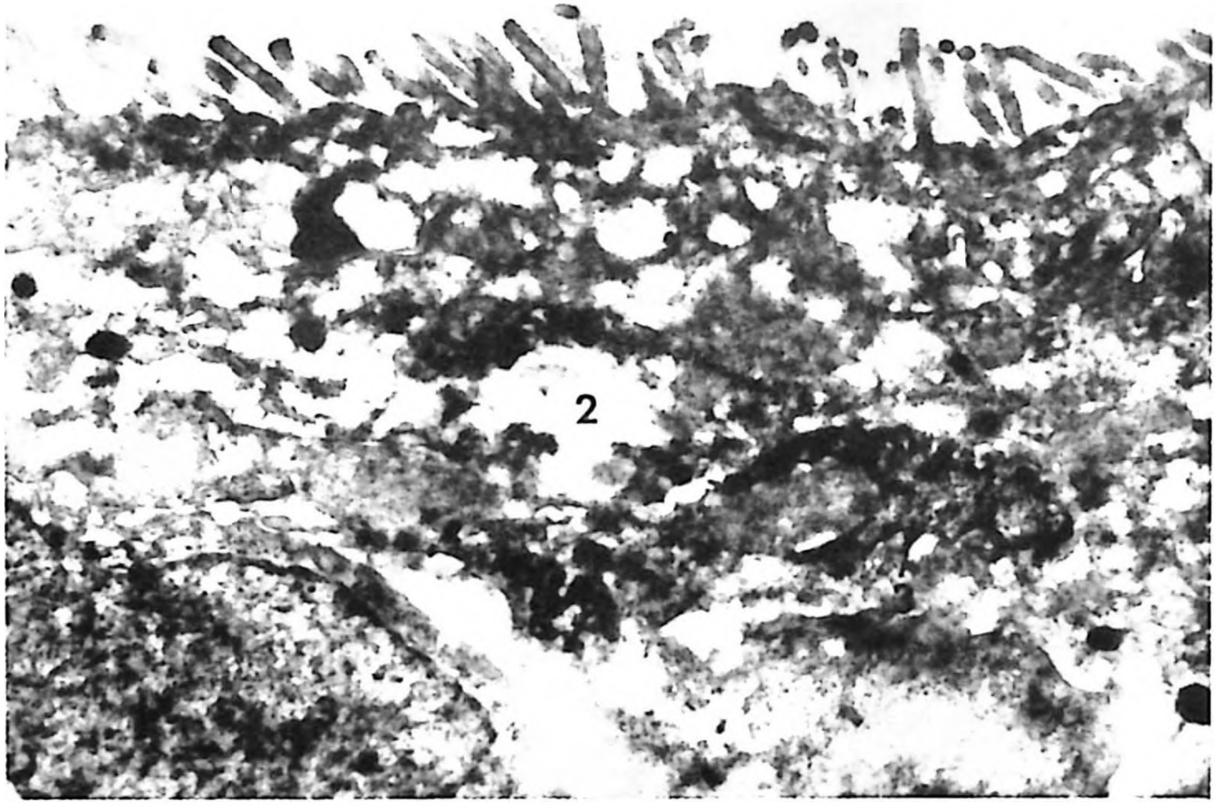

Fig. 3 (Case 9): 1-microvilli, 2-vacuole, 3-nucleus (x10,200). Microvilli are rarefied and irregular, filaments are not seen manifest, terminal web and surface coating are not visible. Nucleus of the epithelial cell on the right is picnotic.

TABLE III: Electron Microscopic Findings of Surface Epithelium in Patients With Iron Deficiency

Case No.	Microvilli lesion	Mitochondrial changes	Increase in lysosomes
8	–	+	+
7	–	–	–
8	+	+	+
9	+	+	+
10	–	–	–
11	–	+	+

in two patients (Cases 8 and 9). In these specimens cristae of the mitochondria were destroyed and the matrices were diluted, in other words, the mitochondria were swollen. In one of these two specimens, the disconnection of epithelial cells attracted our attention (Figs. 4 - 6). In these specimens, an increase of lysosomes was also observed.

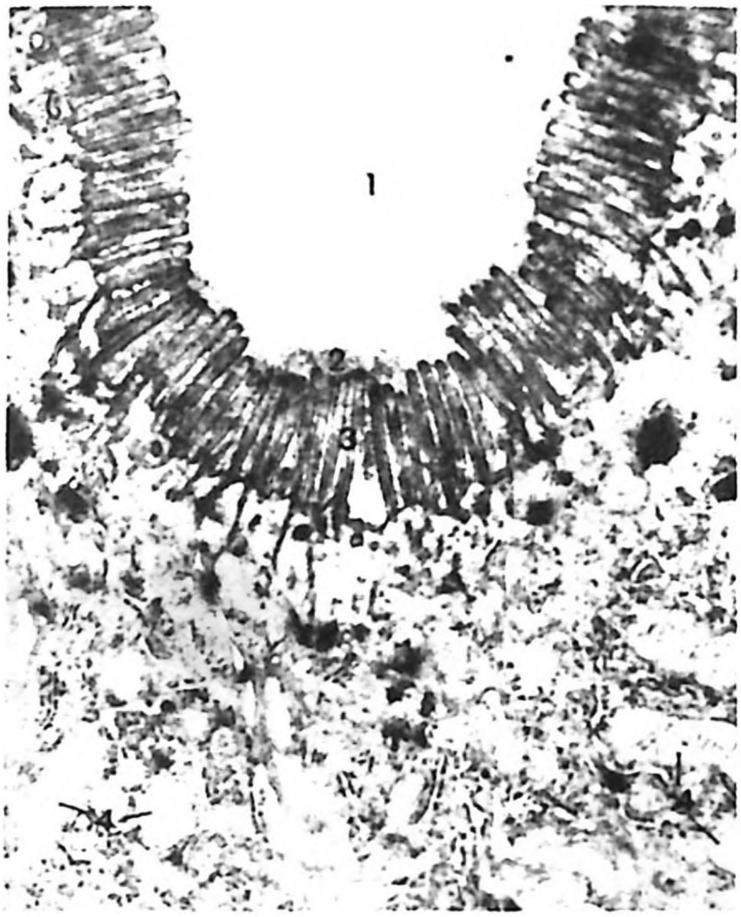

Fig. 4 (Case 9): 1-lumen, 2-surface coating 3-microvilli, 4-mitochondria (x10,200). This electron micrograph was taken from the crypt zone. Surface coating is normal, cristae of the mitochondria are destroyed and the matrix is diluted (mild swelling).

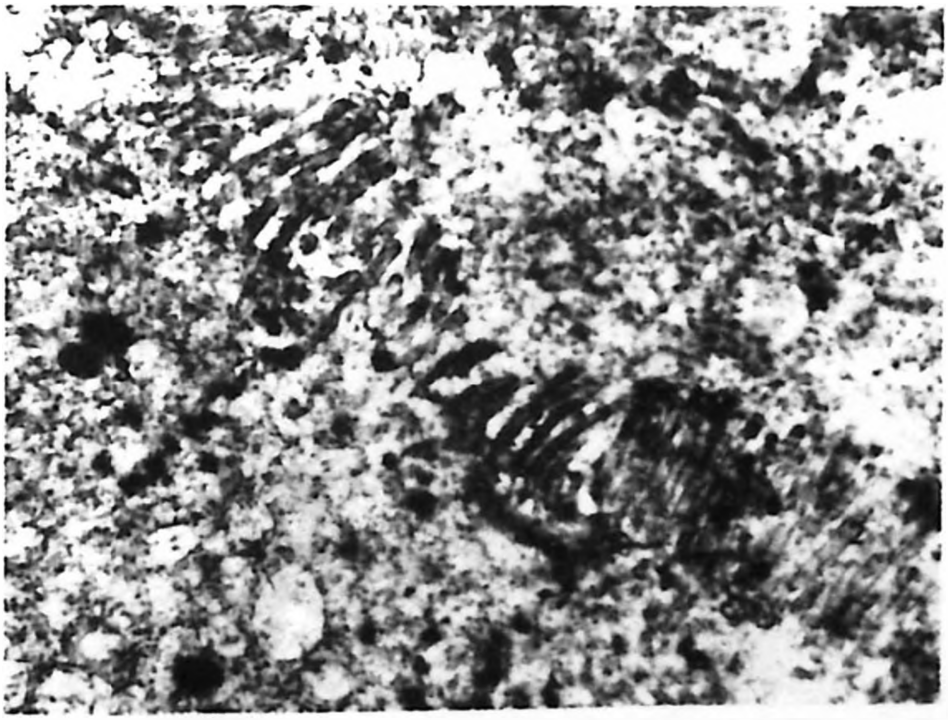

Fig. 5 (Case 8): 1-lumen, 2-microvilli, 3-lysosome, 4-mitochondrion (x10,200). Loss of microvilli and swelling of mitochondrion.

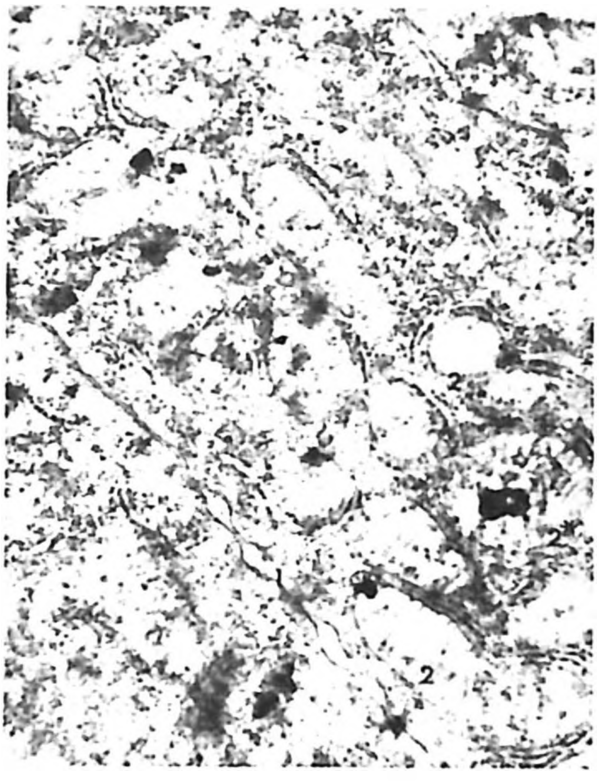

Fig. 6 (Case 8): 1-cell border, 2-mitochondria (x1,000). Disconnection between cells is seen. There is degeneration in a mitochondrion (mitochondrion-lysosome transformation). Mitochondria are swollen.

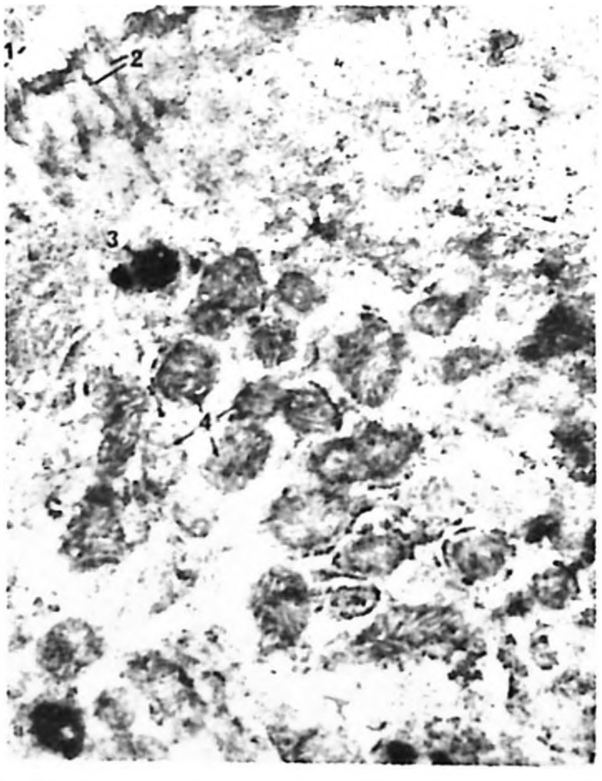

Fig. 7 (Case 6): 1-microvilli, 2-filament, 3-lysosome,4-mitochondria (x16,000). Mitochondria are small, cristae are prominent.

In the other two specimens (Cases 6 and 11), the mitochondria were small, the cristae prominent, and there was a slight increase in the density of the matrices (Fig. 7). In one of these specimens, the mitochondria had a normal appearance after therapy (Fig. 8). Ultrastructural findings are summarized in Table III.

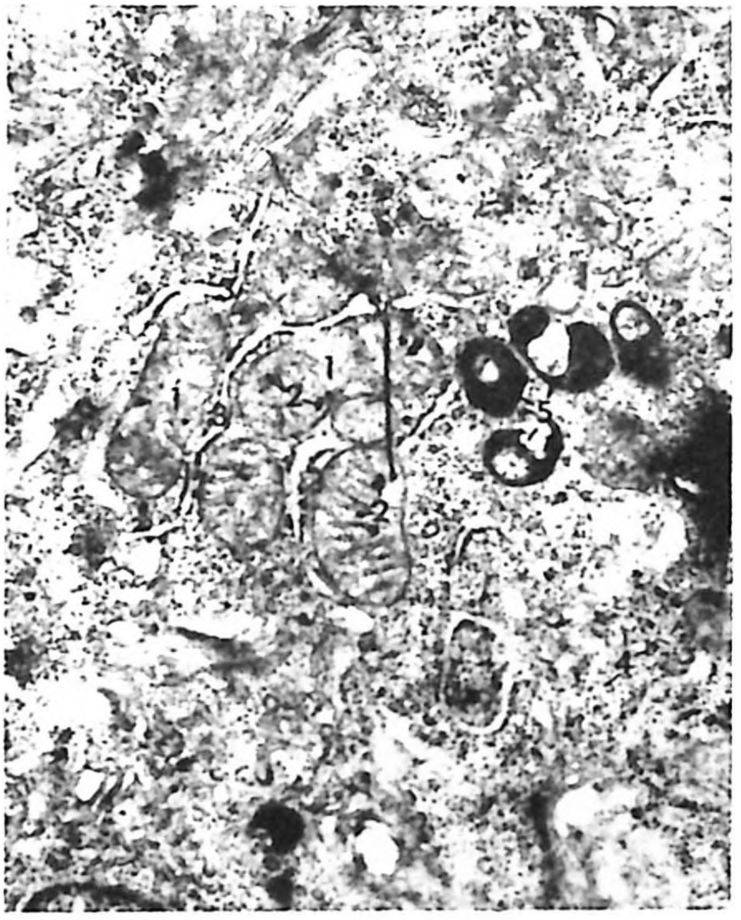

Fig. 8 (Case 6): 1-mitochondria, 2-intramitochondrial granules,3-granular endoplasmic reticulum, 4-free ribosomes, 5-lysosomes (x16,000). After therapy the mitochondria appear normal.

