are made after 18 h at 4° C, using the same criteria as before.

The histone referred to below was prepared from calf thymus by acid extraction[4]; the salmine and lysozyme were pure preparations supplied by I.B.F. and N.B.C., respectively. All were dissolved in 1 per cent sodium chloride in concentrations of 2 mg/ml.; in this concentration the *p*H was between 6·7 and 6·9. The proteins keep in the refrigerator, but solutions must be freshly prepared. The other proteins referred to were purified samples, dissolved similarly.

Adhesiveness. The basic proteins histone and salmine produce increased stickiness of human red cells to each other, that is, increased adhesiveness, in concentrations greater than 0·015 ± 0·01 mg/ml. This effect is virtually irreversible by washing with saline, is unaccompanied by haemolysis in concentrations less than 2 mg/ml., and is almost independent of the red cell concentration in the system; this is probably because, as the concentration increases, the chances of contact between cells increases. These effects on adhesiveness are related to the effect of histone and salmine on the electrophoretic velocity of the red cells, which is rapidly reduced to zero in concentrations of the order of 1 mg/ml.

Lysozyme, in concentrations of 2 mg/ml. or less, has no effect on the adhesiveness of human red cells; in higher concentration it is haemolytic, and in much higher concentration (20 mg/ml.) it reduces the surface charge of human red cells[5].

Effect of fibrinogen and gelatine. The only plasma protein which affects the agglutinating effect of histone and salmine is fibrinogen, which, while not producing an increase in stickiness itself, increases red cell adhesiveness about eight-fold. Serum albumin and serum globulin have no effect in concentrations of 2 mg/ml. or less, although in much higher concentrations they form complexes with histone[5].

Gelatine in concentrations of 2 mg/ml. (at which level it decreases the stickiness of red cells to glass probably because it coats the glass—it is well known that it is not adsorbed on red cells) decreases the adhesiveness of human red cells to each other about four-fold in systems containing histone or salmine.

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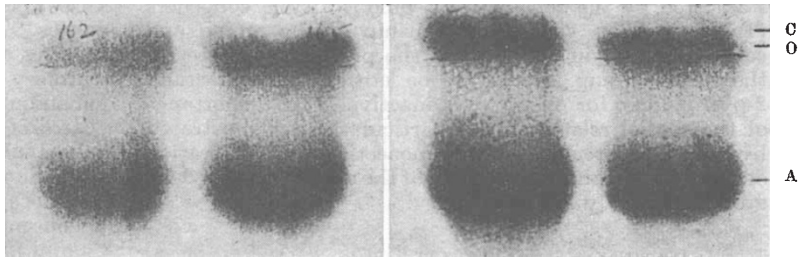

Fig. 1. Paper electrophoresis, barbiturate buffer *p*H 8·6. Three samples of HbA + O and one HbA + C control

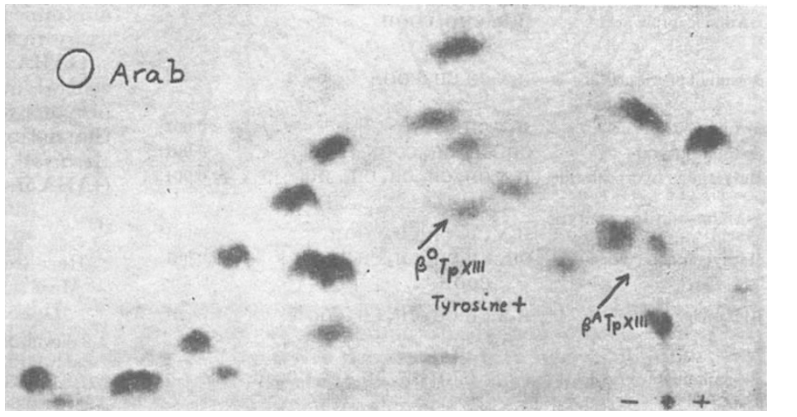

Fig. 2. Fingerprint of the soluble peptides from the tryptic digest of haemoglobin O from the Sudan. It shows the feature of HbO Arab[10]. β^{A}TpXIII is missing and a new tyrosine-containing peptide with additional positive charge can be demonstrated

Haemoglobin O Arab in Sudanese

The Northern and Southern Sudanese differ from an anthropological point of view. The first are primarily Arab, but with varying degrees of African mixture, while the second are Nilotic–Hamitic Africans. The latter can be divided into Northern and Southern Nilotes. Among some groups of Southern Nilotes, haemoglobin S is common[1,2], but it is absent or low in the Northern Nilotes, for example, Dinka, Shilluk and Nuer[3]. Occasionally, also, haemoglobin Stanleyville II is found which relates these populations to their southern and western neighbours[4,5]. In the Northern Sudanese, sickling is found at a low, but constant, rate in Khartoum (182 of 9,100 examined, that is, 2 per cent) and at a more variable rate in Western Sudan[6]. It seems that the Northern Sudanese possess haemoglobin O which relates them to their Arab neighbours. Indeed, haemoglobin O Arab was first reported in an Arab family in Israel[7], but it has recently been found also in Bulgaria[8].

In 1962, we reported the finding of haemoglobin O in a Northern Sudanese woman in Khartoum[9]. Over a 4-year period we have examined 9,300 unselected Northern Sudanese in Khartoum and have found eleven instances of the carrier trait for haemoglobin O Arab. The haemoglobin O was identified in all instances in the Sudan and in England by its electrophoretic (Fig. 1) and other properties[7] and characterized as O Arab in three instances, by examination of the peptides arising from tryptic digestion of the isolated abnormal haemoglobin fraction by fingerprinting[10] (Fig. 2).

