Table I.—Effect of the anti-D incomplete antibodies upon glycolysis of the D-positive red cells. Value are expressed as % of their respective controls.

Number of experiments executed	Experimental conditions (see also footnote)	First sample. Direct Coombs test: + + +	Second sample. Direct Coombs test: + — —
4	Defibrinated blood plus anti-D serum (1)	73 ± 8	77 ± 11
4	Red cells washed and suspended in Tyrode plus anti-D serum (2)	76 ± 9	81 ± 4
5	Red cells sensitized with anti-D serum, then rewashed and suspended in Tyrode (3) . . .	101 ± 15	99 ± 12

Composition of samples: (1) First sample: defibrinated blood, 4 cm³; anti-D serum, 0·30 cm³. Second sample: defibrinated blood, 4 cm³; anti-D serum, 0·06 cm³. (2) First sample: washed red cells suspended in Tyrode solution, 4 cm³ (2 cm³ of packed red cells and 2 cm³ of Tyrode solution); anti-D serum, 0·30 cm³. Second sample: washed red cells suspended in Tyrode solution, 4 cm³; anti-D serum, 0·06 cm³. (3) First sample: 4 cm³ of red cells washed and suspended in Tyrode solution (2 cm³ of packed red cells and 2 cm³ of Tyrode solution) are incubated at 37°C for 1 h with 0·30 cm³ of anti-D serum, then rewashed and resuspended in Tyrode. Second sample: as the first, except that the incubation was made with 0·06 cm³ of anti-D serum.

Results. Our findings are reported in 2 Tables and may be summarized as follows: (1) The addition of anti-D serum to defibrinated blood results in a certain inhibition of glycolysis: a decrease of about 25–30%. (2) Similar results, even if slighty less conspicuous, are obtained when anti-D serum is added to red cells washed and suspended in Tyrode solution. (3) If, however, the washed red cells are incubated at 37°C for 1 h with anti-D serum and then rewashed before resuspending them in Tyrode solution for glycolysis estimation, the resulting values do not significantly differ from the controls. The reason for this behavior remains obscure at this stage, although it can be hypothetically assumed that the presence in the medium of free antibodies or simple serum is needed for glycolysis inhibition. Further work is in progress to elucidate this point.

It is very interesting to note that agglutination *per se* does not affect erythrocyte glycolysis. In fact, the addition of albumin to a sample containing anti-D serum and red cells washed and suspended in Tyrode solution, does not modify the inhibition percentage (Table II). Taking this observation as a starting point, we have also examined the influence of complete antibodies (iso-agglutinin anti-A) on erythrocyte glycolysis, finding no trace of inhibition, whatever the intensity of the agglutination. In some experiments, moreover, the glucose uptake of agglutinated red cells overcomes that of the controls.

Table II.—Influence of agglutination on the inhibition caused by incomplete antibodies on red cells glycolysis. Values are expressed as % of their respective controls.

Number of experiments	First sample (1) Agglutination: — — —	Second sample (2) Agglutination: + + +
First experiment (duration: 3 h)	87	86
Second experiment (duration: 12 h)	77	80

Composition of the samples: (1) Red cells washed and suspended in Tyrode solution, 4 cm³ (2 cm³ of packed red cells and 2 cm³ of Tyrode solution); anti-D serum, 0·30 cm³. (2) Red cells washed and suspended in Tyrode solution containing bovine albumin 20%, 4 cm³ (2 cm³ of packed red cells and 2 cm³ of Tyrode-albumin); anti-D serum, 0·30 cm³.

It remains, of course, to be seen what importance the observed enzymatic inhibition may assume in the pathogenesis of incomplete antibody hemolysis. Our findings, however, may suggest a working hypothesis about the genesis of acquired spherocytosis which may be due to electrolyte unbalance resulting from lowered availability of energy. The observed results are completely independent of the agglutination and are not reproducible with complete antibodies. To the apparent differences between the 2 types of antibodies[13], may be added the capacity possessed only by the incomplete ones to interfere in the red cell energetic metabolism, a difference which may indicate a fundamental diversity in the action mechanism.