Intestinal function (Table IV): D-xylose absorption was impaired in only two patients (Cases 8 and 9), according to the one-hour blood d-xylose levels. Flat iron absorption curves were obtained in three cases (Cases 4, 8 and 9). The curves were elevated in the other patients. The maximal rise in serum iron as graphed on the elevated absorption curves ranged from 150 to 437 μg/dl.

Discussion

The incidence of intestinal mucosal changes, occurring as a result of iron deficiency anemia, has been reported to be 73 and 64 percent by certain investigators[4, 5]. In our study, light microscopic examination revealed intestinal mucosal changes in the specimens of 55 percent of the patients. Similar changes have also been observed in patients with iron deficiency and geophagia[12-15].

TABLE IV: Intestinal Function and Structure in Iron Deficiency

	D-xylose Test		Iron Absorption Test			
Case No.	1. Hour Blood d-xylose level	Grade of Villous Change	Fasting	1. Hour	3. Hours	Maximum Rise Over Baseline Values
	mg x/dl		μ g/dl			
1	75*	I				
2	44*	I	23.3	118.3	341.6	318.3
3	56*	0	57	285	392	335
4	53*	II	3.2	33.3	85	81.3
5	68*	I	27	300	515	215
6	38.3**	0	32	151	256	224
7	—	0	30	216	467	437
8	18.3**	II	34	44	94	60
9	13**	III	38	48	77	39
10	22.6**	0	33	54	183	150
11	25.9**	0	38	241	333	295

* With 0.5 g/kg d-xylose
** With 5 g d-xylose

There are some investigators who have reported no intestinal mucosal lesions in children with iron deficiency but these workers examined the specimens of only three patients[6]. Another group of investigators, who found Grade I and II abnormalities in a minority of their patients, believed these changes to be insignificant because of the geographical area where the study was carried out[7].

In our group of patients, in which intestinal mucosal lesions were detected by light microscopy, the younger patients seemed to have less severe abnormalities than the older ones, possibly due to the fact that the former group had the disease for a shorter period of time than the latter group. The intestinal mucosal changes seen in one of our patients, who was ten years old, were similar to those seen in patients with celiac disease, as reported by Guha et al[4]. Three of the patients with a history of pica had mucosal changes, but these changes were also observed in the patients without a history of pica. There was no significant correlation between pica and the presence of mucosal damage, but pica seems to have a deteriorative effect.

In some of our patients, normal intestinal mucosa was observed by light microscopy, which had no correlation whatsoever with age. When we compared

the group with intestinal mucosal lesions to the group without intestinal mucosal lesions, we found that there were no differences in hemoglobin levels, serum iron concentrations or transferrin saturation indices to explain the differences between the two groups, There was also no correlation between these parameters and severity of the intestinal mucosal lesion. These results are concordant with the observations of other investigators[4, 5].

Several authors have demonstrated that the abnormalities regress or the mucosa returns to normal with iron therapy, even in patients with very severe lesions[4, 5-13], which suggests that iron deficiency is responsible for pathological changes. On the other hand, Arcasoy et al[15] are of the opinion that chronic zinc deficiency caused more morphological abnormalities than iron in their patients with geophagia and iron deficiency, since these changes improved markedly following short-term therapy with zinc alone. One of our two cases, with microvillus lesions, practiced geophagia. The biopsy specimens of these patients revealed Grades II and III villous changes and mitochondrial changes such as swelling and an increase in the number of lysosomes. Such changes in the mitochondria have been observed in hepatic and myocardial mitochondria of iron deficient rats. Mitochondrial swelling, and in some cases, even rupture of the outer membrane of the mitochondria have been seen in peripheral blood lymphocytes in iron deficiency anemia[16].

The electron transport pathway in the mitochondria contains a variety of iron compounds that generate energy. In our patients, the lack of iron compounds along this pathway might have caused the chemical derangement to occur in the mitochondria leading to alterations in membrane permeability which caused the mitochondrial swelling. The mitochondrial changes, increased activity in the crypts, and the villous damage we observed suggest that due to the failure of the system to produce energy, the life of the surface epithelium was shortened and the cells were shed more rapidly than normal, and at a higher rate than repair. The increase in the number of lysosomes might be a sign of this rapid shedding since lysosomes may increase in frequency during the stress of rapid proliferation[17]. Since the gut is a major immunological organ, an increase in lymphoplasmocytic infiltration might be an expected finding in the presence of villous damage[18]. In some of our patients with normal intestinal mucosas, we also observed mitochondrial changes by light microscopy. Since the importance of the mitochondria in cell metabolism is known, mucosal lesions might develop if these patients are not treated. In one of these patients, the mitochondria appeared normal after therapy, which indicates that iron deficiency is responsible for mitochondrial changes.

Recently, Arcasoy et al[19] reported, in a study of patients with Prasad's syndrome, that not many structural alterations were detected on the surface epithelium.

However, in some cells, the microvilli were observed to have formed bunches of varying numbers of microvilli, some of which were branching. In the said study, ultrastructral alterations were mainly observed in Paneth's cells, and as a possible cause of these changes, zinc deficiency was mentioned.

Several studies have shown that there is malabsorption of d-xylose in iron deficiency, and the incidences reported were 13 percent, 65 percent, 18 percent, and 43 percent, respectively[1, 4, 5, 7]. In our patients,the incidence of d-xylose malabsorption was 15 percent. Intestinal mucosal damage and alterations in the structure of the microvilli were present in our patients with d-xylose malabsorption. Changes in the microvilli are believed to markedly reduce the absorptive surface[20]. When the absorption mechanism of d-xylose is taken into consideration, impaired d-xylose absorption in our patients might have been caused by the alterations in the structure of the villi and microvilli. D-xylose absorption was normal in one of our patients (Case 4) with the same grade villous change. We could not examine the microvilli in this patient but milder changes in the surface epithelium, which were detected by light microscopy, attracted our attention.

Schulz et al[21, 22] reported that children with iron deficiency anemia absorb food iron and a ferrous salt more efficiently than do normal children. Gross et al[23] showed that children without iron deficiency demonstrated flat absorption curves. They also showed that iron absorption curves were elevated in most iron deficient children but that some of their patients demonstrated impaired iron absorption. We observed elevated absorption curves in most of our patients. Our three patients with flat absorption curves had either Grade II or Grade III villous changes. Ultrastructural examination carried out in two of these patients revealed microvilli lesions and mitochondrial swelling. Mucosal uptake of iron, the first step in iron absorption, occurs rapidly on the brush border of the mucosal cell which consists of microvilli[24] and where specific receptors are thought to exist[25]. The changes in the microvilli that we observed might have impaired the absorption of iron by affecting these receptors. On the other hand, the appearance of the mitochondria suggests that the mucosal uptake and the transfer of iron from the mucosal cells to the lamina propria might be limited by the failure of sufficient energy production, as both steps probably represent energy dependent active transport processes[25]. In our third patient (Case 4), the fact that the maximal increase in iron concentration was greater than in the other two patients is concordant with the milder changes of surface epithelium and normal d-xylose absorption. In previous studies it was reported that d-xylose and iron malabsorption disappeared with iron therapy and, it was therefore concluded, that iron deficiency resulted in functional changes[4, 5, 23].

The elevated absorption curves observed in our patients with mitochondrial changes, other than the swelling, suggest that these mitochondria are still able to produce sufficient energy.

Kimber and et al[8] found that cytochrome oxidase activity was reduced in the intestinal mucosa of iron-deficient dogs and they believed that a decrease in iron-containing or iron-dependent enzyme systems in the mucosa of iron-deficient subjects might lead to abnormalities of cellular metabolism and a secondary state of malabsorption. This conclusion is similar to that of Dallman et al[9].

In our patients, no relationship was found between certain parameters of iron deficiency, and intestinal functional and morphological abnormalities, but morphological and functional changes were correlated. In one patient, the normal appearance of the mitochondria after therapy and the mitochondrial changes we observed suggest that impairment of cell metabolism due to a decrease of enzymes which contain iron or heme or require iron or heme as a cofactor, as pointed out by other authors[8, 9], might be responsible for the morphological and functional changes. We believe enzyme determinations and examination of ultrastructure in a greater number of iron deficient patients are needed to better understand the pathogenesis of these changes.

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THE EFFECT OF APROTININ (TRASYLOL) ON POSTOPERATIVE BLEEDING IN CYANOTIC CONGENITAL HEART DISEASE*

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SUMMARY: Hazan E, Paşaoğlu İ, Demircin M, Bozer AY. (Department of Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). The effect of aprotinin (trasylol) on postoperative bleeding in cyanotic congenital heart disease. Turk J Pediatr 33: 99-110, 1991.

Despite the widespread clinical success in open-heart surgery, bleeding after cardiopulmonary by-pass (CPB) has been a common problem especially in cyanotic congenital heart disease. Recently, there have been reports demonstrating that treatment with high doses of aprotinin reduces postoperative bleeding.

We studied the effect of aprotinin on postoperative bleeding in patients with tetralogy of Fallot who had undergone total correction in the Department of Thoracic and Cardiovascular Surgery of the Hacettepe University Faculty of Medicine, and compared our results with those in the literature.

Ten patients out of 20 in the study were given high doses of aprotinin and were compared with the remaining 10 patients who had not received the drug. Standard anesthesia, perfusion and surgical techniques were used in all operations. The total amount of bleeding in the aprotinin-treated group was found to be 1530 ml, while in the other group it was 4185 ml ($p < 0.05$). The total quantity of blood transfused in the aprotinin-treated patients was 3250 ml while it was 5865 ml in the control group ($p < 0.05$). No significant effect of aprotinin was found on Hb, Hct, PT, aPTT and thrombocyte counts ($p > 0.05$). However, the effect of the drug on bleeding and coagulation time was found to be statistically significant ($p < 0.05$). *Key words: aprotinin, postoperative bleeding, cyanotic congenital heart disease.*

Cardiopulmonary by-pass (CPB) affects all body systems including the hematopoetic system.

It is known that some coagulation defects in congenital cyanotic heart disease may cause massive, lethal bleeding during and after surgery[1-5]. Coagulation defects, especially thrombocytopenia and hypofibrinogenemia are often seen in patients with elevated hematocrit levels[5-8].

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CPB causes a significant reduction in coagulation factors, thrombocytes and blood cells[9,10]. Therefore, CPB leads to massive bleeding requiring transfusion in the cyanotic patient, already at high risk of bleeding. Excessive transfusions may lead to complications and infectious diseases which are transmitted by the blood.

Recently, there have been reports demonstrating that aprotinin, a protease inhibitor, when used in high doses, reduces postoperative bleeding by the inhibition of kallikrein and plasmin[11-14]. In these studies, aprotinin was administered routinely during open-heart surgery, reoperations and to patients with infective endocarditis who were at high risk of bleeding. Our study was designed to determine how aprotinin affects bleeding in cyanotic congenital heart disease and to compare the results with other studies.

Material and Methods

This study was performed on 20 cyanotic patients diagnosed as having tetralogy of Fallot, who were operated on between May-August 1989, at the Department of Thoracic and Cardiovascular Surgery of the Hacettepe University Faculty of Medicine. Total correction was performed in all patients. Ten patients were given aprotinin and were compared with the control group which included the remaining 10 patients who had not been given the drug. Table I shows the mean ages, sexual distribution and mean weights of the patients.

TABLE I: Mean Age, Mean Weight and Male / Female Ratio of Aprotinin - Treated Patients and Control Group

	Mean Age (yrs)	Male / Female	Mean Weight (kg)
Aprotinin	7.5	4 / 6	20
Control	7.1	4 / 6	18

Standard anesthesia, perfusion and surgical techniques were applied in all patients. Aprotinin was administered to the study group using a single protocol.

Thirty thousand U/kg aprotinin was infused in 20-30 min at the start of anesthesia. After this initial dose, it was infused at 100,000 U/hr until the end of anesthesia. Five hundred thousand units of aprotinin was added for every liter of blood that was perfused through the patient, and 1,000,000 units of aprotinin was added to the priming volume of the oxygenator.

Patients with coagulation disorders and a history of allergy were not included in the study.

Prior to aortic cannulation, 3 mg/kg heparin was administered to the patients. Activated clotting time (ACT) was then maintained at > 400 sec. Heparin was neutralized with protamine sulphate after perfusion was completed.

Cold K^+ cardioplegia, topical cooling and systemic hypothermia at 28 °C was performed in all patients.

The following studies were carried out on the blood samples obtained from the patients.

1– Hb and Hct values (preoperative; 24 hours postoperative, one week postoperative.
2– The thrombocyte count (just after induction; 10 minutes after sternotomy, at the end of CPB; 10 minutes after the initiation of protamine at the end of surgery, 24 hours postoperative.
3– Bleeding time (Preoperative, 90 minutes postoperative).
4– Coagulation time (Preoperative, 90 minutes postoperative).
5– Prothrombin time (Preoperative, 90 minutes postoperative).
6– Activated partial thromboplastin time (preoperative, 90 minutes postoperative).
7– Amount of postoperative bleeding.
8– Quantity of blood transfused postoperatively.

Results

The patients had no problems following surgery. None of the patients had to undergo reoperation due to postoperative massive bleeding. Coagulation parameters, thrombocyte counts, bleeding and coagulation times of all patients were found to be normal preoperatively. Laboratory findings of the study group and the control group are shown in Tables II and III.

1– Hb and Hct values:

Preoperative Hb and Hct vaules of the study and control groups were almost similar. Twenty-four hours after the operation, the Hb values were found to be decreased similarly in both groups. Hb values continued to decline at a slower rate up to the seventh postoperative day. There was no significant difference between the study and control groups (Fig. 1).

