Biochem. J. (1980) **187**, 851–856
Printed in Great Britain

The Serum Competitor of Oestrogen–Rat α_1-Foetoprotein Interactions

IDENTIFICATION AS A MIXTURE OF NON-ESTERIFIED FATTY ACIDS

Geneviève VALLETTE,* Claudine BENASSAYAG,* Lia SAVU,* Jacqueline DELORME,* Emmanuel A. NUNEZ,* Jacques DOUMAS,† Gabrielle MAUME† and Bernard F. MAUME†
**U.E.R. Biomédicale des Saints-Pères, 45 rue des Saints-Pères, 75270 Paris Cedex 06, and †Laboratoire de Biochimie des Interactions Cellulaires, Université de Dijon, Faculté des Sciences, Mirande B.P. 138, 21004 Dijon Cedex, France*

(*Received 12 November 1979*)

The novel endogenous serum ligands of rat α_1-foetoprotein previously demonstrated in different mammalian sera were identified by g.l.c.–mass-spectrometric methods as a mixture of non-esterified long-chain and predominantly unsaturated fatty acids. Detailed comparative analyses of these ligands extracted from foetal- and pregnant-rat sera, rat amniotic fluid and foetal human sera are presented. We also show that an important fraction of these ligands remains associated with the rat α_1-foetoprotein after purification; analyses are given for the composition of this lipid moiety of the foetoprotein. The physiological relevance of these results is discussed.

The demonstration of the oestrogen-binding property of rat and mouse α_1-foetoprotein (Nunez *et al.*, 1971; Savu *et al.*, 1972; Uriel *et al.*, 1972; Nunez *et al.*, 1973; Benassayag *et al.*, 1975; Savu *et al.*, 1977) has suggested numerous hypotheses concerning a possible role of these embryonic proteins in the control of oestrogen activities during foetal or tumour development. A recent systematic study in our laboratory on the hormonal milieu of the perinatal rat has led us to the discovery of new α_1-foetoprotein serum ligands, designated as 'L', able to compete for the α_1-foetoprotein-bound phenol steroids but nonreactive towards anti-oestrogen antibodies (Benassayag *et al.*, 1977; Nunez *et al.*, 1979). The physiological importance of such non-oestrogen endogenous competitors is evident, since their involvement in regulatory endocrine function of the foetoproteins in the rodents seems probable. Moreover, we have shown that these new ligands are also present in species carrying non-oestrophilic α_1-foetoproteins (Nunez *et al.*, 1974), particularly in human sera (Benassayag *et al.*, 1977), thus suggesting the further possibility of an additional role unrelated to their inhibitory action on the α_1-foetoprotein–oestrogen interactions.

The present results concern primarily the g.l.c.–mass-spectrometric identification of the new endogenous serum ligands of rat and mouse α_1-foetoproteins as a mixture of free, predominantly unsaturated, long-chain fatty acids. Comparative data are also given for the quantitative and qualitative compositions of these ligands purified from foetal- and pregnant-rat sera, rat amniotic fluid and human foetuses.

On the other hand, we have shown that rat α_1-foetoprotein still contains, after purification, a lipid fraction able to interfere with its oestrogen-binding properties in a way similar to that of the 'L' serum lipid fraction (Benassayag *et al.*, 1979). We present here data on the chemical nature of the lipid fraction associated with purified rat α_1-foetoprotein.

Material and Methods

Animals and sera

Sprague–Dawley (Charles River France, St-Aubin-les-Elbeuf, France) rats at different developmental stages were used for the preparation of sera, α_1-foetoprotein and α_1-foetoprotein ligands. Human foetal sera originated from 3–4- and 5½-month foetuses after therapeutic abortions.

Fatty acid standards

The following authentic samples of fatty acids were obtained from Nu Chek Prep, Elysian, MN, U.S.A.: myristic acid ($C_{14:0}$); palmitic acid ($C_{16:0}$); stearic acid ($C_{18:0}$); heptadecanoic acid ($C_{17:0}$); palmitoleic acid ($C_{16:1}$); oleic acid ($C_{18:1}$); linoleic acid ($C_{18:2}$); arachidonic acid ($C_{20:4}$); 7,10,13,16-docosatetranoic acid ($C_{22:4}$). The 4,7,10,13,19-docosahexanoic acid ($C_{22:6}$) was purchased from Serdary Research Laboratories, London, Ont., Canada.

