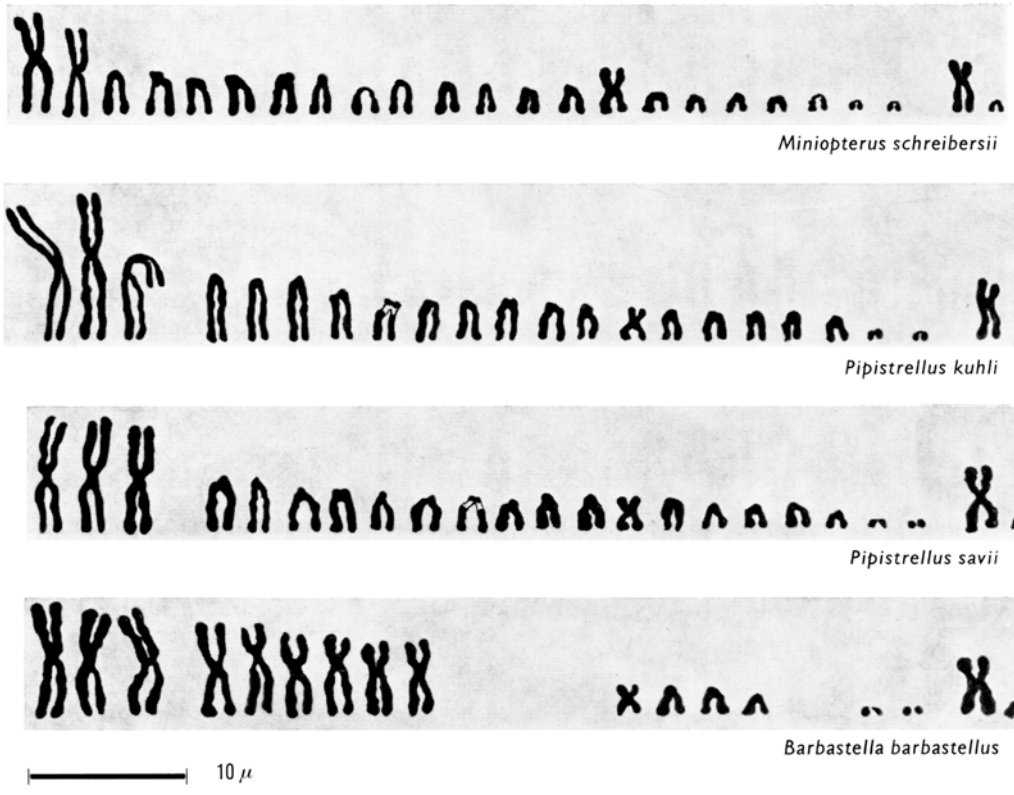

Fig. 3. A comparison between the karyotypes of 4 Vespertilionidae: *Miniopterus schreibersii* $2n = 46$; *Pipistrellus kuhli* $2n = 44$; *P. savii* $2n = 44$; *Barbastella barbastellus* $2n = 32$. Only 1 aploid set and the 2 heterochromosomes are represented.

A recent work of B. DULIC et al.[12] further supports the hypothesis of evolution by centric fusion in the Microchiropteran karyotype. The authors report that in *Nyctalus noctula* the diploid number is $2n = 42$ but the amount of autosomic arms is still 50. In fact there are 4 pairs of large metacentric autosomes. Moreover DULIC identifies all the morphologically peculiarly shaped chromosomes which I found in the 2 species of *Pipistrellus*, besides the morphological identities which I reported in Vespertilionidae.

In conclusion, we may therefore think that the chromomic mutation mechanism of centric fusion is implied in the evolution of karyotype in Microchiroptera.

Riassunto. L'esame condotto dall'Autore sul cariotipo di 7 Microchirotteri (3 Rinolofidi e 4 Vespertilionidi) fa ritenere che nell'evoluzione del cariotipo di questo sottordine sia implicato il fenomeno robertsoniano di fusione centrica.

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[12] B. DULIĆ, B. SOLDATOVIĆ and D. RIMSA, Experientia *23*, 945 (1967).

