

cortisone or with cyclophosphamide are considered to be resistant to the drug. In addition, it is noticeable that there was no significant difference in the cholesterol to phospholipid molar ratio for lymphoid cells between 2 groups of the drug-treated mice within each of the tissues examined¹⁸.

The present results clearly demonstrate that the lymphoid cells from thymus, spleen or mesenteric lymph node of cortisone-treated mice contained significantly high levels of free fatty acids, triglycerides and cholesterol esters as compared to the corresponding cells from normal animals. As described above, cortisone destroyed almost all the thymic leukocytes of mice, resulting in the pronounced decrease of lymphoid cell yields (about 5% of the normal cell yields). This striking cell destruction by cortisone indicates that the lipid composition of the cortisone-sensitive lymphoid cells in mouse thymus is similar to that of the thymic lymphoid cells from normal animals. Thus it appears that in mouse thymus the contents of free fatty acids and their esters are considerably higher in the cortisone-resistant lymphoid cells than in the cortisone-sensitive lymphoid cells. Similarly, the cortisone-resistant lymphoid cells in spleen or mesenteric lymph node of mice appears to contain the markedly high levels of the neutral lipids as compared to the cortisone-sensitive corresponding cells. It has been reported that the cortisone-resistant lymphocytes in mouse thymus are immunocompetent lymphocytes, in which the cortisone-resistant splenic and lymph node lymphocytes are also involved^{4, 5, 9-15}; whereas the cortisone-sensitive lymphocytes in thymus and other lymphoid tissues of mice have been shown to be immunologically incompetent. It is assumed, therefore,

that the lipid composition of immunocompetent lymphocytes differs significantly from that of immunologically incompetent lymphocytes within the respective lymphoid tissues of mice.

The present results also indicate that in mice the high levels of free fatty acids and their esters were found in the cyclophosphamide-resistant lymphoid cells, of which the proportion in lymphoid tissues was less than 20%, and the lipid composition of cyclophosphamide-resistant lymphoid cells from spleen or lymph node was very similar to that of the cortisone-resistant corresponding cells. Histological studies on mouse lymphoid tissues revealed that the lymphocytes present in thymus medulla and in the thymus-dependent areas of spleen and lymph nodes are resistant to corticosteroids as well as to cyclophosphamide^{5, 7, 8, 16}. These histological findings indicate that the population of cortisone-resistant lymphocytes appears to be similar to that of cyclophosphamide-resistant lymphocytes within the respective tissues of mice. Therefore it is conceivable that the lipid composition of cortisone-resistant lymphoid cells in spleen or mesenteric lymph node of mice resembles that of cyclophosphamide-resistant corresponding cells. The reason that a considerable difference was found in lipid composition of lymphoid cells between normal mice and animals treated with cortisone or cyclophosphamide within every one of the tissues examined, is unknown at the present time. However, the present results suggest that the lymphoid cells resistant to immunosuppressive agents differ markedly from the drug-sensitive lymphoid cells in the significantly high levels of free fatty acids and their esters within the respective lymphoid tissues of mice.

Distribution of the A Blood-Group Activity in Porcine Serum¹

A. RADAŠ, J.-N. MEYER and O. W. THIELE

Physiologisch-Chemisches Institut der Universität, Humboldtallee 7, D-3400 Göttingen, and Institut für Tierzucht und Haustiergenetik der Universität, Albrecht-Thaer-Weg 1, D-3400 Göttingen (German Federal Republic, BRD), 5 February 1976.

Summary. The A blood-group activity of pig serum is bound to a lipid, in some cases also to a nonlipid fraction. The major lipidic A activity (roughly 50%) is carried with the HDL class, while the VLDL and LDL classes contain roughly 25% each.

In various mammalian species, certain blood-group active substances are known to occur primarily in blood plasma as soluble constituents and to be secondarily transferred from the plasma onto the erythrocyte membrane. The Lewis substance of man², the J substance of cattle³, the R substance of sheep⁴, and the A substance of pig⁵ are examples of those kinds of blood-group substances. It has been established in previous papers that the J blood-group activity of cattle⁶ as well as the A blood-group activity of pig⁷ can be found in both the total lipid and in a lipid-free preparation of the respective serum. The A system – the first of the 15 porcine blood-group systems detected – comprises 2 factors, the first of which, A, is dominant, the second, 0, is recessive⁸. 2 types of A positive cells are usually discriminated, Ac and Ap. Since Ac cells give strong reactions, and Ap cells weak reactions, it was suggested⁹ to adopt another, more reasonable nomenclature, i.e. simply A instead of Ac, and Aw (w = weak) instead of Ap. The sera of all (Ac) positive pigs contain a soluble A substance giving high inhibition titers. Aw (Ap) sera, however, have either low inhibition titers or none at all.

The distribution of bovine J activity on lipoprotein and protein fractions of serum has been investigated in more detail recently¹⁰. One-third of the total J activity of bovine serum was found in the total lipids, two-thirds in the lipid-free residue precipitated by lipid extraction. Furthermore, one-third of the lipidic J substance was found in the very low density lipoproteins (VLDL), two-thirds in the low density lipoproteins (LDL), while the high density lipoprotein fraction (HDL) of bovine serum is free of J activity. All non-lipidic J activity is present

¹ This work was supported by Forschungsmittel des Landes Niedersachsen.

² J. C. SNEATH and P. H. A. SNEATH, *Nature*, Lond. 176, 172 (1955).

