(decline of absorption at 710 to 715 mμ and increase at 730 mμ), as well as the bleaching at 475 mμ noted by Duysens, remain to be interpreted. Several reversible changes of chlorophyll may occur at once in the cell, for example, the formation of metastable triplet molecules may be superimposed on that of the semiquinone. It will be noted, however, that the effect observed at 475 mμ is opposite in sign to that expected from the formation of metastable chlorophyll-*a* (*20*).

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Clot-Retraction Promoting Factor (Retractin) in Platelets and Tissues

Transfusion of platelet-rich blood or plasma in thrombocytopenic patients is followed by temporary elevation of the number of circulating thrombocytes and by temporary correction of the increased capillary fragility, the prolonged bleeding time, and the poor utilization of prothrombin during clotting that are typical of these patients (*1*). Isolated or preserved platelets fail to survive in the patient's circulation (they are, thus, nonviable), but their administration is equally followed by improvement in prothrombin consumption. This observation led to the isolation of the platelet thromboplastic factor (*2*).

It was observed in the course of unpublished studies that clot retraction also could be favorably influenced by administration of nonviable, preserved platelets. This finding prompted the present investigation (*3*) in which we discuss preliminary evidence for a platelet constituent that is responsible for clot retraction. This factor is referred to as "retractin."

The factor that promotes clot retraction was obtained from either lyophilized or fresh human or bovine platelets. Platelets were collected and separated by the multiple centrifugation technique previously described (*4*). Only preparations were used that contained no white or red cells by microscopic examination. Platelets were washed twice with saline solution at 4°C and packed by final centrifugation at 3500 rev/min for 30 min. Some aliquots were lyophilized by a standard technique, and other aliquots were used fresh.

Three different extraction techniques were used for the separation of retractin: (i) water-acetone extraction procedure; (ii) water-ethyl ether extraction procedure; and (iii) ethyl ether cold precipitation procedure.

1) To 6 mg of lyophilized platelets or to fresh platelets from 50 ml of fresh blood were added 2.5 ml of distilled water. After 24 hr at −20°C, the preparation was brought back to 4°C. Precooled acetone, 7.5 ml in volume, was then added. After it had been shaken for 5 minutes, the mixture was stored at −20°C for 12 hours; it was then centrifuged at 3500 rev/min for 30 min at 4°C to separate all particulate matter. Microscopic examination of the supernatant acetone for platelets, platelet fragments, and ghosts was negative. A 0.5 ml volume of supernatant acetone was then dried at room temperature under 29-in. vacuum aspiration. The dry material was suspended in 0.5 ml of saline solution.

2) To packed, fresh, washed platelets from 50 ml of fresh blood, 3 ml of precooled distilled water and 5 ml of ethyl ether were added. After centrifugation at 3500 rev/min for 30 min at 4°C, three well-differentiated layers were present: (i) an upper layer containing ethyl ether; (ii) a middle layer containing a precipitate, probably of protein material and stroma, and (iii) a bottom layer containing water. The ether layer was aspirated. Aliquots of 0.5 ml were dried at room temperature under 29-in. vacuum, and the dry material was resuspended in 0.5 ml of saline solution.

3) It was observed that storage at −20°C of ethyl ether containing platelets from 50 ml of fresh blood would be followed by formation of a precipitate, which would promptly redissolve at room temperature. The cold precipitate was washed with ethyl ether twice at −20°C; it was finally dissolved in 5 ml of ethyl ether. Aliquots of 0.5-ml were dried and then resuspended in 0.5 ml of saline at room temperature.