ACTUALITAS

Life-Span and Membrane Properties of Erythrocytes

The experimental data showing that in mature erythrocytes there is no synthesis of enzymes and that their enzymatic activities have, at different rates, an exponential decay during their life-span, have been reviewed[1–4] and it seems very probable that the life of the erythrocyte is primarily limited by the depletion of glycolytic enzymes.

Recent studies, however, on the membrane components of erythrocytes suggest that even slight changes in the physicochemical properties of the membrane have an important effect on the life-span of red blood cells. Internal, as well as external factors, can be responsible for the membrane change and ultimately for the erythrocyte removal from the circulation.

The negative charge on the erythrocyte surface is due to the carboxyl group of sialic acid[5–7] and, according to HAYDON and SEAMAN[8], probably also to the α-carboxyl group of a protein-bound amino acid. Sialic acid is a component of a glycoprotein located on the red cell membrane[9–11], and it would seem, following MORAWIECKI's proposal[12] consonant with WINZLER's et al. results[13], that the lipophylic portion of this glycoprotein is associated with the lipid bimolecular leaflet layer of the membrane, while the remaining hydrophylic portion emerges into the aqueous environment of the cell. There are, however, several considerations based on the electrophoretic behaviour of erythrocytes, strongly suggesting

that glycoproteins lie in an extended form above the surface of the cell[14].

It is interesting to note that partial or complete loss of sialic acid produces several important changes on the erythrocyte and has a great influence upon its survival. When erythrocytes are treated with sialidase, their isoelectric point rises to pH 4–5 and their surface charge and electrophoretic mobility are reduced[6,15]. Cleavage of sialic acid in a bound form from the cell membrane resulting in decreased electrophoretic mobility, has also been obtained by incubating erythrocytes with trypsin[16] and other enzymes[15,17].

It has been shown[18,19] that reticulocytes have slower electrophoretic mobility than erythrocytes. On the other hand, younger erythrocytes are migrating more rapidly than older ones[20], the mobility of the latter being comparable to that of reticulocytes[21]. It has been concluded[19] that an increased surface charge accompanied by a decreased adhesiveness may occur during maturation of erythrocytes. This conclusion is in keeping with old observations[22,23] showing that immature cells have a pronounced adhesiveness and with AMBROSE's et al. suggestion[24] that a high charge density, typical of malignant cells, may be correlated with their low adhesiveness.

WALTER et al.[25] have found that the sialic acid content of erythrocytes remains constant throughout their lifespan and have concluded that the reduced surface charge of old cells is due to an alteration of sialic acid per unit surface area. A rearrangement of the membrane components of the ageing red cell resulting in a continuous diminution of surface charge, whether or not due to loss of sialic acid cannot be excluded, although, at the present, it is not clear how it would occur. On the other hand, it is possible that a small percentage of old erythrocytes have, like sialidase-treated ones, a reduced surface charge as the result of a partial loss of sialic acid. Sialic acid could be removed from the erythrocyte surface either by circulating or tissue sialidase: this enzyme has been found in preparations of bovine and human glycoprotein[26], in the chorioallantois of the chick embryo[27], in mouse brain[28], and in various rat organs[29–31].

It is known[2] that old erythrocytes are denser, have less electrolytes, less water, less lipid in the surface[32], and have an altered cell wall[33,34]. Further there is some evidence indicating that old and sialidase-treated erythrocytes present a higher rate of agglutination[35,36], increase in deformability[37] and in adhesiveness, all these changes to be ascribed again to reduction of their electrical surface potential. Finally, they have a shorter half-life than young cells.

Young canine erythrocytes, incubated with influenza virus, had a greatly shortened survival-time[38]. Newcastle disease virus-treated erythrocytes presented also a reduced survival time in the rabbit[39]. Human erythrocytes, incubated with sialidase, maintained their total acetylcholinesterase activity but showed a half-life of only 55 min[40]. Entity of loss of sialic acid was not measured in these studies, but it is likely that erythrocytes had greatly reduced their surface charge.