0306-3275/80/060851-06$01.50/1

Proteins

Rat α_1-foetoprotein was purified by electrophoretic methods as described previously (Benassayag *et al.*, 1975).

Preparation of the lipid serum ligand 'L' of rat α_1-foetoprotein

The extraction of rat and human sera and the chromatographic preparation from these extracts of the corresponding 'L' fractions were performed as described previously (Benassayag *et al.*, 1977; Nunez *et al.*, 1979). The mean recovery was about 80%.

Lipid endogenous ligands of rat α_1-foetoprotein

These were obtained from pure rat α_1-foetoprotein by the extraction procedure described by Parmelee *et al.* (1978).

Reagents

The methylation reagents were dimethylformamide-dimethylacetal ('Methyl-8'; Pierce Chemical Co., Rockford, IL, U.S.A.) or 14% BF_3 in methanol (Sigma Chemical Co., St. Louis, MO, U.S.A.). The silylation reagents were bis(trimethylsilyl)acetamide and trimethylchlorosilane (Supelco, Bellefonte, PA, U.S.A.).

Derivative formation

The methylation was carried out by heating the dried lipid extract for 10 min at 65°C with 0.1 ml of Methyl-8 or 0.1 ml of 14% BF_3 in methanol. In the first case a 1–2 μl portion was directly injected into the packed column of the gas chromatograph, in the second case 1 ml of water was added to the reagent mixture, which was extracted with 1 ml of hexane (three times); after evaporation to dryness with N_2, the residue was taken up with 100 μl of hexane.

The trimethylsilylation was performed by heating the dried lipid extract for 1 h at 65°C with 0.1 ml of bis(trimethylsilyl)acetamide/trimethylchlorosilane (4 : 1, v/v).

Identification by g.l.c.–mass spectrometry

The trimethylsilylated extract was analysed on a quadrupole mass spectrometer coupled with a glass capillary column and monitored by a Finnigan (Sunnyvale, CA, U.S.A.) 3300–6100 computer. The glass capillary column (25 m × 0.21 mm; 110000 theoretical plates) from LKB Instruments (Orsay, France) coated with SE 30 was directly connected to the mass spectrometer source by a deactivated glass line. The spectrometer was equipped with a chemical-ionization source; methane was the reagent gas; the ion source pressure was 93.3 Pa (0.7 Torr), the electron energy 130 eV and the filament current 0.25 mA.

Quantification of fatty acids from serum 'L' extracts and from the lipid associated with pure α_1-foetoprotein

A portion of the methylated sample was analysed with a model 427 Packard Becker (Delft, The Netherlands) chromatograph equipped with an hydrogen flame detector. A silanized glass column (4 m × 3 mm) packed with the methyl silicone SE 30 (Supelco) at 1% on Gas Chrom Q was operated with N_2 flow of 30 ml/min. The temperature was programmed at 1°C/min from 180°C. The response coefficients and concentrations were determined by planimetry for each fatty acid with respect to an internal standard of n-heptadecanoic acid ($C_{17:0}$) i.e. a compound absent from the biological extracts.

Analysis was also carried out with a model 419 Packard gas chromatograph on an SE 30 capillary column (25 m × 0.23 mm) with a temperature program of 175°C with 5°C/min. In this case the response coefficients and concentrations were determined by measuring the heights of the peaks. Similar results were obtained with a polar capillary column of diethylene glycol succinate.

Blanks from all the buffers and solvents used with the biological material were run. No exogenous contaminant was observed.

Results

G.l.c.–mass-spectrometric identification of the extract 'L' from rat amniotic fluid, embryo and pregnancy sera

The fraction 'L' extracted from 19-day-embryo sera was analysed after trimethylsilylation as well as after methylation by g.l.c. on a SE 30 capillary column coupled to a computerized mass spectrometer operating in the chemical-ionization mode. As shown in Table 1, at least nine compounds have been identified as saturated and unsaturated fatty acids: $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{20:4}$, $C_{22:4}$, $C_{22:6}$. Significant amounts of squalene were also observed.