2– Thrombocyte count:

In both groups, the thrombocyte counts began to fall during sternotomy and became more significant during CPB. Although this decline seemed to be

TABLE II: Laboratory Findings of Patients in the Aprotinin-Treated Group

No	Hb (g/dl)	Hct (%)	Bleeding time (min)	Coagulation time (min)	PT (sec)	aPTT (sec)	Blood loss (ml)	Blood transfusion (ml)
	pre-operative / 7 days post-operative		pre-operative / 90 minutes post-operative					
1	14.0 / 14.1	40 / 40	4 / 5	5.5 / 6	15 / 16	38 / 40	150	100
2	16.7 / 14.6	56 / 44	4 / 4.5	6 / 7	15 / 16	38 / 42	50	50
3	15.9 / 15.8	50 / 50	4.5 / 5	6 / 7	15 / 16	37 / 38	280	200
4	18.4 / 19.5	55 / 58	3 / 4	4.5 / 5	14 / 15	35 / 38	50	100
5	14.3 / 13.4	48 / 40	4 / 6	5 / 8	14 / 15	38 / 42	200	300
6	17.5 / 16.3	53 / 48	3.5 / 4.5	5 / 6	14 / 15	42 / 48	180	600
7	14.7 / 13.8	44 / 40	3 / 3.5	5.5 / 6	14 / 15	43 / 46	100	750
8	17.8 / 14.5	54 / 46	3 / 4	4.5 / 6	13 / 14	42 / 42	200	450
9	14.6 / 16.0	42 / 48	3.5 / 7	3 / 4.5	18 / 22	68 / 63	100	250
10	21.0 / 18.0	67 / 54	4 / 5.5	5.5 / 7	13 / 13	42 / 42	220	450

TABLE III: Laboratory Findings of Patients in the Control-Group

No	Hb (g/dl)	Hct (%)	Bleeding time (min)	Coagulation time (min)	PT (sec)	aPTT (sec)	Blood loss (ml)	Blood transfusion (ml)
	pre-operative / 7 days post-operative		pre-operative / 90 minutes post-operative					
1	15.2 / 14.1	51 / 44	4 / 6.5	5 / 9	14 / 16	42 / 48	575	140
2	16.3 / 17.3	54 / 50	4 / 6	5.5 / 8	13 / 14	46 / 48	180	230
3	21.8 / 18.8	62 / 52	4 / 7	5 / 7.5	13 / 15	46 / 48	190	800
4	14.7 / 11.4	47 / 35	3.5 / 6.5	5.5 / 8	13 / 17	46 / 52	350	745
5	15.1 / 14.8	46 / 40	4 / 6.5	5 / 8	13 / 16	40 / 48	350	1150
6	22.2 / 17.3	68 / 52	4 / 7	5.5 / 7.5	14 / 16	42 / 43	470	500
7	14.2 / 14.0	46 / 40	4 / 6.5	5.5 / 8.5	14 / 16	37 / 42	400	600
8	13.8 / 16.5	43.8 / 49	2.5 / 4.5	4 / 8.5	15 / 17	42 / 48	290	500
9	21.2 / 20.5	65 / 61	5 / 7.5	4 / 6.5	13 / 14	46 / 48	630	500
10	16.0 / 13.0	48 / 38	4.5 / 7	5 / 7.5	14 / 15	46 / 48	350	700

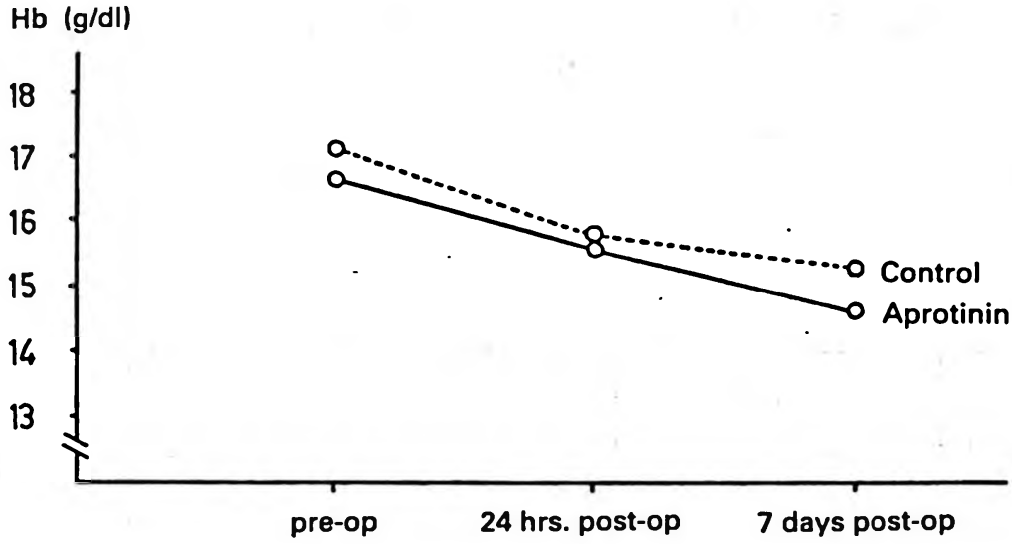

Fig. 1: Hemoglobin variations of aprotinin-treated patients and control group

evidenced more in the control group, there was no significant difference found between the two groups ($p > 0.05$) (Fig. 2).

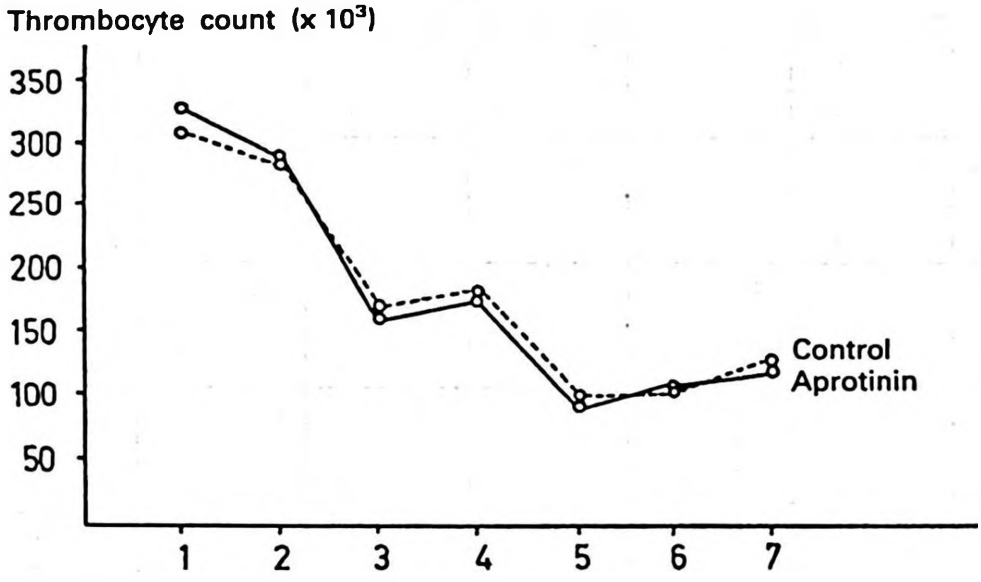

Fig. 2: Thrombocyte counts of aprotinin-treated patients and control group.

1. Induction.
2. Ten minutes after sternotomy.
3. Tenth minute of CPB.
4. End of CPB.
5. Ten minutes after protamine.
6. End of operation.
7. Postoperative 24th hour.

3– Bleeding and coagulation times:

Preoperative bleeding and coagulation times of the patients were normal. Postoperative values were significantly different in the study and control groups. Bleeding and coagulation times in the control group were prolonged significantly, however, this prolongation was very little in the aprotinin treated group. The difference between the two groups was statistically significant ($p < 0.05$) (Fig. 3).

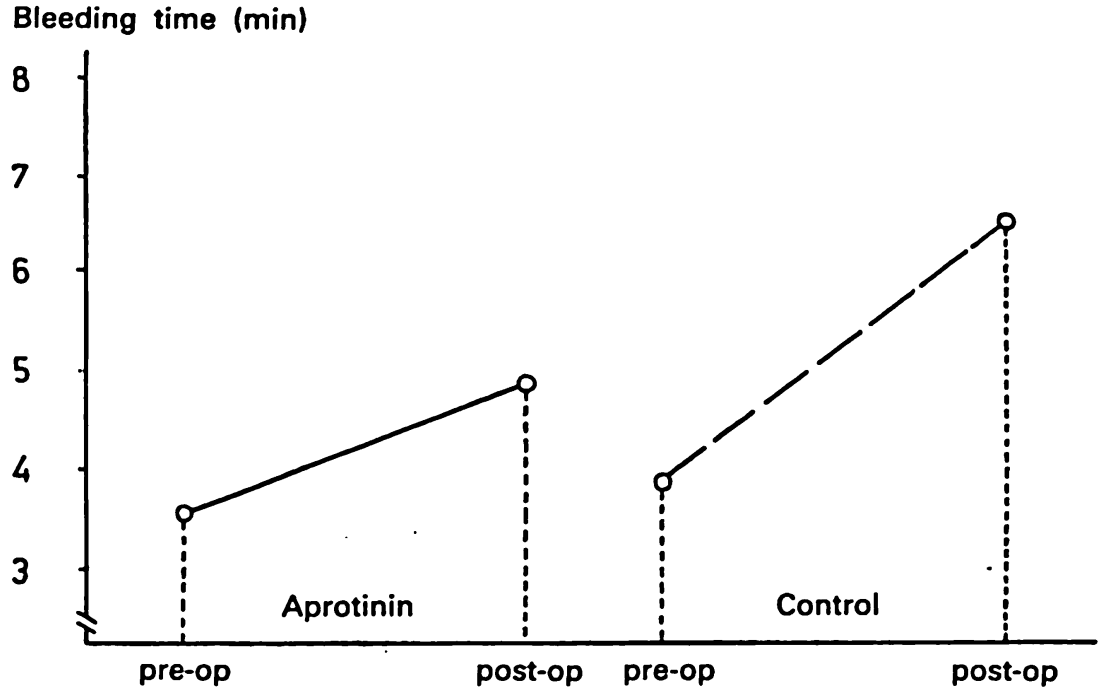

Fig. 3: Bleeding time (min) preoperatively and 90 minutes postoperatively

4– PT and aPTT values:

Preoperative PT and aPTT values of the two groups were not different. Ninety minutes after surgery, the prolongation of these values in the control group was greater than that in the study group, but it was not statistically significant ($p > 0.05$) (Table IV).

TABLE IV: Prothrombin Times (sec) Preoperatively and 90 Minutes Postoperatively

	Pre – op	Post – op
Aprotinin	14.5	15.7
Control	13.6	15.6

5– The amount of postoperative bleeding and quantity of blood transfused:

The amount of postoperative bleeding in the study and control groups seemed to be different. The bleeding from the sternal edge and medulla was significantly very little during the operation of the aprotinin-treated patients. The quantity of blood transfused in the two groups was also different, and was found to be statistically significant ($p < 0.05$) (Fig. 4).

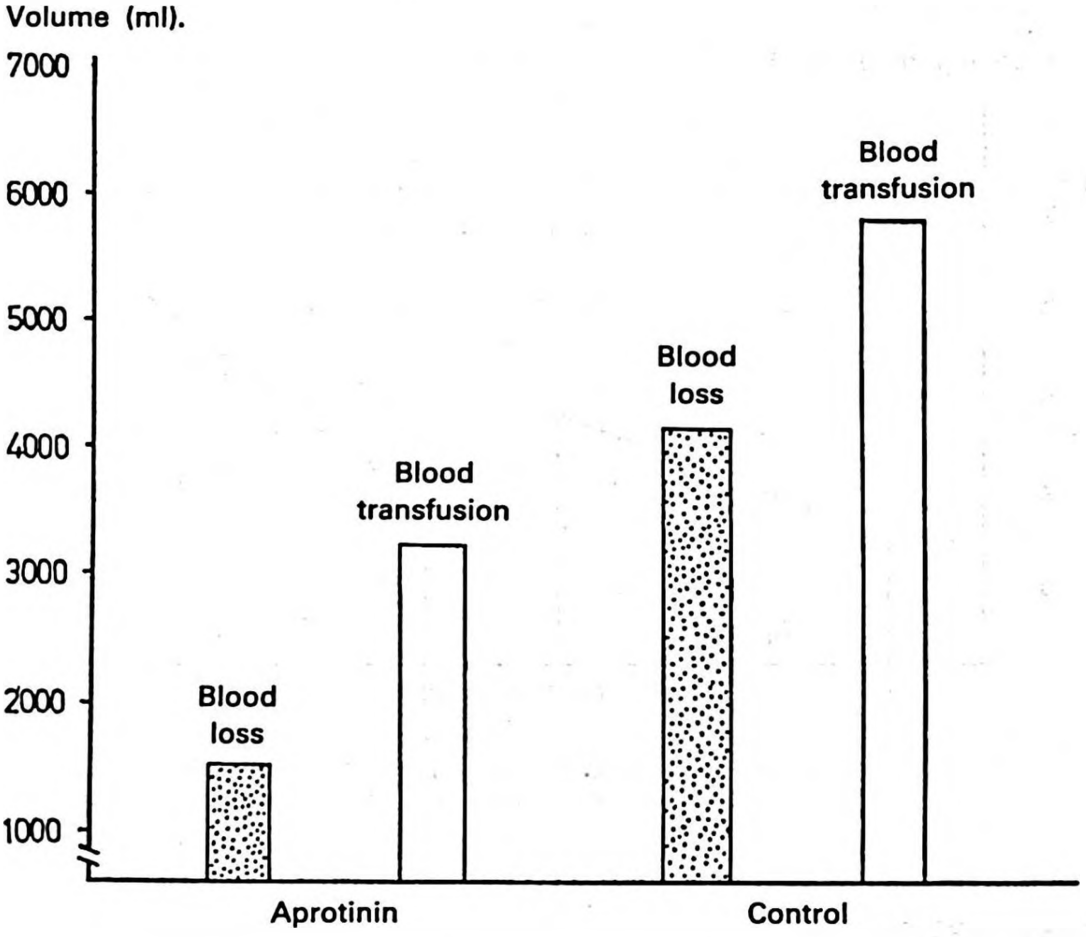

Fig. 4: Postoperative blood loss and need for blood transfusion were significantly decreased in the aprotinin-treated patients

Total body weights, total amount of bleeding, and total quantity of blood transfused were determined, and a blood loss of 7.5 ml/kg and 23 ml/kg was found in the study and control groups, respectively. The volume of blood transfused was 15.8 ml/kg in the study group and 32.5 ml/kg in the control group.

