Experimental Inhibition of Complement

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With 3 Figures

Four methods are available to elucidate the biological significance of the serum-complement system:

1. The demonstration of complement components at the site of the immunological event.
2. Investigation with animals possessing a genetic C defect.
3. Experiments with anticomplement substances and
4. Turnover studies with individual C-components.

We have been interested for some years now in our institute in the inhibition of serum-complement in animal experiments. The motive for these investigations came from experiments designed to study the mechanism of action of heparin. We had found that in animal experiments heparin possessed marked anti-inflammatory properties (1962). It was found, however, that the long established complement inhibiting component of heparin is without significance in the usual chemically induced inflammation models. Also, a fall in serum complement occurs in vivo only at doses at which blood coagulation is already inhibited. The duration of action is very short. Two hours after intraperitoneal injection the complement is again within the normal range. In all these experiments the total complement i.e. the total haemolytic activity of the serum was determined by applying the usual immune-haemolytic methods to the sera obtained by cardiac-puncture using sheep cells and rabbit antiserum.

At one time, working together with H. Fischer and H. G. Siedentopf (1965) we determined the site of action of heparin and also other heparinoids within the complement chain. We found that heparin inhibits the first component of serum complement. At the same time, Borsos, Rapp and Crisler (1965) found that carrageenin, also a heparinoid, inhibits the first complement component by forming heparinoid precipitates with C1. Also they found in vivo an inhibition of complement only at doses at which bleeding time was already considerably prolonged. One must, therefore, conclude that heparin and the heparinoids are not suitable as inhibitors for studies on the biological action of total complement.

We have tested nearly all known in vitro active complement inhibitors in animal experiments, either on the rat or on the guinea pig. These substances were either too toxic for animal tests, or they showed no action.

The action of aggregated human γ-globulin on the serum complement level was of too short duration to carry out animal experiments. Also copper-chlorophyllin which, according to the literature, is active in vivo proved to be too weak in action.
We were not able to examine the C3 inactivating factor which Nelson isolated from cobra venom. In the search for an anticomplement substance which is also active in vivo and non-toxic, we then tested a whole series of new substances both in vitro and in vivo. Some high molecular components made available from our Leverkusen Chemical Laboratories by Dr. Pieper proved to be of particular interest.

Among these the sulphated high-polymers all possessed anticoagulant properties. This type of compound was not investigated further. It was finally found that the poly-N-oxide of the nicotinic acid ester of polyvinyl alcohol was the most active.

\[
\begin{align*}
(-\text{CH}_2\text{-CH}_2\text{-})_n \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{N} & \rightarrow \text{O}
\end{align*}
\]

This substance which was only just soluble in water was highly active in the rat at doses of 10 to 20 mg/kg. A fall in total haemolytic activity of serum complement occurred barely 1 h after i.p. injection, being optimal between 2 to 3 h. After about 6 h the complement level was again normal. This effect can be repeated any number of time by renewed administration; we treated animals up to 3 weeks with one or two daily injections.

![Graph](https://via.placeholder.com/150)

**Fig. 1. Complement inhibition in rats. Male Wistar I rats (breeder Winkelmann, Paderborn) weighting 120 to 140 g, were injected 10 resp. 20 mg/kg Poly-N-oxide of the nicotinic acid ester of polyvinylalcohol (PVA-NA). Each point represents the C-activity of the pooled sera of 10 animals. Estimation of whole C according to Kabat and Mayer**

To some extent it is possible in this way to achieve 100% inhibition of serum complement, i.e. the residual activity lies within the deviation of our method. Such a marked inhibition can, however, only be obtained with the rat. With the guinea-pig we achieved at the most a 50% reduction of serum complement. Increasing the dose further was without effect. We were unable to influence complement levels in the rabbit, dog, and in man — as we discovered later.

The substance which we first used still consisted of a mixture of fractions with a very variable molecular weight. After further separation and purification, it was