Retractin was assayed by the two following experiments. (i) Native platelet-poor human plasma was prepared as previously described (*4*). One milliliter of plasma was then added to 0.5 ml of saline suspension of the acetone or ethyl ether extracts in chemically clean glass test tubes. After mixing of the contents, the tubes were incubated in a water bath at 37°C, and the contents were allowed to clot; retraction was observed at various intervals of time. It occurred within 15 to 40 min in most samples that contained retracin (Fig. 1). (ii) A solution containing 300 mg percent of bovine commercial fibrinogen in saline solution and a solution containing 100 N.I.H. units of bovine thrombin per milliliter were first prepared. In glass test tubes incubated in a water bath at 37°C, 0.5 ml of saline suspension of acetone or ethyl ether extracts, 0.9 ml of fibrinogen solution, and 0.1 ml of thrombin solution were introduced in rapid succession. Clot retraction occurred after approximately 20 min in the samples that contained retractin. In addition to visual inspection, the activity of retractin preparation was evaluated with a semiquantitative technique. This consisted in the measurement of the volume of serum or saline solution expressed by the spontaneous retraction of the clot after 1 hr of incubation at 37°C. The ratio

$$\frac{\text{Volume of serum or saline expressed}}{\text{Total volume of mixture}} \times 100$$

was taken as an index of clot retraction (Table 1).

Clot retraction in test tubes containing native platelet-free plasma was absent or minimal; it never exceeded 10 to 12 percent in test tubes containing fibrinogen solution.

The two ethyl ether preparations were found to be free of thromboplastic factor. On the other hand, the acetone

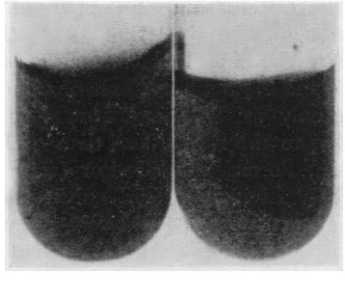

Fig. 1. Clot-retraction promoting effect of an acetone extract of human platelets (retractin) when added to native platelet-poor human plasma: (left) control tube containing 0.5 ml of saline solution; (right) tube containing 0.5 ml of acetone extract of platelets suspended in saline. Photograph was taken 2 hr after incubation of the test tubes in a water bath at 37°C.

Table 1. Clot-promoting effect of various extracts of human platelets on bovine fibrinogen solution clotted by thrombin. Tests were read 1 hr after completion of clotting. Figures are average of several (20) experiments. Clot retraction was measured as the percentage volume of saline obtained from a volume of 1 ml of fibrinogen solution, times 100. Similar results were obtained when native platelet-free plasma was used as substrate.

Extract	Clot retraction
Control (saline)	10.2 ± 1.5
Acetone—fresh platelets	35.7 ± 5.6
Acetone—lyophilized platelets	40.1 ± 4.3
Chloroform	34.8 ± 2.9
Ethyl ether—direct extraction	24.6 ± 6.1
Ethyl ether—precipitation	36.9 ± 3.7
Benzene	12.4 ± 0.8
Ethyl alcohol	11.8 ± 1.4
Water	9.7 ± 1.5

preparation contained some thromboplastic factor activity as assayed by our technique (*2*), although the greater activity remained in the acetone-insoluble residue. Thromboplastic factor in the acetone extract, however, could be destroyed by heating at 56°C for 2 hr and then storing at – 20°C for 48 hr. Retractin itself appeared quite stable at 56°C and, at – 20°C, kept its activity indefinitely.

Other solvents were used for the preparation of retractin from lyophilized platelets. Water, alcohol, and benzene extracts failed to show any retractin activity. Chloroform extracts were of comparable potency to acetone extracts. In addition, retractin could be obtained from tissues other than platelets. Brain supplied a potent preparation; liver and spleen a less active one; erythrocytes and platelet-free plasma failed to yield retractin.

It is generally accepted that intact platelets rather than a constituent of the platelets are needed for normal clot retraction. Glanzman (*5*) and Fonio (*6*), however, have postulated that clot retraction may be the result of a specific platelet factor. More recently, Fenichel and Seegers (*7*) and Ballerini (*8*) have reported the clot-retraction promoting effect of another possible platelet constituent, 5-hydroxytryptamine creatinine sulfate, a finding that we have been unable to confirm (*9*). Our experiments, then, seem to represent the first demonstration that platelets contain a factor promoting retraction of plasma and fibrin clots and entirely distinguishable from other platelet constituents. This factor may be found in other tissues as well and does not seem to require optimal amounts of calcium or the presence of a "serum factor" for its activity, for it will operate in a system that contains only purified thrombin and purified fibrinogen. Studies for the chemical identification of this lipid substance are in progress. *Note added in proof*: Preliminary experiments indicate that its physical, as well as chemical, properties are responsible for the activity of retractin.

