The defects in the normoblasts although at times quite considerable were generally of lesser degree and only a few of these cells were affected. However, severely altered reticulocytes were frequently seen. The most flamboyant morphological alterations, however, were exhibited by rare erythrocytes (Figure 2) in the peripheral blood, the rest of the red cells being completely free of such defects. The paucity of abnormal erythrocytes in the peripheral blood would suggest their rapid destruction.

The underlying cause of this process is not clear. Marked alterations in form of red cells are most commonly observed in situations where normal red cells are altered by an abnormal plasma environment, e.g. acanthocytes and echinocytes[8], or where mechanical factors fragment and disrupt the cells as in schistocyte formation[6,9]. The abnormal form in the present case is, however, totally different from these and also differs from the small tubular protrusions, described by BESSIS and BRICKA[10], in cells undergoing agglutination.

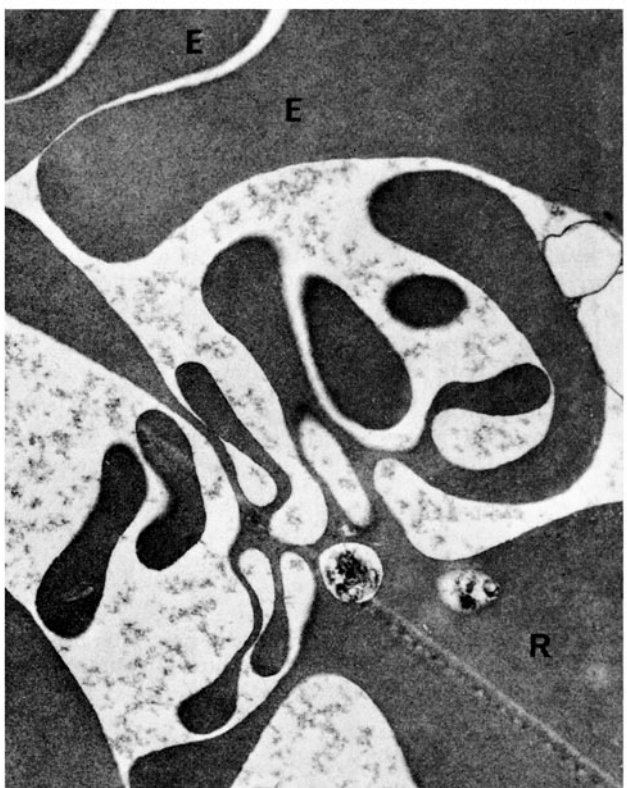

Fig. 2. Some morphologically normal erythrocytes (E) and a ropalocyte (R) with branched and unbranched club-shaped processes found in the peripheral blood. ×17,500.

The defect may lie in the cell membrane but whether this is an entirely intrinsic abnormality of the affected cells or whether extrinsic factors are involved, cannot be ascertained at the present. In keeping with the custom of naming erythrocyte abnormalities we would like to propose a name for this picturesque alteration of erythrocyte morphology. Since the basic defect here seems to be a production of processes which in ultrathin sections appear club-shaped and the greek for club is ropalon, we propose that this abnormality is called ropalocytosis.

Detailed study of the leukaemic and erythropoietic cells from this case are in progress and will be reported later. The purpose of this communication is to draw attention to this intriguing alteration of erythrocyte form, so that it may be further investigated in future cases of this rare condition.

Zusammenfassung. Bei leukämischer Retikulose wurden in Ultradünnschnitten im Elektronenmikroskop abnorm deformierte Erythrozyten, Reticulozyten und Normoblasten mit Einbuchtungen und Pseudopodien-artigen Ausstülpungen, die in gewöhnlichen Ausstrichpräparaten nicht auftreten, nachgewiesen.

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Effects of Some Membrane-Active and Other Compounds on Thrombin-Induced Platelet Aggregation

Thrombin-induced platelet aggregation appears to play a central role in both thrombosis and hemostasis[1]. Numerous agents have been reported to inhibit the aggregation of platelets induced by thrombin, but the mechanisms of action of most such agents are unclear. Recently, increasing evidence has accumulated to indicate that thrombin-induced platelet aggregation is mediated through changes in the platelet plasma membrane, possibly through changes in platelet adenosine triphosphatase (ATPase)[2] or acetylcholinesterase (AChE)[3] activities or by alteration of cyclic adenosine monophosphate (cAMP) levels[4,5]. Accordingly, a group of compounds were tested to determine their effects on thrombin-induced platelet aggregation. This group included steroids, antibiotics, adrenergic blocking agents, antiacetylcholine compounds, and others, many reported to influence plasma membrane phenomena[6,7]. Also tested for comparison were cAMP and dibutyryl cAMP.

