tion for erythrocytes stored more than 30 days decreased. During incubation, 2,3-DPG content did not change significantly, and the content of ATP, an important substance for 24 h-posttransfusion survival of erythrocytes[20,21], also did not change or rather increased slightly in these experimental series. Furthermore, the degree of hemolysis during incubation for 4 h was less than 3%.

The time courses of PLP incorporation and the changes of adenylates in erythrocytes, which were performed for the 16 days-stored erythrocytes, are shown in figure 2. Although the ATP-content increased in the initial stage of the incubation, there were no systematic differences between the control experiment and the PLP experiments, and between the amounts of PLP incorporated, within the experimental error. On the other hand, it seemed that more ADP and AMP, systematically, accumulated in erythrocytes as they were incubated with more PLP. Therefore, systematic changes in ATP content (perhaps incubation with more PLP might result in a greater decrease of ATP) may be concealed in the experimental error, and ATP may partly participate in the incorporation of PLP. In this sense, the decreased incorporation of PLP by erythrocytes stored for more than 30 days in figure 1, b may be explained by the marked decrease of ATP in the erythrocytes. Furthermore, the changes in the membrane properties of erythrocytes during storage may partly affect the degree of PLP incorporation.

The oxygen affinity of erythrocytes treated by PLP (represented by P_{50}) after various periods of storage, is shown in figure 1, c. It is clear that the oxygen transport function of the ACD-stored blood was perfectly improved by PLP, as reflected by the oxygen affinity. Furthermore, it was proved for ACD-stored erythrocytes that the change of the oxygen affinity due to PLP was almost same as that with 2,3-DPG, as already observed for hemoglobin solution[6,7]. However, the Hill's coefficient (n) of erythrocytes containing 5 mM PLP was 2.2, while that of fresh ACD-erythrocytes containing 5 mM 2,3-DPG was 2.6. The decreasing degree of the heme-heme interaction was dependent on the amounts of PLP incorporated, as previously observed[19].

In conclusion, PLP is easily incorporated into ACD-stored erythrocytes without any decrease in ATP content, restores the poor oxygen transport function with a similar effect to 2,3-DPG, and may be applicable for blood transfusion.

1 D.J. Valtis and A.C. Kennedy, Lancet *1*, 119 (1954).
2 O. Åkerblom, C.H. de Verdier, L. Garby and C.F. Högman, Scand. J. clin. Lab. Invest. *21*, 245 (1968).
3 H.F. Bunn, M.H. May, W.F. Kocholaty and C.E. Schields, J. clin. Invest. *48*, 311 (1969).
4 C.R. Valeri, in: The Red Blood Cell, 2nd ed., p.511. Ed. D. Mac Surgenor. Academic Press, New York and London 1974.
5 A. Chanutin and R.R. Curnish, Archs Biochem. Biophys. *121*, 96 (1967).
6 R.E. Benesch, R. Benesch, A. Bank, R. Renthal and B.A. Bray, in: Proc. 1st Inter-Am. Symp. Hemoglobins, p.134, Caracas 1969.
7 R.E. Benesch, R. Benesch, R.D. Renthal and N. Maeda, Biochemistry *11*, 3576 (1972).
8 G.R. Bartlett, Biochim. biophys. Acta *156*, 221 (1968).
9 K. Imai, H. Morimoto, M. Kotani, H. Watari, W. Hirata and M. Kuroda, Biochim. biophys. Acta *200*, 189 (1970).
10 N. Maeda, H. Chang, R. Benesch and R.E. Benesch, New Engl. J. Med. *284*, 1239 (1971).
11 S. Rapoport, J. clin. Invest. *26*, 591 (1947).
12 E. Beutler, A. Meul and L.A. Wood, Transfusion *9*, 109 (1969).
13 R.B. Dawson, Adv. exp. Med. Biol. *6*, 305 (1970).
14 T. Ochiai and Y. Enoki, Jap. J. Physiol. *26*, 375 (1976).
15 K. Yamada and M. Tsuji, J. Vitam. *14*, 282 (1968).
16 R. Suzue and M. Tachibana, J. Vitam. *16*, 164 (1970).
17 B.B. Anderson, C.E. Fulford-Jones, J.A. Child, M.E.J. Beard and C.J.T. Bateman, J. clin. Invest. *50*, 1901 (1971).
18 S.K. Strivastava and E. Beutler, Biochim. biophys. Acta *304*, 765 (1973).
19 N. Maeda, K. Takahashi, K. Aono and T. Shiga, Br. J. Haemat. *34*, 501 (1976).
20 R.J. Dern, G.J. Brewer and J.J. Wiorkowski, J. Lab. clin. Med. *69*, 968 (1967).
21 A.R. Haradin, R.J. Weed and C.F. Reed, Transfusion *9*, 229 (1969).