It is interesting to remember at this point that cortisone, which, as is generally known, is successfully employed in the therapy of acquired haemolytic anaemias, produces an evident rise of the erythrocyte glycolysis[14].

E. Storti, F. Vaccari, and E. Baldini

Department of Medical Pathology, University of Modena, October 3, 1955.

Zusammenfassung

Die unvollständigen Antikörper verursachen eine Hemmung der Erythrozytenglykolyse. Dieser Effekt ist völlig unabhängig von der Eigenschaft der sensibilisierten Erythrozyten, nach Zusatz von Rinderalbumin zu agglutinieren. Die Glykolyse wird durch Agglutinine (vollständige Antikörper) nicht gehemmt. Die Bedeutung dieser Befunde für die Pathogenese der Hämolyse wird kurz diskutiert.

[13] J. V. Dacie, *The Haemolytic Anaemias* (J. & A. Churchill Ltd., London 1954).

[14] F. Vaccari and M. Rossanda, Arch. int. Pharmacodyn. *90*, 421 (1952). – F. Vaccari, M. Rossanda, and G. Malaguti, Arch. int. Pharmacodyn. *99*, 234 (1954).

Thrombozytose und Eosinopenie bei Ratten nach einmaliger 5-Oxytryptamininjektion

In Fortsetzung experimenteller Untersuchungen von Hedinger und Langemann[1] über die endokrine Aktivität der Karzinoide und ihrer 5-Oxytryptaminausscheidung wird bei weissen Ratten der Einfluss einer hohen Einzeldosis von 5-Oxytryptamin auf die Thrombozyten- und Eosinophilenwerte des Blutes untersucht. Als Versuchstiere dienen 180–230 g schwere weisse Ratten einer langjährigen Inzucht des Pathologischen Institutes der Universität Zürich. 5 Tieren wird 250 µMol/kg Körpergewicht 5-Oxytryptaminhydrochlorid[2] in einmaliger Dosis subkutan injiziert. Die Thrombozyten des der Schwanzspitze entnommenen Blutes werden ungefärbt direkt in der Zählkammer mit Hilfe des Phasenkontrast-

[1] Chr. Hedinger und H. Langemann, Schweiz. med. Wschr. *85*, 368 (1955).

[2] Der Firma Dr. A. Wander AG. verdanken wir die zum Versuche verwendeten Mengen von 5-Oxytryptaminhydrochlorid.

mikroskopes, die eosinophilen Leukozyten dagegen gefärbt, ebenfalls direkt in der Zählkammer gezählt[3]. Diese Bestimmungen werden unmittelbar vor, eine halbe Stunde, 4, 8, 12 und 24 h nach der Injektion durchgeführt. Die Resultate sind aus den Abbildungen 1 und 2 ersichtlich. Die Abbildungen zeigen die beiden Mittelwertskurven und die Einzelwerte der Thrombozyten und Eosinophilen mit einem Maximum des Thrombozytenanstieges und des Abfalles der eosinophilen Leukozyten 12 h nach der Injektion von 5-Oxytryptamin. Die Mittelwerte der Thrombozytenzahlen und der Menge der eosinophilen Leukozyten von 5 Kontrolltieren, denen physiologische NaCl-Lösung in einer Menge von 10 ml/kg Körpergewicht subkutan injiziert worden ist, halten sich im Bereiche der normalen Tagesschwankungen.