Discussion

Postoperative bleeding and the maintenance of hemostasis is occasionally a troublesome problem in open-heart surgery, especially in patients with cyanotic congenital heart disease, since some coagulation defects are present preoperatively[1-6, 15]

For several years aprotinin has been used to reduce bleeding. In 1963, Tice and associates[16]treated their patients with aprotinin for bleeding due to hyperfibrinolysis. Subsequently, aprotinin continued to be used for treatment purposes and not as a prophylactic[17-19]. Later, when hyperfibrinolysis was observed in the CPB apparatus, it was realized that aprotinin was not effective when given after by-pass[20]. In 1978, Philipp[21] suggested that if the levels of aprotinin are adequate, inhibition of plasmin and kallikrein can be achieved.

The hemostatic defect associated with CPB is very complex[22-24]. It is known that a transient and occasionally severe defect in platelet function occurs[25-28].

Recent studies have evaluated the effects of other drugs on blood loss after CPB. The administration of desmopressin acetate[29] and dipyridamole[30] resulted in significant reductions in blood loss, but neither drug can eliminate the need for blood transfusion. Prostacyclin was also investigated for this purpose, but its routine use was not suggested[31]. Aminocaproic acid, a synthetic antifibrinolytic drug, reduced blood loss slightly, but a difference in the quantity of blood required for transfusion was not reported[32].

Many studies have reported the efficacy of aprotinin either in routine open-heart surgery or in patients who were at high risk of bleeding because of reoperation or infective endocarditis[11-14].

In this study, aprotinin was used to treat patients with cyanotic heart disease, and its effect was demonstrated in regard to the amount of bleeding and the quantity of blood transfused either during the total correction operation or during the postoperative follow-up period.

With the increase in information regarding the risks inherent in blood transfusions, studies for reducing the amount of bleeding have progressed and auto-transfusion systems have appeared[11].

While at present, the transmission of the human immunodeficiency virus (HIV) is estimated to be of low probability, the same cannot be said of hepatitis B and hepatitis non-A non-B[33, 34].

Mismatched blood transfusions cause acute renal insufficiency or pyrogenic reactions due to an incompatibility of ABO or a subgroup. Moreover, the citrate, which is used as an anticoagulant in bank blood, binds the calcium ions of plasma and causes hypocalcemic tetany and coagulation defects since the ionized calcium is necessary for normal coagulation[35].

This study demonstrated that treatment with high doses of aprotinin reduced postoperative blood loss in cyanotic congenital heart disease, thus decreasing the need for bank blood and transfusion. On a search of the literature, we were unable to find another study illustrating the effect of aprotinin on postoperative bleeding in cyanotic congenital heart disease.

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AN EXPERIMENTAL STUDY ON THE USE OF FIBRIN SEALANTS IN STRABISMUS SURGERY*

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SUMMARY: Erbil H, H, Sınav S, Süllü Y, Kandemir B. (Departments of Ophthalmology and Pathology, Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey). An experimental study on the usage of fibrin sealants in strabismus surgery. Turk J Pediatr 33: 110-116, 1991.

Fibrin sealants were used in place of the classical suture materials in strabismus surgery. Classical recession operations were performed on the right eyes of ten albino rabbits, while the recessed anterior rectus muscles of the left eyes were fixed to the sclera with the tissue sealant. Better histopathological results were obtained with the fibrin sealant than with the classical sutures, but the functional results were worse.
Key words: fibrin sealant, strabismus surgery, extraocular muscles.

Strabismus is a major ophthalmic problem, particularly in children, because it often results in amblyopia, but can also lead to psychological and asthenopical problems. Suturing attempts and suture materials used in surgical techniques to either reduce or induce the strength of extraocular muscles can cause some well known complications. Nurözler et al[1] reported the following postoperative complications, all due to suturing, that they had observed after performing strabismus surgery on 191 horizontal muscles: scleral perforation (1.57 %), acute allergic suture reaction (2.09 %), and chronic suture granuloma (1.04 %). Although these rates are not high, to prevent such complications, disappointing both for the surgeon and the patient, we suggest that tissue adhesives be used instead of sutures in certain surgical procedures.

Tissue adhesives are either synthetic (acrylates, gelatin-formaldehyde-resorchin) or natural (fibrin sealant)[2]. Synthetic tissue adhesives have been found to be too toxic for clinical treatment purposes[3]. Fibrin sealants are highly concentrated homologous cryoprecipitates accelerating natural wound repair[4].

The present study was carried out on experimental animals to ascertain if fibrin sealants could be used in place of sutures when attaching sclera to muscles in strabismus surgery.

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Material and Methods

Recession operations were performed on the anterior rectus muscles of both eyes of ten albino rats under general anesthesia, in which the animals had inhaled ether and had been administered ketamine intramuscularly.

The recessed muscles of the right eyes were fixed to the sclera with 6-0 vicryl sutures and the muscles of the left eyes were attached by using a fibrin sealant. The sealant kit we used (Tisseel) contained fibrinogen, factor XIII, fibronectin, thrombin, aprotinin and $CaCl_2$, and two solutions prepared at 37 °C which were mixed in a special double lumened injection system (duploject system) just before application. In order that the sealant take effect, the muscle end of the eyes was brought next to the scleral tissue and was held there steady for five minutes. All conjunctivas were closed by suturing. Postoperatively, the eyes were left open and antibiotic eyedrops and ointments were applied daily. On the seventh day postoperatively, all the eyes were enucleated and examined anatomically and histopathologically. The histologic sections were stained with hematoxylin and eosin (H + E).

Results

None of the ten eyes in which the muscles were fixed to the sclera by suturing showed anatomical problems. In two of the ten eyes, in which the fibrin sealant was used, the recessed muscles retracted to the orbital space, dislodging from their new insertions upon the release of the muscle forceps. These eyes were not included in the histopathologic examinations. The H + E stained histologic sections prepared from the muscle sclera junctions showed the following microscopic findings: in both suture and sealant groups, tissue integrity was obtained between muscle and sclera (Figs. 1 and 2). Microscopic examination at higher magnifications of the suture group revealed that some of the muscle fibers had atrophied, while others had been replaced with connective tissue, and that there was a decrease in the striations of the muscle fibers (Fig. 3). In the sealant group, the striations were fully preserved, but some muscle fibers had lost their polygonal structures and had formed round shapes (Fig. 4).

Discussion

In all the histologic sections, the muscle tissues of both the adhered and the sutured segments were alive and integral. Microhematomas were observed at the operated sites of the suture group, but were not present in the newly formed insertion areas of the sealant group. Fibrinogen, factor XIII and thrombin included in the sealant were effective in inducing hemostasis.

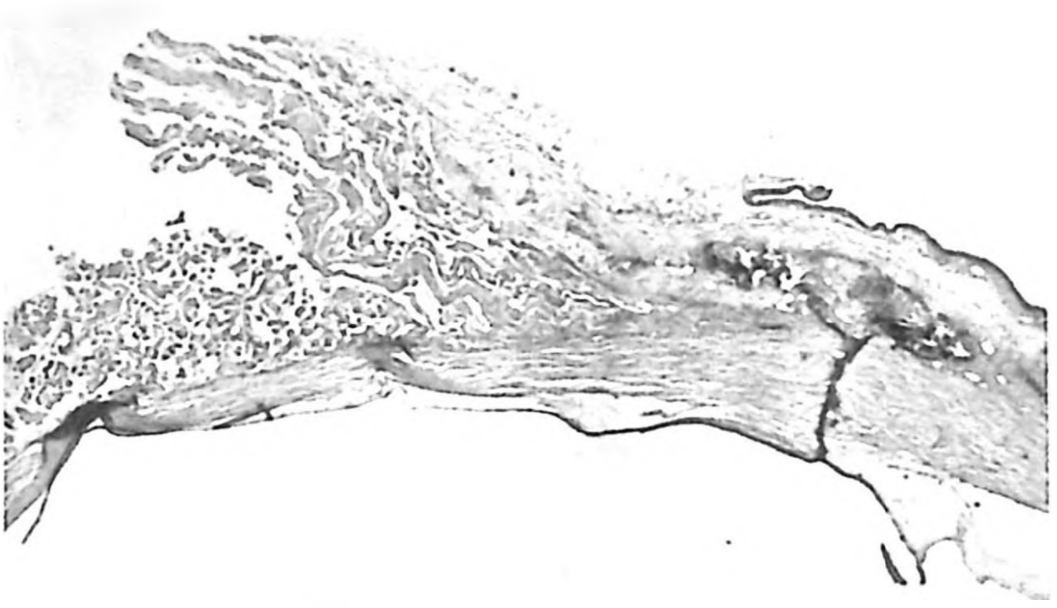

Fig. 1: Complete tissue integrity between muscle and sclera can be seen in the suture group (H + E × 100).

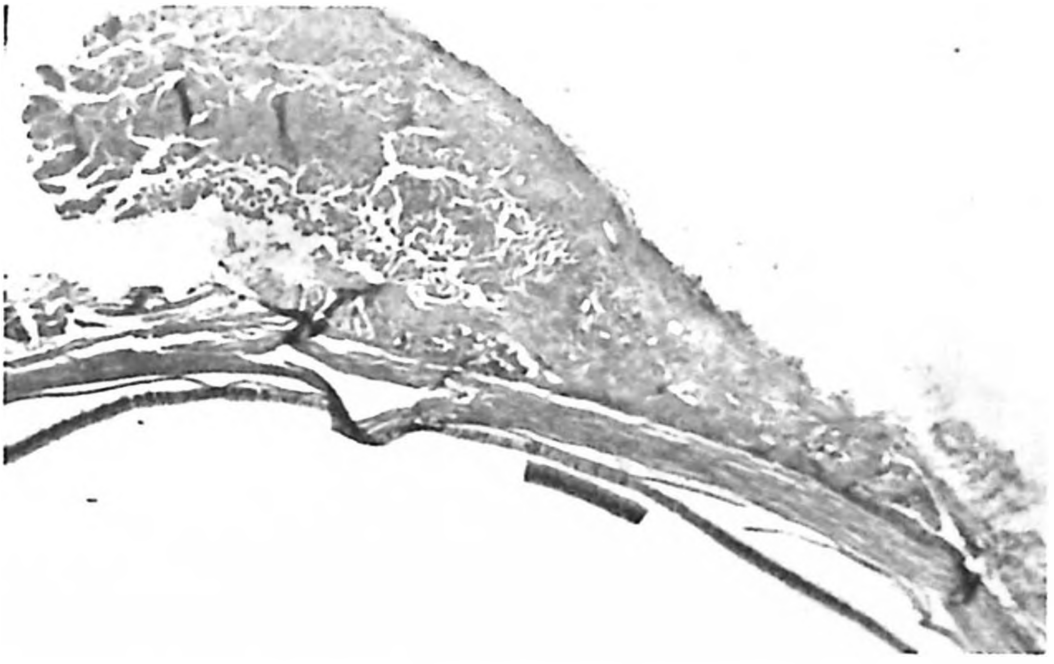

Fig. 2: Complete tissue integrity between muscle and sclera is maintained in the sealant group (H + E × 100).

Tissue destruction and muscle fiber atrophy may occur, to some extent, due to surgical trauma. But the histopathologic sections showed trauma resulting from suturing, which perhaps is caused by the suture material used. This complication was observed much less in the sections of the eyes in which fibrin sealant had been used.

Fig. 3: Higher magnification of Fig. 1 shows lack of some muscle fiber striation and displacement of some fibers with connective tissue (H + E × 200).

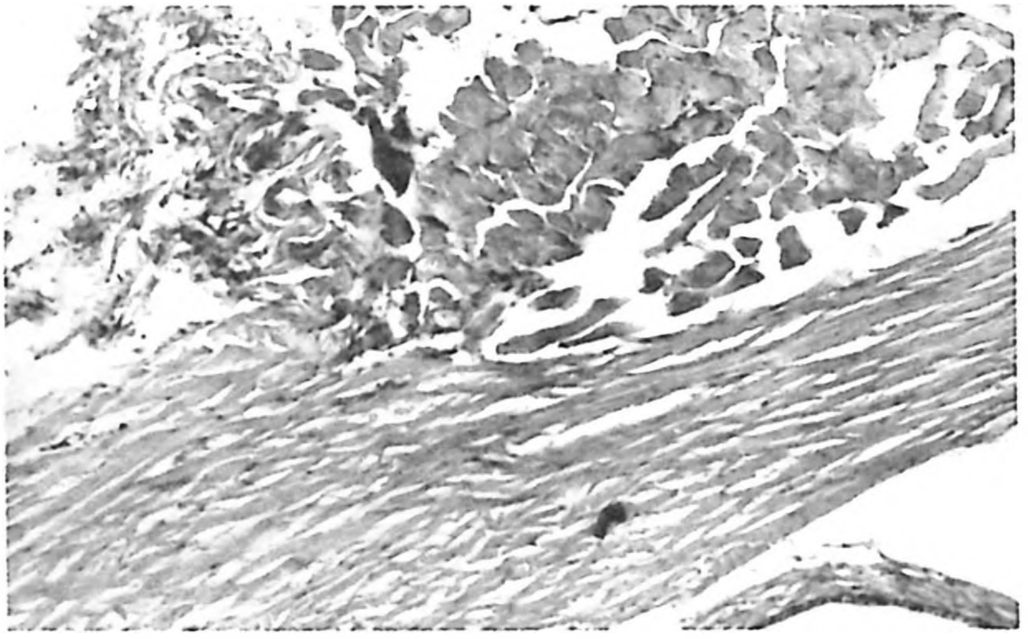

Fig. 4: Higher magnification of Fig. 2 shows, generally, that normal histologic structure of muscle fibers is preserved but some formed round shapes (H + E × 200).