Spherule cells in *Drosophila* species

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Summary. Spherule cells are restricted to the larval stage and make up 5–16% of cells in the hemolymph. Their morphology varies between species, mainly due to the shape of their inclusions which may be oval ('spheroidocytes'), polyhedral ('crystalloid cells'), or clearly crystalline ('crystal cells'). These inclusions are very rich in tyrosine. They liquefy rapidly in vitro, whereby the cells become hyaline (coagulocytes).

Known since 1864 under the name of 'Körnchenkugeln'[2], and subsequently designated as 'granules mûriformes'[3], or 'sphérules de granules'[4–6], spherule cells seem to be of common occurrence in insects[7–10]. There is however some controversy concerning the Diptera: In *Calliphora erythrocephala*, spherule cells are absent according to certain authors[11,12] but have been recognized by others [10,13]. Dennell[14] did not find them in *Sarcophaga falculata*, whereas in *S. bullata* they are reported to be present[15–17]. Concerning the various species of *Drosophila* which have been investigated, most of the authors [17–27] have overlooked this type of cell, while Rizki[28] reported it from *D. willistoni* under the designation 'spheroidocyte'. Spherule cells have more recently been identified in *D. hydei*[29]. We examined several species of *Drosophila* and one species of *Zaprionus* in order to see whether spherule cells are generally present, and whether there are species-specific differences in their morphology.

Material and methods. Fresh hemolymph was collected on refrigerated slides and observed under phase contrast. In *D. hydei*, all phases of larval development from hatching to pupation were examined, in other species selected stages only. Following immediate pre-fixation in 45% acetic acid which is essential for preserving the cells, smears were fixed in either 100% ethanol followed by orcein staining for direct observation, or Carnoy for May-Grünwald Giemsa staining, or 10% neutral formaldehyde for the detection of proteins with a high tyrosine content according to the technique of Glenner and Lillie, modified by Morel and Sisley as quoted by Thompson[30].

Results and discussion. 5 types of hemocytes can be distinguished in *Drosophila hydei*[29], viz.: prohematocytes, plasmatocytes, granular hematocytes, spherule cells, and oenocytoids. It is probable that plasmatocytes and granular hematocytes derive from prohematocytes; the distinction between the latter 2 types is a delicate one and is possible only with living cells[29]. Spherule cells by contrast are easily recognized in fixed as well as in fresh preparations. They appear for the 1st time halfway through the 1st larval instar. It is not clear whether they stem from a separate embryonic line and begin at this time to form spherules. Alternatively, they might derive from plasmatocytes, the latter being present in the hemolymph already at hatching. Contrary to observations by Jones[7], we found that spherule cells are indeed capable of emitting pseudopods, but this occurs infrequently and by preference in young larvae. In the course of larval life spherule cells gradually increase in numbers to attain 16% of the total of hemocytes at 150 h, i.e. 24 h before the onset of metamorphosis. In the intervening period their proportion drops to almost zero, and in the white prepupa not a single spherule cell can be found.

Staining by May-Grünwald Giemsa reveals a strongly basophilic cytoplasm with spherule-to crystal-like inclusions in varying sizes and numbers depending on the species. These inclusions are colourless, or stained a light blue when the staining procedure is prolonged. Nuclei are eosinophilic, of small size and often hidden by the inclusions.

