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LEUKOCYTE PHAGOCYTOSIS DURING CARDIOPULMONARY BYPASS IN ADOLESCENTS*

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It is generally recognized that in patients undergoing extracorporeal circulation the immune defence system is temporarily impaired. The decrease in host resistance against invading or endogenous microorganisms can lead to postoperative infections. In patients undergoing operations involving cardiopulmonary bypass (CBP), infection is higher than in closed heart surgery, and other major surgical procedures[1,2]. The higher infection rate has been suggested to be the result of CPB. Cardiopulmonary bypass is associated with hemolytic anemia, denaturation of circulating blood proteins, and reduced levels of complement and immunoglobulins[1,3,4,5]. Therefore, we planned to investigate the effect of CPB on leukocyte phagocytosis in the adolescent. A recent study involving infants has demonstrated a decreased polymorphonuclear leukocyte (PMNL) function, resulting from immature capacities[6]. Studies performed in adults using different methods have shown contradictory results in this field[7-9].

Although in adolescents, the leukocyte functions have completed their maturity, increasing levels of sex hormones cause total body epithelium to change, and there is a rise in susceptibility to infections when compared to adults[10]. As a result, the effects of operations involving CPB on PMNL phagocytic function may differ from those in adults. Since, as far as we know, no other study has been reported in adolescents, this investigation was undertaken to define the effect of operations involving CPB on phagocytic functions of PMNL in adolescents undergoing complete repair of congenital or acquired heart defects.

We decided to undertake this study, using for the first time in this field a radioactive method which had previously been used in the identification of bacteria and is based on the principle that their presence can be determined by their metabolic activity[11]. Almost all aerobic bacteria which use various carbohydrates release CO_2 as a final froduct[11]. Previously, De Land and Wagner[11] added

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^{14}C-uniform glucose, and later Levin et al[12] used a mixture of ^{14}C-format and ^{14}C-uniform glucose as an energy source in the medium. Thus, they proved that the growth of aerobic bacteria could easily be observed in a short period of time. Subsequently, this method had been employed to determine whether or not biological life existed on the planet, Mars[12].

Material and Methods

Fifty adolescents in which absolute PMNL counts and phagocytosis were assessed were included in this study. They were divided into two groups. The first group consisted of thirty adolescents between 13-19 years of age who had undergone open-heart surgery because of congenital and rheumatic valve disease. The second group, the control group, consisted of twenty adolescents undergoing closed heart surgery.

Anesthesia was induced by use of intramuscular ketamine hydrochloride 5 mg/kg with atropine sulphate and was maintained with fentanyl citrate 50 µg/kg. Muscle relaxation was induced with pancuronium bromide. Ventilation was accomplished by an air/oxygen mixture. In the open operations, heparin 300 U/kg was administered before CBP. Cardiopulmonary bypass was performed with a roller De Bakey pump, nonpulsatile perfusion and a disposable bubble oxygenator (Rygg-Kyvsgaard), with a built-in heat exchanger. The pump was primed with electrolyte solution and complete hemodilution was applied. No blood was used during the operation. None of the patients received preoperative medication and no antibiotics were administered pre-or postoperatively to the patients or to the pump prime during this study. In control cases, closed heart surgery was performed.

The following assays were performed to examine each step in the process. Absolute PMNL count and their phagocytic functions were assessed. Total white blood cell counts were determined by a coulter counter and absolute values for PMNL counts were obtained from Wright-stained smears from the same sample and read by an observer who was unaware of the timing of the sample. The counts were corrected for hemodilution by means of the hemoglobin (Hb) concentration.

$$\text{corrected absolute PMNL counts} = \frac{\text{sample PMNL counts} \times \text{control Hb}}{\text{sample Hb}}$$

At least two samples were analyzed at each blood flow.

The following assays were performed to determine phagocytic functions of PMNL.

Blood collection: A total 15 ml of heparinized venous blood was collected from each patient prior to bypass, within the first three minutes on bypass, off bypass and three days post bypass; in control cases the collection occurred before and after the operation. Blood samples were placed in three groups of specially vacuumed tubes.

Preparation of bacteria: *Staphylococcus aureus* coagulase positive and catalase positive were used (*S. aureus* 502A). A stock culture of *S. aureus* 502A, stored at-70°C in trypticase soy broth with 20% glycerol was thawed and incubated for 18 hours at 37°C. The culture was centrifuged and washed with Hanks balanced salt solution and adjusted to $2\text{-}5\times10^8$ bacteria per millimeter.

