A C T A U N I V E R S I T A T I S L O D Z I E N S I S
FOLIA BIOCHIMICA ET BIOPHYSICA 6, 1988

Jadwiga Gniot-Szulżycka, Anna Jakubowska

OESTRONE SULPHATE SULPHOHYDROLASE AND ARYLSULPHATASE C ACTIVITIES OF HUMAN PLACENTA CELL NUCLEI AND NUCLEAR SUBFRACTIONS

The procedure of isolation of cell nuclei and nuclear envelopes from human placenta was described. The nuclear preparations consist of 56.4% of protein, 37% of DNA, 5.02% of phospholipid and 1.42% of RNA. The protein to DNA ratio varies from 1.20-1.94; the E_{230}/E_{260} and E_{260}/E_{280} ratios are 1.34 and 0.689, respectively. The purified cell nuclei were subfractionated into: nuclear sap, histone rich fraction and chromatin-membrane fraction. Successive digestion with deoxyribonuclease of the chromatin-membrane fraction lead to getting the nuclear envelopes which contained: 59.4% of protein, 1-12% of DNA and 28.6% of phospholipid.

There is evidence that oestrone sulphohydrolase and arylsulphatase C are integral parts of human placenta cell nuclei. The enzyme activities are associated with the nuclear envelopes and with non-histone chromatin proteins tightly bound with DNA.

INTRODUCTION

The subdivision of arylsulphate hydrolysing enzymes into type I (microsomal) and type II (lysosomal) outcomes from experiments on subcellular distribution of the enzyme activities. The use of 2-hydroxy-5-nitrophenyl sulphate (NCS) at acidic pH indicated the arylsulphatases as lysosomal enzymes (R o y [27], P e r u m a l et al. [22]), while the use of p-nitrophenyl sulphate (NPS), as substrate pointed to microsomal localization of arylsulphatases (D o g s o n et al. [6], D o l l y et al. [7]). The presence of arylsulphatase activities of type II in the nuclear

fraction was announced by Pokrovsky et al. [24] Makita, Sandborn [17] and Lewicki, Trzeciak [15]. The arylsulphatase activities of type I and sterolsulphohydrolase activities were shown in nuclear fractions in several laboratories (Dolly et al. [7], Kishimoto, Sostek [14], Balasubramanian [2] Gniot-Szulżycka, Jakubowska [11, 12]).

According to the preliminary data presented in our previous paper, the arylsulhpatase C and oestrone sulphate sulphohydrolase activities were associated with the chromatin-membrane fraction (Gniot-Szulżycka, Jakubowska [12]).

The present work has been aimed at isolating of highly purified cell nuclei and nuclear subfractions from human placenta as well as to test them for sulphohydrolase activities. It is not unlikely that sulphohydrolase play an important role in nucleo-cytoplasmic exchange of various compounds.

MATERIAL AND METHODS

Reagents: The following reagents were purchased from Serva Heidelberg, Germany: Triton X-100, p-nitrophenylsulphate dipotassium salt; 2-deoxyribose; deoxyribonucleic acid from calf thymus; 2-(4-iodophenyl)-3-(-4-nitrophenyl)-5-phenyltetrazolium chloride (INT). Glucose-6-phosphate, barium salt and oestrone sulphate, sodium salt were from Sigma Chem. Co., St. Louis USA Deoxyribonuclease (amorphous) from ox bovine pancreas was from Schuchardt, Munchen, Germany. p-Nitrophenyl phosphate dipotassium salt was from Koch Light Labs., Colnbrook-Bucks, England. Ribose was from BDH Biochemical Division LTD, Poole England.

Enzyme activity determination. Arylsulphatase C was determined in the presence of 10 mM p-nitrophenyl sulphate (NPS) or 10 mM 2-hydroxy-5-nitrophenyl sulphate (NCS) in 0.25 mM Tris-HCl buffer pH 7.5. The reaction was terminated after appropriate time of incubation at 37°C by the addition of 3 ml of 5% NaOH. The liberated p-nitrophenol or 4-nitrocatechol was measured at 405 and 510 nm. The steroid sulphohydrolase activity was determined at 0.25 mM oestrone sulphate concentration and 0.25 mM Tris-HCl buffer pH 7.5. The unhydrolysed substrate was estimated by the procedure of Roy [26].