Analysis of 10 species of *Drosophila* and of *Zaprionus vittiger* (which belongs to the same family) has shown that only *D. melanogaster* and *D. simulans* (figure 1, A and B) have regular crystalline inclusions. These are large and few in number (often single) in the former species, but small and numerous in the latter, so that on the basis of this distinctive character any individual can be unambiguously assigned to one or the other of the 2 sibling species which are difficult to separate on the grounds of standard morphological criteria. In 8 other species of *Drosophila,* and in *Z. vittiger,* inclusions are in the shape of more or less globular spherules (figure 1, C–K). Although there are differences between the species in cell size and also in the size and number of spherules, as suggested by the photographs, indivudual variation in these cells is so important that it was not feasible to establish a catalogue of clearcut distinctions.

In live cells in vitro, the spherules or crystals, opaque under phase contrast, liquefy rapidly and disappear, whereby the cells become uniformly hyaline. At this stage the cells correspond exactly to the so-called coagulocytes of insect hematology[31]. The speed of transition to the hyaline state depends on larval age: it is relatively moderate in the hemolymph of young larvae, whereas at advanced stages it is so rapid that many of the spherule cells have turned into coagulocytes by the time the preparation has been focused under the microscope. The transformation in vitro can take 3 different courses. Usually the inclusions dissolve within the cell which then becomes enlarged and transparent (figure 2, A and C). A small proportion of cells first emit lobes of cytoplasm (lamellopodia[32]) which become charged with dissolving spherule material. Such cells finally also assume the oval shape of typical coagulocytes (figure 2, B). A 3rd mode of behaviour takes place in the hemolymph of mature larvae: the cell membrane breaks open, the spherules are discharged from the cell and immediately dissolve.

A comparative study of the different species has shown that the 'crystal cells' of *D. melanogaster*[18–20,23] and *D. simulans* in general features and behaviour are remarkably similar to the spherule cells of other species. 'Crystal cells' as illustrated in figure 2, A, for *D. melanogaster,* and spherule cells as illustrated in figure 2, C, for *D. hydei,* during their transformation in vitro pass through similar stages and end up as