Preparation of radioactive material: The radioactive substances, ^{14}C-uniform-glucose and ^{14}C-format mixture which have 3 mCi/ml radioactivity were specifically selected. We used 0.2 ml of this radioactive solution which had 1 mCi per millimeter.

Blood samples, bacteria and radioactive substances were placed in three groups of specially vacumed tubes as follows:

Material	Group I	Group II	Group III
Blood samples (leukocytes)	5 ml	5 ml	5 ml
Bacteria	0.5 ml	–	0.5 ml
Radioactive substance	0.2 ml	0.2 ml	0.2 ml

Tubes in Group I contained leukocytes of patients, bacteria, and a mixture of ^{14}C-format in which the leukocyte function was assessed. The tubes in Group II contained only leukocytes and radioctive substances which indicated whether or not the blood samples of the patient and the intravenous infusion liquids had been contaminated with bacteria. The tubes in Group III were primarily frozen at −20°C and later thawed at room temperature. Thus, the leukocytes in the blood sample were broken down. This group of tubes indicated whether or not the bacteria were alive. Tubes in Groups II and III were control tubes of Group I and of the method which was used. The tubes in Groups I, II and III were placed in a special mixture-heater machine whose temperature was stable at 37°C. Thus, optimal conditions were obtained so that the bacteria could easily reproduce. The $^{14}CO_2$ level which is produced as a result of the bacteria reproducing was counted with a special "Bacted" machine. It has been established that the volume counted is satisfactory within a 12-hour period. The $^{14}CO_2$ volume which is obtained in the medium is assessed as vol/min. It has been demonstrated that bacteria in the

medium is unable to reproduce in cases where the $^{14}CO_2$ volume is low. This shows that the leukocyte phagocytosis is effective. In cases where the $^{14}CO_2$ volume is high, the bacteria in the medium is found to multiply rapidly and leukocyte phagocytosis is inhibited.

Results

The effect of CPB on the absolute PMNL count is presented in Fig 1 a. Initially, the absolute PMNL count was 3.81 ± 0.43 /mm^3, and had decreased on bypass and off bypass periods; it exceeded the baseline level significantly on the third post bypass day ($p < 0.05$). The values in closed heart surgery did not show any significance ($p > 0.05$; Fig. 1 b).

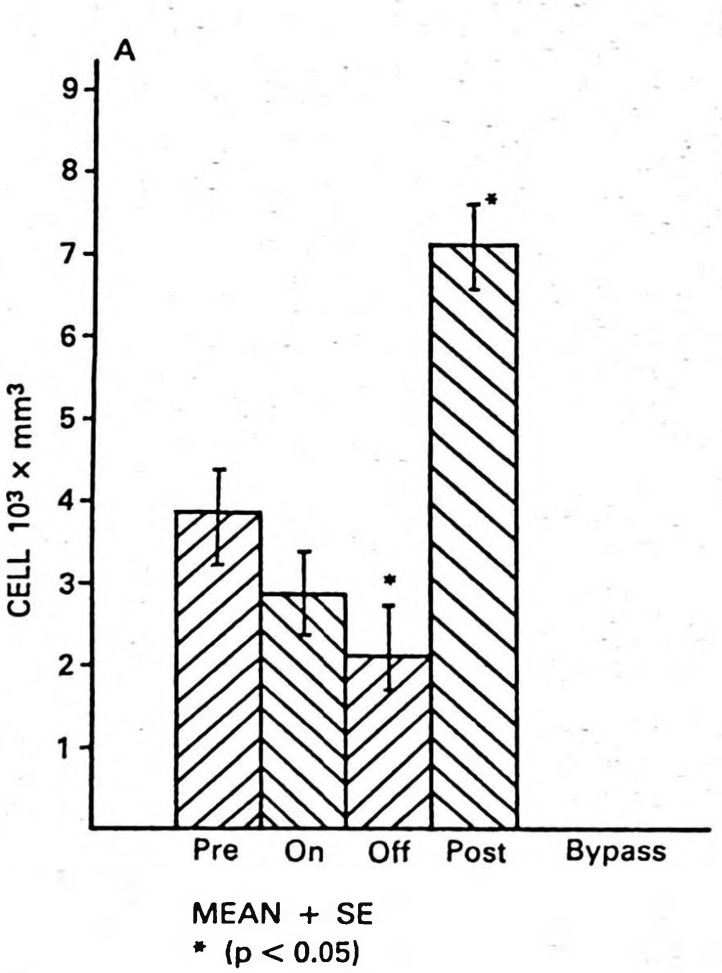

Fig. 1 a: Effects of CPB on absolute PMNL count; it dropped in the on and off bypass periods, and then increased approximately two-fold in the post bypass period.

