tent will explain why the iron analyses of various preparations may differ somewhat from each other. Taking 23 per cent. Fe as the maximum value, one may say that 23 per cent. dry weight of the crystals is iron. On removing Fe^{+++}, one may either imagine their replacement by protons, or their removal in the form of a hydroxide such as FeO(OH). Accordingly, either 23 per cent. or 36 per cent. of the mass of the crystal can be removed without essentially altering the very characteristic crystal shape. One may imagine how large is the volume occupied by non-protein interstices within the crystal lattice made up by the protein part. These interstices may be filled either by water or by ferric hydroxide without any obvious influence on the crystal lattice. One may imagine what amount of "impurities" or non-protein constituents can adapt themselves into the interstices of a protein crystal, and how critical one should be with respect to the homogeneity and "purity" of a crystallized protein. This critical aspect, of course, is not all novel. So, pepsin may be more or less acetylated[5] without influence on the shape of the crystals. Yet the present case shows this phenomenon to an extent unappreciated heretofore.

On the other hand, it would be quite absurd to consider the iron of ferritin simply as a contamination of the protein. Rather may one say that the specific structure of apoferritin is adapted to the task of fixing iron atoms in the ratio of about one half to one Fe to each peptide link, without losing the specific structure. It is easy to understand that the space between the four N atoms of a porphyrin allows one Fe atom to be enclosed without loss of the porphyrin structure, but it is hard to imagine a structure of a protein allowing the building in of iron to that enormous extent as found in ferritin. One can not exclude the possibility that a cluster of many molecules of ferric hydroxide such as are known to represent a micelle of a colloidal ferric hydroxide solution, form a center surrounded by apoferritin molecules, reminding one of the very open structures of the zeolites. Such an assumption would dispense with the necessity of linking almost each peptide group with an Fe atom. Study of the crystal structure may be able to decide this point.

The iron content of ferritin is rather constant, *i.e.*, within 2–3 per cent. Ferritin from a normal horse was compared with that from a horse which had been subjected to many bleedings. Although the yields of ferritin obtainable from the two horses differed by 10 : 1, the Fe content of the ferritin was the same. At present we have no evidence for the existence of apoferritin as such in the spleen. That ferritin is not an artefact due to the method of preparation can be seen from the fact that the juice of spleen mixed with $CdSO_4$ without any other treatment yields crystals of ferritin.

S. GRANICK
L. MICHAELIS

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK

[5] Northrup, "Crystalline Enzymes," Columbia University Press, 1939.

THE PRODUCTION OF ANTIBODIES IN VITRO

BY following the general procedure proposed in connection with a theory of serological phenomena,[1] we have succeeded in making antibodies in the laboratory.

The procedure consists in subjecting normal globulin or other protein to the action of denaturing reagents or conditions in the presence of an antigen. The protein molecule unfolds, and then refolds in such a way as to assume a configuration complementary to that of the antigen, thus acquiring the properties of a specific homologous antibody.

Bovine γ-globulin was the protein used in most of our experiments. Some success was obtained also with other serum globulin fractions and with serum albumin. As antigens there have been used the triphenylmethane dye methyl blue (a mixture of the *p*-trisulfonated and disulfonated triphenylpararosanilines), the azo dye 1,3-dihydroxy-2,4,6-tri(*p*-azophenyl-arsonic acid) benzene, and pneumococcus polysaccharide type III. Successful experiments were made by addition of alkali (to pH 11) and slow return to neutrality, by addition and slow removal of urea, and by heating to about 65° and slowly cooling; some success was also obtained by surface denaturation. The most satisfactory of the treatments tried was that of holding a solution of protein and antigen for several days at about 57°; this temperature seems to be high enough to cause the protein chains to unfold and to refold under the influence of the antigen into specific complementary configurations.

In one experiment a solution containing 0.01 per cent. of the azo dye mentioned above and 1 per cent. of bovine γ-globulin was held at 57° for about 14 days, then removed from the bath, and dialyzed through Cellophane against 1 per cent. salt solution. Some precipitate formed during the heating and more during the dialysis. The mixture was then dialyzed against a 1 per cent. solution of the haptene arsanilic acid, which was changed several times. Most of the precipitate dissolved during this dialysis; that which remained was discarded. The solution, which was free of dye, was then dialyzed against 1 per cent. salt solution to remove the haptene.

[1] L. Pauling, *Jour. Am. Chem. Soc.*, 62: 2643, 1940.