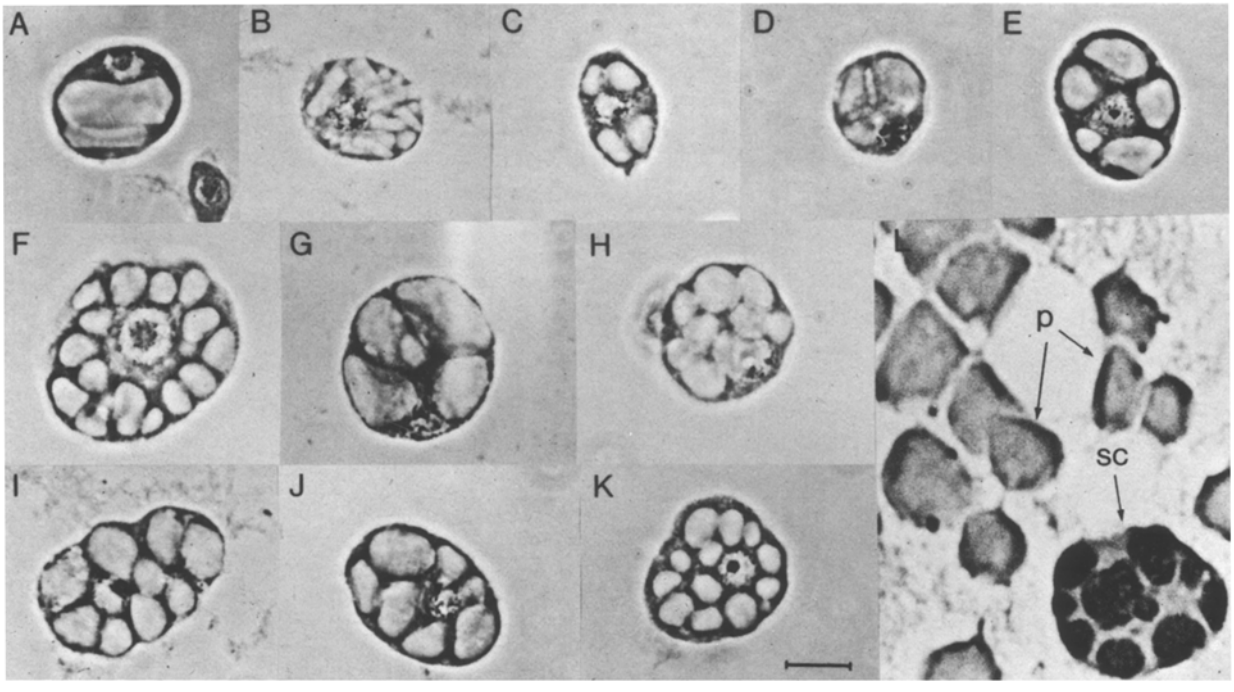

Fig. 1. *A–K* Phase contrast photographs of spherule cells from several species of *Drosophila (A–D, F–K)* and from 1 species of *Zaprionus* (E), after pre-fixation in 45% acetic acid, fixation in 100% ethanol, and staining in acetic-orcein. *A D. melanogaster, B D. simulans, C D. subobscura, D D. busckii, E Z. vittiger, F D. virilis, G D. mercatorum, H D. immigrans, I D. funebris, J D. 'zomba',* a nonidentified species close to *D. repleta* (from Zomba, Malawi, provided by H. Feijen, Leiden, The Netherlands). *K D. hydei.* Magnification is the same in all photographs; the bar (K) represents 10 µm. *L* A spherule cell (sc) of *D. hydei* surrounded by plasmatocytes (p) after formaldehyde fixation and specific coloration for tyrosine following the Glenner and Lillie method, as modified by Morel and Sisley[30].

typical coagulocytes. This already indicates that they are one and the same type of cell. Moreover, the inclusions present in these cells, although varying between species in shape, size and number, all respond positively to a specific staining procedure[30] which reveals tyrosine-rich compounds. They stain an intense violet while other hemocytes take on a very light colour only, as shown for *D. hydei* in figure 1, L.

Hematological nomenclature in *Drosophila* is contradictory and confusing, in the first place because several new terms have been introduced, viz. 'spheroidocytes', 'cristalloid cells'[28], 'cristal cells' and 'lamellocytes'[19], but also because of doubtful statements about the origin and transformations of hemocytes[18]. Since these notions have been adopted by subsequent authors, *Drosophila* has become a hematological monstrosity among other insects. The situation is further complicated by inadequate homologation. Jones[7,16] e.g. considers the 'crystal cells' of *Drosophila*[17] as equivalent to the oenocytoids of *Sarcophaga.