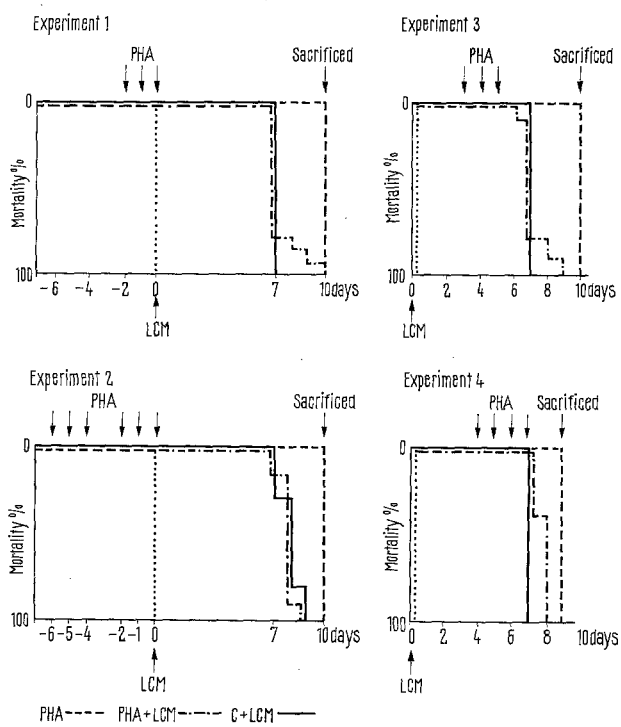
typical neurological symptoms of the virus infection were apparent after the usual incubation period in the mice of the PHA + LCM group also, and the animals died at the same rate as those of the infected control (C + LCM) group, on the 7th–9th days. Histological analysis showed in their brain the typical symptoms of lymphocytic choriomeningitis. In the non-infected groups treated with PHA none of the animals were lost, they were apparently healthy and no loss of weight could be observed as compared with the controls. The phytohaemagglutinin treatment did not cause any appreciable change of the number of circulating lymphocytes. Having lost the animals infected with the LCM virus, the mice of the PHA and control groups were sacrificed. *Splenomegaly* was observed in the mice which were killed 2 days after the administration of phytohaemagglutinin (experiment 4). *Splenomegaly* could not be shown in the animals which were sacrificed on the 5th–10th days after the astl phytohaemagglutinin injection (experiments 1, 2 and 3). This observation is consistent with the earlier findings which showed *Splenomegaly* to be the most pronounced on the 3rd day after the phytohaemagglutinin injection[9,10].



Mortality rate of mice inoculated with LCM virus and treated with phytohaemagglutinin.

It turned out from the present experiments that the phytohaemagglutinin treatment did not affect the development of intracerebral LCM virus infection, the animals died after exhibiting the typical neurological symptoms and in the cerebral tissues the histological characteristics of lymphocytic choriomeningitis could be identified. Our earlier observations as well as those of other authors have shown that there exists a relationship between the neurological symptoms of the LCM virus infection with subsequent development of the fatal hypersensitivity reaction and the number of circulating lymphocytes[6,8,11]. Since the phytohaemagglutinin treatment does not induce any permanent decrease in the number of circulating lymphocytes, this may be the reason why the development of fatal lymphocytic choriomeningitis could not be suppressed by the phytohaemagglutinin treatment.

The results of the present investigation, which showed that the phytohaemagglutinin treatment does not suppress the immunological reactions of mice to intracerebral LCM virus infection, confirm the opinion of those who do not believe in the immunosuppressing effect of phytohaemagglutinin.

Zusammenfassung. Es wurde festgestellt, dass die Virusinfektion in mit LCM-Virus intrazerebral infizierten Mäusen bei Phytohämagglutinin-Behandlung gleich verläuft wie in unbehandelten Kontrolltieren. Dies stützt die Annahme, Phytohämagglutinisierung vermindere die immunologische Reaktivität des Organismus nicht.

Zs. Bános, I. Szeri,
P. Anderlik and B. Radnai

Institute of Microbiology, University Medical School and Pathology Department, 'István' Hospital, Budapest IX (Hungary), 30 June 1969.

[9] C. N. Gamble, Blood *28*, 175 (1966).
[10] L. B. Epstein and C. W. Smith, J. Immun. *100*, 421 (1968).
[11] J. Hotchin, Cold Spring Harbor Symp. quant. Biol. *27*, 479 (1962).