Glucose-6-phosphatase activity determination was performed according to the procedure of S w a n s o n [29].

Succinate dehydrogenase was determined according to the procedure described by L e ź n i c k i et al. [16].

Acid phosphatase activity was determined in the presence of 10 mM concentration of p-nitrophenyl phosphate in 0.5 M acetate buffer pH 5.0. After 5 min. incubation the reaction was terminated by the addition of 3 ml of 5% NaOH. The liberated p-nitrophenol was measured at 405 nm.

DNA and RNA were separated according to M u n r o, F l e c k [21] DNA was estimated by the Dische procedure (S c h n e i d e r [28]). RNA was determined spectrophotometrically according to M u n r o, F l e c k [21].

Protein content was determined by the biuret procedure |19| or, if the interfering material was present (Triton X-100), the protein content was determined by the B e n s a d o w n, W e i n s t e i n procedure [3].

Phospholipid Pi was estimated by the procedure of F i s k e, S u b b a r o w [8]. For extraction of phospholipids a mixture of etanol-ethyl ether (1:3) was used. The phospholipid content was calculated by multiplying the determined amount of phosphorus by [26].

The preparation of nuclear fraction from human placenta. The procedure of P h i l p o t, S t a n i e r [23] was addapted for the preparation of human placenta cell nuclei. The placenta tissue, shortly after delivery, was washed with 0.15 M NaCl and homogenized in a medium consisting of 0.3 M sucrose, 0.001 M $MgCl_2$ at pH 7.0. The placenta tissue was diluted 1:10 (w/v) for homogenization and homogenized. The resulting suspension was filtered through a nylon cloth. The filtrate was diluted with an equal volume of the homogenizing medium containing 80% glycerol. All the solutions used were kept at 0-5°C. The centrifugation at 700 x g for 10 min resulted in precipitation of crude nuclear fraction, which contains 72% of DNA present in the homogenate. The pellet was suspended in an equal volume (w/v) of the homogenization medium. 15 ml aliquotes were layered over 70 ml of the homogenization medium containing 40% glycerol and centrifuged for 10 min at 700 x g in Heintz-Janetzki refrigerated centrifuge. The pellets were resuspended in homogenization medium and the layering and centrifugation operation was repeated once again. The purified nuclear fraction contained 53% of the DNA present in the

homogenate. Additional purification of the nuclear fraction was done by repeating the layering and the centrifugation operation. The final preparation contained 46% of the DNA present in the homogenate. The precipitate of the nuclear preparation was suspended in the homogenization medium containing 40% glycerol and stored at - 15°C.

The subfractionation of the nuclei and preparation of nuclear membranes.

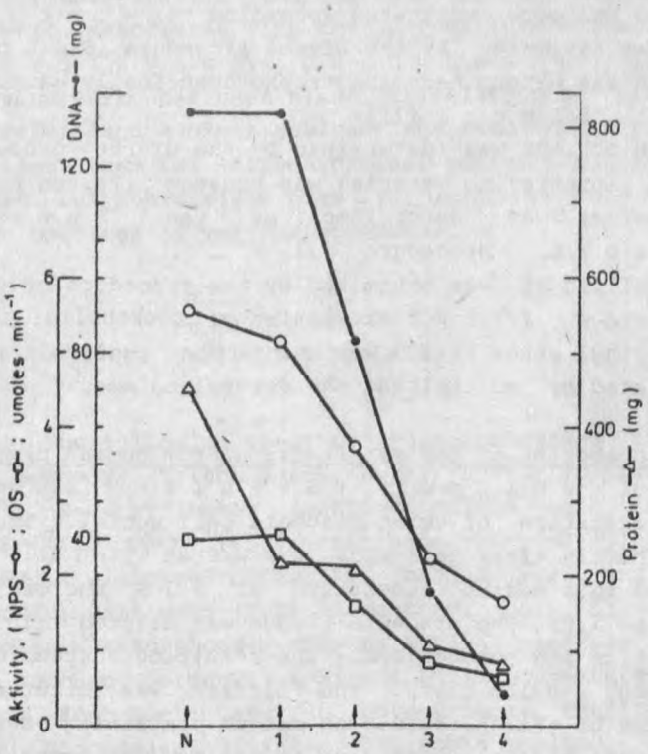

Fig. 1. DNA and protein content and sulphohydrolase activities in purified nuclear fraction, chromatin-membrane fraction and envelope fractions after deoxyribonuclease digestion

