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Serum Galactosyltransferase Activity with Three Acceptors in Ovarian Cancer Patients

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Abstract—*Several laboratories have demonstrated the usefulness of serum galactosyltransferase as a biological marker for ovarian neoplasms. However, contradictory results have been published recently, which might be partially explained by differences in methodology. We thus decided to measure serum galactosyltransferase activity in patients with ovarian cancer and benign gynecological diseases using three different assay systems. A very good correlation was obtained between the results of these assays. Furthermore, we confirm that serum GT is frequently elevated in cancer patients, and is of potential value for their follow-up.*

INTRODUCTION

An elevated level of galactosyltransferase (GT) (EC 2.4.1.22) has been found in the serum of certain cancer patients [1, 2] especially those with ovarian neoplasms [3]. This has led to a growing interest in GT as a potential tumor marker [4–9]. Galactosyltransferase assay procedures are yet to be standardized: a variety of acceptors are used, and incubation conditions differ [10, 11]. Differences in methodology have been invoked as a possible source of contradictory results [12, 13]. In the present communication, we report the results of GT assays performed on the sera of ovarian cancer patients, patients with benign gynecological diseases and normal controls. Serum GT activity was measured in each population with three different acceptors: ovomucoid, asialo-agalacto fetuin and *N*-acetylglucosamine, and the influence of some reaction parameters investigated.

MATERIALS AND METHODS

Materials

UDP-[^{3}H]galactose (16 Ci/mmol) was obtained from Amersham, U.K. Ovomucoid (trypsin inhibitor type III-O), fetuin (type III) and all other chemicals were purchased from Sigma Chemical Co. The anion exchange resin AG 1-X8 (200–400 mesh, chloride form) was obtained from Bio-Rad Laboratories. Asialo agalacto-fetuin was prepared by sequential removal of sialic acid and galactose from fetuin [12].

Patients

Two groups of patients with ovarian cancer were studied. The first group comprised 137 patients who were included in an extensive study on the biological markers of ovarian cancer conducted in our laboratory over the past four years. One sample was collected from each patient at the beginning of the follow-up period. Twenty-three patients with advanced disease (Stages III and IV according to International Federation of Gynecology and Obstetrics staging) had not yet undergone treatment, 54 were tumor-positive despite of therapy and 60 were in clinical remission after therapy.

In order to accurately compare the three assay techniques, another group of 28 patients was selected to give an extensive panel of GT activities. This group was not a random sampling of the whole ovarian cancer population treated at our Center. Three patients were in clinical remission, five showed a regression of their disease and 20 were tumor-positive. In addition, GT activity was analyzed in 10 patients with benign gynecological disorders, and nine healthy women.

Serum collection

Sera were collected and stored as previously described [6]. GT activities of three serum mixtures

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remained unchanged over a storage period of at least 1 year at −20°C.

Assays of serum galactosyltransferase

GT activity was determined by measuring the transfer of [^{3}H]galactose from UDP-[^{3}H]galactose to the terminal *N*-acetylglucosaminyl residues of the oligosaccharide chains of a glycoprotein, or to free *N*-acetylglucosamine.

For the first group of ovarian cancer patients, serum GT was evaluated with ovomucoid as glycoprotein acceptor, as described previously [6].

For the comparative study, we used ovomucoid, asialo agalacto-fetuin (SGF-fetuin) and free *N*-acetylglucosamine (GlcNAc).

A disk assay method modified from Baxter and Durham [14] was employed for glycoprotein acceptors. The standard assay mixture (105 μl) contained: 10 μl of serum, 70 μl of buffer including $MnCl_2$ (final concentration: 20 mM), 15 μl of acceptor solution and 10 μl of UDP-[^{3}H]galactose (final concentration: 70 μM, 56 mCi/mmol). After a 30 min preincubation period, the reaction was initiated by addition of serum. The reaction was stopped by adding 400 μl of cold EDTA 0.1 M. A 50 μl aliquot was spotted onto a 2.4 cm Whatman I paper disk. After drying, the disk was treated and radioactivity counted by liquid scintillation, as described previously [15]. Counting efficiency was 35%. Radioactivity obtained by terminating the reaction at 0 min was subtracted from the total radioactivity. No GT activity was observed in the absence of exogenous acceptors. The buffers for the ovomucoid and SGF-fetuin assays were respectively 25 mM sodium cacodylate pH 7.2, 40 mM NaCl and 50 mM Tris–maleate pH 6.2, 20 mM ATP, 0.5% Triton X-100. The saturating quantities of acceptor in the final mixture were 1.5 mg ovomucoid and 1 mg SGF-fetuin. The incubation was performed at 37°C for 30 min (ovomucoid) and at 25°C for 4 h (SGF-fetuin). For SGF-fetuin the assay conditions were similar to those described by Belfield and Pledger [12].

