TABLE V. Effect of 2:4-Dinitrophenol and Fluorocompounds on Mitochondrial Respiration and Citrate Accumulation.

Additions to system	Q_{O_2} (N)	Citrate, μMole/mg N
None	94	.07
DNP	120	.08
FAc	75	1.35
" + DNP	70	.92
FC	39	3.35
" + DNP	26	2.51
No. experiments	4	

Conditions and substrate concentrations were same as in Table II. DNP was .1 μMole/flask.

ported by them was sufficient to produce complete inhibition of fluoroacetate conversion to fluorocitrate. In our system we were unable to show a reactivation of respiration when DNP and fluoroacetate were incubated together, even at higher DNP concentrations.

Summary. In presence of fumarate, rat liver mitochondria responds with citrate accumulation to fluoroacetate poisoning. This response is not sex-dependent. There is a time lag to the inhibitory effect of fluorocompounds on respiration. Ratios of respiration of fluoroacetate and control system of starved and fed animals were in close agreement. Testosterone did not abolish the effect of fluoroacetate on citrate accumulation, whereas progesterone significantly reversed it only on liver mitochondria of male rats. Progesterone, like DNP, when in combination with fluoroacetate, inhibited respiration. In normal nutritional states irradiation does not significantly increase citrate accumulation in the liver mitochondria of male rats.

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Buffy Coat Cell Migration from Symmetrical Explants in Presence and Absence of Clotted Plasma.* (25537)

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Difficulties most commonly encountered in work concerned with buffy coat cell migration are (a) it is very difficult, if not impossible, to obtain symmetrical and reproducible explants using conventional technics, (b) migration is slow when cells are imbedded in clotted plasma. Our purpose was to develop a simple and reproducible procedure to obviate these difficulties.

The method consists essentially of filling small bore polyethylene tubing with material to be studied and then cutting it into small pieces handled as if they were tissue explants. Intradermic polyethylene tubing formulation PH-F-Adams PE-50† was sterilized by filling

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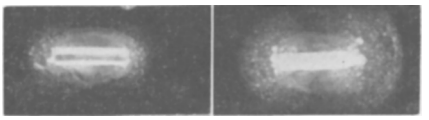
† Sterile tubing obtained from Clay Adams.

it with 1:1000 zephiran chloride. After 18 hrs, tubing was washed with several changes of sterile distilled water, cut into 6 inch long pieces then placed in test tubes and dried in incubator at 37°C. Glass chambers were sealed together with petrolatum-paraffin-wax mixture,‡ and sterilized under ultraviolet germicidal tube. Ten ml of heparinized§ blood were placed in siliconed tubes, kept for 2 hrs at room temperature, then centrifuged 10 min at 1500 rpm with radius of 20 cm. Supernatant plasma was discarded, buffy coat harvested, transferred to hematocrit tubes (made from pyrex glass tubing of 6 mm internal diameter) and centrifuged as before. Supernatant plasma was pipetted off and buffy coat transferred to another tube and mixed gently. Tip of plastic tube was dipped into buffy coat cell suspension and filled by slow gentle suction applied with automatic 2 ml syringe through 23 gauge needle. One end of tube was softened with small flame and sealed by crimping tightly with fingers. Plastic tubes were then inserted individually into 6″ long, 16 gauge metal tubes (like those used for filling Winthrop hematocrit) and again centrifuged 10 minutes at 3000 rpm to pack the cells. The sealed tubes were sterilized by immersion in 95% alcohol and then left at room temperature until alcohol evaporated. Tubes were cut aseptically into small pieces about 3.6 mm long containing approximately 1 mm^3 of packed cells. The small pieces were moistened with chick embryo extract and placed in chambers, as if they were tissue explants, in tiny drop of chicken plasma which clotted rapidly and fixed them to the glass. Sealed chamber was then filled with Hanks serum (7 parts Hanks and 3 parts pooled guinea pig serum) or a semisolid Hanks-serum agar medium (HSAM), with 0.5% agar, using syringe and needle. Needle was introduced through one of 2 perforations made with inoculating wire and sealed subsequently. When cell-plasma clot explants were desired, cell-plasma-embryo extract mixture was drawn rapidly into plastic tubing, allowed to clot, sliced and

‡ Petrolatum 0.5 part, paraffin 1 part, wax 3 parts by weight.

§ 5 USP units/ml blood.