N - nuclear fraction, 1 - chromatin-membrane fraction, 2 envelopes after the first DN-ase digestion, 3 - envelopes after the second DN-ase digestion, 4 - envelopes after the third DN-ase digestion

Rys. 1. Zawartość DNA i białka oraz aktywność sulfohydrolaz w oczyszczonych jądrach komórkowych, frakcji chromatyczno-błonowej oraz frakcjach otoczek jądrowych po trawieniu dezoksyrybonukleazą

N - frakcja jądrowa, 1 - frakcja chromatynowo-błonowa, 2 - otoczki jądrowe po pierwszym trawieniu DN-azą, 3 - otoczki jądrowe po drugim trawieniu DN-azą, 4 - otoczki jądrowe po trzecim trawieniu DN-azą

The nuclear fraction was subjeted to osmotic swelling followed by mechanical shrinking, generally according to the B u s c h and M a u r i t z e n procedure [5]. The whole nuclei were swollen by resuspending the nuclear pellets in 0.14 M NaCl and 0.05 M Tris-HCl buffer pH 7.6 containing 1 mM $MgCl_2$ and afterwards they underwent a carefull shearing in Potter glass homogenizer. The sheared nuclei were centrifuged at 40 000 x g for 1 h for separation of the nuclear sap. The debris from the centrifugation was extracted with 2 M NaCl at pH 7.6, kept in ice bath for 1 h, and centrifuged at 16 000 x g for 20 min.

This procedure was supposed to separate most of the histone proteins in supernatant and chromatin-membranes in pellets. The pellets of the chromatin-membranes (Fig. 1, fraction 1) were suspended in the homogenizing buffer to a final protein concentration of about 5 mg per ml; deoxyribonuclease was added (100 µg per ml) and the digestion was continued for 16 h at 2°C. The suspension was centrifuged at 36 000 x g for 15 min. The pellet represented nuclear envelope fraction, designated as fraction 2; the supernatant represented chromatin fraction 2. The second and third additional digestion with deoxyribonuclease made possible to obtain the nuclear envelopes with low DNA content, designated as fraction 3 and 4 and the respective chromatin fractions.

RESULTS

Enzymatic activities and the data on protein and DNA contents in the crude (A), purified (B) and additionally purified (C) nuclear fractions are presented in Tab. 1.

The nuclear preparations A, B, and C contained respectively: 72%, 53% and 45.7% of the DNA and 13%, 7.8% and 4% of the protein present in the original homogenate. The ratio of protein: DNA, revealing the value of 4.14 in the less purified nuclear preparations, was lowered to 1.94-1.54 in the purified nuclear preparations (Tab. 1). According to R o o d y n [25] the value of protein: DNA ratio varies from 2.4-22; the highly purified nuclear preparations revealed lower values (2.4-5.8) while haevily contaminated nuclear preparations showed relatively high protein: DNA ratios (12-22).

Table 1

DNA and protein content as well as marker enzyme and sulphohydrolase activities in the nuclear fraction from human placenta

Zawartość DNA i białka oraz aktywność enzymów markerowych i sulfohydrolaz we frakcji jąder komórkowych z łożyska ludzkiego