Differences Between Plasma and Serum Mediated Chemotaxis of Leukocytes

We have recently shown in experiments in vitro that sera from normal rabbits contain chemotactic activity for both macrophages and neutrophils[1]. The specific activity in normal sera for macrophages was due to a fraction with the approximate molecular weight of 200,000, whereas the distinct main activity for neutrophils was located in a fraction with a molecular weight between 5000 and 35,000. Such low molecular weight neutrophil cytotaxins have also been observed by other workers and were found to be split products of complement components[2–4].

So far no explanation has been put forward for the variable presence of chemotactic activity in normal serum. The question arises whether these cytotaxins are already present in plasma or whether they are formed during the blood clotting process. In the latter case it is likely that a link between chemotaxis, blood coagulation and complement activation exists.

[1] P. C. Wilkinson, J. F. Borel, Vera J. Stecher-Levin and E. Sorkin, Nature *222*, 244 (1969).
[2] S. Shin, R. Snyderman, E. Friedmann, A. Mellors and M. Mayer, Science *162*, 361 (1968).
[3] P. A. Ward, C. G. Cochrane and H. J. Müller-Eberhard, Immunology *11*, 141 (1966).
[4] V. A. Bokisch, H. J. Müller-Eberhard and C. G. Cochrane, J. exp. Med. *129*, 1109 (1969).

We have shown earlier, that reaction of normal sera with immune complexes results in an appreciable increase in chemotactic activity for neutrophils and a concomitant decrease for macrophages[1]. The present note reports on numerous experiments comparing the chemotactic activity of plasma and serum for macrophages and neutrophils both in the presence and absence of immune complexes.

Chemotaxis was measured by the method of BOYDEN[5] as modified by KELLER[6]. Chemotactic activity present in individual samples and in pools of normal rabbit serum (NRS) and normal rabbit plasma (NRP) was evaluated both before and after treatment with immune complexes consisting of a 2% mixture of HSA and rabbit anti-HSA serum at equivalence. Neutrophils were obtained from the peritoneal cavity of rabbits after injection of 40 ml of a 3.5% solution of sodium caseinate at pH 7 in physiological saline. The peritoneal exudate was collected $3^1/_2$ h later and contained an average of 98% neutrophils. Macrophages were obtained by one i.p. injection of 50 ml of sterile heavy paraffin oil. The exudate cell population collected 4 days later consisted of approximately 85% large mononuclear cells, the remaining cells being mainly neutrophils.

Table I shows the chemotactic activity for rabbit neutrophils and macrophages present in NRP and NRS before and after treatment with immune complexes. The numbers constitute the average figures of several experiments using 6 different rabbits as plasma and serum donors; citrated as well as heparinized NRP and also NRS from individual rabbits were compared. The results obtained with several pools of NRP and NRS agreed well with those from samples of individual animals.

The results shown in this Table indicate a number of points: (a) plasma alone is not chemotactic for either cell type; (b) serum alone is chemotactic for both types of leukocytes; (c) immune complexes generate chemotactic activity in plasma for both cell types; (d) immune complexes increase the serum activity for neutrophils only and decrease the activity for macrophages.

Neither citrated nor heparinized plasma showed any activity for neutrophils or macrophages. On the other hand, plasma incubated with immune complexes revealed a strong chemotactic effect for both types of leukocytes. Among the 13 NRP tested, we found only 1 sample drawn from an individual animal, which after treatment with immune complexes failed to be enhanced in its activity for macrophages. Conversely, NRS alone contained a low to sometimes moderate activity for neutrophils, while it usually had a marked chemotactic effect on mononuclear cells. The treatment of NRS with immune complexes always led to an impressive increase of neutrophil chemotaxis as compared to NRS alone. In marked contrast to this stands the 'naturally-occurring' high activity of NRS for macrophages, which was not enhanced by activation with immune complexes but regularly significantly depressed. Only 1 exception out of 20 NRS samples was encountered in this series of experiments when serum from a single normal rabbit could repeatedly be shown to contain a strikingly increased chemotactic activity for macrophages after its treatment with immune complex. These clearcut results and our previous ones[7] do not agree with those published by WARD[8], who reported generation of chemotactic activity for mononuclear cells after treatment of NRS with immune complexes. The finding is also noteworthy that the small minority of neutrophils present in the mixed cell population of oil-induced exudates migrated towards the antigen-antibody activated serum just as well as the neutrophils present in almost pure granulocyte exudates elicited with sodium caseinate. Thus macrophages, which migrated little under such conditions, did not influence the chemotactic behaviour of the neutrophils adversely.