Further, these results were confirmed by comparing the unknown compounds with known reference fatty acids by g.l.c. of methylated derivatives on a 1% SE 30-packed column. The same techniques were applied to 'L' fractions from amniotic fluid, pregnant-rat serum and human foetal serum. These biological fluids contain the same fatty acids as the rat foetal serum but important quantitative differences may be observed, and these are documented below.

Quantification by g.l.c. of the fatty acids derived from rat amniotic fluid, foetal-rat and pregnant-rat sera

A quantitative comparative analysis of 'L' fractions from the different biological fluids was per-

Table 1. *Mass (m/e values) and relative abundance of ions observed in methane-chemical-ionization mass spectrometry from fatty acids of embryonic-rat serum 'L' extract*

Fatty acids were observed as trimethylsilyl derivatives. The mass-spectrometric conditions are given in the text.

Spectrum no. in analysis of extract 'L'	Fatty acid identified	Molecular weight	Fragment ...	Mass (*m/e*) and relative abundance (in parentheses)							
				M+41	*M*+29	*M*+H	*M*−H	*M*−15	*M*H−72	*M*H−90	Other fragments
34	$C_{14:0}$	300		341 (4)	329 (10)	301 (100)	299 (35)	285 (25)	229 (4)	211 (15)	
98	$C_{16:1}$	326		367 (4)	355 (13)	327 (100)	325 (28)	311 (24)	255 (4)	237 (8)	
112	$C_{16:0}$	328		369 (5)	357 (12)	329 (100)	327 (60)	313 (35)	257 (4)	239 (12)	
201	$C_{18:2}$	352		393 (4)	381 (14)	353 (100)	351 (27)	337 (28)	—	263 (23)	
210	$C_{18:1}$	354		395 (4)	383 (13)	355 (100)	353 (35)	339 (26)	283 (2)	265 (10)	
229	$C_{18:0}$	356		397 (5)	385 (15)	357 (100)	355 (67)	341 (34)	285 (4)	267 (11)	132 (6.5), 117 (5.2)
304	$C_{20:4}$	376		417 (5)	405 (16)	377 (100)	375 (16)	361 (19)	305 (2)	287 (22)	269 (7.8), 191 (6.8), 177 (6), 163 (8.3), 137 (12), 121 (8.1)
432	$C_{22:6}$	400		441 (7)	429 (21)	401 (100)	399 (11)	385 (24)	329 (4)	311 (29)	293 (16.7), 269 (18.3), 241 (11), 215 (24.6), 201 (25.1), 197 (20.4), 175 (34.5), 163 (25.1), 161 (35.6), 149 (37.1), 135 (53.4), 121 (42.9), 109 (13.4)
440	$C_{22:4}$	404		445 (4)	433 (19)	405 (100)	403 (31)	389 (23)	—	315 (13)	203 (13), 191 (9.4), 179 (11.5), 177 (7.8), 165 (9.4), 151 (14.6), 137 (14.6), 121 (25.1), 109 (16.7), 107 (14.6)

Table 2. *Relative percentage of the endogenous non-esterified fatty acids of different rat sera*

The quantification was performed by gas chromatography with a Packard model 427 instrument on a packed-1% SE 30 column on six pools from pregnant rats at day 19 of gestation. Abbreviations used: TFA, total fatty acid; Sat FA, saturated fatty acid; Mono, monounsaturated; Di, diunsaturated; Poly, polyunsaturated.

Fatty acid ...	Relative percentage									TFA (nmol/ml)	Sat FA (%)	Mono + Di FA (%)	Poly FA (%)
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:4}$	$C_{22:6}$				
19-Day embryonic-rat serum	2.5±0.6	23±5	8±1.5	7±1.5	21±1.5	13±0.6	10±1.5	2±0.3	13.5±1.5	260±36	33±4	42±2	25±5
19-Day-pregnant-rat serum	1±0.1	24.5±1	4±0.6	5±0.4	30±4	23.5±1.5	5.5±1.5	1.5±0.3	5±1.5	1220±150	31±1.5	57±3	12±2
19-Day-pregnant-rat amniotic fluid	2±0.4	17±4.5	19±9	6±3	21±5.5	18±2	6±0.9	2.5±0.6	8.5±1	24±4	25±9	58±9	17±3

formed on a 1% SE 30 column. The relative percentages of the different fatty acids are shown in Table 2.