For other cells, at least, sialic acid seems neither to control cell morphology and viability[41], nor to influence transport across cell membrane of positively charged substances[42], but, in the case of erythrocytes, a strongly negative surface charge appears to be important in preventing erythrophagocytosis.

Phagocytosis of sialidase- or virus-treated erythrocytes was negligible if the phagocytizing cells were leucocytes[43], whereas it was enhanced in the presence of spleen macrophages[44,45]. These results support the hypothesis[46] that

[1] A. C. ALLISON, Nature *188*, 37 (1960).
[2] J. H. JANDL, *Kernicterus* (University of Toronto Press, Toronto 1961), p. 41.
[3] C. BISHOP, in *The Red Blood Cell* (Academic Press, New York 1964), p. 147.
[4] R. G. CHAPMAN and L. SCHAUMBURG, Br. J. Haemat. *13*, 665 (1967).
[5] G. M. W. COOK, D. H. HEARD and G. V. F. SEAMAN, Nature *191*, 44 (1961).
[6] E. H. EYLAR, M. A. MADOFF, O. V. BRODY and J. L. ONCLEY, J. biol. Chem. *237*, 1992 (1962).
[7] R. M. GLAESER and H. C. MEL, Biochim. biophys. Acta *79*, 606 (1964).
[8] D. A. HAYDON and G. V. F. SEAMAN, Archs Biochem. Biophys. *122*, 126 (1967).
[9] R. H. KATHAN, R. J. WINZLER and C. A. JOHNSON, J. exp. Med. *113*, 37 (1961).
[10] A. H. MADDY, Biochim. biophys. Acta *117*, 193 (1966).
[11] A. F. REGA, R. I. WEED, C. F. REED, G. G. BERG and A. ROTHSTEIN, Biochim. biophys. Acta *147*, 297 (1967).
[12] A. MORAWIECKI, Biochim. biophys. Acta *83*, 339 (1964).
[13] R. J. WINZLER, E. D. HARRIS, D. J. PEKAS, C. A. JOHNSON and P. WEBER, Biochemistry *6*, 2195 (1967).
[14] E. J. AMBROSE, Prog. Biophys. and molec. Biol. *16*, 243 (1966).
[15] G. V. F. SEAMAN and G. UHLENBRUCK, Archs Biochem. Biophys. *100*, 493 (1963).
[16] E. PONDER, Blood *6*, 350 (1951).
[17] R. M. GLAESER and H. C. MEL, Archs Biochem. Biophys. *113*, 77 (1966).
[18] J. G. STEPHENS, J. Physiol. *99*, 30 (1940).
[19] F. DOLJANSKI and N. SCHULMAN, Expl Cell Res. *36*, 605 (1964).
[20] D. DANON and Y. MARIKOVSKY, C. r. hebd. Séanc. Acad. Sci., Paris *253*, 1271 (1961).
[21] H. WALTER and F. W. SELBY, Biochim. biophys. Acta *112*, 146 (1966).
[22] J. A. KEY, Archs intern. Med. *28*, 511 (1921).
[23] R. ISAACS, Folia Haemat. *40*, 395 (1930).
[24] E. J. A. AMBROSE, A. M. JAMES and J. H. B. LOWICK, Nature *177*, 576 (1956).
[25] H. WALTER, R. WINGE and F. W. SELBY, Biochim. biophys. Acta *109*, 293 (1965).
[26] L. WARREN and C. W. SPEARING, Biochem. biophys. Res. Commun. *3*, 489 (1960).
[27] G. L. ADA and P. E. LIND, Nature *190*, 1169 (1961).
[28] R. T. KELLY and D. GREIFF, Biochim. biophys. Acta *110*, 548 (1965).
[29] R. CARUBELLI, R. E. TRUCCO and R. CAPUTTO, Biochim. biophys. Acta *60*, 196 (1962).
[30] B. H. TAHA and R. CARUBELLI, Archs Biochem. Biophys. *119*, 55 (1967).
[31] S. MAHADEVAN, J. C. NDUAGUBA and A. L. TAPPEL, J. biol. Chem. *242*, 4409 (1967).
[32] T. A. J. PRANKERD, J. Physiol. *143*, 325 (1958).
[33] D. DANON and Y. MARIKOVSKY, C. r. Séanc. Soc. Biol. *155*, 12 (1961).
[34] D. DANON and K. PERK, J. cell. comp. Physiol. *59*, 117 (1962).
[35] P. SACHTLEBEN and G. RUHENSTROTH-BAUER, Nature *192*, 982 (1961).
[36] Y. MARIKOVSKY, D. DANON and A. KATCHALSKY, Biochim. biophys. Acta *124*, 154 (1966).
[37] L. WEISS, J. Cell Biol. *26*, 735 (1965).
[38] W. B. STEWART, C. W. PETENYI and H. M. ROSE, Blood *10*, 228 (1955).
[39] E. GARDNER, C. WRIGHT and B. Z. WILLIAMS, J. Lab. clin. Med. *58*, 743 (1961).
[40] G. PERONA, S. CORTESI, P. XODO, C. SCANDELLARI, G. GHIOTTO and G. DE SANDRE, Acta Isotop. *4*, 287 (1964).
[41] P. M. KRÄMER, J. cell. comp. Physiol. *68*, 85 (1966).
[42] J. L. GLICK and S. GITHENS, Nature *208*, 88 (1965).
[43] W. N. JENSEN and J. MORENO, Nouv. Revue Fr. Hémat. *4*, 483 (1964).
[44] N. G. BRANDT, J. A. BASS, M. C. DODD and C. WRIGHT, Fedn Proc. Fedn Am. Socs exp. Biol. *11*, 462 (1952).
[45] C. WRIGHT, M. C. DODD, J. A. BASS and N. G. BRANDT, Anat. Rec. *115*, 457 (1953).
[46] L. WEISS, E. MAYHEW and K. ULRICH, Lab. Invest. *15*, 1304 (1966).