It has been shown elsewhere[9] that incubation of the granular fraction from neutrophils with normal serum resulted in a high chemotactic activity for neutrophils. As can be seen from Table II this does not hold true for macrophages. A pronounced chemotactic activity for macrophages is produced, however, if plasma instead of serum is incubated with the same granular fraction from neutrophils. Granules and immune complexes seem thus to exert similar effects on plasma resulting in generation of factors which are chemotactic for macrophages. Whether the mechanism underlying this cytotaxin formation is the same is not known.

The question as to the origin of the chemotactic activity for macrophages and neutrophils in normal serum is of interest. Since plasma showed no such activity it is likely

Table I. Chemotactic activities for rabbit neutrophils and macrophages before and after activation of plasma and serum with immune complex (HSA/anti-HSA)

Agents in Gey's solution	Neutrophils per field	Macrophages per field
Controls		
Gey's solution	0	1
Immune complex (2%)	1	5
Citrated plasma		
Plasma alone (10%)	0	0
Plasma + immune complex	303	49
Heparinized plasma		
Plasma alone (10%)	0	0
Plasma + immune complex	220	36
Serum		
Serum alone (10%)	62	104
Serum + immune complex	344	45

The 2 plasmas (citrate and heparin) and the serum are from the same rabbit.

Table II. Chemotaxis of peritoneal macrophages induced by neutrophil granules in the presence of plasma and serum

Agents in Gey's solution	Macrophages/field
Controls	
Gey's solution alone	0
Granular fraction of neutrophils (50 γ protein/ml)	6
Heparinized rabbit plasma	
Plasma alone (10%)	4
Plasma + granular fraction of neutrophils	70
Normal rabbit serum	
Serum alone (10%)	27
Serum + granular fraction of neutrophils	26

[5] S. V. BOYDEN, J. exp. Med. *115*, 453 (1962).
[6] H. U. KELLER, Immunology *10*, 225 (1966).
[7] H. U. KELLER and E. SORKIN, Int. Arc. Allergy *31*, 575 (1967).
[8] P. A. WARD, J. exp. Med. *128*, 1201 (1968).
[9] J. F. BOREL, H. U. KELLER and E. SORKIN, Int. Arch. Allergy *35*, 194 (1969).

that it is generated during blood coagulation. It would seem an attractive hypothesis that the proteolytic enzymes plasmin or thrombin formed during blood coagulation might be involved in the generation of this serum chemotactic activity. Indeed, it has already been shown that plasmin splits complement components into chemotactically active agents[4,10]. Alternatively, one may assume that analogous to our findings with chromatographed serum, chemotactic factors are already present in plasma, but masked by inhibitors or otherwise, or that they are unmasked by their interaction with the immune complexes[1].

The relation of these findings in vitro to the accumulation of granulocytes and macrophages in inflammatory sites is unknown. However, immune complex induced accumulation of both neutrophils and macrophages does occur in vivo. Since the accumulation of the 2 cell types has been shown to be independent of each other[11,12], it is tempting to speculate that the interaction of plasma with antigen-antibody complexes results in the formation of different cytotaxins specific for both types of leukocytes.

In conclusion, the chemotactic activity of serum differs from that of plasma in the following ways: (1) Normal serum contains chemotactic factors for both neutrophils and macrophages. Normal plasma has no chemotactic activity for either neutrophils or macrophages. (2) If fresh serum is incubated with antigen-antibody complexes, a great rise in neutrophil chemotactic activity is seen but a drop is observed in macrophage chemotactic activity. On the other hand, the incubation of plasma with antigen-antibody complexes causes a rise in activity for both neutrophils and macrophages. Neutrophil granules upon incubation with plasma generate chemotactic activity for macrophages, but not upon incubation with serum[13,14].