The highest concentration of total non-esterified fatty acids is observed in the pregnant-rat serum: it is four or five times higher than that of the rat embryo and about 50 times that measured in the amniotic fluid.

Additional differences between the foetal and maternal compartments are manifest when comparing the relative proportions of the individual fatty acids (Table 2). In particular, it is apparent that the foetal serum contains the highest percentage of polyunsaturated fatty acids.

In Table 3 we show the molar ratios (foetus versus mother) for each fatty acid. These are particularly elevated for the $C_{14:0}$, $C_{16:1}$, $C_{20:4}$ and especially for $C_{22:6}$ fatty acids, with a foetus/mother value of 0.6. It is also noteworthy that the ratios are particularly low for the fatty acids that are most abundant in the maternal serum: $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$. These results suggest that the fatty acids may not be distributed passively between the foetal and maternal compartments.

Quantification of fatty acids extracted from human sera

Our studies on the non-oestrogen-binding human foetal sera have evidenced compounds analogous to the 'L'-serum fractions derived from rat and mouse with a strong inhibitory effect on the rat α_1-foetoprotein–oestrogen interaction (Benassayag *et al.*, 1977). It was deduced that the presence of such inhibitors bears no relation to the oestrophilic character of the α_1-foetoprotein of the studied species.

Table 4 gives composition of 'L' fractions from three human foetal sera at three developmental stages. The percentage of unsaturated fatty acids is noteworthy, and is similar to the situation in the rat; it represents 65–75% of the total non-esterified fatty acids. The major polyunsaturated fatty acid in the human is arachidonic acid; it constitutes 70% of the polyunsaturated species. Significant quantities (24%) of $C_{22:6}$ fatty acid are also found. It is interesting to compare these values with the relative proportions of these two fatty acids in the rat, where, in contrast, the $C_{22:6}$ predominates over the $C_{20:4}$ fatty acid (60 and 40% respectively).

Identification and quantification of the fatty acids extracted from purified rat α_1-foetoprotein

The lipids extracted from four different α_1-foetoprotein preparations have been analysed by g.l.c. on a 1% SE 30-packed or SE 30 capillary column. All the fatty acids identified in the embryo serum extracts were found in the lipids from the purified α_1-foetoprotein.

Table 5 shows the total and individual fatty acid concentrations in the different preparations. Under our purification conditions, 1–3 mol of fatty acid/mol of α_1-foetoprotein remains closely attached to the protein. The unsaturated species represent the bulk of these α_1-foetoprotein-associated fatty acids, i.e. about 60–70% of the total lipid moiety. Although the relative percentages of the majority of the fatty acids vary from one preparation to another, some predominant ligands are evident in all the studied batches; these are the $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{22:6}$ fatty acids.

Discussion and Conclusions

Previous studies from our laboratory have demonstrated in rat, mouse and human embryo sera a non-oestrogen lipid fraction, acting nonetheless as a physiological competitor with respect to the oestrogen–rat α_1-foetoprotein interactions (Benassayag *et al.*, 1977, 1979; Vallette *et al.*, 1979).

The present work identifies this fraction, by g.l.c.–mass-spectrometric techniques, as a mixture of non-esterified fatty acids, mostly unsaturated. All the identified fatty acids are found in the maternal and foetal sera as well as in the amniotic fluid of the rat, but significant quantitative differences may be observed. In particular the relative amounts of the polyunsaturated compounds are highest in the embryo.

Our present studies also show that the pattern of the lipid portion of purified α_1-foetoprotein is

Table 3. *Ratios (foetus/pregnant, amniotic-fluid/pregnant and amniotic-fluid/foetus) for the molar concentrations of rat serum fatty acids*

Abbreviation used: TFA, total fatty acid.

	Molar-concentration ratio									
Fatty acid ...	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:4}$	$C_{22:6}$	TFA
Foetus/pregnant	0.53	0.2	0.43	0.30	0.15	0.12	0.39	0.28	0.57	0.21
Amniotic-fluid/pregnant	0.04	0.01	0.09	0.02	0.01	0.01	0.02	0.03	0.03	0.02
Amniotic-fluid/foetus	0.07	0.07	0.21	0.08	0.09	0.13	0.06	0.12	0.06	0.09

Table 4. *Non-esterified-fatty-acid concentrations in sera of human foetuses of different ages*
For further details, see the legend to Table 2.