All studies were conducted using the macroscopic platelet aggregation test of BRINKHOUS et al.[8], which is sensitive to small changes in the degree of aggregation of platelets. The test system contained thrice washed human platelets[8] mixed with equal parts of 1.08 m*M* $CaCl_2$, bovine thrombin 0.5 units per ml, 50 m*M* *tris* (hydroxymethyl) aminomethane buffer pH 7.4 (*Tris* buffer), and test agent in *Tris* buffer. Test agents were incubated at 25°C for 15 min with platelets prior to addition of calcium and thrombin. Aggregation was

quantitated visually at 25 °C over a 2 min period with a final check by phase-contrast microscopy for aggregated and single platelets. Test agents were obtained from Sigma Chemical Co. or from appropriate pharmaceutical firms and were devoid of additives in most cases. Each test agent was investigated at high concentration ($10^{-4}M$ or greater) initially and, if it displayed inhibitory activity, at progressively lower concentrations to determine the lowest concentration producing detectable inhibition of aggregation. A decrease of 50% or more in the number of aggregated platelets is termed inhibition in the present study.

A number of compounds had no effect on thrombin-induced platelet aggregation even when present in high concentration (Table A). This group included 4 antibiotics and isoniazid, 2 glucocorticoids and prednisone, and 5 unrelated compounds. Lack of inhibitory effect on the part of penicillin and streptomycin is of interest, since these agents are thought to produce changes in membrane lipids[7]. Lack of inhibition by chloramphenicol is not surprising, since this agent inhibits protein synthesis and might require additional incubation time to produce an effect[7].

One group of test agents inhibited aggregation only at relatively high concentrations (Table B). Included were cAMP and dibutyryl cAMP, agents thought to act only after entering the platelet; atropine and tubocurarine, both antiacetylcholine compounds; propranolol, a β-blocker; amytal, colchicine, and morphine, all analgesic agents to varying degrees; and diphenylhydantoin, a compound thought to influence membrane permeability.

Effects of test agents on thrombin-induced aggregation of washed human platelets

A. No inhibition of aggregation (test agents at final concentration of $10^{-4}M$ or greater)

Chloramphenicol	Penicillin-G
Cortisone	Phenacetin
Diamox	Prednisone
Digitoxin	Streptomycin
Hydrocortisone	Tetracycline
Isoniazid	Thiouracil
Nicotinic acid	

B. Inhibition of aggregation (test agents at final concentration of $10^{-4}M$ or greater)

Amytal	Diphenylhydantoin
Atropine	Morphine
Cyclic AMP	Propranolol
Colchicine	Tubocurarine
Dibutyryl cyclic AMP	

C. Inhibition of aggregation (test agents at final concentration of less than $10^{-4}M$)

	Final concentration of test agent (M)
Chloroquin	2×10^{-5}
Corticosterone	1.3×10^{-5}
Dichloroisoproterenol	2×10^{-5}
Dipyridamole	2×10^{-6}
Estradiol	1.3×10^{-5}
Glucagon	1×10^{-6}
Testosterone	1×10^{-5}
Tetraethylammonium	6×10^{-5}

Of perhaps greater interest was a 3rd group of test agents which inhibited aggregation at concentrations of less than $10^{-4}M$ (Table C). This group included the mineral corticoid corticosterone as well as an estrogen and an androgen; tetraethylammonium, an antiacetylcholine agent; dichloroisoproterenol, a β-blocker; and chloroquin, an antimalarial sometimes used to treat cardiac arrhythmias. Also included were the 2 agents active at lowest concentration, dipyridamole, a vasodilator which inhibits nucleotide breakdown, and glucagon, a hormone reported to produce increased intracellular levels of cAMP.

Agents which inhibited thrombin-induced platelet aggregation fell into the following 3 major groups: 1. steroid membrane stabilizers, 2. agents influencing membrane electrical properties or permeability or both, and 3. agents reported to produce elevated intracellular cAMP levels. All 3 groups may eventually prove to be more closely related than appears to be the case at first glance, since all influence plasma membrane phenomena.

Of the steroids tested, only those with little or no anti-inflammatory effect inhibited thrombin-induced platelet aggregation. The anti-inflammatory steroids, cortisone and hydrocortisone, as well as prednisone were without inhibitory effect. The steroids which inhibited aggregation were effective at low concentration; high levels of these compounds have been reported to lyse cells[6].

The group of inhibitors of aggregation which influence membrane electrical properties is a diverse one. Included are antiacetylcholine agents, analgesics, an anitconvulsant, an antiarrhythmic, and a vasodilator. The precise mechanism of action of each agent on platelets is not known, but our findings coupled with those of others[1-3,9-11] strongly suggest that alteration of plasma membrane electrical or permeability phenomena or both are essential to induction of platelet aggregation by thrombin. AChE activity[3] recently has been suggested to play a role in platelet aggregation. Several workers have related change in platelet surface charge to aggregation induced by thrombin[9,12].

