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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References and Notes

1. M. Stefanini *et al.*, *Blood* 7, 53 (1952).
2. M. Stefanini and E. W. Campbell, *Rev. hématol.* 9, 576 (1954).
3. Communication No. XV of a series of "Studies on platelets." This work was supported by grant H-2132 from the National Institutes of Health, U.S. Public Health Service, and by a grant-in-aid from the American Heart Association.
4. M. Stefanini and W. Dameshek, *New Engl. J. Med.* 248, 797 (1953).
5. E. Glanzmann, *Ann. Paediat.* 88, 113 (1918).
6. A. Fonio, *Acta Haematol.* 8, 363 (1952).
7. R. L. Fenichel and W. H. Seegers, *Am. J. Physiol.* 181, 19 (1955).
8. G. Ballerini, *Atti accad. sci. Ferrara* 32, 1 (1954–55).
9. S. I. Magalini and M. Stefanini, *Proc. Soc. Exptl. Biol. Med.* 90, 615 (1955).

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6 January 1956

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-

Table 1. Effect of *p*-nitrophenol (pNP) on aerobic phosphorylation and lactic acid formation by guinea pig brain homogenates.

Concn.	Q_{O_2}	Q_P	$Q_{lactate}$
Expt. No. 1			
Control	18.1	− 4.0	6.8
$10^{-4}M$ pNP	20.2	4.0	8.6
$2 \times 10^{-4}M$ pNP	18.1	10.4	10.6
Expt. No. 2			
Control	17.7	− 16.8	10.7
$10^{-4}M$ pNP	20.3	− 5.8	11.0
$2 \times 10^{-4}M$ pNP	21.3	6.9	14.2
Expt. No. 3			
Control	18.0	− 11.6	13.2
$5 \times 10^{-5}M$ pNP	20.5	− 8.2	14.0
$10^{-4}M$ pNP	21.3	− 0.3	15.8
$2 \times 10^{-4}M$ pNP	20.5	9.8	18.2

enate and was corrected for the salt content of the medium. Each vessel contained 35 to 45 mg (dry weight) of tissue.

The effect of the Lipo-Adrenal Cortex, which is a cottonseed oil preparation, was determined by adding the desired volume of this material to the fluid in the vessel. Control vessels contained an equal volume of the cottonseed oil vehicle. In order to assess the significance of our results and to compare them with those of Terner, we also studied the influence of *p*-nitrophenol (pNP). This was an Eastman Kodak Company product that was recrystallized twice from water.

Q_{O_2} and $Q_{lactate}$ are the standard metabolic quotients. For purposes of convenience, aerobic phosphorylation has been expressed as Q_P. This represents microliters of H_3PO_4/mg (dry weight)/hr, according to which 1 μmole of P represents 22.4 μl. A negative value in Q_P represents disappearance of inorganic P from the medium, and a positive value, liberation of inorganic P from organic substrate.

Table 2. Effect of Lipo-Adrenal Cortex on aerobic phosphorylation and lactic acid formation by guinea pig brain homogenates.

Lipo-adrenal cortex (ml/4 ml)	Q_{O_2}	Q_P	$Q_{lactate}$
Expt. No. 1			
0	17.0	− 8.6	11.3
0.05	15.4	− 8.7	11.1
0.1	14.5	− 4.1	11.6
Expt. No. 2			
0	19.9	− 6.4	13.1
0.05	17.0	− 3.1	12.7
0.1	14.6	0.9	14.7
Expt. No. 3			
0	15.9	1.3	6.6
0.1	13.9	3.5	6.8
Expt. No. 4			
0	17.3	− 10.1	13.2
0.1	15.2	− 2.4	15.1
0.2	13.2	1.5	15.2
Expt. No. 5			
0	15.6	− 11.0	10.7
0.2	11.5	0	12.7
Expt. No. 6			
0	15.8	− 1.2	6.8
0.2	11.6	5.5	8.8

In confirmation of the results reported by Terner (*6*), Table 1 shows that pNP stimulates respiration and inhibits aerobic phosphorylations. Effective concentrations range from $5 \times 10^{-5}M$ to $2 \times 10^{-4}M$. Aerobic glycolysis is stimulated by $2 \times 10^{-4}M$ pNP, and in 2 of 3 experiments by $10^{-4}M$ pNP. The degree of stimulation is, roughly, a function of the inhibition of aerobic phosphorylation. It is apparent from experiment 2, however, that aerobic phosphorylation can be markedly inhibited without significant change in aerobic glycolysis. In most of our experiments, the controls showed higher phosphorylation and glycolysis than Terner reported. The reason for these discrepancies is not apparent; they may be due to the size or strain of guinea pig used.