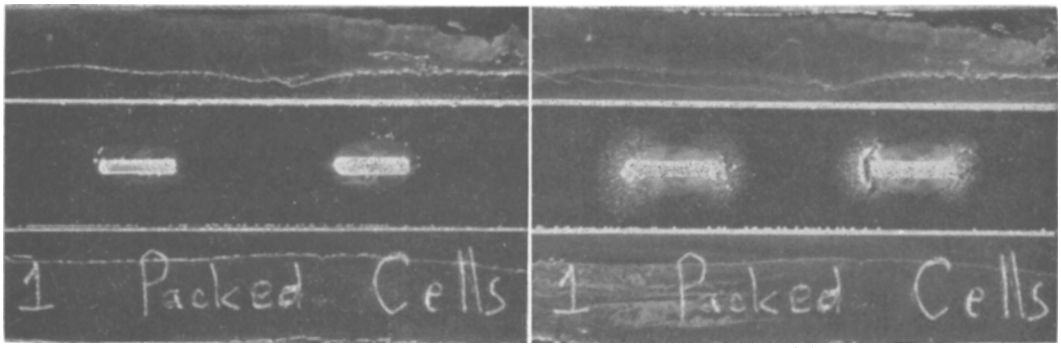
(A) (B)

FIG. 1. Differences in cell migration from buffy coat explants (A) with, and (B) without plasma clot.

explanted to a chamber as described above. Chambers were incubated at 37°C and examined at regular intervals to 18 hrs for cell migration at both ends of tube (Fig. 1-A, B). The cross sectional area of tube, not its length, is the critical factor in migration.

Chambers are made with 3 standard (3 x 1″) microscope slides as follows: One is split lengthwise and strips held ⅜″ apart with spring clips attached to flat-ground metal plate. A second slide is centered on top of strips and 2 drops of melted wax-paraffin-vaseline mixture applied to each side. Plate is heated until mixture flows evenly by capillarity after which plate is cooled and chamber removed. Two plugs about ¼″ long are gradually built-up, in trough over ends of chamber, with melted drops of a paraffin-vaseline (1:2) mixture until raised above level of depression. After solidification, excess is cut flush with razor. Chamber is now ready for washing, drying and ultraviolet light sterilization. Following insertion of explants, a third, sterile slide is placed on top and sealed with wax-paraffin-vaseline mixture.

Results. 1. Cell migration is obtained usually at both ends of tube with both packed cells and cells incorporated into a plasma clot. This enables observer to take 2 readings from a single explant, one at each end. 2. There are individual differences in cell migration with material from apparently normal persons at end of 18 hrs but these were minimized when packed cells in absence of plasma clot were used. 3. Packed cells migrated faster and for longer distances in Hanks serum than did those from plasma clot explants, or than cells incorporated into HSAM (Fig. 2-A, B). 4. Cells remain active for significantly longer time and their phase image shows more contrast when suspended in HSAM than when suspended in plain liquid Hanks-serum medium.



(A) (B)

FIG. 2. Polyethylene tubes with packed cells, without plasma clot inside closed chamber containing Hanks-serum medium (A) before, and (B) after 18 hr incubation at 37°C. Notice migration at both ends.

Migration studies were carried out with buffy coats from 25 blood bank donors with both packed cells and cells incorporated into plasma clot. Three similar explants were used in each instance and readings made, measuring distance from end of tube to advancing front of migrating cells. In Table I, each value represents average of 6 readings. Additional determinations were made in 75 blood bank donors using packed cells without plasma with mean migration of 3.73 mm after 18 hrs. Individual differences in migration are minimized when packed cells without clotted plasma are used. There is a statistical difference in cell migration from tubes with packed cells and tubes containing cells embedded in plasma clot as evidenced by lack of overlapping of corresponding ranges.

TABLE I. Human Buffy Coat Cell Migration after 18 Hr Incubation Using Polyethylene Tube (PE) Technic in Presence and Absence of Plasma Clot.

Cells suspended in chicken plasma clot in PE tube	Cells packed in PE tube by centrifugation in absence of plasma clot	Cells suspended in chicken plasma clot in PE tube	Cells packed in PE tube by centrifugation in absence of plasma clot
(migration in mm)		(migration in mm)	
2.75	3.30	3.25	3.79
2.82	3.27	2.63	4.05
1.80	3.84	1.20	3.86
3.35	3.79	1.92	4.05
1.89	3.39	2.25	3.65
1.59	3.15	2.90	3.68
1.29	3.05	1.76	3.30
1.50	3.84	2.10	3.28
1.71	3.30	1.50	3.56
1.05	4.95	1.05	3.33
2.70	4.20	1.85	3.75
2.78	3.35	2.25	4.05
1.83	5.10		
Mean ± S.D. (mm)		2.07 ±.67	3.72 ±.51
Range		1.05-2.90	3.05-5.10

Cell migration from bone marrow can be studied by this method. Bone marrow can be easily aspirated into the tube, or marrow is placed into small syringe and forced into tubing through Clay-Adams polyethylene tubing syringe adapter of suitable bore. Tubes are centrifuged to pack material and then handled as described above for buffy coat.

Summary. 1. A simple and easily reproducible technic for study of cell migration from explanted buffy coat and bone marrow is described. 2. There are individual differences in cell migration with buffy coat from normal persons at the end of 18 hrs, but these are minimized when packed cells, in absence of plasma clot, are used.

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