6 ml. I.P. of the precipitated antigen suspension on approximately six successive days. In some cases the rabbits also received additional doses of native serum and were bled 2-3 weeks later if the titres were satisfactory.

(2) An alum-precipitated antigen prepared by the method of Proom⁴ was also used. The rabbits receiving this antigen were injected with 10 ml. I.M. into both hind legs, and were finally bled approximately three weeks later.

Both methods of immunization gave satisfactory precipitating sera, and these were used in the tests reported below. The antisera on testing mostly gave positive precipitin ring tests with the homologous test serum when the latter was used at dilutions of 1/3,000-1/20,000. However, none of the rabbit anti-sera used by me when tested with *Rh* sensitized cells, after absorption with *A*, *B* and *O* cells, gave titres as good as those reported by Coombs *et al.*

The rabbit anti-sera which had been prepared during 1943-44 contained 0.02 per cent 'Merthiolate' as a preservative and had been Seltz-filtered through E.K. pads, ampouled and stored at 5° C.

Results. The cells employed in the following tests were the washed red cells of a baby of Group *O* sensitized *in vivo*; normal cells of Group *O*; cells of Group *A* sensitized *in vitro* with a human serum of Group *A* containing pure anti-*Rh₀* (Δ) 'blocking' or 'incomplete' antibody; normal cells of Group *A*, and normal cells of Group *B*.

Some rabbit anti-sera tested were first absorbed with *A*, *B* and *O* cells, and others were used diluted beyond the range of hetero-agglutination which, in most cases, did not extend beyond a dilution of 1/5. With some animal anti-sera heteroagglutination was seen only in the undiluted serum and then was extremely weak.

The following precipitating anti-sera, prepared in rabbits gave positive results with *Rh* sensitized cells, and negative results with normal cells.

Rabbit anti-human	(2 lots)
Rabbit anti-porcine (pig)	(2 lots)
Rabbit anti-feline (cat)	(1 lot)
Rabbit anti-equine (horse)	(1 lot)
Rabbit anti-caprine (goat)	(2 lots)
Rabbit anti-canine (dog)	(1 lot)
Rabbit anti-bovine (ox)	(1 lot)

Negative results with *Rh* sensitized cells were obtained with rabbit anti-galline (fowl) (3 lots). Two of these anti-sera showed no evidence of hetero-agglutinins for normal *A*, *B* and *O* cells, while the other, prepared by the alum-precipitated method, showed the presence of heteroagglutinins only undiluted. Tests made on these anti-sera as a further check showed that they had not deteriorated as precipitating sera on storage for over two years at 5° C.

The above results are reported because they are interesting, and because data of this kind may help to throw further light on the immunological basis of the test described by Coombs, Mourant and Race. The test is one which promises to have wide applications and general use. It should be noted that Coombs *et al.* have shown that the test is not confined to *Rh* sensitization, but has been the means of detecting a new antigen and antibody. The test to date has been found to give negative results when the baby has haemolytic disease due to blood group agglutinins anti-*A* or anti-*B*, and the reason for this has not yet been proved.

R. T. SIMMONS

The Commonwealth Serum Laboratories,
Melbourne, N.2.
Aug. 16.

¹ Coombs, R. R. A., Mourant, A. E., and Race, R. R., *Lancet*, ii, 15 (1945).

² Coombs, R. R. A., Mourant, A. E., and Race, R. R., *Brit. J. Exp. Path.*, 26, 255 (1945).

³ Coombs, R. R. A., Mourant, A. E., and Race, R. R., *Lancet*, i, 264 (1946).

⁴ Proom, H., *J. Path. Bact.*, 55, 419 (1943).

The Incomplete Antibody: a Quantitative Aspect

It is widely believed that the titre of the incomplete or blocking (Δ) antibody, as determined by the quantitative 'saline test'¹, is dependent on the dilution of the anti-*Rh₀* (Δ) serum added to the mixture of red cells and unknown serum². Coombs, Mourant and Race³ stated in a recent article, "It is remarkable that the 'blocking' effect at this time still remained proof against our strongest Δ serum". However, if the incomplete antibody is 'univalent', as suggested by Wiener⁴, and if the 'blocking' effect is a quantitative reaction, the presence or absence of agglutination in the 'saline test' would be expected to be dependent on the absolute amount of incomplete antibody (Δ) present and not on the amount of the anti-*Rh₀* (Δ) serum added.

The results of the 'saline tests' with various dilutions of serum containing the anti-*Rh₀* (Δ) serum provide some information of the nature of the reaction.

The serum containing incomplete antibody (Δ) was serially diluted with saline and to each dilution was added an equal volume of a 1 per cent suspension of *Rh₀* (*cDe*) red cells. The mixtures, which were made in small test tubes, were incubated at 37° C. for thirty minutes. A volume (equal to that already contained in the tube) of serum containing the anti-*Rh₀* (Δ) agglutinin in high titre was then added to each tube, and the tubes incubated for a further sixty minutes. At the end of this time a small quantity of the sedimented red cells was carefully removed from each tube with a fine capillary pipette and placed on a microscope slide. The cells were examined under the low power of the microscope for the presence or absence of agglutination. The titre of the incomplete antibody (Δ) was taken to be the last dilution of the unknown serum in which agglutination was absent.