* The same author[7] identifies the spherule cells of *Sarcophaga* with the 'lamellocytes'[29] of *D. melanogaster*[20,33]. Nappi[24] using Jones'[7] terminology, correspondingly places the spherule cells of *D. euronotus* with the 'lamellocytes'. A recent[23] and exceptionally clear illustration of 'lamellocytes' makes us aware of the fact that in a previous publication[29] we had erroneously assumed that this term was meant to describe the 'oenocytoid' cells of the so-called lymph gland.

Our observations show that 'spheroidocytes', 'crystalloid cells' and 'crystal cells' in reality all are identical to the original 'spherule cell' as defined by Weismann[2]. While the term 'crystal cells' might seem justified because of the very characteristic inclusions (so far restricted to *D. melanogaster* and *D. simulans*), it should nevertheless be emphasized that this term as well as the names 'crystalloid cell' and 'spherocytoid' introduced by Rizki[18,28], actually all refer to one and the same type of cell, known since 1864[2] as spherule cells, the original description of which fits most of the Drosophilid species examined to-day. We propose to retain the name spherule cells exclusively, at least for Dipteran species, especially since insect hematology is exceedingly confused already, with more than 70 different terms applied to the few distinct types of hemocytes. In vitro, spherule cells are seen to rapidly liquefy and/or discharge their inclusions following one or the other of the 3 modes described above. This process accomplished, the cells become large, flattened and hyaline (figure 2). Cells in this (probably moribund) state are known in Diptera[31] as coagulocytes, and in other insects as cystocytes[7,8]. For *Drosophila,* the term 'lamellocyte' has been introduced by Rizki[19,23] but we prefer for simplicity to retain the name coagulocyte[31] exclusively. Also, it should be emphasized that these cells are not a class of hemocytes in its own right. Rather, they represent a state of transformation of the spherule cells (figure 2).

