SUCCINYLDICHOLINE ESTERASE ACTIVITY IN STORED CITRATED BLOOD

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A recent review[1] of our experience with postanesthetic apnea and respiratory insufficiency suggested that among the many etiologic factors which may contribute to this complication, one was related to multiple blood transfusions. Postoperative apnea seemed to occur frequently in those patients who had received both succinyldicholine and large quantities of citrated blood to replace blood lost following acute trauma or during extensive vascular surgery. The mechanism postulated to explain this relationship was based on changes in the cholinesterase activity of transfused blood. If the enzyme responsible for succinylcholine hydrolysis decreased in the plasma of stored blood, then failure of detoxification and prolonged apnea could result from dilution or washout of the patient's cholinesterase by large quantities of transfused blood.

Changes in plasma cholinesterase activity (nonspecific cholinesterase, pseudocholinesterase) have been studied in a wide variety of normal and pathologic circumstances.[2] However, no information was available as to changes in plasma cholinesterase or in the specific enzyme, succinyldicholine esterase,* during storage of blood. This study was designed to measure changes in succinyldicholine esterase activity in whole blood during storage as citrated blood and to test the hypothesis that one cause of prolonged apnea after succinylcholine was the dilution of the hydrolyzing enzyme by multiple transfusions.

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* The term "succinyldicholine esterase" has been used for convenience throughout this paper to distinguish between enzyme activity measured with acetylcholine as the substrate ("cholinesterase") and activity measured with succinyldicholine as the substrate ("succinyldicholine esterase"). The term is used in the same sense as "procaine esterase," without implying that the enzyme has been isolated or that its specificity for succinyldicholine has been established.

Method

Under aseptic conditions 100 ml. samples of blood were collected from 17 male patients who were without systemic disease and were convalescing from minor surgical procedures. Samples were collected in plastic bags containing 25 ml. of A C D Solution "B." Each 100 ml. of A C D Solution "B" contained sodium citrate 1.32 Gm., citric acid 0.44 Gm., and dextrose 1.47 Gm. The samples were stored at 6 C. under blood bank conditions for the duration of the study. On the day of collection and at 3 to 5-day intervals of storage up to 25 days, 10-ml. aliquots were withdrawn and the plasma succinyldicholine esterase activity determined. Not all 17 blood samples were studied at each interval of storage.

The Warburg manometric technique described by Tsuji, Foldes and Rhodes[3] was modified and used to determine succinyldicholine esterase activity. Ringer's bicarbonate solution (100 ml. 0.90 per cent NaCl, 30 ml. 1.26 per cent $NaHCO_3$ and 2 ml. 1.26 per cent $MgCl_2.6H_2O$) was used as the buffer after equilibration with 5 per cent CO_2-95 per cent nitrogen. Undiluted plasma (0.5 ml.) obtained from the whole blood aliquot and 1.3 ml. of Ringer's solution were placed in the main chamber. Succinyldicholine chloride (0.2 ml. containing 50 μg. in Ringer's solution) was placed in the side arm. Hydrolysis was carried out in a 5 per cent CO_2-95 per cent nitrogen gas phase at 37 C. After mixing for 10 minutes and equilibration, the CO_2 produced during the next hour was measured at 10-minute intervals. All determinations were carried out in duplicate and with each determination two control manometers were used. In one manometer, substrate and in the other, plasma was omitted and an equal volume of buffer was added. The CO_2 of the control manometers was subtracted from that produced in the manometer containing all three components to give succinyldicholine esterase

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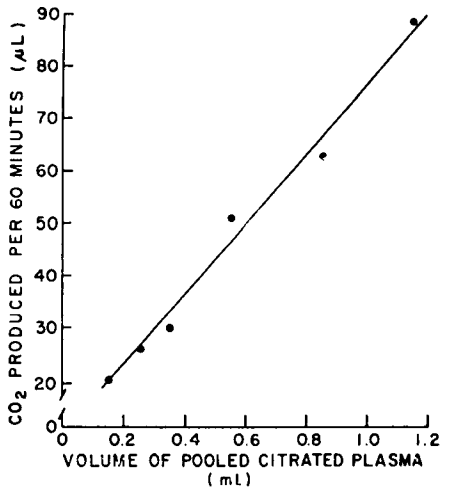

FIG. 1. Relationship between enzyme concentration (ml. plasma) and CO_2 production per hour by Warburg technique using 50 μg. of succinyldicholine chloride as substrate in Ringer's solution. Total volume was 2.0 ml.

activity as μl. CO_2 per hour per milliliter of plasma.

The sensitivity of this technique was determined by measuring CO_2 production when varying volumes of plasma (*i.e.*, enzyme) were added to a constant amount of substrate diluted to the same final volume with buffer. These data indicate a linear relationship between CO_2 production and enzyme concentration up to 1.15 ml. of plasma and 90 μl. CO_2/hour (fig. 1). The slope of this relationship ($y = 74x + 5$) is almost identical to that calculated from the data of Tsuji, Foldes and Rhodes[3] ($y = 77x - 1$) who used a similar technique.