Specification	A	B	C
DNA recovery (%)	72.00	53.25	45.70
DNA mg per g of fresh tissue	1.77 ± 0.31	1.31 ± 0.41	1.12 ± 0.69
Protein recovery (%)	13.31	7.76	4.02
Protein mg per g of fresh tissue	7.31 ± 0.86	4.27 ± 0.95	2.21 ± 0.53
Protein: DNA ratio	4.14	3.26	1.94
Succinate dehydrogenase umole of substrate · min^{-1} · · mg $protein^{-1}$	0.025 ± 0.01	0.014 ± 0.001	0.012 ± 0.007
Glucose-6-phosphatase umoles of substrate · min^{-1} · · mg $protein^{-1}$	0.163 ± 0.06	0.129 ± 0.040	0.031 ± 0.020
Acid phosphatase umoles of substrate · min^{-1} · · mg $protein^{-1}$	14.74 ± 5.44	17.59 ± 5.62	13.99 ± 1.23
Arylsulphatase C nmoles of substrate · min^{-1} · · mg $protein^{-1}$	4.71 ± 0.48	5.79 ± 1.24	9.06 ± 2.74
Oestrone sulphate sulphohydrolase nmoles of substrate · min^{-1} · · mg $protein^{-1}$	2.45 ± 0.32	2.04 ± 0.51	3.33 ± 1.62

Note: A - crude nuclear fraction, B - purified nuclear fraction, C - additionally purified nuclear faction. The data are mean of 4-5 series of experiment.

The specific activity of succinic acid dehydrogenase, which is the marker enzyme of the mitochondrial fraction, is very low; and is beeing lovered during the course of the purification of the nuclear fraction from the value of 0.025 to 0.012. This indicates that the contamination with mitochondrial particles of the purified nuclear preparations (C) is very low.

Since the nuclear membrane is contineous in certain regions with endoplasmic reticulum (Mirsky, Osawa [20];

Franke, Scheer [9]; Arion et al. [1]) it is quite likely that certain enzymes in the nuclear fraction are of microsomal origin. The most likely "marker" for contamination with microsomes is glucose-6-phosphatase. The data presented in Tab. 1, show that the specific activity of the glucose-6-phosphatase in the purified nuclear fraction is very low. The purification procedure resulted in a five-fold diminishing of the specific activity of the enzyme.

In contrast to the data for succinic acid dehydrogenase and glucose-6-phosphatase, the specific activity of acid phosphatase was not reduced during the course of the purification procedure, which suggeste that the enzyme is an integral component of the nuclear fraction. This statement is in according with the data given in the literature, indicating that acid phosphatase (a low molecular form) is present in the nuclear preparations (Gałka et al. [10]; Szeszak, Pinna [30]).

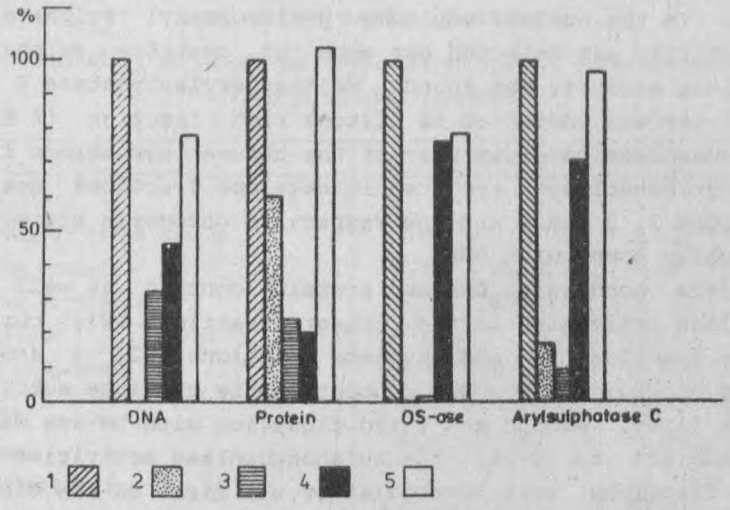

Fig. 2. DNA and protein content and sulphohydrolase activities in subnuclear fractions

1 - purified nuclei, 2 - nuclear sap, 3 - histone rich fraction, 4 - chromatin-membrane fraction, 5 - recovery

Rys. 2. Zawartość DNA i białka oraz aktywność sulfohydrolaz we frakcjach subjądrowych

1 - jądra oczyszczone, 2 - sok jądrowy, 3 - frakcja bogata w histony, 4 - chromatynowo-membranowa frakcja, 5 - odzysk

The specific activities of sulphohydrolase C and oestrone sulphate sulphohydrolase were beeing raised after purification of the nuclear fraction (Tab. 1). The respective data of specific activities in nuclear preparations A, B and C are: 4.75, 5.79 and 9.06 in the presence of NPS as substrate and 2.45, 2.04 and 3.33 in the presence of oestrone sulphate as substrate. These results indicate the sulphohydrolases as the true nuclear enzymes.