The effect of Lipo-Adrenal Cortex is indicated in Table 2. Concentrations as low as 0.05 ml of the cottonseed oil preparation in a final volume of 4 ml may inhibit aerobic phosphorylation. The inhibition is considerably more marked with 0.1 and 0.2 ml, and, in most experiments, is accompanied by small but significant increases in aerobic glycolysis, 12 to 30 percent. As with pNP, inhibition of aerobic phosphorylation is not always accompanied by increased glycolysis. By comparison of Tables 1 and 2, it can be seen that the degree of stimulation of glycolysis by pNP and Lipo-Adrenal Cortex is essentially the same for the same degree of inhibition of aerobic phosphorylation. This suggests a very direct relationship between these two phenomena.

Unlike pNP, Lipo-Adrenal Cortex inhibits respiration. This inhibition did not exceed 26 percent in any of the experiments reported here. With whole cell preparations, where smaller amounts of tissue and lower concentrations of Lipo-Adrenal Cortex were effective, stimulation of glycolysis was observed without any inhibition of respiration (*4*). This suggests that the factor affecting glycolysis may be different from that which influences respiration. Unpublished experiments (*11*) on the fractionation of Lipo-Adrenal Cortex indicate that these factors can be separated.

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References and Notes

1. R. Schrek, *Endocrinology* **45**, 317 (1949); O. Hechter and S. Johnson, *ibid.* **45**, 351 (1949); J. Feldman, *ibid.* **46**, 552 (1950).
2. Z. Miller, *Federation Proc.* **12**, 247 (1953).
3. ———, *Endocrinology* **54**, 431 (1954).
4. ———, *J. Biol. Chem.* **208**, 327 (1954).
5. O. Meyerhof and S. Fiala, *Biochim. et Biophys. Acta* **6**, 1 (1950).
6. C. Terner, *Biochem. J. London* **56**, 471 (1954).
7. This work was aided by a grant from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service. I wish to acknowledge the technical assistance of Nancy McIntosh and Mary Hannum.
8. O. H. Lowry and J. A. Lopez, *J. Biol. Chem.* **162**, 421 (1946).
9. B. F. Miller and J. A. Muntz, *ibid.* **126**, 413 (1938).
10. S. B. Barker and W. H. Summerson, *ibid.* **138**, 535 (1941).
11. O. Hechter and Z. Miller, unpublished.

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18 October 1954

Tergal and Cercal Secretion of Blatta orientalis L.

A greyish viscous secretion (Fig. 1, bottom) accumulates on the terminal abdominal segments of adult females and nymphs of both sexes of *Blatta orientalis* (*1*) and nymphs of *Nyctibora lutzi* Rehn and Hebard (*2*), and on the cerci of nymphs of *Blattella germanica* (L.) (*3*).

We and George Riser, formerly of this laboratory, observed that this mucouslike secretion accumulated on the cerci and terminal abdominal segments of both sexes of nymphs of the following oviparous species of cockroaches, particularly when the insects were isolated or when small numbers were kept together in a large container: *Blattella germanica, B. vaga* Heb., *Periplaneta americana* (L.), *P. brunnea* Burm., *P. australasiae* (Fab.), *Supella supellectilium* (Serv.), *B. orientalis, Parcoblatta pensylvanica* (Deg.), *Neostylopyga rhombifolia* (Stoll), *Eurycotis floridana* (Walk.), and *Ectobius livens* (Turt.) (*4*). We have not found the secretion on isolated nymphs of the viviparous species *Diploptera dytiscoides* (Serv.) or on the following false ovoviviparous species: *Blaberus craniifer* Burm., *Pycnoscelus surinamensis* (L.), *Leucophaea maderae* (Fab.), and *Nauphoeta cinerea* (Oliv.).

In *Blatta orientalis,* the material is secreted by the cerci and by glandular cells in tergites 6 and 7. We removed the cerci of oriental cockroach nymphs, and the secretion built up quickly on the tergites.

We collected secretion weekly from isolated nymphs and, after several months, had accumulated enough for analysis. The dried secretion was tan-colored and amorphous. It became soft and moist at 166°C and began to decompose by charring at approximately 205°C. It