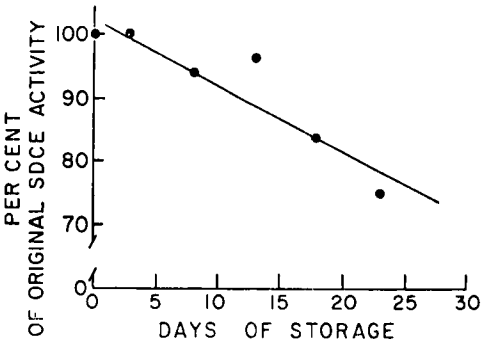

FIG. 2. Decrease in plasma succinyldicholine esterase activity with time of storage as citrated blood at 6 C.

RESULTS

The mean values of the 65 duplicate determinations on the 17 original blood samples after varying intervals of storage are presented in table 1 and plotted in figure 2. Because of the large variation between individuals in the initial absolute values for succinyldicholine esterase activity (mean ± S.E. 103 ± 6.2, range 69–158 μl. CO_2/hour/ml.), all values have been expressed as percentage of activity present in the fresh blood. The relationship between the days of storage and the decrease in enzyme activity is statistically significant if a linear relationship is assumed ($y = 103 - 1.1x$, $P < 0.01$).[4] From the equation one could estimate that the succinyldicholine esterase activity would disappear in 103 days. It is highly unlikely however that the relationship would remain linear. In any case, the loss of activity is small. At the end of 21 days of storage, more than 80 per cent of the original activity remained.

TABLE 1

SUCCINYLDICHOLINE ESTERASE (SDCE) ACTIVITY IN STORED CITRATED BLOOD AFTER VARYING PERIODS OF STORAGE UNDER BLOOD BANK CONDITIONS

Days of Storage	Number of Samples	SDCE Activity as Per Cent of Fresh Blood Activity ± Standard Error of Mean
0	17	100 ± 0
1–5	4	100 ± 0.3
6–10	11	94.0 ± 3.5
11–15	16	96.5 ± 4.0
16–20	6	83.8 ± 6.4
21–25	11	75.0 ± 4.1

TABLE 2

PLASMA SUCCINYLDICHOLINE ESTERASE (SDCE) ACTIVITY IN μL. CO_2/HOUR/ML. FOLLOWING MULTIPLE TRANSFUSIONS OF CITRATED STORED BLOOD IN SIX PATIENTS

Patient	Pre-operative SDCE	Post-operative SDCE	Blood Received (ml.)
1	80	99.9	6,000
2	126	124.0	5,500
3	102	90.8	3,000
4		86.0	3,500
5		97.3	7,500
6		76.8	11,000

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To confirm these results, blood was sampled from 6 patients who had received multiple, rapid transfusions of citrated banked blood and their succinyldicholine esterase activity was determined. In 3 patients it was possible to obtain blood both preoperatively and immediately postoperatively. These data are presented in table 2. There was little difference in the succinyldicholine esterase activity following transfusion of moderate amounts of citrated blood. In patients whose blood was sampled postoperatively only, the mean value (86.7 μl. CO_2/hour/ml.) approximated our normal values for fresh plasma (103 $\pm$ 6.2 μl. CO_2/hour/ml.). Our normal values are essentially the same as that reported by Tsuji, Foldes and Rhodes (120 μl. CO_2/hour/ml.).

Discussion

Although succinyldicholine esterase activity does decrease in stored banked blood, the change is too small to be responsible for failure of succinylcholine hydrolysis and prolonged apnea following massive transfusion. However, several other reasonable hypotheses could explain the relationship between multiple transfusions and respiratory insufficiency in the presence of normal enzyme concentrations. For example, the citrate of banked blood may deplete ions such as calcium or magnesium which may be necessary for enzymatic hydrolysis. A relationship between calcium ion and succinylcholine apnea has already been suggested.[5] Possibly metabolism of infused citrate may lead to accumulation of succinate which would prevent or retard the enzymatic reaction toward succinic acid, even though *in vitro* inhibition by succinate is slight.[3] It is also possible that the metabolic acidosis accompanying the rapid infusion of banked blood may inhibit hydrolysis. Finally, a prolonged action of succinylcholine could be related to the inadequate tissue circulation associated with circumstances requiring multiple rapid transfusions. Continuing studies in this laboratory are investigating these possible mechanisms.

Summary

The plasma succinyldicholine esterase activity of 17 healthy subjects was determined as fresh citrated blood and after intervals of storage up to 25 days. Enzyme activity decreased with time of storage, but 80 per cent of the original activity remained at the end of three weeks. It is unlikely that washout of succinyldicholine esterase by banked citrated blood is a cause of prolonged apnea following the use of succinylcholine together with multiple blood transfusions.

Crystalline succinyldicholine chloride was supplied by Burroughs Wellcome & Co., Inc., Tuckahoe, New York.

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