We found that the greatest drawback in using the sealant was its insufficient adhesive properties which resulted in the retraction of the muscle from its original insertion to the retroocular orbital tissue. Although tissue adhesives provide a better microscopic impairment base, it seems that they must be used in

combination with sutures, at least in strabismus surgery. It may be possible to use sutures and sealants together, since the sealant aids in maintaining hemostasis and in accelerating wound healing, but we did not attempt this technique. Aichmair, et al[5] reported successful experimental results with this combined surgical procedure, but they did not include a control group in their study in which only suturing was used.

The second major drawback that we discovered in using the sealant is that there is broader adhesion of the muscle to sclera than required because the sealant spread on the new insertion line seeps down into the muscle pathway. The resulting amount of recession (or resection) will over or under correct the deviation which the surgeon plans and in spite of excellent surgical planning, unsuccessful results are not a surprise. In addition, sliding of the insertion back from the equator will produce restrictions of eye movements. Flick[6] who was rather sceptical about this method, pointed out in his report that sealing was sometimes so rapid that a correction could not be made during the operation.

In ophthalmology, fibrin tissue sealants have been used experimentally in the reconstruction of corneal and conjunctival wounds[7, 8], and lens capsule perforations[9], but reports of gluing extraocular muscles with fibrin in strabismus surgery are extremely rare.

Although our results showed positive effects with fibrin sealants, we believe that it is still too early to use these adhesives in strabismus surgery. Careful microsurgery and advanced suture materials will further reduce the complications of classical surgical attempts.

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A DIFFERENT APPROACH TO SLEEP PROBLEMS OF INFANCY: SWADDLING ABOVE THE WAIST*

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SUMMARY: Çağlayan S, Yaprak I, Seçkin E, Kansoy S, Aydınlıoğlu H. (Department of Pediatrics, Tepecik Social Security Teaching Hospital, Izmir, Turkey). A different approach to sleep problems of infancy: swaddling above the waist. Turk J Pediatr 33: 116-119, 1991.

Daytime sleep and wake periods of ten swaddled infants were periodically recorded by their mothers. The babies were swaddled above the waist for the first four days of the second, third and fourth months of life. During the next four days of the same months, the same infants were monitored with no swaddling. Comparison of the two sleep situations (swaddled and unswaddled) demonstrated that the increase in the total daytime sleep was statistically significant when the baby was loosely swaddled above the waist. *Key words: sleep, problems, swaddling.*

Sleep disorders are considered important complaints in the first four months of life. Several studies have documented the sleep problems in infants[1-3] and children[4, 5]. Temperament[5, 6], perinatal complications[7], neonatal irritability[2], and daytime separation from the mother[4] were all cited as causes of frequent waking or sleep problems of infants.

During the first few months of postnatal life, any internal or external stimulus during sleep can activate the Moro reflex, resulting in the waking of the infant. If the arms of the infant are loosely swaddled in front of the body, the action involved in the Moro reflex will be suppressed by external compression, and the infant will remain asleep. Additionally, a loose wrapping may provide confidence for the sleeping infant by preventing disturbing, and perhaps mentally traumatizing, Moro reflex activity.

The ancient custom of swaddling the infant during the first year of life is a common practice in rural Turkey and is believed to increase the sleeping periods of babies. In a population health survey conducted in Turkey[8], it was found that the practice of swaddling was quite common throughout the country and that 93.1% of mothers swaddled their infants. In the same study, it was also found that there was an inverse relationship between the practice of swaddling and the

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educational level of mothers; the more educated, the less likely they were to swaddle their infants, but still the percentage of swaddling was high (74.2 %).

There are multiple risks involved in tightly swaddling the entire body; some of the dangerous ones being an increased tendency for hip dislocation, suffocation, and aspiration of vomited materials. Bearing this in mind, loosely swaddling the infant above the waist might prolong the infant's sleep periods.

This study was designed in an out-patient setting among breastfed infants to determine whether swaddling an infant above the waist contributes to the prolongation of sleep durations.

Material and Methods

From February 1988 to January 1989, ten infants were periodically monitored by their mothers. During the first four days of the second month (31^{st} - 34^{th} day), third month (61^{st} - 64^{th} day), and fourth month (91^{st} - 94^{th} day) of life, the babies were monitored with swaddling clothes. During the next four days of the same months (35^{th} to 38^{th}; 65^{th} to 68^{th}, and 95^{th} to 98^{th} day,respectively) the babies were not swaddled for comparisons. In both groups, the sleep and wake periods of the babies were recorded by the mothers in the daytime from 06 a.m. to 18 p.m.

To be eligible for the study the mothers had to be a minimum of 20 years of age, cooperative, had to care for their infants on a full-time basis during the observation period, and had to breast feed their babies without giving them any supplementary food feedings. While the infants could not have colic, could not be the first-born infant in a family, or have insufficient records of sleep periods, the socioeconomic status of the families had to be similar. If an infant-mother pair did not conform with the above criteria, they were excluded from the study at the very outset.

A sleep episode was defined as a period of at least 15-minutes of quiet sleep with closed eyelids, delimited by at least 15-minutes of an awake period. For uniformity, all the mothers were given instructions on how to swaddle their infants above the waist. Before going to sleep, the infants were loosely swaddled with their hands meeting in front of their body. The legs were not wrapped (Fig. 1).

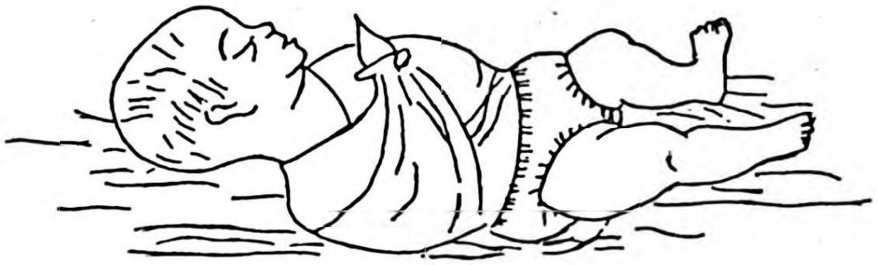

Fig. 1: Swaddling above the waist.

Results

Table I shows the average values of total daytime sleep during observation periods in the second, third and fourth months of life. Statistical analysis revealed significant differences between the average daytime sleep of the swaddled and unswaddled infant groups (P values for the second, third and fourth months are < 0.05, < 0.01, and < 0.05, respectively). Statistically significant differences ($P < 0.05$) were also found between total daytime sleep of the swaddled and unswaddled infant groups.

TABLE I: Comparison of Average Daytime Sleep Periods in Both Groups

NS		S		P Values
SLD	ADS	SLD	ADS	
31-34	7.96	35-38	8.46	$P < 0.05$
61-64	7.70	65-68	9.94	$P < 0.01$
91-94	5.60	95-98	6.29	$P < 0.05$

NS : unswaddled group.
S : swaddled group.
SLD : sleep recording (days of life).
ADS : average daytime sleep (in hours).

Discussion

Complete body swaddling is an inconvenient procedure for the infant, since it may create orthopedic problems such as unstable hip joints, and by preventing the infant's protective movements may result in life-threatening aspiration of vomited material. The face of a swaddled infant is also covered with a piece of cloth, which increases the risk of suffocation. Swaddling is an extremely popular practice in Turkey[8]. It is known that the piece of cloth that is placed over the infant's face while asleep increases the risk of expected infant death, even though there is no available data in this regard.

Sleep disorders are not only problems of infancy but occur in children of all age groups. Although many etiologic factors have been described[1-7], attempts at finding solutions for these problems have not met with success. In our study, the infants were swaddled above the waist for the first four months of life to provide a quiet, long sleep. We observed that if the infant's arms were loosely wrapped in front of the body, it could help the infant prolong daytime sleep episodes and, therefore, increase the total sleep duration.

Two factors can be mentioned regarding the cause for the increase in the total sleep period. The first is neurological; external stimuli, such as touching or speaking loudly can activate the Moro reflex during the first few months of life. Swaddling probably restrains the extention of the arms and prevents waking of the infant. The second is psychological; the infant's feeling of separation from the mother can be exaggerated with free, unwrapped hands. From this point of view, swaddling may support the infant's feeling of confidence.

Even though we did not evaluate night sleep patterns of swaddled infants, the daytime records convinced us that swaddling above the waist can prolong the total daily sleep of the infant.

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NEONATAL THERMOREGULATION*

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SUMMARY: Risbourg B, Vural M, Kremp O, de Broca A, Leke L, Freville M. (Departments of Pediatrics and Physiology, Picardie University Faculty of Medicine, Amiens, France). Neonatal thermoregulation. Turk J Pediatr 33: 121-134, 1991.

The human being is a homeotherm. Homeothermy is a result of thermoregulation which includes many physiological processes. Thermoregulation maintains an equilibrium between heat production (thermogenesis) and heat loss (thermolysis). There are three principal modes of heat production:

1. **Voluntary muscle activity.**
2. **Involuntary tonic or rhythmic muscle activity known as "shivering".**
3. **Non-shivering thermogenesis (NST) essential for newborns.**

Heat loss occurs in two stages:

1. **The flow of heat from the center of the body to its surface.**
2. **The flow of heat from the body surface to the environment by conduction, convection, radiation or water evaporation.**

Even in the very small premature baby, we find that metabolic and vasomotor control responses are developed.

To protect the newborn from stress resulting from hypo or hyperthermia, one should take into consideration the concept of the neutral temperature range which is also called the "Thermoneutral Zone" in (TNZ) or "Thermal Neutrality". Curves, proposed in 1971 by Hey[16] are essential for keeping newborns in the TNZ. *Key words: thermoregulation, neonate.*

The human being is a homeotherm. His internal temperature varies within the limits of 37 ± 1 °C. Homeothermy is a result of thermoregulation which includes many physiological processes. Thermoregulation maintains an equilibrium between heat production (thermogenesis) and heat loss (thermolysis). The maintaining of a constant body temperature is a result of the thermoregulatory function of the cells, particularly those of the nervous system.

The notion of homeothermy has been known in medicine since antiquity. It is interesting to note that in the past pediatricians ignored this fact when dealing

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with newborns and considered them to be poikilothermic organisms (body temperature showing wide fluctuations which depend on changes in environmental temperature). In fact, it was only in the beginning of the sixties that the importance of this concept was accepted in neonatal medicine.

In the last two decades of the 19th century, Pierre Budin[1] (Paris) demonstrated the importance of the thermal environment on newborn infants. He observed in premature infants weighing less than 2000 g, that if their temperatures fell to 32 °C, 98 percent of them died and if their temperatures were between 32.5 °C and 33.5 °C, 90 percent of them died. While on the contrary, if their temperatures were maintained between 36 °C and 37 °C , the survival rate rose to 77 percent.

Based on these observations, Budin produced an incubator that had the same operating principles as the modern incubators of today[2]: circulation of humidified and warmed air; control of ambient temperature; infant dressed with a cap on its head and placed over a support which is covered by a sheet. Subsequent studies by Blacfan et al[3], Hill[4], Buetow et al[5], and Day et al[6] demonstrated the following:

1. Importance of a thermal control of the environment especially for premature infants;
2. Fundamental role of thermoregulation during the first days of life.

Physiology of Thermoregulation

Thermoregulation is essentially controlled by the nervous system. It originates in the deep and superficial thermoreceptors that send nerve impulses to the spinal cord and brain. The physiologic adaptive reactions originating in the hypothalamus and behavioral (flexion position, clothing, etc.), and technological responses, functions of the cerebral cortex, are elaborated herein.

Thermoreceptors: They consist of the following two types which are sensitive to heat and cold:

1. Superficial receptors (cutaneous and mucosal)
2. Deep receptors which are present in the abdominal cavity, medulla, and also in the anterior hypothalamus.

Hypothalamus and spinal cord: The spinal cord plays an important role in vasomotor reactions and in the reflex. The hypothalamus is the most important part of the thermoregulatory system. It has been demonstrated that entire destruction of the hypothalamus does not eliminate thermoregulatory reactions, but makes them much less adaptive. It has also been shown that there are two groups of cells in the hypothalamus: a posterior and an arterior group. The posterior group coordinates reactions to cold while the anterior group coordinates reactions to heat.

Orders for thermoregulation are sent along two neural pathways: the somatomotor system and the sympathetic system. The somatomotor system, originating in the posterior hypothalamus, acts on the skeletal muscles and produces heat by the mechanism of shivering, while, the sympathetic system acts on the sweat glands, vessels, and brown adipose tissue.

The Role of the Endocrine System: Exposure to cold stimulates the secretion of adrenaline. This hormone increases energetic metabolism by hepatic glycogenolysis and lipolysis in adipose tissue (brown adipose tissue in neonates) and activates cutaneous vasoconstriction and directs blood from the skin to the deep parts of the body. The role of the thyroid hormones (T3, T4) are uncertain. It is thought that these hormones adapt the body to cold by increasing general metabolic activity. Their action is synergic with that of adrenaline.

It is accepted that there is a thermostat in the hypothalamus, which keeps the body temperature within the limits of 37 ± 1 °C. This set-point can be increased or decreased as a result of various causes. Whatever the level of this set-point temperature, the thermoregulatory system maintains this temperature as the body temperature.

Deviations from this temperature will actuate one or more appropriate effector systems to restore the temperature field by extra heat production or by heat loss.

Modes of Extra Heat Production

There are three principal modes of heat production:

1. Voluntary muscle activity.
2. Involuntary tonic or rhythmic muscle activity known as "shivering".
3. Non-shivering thermogenesis (NST)

* Though voluntary muscle activity can be a good source of energy for an adult, the newborn infant is not capable of using this mechanism.

* Normally, in the adult male, shivering is quantitatively the most important involuntary mechanism of thermoregulatory heat production. NST develops to a greater extent only after long-term exposure. On the contrary, in the newborn infant, NST is an important and effective mechanism of heat production. This system is controlled by two mechanisms: neural and hormonal. In the newborn infant, brown adipose tissue is the principal element of NST. It constitutes 2-6 percent of the body mass in full-term newborn infants[7]. Only in the early sixties, was it demonstrated that in vitro metabolic activity of brown adipose tissue was much higher than that of white fat. Two important features can be recognized by electron microscopy:

(1) The richness in mitochondria, and (2) The synaptic contact between sympathetic nerve terminals and cell membranes.

