A Modification of LOVELL, PRYCE, and BOAKE'S Guinea-Pig Skin Test, its Possible Use in the Semi-Quantitative Evaluation of the Activity of Various Diseases, Including Collagen Disease

There still seems to be a need for a simple test enabling the clinician to detect rheumatoid arthritis in its early stages and making it possible to evaluate the degree of severity of the disease more adequately than do the serological tests at present available (FELLINGER[1], VAUGHAN[2]). In 1954 LOVELL, PRYCE, and BOAKE[3] and BOAKE and LOVELL[4] described the necrotising effect of human sera following intracutaneous injection into the guinea-pig. A toxic action of certain sera had been discovered in principle already by DOERR and MOLDOVAN in 1907[5]. Although BOAKE and LOVELL[4] obtained positive results with sera of patients suffering from rheumatoid arthritis, their test did not, until very recently, receive clinical attention as a routine method for detecting rheumatoid arthritis and related disorders. Thus, KLEMPERER, FRIED, and STARK[6] were able to confirm generally the findings of the British authors; a considerable number of positive results were also obtained by KLEMPERER, FRIED, and STARK in malignant disease. In view of the simplicity of the guinea-pig skin test, we decided some time ago to evaluate its use as a means of testing anti-inflammatory substances. We therefore worked out a semi-quantitative modification which seems capable of reflecting the actual stage of the disease more appropriately than do serological tests. The following is a short account of the experience we have had so far with the modified test.

Methods. Blood was obtained by venipuncture from patients suffering from various diseases, as well as from apparently healthy adults, and was left to clot. In most cases, serum was injected within 6–8 h. Male albino guinea-pigs weighing 250–500 g were depilated on the back with Buto® 2–3 h prior to testing the sera. After preliminary tests had shown that most active sera were positive in amounts ranging from 0.05 to 0.2 ml, it was decided to inject 0.05, 0.1 and 0.2 ml intracutaneously into three sites of the back on both sides of the midline and at least 3 cm apart from each other. Readings of the double skin-fold and of the diameter of the haemorrhagic and necrotic lesions were made 2, 5, 24, and 48 h after injection. The four separate readings were classified using a + to +++ scale according to the following schedule:

Double skin-fold (increase above normal control skin)		Diameter of haemorrhage/ necrosis	
1–1.9 mm	+	< 2 mm	+
2–2.9 mm	++	2–4 mm	++
> 3 mm	+++	> 4 mm	+++

With positive sera, haemorrhage and incipient necrosis were usually clearly evident 2 h after injection; strongly positive sera produced haemorrhagic lesions as early as 5–10 min following the injection, and when observed over a sufficiently long period (> 72 h), gave rise to central scarring. The lesions observed were thus essentially similar to those described by BOAKE and LOVELL[4]. Sera producing haemorrhagic and necrotic lesions with the smallest amount used (0.05 ml) were assigned a +++, those producing this effect with 0.1 ml a ++ activity, etc. Since more than 70% of non-necrotising sera produced an increase in skin-fold thickness, particularly in the 2–5 h following the injection, swelling of the skin was not taken into account for the final evaluation of the activity.

Results. A total of 108 sera from patients suffering from various diseases as well as 36 normal sera were examined. The sera of 97 out of the 108 patients, i.e. 89%, produced skin lesions rating from + to +++, 2 other such sera gave rise to transient reactions consisting mainly in some petechiae appearing in the skin site 5 h following injection of the highest amount of serum used (0.2 ml). Eleven sera, including 4 from patients suffering from rheumatoid arthritis, were negative. Of the 36 normal sera examined, three were slightly positive whereas 33 produced no haemorrhage or necrosis. The proportion of sera exhibiting medium (++) to strong (+++) necrotising activity was greatest among patients suffering from rheumatoid arthritis, psoriatic and gouty arthritis, ankylopoietic spondylitis, systemic *Lupus erythematosus*, arteritis, tuberculosis and malignancy, i.e. among sera from a variety of etiologically unrelated diseases. The results are summarised in the Table. In connexion with the positive results obtained with the sera from patients suffering from rheumatoid arthritis, it is worth noting that the knee-joint exudate of such patients gave negative reactions in 9 out of 10 cases despite the fact that in 8 of these patients the serum displayed a ++ to +++ activity in the guinea-pig skin. The necrotising effect of rheumatoid or malig-

Clinical diagnosis	Total examined	Necrotising activity (number of cases) nil	+	++	+++	Total positive
Rheumatoid arthritis						
(a) III–IV[a]	13	1	1	3	8	12
(b) II–III[a]	38	2	2	23	11	36
(c) incipient–I[a]	4	1	2	1	—	3
(d) questionable[a]	3	—	2	1	—	3
Reiter's disease	2	—	—	2	—	2
Monarthritis/'Milkman's' arthritis	2	—	—	2	—	2
Gouty arthritis	2	—	1	1	—	2
Psoriatic arthritis	3	—	1	—	2	3
Ankylopoietic spondylitis	6	1	1		4	5
Rheumatic fever	1	—	1[b]	—	—	1
Systemic lupus erythematosus	6	—	2	3	1	6
Arteritis	2	—	—	1	1	2
Scleroderma	2	1	—	—	1	1
Spondylosis/Arthrosis	5	—	2	1	2	5
Oesteoporosis/Pathologic bone fracture/Herniated disc	2	1	1[b]	—	—	1
Tuberculosis/Infection of undefined origin	3	—	—	1	2	3
Typhoid fever	4	2	2	—	—	2
Malignancy	10	2	1	6	1	8
Healthy individuals	36	33	3	—	—	3

[a] Clinical assessment of the stage.
[b] Transient 5 h-reaction consisting of petechiae.