the reduction in surface charge density on the cell after sialidase treatment enhances phagocytosis. The role of the spleen in sequestration of erythrocytes has been assessed by GARDNER et al.[39] and by OWEN et al.[47].

It has also been shown[48,49] that macrocytic erythrocytes disappear from the circulation at a faster rate than normocytic erythrocytes and this high susceptibility to destruction might be due to a physical defect and metabolic anomaly as well as to a low surface charge.

In conclusion it can be envisaged that erythrocytes at different degrees of maturity have a different surface charge which is regulated by the cell internal metabolism and possibly by the action of plasma or tissue sialidase. A low surface charge and increased tendency to adhere to reticuloendothelial cells may be the condition that determines the removal of a large part of macrocytes[49] and old erythrocytes from the circulation[50].

Riassunto. Le proprietà della membrana dell'eritrocita variano in rapporto all'età della cellula e probabilmente all'azione della sialidasi. Una diminuzione della carica elettrica dell'eritrocita appare essere la condizione determinante l'eritrofagocitosi.

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[47] C. A. OWEN, A. L. ORVIS and J. M. KIELY, Am. J. Physiol. *211*, 273 (1966).
[48] F. STOHLMAN, Proc. Soc. Biol. Med. *107*, 884 (1961).
[49] R. T. CARD and L. S. VALBERG, Am. J. Physiol. *213*, 566 (1967).
[50] Supported by a grant from the Consiglio Nazionale delle Ricerche, Roma.

STUDIORUM PROGRESSUS

The Effect of Insulin on the Glycogen Metabolism of Isolated Fat Cells

LEONARDS and LANDAU[1] showed that insulin plus low concentrations of glucose stimulated the incorporation of glucose C-1 and C-6 into glycogen and fatty acids, and the oxidation of glucose C-1 to carbon dioxide by epididymal adipose tissue in vitro. High concentrations of glucose favoured the oxidation of glucose C-6 to carbon dioxide and the incorporation of glucose carbon into the glycerol moiety of triglycerides.

In the presence of insulin and (U^{14}C)glucose the rat epididymal adipose tissue and the mouse hemidiaphragm in vitro synthesize more glycogen than can be accounted for by incorporation of exogenous glucose into glycogen[2]. In the absence of glucose, insulin can stimulate the synthesis of glycogen by the mouse hemidiaphragm in vitro[2].

