Table 2

Sample of insulin	C (gm./l.)	S^{W}_{20} (svedbergs)
Dissolved directly	5·1	$3·3_8$
	2·9	$2·9_8$
Recovered from N,N-dimethyl-formamide	5·1	$3·1_5$
	2·5	$2·8_4$

Although minimal molecular weights of 6,000 and 12,000 have been reported, there is no inconsistency necessarily involved. The value of 12,000 has been found in aqueous solutions, in which it is not unlikely that the dimer persists. Two reports of the value 6,000 have now been obtained independently by Harfeneist and Craig[3] and in this communication, in non-aqueous solvents, in which the aggregation reactions are apparently less favoured, and the true minimal molecular weight is observed.

This work is part of a study of the solubilities and properties of proteins in non-aqueous solvents, which will be reported in detail elsewhere.

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Inhibition of Complement Activity by Di-*isopropyl* Fluorophosphate

It is generally agreed that di-*iso*propyl fluorophosphate acts specifically to inhibit enzymes with esterase activity[1]. In the light of this conclusion, the finding that it inactivates guinea pig complement (C') is felt worthy of this brief communication. (In a personal communication, Dr. Laurence Levine reported that he has independently observed this same finding.)

When di-*iso*propyl fluorophosphate in concentrations of $8·0 \times 10^{-3}$ to $1·0 \times 10^{-4}$ M was incubated with diluted or undiluted guinea pig serum up to 4 hr. at 37° C., or overnight at 26° or 4°, and then the serum diluted and tested for complement activity, no effect of the fluorophosphate could be detected. When, however, $8·0 \times 10^{-3}$ M di-*iso*propyl fluorophosphate was incubated for ½ hr. at 37° with varying concentrations of 1–100 diluted guinea pig serum in the presence of sheep cells sensitized with rabbit hæmolysin, 75–80 per cent inhibition of the hæmolytic activity of complement was noted. In the same circumstances, $3·5 \times 10^{-3}$ M di-*iso*propyl fluorophosphate gave 10 per cent inhibition. On testing the dialysed supernatants from the latter reaction for the presence of the four components ($C'1$, $C'2$, $C'3$, $C'4$) of complement by the usual methods[2], only the first component ($C'1$) was found to have been appreciably inactivated.

That the effect of di-*iso*propyl fluorophosphate is on $C'1$ was confirmed by using sensitized sheep cells containing the first, second and fourth components (*Ea* $C'1$, 4, 2). These were prepared as described by Levine *et al.*[3] and incubated overnight at 20° with di-*iso*propyl fluorophosphate, then washed, resuspended in buffer and tested for activity with reagents specific for the respective components. As can be seen in Table 1, the concentrations required for inhibition of the $C'1$ activity of these cells varied from $2·7 \times 10^{-3}$ to $8·3 \times 10^{-5}$ M. This is considerably less than the molar concentrations ($8·0 - 3·5 \times 10^{-3}$) required for inhibition of C' for whole serum. The $C'4$ activity was not affected by this treatment. The effect of di-*iso*propyl fluorophosphate on $C'2$ could not be tested, since $C'2$ was spontaneously inactivated on standing in buffer alone. Partial reversal of the inhibition of $C'1$ activity could be obtained by allowing the cells treated with fluorophosphate to stand overnight in the presence of $0·1$ M nicotinohydroxamic acid[4].

Table 1. Inhibition of $C'1$ on *Ea*C' 1, 4, 2, incubated with Di-*iso*-propyl Fluorophosphate

$1·0 \times 10^8$ cells/ml. were incubated at 30° C. overnight with saline–veronal–bicarbonate buffer to which was added the di-*iso*propyl fluorophosphate, $0·001$ M calcium ions, $0·003$ M magnesium ions, and $0·04$ M imidazole buffer *p*H 7·6. The cells were then centrifuged, washed, resuspended in buffer and reacted with R_1, the reagent for testing the $C'1$ reaction (ref. 2).

Molar concentration of DFP ($\times 10^5$)	270	41	21	8·3	0
Time (min.) required to attain 50 per cent hæmolysis	>30	29	9	4	1

From this it is concluded that the first component of complement $C'1$ is an enzyme with esterase activity which exists in serum in a precursor form resistant to the action of di-*iso*propyl fluorophosphate. In the course of the immune hæmolysis reaction, the $C'1$ precursor is changed to an active form of $C'1$ which is now capable of being inhibited by the fluorophosphate. The $C'1$ on the 1, 2, 4 cells apparently exists in such an active form, or was possibly changed into such an active form, during incubation with the fluorophosphate.

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Occurrence of an L(+)-Alanine-Dehydrogenase in *Bacillus subtilis*

The metabolic analysis of mutant strains of *B. subtilis* has shown that the ability to use the ammonium ion as the sole source of nitrogen for growth corresponds to the presence of a glutamic-dehydrogenase[1]. However, a mutant unable to use the ammonium ion (strain S^-), lacking glutamic-dehydrogenase, oxidizes glutamic acid very actively. This strain may grow, with aeration, in a culture medium containing: $l(+)$-glutamate $0·01$ M, ammonium sulphate $0·01$ M, phosphate buffer (KH_2PO_4–Na_2HPO_4) $0·02$ M, *p*H 7·5, sodium chloride $0·01$ M, potassium sulphate $0·01$ M, magnesium sulphate $0·001$ M and ferric citrate $0·0004$ M.