Brown adipose tissue is located around the neck and on the back between the scapulae, in the axillae, on the surface of the heart and kidneys and adjacent to the large vessels within the thoracic cavity. It is shown that the venous blood of the cervical and thoracic interscapular adipose tissue is partly drained into the inner vertebral sinus (plexus venousus vertebralis internus) which surrounds the spinal cord like a heat exchanger[8]. In this manner, the two effector systems, NST and shivering are interlocked. With external cooling, NST is initiated by the cooling of thermoreceptors in the skin, which leads to an increase in the temperatures of the interscapular brown adipose tissue and of the cervical vertebral canal (by heat flow from the adipose tissue to the spinal cord). As a result of the temperature of the spinal cord being maintained at a high level, shivering remains suppressed.

However, if we block NST, the temperature of the cervical spinal cord begins to fall and shivering occurs. Thus, under natural conditions, shivering occurs only after NST is evoked to its fullest extent. The neonate, therefore, shivers only when subjected to the stress of severe cold. On the contrary, in the adult, since brown adipose tissue does not exist, NST is not an important source of heat production, and occurs only when shivering is not sufficient to increase the body temperature. Fig. 1 shows the importance of NST and shivering during the first 12 months of life[9].

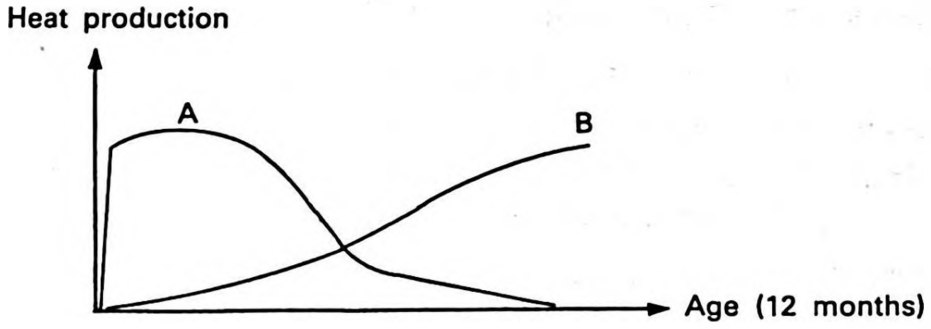

Fig. 1: Importance of NST (A) and shivering (B) during the first 12 months of life.

Modes of Heat Loss

Heat loss occurs in two stages:

1. The flow of heat from the center of the body to the surface.
2. The flow of heat from the body surface to the environment.

1* According to the equation:

$$H_{int} = A.C\ (T_{re} - T_s) \quad \text{in kcal / h or in W}$$

Where H_{int} represents internal heat flow; A the body surface area, W the number of watts, T_{re} the deep body (rectal) temperature, T_s the mean skin temperature,

and C the thermal conductance. Heat transfer to the body surface is determined by the internal temperature gradient (Tre - Ts) and thermal conductance. This compound quantity "C" is determined mainly by three factors:

1. specific heat conductivity of these tissues;
2. absolute thickness of these tissues, particularly the thickness of subcutaneous fat, which has relatively low heat production,
3. the rate of blood flow to the surface of the trunk and extremities.

Thus, cutaneous vasoconstriction diminishes heat loss and vasodilatation increases it. But vasoconstriction, as a cold defence reaction, is much less effective in the neonate than in the adult, due merely to differences in body mass. Thin subcutaneous fat in the newborn increases the conductance of the skin and heat loss.

2* Heat transfer from the body to its surroundings is a complex process. For quantitative consideration, we distinguish four components: heat transfer by conduction, convection, radiation and water evaporation expressed as follows.

$$H_{ext} = H_k + H_c + H_r + H_e$$

Conductive heat loss is a result of simple thermal conductibility between the body surface and the objects with which it is in contact. It occurs, for example in the small area of the back when the infant is in contact with the support. Depending on the heat insulation of the support, conductive heat loss may be very important, but it may also be so small as to be negligible if the baby is laid on a well-insulated mattress.

The parts of the body that are neither in contact with the support nor covered by clothes are enveloped in a layer of still air that is termed the "boundary layer". If there is no wind outside this layer, which may be as thick as 4-8 mm, the moving air carries heat by mass flow. This phenomenon is called heat transport by convection. Within the boundary layer, heat is transported by conduction.

Heat loss by radiation occurs by means of electromagnetic waves. There is an interchange of heat between the naked newborn infant and its environment (walls, windows, people). Therefore, it can easily lose its body heat even when it is placed in a warm incubator if the walls of the incubator are cold. This kind of heat loss can be reduced in the following manner:

- Insulating the walls of the incubator.
- Placing a plastic tunnel over the child.
- Placing a radiant heater over the incubator.
- Heating the room.

In the adult male, under conditions of thermal neutrality, about 20-25 percent of the total heat loss is due to evaporation from the skin and respiratory tract

(insensible water loss). Similar values have been found in two to ten day-old newborn infants upon exposure to neutral temperatures and a Relative Humidity (RH) of 50 percent. The driving force for evaporation is the difference between the vapor pressure and the environment. In one study, when RH fell from 80 to 20 percent, heat loss doubled in full-term newborn infants. The metabolic rate was found to be at a minimum of 50 % RH under these conditions. Figure 2 shows the different types of heat loss of a premature infant weighing less than 1500 g. under different conditions[9].

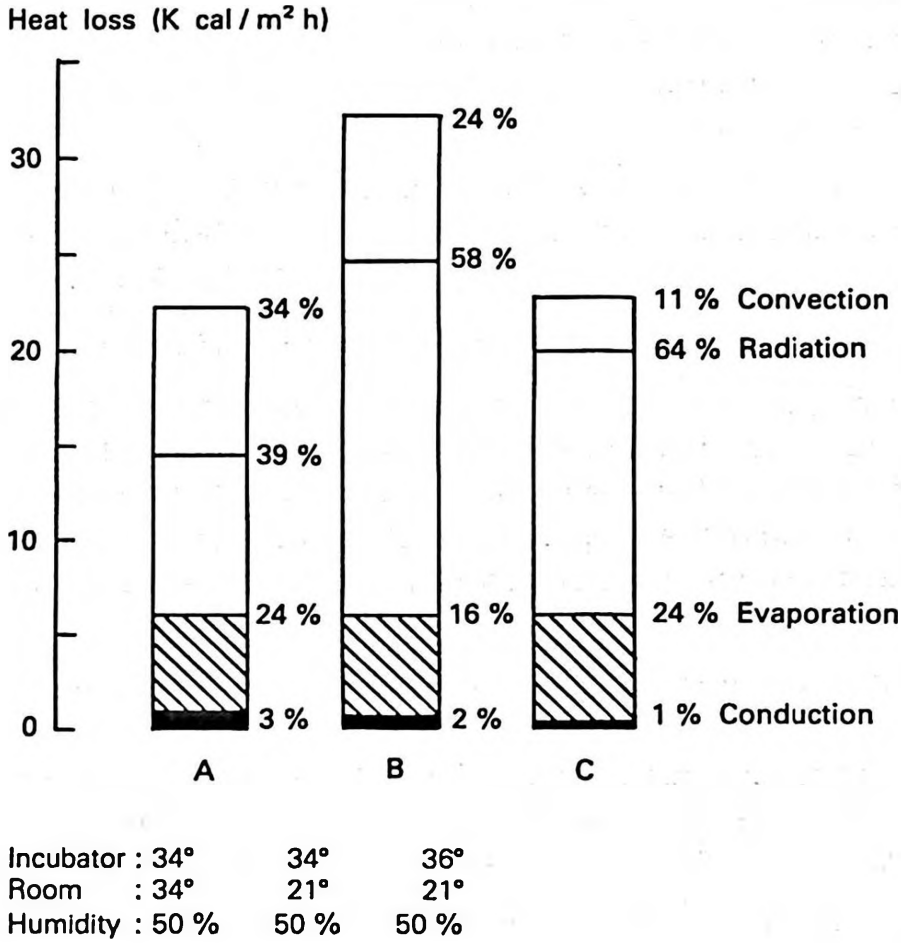

Fig. 2: Thermal environment and heat loss of a premature infant weighing less than 1500 g with a rectal temperature of 37 °C

Evaporative heat loss can be increased considerably by sweat secretion and by an increasing respiratory rate. In a hot environment, evaporative heat loss may represent the only, but a very effective avenue, of heat loss. Evaporation of one liter of sweat dissipates about 580 kcal (2430 J) from the body. The most effective to date heat-dissipation mechanism is sweat secretion in the newborn infant as well as in the adult. Maximum sweat secretion is slightly different in the full-term

infant and in small-for-dates and slightly premature infants, but there is a considerable difference in the threshold esophageal temperature for the onset of sweating in smaller and larger infant groups. (Fig. 3)[10].

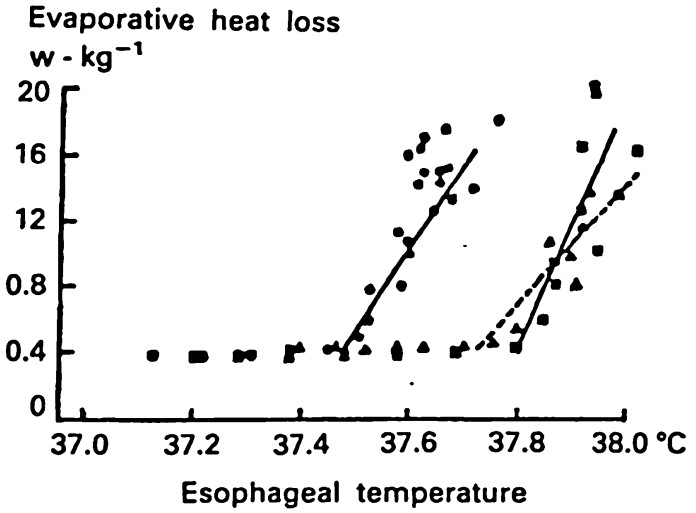

Fig. 3: Evaporative heat loss in relation to esophageal temperature in 22 full-term (●), 9 small-for-dates (■), and 8 premature (▲) infants. Note the equal maximum response but increased threshold in the small-size infants.

Sweating due to thermal stimuli could not be detected in infants less than 210 days postconception, even when the rectal temperature rose as high as 37.8 °C. In such infants, the sweat glands did not even respond to intradermal injection of acetylcholine[11]. It is thus assumed that the failure of the sweat response is due mainly to an immaturity of the sweat glands. In comparison to adults, the maximum sweat response is minimal in the neonate. Thus, it can be said that the newborn infant is markedly less heat-resistant than the adult.

Stage of Maturity of the Thermoregulatory System at Birth

Temperature regulation is frequently said to be "not fully developed" at the time of birth. Caution should be used in asserting that the control centers are immature, even though the neonate shows more fluctuations in body temperature than the adult. Even in the very small premature infant, metabolic and vasomotor control responses are developed. Thus, instability of the body temperature seems to be due, to a large extent, to the discrepancy between the efficiency of the effector system and body size; growth alone will slowly enlarge the control range.

Pathophysiology and Clinical Aspects

Hypothermia : The fetus lives in a constant thermal environment. Its body temperature is approximately 0.5 °C higher than that of its mother. At birth, the

newborn infant finds himself in an environment which is 12 °C colder than that of intrauterine life (25 °C vs. 37 °C), wet with amniotic fluid, and loses heat by evaporation, radiation, convection and conduction.

In addition to these environmental factors, birth trauma (intracranial pathologies), cerebral malformations, and certain drugs play an important role in disturbing the regulation of normal neonatal temperature, thus resulting in hypothermia. Morever, respiratory deficiency leading to hypoxia and hypercapnia may contribute to a rapid development of hypothermia. As a result of many experiments, it has been shown that the extra heat produced in the brown adipose tissue is especially susceptible and suppressed by hypoxia and hypercapnia.

Signs and symptoms :

- Drop in rectal temperature.
- Apathy.
- Refusal of food.
- Edema of the extremities.
- Coloration of the extremities may change; they may be pale or cyanotic or deceivingly red (dissociation of oxyhemoglobin is restricted).
- Respiration may become slow, superficial or irregular and bradycardia may occur. However, the neonate is generally thought to be less susceptible to the dangerous corollaries of hypothermia such as cardiac arrest and respiratory distress.
- Risk of intraventricular hemorrhage.
- Digestive problems (distension, vomiting) are frequent.
- Oliguria is the rule.
- Metabolic disorders such as metabolic acidosis, hypoglycemia, hyperkalemia, hyperazotemia are common.

In the prolonged and severe forms, generalized bleeding (especially intrapulmonary) is seen as a result of Disseminated Intravascular Coagulation.

Prevention of Hypothermia

Delivery room : Immediately after birth, the skin temperature drops considerably, and is followed by a drop in deep-body temperature. Heat loss occurs especially as a result of water evaporation from the moist skin, but there is also significant heat loss which occurs as a result of convection and radiation. Under normal conditions in a delivery room, and if no precautions are taken, the deep-body temperature of the newborn may drop two to three degrees in half an hour. Figure 4 shows the change in rectal temperature during the first thirty minutes of life under varied conditions[12].

