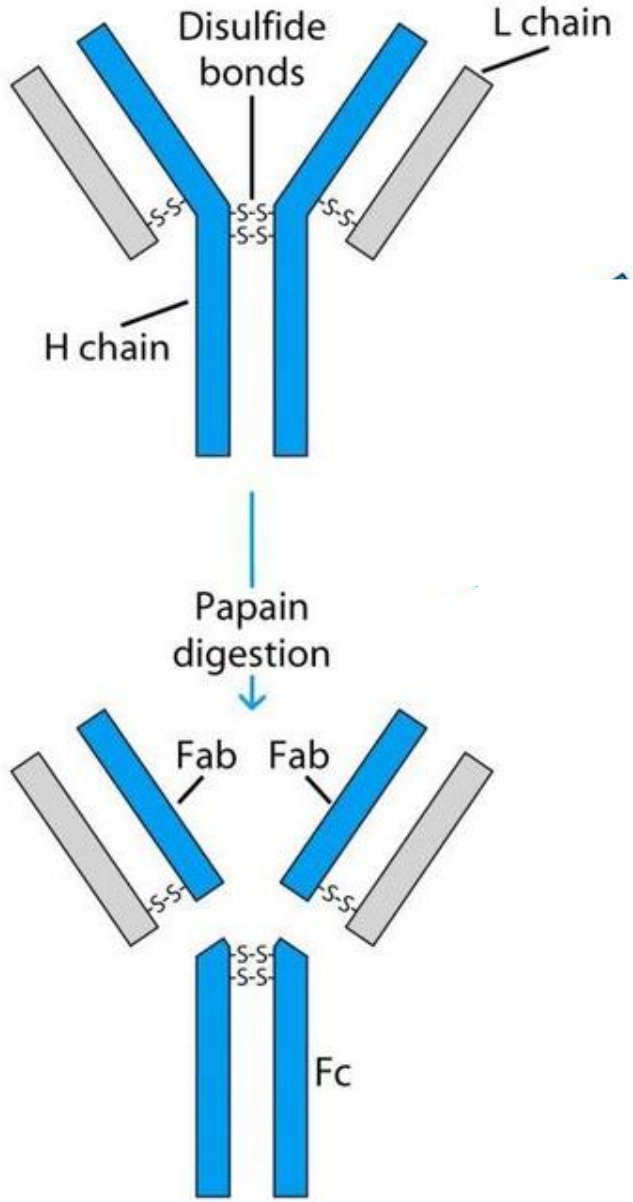
Chemical and enzymatic methods that revealed antibody structure

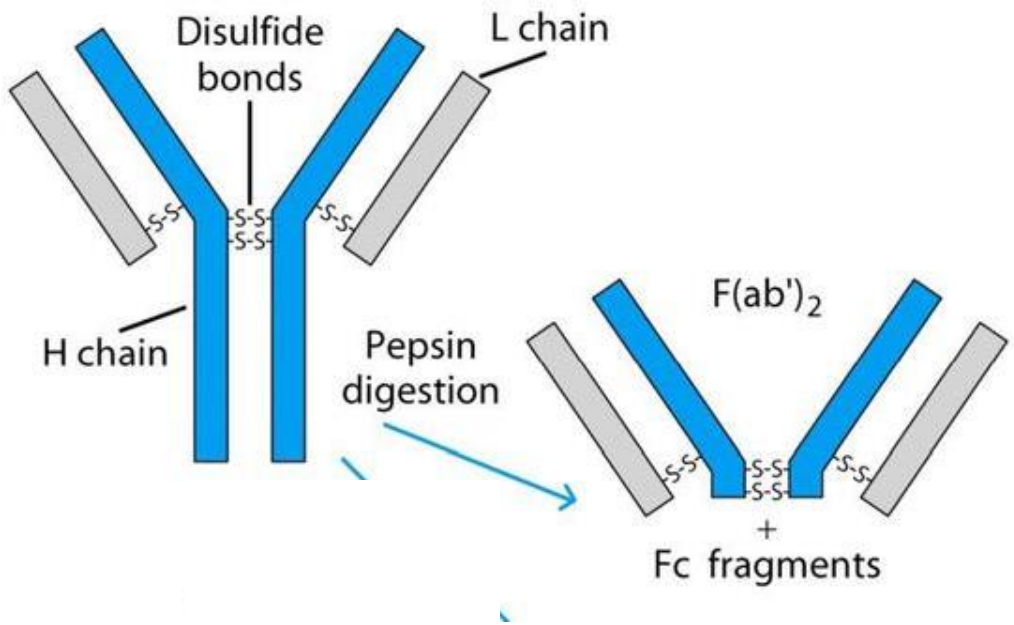
Papain digestion

- digestion of IgG with the enzyme papain produced three fragments,
- two of which were identical fragments and a third that was different .
- The two identical fragments(each with a MW of 45,000), had antigen-binding activity and were called **Fab fragments** (“fragment, antigen binding”).
- The other fragment (MW of 50,000) had no antigen binding activity at all. Because it was found to crystallize during cold storage, it was called the **Fc fragment** (“fragment,crystallizable”).


