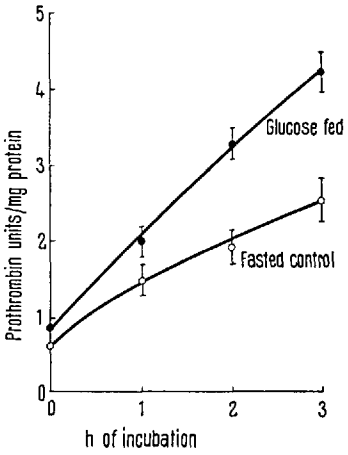
groups were homogenized and the isolated heavy microsomes were incubated as described above. The graph shows that prothrombin activity accumulated much more rapidly in the microsome preparations made from the carbohydrate-fed animals than in the microsome prepared from fasting animals. The vertical bars show the standard errors for variation in replicate experiments; this indicates that the rate of prothrombin accumulation in glucose-fed animals is significantly greater than in the control series.



Prothrombin activity of rat liver microsomes during incubation in Krebs-Ringer solution. The results are the mean of three experiments in which one group of rats were kept fasting and the other group received glucose 2 h before killing.

These findings suggest that intracellular energy supply may influence subsequent prothrombin release from microsomes. The factor involved was not identified. Although level of energy intake has a considerable effect on the ATP concentration in the liver[6], addition to the incubation of ATP, ADP, NAD and NADH at concentrations between 0.5 and 1.5 μmoles/ml of incubation medium inhibited prothrombin accumulation. Addition of NADP and NADPH did not affect prothrombin increment during incubation. Addition of dinitrophenol to the medium and incubation in the presence of nitrogen in place of oxygen had no effect for the first 2 h of incubation, and were somewhat inhibitory during the third hour.

Résumé. Les microsomes isolés du foie continuent à relâcher le prothrombin pendant l'incubation de façon désavantageuse pour la synthèse totale des protéines. L'accumulation est plus rapide quand les rats, soumis à un jeûne bref, étaient nourris de glucose avant le sacrifice.

H. N. Munro and P. Goswami

Institute of Biochemistry, The University, Glasgow (Scotland), October 15, 1964.

[6] C. M. Clark, G. A. J. Goodlad, J. Chisholm, and H. N. Munro, Nature *186*, 719 (1960).

Attempts to Isolate C'_3 Activity from Pig Serum

In a previous investigation (Cavallo, Pontieri, and Imperato[1]) the ability of lysozyme to reconstitute the lytic activity of a reagent used for the titration of the activity of the third component of complement (C'_3), prepared with 'Liquoid' (sodium polyanethol sulphonate, Hoffmann-La Roche) treated serum was demonstrated.

Researches on the mechanism of this activity, played by either lysozyme or other basic proteins (Pontieri, Imperato, and Cavallo[2]), showed that the phenomenon was due to the formation of complexes between basic proteins and 'Liquoid' fixed in the reagent to the serum globulins of C'_3 activity furnished. The displacement of the inactivating agent from these determined the appearance of C'_3 activity which was inhibited in the reagent.

In the course of the above-mentioned work, it was observed that formation of insoluble complexes, which precipitate, takes place when 'Liquoid' is added to either guinea-pig or human serum. It seemed, therefore, of interest to determine whether or not C'_3 was carried down in the precipitate and, if such were the case, whether or not the recovery of C'_3 activity from the precipitate itself was possible.

Pig serum was selected as source of C'_3 because of its high content in this component of complement.

Figure 1 shows the degree of precipitate formation which takes place in pig serum when added with various amounts of 'Liquoid'. For all the further determinations 600 μg of 'Liquoid' were constantly added to each ml of pig serum.

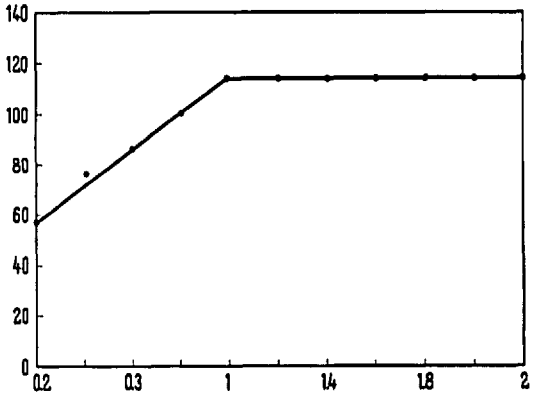


Fig. 1. Formation of insoluble complexes following addition of *'Liquoid'* to pig serum. Abscissae μg of *'Liquoid'*/ml pig serum. Ordinates: turbidimetric Klett Units.

[1] G. Cavallo, G. Pontieri, and S. Imperato Exper. *19*, 36 (1963).
[2] G. Pontieri, S. Imperato, and G. Cavallo G. Microbiol. *10*, 93 (1962).

In order to examine the precipitate for the presence of components of complement (C′), this was washed twice with veronal buffer (MAYER, CROFT, and GRAY[3]) and brought into solution by addition of 0.1 *N* NaOH, followed by neutralization with 0.1 *N* HCl. The resulting solution (S) was tested for hemolytic activity on sensitized erythrocytes (EA) and on partially complemented cells ($EAC'_{1,4,2}$) in the presence of 0.01 *M* EDTA. Red cells in the state of $EAC'_{1,4,2}$, when suspended in a buffer containing a chelating agent such as EDTA, undergo lysis only after fixation of the component of C′ that can be fixed to sensitized erythrocytes in the absence of cations (C'_3). The supernatant S resulted inactive in promoting lysis of both EA and $EAC'_{1,4,2}$: such inactivity could be dependent on (a) absence or (b) inactivation of C′ components.