The incidence of haemoglobin O Arab in Northern Sudanese is admittedly low (about 0·1 per cent), but when viewed in the light of other surveys numbering many thousands of persons in Africa, it assumed some significance. It is tempting to consider the haemoglobin to be an Arab feature, particularly since it was first found in an Arab family. However, one of the large-scale surveys which failed to reveal the presence of haemoglobin O Arab was conducted in Saudi Arabia[11]. It is thus possible that the haemoglobin O Arab of the Northern Sudanese may be part of their non-Arab heritage related to pre-simitic Egypt. It is worth recalling that sickling was also present in the Arab family in which haemoglobin O Arab was first described, and that sickling is not a feature of pure Arab ('Bedouin') stock, and that haemoglobin O Arab has recently been reported in three generations of an American Negro family[12]. Kantchev *et al.*[8] have suggested that haemoglobin O Arab in Bulgaria may be the outcome of an independent mutation. Before accepting this unreservedly, one has to consider carefully

the links between Bulgaria and various parts of the middle-east and north-east Africa provided by the movement of armies during the prolonged Turkish occupation of these regions.

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Haemoglobin Typing of the Kerry Breed of Cattle

Two types of haemoglobin, designated Bov A and Bov B, have been identified in the blood of cattle by Bangham[1]. These are distinguished by their motility in paper electrophoresis; Bov A is the slow-moving and Bov B the fast-moving component. The type of haemoglobin which occurs in an animal is controlled by two simple allelic autosomal genes which give rise to three phenotypes A, AB and B.

In a survey of stud bulls in Britain, Bangham[1] found that with the exception of the Jersey, Guernsey and South Devon breeds all other British breeds exhibited A type haemoglobin exclusively, while in the case of Channel Island and South Devon bulls both B and AB types were encountered in addition to A type haemoglobin. In a subsequent survey of some cattle breeds of Europe and Africa, Bangham and Blumberg[2] found that B type haemoglobin occurred in breeds located geographically along a line running southwards through France into Africa, while cattle of north-east France, Holland and Denmark showed A type haemoglobin exclusively. This finding tended to support the suggestion of Boston[3] that one ancestral line of the Jersey breed originated in the Indus valley and permeated through Africa to Europe. The type of haemoglobin occurring in Kerry cattle—a breed native to Ireland—has not previously been reported and it was felt that haemoglobin typing might throw some light on the views of Moyles[4], who has suggested that the Kerry breed originated in mid-Asia.

The standards for the different types of haemoglobin were established according to the method of Bangham[1] using blood samples from different breeds, including Friesians, Shorthorns, Herefords, Guernseys and Jerseys, which exhibited the A, AB and B types. Blood samples were then collected from 25 Kerry cattle in a pure-bred Kerry herd and these were run on paper electrophoresis side by side with samples from other breeds. All twenty-five samples from the Kerry cattle contained the commonly occurring A type haemoglobin. This finding has more recently been confirmed by Dabczewski[5].

Haemoglobin typing, therefore, would suggest that the Kerry breed of cattle has no link with that ancestral line of Channel Island cattle but would appear to have an origin resembling that of other northern European breeds.

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IMMUNOLOGY

Active Immunization of Mice with Chagastoxin

Previously we reported on the immunology, immunochemistry and immunochemotherapy of experimental *Trypanosoma cruzi* infection in mice. *T. cruzi* has at least 9 antigens. Sonic lysates of the cultures contain antigens A, B, C, D, E, F, G and H, and the sediment contains B, E, D and X. Living trypanosomes have antigens B, E and G. Saline supernatant has antigens A, B and F, while distilled water supernatant has A, B and E, and sediment antigens B and X. The recently phenolized cultures have A, B, G and X, and old phenolized cultures have only B. 50 per cent extraction with ammonium sulphate yields A, B, E, G and F. 100 per cent saturation still extracts B, while precipitation with alcohol after full saturation with ammonium sulphate still extracts antigen B. The phenol extract and sediment (Westphal) contain no antigens, but phenol aqueous extract contains antigen B. The antigens B, E and G are major antigens. The agglutinin titre of hyperimmune rabbit serum was 1 : 10,240 and that of horse serum was 1 : 327,600. Both rabbit and horse hyperimmune sera had 1 : 4 titre of agar-gel precipitin. Immunotherapy with hyperimmune rabbit serum prolonged the survival time in fatal *T. cruzi* infection in mice two- to three-fold. Chemotherapy with 1-furaltadone, in doses of 50 mg/kg/day for two weeks, followed by 12·5 mg/kg/day for 2·5 months, protected 86·7 per cent of infected mice, while combined immunochemotherapy with hyperimmune rabbit serum plus 1-furaltadone protected all mice for at least 5 months[1–3].

The present report deals with the toxicity and immunizing properties of the lipopolysaccharide, chagastoxin.

Phenol extraction. Chagastoxin is prepared or extracted using the following modified Westphal method[4], used for the extraction of lipopolysaccharides from the cell walls of Gram-negative rods. *Trypanosoma cruzi* (Tulahuan) is grown for 10–12 days on brain heart blood agar (Difco), containing 5–8 per cent citrated human blood obtained from the blood bank, in Roux bottles. The cultures are pooled, should contain no bacteria and erythrocytes, and are sonically lysed for 90 sec in Raytheon sonic oscillator model *DFO*1, 10 kc/s, 250 W, 115 V and 60 c/s. After spinning, the sediment and supernatant are treated with an equal volume of 88 per cent phenol for 30 min in the refrigerator. It is then centrifuged at 3,000–4,000 r.p.m., and the supernatant is then dialysed for 3 days in running tap water and for 24 h against distilled water. The sediment is precipitated with alcohol or is directly lyophilized. The phenol fraction is precipitated with 95 per cent alcohol (6–10 volumes); the sediment is washed in saline and then lyophilized. Gentle handling is