Disulfide bonds
L chain
H chain
Papain digestion
Fab
Fab
Fc

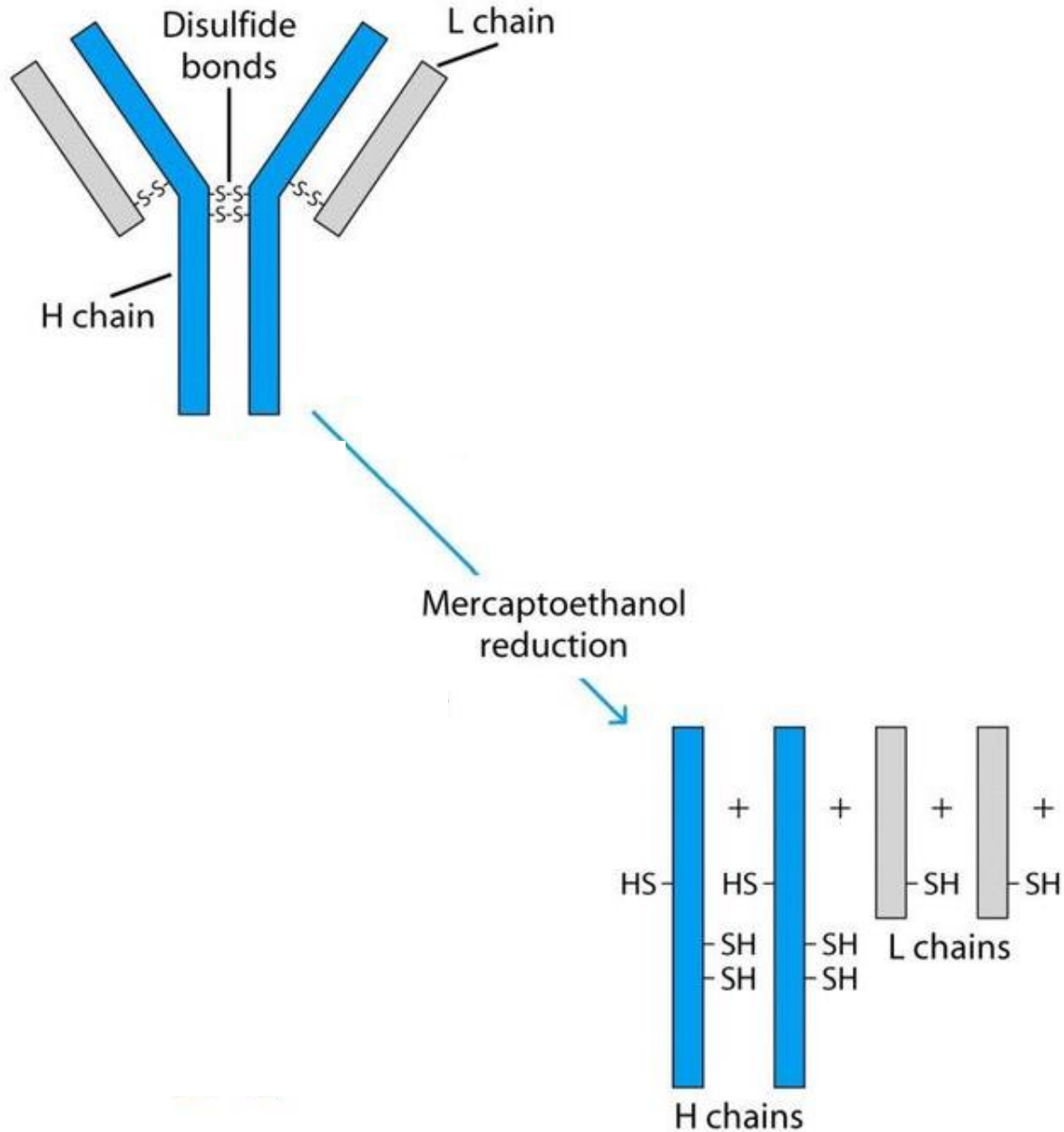
Pepsin digestion

- Digestion with pepsin, a different proteolytic enzyme, also demonstrated that the antigen-binding properties of an antibody can be separated from the rest of the molecule.
- Pepsin digestion generated a single 100,000-MW fragment composed of two Fab-like fragments designated the **F(ab)2 fragment,** which binds antigen.
- The Fc fragment was not recovered from pepsin digestion because it had been digested into multiple fragments.