Zusammenfassung. Die chemotaktische Aktivität von Kaninchenplasma und -serum für neutrophile Leukozyten und Makrophagen ist verschieden. Normales Serum enthält im Gegensatz zum Plasma chemotaktische Faktoren für Neutrophile und Makrophagen. Inkubation von Serum mit einem Immunkomplex bewirkt einen erheblichen Anstieg der chemotaktischen Aktivität für Neutrophile, dagegen eine Verminderung für Makrophagen. Die Inkubation von Plasma mit Immunkomplex bewirkt einen starken Anstieg für beide Zelltypen. Nach Inkubation einer Granula-Fraktion aus Neutrophilen mit Plasma – nicht aber mit Serum – entsteht chemotaktische Aktivität für Makrophagen. Diese Befunde lassen einen möglichen Zusammenhang zwischen Chemotaxis und Koagulation vermuten.

J. F. Borel and E. Sorkin

Schweizerisches Forschungsinstitut,
Medizinische Abteilung,
CH-7270 Davos-Platz (Switzerland), 18 September 1969.

[10] P. A. Ward, J. exp. Med. *126*, 189 (1967).
[11] J. H. Humphrey, Br. J. exp. Pathol. *36*, 268 (1955).
[12] C. G. Cochrane, E. R. Unanue and F. J. Dixon, J. exp. Med. *122*, 99 (1965).
[13] This work was supported by the Swiss National Foundation for Scientific Research, Grant No. 4518.
[14] We thank Miss M. Fehlmann and Mr. W. Theilkäs for their skilful technical assistance.

Production of Antibody for Rat Tail Tendon Collagen

Evidence for direct antigen-antibody reactions as the auto-immune basis for many connective tissue diseases has been largely circumstantial[1]. Though collagen and related proteins have been considered to be immunologically inert, recent studies[2–4] have shown that even in the absence of accepted antigenic determinants, collagen does possess a degree of antigenicity. The present study was undertaken to produce purified antibodies against specific fractions of collagen as the prelude to determining whether such antibodies might induce changes in newly proliferating connective tissue in experimental animals similar to those noted in human diseases associated with the auto-immune response.

Materials and methods. A total of 9 guinea-pigs (without consideration of sex) were given periodic injections of 0.15 *M* citrate buffer (pH 3.8) extracted rat tail tendon collagen according to the schedule outlined in the Table. Blood was collected retro-orbitally, twice weekly from alternate eyes, about 3 ml from each eye per collection, permitted to clot at 37 °C for 4 h and the serum obtained after rimming and centrifugation at 1000 × g for 20 min.

The collagen used as the antigen was prepared from tail tendons of laboratory rats (175–225 g; Holtzman, Madison, Wisc.). The well-minced tendons were washed at 4 °C for 24 h with 50 ml/g tendon of a 3 g/l sodium chloride solution. The tendons, recovered by decantation, were extracted with 1.0 *M* sodium chloride (50 ml/g tendon) for 24 h at 4 °C. The solution thus obtained contained the neutral salt soluble collagen (NSS). The tendons then were subjected twice to further extractions with 0.15 *M* citrate buffer, pH 3.8 at 4 °C for 24 h. After dialysis against cold running tap water, the precipitated acid soluble collagen (AS) was suspended in 1% saline (2 mg/ml on a dry weight basis). Only the AS fraction was used as an antigen in this experiment.

The sera were qualitatively assayed for the presence of antibody against NSS or AS collagen by the Ouchterlony method[5]. Fractionation of the pooled guinea-pig sera on Sephadex G-200 was according to the general procedure of Killander[6]. Nitrogen determinations on the antigen and antibody fractions were performed with a Coleman Nitrogen Analyzer.

Results. The presence of anti-rat tail tendon collagen antibody in guinea-pig serum was indicated after 24 h by the typical Ouchterlony reaction as evidenced in the Figure (a–f). A strong reaction was given against the AS tendon collagen whether the collagen antibody was at the pH of extraction (3.8), near neutrality or at pH 5.5. The best response was given at pH 5.5. (d). A positive antigen-

[1] L. E. Glynn, in *International Review of Connective Tissue Disease* (Ed. D. A. Hall; Academic Press, New York 1964), p. 214.
[2] F. O. Schmitt, L. Levine, M. P. Drake, A. L. Rubin, D. Pfahl and P. F. Davison, Proc. natn. Acad. Sci., USA *51*, 493 (1964).
[3] S. Hisa, J. Allergy Tokyo *9*, 555 (1962).
[4] J. P. Bray, J. A. Bass and F. L. Estes, Tex. Rep. Biol. Med. *22*, 220 (1964).
[5] O. Ouchterlony, Acta path. microbiol. scand. *32*, 231 (1953).
[6] J. Killander, Biochem. biophys. Acta *93*, 1 (1964).