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nancy sera was not antagonised by pretreating the test animals with rather large doses of corticosteroids, salicylate or aminopyrine. This refractoriness to known anti-inflammatory agents is all the more astonishing when compared with the extreme lability of the necrotising action. Thus, mere heating of active sera for as little as 5 min at 56°C, or mixing active sera with equal volumes of knee or pleural exudate, normal serum or Tyrode's solution abolished the necrotising effect either completely or to a considerable extent. In some cases knee exudate was very active in respect of its 'neutralising' effect in that as little as 2 parts of exudate added to 8 parts of a +++ serum were able to abolish the necrotising capacity of the serum. On the other hand, no such loss was observed when equal volumes of two active rheumatoid sera or of rheumatoid and malignancy serum were mixed.

In accordance with BOAKE and LOVELL[4], it was also observed that the factor causing skin necrosis is not identical with Forssman's antibody or complement, nor were attempts to produce skin lesions with precipitates prepared in the cold according to SVARTZ[7,8] successful. No correlation was found between serological tests and the guinea-pig skin activity, the latex test for example being negative in a considerable number of cases with sera producing +++ lesions. The necrotising activity seemed to run to some degree parallel with the erythrocyte sedimentation rate, an observation also made by KLEMPERER et al.[6]. On the whole, the guinea-pig skin test reflected the activity of a given disease far more than the stage to which it had progressed. Furthermore, no appreciable change in the necrotising activity was observed when sera from patients were tested before and after corticosteroid treatment. The sera of 3 patients with typhoid fever in remission were tested for their capacity to induce a local Shwartzman phenomenon in the rabbit. All three sera were able to prepare the skin as well as provoke the reaction when injected intracutaneously in amounts of 0.2 to 0.5 ml followed 24 h later by an intravenous injection of 0.5 ml/kg. These sera were also able to prepare the skin for the provocative injection of *Proteus* endotoxin or to elicit the reaction in a skin site prepared with *Proteus* endotoxin. No such Shwartzman activity was noticed when serum was used which had been obtained from a patient with fresh typhoid fever. In accordance with LOVELL, PRYCE, and BOAKE[3] but in contrast to KLEMPERER et al.[6], no skin necrotising activity was observed when rheumatoid arthritis sera were injected intracutaneously in either rabbit or rat[9].

Zusammenfassung. Es wird eine semi-quantitative Abwandlung des Hautnekrosetests von BOAKE, PRYCE und LOVELL beschrieben. 108 Seren von Patienten, zur Hauptsache solchen mit primär-chronischer Polyarthritis, und 36 Seren von klinisch Gesunden wurden auf diese Weise am Meerschweinchen auf ihre Nekrose erzeugende Eigenschaft geprüft. Unter den pathologischen Seren erzeugten 89% eine positive Hautreaktion, wogegen weniger als 10% der Seren von Gesunden einen schwach positiven Ausfall der Hautreaktion hervorriefen.

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Ferrous Complexes in the Catalase Reaction

WIESNER[1] has recently suggested that new evidence from polarography supports the mechanism for catalase action put forward by WESTHEIMER[2]. According to WIESNER[3], HANUS[4] has shown that in the haemin-catalysed reduction of hydrogen peroxide[5,6] reversible haem-peroxide complexes ($Fe_h^{2+} - H_2O_2$) are involved. There are, however, some serious objections to WESTHEIMER's theory, and there is also a need for considerable caution in adopting the kind of analogy WIESNER proposes.

The polarographic evidence refers only to free haematin in strongly alkaline solution. Catalase itself[7] and blood haemolysates[5] remove the hydrogen peroxide 'wave' in the electrolytic reduction of oxygen by destroying the peroxide catalatically in solution (Equation 1):

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad (1)$$

But no intermediates active at the electrode in such systems have been detected. Even in the case of haemin, the only intermediates chemically identifiable are the ferrous (Fe_h^{2+}) and ferric (Fe_h^{3+}) forms. Under certain conditions[1], the half-wave potential of the catalysed reaction is that of the Fe_h^{2+}/Fe_h^{3+} couple. The oxidation of ferrous haem by peroxide may therefore play an important role in the catalysis, despite the calculated discrepancies in rate constants[1], because the apparent velocity constants obtained polarographically often exceed the true constants obtained by conventional chemical techniques[8]. Furthermore, under the conditions employed the rate limiting step may not be that of Equation 2, the usual reaction of haem with peroxide; other reactions can be involved in the reoxidation of the ferrous haem.

$$Fe_h^{2+} + H_2O_2 \rightarrow Fe_h^{3+} + OH^- + OH\cdot \qquad (2)$$

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