Four different sera containing an incomplete antibody (Δ) were tested against various dilutions of the anti-*Rh₀* (Δ), and all gave consistent results. A typical protocol is shown in the accompanying table.

Dilution of anti- <i>Rh₀</i> (Δ) serum	Dilution of serum containing incomplete antibody (Δ)				
	1/2	1/4	1/8	1/32	1/128
1/2	—	—	+	+	+
1/4	—	—	+	+	+
1/8	—	—	+	+	+
1/10	—	—	+	+	+
1/20	—	—	+	+	+

The titre of the incomplete antibody (Δ) in the other three sera was similarly independent of the dilution of the serum containing anti-*Rh₀* (Δ) agglutination.

The results are consistent with the concept that there is a common receptor on the surface of the *Rh*-positive red cell for anti-*Rh₀* (Δ) agglutinin and the incomplete antibody (Δ). If the incomplete antibody (Δ) forms a union with all these receptors then even large amounts of the anti-*Rh₀* (Δ) serum will not produce agglutination. On the other hand, if the amount of the incomplete antibody (Δ) present is insufficient to unite with all the receptors, added anti-*Rh₀* (Δ) serum will unite with the remaining receptors and produce agglutination of the red cells. However, although the results are consistent with this quantitative concept, they do not necessarily provide confirmation of this theory.

RUTH A. SANGER

N.S.W. Red Cross Blood Transfusion Service,
Sydney.
Aug. 24.

¹ de Burgh, P. M., Sanger, Ruth A., and Walsh, R. J., in the press.

² Henry, N. R., and Simmons, R. T., personal communications.

³ Coombs, R. R. A., Mourant, A. E., and Race, R. R., *Lancet*, i, 264 (1946).

⁴ Wiener, A. S., *Proc. Soc. Exp. Biol. and Med.*, 51, 173 (1944).

Testicle and Spermatic Tract Lesions in Lymphogranuloma Venereum

In a report on venereal diseases in West Africa by Lieut.-Colonel R. R. Willcox¹, when referring to other tropical conditions, he says: "Hydrocoele are very common in the African though considering the amount of gonorrhoea it is not surprising". According to him, lymphogranuloma venereum (inguinale) is also very common among these natives.

L.V. is responsible for a certain number of cases of epididymal inflammations, many of them of a subclinical type²⁻⁴, others, especially when the infection with *Neisseria gonorrhoea* has been simultaneous, with intense inflammatory symptoms. Some of these cases are accompanied by vaginalitis, which is rapidly reabsorbed; others follow a different course and hydrocoele is installed. Both subclinical or associated L.V.-gonorrhoea cases may follow a slow course and occasionally small epididymal abscesses adhere to the scrotum, break down and leave draining sinuses that heal spontaneously or under treatment. In all mentioned types, L.V. infection is seldom suspected⁵.

In some cases the process mainly affects the blood-vessels and lymphatics of the spermatic cord; the vas or ductus deferens remaining normal or slightly enlarged. Thrombo-angiitis, phlebitis and lymphangitis with micro-abscess formation can be found on study of sections of these structures.

Lesions of L.V. nature of the testicle proper have also been recorded^{6,7}.

Our observations have all been made in a country where filariasis is unknown.

W. R. COUTTS

Department for Social Hygiene,
National Service of Health,
Santiago, Chile. Aug. 2.

¹ Willcox, R. R., *Nature*, 157, 416 (1946).

² Coutts and Vargas Zalazar, *Ann. Malad. Venér.*, 31, 895 (1936).

³ Sato Akira, *Japan. J. Dermat. and Urol.*, 39, 75 (1936).

⁴ Bizzozero and Franchi, *Minerva Med.*, 2, 241 (1937).

⁵ Coutts and Martini Herrera, *Ztschr. f. Urol.*, 32, 439 (1938).

⁶ Huard and Joyeux, *Ann. d'Anat. Pathol.*, 16, 228 (1939).

⁷ Coutts, *Brit. J. Ven. Dis.*, 19, 37 (1943).

⁸ Bastos de Sequeira, *Bol. Soc. Med. e Cir. de Sao Paulo*, 26, 13 (1942).

⁹ Midana, *Dermatologica*, 85, 403 (1942).

DR. COUTTS'S letter is very interesting as the writer has made a particular study of intra-urethral infections of lymphogranuloma venereum (inguinale).

As regards hydrocoele in West Africa, however, the matter of their origin is difficult to determine. Gonorrhoea is extremely prevalent there and, apart from lymphogranuloma venereum, which is also very common, there are also filariasis, dracontiasis and schistosomiasis, as well as other genito-urinary conditions, which may subscribe to their production.

As a basis of probability, gonorrhoea is singled out as the most likely antecedent while the other diseases mentioned are probably more common causes than the comparatively rare one of lymphogranuloma venereum. This matter is very hard to prove as a hydrocoele in a patient showing a positive Frei test is by no means proof that the hydrocoele is due to lymphogranuloma venereum, as the Frei test may remain positive for a very long time after infection.

R. R. WILLCOX

Military Isolation Hospital,
283 Harrow Road,
London, W.9.