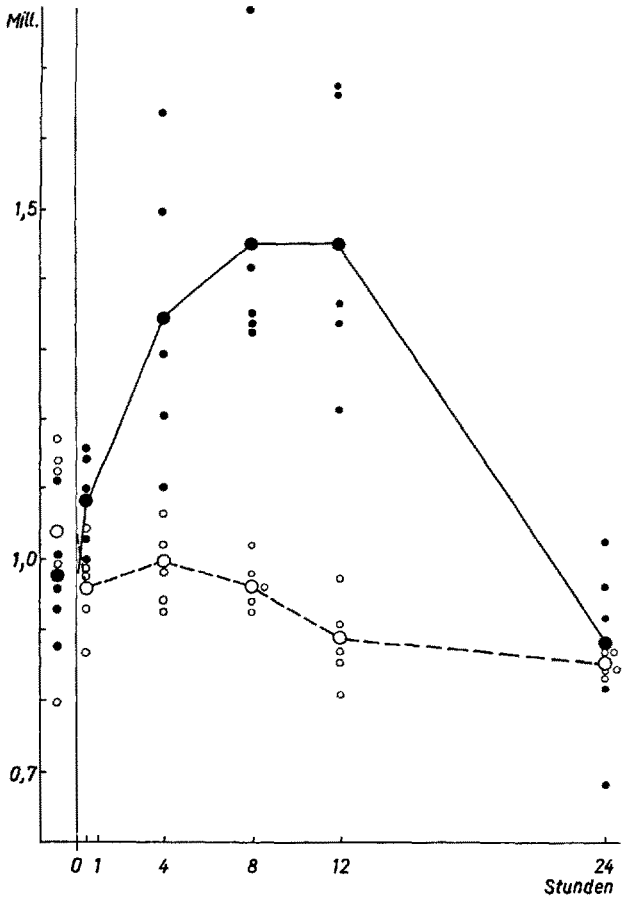

Abb. 1. Thrombozytose im peripheren Blut von Ratten bei einmaliger Injektion von 5-Oxytryptamin (250 µMol/kg Körpergewicht). Abszisse: Bestimmungszeiten unmittelbar vor und nach der Injektion. Ordinate: Thrombozyten pro mm³ Blut in Millionen.
–●–●– Mittelwerte der 5-Oxytryptamintiere, • Einzelwerte.
–○–○– Mittelwerte der Kontrolltiere, ○ Einzelwerte.

Die Thrombozyten zeigen somit nach einmaliger Injektion einer hohen Dosis 5-Oxytryptamin einen deutlichen Anstieg, die eosinophilen Leukozyten einen ausgesprochenen Abfall, wobei Maximum des Thrombozytenanstieges und Minimum der Eosinophilenzahlen zeitlich übereinstimmen. Eine wesentliche unspezifische Reaktion, verursacht durch das Trauma der Injektion, lässt sich ausschliessen, wie die Kontrollversuche zeigen. Da die Maxima des Thrombozytenanstieges und des Abfalles der eosinophilen Leukozyten gleichzeitig nach 12 h erreicht werden, muss in erster Linie ein Wirkungsmechanismus gesucht werden, der beide Phänomene erklärt. HALBERG[4] wies schon früher nach, dass 5-Oxytryptamin und Cortison an Mäusen synergistisch auf die eosinophilen Leukozyten im Blut wirken und eine Eosinopenie erzeugen. KOLLER und ZOLLIKOFER[5] haben gezeigt, dass ACTH-Injektionen im peripheren Blut eine Thrombozytose und gleichzeitig einen Eosinophilenabfall