Serum extract	Fatty acid	Concentration (nmol/ml) ...$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:4}$	$C_{22:6}$	TFA	Sat FA (%)	Mono + Di FA (%)	Poly FA (%)
Embryo serum (3 months)		1.5	24	8	17	71	5.5	40	6	10	183	23	46	31
Embryo serum (4 months)		3	70	10	20.5	120	4	12	2	9	250.5	37	54	9
Embryo serum (5½ months)		8	139	24	41.5	111.5	94.5	80.5	4	27	530	36	43	21

Table 5. *Molar amounts and relative percentages of the endogenous fatty acids with respect to pure rat α_1-foetoprotein*
The quantification was performed by g.l.c. with a packed 1% SE 30 column or a SE 30 capillary column(*). Abbreviations are defined in Table 2.

'L' extracts from purified α_2-foetoprotein	Fatty acid	Molar amount [10^{-3} × (mol of fatty acid/mol of α_1-foetoprotein)] or relative percentage (in parentheses) ... $C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:4}$	$C_{22:6}$	TFA (mol of FA/mol of α_1-foetoprotein)	Sat FA (%)	Mono + Di FA (%)	Poly FA (%)
Preparation I		21.5	355	113	74	347	175	48	53	191	1.4	32.5	46	21.5
		(1.5)	(26)	(8)	(5)	(25)	(13)	(3.5)	(4)	(14)				
Preparation II		30	409	96	155	248	62	87	27	198	1.3	45	31	24
		(2)	(31)	(7)	(12)	(19)	(5)	(7)	(2)	(15)				
Preparation III		92	764	231	189	619	230	195	42	315	2.7	38	41	21
		(3)	(28)	(9)	(7)	(23)	(9)	(7)	(2)	(12)				
Preparation IV*		150	600	100	400	380	200	80	40	570	2.5	46	27	27
		(6)	(24)	(4)	(16)	(15)	(8)	(3)	(1.5)	(22.5)				

analogous to that of the lipids extracted from whole sera. As shown recently by equilibrium-dialysis studies, the major polyunsaturated fatty acids of this lipid moiety display particularly high association constants for the protein (Benassayag *et al.*, 1980).

Taken together, these results suggest a selective transfer of polyunsaturated compounds from mother to foetus, with perhaps the α_1-foetoprotein playing the role of a trap. It may alternatively be envisaged that the placenta metabolizes some fatty acids to their superior polyunsaturated homologues and/or that a specific foetal lipogenesis is involved.

Important analogies may be observed between the fatty acids of rat α_1-foetoprotein and those identified by us in human foetal sera or described by Parmelee *et al.* (1978) for purified human α_1-foetoprotein. The predominance of $C_{20:4}$ and $C_{22:6}$ fatty acids in both species is particularly remarkable. These results point, for the first time, to a common functional property for the rat and human α_1-foetoproteins, namely an affinity for the polyunsaturated long-chain fatty acids, in contrast with their different binding behaviour towards oestrogens (Nunez *et al.*, 1974).

Much recent research and many clinical observations have shown the importance of the essential polyunsaturated fatty acids for foetal development. Dietary deficiences of these compounds in the pregnant animals or disorders of their placental metabolism seem to be responsible for certain foetal hypotrophies (Friedman & Byers, 1961; Hansen *et al.*, 1965; Degrelle-Cheymol, 1972). It is also noteworthy that the polyunsaturated fatty acids that preferentially interact with α_1-foetoprotein (Benassayag *et al.*, 1979) are essential to the building up of the embryo nervous system (Crawford *et al.*, 1976, 1977) and seem to play important roles in the development of chemically induced tumours (Gammal *et al.*, 1967; Hopkins & West, 1978; James *et al.*, 1979) as well as in the lymphocyte function (Tonkin & Brostoff, 1978).

Our results suggest a central role of α_1-foetoprotein for concentrating in the foetus the necessary fatty acids; the foetal antigen might thus perform complex biological functions, modulating not only hormone but also fatty acid distribution and activities during periods of intensive cellular development.