These data support the hypothesis that insulin has a direct effect on the metabolism of glycogen in certain tissues in vitro. This paper reports the effects of different concentrations of insulin on the conversion of (1^{14}C)glucose to (^{14}C)glycogen, (^{14}C)triglycerides and (^{14}C)O_2 by isolated fat cells.

Materials and methods. Male Wistar rats 110–130 g (Novo Terapeutisk Laboratorium A/S, Copenhagen) were allowed food and water ad libitum until used. Human serum albumin (Swiss Red Cross Blutspendedienst, Bern, Switzerland) was purified by dialysis[3]. Ten times crystallized bovine insulin (24.4 U/mg, lot No. 0818864) was obtained from the Novo Research Institute. Unless otherwise stated, the chemicals used throughout this work were of analytical grade (Merck AG). (1^{14}C)glucose (2.96 mC/mmole) was purchased from The Radiochemical Centre, Amersham, England. Collagenase was bought from the Sigma Company, USA.

Free fat cells were prepared by disruption of rat epididymal adipose tissue with collagenase (RODBELL[4], GLIEMANN[5]). The suspension was diluted to about 10^5 cells/ml in bicarbonate buffer (KREBS and HENSELEIT[6]), pH 7.4, which contained 10 mg of albumin/ml and 0.55 m*M* glucose. The concentration of cells in the suspension was measured[5]. In some experiments, the triglycerides were extracted from aliquots of the cell suspension, dried and weighed[5]. Standard concentrations of insulin were prepared according to GLIEMANN[5].

Twelve flasks were prepared with insulin-free buffer, and 6 with each of the insulin standards. (1^{14}C)glucose was added to the cell suspension to a final specific activity of 70–80 nC/mmole of glucose. 1 ml of the cell suspension was then pipetted into each incubation flask. The flasks were gassed with 95% O_2/5% CO_2 and stoppered. Immediately after the addition of the cell suspension, the contents of 6 of the insulin-free flasks were analysed. The ^{14}C recovered in each metabolite from these flasks – the cell blanks (CB) – served as the background for the determination of ^{14}C. The remaining flasks were incubated for 2 h at 37 °C, with shaking (50 strokes/min). Three flasks from each group were used for the isolation of (^{14}C)O_2 and the (^{14}C)triglycerides. The remaining 3 flasks were used for the preparation of the (^{14}C)glycogen. The concentration of glucose in the medium was determined by the glucose oxidase method[7], and the ^{14}C by liquid scintillation counting[5]. The (^{14}C)O_2 and (^{14}C)triglycerides were recovered and determined according to GLIEMANN[5].

After the incubation, the cell suspension was filtered through Oxoid filters (22–24 mm diameter, pore size 0.45 μ). The flasks were washed once with bicarbonate buffer[6] containing 10 mg albumin/ml and once with albumin-free bicarbonate buffer, and the washings were transferred to the filters. The filters were then washed with 2 ml of triglyceride extraction medium[8]. Each filter was transferred to 3 ml of KOH (30 g/100 ml containing 100 μg of glycogen/ml) in a polypropylene centrifuge tube.

[1] J. R. LEONARDS and B. R. LANDAU, Archs Biochem. Biophys. *91*, 194 (1960).
[2] A. J. MOODY and J.-P. FELBER, Diabetes *15*, 492 (1966).
[3] J. GLIEMANN, Diabetes *14*, 643 (1965).
[4] M. RODBELL, J. biol. Chem. *239*, 375 (1964).
[5] J. GLIEMANN, Diabetologia *3*, 382 (1967).
[6] H. A. KREBS and K. HENSELEIT, Hoppe-Seyler's Z. physiol. Chem. *210*, 33 (1932).
[7] H. U. BERGMEYER and E. BERNT, *Methods of Enzymatic Analysis*, 1st edn. (Ed. H. U. BERGMEYER; Academic Press Inc., New York 1963), p. 123.
[8] V. P. DOLE and H. MEINERTZ, J. biol. Chem. *235*, 2575 (1960).