As to the question of the origin of the spherule cells, Rizki[22] states that the lymph gland (also known as 'blood-forming organ') contains the 'crystal cells' besides the plasmatocytes. Similarly, according to Gateff[26,27] the lymph gland gives rise to 2 cell lines which evolve into plasmatocytes and 'crystal cells' respectively. Yet, such an interpretation is untenable because in very young larvae the gland consists of a few anlage cells only, while at the same time the

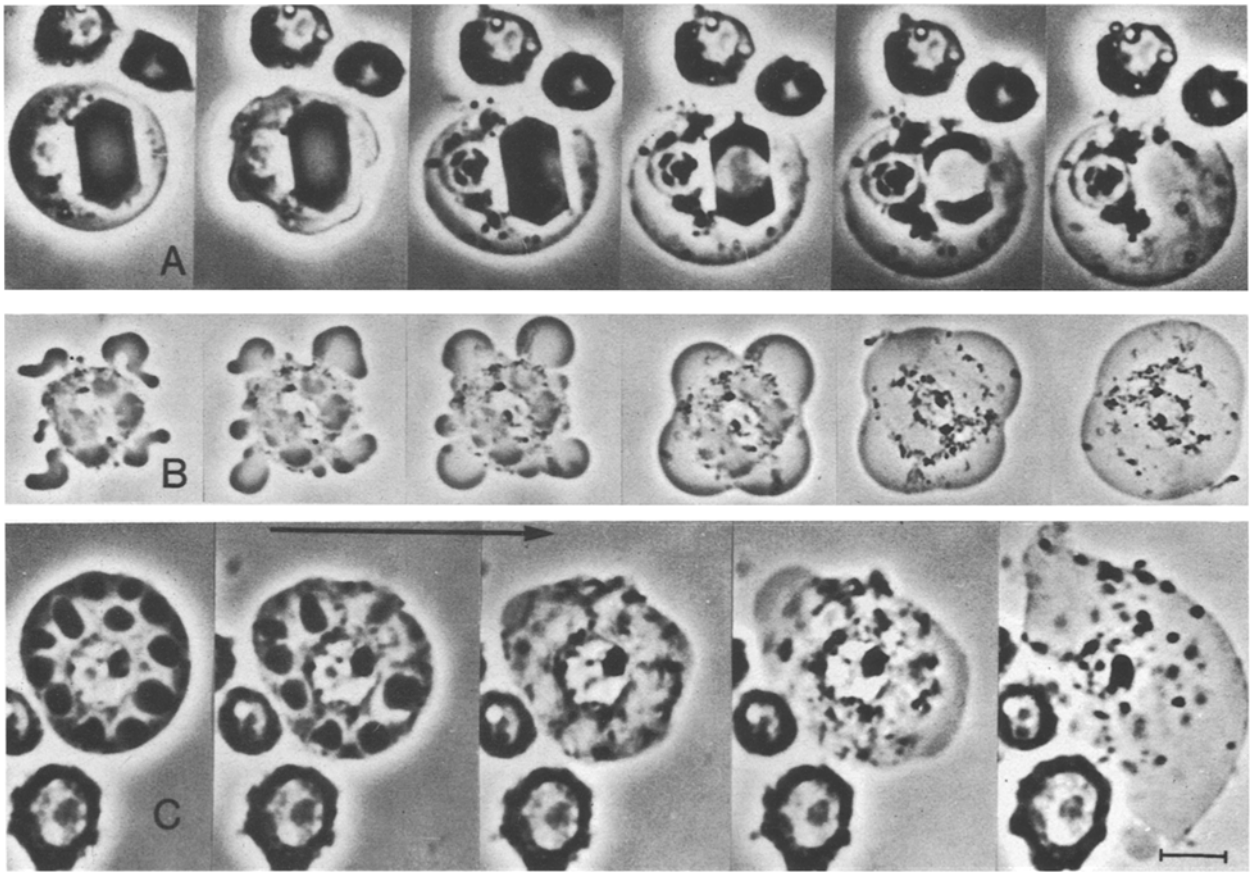

Fig. 2. The transition in vitro of spherule cells into 'coagulocytes' as observed under phase contrast on refrigerated slides. *A D. melanogaster* larva of 70 h. *B, C D. hydei* larvae of 150 h. In both species, changes as those shown in these series of photographs take place within 1-3 min. The bar represents 10 µm.

2 hemocyte types in question are already present in the hemolymph.

Steps in hematopoiesis imply, according to Rizki[18,19], the transformation of prohematocytes into 'crystal cells' and plasmatocytes, and the transformation of the latter into 'podocytes' and finally 'lamellocytes'. However, in our experience spherule cells (= 'crystal cells') transform directly into coagulocytes (= 'lamellocytes'), while plasmatocytes which by the emission of pseudopods may turn into 'podocytes', never end up as coagulocytes (= 'lamellocytes'). We have further shown[29] that the lymph gland which grows progressively like an imaginal disc, releases at the end of larval life a single type of cell, viz. the oenocytoids. Thus, spherule cells more likely stem from embryonic mesoderm directly as seems to be the case with prohematocytes and plasmatocytes[7,9,34,35]. Or, alternatively, they might branch off from plasmatocytes at a later stage. Spherule cells appear to be implicated in the process of coagulation[31], in the encapsulation of parasites[25], and in the formation of melanotic tumours[21]. Moreover, they are capable of phagocytosis, as are all other hemocytes which presumably stem from embryonic mesoderm. We found the oenocytoids of the lymph gland to be the only element incapable of phagocytizing heat-killed, stained yeast cells. In any case it sems likely that the essential function of spherule cells is the stocking of compounds highly charged with tyrosine, as indicated by the Millian reaction in Rizki's[20] observations and by the Glenner and Lillie[30] reaction in our own observations (figure 1, L). Significantly, spherule cells in *D. melanogaster*[20,33] and in *D. hydei* alike, disappear abruptly at the approach of metamorphosis. This could mean that they contribute to the sclerotization of the pupal case. Biochemical analyses of hemolymph have shown that tyrosine derivatives, in particular tyrosine-o-phosphate in *D. melanogaster*[36] and β-glucosyl-o-tyrosine in *D. busckii*[37], increase steadily through larval life, but diminish considerably just before pupation. This suggests that spherule cells function as a reservoir of tyrosine.