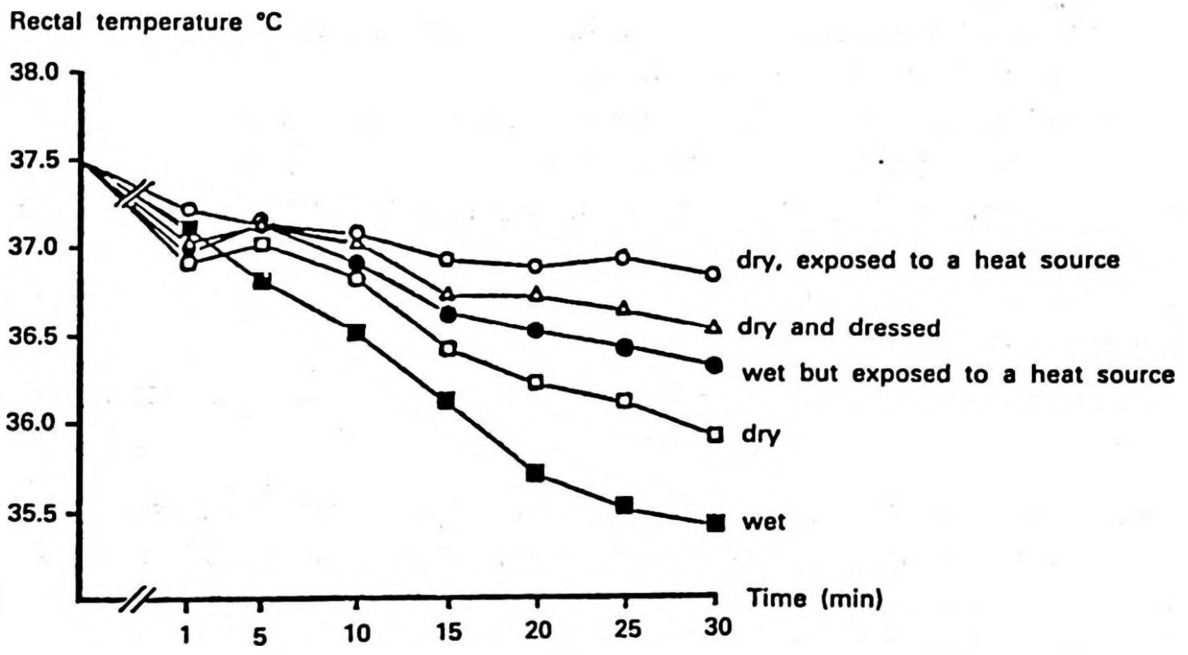

Fig. 4: Change in rectal temperature during the first 30 minutes of life.

In fact, the drop in temperature can play a beneficial role at birth by stimulating skin receptors thereby initiating respiration. At the same time, increased peripheral vasoconstriction will reduce the right-to-left shunt of ductus arteriosus. However, it is preferred that the newborn be kept in a warm atmosphere, adequate enough to prevent the dangerous effects of cold. The following measures should be taken to prevent hypothermia from occurring in the delivery room.

- Ambient temperature should be 22 °C (25 °C is more efficient) with a RH of 60 to 65 %, and devoid of air currents.
- Skin of the child (especially face, head and hair) should be dry. This precaution will diminish evaporative heat loss.
- Conductive heat loss should be diminished by placing the newborn on a dry, warmed sheet.
- The newborn should be systematically exposed to a radiating heat source, especially if reanimation is necessary.
- The baby's first bath should be delayed because washing a baby soon after birth contributes to a fall in body temperature[13].

During Transport :

- An incubator having a convection heating system is almost always used. An insulator is used to cover the incubator for decreasing the heat loss caused by radiation.
- If the child is on mechanical ventilation or oxygen, the gases should be heated and humidified.

- The ambient temperature of the vehicle should be between 25 ° and 28 °C (which is difficult to attain in winter).
- Head and hair of the child should be covered with a cap.
- The rectal temperature should be monitored at least two times during transport (at the beginning and at the end). If the infant is transported over a long distance, the rectal temperature should be taken regularly.
- Since the newborn infant is in need of energy, glucose should be given per os or intravenously.
- If severe hypothermia is present, the temperature should be increased slowly (2°/hour).

Hyperthermia : As mentioned previously, the hypothalamus, more precisely, the anterior hypothalamus (Pre-Optic Anterior Hypothalamus = POAH) is the neural center which receives different signals from the periphery and initiates mechanisms of heat loss or gain. According to Ranson[14] this region functions as a "thermostat". It is set around 37 °C. It should be emphasized that fever is not hyperthermia and hyperthermia is not fever.

Fever is the augmentation of the set-point of the human thermostat. The body activates all of its functions to be able to increase its temperature to the level of the thermostat. Fever is most frequently produced by such stimuli as bacteria, viruses and yeasts, but, it is also known to occur as a result of immune reactions, hormonal substances and drugs. It has been postulated that these stimuli liberate an intermediary substance termed endogenous pyrogen (E P). EP is known to be identical to interleukin-1 (IL-1), a product of monocytes and macrophages which initiates many of the reactions termed collectively the "acute phase response". Within the hypothalamus EP/IL-1 appears to act by inducing the synthesis of prostaglandins of the E series, particularly prostaglandin E2 (PGE2) (Fig. 5)[14]. Endogenous pyrogens shift the set-point of the thermoregulatory system to a higher level. In the neonate there is an unresponsiveness to pyrogen in the hypothalamus, which explains the absence of fever in infection.

Hyperthermia appears when metabolic heat production exceeds the capacity of thermolysis. In the neonate, it is observed in the case of thermoregulatory center perturbation, for example after peri-natal central trauma. It may also be seen when the incubator is exposed to the sunshine or in the case of phototherapy for the treatment of jaundice.

Signs and symptoms :
- The first sign is hyperpnea.
- tachycardia.
- red color of extremities (vasodilatation).
- increased sweating.

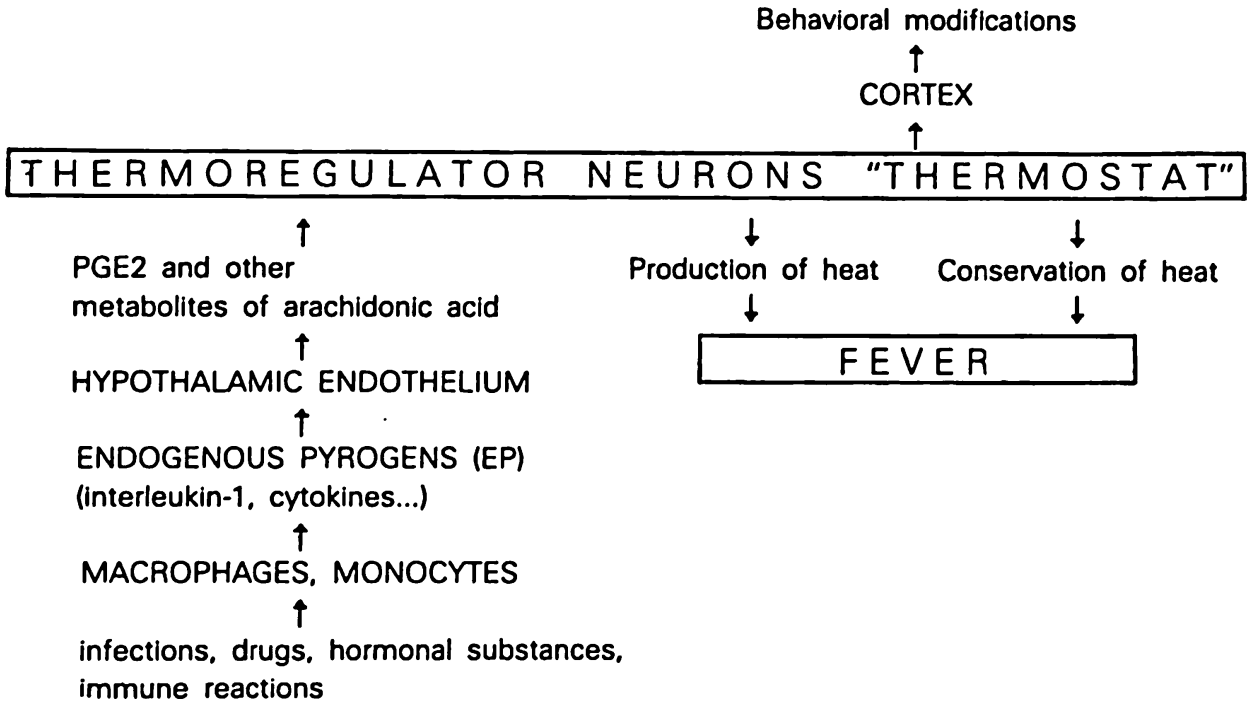

Fig. 5: Physiopathology of fever

Treatment: Cooling should be done slowly and simply by decreasing the temperature of the incubator. In order to protect the newborn infant from the stress of hypo or hyperthermia, one should take into consideration the concept of a neutral temperature range (also termed "Thermoneutral Zone" (TNZ) or "Thermal Neutrality". The Thermoneutral Zone (Fig. 6)[15] is defined as the range of ambient temperature within which:

- temperature regulation is achieved only by a nonevaporative physical process (sweat gland activity = 0).
- Oxygen consumption is the least
- The metabolic rate is at a minimum.

This range is delineated by the symbols T3, T4 (Fig. 6). In small premature infants, the lower limit of TNZ may be as high as 35 °C. The ambient temperatures which are necessary to create thermal neutrality in naked and dressed neonates are shown in Fig. 7[16].

These curves were proposed in 1971 by Hey[16]. These temperatures are valid under the following conditions:

- still air.
- RM = 50 %
- Child covered by another plastic tunnel or an incubator with double walls.

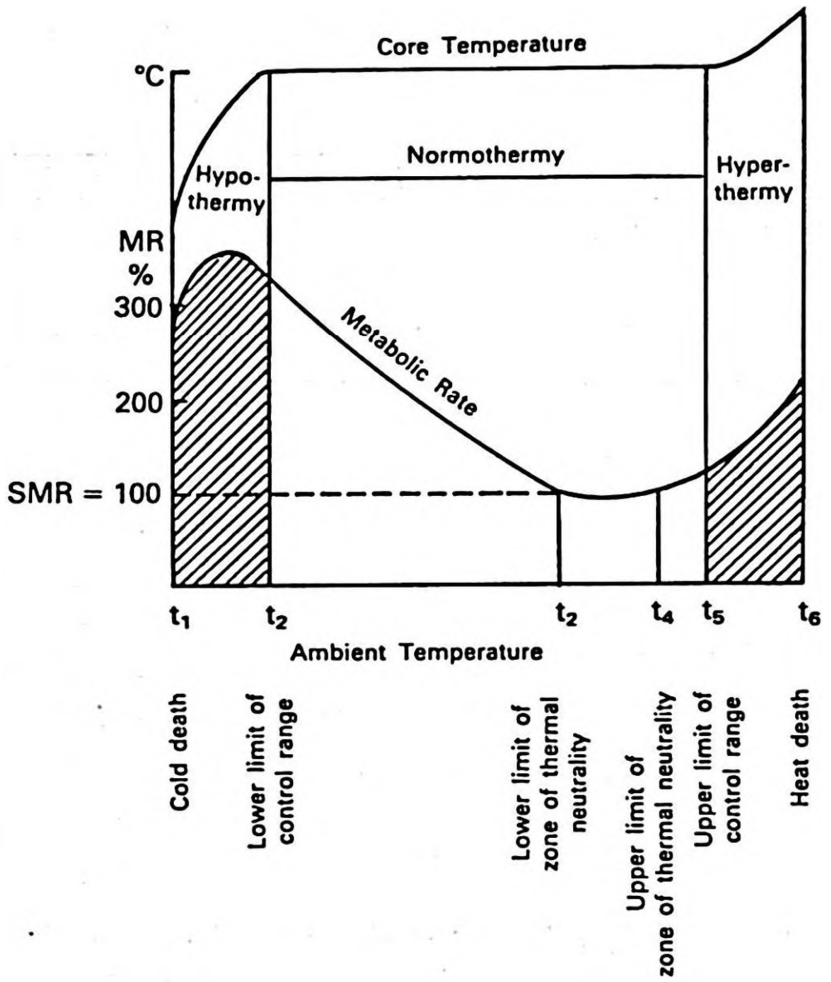

Fig. 6: A schematic representation of the relationship between metabolic rate and ambient temperature in homeotherms; zone of thermal neutralitiy (t3...t4).

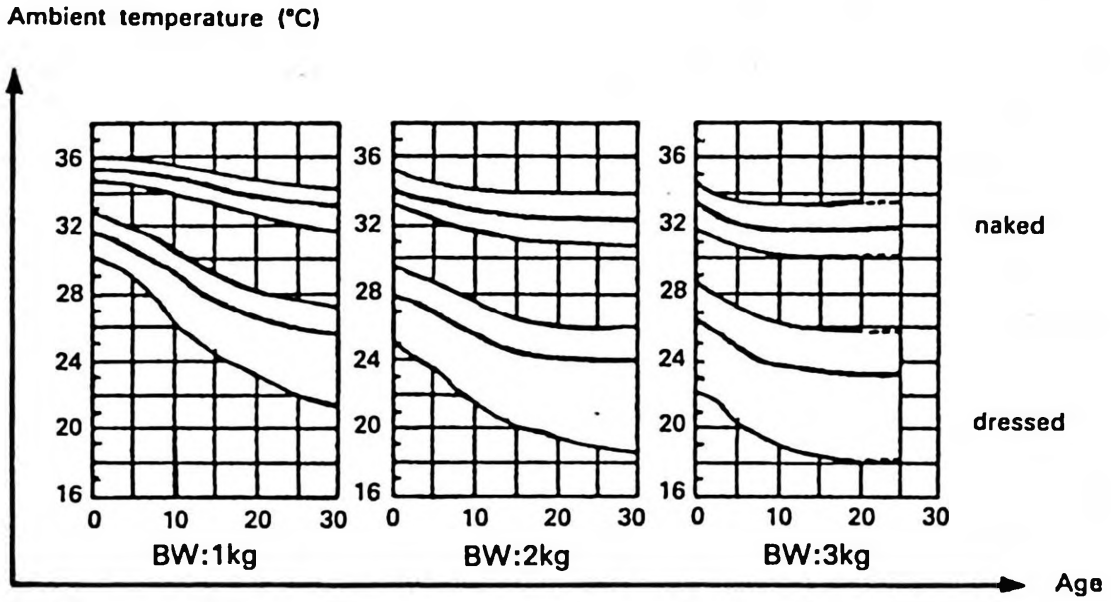

Fig. 7: Diagrams showing the optimal ambient temperature for a neonate as a function of weight, age and state of dress in the incubator.

These values can be changed :

- If RM is increased, the temperature should be decreased 0.5 °C for the naked child and 1 °C for the dressed child.
- If the incubator has one wall, the temperature should be increased 1 °C for each 7 °C of difference between the temperature of the incubator and the temperature of the room.
- In case of convulsion, agitation or left-to-right shunt, temperatures should be reduced.

In conclusion, the newborn is capable of thermoregulation within limits by ways different from that of the adult. It is usually exposed to hypothermia. Premature infants are at a greater risk of hypothermia. However, we can help in the maintenance of TNZ, essential for the optimal functions of body cells, by adhering to certain simple procedures.