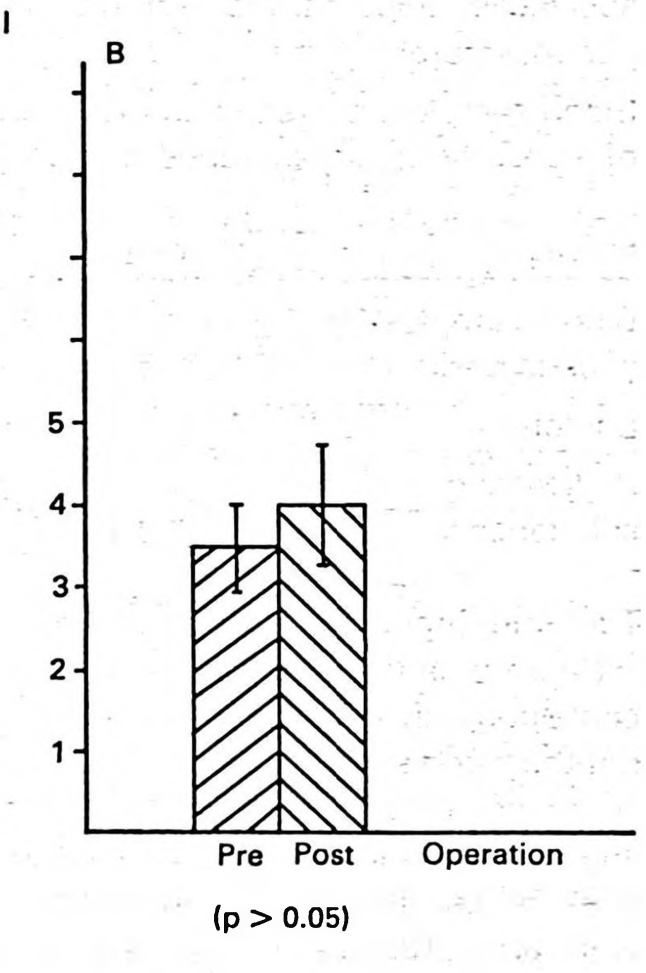

Fig. 1 b: Effects of closed operations on absolute PMNL count.

In the open operations the phagocytic function of PMNL is shown in Table I. The tubes in Group I show the $^{14}CO_2$ volume indicating phagocytosis of PMNL to be normal before the operation. However, the phagocytic function began to alter on

TABLE I: $^{14}CO_2$ Values (cc/min) in Patients Undergoing Open-Heart Surgery (n: 30)

	Pre-Bypass			On Bypass			Off Bypass			3 days Post-Bypass		
Group	I	II	III	I	II	III	I	II	III	I	II	III
Mean±SD	0	0	850±20.4	8.4±2.3	0	780±18.2	254±22.8	0	625±28.4	7.8±0.6	0	930±31.8

$p < 0.05$ $p < 0.05$

bypass. Immediately off bypass the same activity was significantly decreased as compared with the beginning on bypass ($p < 0.05$). The low $^{14}CO_2$ volume in Group tubes I on the third post bypass day showed that the growth of bacteria was inhibited because of active phagocytosis. The $^{14}CO_2$ volume in Group tubes II had been zero before surgery, at the start on bypass, off bypass and in the post bypass period. This result demonstrated that the patient's blood and the perfusion system used in the operation had not been contaminated with bacteria. Due to the fact that the results obtained in Group II tubes yielded a value of zero with regard to $^{14}CO_2$ for each period, no statistical comparison was made. We obtained a rather higher volume of $^{14}CO_2$ in the third group of tubes during the four periods of study (pre-bypass, on bypass, off bypass and post bypass). Indicating that the bacteria were alive and that they could easily multiply. This leads to the conclusion that no technical fault was found in the method used. For this reason, we felt that there was no need for a statistical study involving Group III which constituted the control tubes of the perfusion system as well as of the method.

In control cases who underwent closed heart surgery, the phagocytic function of PMNL is shown in Table II. The tubes in Group I show the volume of $^{14}CO_2$ which indicates the PMNL phagocytosis to be at a normal level in the pre-and postoperative periods.