Platelet aggregation has been associated with decreased levels of cAMP, while inhibition of aggregation has been associated with elevated cAMP levels[4,5]. The present study reports the inhibition of aggregation produced by β-blockers in a test system designed to detect small changes in the degree of aggregation of washed platelets. β-blockers generally produce increased levels of intracellular cAMP[4]. Further, glucagon, a hormone reported to elevate cAMP levels in certain tis-

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sues[13–15] has been found to inhibit aggregation. Insulin, also tested, inhibited aggregation only at high concentration (40–80 U/ml).

These studies, taken in light of the work of others, suggest that thrombin may act by producing change in the platelet plasma membrane. Thrombin is known to alter platelet membrane charge[12] as well as the permeability of the membrane to K^+[16]. Recently thrombin has been shown to alter platelet cAMP levels[4,5] and platelet ATPase[2] and AChE[3] activities. One or more proteins of the platelet plasma membrane are changed following exposure to thrombin[17,18]. Others have reported that intracellular Ca^{++} and cAMP levels can be influenced by AChE and ATPase activities of the cell[19–21]. Thus thrombin may alter platelet cAMP levels not only by action on the adenyl cyclase-phosphodiesterase system which does not appear to be located in the plasma membrane but also by influencing membrane associated ATPase and AChE activities. These findings illustrate that the actions of thrombin on the platelet plasma membrane are diverse, a fact supported by our present data with inhibitors of thrombin-induced aggregation[22].

Zusammenfassung. Substanzen, welche die thrombininduzierte Plättchenaggregation hemmen, lassen sich in drei Hauptgruppen aufteilen: 1. membranstabilisierende Steroide, 2. Substanzen, welche die elektrischen Eigenschaften der Membran oder die Permeabilität oder beide beeinflussen, und 3. Substanzen, die eine Erhöhung des intrazellulären cAMP-Spiegels bewirken sollen. Eine Hypothese über die Hemmung der Plättchenaggregation durch Thrombin wird vorgeschlagen.

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Occurrence of Insulin in Rat Duodenum and its Depletion with Alloxan

The pyloric antrum, duodenum and pancreas have been referred to by the collective name of 'the abdominal endocrine organ'[1]. All the endocrine cells of this region are believed to arise from a limited area of the primitive foregut, and their hormones are without exception concerned with the regulation of digestion, absorption and utilization of food[1–3]. The hormones of 'the abdominal endocrine organ' are polypeptides such as gastrin, secretin, cholecystokinin-pancreozymin and enteroglucagon in the antro-duodenal mucosa, and insulin and glucagon (possibly also gastrin[4]) in the pancreatic islets. The chemical similarity between several of these polypeptide hormones (gastrin and cholecystokinin[5], secretin, glucagon and insulin[5,6]) supports the concept of a closely integrated hormone-producing locus, comprising several endocrine glands, which conceivably are developed from the same primordial origin. Moreover, the possible existence of an 'enteroinsular axis', whereby insulin-releasing agents from the gut control the insulin release, emphasize the close functional coordination of this particular region[7–12]. There is also some evidence that endocrine cells of this region may have a dual distribution. Thus, glucagon or glucagon-like agents (enteroglucagon) have been demonstrated in the intestinal mucosa[7–12], and gastrin-storing cells can be recognized both in the pyloric gland area of the gastric mucosa[13] and in the pancreatic islets[4]. We decided to investigate the possibility that insulin might have a similar dual distribution.

The musosa was scraped off various regions of the bowel of freely fed rats (male Wistar rats weighing 200–300 g; material from 4–5 rats was pooled), rabbits, cats and dogs. Care was taken in dissection and in the collection of mucosa to avoid contamination with pancreatic tissue. The mucosa was homogenized in acidified ethanol (96 vol. ethanol, 2.4 vol. conc. H_2SO_4, 18 vol. water) in a concentration of 50 mg tissue (wet weight) per ml. The homogenate was allowed to stand overnight at +4 °C. The homogenate was then neutralized with 5 *N* ammonium hydroxide and centrifuged at $1500 \times g$. The supernatant was dialyzed overnight at +4 °C against 2 changes of acetate buffer (pH 4.5), 0.1 and 0.02 *M* respectively, and subsequently assayed for immunoreactive insulin (IRI)[14]. After further dialysis overnight against Ca^{++}-free Krebs-Ringer bicarbonate solution, the insulin-like activity (ILA) of the ex-

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