The data on DNA and protein content, as well as p-nitrophenylsulphate and oestrone sulphate hydrolysing activities in subnuclear fractions are presented in Fig. 2.

The overal recovery of DNA and protein after subfractionation of highly purified cell nuclei is 78% and 104%, respectively. The oestrone sulphate sulphohydrolase and arylsulphatase C are recovered in the range of 80-97%. It is worth mentioning that subfractionation of the nuclear fraction with biochemical characteristics similar to the nuclear fraction B gave lower recovery of oestrone sulphohydrolase activity. Almost all of the sulphohydrolase activities appeared in the "chromatin-membrane" fraction (Fig. 2). In the nuclear sap some p-nitrophenyl sulphate hydrolysing activity was detected but none of oestrone sulphate sulphohydrolase activity was found. Neither arylsulphatase C nor OS--ase activity was detected in histone rich fraction (2 M NaCl).

Three successive digestion of the chromatin-membrane fraction with deoxyribonuclease resulted in envelope fractions designated as fractions 2, 3 and 4 and the respective chromatin protein fractions tightly bound with DNA.

The data concerning DNA and protein content as well as sulphohydrolase activities in the nuclear fraction (N), chromatin--membrane fraction (1) and envelope fractions (2, 3 and 4) are presented on Fig. 1. The DNA content in the envelope subfractions after the first, second and third digestion with DN-ase was diminished from 12% to 0-1%. The sulphohydrolase activities in the envelope fractions were lowered after the first DN-ase digestion. No change of sulphohydrolase activities appeared after the second and third successive digestion with DN-ase. These results suggest that sulphohydrolase are both integral part of the nuclear envelope, and are constituents of the chromatin proteins fraction tightly bound with DNA. The overal recovery of the sulphohydrolase activities in the envelope fraction and chromatin fractions tightly bound with DNA are found in the range of 91%-96%.

Composition analysis of the nuclear preparations (C) and

nuclear envelope preparations (after single digestion with DN-ase of the chromatin-membrane fraction) is presented in Tab. 2 and 3.

Table 2

Chemical composition of nuclear fraction and nuclear envelope fraction (in %)
Skład chemiczny frakcji jąder komórkowych i frakcji otoczek jądrowych (w %)

Specification	Protein	DNA	RNA	Phospholipid	RNA : DNA
Cell nuclei[a]	56.37	37.04	1.41	5.02	0.04
Envelopes[b]	59.43	12.12	0	28.59	-

[a] The data are mean of 8 series of experiment.
[b] The data are mean of 3 series of experiment.

Table 3

Chemical composition and sulphohydrolase activities of nuclear fraction and nuclear envelope fraction

Skład chemiczny i aktywność sulfohydrolaz we frakcji jąder komórkowych i frakcji otoczek jądrowych

Specification	Nuclear fraction	Nuclear envelopes
Protein		
mg per g of fresh tissue	3.600 ± 1.00	0.454 ± 0.104
DNA mg/mg protein	0.554 ± 0.262	0.154 ± 0.039
RNA mg/mg of protein	0.034 ± 0.020	0
Phospholipid		
mg/mg of protein	0.093 ± 0.031	0.484 ± 0.047
E_{230}/E_{260}	1.337 ± 0.135	2.019 ± 0.332
E_{260}/E_{280}	0.689 ± 0.051	0.820 ± 0.102
Arylsulphatase C		
nmoles · min^{-1} · mg $protein^{-1}$	10.38 ± 3.56	14.32 ± 4.69
nmoles · min^{-1} · mg DMA^{-1}	18.53 ± 6.43	92.98 ± 30.45
Oestrone sulphate sulphohydrolase		
nmoles · min^{-1} · mg $protein^{-1}$	3.53 ± 1.08	5.46 ± 1.58
nmoles · min^{-1} · mg DNA^{-1}	6.37 ± 1.95	35.45 ± 10.26

Note: The data are mean of 5 series of experiments.