TABLE II: $^{14}CO_2$ Values (cc/min) in Patients Undergoing Closed Heart Surgery (n: 20)

	Preoperative			Postoperative		
Groups	I	II	III	I	II	III
Mean±SD	0	0	680±4.81	7.3±1.21	0	6.20±5.24

As a result of this study it has established that the absolute PMNL count as well as the phagocytic functions were significantly decreased in off bypass adolescents who had undergone CPB as compared to the cases who had undergone closed heart surgery. This decrease in PMNL phagocytic causes adolescents to be susceptible to infection after open heart surgery. Indeed, of the thirty adolescents, fourteen had various types of infections in the postoperative period. The majority of the cases (nine) had genitourinary tract infections but there were also three pulmonary and two wound infections. Another point of interest found in this study was that in the cases in which a clinical infection picture was seen after surgery, there was a longer duration on bypass and a higher inhibition of leukocytic phagocytosis. The correlation between the duration on bypass and the $^{14}CO_2$ volume related to the inhibition of leukocyte phagocytosis could be shown as a trend (Fig. 2).

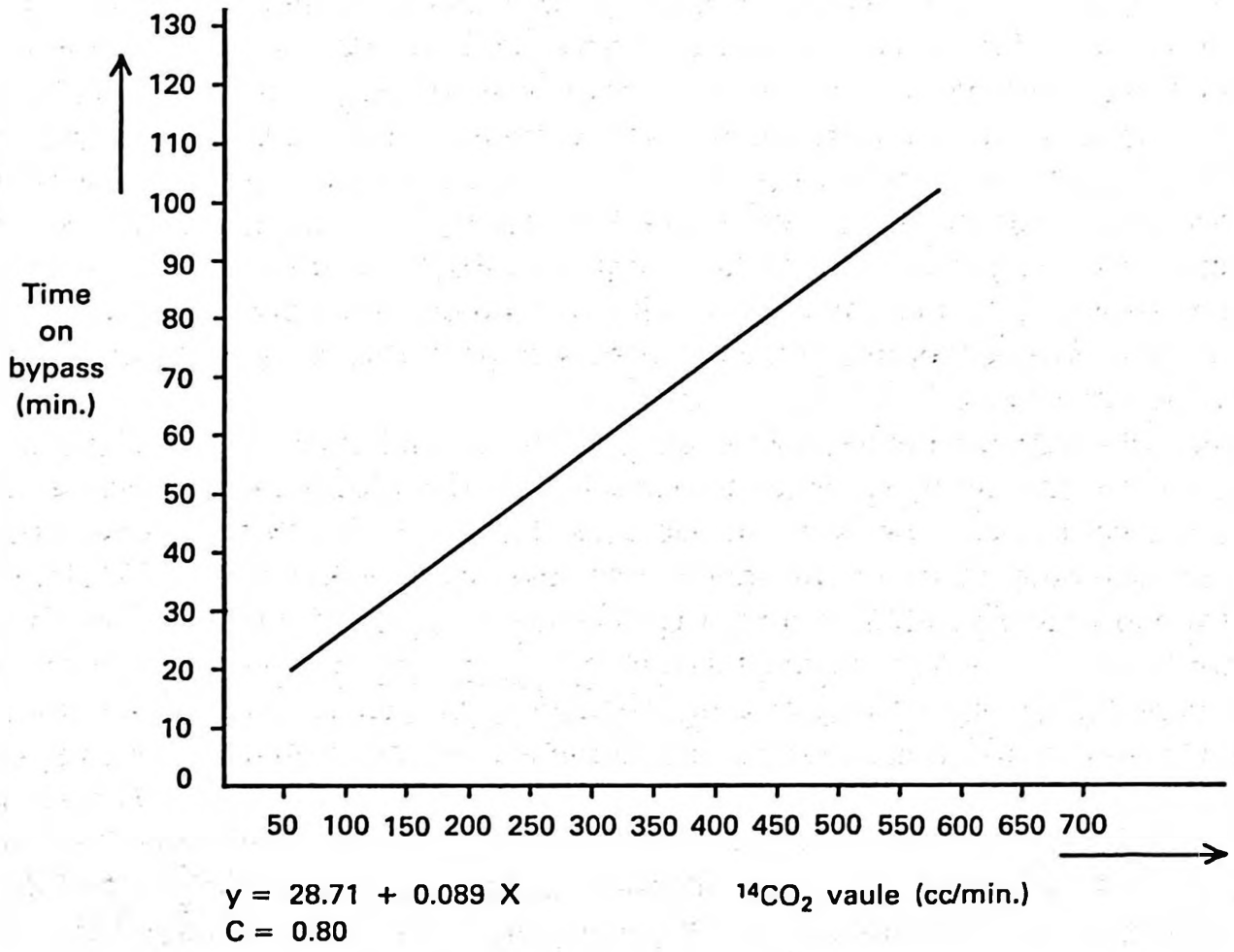

Fig. 2: Correlation between the duration of bypass and $^{14}CO_2$ values with relation to the inhibition of leukocytic phagocytosis.