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2 A. Weismann, Z. wiss. Zool. *14,* 187 (1864).
3 E. Metchnikoff, Arb. zool. Inst. Univ. Wien *5,* 141 (1887).
4 H. Viallanes, Annls Sci. nat. *14,* 1348 61882).
5 C. Pérez, C.r. Soc. Biol. *56,* 781 (1904).
6 A.C. Hollande, Archs Zool. exp. gén. *6,* 283 (1911).
7 J.C. Jones, Am. Zool. *2,* 209 (1962).
8 J.C. Jones, The circulatory system of Insects, Charles C. Thomas, Springfield, Ill. 1977.
9 J.W. Arnold, in: Physiology of Insecta, p. 201. Ed. M. Rockstein. Academic Press, New York and London 1974.
10 C.D. Price and N.A. Ratcliffe, Z. Zellforsch. *147,* 537 (1974).
11 M. Rooseboom, Archs néerl. Zool. *2,* 432 (1937).
12 D. Zachary and J.A. Hoffmann, Z. Zellforsch. *141,* 55 (1973).
13 B. Åkesson, Ark. Zool. *6,* 203 (1953).
14 R. Dennell, Proc. R. Soc. Lond. B *134,* 79 (1947).
15 J.C. Jones, J. Morph. *99,* 233 (1956).
16 J.C. Jones, J. Insect Physiol. *13,* 645 (1967).
17 J.M. Whitten, J. Insect Physiol. *10,* 447 (1964).
18 M.T.M. Rizki, in: Physiology of Insect Development, p. 91. Ed. F.L. Campbell. Chicago University Press 1956.
19 M.T.M. Rizki, J. Morph. *100,* 437 (1957).
20 M.T.M. Rizki, J. Morph. *100,* 459 (1957).
21 M.T.M. Rizki, J. Morph. *106,* 147 (1960).
22 M.T.M. Rizki, Am. Zool. *2,* 247 (1962).
23 M.T.M. Rizki, in: The Genetics and Biology of *Drosophila,* p. 397. Ed. M. Ashburner and T.R.F. Wright. Academic Press, London, New York and San Francisco 1978.
24 A.J. Nappi, Ann. ent. Soc. Am. *63,* 1217 (1970).
25 A.J. Nappi and F.A. Streams, J. Insect Physiol. *15,* 1551 (1969).
26 E. Gateff, Science *200,* 1448 (1978).
27 E. Gateff, in: The Genetics and Biology of *Drosophila,* p. 181. M. Ashburner and T.R.F. Wright. Academic Press, London, New York and San Francisco 1978.
28 M.T.M. Rizki, J. exp. Zool. *123,* 397 (1953).
29 Ž. Srdić and H. Gloor, Revue suisse Zool. *85,* 11 (1978).
30 S.W. Thompson, Selected Histochemical and Histopathological Methods, Charles C. Thomas Pub. Springfield, Illinois (1966).
31 C. Gregoire, in: Physiology of Insecta, p. 309. Ed. M. Rockstein. Academic Press, New York and London 1974.
32 A.C.S. Crossley, in: Advances in Insect Physiology, p. 117. Ed. J.E. Treherne, M.J. Berridge and V.B. Wigglesworth. Academic Press, London and New York 1975.
33 M.T.M. Rizki and R.M. Rizki, J. biophys. biochem. Cytol. *5,* 235 (1959).
34 M. Demerec, Biology of *Drosophila,* John Wiley, New York 1950.
35 J.C. Jones, in: Regulation of Hematopoiesis, vol. 1, p. 7. Ed. A.S. Gordon. App. Century Crofts, New York 1970.
36 H.K. Mitchell and K.D. Lunan, Archs biochem. Biophys. *106,* 219 (1964).
37 P.S. Chen, H.K. Mitchell and M. Neuweg, Insect Biochem. *8,* 279 (1978).

Cerebrospinal fluid immune complexes in multiple sclerosis

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Summary. Using a C1q binding test, immune complexes have been detected in one half of cerebrospinal fluid samples from patients with multiple sclerosis. These results provide additional evidence for the participation of an immune reaction in the disease process.

Several groups have reported on the presence of immune complexes (IC) in blood sera of patients with multiple sclerosis (MS)[1-3]. Using the C1q binding test (C1q BT)[4], we have examined cerebrospinal fluid (CSF) samples from 32 MS patients taken during an active phase of their disease as determined by a standardized clinical documentation system[5]. For the IC assay, CSF samples were mixed with one volume of normal serum (itself negative in the C1q BT). Amounts of IC were expressed in IC units, obtained by dividing the percent binding values of the patient's sample by the arithmetic mean percent binding value of a larger pool (n = 118) of negative control sera from healthy individuals, and multiplying the results $\times 10^6$. Immune complexes were detected in 15 CSF samples, i.e. in 46.9% (figure). Positive results were also obtained in 6/64 control CSF from miscellaneous neurological disorders including several encephalitis cases (all IC −ve). Among the positive control CSF, 1 case of SLE with chorea is noteworthy.