The GlcNAc acceptor assay used an optimal concentration of GlcNAc (20 mM) and a saturating concentration of UDP-[^{3}H]galactose (400 μM, 16 mCi/mmol). All other parameters were identical to those of the ovomucoid assay. The reaction was terminated by the addition of 500 μl cold water. The amount of [^{3}H]galactose transferred to free GlcNAc was determined by separating tritiated *N*-acetyl-lactosamine from UDP-[^{3}H]galactose on AG 1-X8 anion-exchange columns as described by Paone *et al.* [16]. Each column contained 0.8 ml resin. The 2.5 ml column eluant was collected in a scintillation vial and radioactivity counted after the addition of 15 ml of Picofluor 15 (Packard). Counting efficiency was 39.5%. A control assay without exogenous acceptor was performed for each serum, and the radioactivity recovered from the column was subtracted from the radioactivity obtained in the presence of GlcNAc. Its value did not exceed 15% of the latter.

All enzyme assays were carried out in duplicate on each serum sample. In our experimental conditions, kinetics of galactose incorporation were linear during at least 45 min at 37°C, and for enzyme concentrations up to 30 μl of serum. Enzyme activity was expressed as nmol/h/ml galactose transferred.

RESULTS

Figure 1 shows GT activities in the first group of ovarian cancer patients. Normal values were between 6 and 12 nmol/h/ml. Twenty of the 23 (87%) untreated patients and 25 of the 54 (46%) treated patients had GT activity levels above the normal value. In contrast, only seven of the 60 (12%) patients in clinical remission showed an increase in GT activity.

None of the sera showed an activity below 6 nmol/h/ml. Given that conflicting results could have been published elsewhere [12], we sought to determine whether the variance could be attributed to differences in assay technique or the use of another acceptor. We thus measured serum GT activities in the second group of cancer patients and the control groups under experimental conditions similar to those described by Belfield *et al.* [12], using asialo agalacto-fetuin as acceptor (Fig. 2). Our healthy population had a mean GT activity of 15.4 nmol/h/

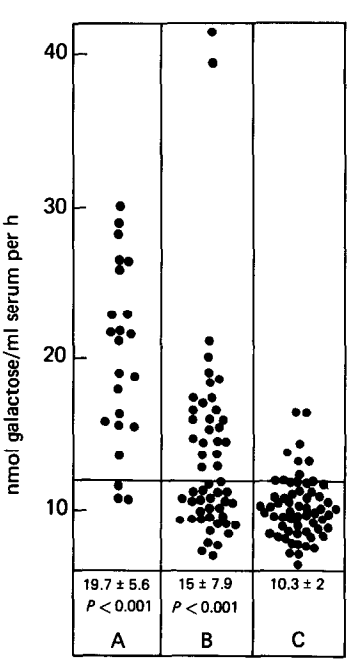

Fig. 1. Serum galactosyltransferase activities in ovarian cancer patients with ovomucoid as acceptor. A: untreated patients; B: tumor positive despite therapy: C: clinical remission. Means ± S.D. are given. P values are in comparison with the group of patients in clinical remission.