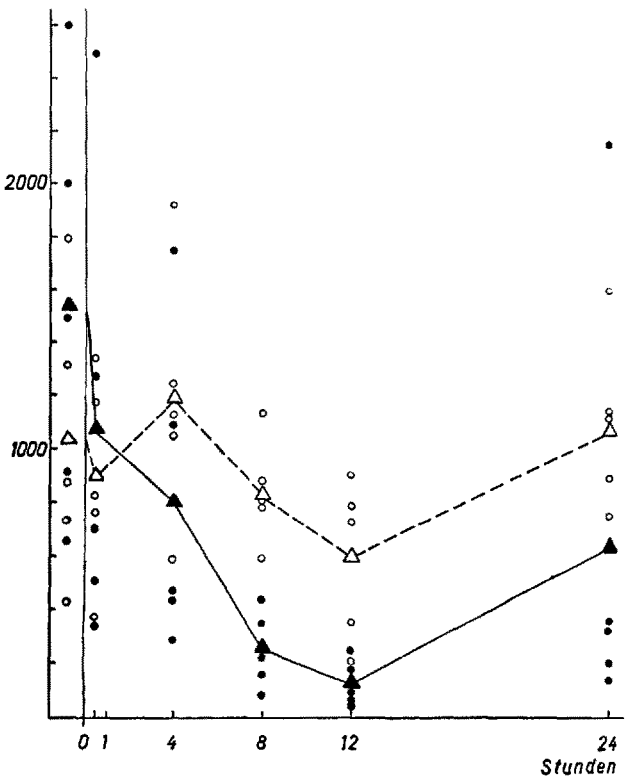

Abb. 2. Abfall der eosinophilen Leukozyten im Blut von Ratten bei einmaliger Injektion von 5-Oxytryptamin (250 µMol/kg Körpergewicht). Abszisse: Bestimmungszeiten unmittelbar vor und nach der Injektion. Ordinate: Eosinophile Leukozyten pro mm³ Blut.
–▲–▲– Mittelwerte der 5-Oxytryptamintiere, • Einzelwerte.
–△–△– Mittelwerte der Kontrolltiere, ○ Einzelwerte.

auslösen. Es wäre daher möglich, dass bei dem vorliegenden Versuche keine unmittelbare 5-Oxytryptamin-Wirkung, sondern eine mittelbare über die Ausscheidung von ACTH und Cortison im Spiele ist, im Sinne einer Alarmreaktion. Um diese Frage näher abzuklären, führen wir zur Zeit entsprechende Versuche an nebennierenlosen Ratten durch. Es muss aber auch die Möglichkeit in Betracht gezogen werden, dass dem Thrombozytenanstieg und dem Abfall der eosinophilen Leukozyten verschiedene Wirkungsmechanismen zugrunde liegen und dass das zeitliche Zusammentreffen beider Erscheinungen nur einen einheitlichen Wirkungsmechanismus vortäuscht. So wäre es denkbar, dass die Thrombozytose eine 5-Oxytryptamin-Reaktion darstellt, um das zusätzliche 5-Oxytryptamin wegzuschaffen, und dass nur die Eosinopenie durch Rindenhormone verursacht würde.

F. A. STEINER und CHR. HEDINGER

Pathologisches Institut der Universität Zürich, den 29. Dezember 1955.

[3] Frl. C. SANDRI danken wir für ihre Mithilfe bei den Blutuntersuchungen.

[4] F. HALBERG, Amer. J. Physiol. *179*, 309 (1954).
[5] F. KOLLER und H. ZOLLIKOFER, Exper. *6*, 299 (1950).

Summary

After injection of a high dose of 5-hydroxytryptamine into rats, the blood reveals a strong increase of the blood platelets and a definite decrease of the eosinophiles. The maxima of increase and decrease are in temporal accordance.

Does Thyro-Parathyroidectomy Abolish the Antiphlogistic Actions of Cortisone?

Extensive animal experiments, reported in this Journal a short time ago[1], led to the conclusion that the antiphlogistic effects of cortisone are abolished by thyro-parathyroidectomy. Such a complete dependence of antiphlogistic corticoid actions upon the thyroid apparatus would require a radical reorientation of our views on the role played by hormones in inflammatory disease. We had learned that the nephrosclerotic effect of desoxycorticosterone is abolished after hypophysectomy[2] or thyro-parathyroidectomy[3] while it is aggravated by thyroxin[2]; yet we considered the actions of antiphlogistic corticoids upon inflammation to be more direct and neither relayed through other glands, nor strictly dependent upon "conditioning" by hypophyseal and thyroid hormones[4]. The present communication deals with experiments designed to verify this point, using three procedures for the objective assessment of inflammation: the "granuloma pouch", and the "topical irritation arthritis" technique, performed with formalin and with dextran.

Methods. Forty female Sprague-Dawley rats were subdivided into 4 groups of 10 animals—each with an initial mean body-weight of 130 g (range: 125–137 g)—as indicated in the Table. Thyro-parathyroidectomies were performed in Groups III and IV, under ether anesthesia, on the first day of the experiment. At the same time treatment with cortisone was initiated in Groups II and IV. This hormone was given in the form of microcrystals of its acetate, at the daily dose of 5 mg, in 0·2 ml aqueous suspending agent ("Cortone", Merck & Co., Inc.), subcutaneously in the groin, throughout the period of observation.