It was therefore decided to observe whether or not the addition of lysozyme to the suspension of the precipitate formed when 'Liquoid' was added to pig serum, would be followed by appearance of hemolytic activity with the mechanism previously observed with the whole serum inactivated by 'Liquoid'.

Various amounts of lysozyme were then added to different samples of washed precipitate and the mixtures were incubated for 15 min at 37°C. After centrifugation at +4°C, the supernatants (S_1) were tested for C'_3 activity on $EAC'_{1,4,2}$ in the presence of EDTA.

Figure 2 summarizes the results showing that the recovery of C'_3 activity is proportional to the amount of lysozyme added. On the other hand, it was necessary to ascertain whether or not other components of C′ were brought into the precipitate and then reactivated by lysozyme.

The Table shows that the supernatant obtained after treatment of the precipitate with Fleming's enzyme is inactive in promoting lysis of EA in the absence as well as in the presence of reagents for the titration of C'_1, C'_2 and C'_4 (R_1, R_2 and R_4). Lysis is, however, obtained when the supernatant (S_1) is added to EA in the presence of R_3. Three different reagents for the titration of C'_3 were used, respectively prepared with pig serum inactivated with zymosan (insoluble residue of yeast cell-wall), 'Liquoid', and formaldehyde. PONTIERI and PLESCIA reported in another paper[4] results suggesting the use of different reagents for the titration of C'_3 activity in a serum under investigation. Methods for the titration of activity of whole C′ as well as of its components are described by PONTIERI, IMPERATO, and CAVALLO[2].

Experiments for the purification of C'_3 obtained with this method are under way, as well as attempts to ascertain which are the components of the C'_3 complex present in the precipitate.

It is relatively easy to deprive a given serum of the activity of one component of C′, leaving almost unmodified the activity of the others, and such a specific inactivation is routinely used for obtaining the so-called reagents which, following BIER et al.[5], are used for the titration of the components of C′. On the other hand, when a certain reagent is exposed to the procedure used for the preparation of another reagent, a serum lacking in two of the components results. It is difficult, however, to obtain inactivation of three components, leaving the serum with the activity of only one. By use of the present method, it is possible easily to obtain C'_3 isolated from the activity of other components of C′.

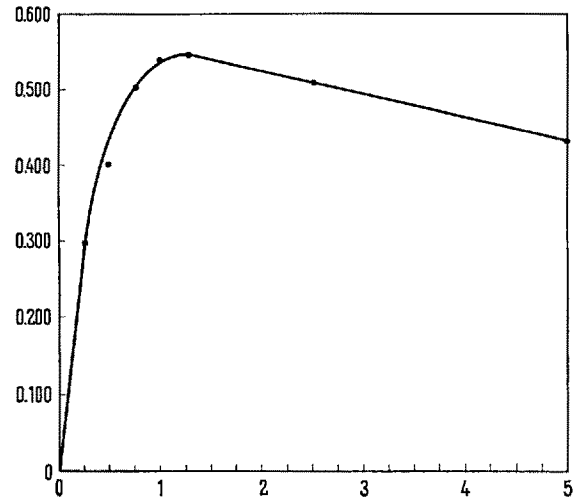


Fig. 2. Hemolytic activity of S_1 (supernatant obtained by addition of lysozyme to samples of suspension of precipitate formed by treatment of pig serum with *'Liquoid'* 600 μg/ml). Abscissae: mg of lysozyme/ml of precipitate suspension. Ordinates: Optical Density at 5410 Å of the supernatants of hemolytic systems constituted by 0.2 ml $EAC'_{1,2,4}$ (1.10^9 cells/ml) + 1 ml S_1 + 1.4 ml veronal buffer.

C'_3 activity in the supernatant (S_1) obtained after addition of lysozyme to a suspension of precipitate formed when pig serum was treated with *'Liquoid'* (600 μg/ml)

Reactants	Optical density at 5410 Å
S_1 + $EAC'_{1,4,2}$ (EDTA)	0.600[a]
S_1 + EA	0.020
S_1 + EA + R_1	0.030
S_1 + EA + R_2	0.025
S_1 + EA + R_3 (*'Liquoid'*)	0.600
S_1 + EA + R_3 (Formaldehyde)	0.600
S_1 + EA + R_3 (Zymosan)	0.600
S_1 + EA + R_4	0.020

[a] At complete lysis of the red cells the optical density was 0.600.

Riassunto. Gli autori descrivono un metodo per l'isolamento del terzo componente del complemento dal siero di maiale, basato sulla possibilità di provocare, per aggiunta di lisozima, passaggio in soluzione del C'_3 precipitato insieme ad altre proteine in seguito all'aggiunta di opportune quantità di «Liquoid» al siero.

G. M. PONTIERI, M. COTRUFO, F. CICCIMARRA, and G. TOLONE

Istituto di Patologia Generale, Università di Palermo (Italy), September 10, 1964.

[3] M. M. MAYER, C. C. CROFT, and M. M. GRAY, J. exp. Med. *88*, 427 (1948).
[4] G. M. PONTIERI and O. J. PLESCIA, Exper. *21*, 81 (1965).
[5] O. G. BIER, G. LEYTON, M. M. MAYER, and M. HEIDELBERGER, J. exp. Med. *81*, 449 (1948).