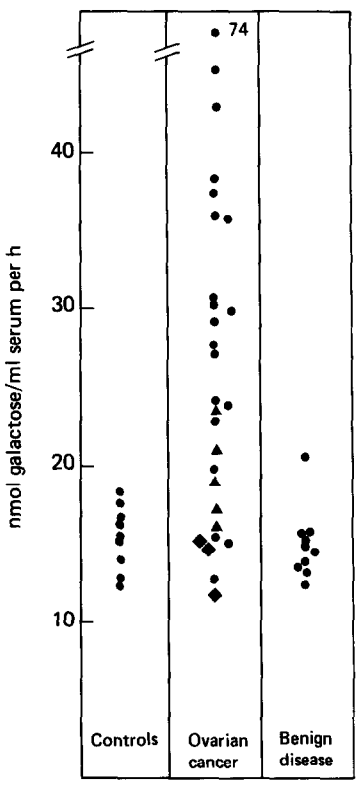

Fig. 2. Serum galactosyltransferase activities with asialo agalacto-fetuin as acceptor in ovarian cancer patients, patients with benign gynecological diseases and healthy females. Patients with ovarian cancer are as follows: (●) *active disease,* (▲) *regression of disease,* (◆) *clinical remission.*

ml (S.D. 2), very close to that given by these authors [13.35 nmol/h/ml (S.D. 1.55)]. The patients with benign gynecological diseases and ovarian cancer had mean GT activities of 15 nmol/h/ml (S.D. 2.5) and 25 nmol/h/ml (S.D. 9.5), respectively. One of the cancer patients had a very high GT level (74 nmol/h/ml), and was not included in the calculation. The three patients in clinical remission and three of the five patients in partial remission had normal values. None of the cancer patients had an activity below the normal value.

Serum GT activity was measured in the same three groups using ovomucoid and GlcNAc as acceptors. Figure 3 shows that the distribution of activity obtained with these two acceptors was similar to that obtained with SGF-fetuin. Correlation coefficients r were 0.99 for the whole population, the linear regression equations established for the three groups of sera being very close to each other.

The influence of the buffer, ATP, Triton X-100, temperature and time of incubation on GT activity was studied in two serum samples with each of the three acceptors (Fig. 4 and Table 1). GT activities were always higher in cacodylate than in the Tris-maleate buffer, probably because the pH of the latter was not optimal and Tris ions are less favorable to GT activity than cacodylate [17]. The additional inhibitory effect observed in the GlcNac assay as compared to the glycoprotein assays probably reflects differences in pH dependence, as reported by Davey *et al.* [18]. ATP and Triton X-100 had little effect on serum GT activity, especially when added simultaneously. Figure 1 shows that the GT activities from the two serum samples were equally affected by the various parameters: their ratio remains constant (1.46, S.D. 0.047).

DISCUSSION

Serum GT activity has been studied by numerous investigators using various techniques with different acceptors. Significant elevations of serum GT activity in ovarian carcinoma patients were reported