Discussion

The phagocytic function of leukocytes has an important effect on the defence mechanism of the body[1]. There have been contradictory opinions related to the function of leukocytes during operative procedures involving cardiopulmonary bypass[7-9]. However, in these studies various methods have been used in evaluating the function of leukocytes[7-9]. In this study we have assessed the phagocytic function of leukocytes by using a radioactive method which differs from other studies in this field[11]. In the method we used it is unnecessary to obtain the leukocytes from the blood medium[12]. Furthermore, only a 12-hour period is sufficient for the growth of bacteria whereas 72-96 hours are required in some other methods[3]. It is also possible to complete the experiment by measuring the $^{14}CO_2$ volumes every hour without cancellation of the medium. Thus, this method can easily and simply be applied in this field. In our study it has been shown that the phagocytic function of leukocytes were normal at the beginning of the operation, then, with the start of perfusion, gradually diminished.

Although, the phagocytic functions of leukocytes decreased significantly off bypass, these functions returned to normal in three days. However, similar studies in adults have shown contradictory results[6-9,11-17]. For instance, Lunström et al[13], have reported a decrease in phagocytosis which lasted up to 8 days after CPB, although no information was reported regarding the anesthesia or CPB techniques. In contrast Kaplan et al[8], and Ros et al[14] in their studies have reported no change in phagocytosis in adults undergoing CPB. In our opinion, their findings can be explained by the transfusion of fresh blood elements during perfusion. In our system, we neither used blood elements in the perfusion system nor we did transfuse any blood.

In another study, Silva et al[9], who evaluated PMNL in adults following CPB using nitroblue tetrazolium dye reduction, found that the dye reduction was decreased. This finding implies a decrease in bactericidal capacity, which agrees with our results. The other studies performed in this field by Bubenik et al[15] and Mayer et al[16] found a change attributed to a CPB-induced defect of the PMNL in adult patients with decreased phagocytosis. However, our method differs from those mentioned above in that we could not see a defect in our patient. In their study, Van Oeveren et al[17], measured the opsonic activity of serum after CPB with an oil red uptake assay, a method not entirely comparable to our technique. Consequently, they found that there was a decrease in phagocytosis, which agrees with our study. A recent study involving infants has demonstrated a decrease in PMNL functions, which are immature at birth[6]. In our study, as compared to others, no antibiotics were added to the perfusion system nor were they given to the patient prior to surgery. This has proven to be assuring with regard to our findings. We have demonstrated that the influence of surgery involving cardiopulmonary bypass has an adverse effect on polymorphonuclear leukocyte phagocytosis. Another point of interest is that in the cases in which postoperative infections occurred there was a longer duration on bypass and a higher inhibition of leukocytic phogocytosis. This decrease in the PMNL phagocytic functions might be due to the decrease in the absolute PMNL count when on and off bypass and the deleterious effect of CPB in which the blood components are subjected to mechanical trauma as well as the drugs used in the perfusion system.

Summary

This study was performed to determine the absolute PMNL count as well as phagocytic functions in adolescents who had undergone CPB; a radioactive method was used for the first time in this field. Although CPB causes a decrease in the absolute PMNL count when the subject is off bypass, this value exceeded the baseline level within three days. A day prior to surgery PMNL phagocytosis was found to be normal and was unaffected within the first minutes on bypass. Whereas, PMNL phagocytosis decreased significantly.off bypass. However, the

decrease was transient and returned to normal within three days. Another interesting finding obtained as a result of this study was that in the cases in whom the clinical infection picture was seen, the patient had a longer duration on bypass and a higher inhibition of leukocyte phagocytosis. The correlation between the duration on bypass and inhibition of leukocyte phagocytosis could be shown as a trend. Thus it may be concluded that the longer the on bypass period the higher the inhibition of leukocytic phagocytosis and the higher the infection rate in the postoperative period.

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