With the assumption that % protein + % DNA + % RNA + % phospholipid = 100%, the nuclear fraction contained: 56.4% of protein, 37% of DNA, 5.02% of phospholipid and 1.42% of RNA. The protein: DNA, phospholipid : DNA, RNA : DNA ratios as well as the ratios of E_{230}/E_{260} and E_{260}/E_{280} (Tab. 3), are comparable with those of the most highly purified nuclear preparations (R o o d y n [25]).

The nuclear envelope fraction contained: 59.4% of protein, 12.12% of DNA and 28.58% of phospholipid. No RNA was detected in the envelope fraction. The data of protein and phospholipid content (Tab. 2 and 3) are very characteristic for nuclear envelope preparations (B e r e z n y et al. [4], M a l e c [18], K a s p e r [13]), but the DNA content after the single digestion with DN-ase is relatively high (9-12%). These results prooved the general statement that the most significant differences in envelope composition from different sources is the variation in DNA content which varies from 0-8% (Kasper [13]).

DISCUSSION AND CONCLUSIONS

The analitycal data concerning DNA, protein, RNA and phospholipid concentration, as well as the data on the marker enzyme activities show that the nuclear fraction isolated from human placenta by the procedure described in this paper are of high purity (R o o d y n [25]).

As concluded from the specific activities of succinic acid dehydrogenase and glucose-6-phosphatase (Tab. 1), the first step of the purification procedure made possible to separate most of the mitochondrial impurities, while the second additional step allowed to eliminate the microsomal contaminations. It is also unlikely that the outer leaflet of the envelope, that revealed considerable activity of glucose-6-phosphatase, was cleared away after the third additional step of purification (A r i o n et al. [1]). In contrast to this, neither the first nor the second and third step of the purification procedure effected in lowering of the oestrone sulphate sulphohydrolase and arylsulphatase C activities: the specific activities of both enzymes are raised after each step of purification; this indicates that the two sulphohydrolases may be considered as true placental cell nuclear

enzymes. An open question at present is, did the enzymes represent activities common to the nuclei originated from all tissues or wether are they characteristic only for cell nuclei of very distinct tissues or organs? It should be stressed that the specific activities of arylsulphatase C and oestrone sulphate sulphohydrolase of the purified placental cell nuclei and envelopes are of such magnitude that they cannot be ignored.

The preliminary data presented in this paper showed that sulphohydrolase activities are associated with the nuclear envelope and non histone chromatin proteins tightly bound with DNA.

The biochemical significance on the presence of OS-hydrolysing activity and arylsulphatase C activity is not, at present, clear; it might not be ruled out that they play and important role in nucleo-cytoplasmic exchange of various compounds.

REFERENCES

[1] Arion W. J., Schulz L. O., Lange A. J., Telford J. N., Walls H. F. (1983), J. Biol. Chem., 258, 12661-12668.

[2] Balasubramanian A. S. (1976), Indian J. Biochem. Biophys., 12, 325-330.

[3] Bensadown A., Weinstein D. (1976), Anal. Biochem., 70, 241-250.

[4] Berezny R., Funk L. K., Crane F. L. (1970), Biochim. Biophys. Acta, 203, 531-546.

[5] Busch H., Mauritzen Ch. M. (1967), [in:] Methods in cancer research, Academic Press, New York, Vol. III, 391.

[6] Dodgson K. S., Spencer B., Thomas J. (1954), Biochem. J., 56, 177-188.

[7] Dolly J. O., Dodgson K. S., Rose F. A. (1972), Biochem. J., 128, 337-345.

[8] Fisce C. H., Subbarow J. (1925), J. Biol. Chem., 66, 375-400.

[9] Franke W. W., Scheer U. (1974), [in:] The cell nucleus, ed. E. H. Busch, Academic Press, New York, San Francisco, London, Vol. II, 232-256.

[10] Gałka M., Dziembor-Gryszkiewicz E., Kos S., Ostrowski W. (1980), Acta Biochem. Pol., 27, 281-283.

[11] Gniot-Szulżycka J., Jakubowska A., Janu-szewska B. (1984), 16-th Meeting of the Federation of European Biochemical Societies, Moscow, Abstract II-188, 191.