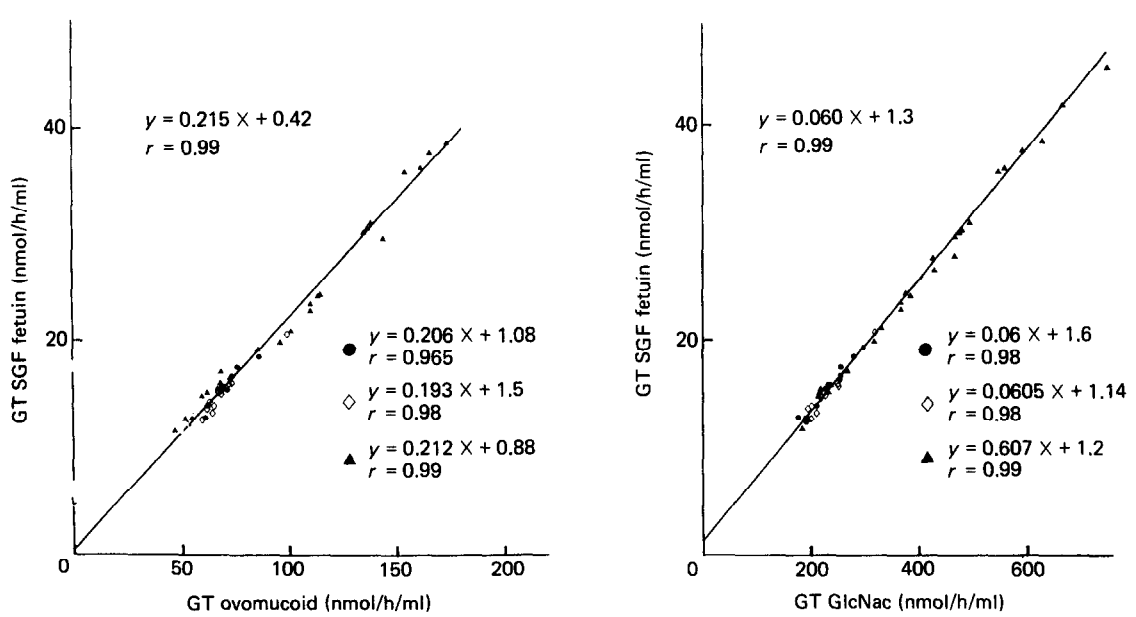

Fig. 3. Correlation between galactosyltransferase acceptor assays. (▲) *Ovarian cancer patients;* (◇) *patients with benign gynecological disease;* (●) *healthy females. Linear regression equations are given for the three groups of sera.* r: *correlation coefficient.*

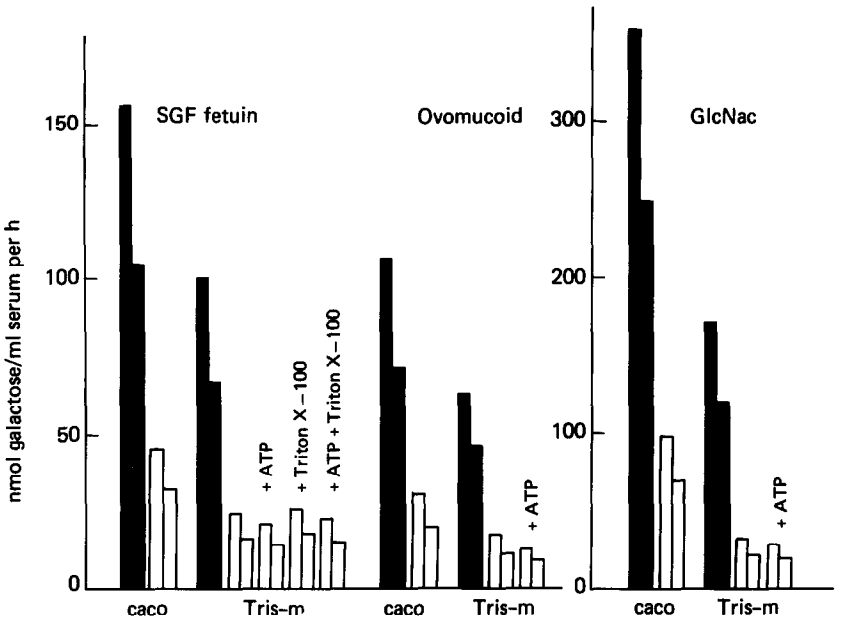

Fig. 4. Influence of various parameters on serum GT activity measured with SGF-fetuin, ovomucoid or N-acetylglucosamine as acceptor. The results obtained under the same conditions for two serum samples are depicted by coupled vertical bars. Incubation temperature was 37°C *(filled bars) or* 25°C *(open bars). caco:* 25 mM *cacodylate buffer pH* 7.2 *Tris-m:* 50 mM *Tris meleate buffer* pH 6.2.

Table 1. *Effect of variations in the incubation mixture on galactosyltransferase activity**

Acceptor	Incubation temperature	Additions: None	ATP	Triton X-100	ATP + Triton X-100
SGF-fetuin	37°C	64	—	—	—
		64			
	25°C	53	47	57	49
		52	45	55	47
Ovomucoid	37°C	59	—	—	—
		65			
	25°C	54	43	—	—
		54	47		
GlcNac	37°C	47	—	—	—
		48			
	25°C	33	28	—	—
		32	28		

*The results are expressed as a percentage of the activity measured in cacodylate buffer at the same temperature for two sera.

by several groups of workers, and GT was proposed as a useful marker for the follow-up of the disease [3–7]. This conclusion is supported by the results obtained in our laboratory over the past few years, as illustrated in Fig. 1 which summarizes initial GT assays for 137 patients.

Belfield *et al.* [12] found that seven of 18 ovarian cancer patients had serum GT activities lower than controls, and eight had normal values. We consider these results to be controversial and wondered whether they might be partly due to different assay conditions. The acceptor SGF-fetuin has already been used by Bhattacharya *et al.* [3] in their demonstration of the usefulness of serum GT as a marker for ovarian carcinoma. However, a lower temperature and four-fold higher incubation time are used by Belfield and Pledger [12]. In addition, both laboratories use an assay mixture which differs markedly from ours.

