THE ABSORPTION SPECTRA OF IMMUNE PROTEINS

BY EMIL L. SMITH* AND N. H. COY

(From the Biological Laboratories, E. R. Squibb and Sons, New Brunswick)

(Received for publication, March 29, 1946)

Since the absorption spectra of simple proteins are known to depend on their content of the three aromatic amino acids, tyrosine, tryptophane, and phenylalanine, it was of interest to examine the group of purified immune proteins which were available in order to determine whether differences in their composition could be found by this method. These proteins, all of which are associated with immune properties in the plasma of the cow, horse, and human and the colostrum of the cow, have been described in terms of the method of isolation employed and of their physical and chemical properties (1, 2). It has already been shown that, even in the same species, proteins of quite different composition are associated with immune properties. These differences are also reflected in the absorption spectra of the proteins.

EXPERIMENTAL

The preparation and properties of the proteins used in this study have already been described (1, 2). For measurement of the absorption spectra, the proteins were dissolved in 0.15 M NaCl, adjusted with 0.1 M NaOH to pH 7.0 ± 0.3, and then made up to a definite volume. The protein concentrations were calculated on an ash- and moisture-free basis, and checked by nitrogen determinations. The measurements were made at room temperature with a Beckman spectrophotometer having a hydrogen lamp as light source.

The absorption spectra of the proteins studied are shown in Figs. 1 and 2. Since all of these proteins have been shown to contain tyrosine, tryptophane, and phenylalanine, the typical protein absorption band with a broad maximum at about 280 m\(\mu\) was to be expected. No evidence was found for the presence of groups that absorb light in the visible or ultraviolet regions of the spectrum other than those of the three aromatic amino acids.

The two human \(\gamma\)-globulin fractions have already been shown to differ slightly in their content of tryptophane, 2.81 per cent for the II-3 and 2.56 for the II-1 fraction. Interestingly enough, this difference is shown in the

* Present address, School of Medicine, University of Utah, Salt Lake City.

1 All of these proteins have been analyzed for tryptophane and phenylalanine (2), and tyrosine has been qualitatively demonstrated (unpublished observations).
two absorption spectra (Fig. 1). The two horse serum proteins, which are quite dissimilar in composition and other properties, show this in their absorption curves only in the extinction coefficients and not in the character of the curves.

The most striking differences have been found for the bovine proteins (Fig. 2). The T component is clearly and radically different in its spectrum from either the γ-globulin or the colostrum proteins. Since the absorption band in the region 240 to 250 μm is attributed mainly to phenylalanine (3, 4), the steep end-absorption of the T-globulin indicates a higher phenyl-

![Graph showing ultraviolet absorption spectra of horse and human immune globulins.](http://www.jbc.org/)

Fig. 1. Ultraviolet absorption spectra of horse and human immune globulins

alanine content than the other proteins. This conclusion is supported by the analytical results, since the preparation contains 4.5 per cent compared with 3.2 per cent for the γ-globulin, and 3.7 per cent for the colostrum globulins. The large differences in extinction coefficients for the total compared with the pseudoglobulin and euglobulin fractions from other animals are to be expected from the earlier finding of differences in tryptophane content.

Coulter, Stone, and Kabat (4) compared the ultraviolet absorption curves of horse pseudo- and euglobulins with pneumococcus Type I antibody prepared by the method of Felton. They found that the antibody re-
sembled the normal pseudoglobulin but showed some characteristic differences. Since the sulfate precipitation method used by these investigators for the preparation of the horse globulins is now known to yield mixtures of α-, β-, and γ-globulins (5), their results cannot be regarded as evidence of a difference between the antibody and normal globulin. This is likewise true for all of the earlier comparative analyses of normal and immune proteins. Such data are valid only when completely homogeneous preparations of the same globulin fraction from normal and hyperimmune animals are compared. The present results have clearly shown large differences between the different globulin fractions associated with immunity in the same species, and have failed to indicate significant differences in the composition of bovine γ-globulins from normal and hyperimmune plasmas (2).

**SUMMARY**

The ultraviolet absorption spectra of electrophoretically homogeneous proteins associated with immunity from the horse, cow, and human have
been measured. No evidence was found for the presence of light-absorbing groups other than the three aromatic amino acids, phenylalanine, tyrosine, and tryptophane. The differences in spectra measured for these proteins are readily accounted for in terms of their content of these amino acids.

BIBLIOGRAPHY

THE ABSORPTION SPECTRA OF IMMUNE PROTEINS
Emil L. Smith and N. H. Coy