tetanus toxoid. Inhibition of precipitation was observed in one of the first three high-neutralizing sera tested by immunodiffusion. An undigested sample of the same γ-G fraction did not alter precipitation. This precipitating, high-neutralizing serum was probably specific for no more than two determinants and inhibition was probably caused by the blocking of one of these determinants by the univalent antibody digest.

These experimental findings appear to agree with our previous conclusion. We therefore suggest that the combined use of precipitation and passive agglutination could be useful in the recognition of antisera specific for a single antigenic determinant.

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Immunoglobulins in Tibetans

INFORMATION about increased gamma-globulins in certain ethnic communities is rare and as far as we know extends only to Navajo Indians, Puerto Ricans and certain American Negroes[1–3]. There has been no convincing explanation for this phenomenon. We discuss here racial differences in the synthesis of gamma-globulins, and increased exposure to chronic infections, the latter resulting in an increased production of humoral antibodies. Chronic liver disease can easily be excluded as a cause.

We have found low serum lipids as well as generally increased gamma-globulins in Tibetans in Nepal and Switzerland[4]. Now we have examined several serum components of a group of Tibetan refugees resettled in Switzerland by the Swiss Red Cross.

This communication presents the results of an examination of the immunological components of the serum proteins in seventy Tibetan males and females aged between 30 and 50. The results are compared with those of twenty-one matched Swiss controls of a similar age and without known illness.

Total proteins were determined in fasting serum samples; paper electrophoresis was performed, and immunoglobulins were separated on a semi-quantitative basis by double diffusion in agar-gel (differentiation of gamma-A and gamma-M).

Table 1

	Tibetans	Swiss	*P*-value
Serum samples (N)	41	22	—
Age ($\bar{x}$)	38·5	34·5	—
Total proteins (g per cent)	7·07 ± 0·46	6·86 ± 0·33	0·05
Gamma-globulins (g per cent)	1·58 ± 0·25	1·20 ± 0·14	0·001
Gamma-A-globulins (mean titre)	1 : 236	1 : 195	0·05
Gamma-M-globulins (mean titre)	1 : 37	1 : 31	0·05

Table 1 shows the results as mean values with the standard deviation. A further analysis was made according to age and sex, but this analysis did not yield any further information.

The total gamma-globulins in Tibetans are greater than in the Swiss controls, the difference being statistically significant ($P < 0·001$). The mean value for the immunoglobulins gamma-A and gamma-M is also greater in the Tibetans but is less pronounced and these differences do not attain statistical significance. The more marked increase of the total gamma-globulins in comparison with the gamma-A and gamma-M values might be largely the result of a higher gamma-G subfraction.

We have no explanation for these findings. A substantial rate of manifest infection or liver disease in the Tibetans can be excluded. Racial and environmental factors require consideration, and further elucidation may be obtained by observation and re-examination of Tibetans abroad.

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Post-embedding Staining with Ferritin Labelled Antibodies

THE ferritin labelled antibody technique[1] has been widely used for the location of antigens at the cellular and subcellular level[2]. There are two possible methods of applying the technique: (*a*) pre-embedding staining in which antigen is allowed to react with the labelled antibody before the cells are processed and sectioned in preparation for electron microscopy; and (*b*) post-embedding staining in which labelled antibody is applied to and reacts with the antigen in suitably fixed and embedded thin sections. Almost all published investigations have been carried out using the pre-embedding staining technique. Surface antigens are readily located by this method, but intracellular antigens can only be located after treatments such as freezing and thawing and disintegration which enable the labelled antibody to penetrate within the cell. The technical difficulties associated with the post-embedding staining technique are complicated by the non-specific attraction for ferritin of all commonly used embedding media, including butyl methacrylate–ethyl methacrylate co-polymer, polyglycol methacrylate, vestopal and epon[3]. The phenomenon was attributed by these workers to the non-ionic character of the embedding media, and they attempted to develop a new embedding medium with ionic charges which allowed wetting, thus reducing non-specific adsorption. Preliminary results with viral antigens in the polyampholyte embedding medium which they developed were very encouraging.

The present communication deals with our experience using this embedding medium in the location of bacterial antigens.

The strain used throughout this work was *Bacillus cereus*, variety terminalis. Antisera were prepared against both spores and vegetative cells[4] and coupled to ferritin using toluene 2 : 4 di-isocyanate[5]. A young vegetative cell suspension was prepared by thoroughly washing the deposit from a 12 h broth culture fixed overnight in 10 per cent buffered formalin, *p*H 7·2, or in 2·5 per cent buffered glutaraldehyde *p*H 7·2–7·4 for 2 h. After thorough washing in saline the organisms were embedded in the polyampholyte medium using the methods described by Singer and McLean[3]. Polymerization was initiated by heating at 60° C overnight and completed by a further period of curing for 3 days at room temperature. Sections