In their recent paper, Midiyalakan *et al.* [13] criticize the use of high molecular weight acceptors of complex and unrelated oligosaccharide structure for the measurement of serum GT during clinical investigations. Using a well-defined synthetic oligosaccharide substrate, they found a significant elevation of GT in ovarian cancer patients with active disease. However, one third of their cured patients still had slightly elevated levels. Similar results were reported by Waalkes *et al.* [5] who used free *N*-acetylglucosamine as acceptor.

In order to examine whether differences in methodology might account for the conflicting results, we measured GT levels in the sera of ovarian cancer patients and normal controls using three different acceptors, and investigated the influence of various assay parameters. The correlation between the three types of acceptor assays was very satisfactory, suggesting that the same enzyme activity is evaluated. This conclusion is supported by experiments showing that the monosaccharide competitively inhibits the transfer of galactose toward the glycoprotein acceptors (data not shown). The ability to use free GlcNAc as acceptor and strong inhibition we observed at high concentrations of this monosaccharide are characteristics of UDP-galactose: GlcNAc(β1-4)GT [10]. As discussed above, it is most likely that, under our experimental conditions, this particular enzyme also catalyzes the transfer of galactose toward ovomucoid and SGF-fetuin, even if in other cases these glycoproteins might serve as substrates for different galactosyltransferases [10]. Davey *et al.* [18] presented a similar correlation between various GT assays in a group of patients with solid tumors, and extensively discussed their results in terms of acceptor structures.

Hence it appears to be possible to study serum GT variations in ovarian cancer patients in comparison to control populations with any one of the acceptors we used. It is, of course, important to perform measurements at an optimal pH (around 7.0) in a suitable buffer (such as cacodylate) to obtain the highest values of GT activity. An incubation temperature of 37°C lowers the duration of the assay without altering the conclusions. The addition of ATP and/or Triton X-100 to the incubation mixture does not seem necessary. Under the different experimental conditions studied here, the ratios of activity between the various sera remained unchanged.

As the *N*-acetylglucosamine assay is easier and faster to perform, it will be used in our laboratory for analyzing large series of samples. Furthermore, with this method, competing reactions that liberate radiolabeled galactose or some of its derivatives [19, 20] can be detected. In a current investigation, radioactivity eluted from the anion-exchange column after incubation without exogenous acceptor was found to be elevated in only seven of 125 samples.

In our assays performed under the same experimental conditions and with the same acceptor as Belfield and Pledger [12], none of the cancer patients showed serum GT activity lower than normal. The few technical differences between our assay with SGF-fetuin and that described by Belfield cannot explain the discrepancy. In several samples, we compared the results obtained when the reaction was started by adding UDP-gal to the incubation mixture with those obtained when serum was added, and found that the GT activity measurement remained unchanged. The method used by Belfield and Pledger for the participation of SGF-fetuin was different from ours but was similar to those used by other workers [3, 17]. Furthermore, the mean value for the healthy control population in our study was consistent with that reported by Belfield and Pledger. The differences might be linked to the origin of the serum samples. The 18 ovarian cancer patients studied by this author had not undergone treatment. If we examine the results we obtained with the first group of 137 patients, 87% of untreated cases had elevated GT activity (Fig. 1) and none had a low level. In a previous paper [6], we reported that a similar group of patients showed a significant drop in GT level after the first course of chemotherapy. In treated patients, GT activity remains an interesting marker: when GT levels in the sera of tumor-bearing patients were compared to those of the patients clinically free of disease, a statistically significant elevation was observed.

Our observations suggest that although a standardized method had not yet been agreed upon, if the assay is well defined, GT measurement can be useful in the monitoring of ovarian cancer patients, especially when the antigen CA-125 cannot be detected. Monthly measurements are currently being performed in our laboratory for both markers, and the results, which concern an extensive population followed for a minimum of 2 years, will be published at a later date.