The "topical irritation arthritis" test with dextran was performed on the seventh day. The commercial (6%) dextran ["Intradex", Glaxo (Canada) Ltd.] was diluted with 35 times its volume of water and 0·1 ml of the resulting solution was injected under the plantar aponeurosis of the right hind paw. On the ninth day, a similar topical irritation arthritis was produced by injecting 0·1 ml of a 3% aqueous formaldehyde solution under the plantar aponeurosis of the left hind paw. In both instances the degree of inflammation was assessed by determining the "index of swelling"[5]. First, the normal thickness of the hind paw is measured (with calipers) between the dorsum pedis and the planta, at the origin of the toes, in the metatarsal region; a second measurement establishes the greatest lateral thickness of the ankle. The figures so obtained are multiplied by each other. The product is a function of the general thickness of the paw, which we found to be extremely constant under normal conditions. These measurements are subtracted from the product of the corresponding two thicknesses, taken 2½ h after the injection; the difference gives the "index of swelling" which is listed in our Table.

The "granuloma pouch" was prepared on the 11th day, by injecting (under ether anesthesia) first 25 ml of air and then introducing 0·5 ml of a 1% croton-oil solution (in corn oil) into the air-sac thus created[6]. The amount of inflammatory exudate produced was measured by aspiration into a graduated syringe 7 days later.

All animals were maintained exclusively on "Purina Fox Chow" and tap water. Rats rarely develop manifest tetany during the first weeks after parathyroidectomy, unless their diet is extremely rich in phosphates or deficient in calcium. It was therefore considered best not to complicate the results by administering an excess of calcium-salts since these have an antiphlogistic effect of their own.

Results. All animals were killed on the 18th day, and the principal findings are summarized in our Table.

Perhaps thyroidectomy in itself had slightly diminished the phlogistic potential of our rats (at least with respect to dextran and croton oil), but cortisone undoubtedly exerted a marked antiphlogistic effect, both in the normal and in the thyroidectomized animals.

It is difficult to understand why our results were so strikingly different from those of DOMENJOZ *et al.*[1]. It is noteworthy, however, that the latter investigators used only formalin arthritis as an indicator. This is a much less sensitive test for antiphlogistic corticoids than the granuloma pouch. Furthermore, DOMENJOZ *et al.* administered ample calcium-supplements (100 mg/kg of calcium Sandoz subcutaneously daily, and 1% calcium-lactate as a drinking fluid); in the thyro-parathyroidectomized animals, whose phlogistic potential is low to start with, this may have brought inflammation down to a level at which cortisone could not induce further

[1] R. DOMENJOZ, H. NAUMANN, and E. G. STENGER, Exper. *11*, 403 (1955).

[2] H. SELYE, J. clin. Endocrinol. *6*, 117 (1946).

[3] E. SALGADO, Endocrinology *55*, 377 (1954).

[4] H. SELYE, *Stress. The Physiology and Pathology of Exposure to Stress* (Acta Inc., Med. Publ., Montreal, 1950). – H. SELYE and G. HEUSER, *Fifth Annual Report on Stress* (M D Publ., Inc., New York, 1955).

[5] H. SELYE and G. HEUSER, Int. Arch. Allergy *5*, 52 (1954).

[6] H. SELYE, J. Amer. med. Assoc. *152*, 1207 (1953).

Effect of thyro-parathyroidectomy upon the antiphlogistic actions of cortisone

Group	Treatment	Topical irritation arthritis (Swelling index)		Granuloma Pouch (Exudate in ml)	Final bodyweight (g)
		Dextran	Formalin		
I	Intact control	34·1 ± 1·6	20·9 ± 2·8	8·3 ± 0·7	178 ± 1·5
II	Intact cortisone	5·3 ± 1·7	11·9 ± 1·7	0	121 ± 1·5
III	Thyro-parathyroidectomy control	17·2 ± 3·7	27·5 ± 2·9	6·7 ± 0·7	148 ± 2·9
IV	Thyro-parathyroidectomy + cortisone	6·8 ± 1·8	11·0 ± 2·5	0	109 ± 2·0