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Inhibition of Aerobic Phosphorylation and Pasteur Reaction by an Adrenal-Cortical Extract

A commercial adrenocortical extract, Lipo-Adrenal Cortex (Upjohn), has been reported to possess hormonal potency, *in vivo* and *in vitro*, that is not manifested by pure adrenal corticosteroids (*1*). One of these properties is lymphocytolytic activity *in vitro*. Attempts to correlate this lymphocytolytic activity with alterations in some specific enzymatic system have led to the observation that Lipo-Adrenal Cortex markedly stimulates the aerobic glycolysis of rat thymus lymphocytes and several other normal tissues (*2–4*). No stimulation of tumor glycolysis has been observed. The increase in aerobic glycolysis appears to be a true inhibition of the Pasteur reaction by a physiological preparation, for it is manifested at concentrations that have no significant influence on either respiration or anaerobic glycolysis. It has been demonstrated (*4*) that the effect is not attributable to known cortical steroids, oxygenated at the C_{14} position, that are present, for these are ineffective at the concentrations employed.

In the experiments originally reported, stimulation of glycolysis was found only with intact cells or tissue slices, and attempts to demonstrate it in homogenates were unsuccessful. It was therefore suggested (*4*) that Lipo-Adrenal Cortex may function by altering the permeability of the cell to glucose. Because permeability would not be a limiting factor in homogenized preparations, the material might fail to stimulate glycolysis in homogenates. However, an alternative possibility was that, for reasons that are obscure, the Pasteur effect could not be demonstrated with broken cell preparations of any kind. Recently, however, Meyerhof and Fiala (*5*) and Terner (*6*) reported that aerobic phosphorylation and the Pasteur effect can be demonstrated with a dried yeast preparation and with concentrated homogenates of guinea pig mammary glands and brain. Furthermore, their experiments reveal that inhibition of the Pasteur reaction by *p*-nitrophenol is accompanied by inhibition of aerobic phosphorylation.

We have therefore investigated the effect of Lipo-Adrenal Cortex on aerobic phophorylations and lactic acid formation by guinea pig brain homogenates in an effort to elucidate the mechanism of action of this preparation (*7*).

The procedure followed was essentially that described by Terner (*6*). Guinea pig brain was homogenized for 30 sec in a Potter-Elvehjem glass homogenizer in 2 volumes of isotonic KCl containing 0.024*M* $KHCO_3$ and 0.02*M* nicotinamide. The concentrated suspension was further diluted with 2 volumes of isotonic KCl. The reaction vessels contained 0.0075*M* $MgCl_2$, 0.02*M* nicotinamide, $2 \times 10^{-4}M$ diphosphopyridine nucleotide, 0.001*M* adenosine triphosphate (K salt), $10^{-5}M$ cytochrome *c*, 0.01*M* glucose, 0.005*M* hexose diphosphate (K salt), 0.00375*M* potassium phosphate buffer at *p*H 7.4, and 0.02*M* glycyl-glycine buffer at *p*H 7.4, all in isotonic KCl. The total volume was 4 ml, which included 1 ml of tissue homogenate that was tipped in from the side arm when the Warburg vessels were placed in the 37°C water bath. After a 10-minute equilibration period, readings were taken at 5-minute intervals for 20 minutes. The vessels were then iced, and protein was precipitated with either 1.5*N* perchloric acid or 10-percent trichloroacetic acid. Phosphorus was determined according to Lowry and Lopez (*8*), and lactic acid was determined by the method of Miller and Muntz (*9*) as modified by Barker and Summerson (*10*). All experiments were performed in duplicate, and all analyses were done on duplicate samples. Initial controls were precipitated at the start of the incubation period, and all experimental values were calculated by extrapolating to zero time. Initial phosphorus values were approximately 600 μg.

Dry weight was determined on a representative sample of the tissue homog-