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REFERENCES

1. Kim KS, Perdomo J, Whitehead JS, Curtis KJ. Glycosyltransferases in human blood. II. Study of serum galactosyltransferase and *N*-acetylgalactosaminyl transferase in patients with liver diseases. *J Clin Invest* 1972, **51**, 2033–2039.
2. Weiser MM, Wilson JR. Serum levels of glycosyltransferases and related glycoproteins as indicators of cancer: biological and clinical applications. *CRC Crit Rev Clin Lab Sci* 1981, **14**, 189–239.

3. Bhattacharya M, Chatterjee SK, Barlow JJ. Uridine 5′-diphosphate-galactose:glycoprotein galactosyltransferase activity in the ovarian cancer patient. *Cancer Res* 1976, **36**, 2096–2101.
4. Chatterjee SK, Bhattacharya M, Barlow JJ. Correlation of UDP-galactose glycoprotein: galactosyltransferase levels in the sera with the clinical status of ovarian cancer patients. *Cancer Lett* 1978, **5**, 239–244.
5. Waalkes TP, Rosenshein NB, Shaper JH *et al.* A feasibility study in the development of biological markers for ovarian cancer. *J Surg Oncol* 1982, **21**, 207–214.
6. Gauduchon P, Tillier C, Guyonnet C, Heron JF, Bar-Guilloux E, Le Talaer JY. Clinical value of serum galactosyltransferase levels in different histological type of ovarian carcinoma. *Cancer Res* 1983, **43**, 4491–4496.
7. Verdon B, Berger EG, Salchli S, Goldhirsch A, Gerber A. An enzyme-linked immunosorbent assay for lactose synthase (galactosyltransferase) in serum and is application as a tumor marker in ovarian carcinoma. *Clin Chem* 1983, **29**, 1928–1933.
8. Kessel D. Glycosyltransferases as marker enzymes in neoplasia. *Prog Clin Biol Res* 1986, **204**, 21–30.
9. Davey RA, Milliken ST, Harvie RM *et al.* Serum galactosyltransferase as a prognostic marker in patients with solid tumors. *Cancer Res* 1986, **46**, 5973–5975.
10. Berger EG, Roth J, Strous GJ. Galactosyltransferase: biology and clinical applications. *Gann* 1983, **29**, 207–220.
11. Pohl AL. Critical comments on galactosyltransferase. *Cancer Detect Prev* 1984, **7**, 299–310.
12. Belfield A, Pledger DR. The specificity of serum uridine 5′-diphosphogalactose:glycoprotein galactosyltransferase activity in the diagnosis of ovarian cancer. *Eur J Cancer Clin Oncol* 1986, **22**, 101–104.
13. Madiyalakan R, Piskorz CF, Piver MS, Matta KL. Serum β-(1-4)-galactosyl-transferase activity with synthetic low molecular weight acceptor in human ovarian cancer. *Eur J Cancer Clin Oncol* 1987, **23**, 901–906.
14. Baxter A, Durham JP. A rapid, sensitive disk assay for the determination of glycoprotein glycosyltransferase. *Anal Biochem* 1979, **98**, 95–101.
15. Barbé D, Gauduchon P, Bar E, Héron JF, Le Talaer JY. Isoenzymatic patterns of human galactosyltransferase from normal and cancer patients. *Cancer Detect Prev* 1985, **8**, 279–285.
16. Paone JF, Waalkes TP, Baker RR, Shaper JH. Serum UDP-galactosyltransferase as a potential biomarker for breast carcinoma. *J Surg Oncol* 1980, **15**, 59–66.
17. Kim KS, Perdomo J, Whitehead JS. Glycosyltransferases in human blood. I. Galactosyltransferase in human serum and erythrocyte membranes. *J Clin Invest* 1972, **51**, 2024–2032.
18. Davey R, Harvie R, Cahill J. The heterogeneity and acceptor specificity of human serum galactosyltransferase. *Biochem Int 1984*, **8**, 687–696.
19. Chatterjee SK, Bhattacharya M, Barlow JJ. Glycosyltransferase and glycosidase activities in ovarian cancer patients. *Cancer Res* 1979, **39**, 1943–1951.
20. Spik G, Six P, Montreuil J. Chemical and enzymatic degradations of nucleoside mono- and diphosphate sugars. I. Determination of the degradation rate during the glycosyltransferase assays. *Biochim Biophys Acta* 1979, **584**, 203–215.