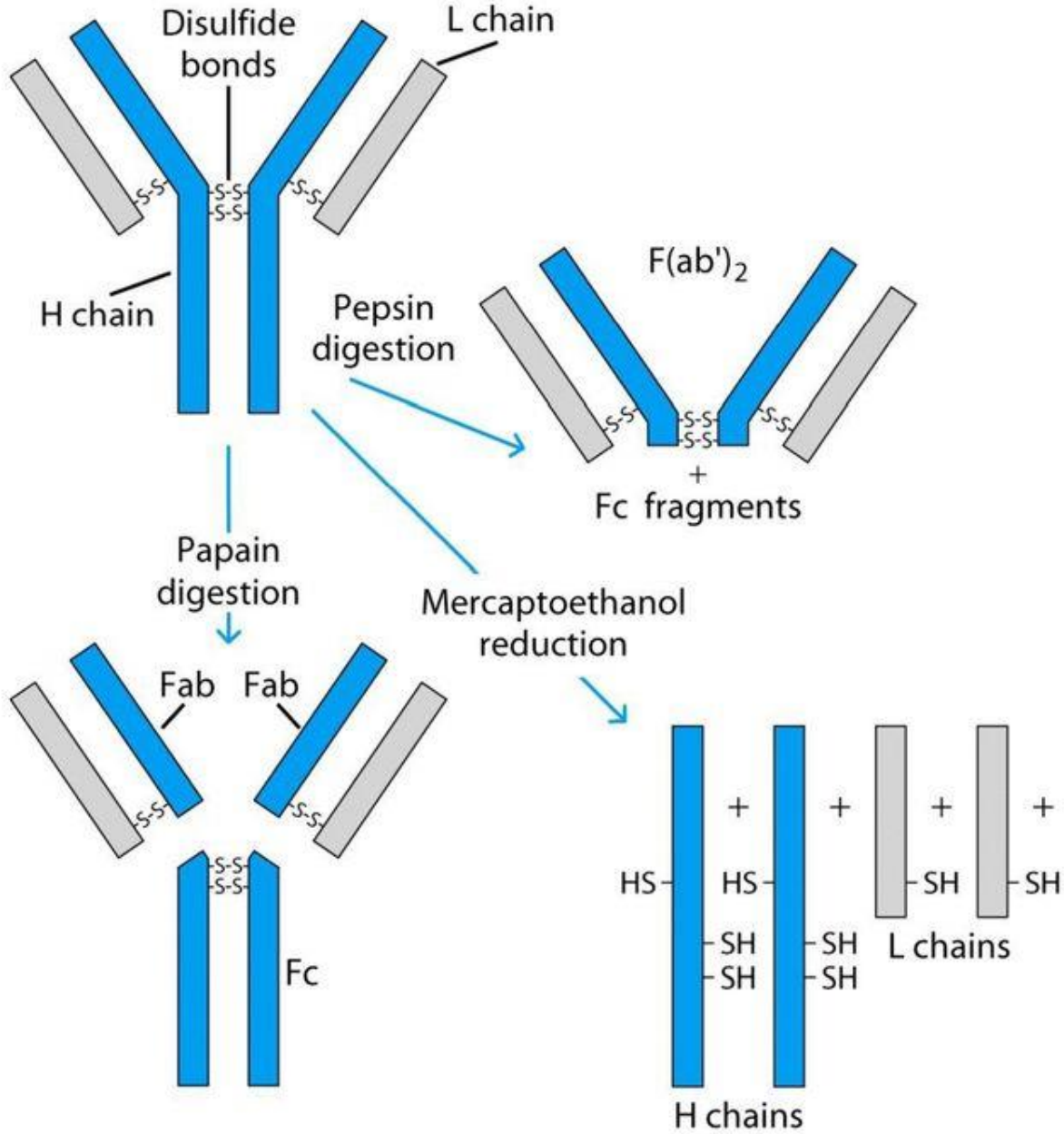

Disulfide
bonds
L chain
H chain
Pepsin
digestion
F(ab')2
+
Fc fragments

mercaptoethanol reduction and alkylation

- chemical treatment that irreversibly cleaves disulfide bonds
- If the sample is chromatographed on a column that separates molecules by size following cleavage of disulfide bonds, it is clear that the intact 150,000-MW IgG molecule is, in fact, composed of subunits.
- Each IgG molecule contains two 50,000-MW polypeptide chains, designated as heavy (H) chains, and two 25,000-MW chains, designated as light (L) chains


Disulfide bonds
L chain
H chain
Mercaptoethanol reduction
HS
HS
SH
SH
SH
SH
SH
SH
L chains
H chains

using antisera from goats that
had been immunized with either the Fab fragments or the Fc
fragments of rabbit IgG. The antibody to the Fab fragment
could react with both the H and the L chains, whereas antibody
to the Fc fragment reacted only with the H chain. These
observations led to the conclusion that the Fab fragment
consists of portions of a heavy and a light chain and that Fc
contains only heavy-chain components. From these results,
and those mentioned above, the structure of IgG was identified
. According to this model, the IgG
molecule consists of two identical H chains and two identical
L chains, which are linked by disulfide bridges. The enzyme
papain cleaves just above the interchain disulfide bonds linking
the heavy chains, whereas the enzyme pepsin cleaves just
below these bonds, so that the two proteolytic enzymes generate
different digestion products. Mercaptoethanol reduction
and alkylation allow separation of the individual heavy
and light chains.


Disulfide bonds
L chain
H chain
Pepsin digestion
F(ab')2
+
Fc fragments
Papain digestion
Mercaptoethanol reduction
Fab
Fab
Fc
HS
HS
SH
SH
SH
SH
SH
SH
L chains
H chains