[12] Gniot-Szulżycka J., Jakubowska A. (1984 accepted for publication), Acta Univ. N. Copernici, XXXI.

[13] Kasper Ch. B. (1974), [in:] The cell nucleus, ed. E. H. Busch Academic Press, New York, San Francisco, London, Vol. II, 349-384.

[14] Kishimoto J., Sostek R. (1972), J. Neurochem., 19, 123-130.

[15] Lewicki J., Trzeciak W. H. (1972), Am. J. Obst. and Gynecol., 112, 886-889.

[16] Leźnicki A., Rożańska M., Dymecki J. (1976), Neuropat. Pol., 14, 41-55.

[17] Makita T., Sandborn E. B. (1971), Experientia, 27, 187-189.

[18] Malec J. (1971), Postępy Biochemii, 17, 195-208.

[19] Mejbaum-Katzenellenbogen W., Mochnacka I. (1966), [in:] Kurs praktyczny z biochemii, Warszawa, 167-168.

[20] Mirsky A. E., Osawy S. (1961), [in:] The cell, eds J. Brachet, A. E. Mirsky, Academic Press, New York, Vol. II, 677.

[21] Munro H. N., Fleck A. (1967), [in:] Methods of biochemical analysis, ed. E. Glick, Interscience Publ., New York, London, Sydney, Vol. XIV, 113-176.

[22] Perumal A. S., Lakshman M. R., Cama H. R. (1968), Biochim. Biophys. Acta, 170, 399-408.

[23] Philpot J. S. L., Stanier J. E. (1956), Biochem. J., 63, 214-223.

[24] Pokrowskij A. A., Zbarskij J. B., Tytelia B. A., Perewoszczikowa K. A., Laszewa N. W., Delektorskaja L. N. (1968), Dokłady Akademii Nauk SSSR, 5, 1280-1283.

[25] Roodyn D. B. (1963), [in:] Biochemical symposia No 23 - Methods of separation of subcellular structure components Cambridge Univ. Press, 20-36.

[26] Roy A. B. (1956), Biochem. J., 63, 294-300.

[27] Roy A. B. (1960), Biochem. J., 77, 380-386.

[28] Schneider W. C. (1957), [in:] Methods in enzymology, eds S. P. Colowick, N. O. Kaplan, Academic Press, New York, Vol. III, 680--684.

[29] S w a n s o n M. A. (1955), [in:] Methods in enzymology, eds S. P. Colowick, N. O. Kaplan, Academic Press, New York, Vol. II, 541-543.
[30] S z e s z a k F., P i n n a L. A. (1980), Mol. Cell. Biochem., 32, 13-20.

Department of Biochemistry
Institute of Biological
University N. Copernicus
in Toruń

Jadwiga Gniot-Szulżycka, Anna Jakubowska

AKTYWNOŚĆ SULFOHYDROLAZY SIARCZANU ESTRONU I ARYLOSULFATAZY C W JĄDRACH KOMÓRKOWYCH Z ŁOŻYSKA LUDZKIEGO I SUBFRAKCJACH JĄDROWYCH

Opisano metodę otrzymywania jąder komórkowych i otoczek jądrowych z tkanki łożyska ludzkiego. Preparaty jąder komórkowych zawierały: 56,4% białka, 37% DNA 5,02% fosfolipidów i 1,42% RNA. Stosunek białka do DNA wahał się w granicach 1,20-1,94; E_{230}/E_{260} i E_{260}/E_{280} wynosiły odpowiednio 1,34 i 0,689. Z oczyszczonych jąder komórkowych uzyskiwano: sok jądrowy, frakcję bogatą w białka histonowe i frakcję chromatynowo-błonową. Trawienie tej ostatniej dezoksyrybonukleazą pozwoliło na otrzymanie frakcji otoczek jądrowych o składzie: 59,4% białka, 1-12% DNA, 28,6% fosfolipidów.

Przedstawiono dane świadczące, iż sulfohydrolaza C i sulfohydrolaza siarczanu estrone są integralnymi składnikami jąder komórkowych łożyska ludzkiego. Aktywność enzymatyczną stwierdzono w otoczkach jądrowych i frakcji białek chromatynowych silnie związanych z DNA i częściowo pozbawionych białek histonowych.