We gratefully acknowledge grants (nos. 9 and 34) from the 'Formation de Recherche Associée of the Institut National de la Santé et de la Recherche Médicale' and a grant (no. 267) from the 'Equipe de Recherche Associée of Centre National de la Recherche Scientifique'. We also are indebted to the 'Ministère des Universités' for the g.l.c. instrument endowment as well as to the 'Délégation Générale à la Recherche Scientifique et Technique' (Contract no. 78-7-2738 and grant for J.D.), the 'Fondation pour la Recherche Médicale' and the 'Université Paris VII' for their financial support. The skilled technical assistance of Mme. Claire Millot, Mme. Odile Bonnard, Mme. Nevena Christeff and Mme. Michelle Maya is acknowledged.

References

Benassayag, C., Vallette, G., Cittanova, N., Nunez, E. A. & Jayle, M. F. (1975) *Biochim. Biophys. Acta* **412**, 295–305

Benassayag, C., Vallette, G., Delorme, J., Savu, L., Nunez, E. A. & Jayle, M. F. (1977) *Steroids* **30**, 771–785

Benassayag, C., Savu, L., Vallette, G., Delorme, J. & Nunez, E. A. (1979) *Biochim. Biophys. Acta.* **587**, 227–337

Benassayag, C., Vallette, G., Delorme, J., Savu, L. & Nunez, E. A. (1980) *Oncodevelopmental Biology and Medicine* in the press

Crawford, M. A., Hassam, A. G., Williams, G. & Whitehouse, W. (1976) *Lancet* **i**, 452–453

Crawford, M. A., Hassam, G., Williams, S. G. & Whitehouse, W. (1977) *Adv. Exp. Med. Biol.* **83**, 135–143

Degrelle-Cheymol, C. (1972) Thèse de Doctorat en Pharmacie, Université René Descartes de Paris

Friedman, M. & Byers, O. (1961) *Am. J. Physiol.* **201**, 611–616

Gammal, E. B., Carrol, K. K. & Plunkett, E. R. (1967) *Cancer Res.* **27**, 1737–1742

Hansen, A. E., Wiese, H. F., Adam, D., Boelshe, A. N., Haggard, M. E., Davis, H., Newson, W. T. & Pesut, L. (1965) *Am. J. Clin. Nutr.* **15**, 11–19

Hopkins, G. & West, C. E. (1978) *J. Natl. Cancer Inst.* **60**, 849–853

James, N., Dewille, P. J., Fraker, P. M. & Daler, R. R. (1979) *J. Nutr.* **109**, 1018–1027

Nunez, E. A., Engelmann, F., Benassayag, C. & Jayle, M. F. (1971) *C.R. Hebd. Séances Acad. Sci. Ser. D.* **273**, 831–834

Nunez, E. A., Benassayag, C., Engelmann, F., Vallette, G., Hurst, L. & Jayle, M. F. (1973) *Biomedecine* **18**, 515–520

Nunez, E. A., Vallette, G., Benassayag, C. & Jayle, M. F. (1974) *Biochim. Biophys. Res. Commun.* **57**, 126–133

Nunez, E. A., Benassayag, C., Savu, L., Vallette, G. & Delorme, J. (1979) in *Carcino-Embryonic Proteins: Chemistry, Biology, Clinical Applications* (Lehman, F. G., ed.), vol. 1, pp. 171–180, Elsevier/North-Holland Biomedical Press, Amsterdam, New York and Oxford

Parmelee, D. C., Evenson, M. A. & Deutsch, H. F. (1978) *J. Biol. Chem.* **253**, 2114–2119

Savu, L., Crepy, O., Guerin, M. A., Nunez, E. A., Engelmann, F., Benassayag, C. & Jayle, M. F. (1972) *FEBS Lett.* **22**, 113–116

Savu, L., Benassayag, C., Vallette, G., Nunez, E. A. & Jayle, M. F. (1977) *Biochimie* **59**, 323–328

Tonkin, C. H. & Brostoff, J. (1978) *Int. Arch. Allergy Appl. Immun.* **57**, 171–176

Uriel, J., De Néchaud, B. & Dupiers, M. (1972) *Biochim. Biophys. Res. Commun.* **46**, 1175–1180

Vallette, G., Benassayag, C., Savu, L., Delorme, J. & Nunez, E. A., Doumas, J., Maume, G. & Maume, B. F. (1979) *Protides Biol. Fluids* **27**, 147–152