The resulting protein solution was found to have many properties of an antiserum specific to the phenylarsonic acid group. It gave precipitates with multihaptenic dyes and azoproteins containing this group, and not with other dyes or azoproteins, and the specific precipitates were dissolved by excess of dyes or haptenes containing this group but not by other dyes or haptenes, such as those containing the phenylsulfonic acid group.

A solution of 1 per cent. pneumococcus polysaccharide type III and 1 per cent. bovine γ-globulin was similarly held at 57° for 14 days. Some precipitate formed, which was removed. The resultant solution was found to precipitate type III polysaccharide but not types I or VIII (cross-reaction with type VIII was thus not shown) and to agglutinate pneumococci type III but not types I or II. Purified antibody solutions were made by adding 15 per cent. salt and bringing to pH 4 or adding 15 per cent. salt, calcium chloride, and calcium hydroxide to pH 8, thus precipitating polysaccharide and some protein. Each of the two solutions, after dialysis against 1 per cent. salt solution, was found to precipitate type III polysaccharide but not types I or VIII and to agglutinate type III pneumococci but not types I or II. Mouse protection tests and swelling tests have not yet been carried out.

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Our experiments are being continued.

LINUS PAULING
DAN H. CAMPBELL

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF THE COMPLEMENT FIXATION TEST IN ROCKY MOUNTAIN SPOTTED FEVER

THE clinical differentiation between typhus fever and Rocky Mountain spotted fever may be difficult, especially when both diseases occur in the same region. The laboratory diagnosis may be made by the isolation of the virus from the patient, but this can only be satisfactorily performed if guinea pigs are inoculated immediately upon withdrawal of the blood. The agglutination test, using either typhus or Rocky Mountain spotted fever Rickettsiae is not satisfactory, while the Weil-Felix agglutination with Proteus OX 19 may indicate the presence of a Rickettsial disease, but a differentiation between typhus and Rocky Mountain spotted fever can not be made. It is for these reasons that a specific complement fixation reaction may be of value in distinguishing these two diseases.

Castaneda[1] prepared an antigen from the peritoneal washings of x-rayed rats inoculated with endemic Rickettsiae and reported positive complement fixation in typhus fever. Bengtson[2] described a specific complement fixation reaction for "Q" fever and endemic typhus, using an antigen prepared with Rickettsiae grown in the yolk sac of developing chick embryos (Cox method).[3] No satisfactory antigen for Rocky Mountain spotted fever had previously been prepared and it is the purpose of this communication to describe a specific complement fixation test which may be used in this disease.

The sera used in the tests were obtained from recovered human cases of Rocky Mountain spotted fever, "Q" fever and Brill's disease and from guinea pigs recovered from infections with Rocky Mountain spotted fever, endemic typhus, European typhus and "Q" fever. As controls, human sera were obtained from several different febrile diseases, strongly Wassermann positive sera, as well as normal sera. Likewise, normal guinea pig sera were used.

Two antigens, which were equally satisfactory, were prepared from tissue cultures grown by the semi-solid agar method in Kolle flasks.[4,5] The cultures were initiated with infected guinea pig spleen and grown in contact with ten-day chick embryo cells. Transplants were made every seven days by scraping off the infected cells from the surface of the medium and grinding in a tube with alundum. This material was again brought in contact with normal chick embryo cells. After several transplants, the cultures became exceedingly rich, so that a considerable number of Rickettsiae were seen in every field. The antigens employed were made from the 25th to the 35th transplant. Five cubic centimeters of a 1:10,000 merthiolate solution in saline was used to wash off the cells from each Kolle flask. When a certain amount of material was obtained, the Rickettsiae were extracted in the following manner. One batch was ground up in the Waring Blender and then frozen and thawed five times. The other was shaken in a large Pyrex flask with glass beads. The Rickettsial suspensions were then centrifuged in an angle centrifuge at 1,500 r.p.m. for 30 minutes. The supernatant fluid was removed and centrifuged at 4,500 r.p.m. for one hour. The sediment was then resuspended in 0.85

[1] M. Castaneda, *Jour. Immunology*, 31: 285–291, 1936.
[2] I. Bengtson, *Proc. Soc. Exp. Biol. and Med.*, 46: 665–668, 1941; *Pub. Health Reports*, 56: 649–653, 1941; *Pub. Health Reports*, 56: 1723–1727, 1941.
[3] H. R. Cox, *Pub. Health Reports*, 53: 2241–2247, 1938.
[4] Zinsser, Fitzpatrick and Wei, *Jour. Exp. Med.*, 69: 179, 1939.
[5] Zinsser, Plotz, Enders, SCIENCE, 91: 51–52, 1940.