³ C. STORMONT, *Proc. natn. Acad. Sci., USA* 35, 232 (1949).

⁴ J. RENDEL, *Acta agric. scand.* 7, 224 (1957).

⁵ E. ANDRESEN, *Ann. N.Y. Acad. Sci.* 97, 205 (1962).

⁶ J. SCHRÖFFEL, A. RADAŠ, O. W. THIELE and J. KOCH, *Eur. J. Biochem.* 22, 396 (1971).

⁷ O. W. THIELE and J. HOJNÝ, *Experientia* 27, 447 (1971).

⁸ L. M. SPRAGUE, *Genetics* 43, 906 (1958).

⁹ J. HOJNÝ, *Anim. Blood Grps. biochem. Genet.* 5, 3 (1974).

¹⁰ A. RADAŠ and O. W. THIELE, *Eur. J. Biochem.* 55, 583 (1975).

Protein and lipid composition of lipoprotein fractions of A-positive pig serum. A activity of lipids and nonlipids prepared from those lipoprotein fractions

Fraction	Protein (mg/100 ml original serum)	Lipids (mg/100 ml original serum)	Protein (% of the respective lipoprotein class)	Approximate A activity in lipids in nonlipids (% of original serum)	
A-positive serum	5,120	296.5	—	20	80
VLDL	4.6	41.5	10	5	0
LDL	53.0	169.0	24	5	0
HDL	75.8	80.0	49	10	0
Lipoprotein-free residue	4,871	—	—	—	80

in the lipid-free protein. No J activity was found in the apoproteins of the lipoprotein fractions.

A similar study was carried out on A active pig serum. Among 23 miniature pigs (Göttingen bred), 16 (i.e. 70%) turned out to have A positive serum as examined by agglutination-inhibition tests in the homologous A system. All of these A positive animals carried the A substance in the total lipid fraction of serum, while the lipid-free residue precipitated by the lipid extraction procedure was A active in 2 animals only. Among 6 A positive pigs, 5 animals carried also the A substance on their erythrocyte membranes. This cellular A activity was found in the total lipids extracted from the stroma, while the nonlipid residue was highly A active in one case only; weak activities were observed in the remaining 4 cases.

The distribution of A activity on various fractions of serum was studied in a pig whose serum was A active in both the total lipids and the nonlipid residue obtained after lipid extraction of serum. Semiquantitative assays of A activity were performed by agglutination-inhibition tests and calculated on the basis of the volume of original serum from which the respective sample was derived. Compared with the quantitative hemolysis-inhibition tests of bovine J activity described earlier¹¹, the agglutination-inhibition tests of porcine A activity gives relatively rough results. We found about 20% of the original total serum A activity to be present in the total lipids, and 80% in the non-lipid fraction.

The serum was fractionated by ultracentrifugal flotation at different densities as described previously¹⁰ for bovine serum. 4 fractions were obtained by this procedure: VLDL, LDL, HDL, and lipoprotein-free residue. These fractions were characterized by their protein and lipid contents. The values shown in the Table are in agreement with the data obtained in pig serum by JANADO et al.¹² Lipids were extracted from all fractions obtained. Lipid and non-lipid fractions were checked for Ac activity. As shown in the Table, no A activity was detected in the apoproteins of any lipoprotein class, thus being in agreement with the distribution of bovine J activity. In contrast to bovine J activity, the major lipidic A activity (roughly 50%) is carried with the HDL class of serum, while the VLDL and LDL classes contain roughly 25% each.

The above results show that the distribution of procine A activity on lipid and nonlipid fractions of serum and erythrocyte membranes, and that of the lipidic A activity on various serum lipoprotein classes, is in sharp contrast to the distribution of bovine J activity on the respective fractions.

¹¹ J. SCHRÖFFEL, O. W. THIELE and J. KOCH, Eur. J. Biochem. 22, 294 (1971).
¹² M. JANADO, W. G. MARTIN and W. H. COOK, Can. J. Biochem. 44, 1201 (1966).

Preparation of Specific Antiserum against *Rana esculenta* pre- α Lens Crystallin

S. K. BRAHMA and W. J. VAN DOORENMAALEN¹

Department of Medical Anatomy and Embryology, The State University, Janskerkhof 3A, Utrecht (The Netherlands), 26 January 1976.

Summary. Specific antiserum against *Rana esculenta* lens pre- α crystallin was prepared in a rabbit by injecting antigen-antibody precipitate of this crystallin obtained from immunoelectrophoresis of *esculenta* total soluble lens proteins against homologous antiserum.

Major heterogeneous soluble crystallins from vertebrate lens are now classified into α -, β -, γ -, and δ -crystallins; VAN DAM² observed in bovine lens another crystallin fraction, which migrated faster than α -crystallin during electrophoresis but was eluted with a peak which also contained some β -, and γ -crystallins during gel filtration with Sephadex G200. According to SWANBORN³, this pre- α fraction is restricted to mammals only, but later this fraction was also found to be present in some anuran and urodele amphibians⁴⁻⁶.

BOURS and BRAHMA⁷ reported that the elution profiles of the pre- α crystallin from bovine and *Rana esculenta* lens were identical in gel permeation chromatography with Sepharose 6B. VAN DEN BROEK, LEGET and BLOEMENDAL⁸ isolated pre- α crystallin from bovine lens by chromatography and preparative isoelectric-focusing. They found that at an alkaline pH this fraction had relatively high mobility and the molecular weight was about 14,500. Amino acid analysis showed no relationship with the α -crystallin, so they named this fraction as