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The Turkish Journal of Pediatrics 33: 135-138, 1991

CONTRAST MATERIAL – INDUCED ACUTE RENAL FAILURE IN A DIABETIC PATIENT*

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SUMMARY: Beşbaş N, Topaloğlu R, Saatçi Ü. (Pediatric Nephrology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine). Contrast material-induced acute renal failure in a diabetic patient. Turk J Pediatr 33: 135-138, 1991.

A 16-year-old girl diagnosed as having type 1 diabetes mellitus and oliguric acute renal failure which developed 24 hours after computerized tomography with the infusion of contrast material is presented. She required short-term hemodialysis and in two weeks her renal functions returned to normal. All the findings in this high-risk patient suggest the development of contrast material-induced nephropathy. We propose that there should be adequate hydration before radiocontrast material studies are performed on diabetic patients. *Key words: contrast material, acute renal failure, type 1 diabetes mellitus.*

The infusion of radiographic contrast material is considered to be the third leading cause of iatrogenic acute renal failure (ARF)[1]. The incidence of ARF after the administration of radiocontrast material is high in high-risk patients such as those with diabetes mellitus[1,2]. We report a case of type 1 diabetes mellitus (DM) in which ARF was induced by infusion of radiocontrast material.

Case Report

A 16-year-old girl was admitted to the Ankara Social Security Hospital in a semi-comatose state of two days' duration. The patient underwent computerized brain tomography with the infusion of contrast material. A diagnosis of brain edema and encephalitis was established and she was referred to the Hacettepe University Children's Hospital for further evaluation.

Physical examination revealed a stuporus, acidotic girl. The temperature was 36 °C; respiration rate, 35/min; pulse rate, 120/min, and blood pressure, 120/70 mm Hg.

Laboratory studies revealed a hemoglobin of 13 g/dl and white blood cell count of 14,000/mm^3, with normal red blood cell morphology. Urinalysis revealed a specific gravity of 1028, 2 + protein, 4 + sugar, and a urine sediment with

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numerous erythrocytes and rare, fine granular casts. Blood sugar was 600 mg/dl, serum sodium 131 mEq/L, potassium 5.7 mEq/L, and arterial blood pH 6.95. In view of the physical and laboratory findings, a diagnosis of diabetic ketoacidosis was made and she was admitted to the hospital.

Therapy was started which consisted of an appropriate dose of insulin and the infusion of fluids and electrolytes. Despite loading with 400 ml/m^2 intravenous fluids on two occasions, and the administration of 3600-4200 ml/m^2/day maintenance fluids, the patient had a urinary output of only 180 ml/day. The blood sugar was put under control but not the acidosis. THer BUN was 86 mg/dl and the serum creatinine concentration 5 mg/dl; these values were progressively increasing. Other laboratory studies indicated an erythrocyte sedimentation rate (ESR) of 13 mm/h, antistreptolysin O (ASO) titer 625 Todd units, C-reactive protein (CRP) negative, latex fixation test, negative, complement components: C3 76 mg/dl (normal 60-120 mg/dl); C4 24 mg/dl (normal 20-40 mg/dl), anti-nuclear antibody (ANA) negative and anti-DNA 10 U/ml. Urine osmolarity was 320 mOsml, blood osmolarity 294 mOsml, urine sodium 61 mEq/L, fractional sodium excretion 3.1 %, and the urine urea nitrogen/blood urea nitrogen ratio was 2. The renal insufficiency index was compatible with acute renal insufficiency.

Central venous pressure was 17 cm H_2O. The chest X-ray revealed signs of hypervolemia and ultrasonography showed bilateral enlarged kidneys. Two doses of furosemide 5 mg/kg were administered, but there was no response to treatment. The patient was then placed on hemodialysis. After two sessions of hemodialysis, she started to urinate and the BUN and serum creatinine levels progressively fell to 21 and 0.9 mg/dl, respectively within two weeks.

Discussion

Hyperglycemic hyperosmolar nonketotic coma, diabetic ketoacidosis, radiocontrast material-induced nephropathy, renal papillary necrosis, glomerulonephritis and septicemia are common causes of ARF in diabetic patients[2]. ARF is a rare complication of diabetic ketoacidosis and occurs as a result of hypovolemia due to profound dehydration. The development of ketoacidosis causes patients to present to the hospital sooner than those with hyperglycemic hyperosmolar nonketotic coma, and thus, dehydration is less severe and hypotension less common, as was the case in our patient[2, 3].

Thus the possibility of renal papillary necrosis should always be considered in a diabetic patient with rapidly progressive renal failure. Elderly female diabetics are most frequently affected, and usually present with recurrent fever, hematuria and loin pain. Ultrasonography may indicate obstruction and loss of medullary tissue[2, 4]. These findings however were not observed in our patient.

Renal disease, other than that caused by diabetic glomerulosclerosis, may occur in three percent of diabetics and may lead to acute renal failure[2]. Idiopathic membranous nephropathy, acute post-infectious glomerulonephritis and rapidly progressive glomerulonephritis are most commonly found in diabetic patients[5]. With such laboratory findings as mild proteinuria, normal C3, C4, and anti-DNA levels, negative ANA and the serum creatinine level and urine analysis returning to normal within two weeks, glomerulopathies were easily excluded.

The incidence of contrast material-induced nephropathy in normal patients following intravenous urography, angiography or computerized tomography scanning ranges between two to five percent in normal patients and nine to 16 percent in high-risk patients with such conditions as pre-existing renal insufficiency, DM and congestive heart failure[6, 7]. The mechanism of renal damage is uncertain, but it is probably related to direct tubular toxicity and to a reduction in renal blood flow, causing renal ischemia and a decrease in the glomerular filtration rate[8].

Typical contrast material-induced ARF is a mild, reversible condition of short duration. Serum creatinine levels begin to rise within 24 h and peak in 3-5 days returning to normal within 7-10 days[2, 8]. Patients may require short-term dialysis, but irreversible renal failure is rare[9]. Our patient developed oliguric ARF 24 h after infusion of the contrast material and the condition progressed in 48 hours. She required hemodialysis. After two sessions of hemodialysis, she started to urinate and the renal functions returned to normal within two weeks.

The findings and clinical course of this particular high-risk patient strongly suggest that the contrast material used had induced the ARF.

In conclusion, we propose that radiologic studies with contrast material be avoided in diabetics, but if contrast material has to be administered, proper precautions should be taken such as ensuring adequate hydration prior to injection.

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A CASE OF NEURENTERIC CYST*

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SUMMARY: Turgutalp H, Özoran Y, Özoran AS, Aktürk F, Süzen B, Komşuoğlu SS. (Departments of Pathology, Morphology, Neurology and Neurosurgery, Karadeniz Technical University Faculty of Medicine, Trabzon;Turkey). A case of neurenteric cyst. Turk J Pediatr 33: 138-141, 1991.

An eight-month-old female infant with a neurenteric cyst situated in the lumbar region is presented because of its rarity and because it can mimic other neurological malformations which occur on the same site. Microscopic examination of the surgically excised cyst revealed Water-Paccini corpuscles, lymphoid follicles, intestinal mucosa and meningeal tissue which originated from two ectoderm and endoderm-germ layers. *Key words: neurenteric cyst, enterogenous cyst, intestinoma, teratoid cyst.*

Neurenteric cysts are rare malformations caused by the disturbance in closure of the neurenteric canal occurring between the 18th - 21st day following conception. The exact etiology of this complex malformation remains unknown[1]. It is suggested that the failure of interposition of the mesoderm which forms the normal covering around the spinal cord and persistent opposition of endodermal and ectodermal derivatives give rise to these cysts[2]. Smith[3] called these malformations "dorsal fistula remnants", but they have also been termed "teratoma-like-cyst", "enterocystoma", "foregut cyst", "gastrogenic cyst", "intestinoma", "enterogenous cyst", etc.[1,4]. These cysts are always situated between the gastrointestinal tract and the skin of the back. They may be prevertebral, postvertebral and occasionally intraspinal in type. It is reported that intracranial localization is very rare. The pons and medulla oblongata are the sites of localization of this malformation[1].

Neurenteric cysts are occasionally associated with gastrointestinal, vertebral and central nervous system malformations[1-5].

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In this study, we wish to report a case of neurenteric cyst because it is a rare entity and resembles other neurological malformations.

Case Report

An eight-month-old female infant was admitted to the Karadeniz Technical University Farabi Hospital because of a swelling in the lumbar region. Physical examination revealed a soft, tumoral mass located in the lumbar region and covered by skin. The remainder of the physical examination was within normal limits. On computerized tomography posterolateral laminar defects were detected at the 2nd and 3rd lumbar vertebrae. After a diagnosis of meningocele was established, the mass was excised. Macroscopic examination of the excised biopsy material revealed an off-white nodular mass, 5 x 4 x 2 cm in diameter. Microscopic examination of the mass revealed lymphoid follicles, intestinal mucosa, lipoid tissue and Water-Paccini sensitive nerve endings covered by meningeal tissue and skin, which were regularly organized (Figs. 1a, b).

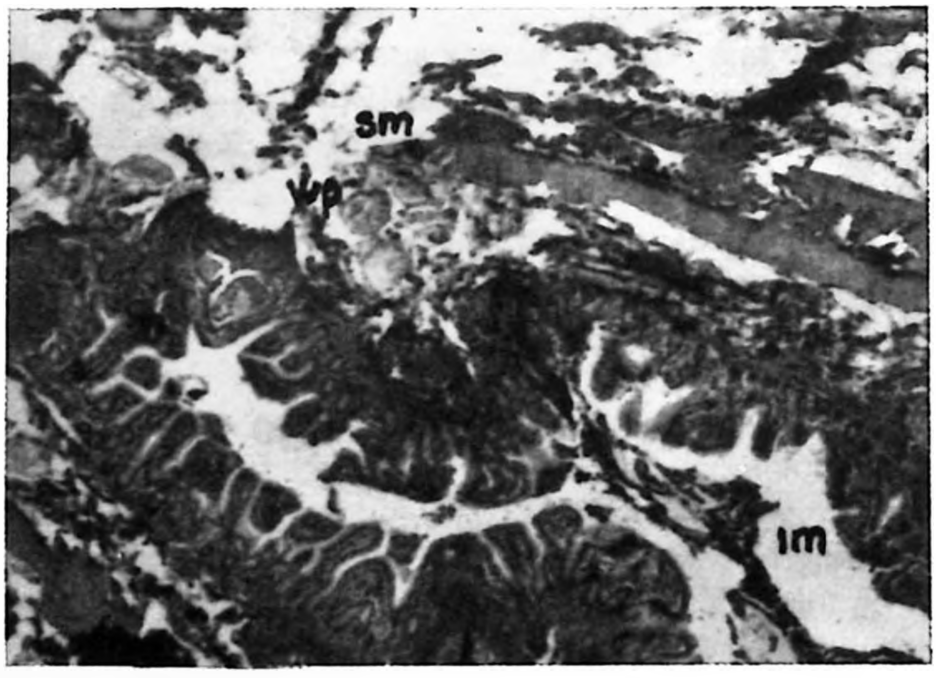

Fig. 1a.: Microscopic examination of excised cyst.

Discussion

Neurenteric cysts are rare congenital malformations and the largest well documented series (19 cases) has been published by The Hospital For Sick Children, Toronto[5].

Neurenteric canal closure defect is the main cause of the disease[5]. The presence of female sex chromosomes in the cysts in male patients strongly suggests their

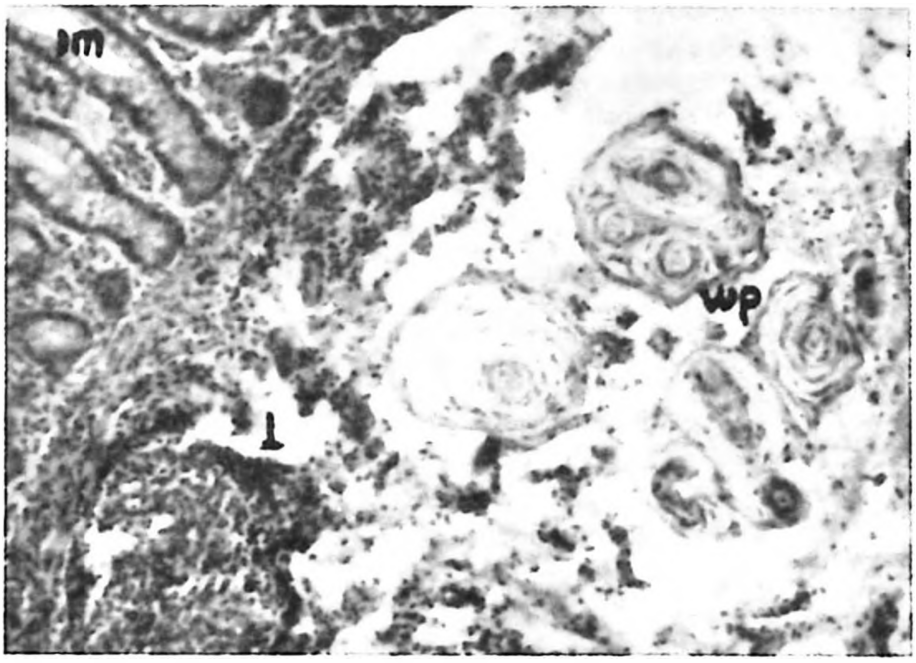

Fig. 1b: Microscopic examination of excised cyst.

teratomatous origin. There is a moderate male preponderance[3]. Their most common site is the cervico-thoracic vertebral region. However, they may also be situated in the medulla oblongata, pons, posterior mediastinum, and in the pelvis[2]. In our case, the cyst was located in the lumbar region. Localization is usually extramedullary, as in our patient[1]. These cysts rarely mimic multiple sclerosis, other cerebromotor dysfunctions, mediastinal, veretebral and gastro-intestinal malformations or hydrocephalus. A preoperative diagnosis of meningo-cele was made in our patient Other authors have also established such a diagnosis in these cases[3,6]. Ciliated pseudostratified or cubic Periodic-Acid-Schiff positive gastrointestinal-type epithelial covering of the cysts and neuroglial elements are the main elements[1,7]. As was described by Hecker[8], we also observed Water-Paccini corpuscles in our patient. In addition to these elements, we detected lymphoid follicles. The main therapeutic approach is surgical excision of the cyst, which was carried out in our patient.

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