

The Sd^a Blood Group in Human Secretions

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Summary. The authors, employing the absorption-inhibition technique, have further extended their experiments on human biologic fluids to investigate the Sid substance.

It was located in tears, sweat, nasal and tracheobronchial mucus, cerebrospinal fluid, and in aqueous humor while it was not found in bile (in accordance with its absence in liver tissue).

The authors emphasize the significance that the verification of the Sid substance may assume in forensic investigations with the purpose of individual diagnosis in stains of the above mentioned secretions, particularly tears, sweat, and nasal mucus.

Key words: Blood groups, Sd^a in human secretions – Sd^a in human secretions – Human secretions, Sd^a group

Zusammenfassung. Die Autoren haben mit Hilfe der Absorptions-Inhibitions-Technik die Skala der menschlichen biologischen Flüssigkeiten, in denen man die Substanz Sid sucht, noch erweitert.

Sie haben auf diese Weise die Präsenz in der Tränenflüssigkeit, im Schweiß, im Nasen- und Luftröhrenschleim, im Liquor und im Kammerwasser nachgewiesen, während die Substanz sich nicht in der Galle befindet (entsprechend der gegebenen Negativität des Lebergewebes).

Die Autoren unterstreichen die Bedeutung, die der Nachweis der Substanz Sid im gerichtsmedizinischen Labor einnehmen kann mit der Perspektive einer individuellen Diagnose in Flecken der besagten Sekrete, besonders von Tränen und Schweiß.

Schlüsselwörter: Blutgruppen, Sd^a in menschlichen Sekreten – Sd^a Gruppenantigen in menschlichen Sekreten – Menschliche Sekreten, Sd^a Blutgruppe

Macvie et al. [1] and Renton et al. [2] identified in 1967 the human blood group Sd^a, present on red cells and also in most tissues and secretions, with the highest concentration in meconium and urine [3].

Sd^a character is inherited as dominant and individuals can be better classified into the two phenotypes Sd(a+) and Sd(a-) with the urinary test because individually there are considerable variations in the strength of agglutination of Sd^a antigen on red cells: normally, the pattern of agglutination of the Sd^a blood group is similar to a mixed field; only Cad Sd(a++) or super Sid—a very strong form of Sd^a—is well agglutinable.

On red cells Sd^a is absent at birth and often disappears in pregnancy.

Sd^a activity seems to depend on a terminal N-acetyl-D-galactosamine [4] and according to Morgan et al. [5–7] it is closely associated with the Tamm and Horsfall (T-H) urinary glycoprotein.

Recently, employing absorption-inhibition techniques, Conte and Pappalardo [8] demonstrated the presence of Sd(a+) also in semen and vaginal secretion with positive findings in 11-year-old semen stain.

Moreover, the same authors [9] pointed out the utility in forensic laboratory of such a stable marker with the purpose of typifying also individual urine stains.

In this paper we report the results of some research studies on the Sd^a activity in other previously untested biologic fluids.

Materials and Methods

Samples of urine, saliva, sweat and tears were obtained from healthy adults: 14 Sd(a+) and 1 Sd(a-). Insoluble material was removed as soon as possible after collection by centrifuging at 3,000 rpm/10 min for the large volumes of urine and saliva; at 10,000 rpm/10 min with microhematocrit centrifuge in microtubes Kartell for the small volumes of sweat and tears. All samples were kept frozen until examination. In seven cases with Sd(a+) only sweat and tears were sufficient to estimate also inhibition titers of the substance: comparison with the titers of corresponding urines and saliva was limited to this group.

Normal cerebrospinal fluids of 5 Sd(a+) leukaemic children were made available by Clinical Pathology Laboratories of S. Orsola Hospital, Bologna, where they had been examined and meningopathy excluded.

Other secretions: aqueous humor, bile and tracheobronchial mucus were obtained at post-mortem from thirteen corpses, 12 Sd(a+) and 1 Sd(a-) as tested in urine from gall bladder puncture, selected for absence of pathologic changes in the districts of drawing. Tracheobronchial secretions removed clean by glass stick were immediately diluted about v/v in saline. All specimens were centrifuged with the aid of micro haematocrit centrifuge and the supernatant was frozen until examination.

Nasal secretions were obtained from 5 Sd(a+) normal volunteers by inserting 2–3 times into the nose swabs of cotton soaked in saline, then squeezed in microtubes. Insoluble material was removed by centrifuging at 10,000 rpm/10 min and residual liquid frozen.

Moreover, for two persons treated on another day the method of collection consisted in instilling into each nostril 5 ml of water while the subject held his head hyperextended, his tongue elevated, and the glottis closed. The washing fluid was then partly recovered in a Petri dish when the head was tilted forward; after centrifugation at 10,000 rpm/10 min, the supernatant was lyophilized and diluted with 0.2 ml of saline upon examination.

Anti-Sd^a serum, obtained from an unimmunized blood donor (B. Nat.) identified in 1976 by the Transfusion Service, S. Orsola Hospital, Bologna, and kindly controlled by Renton and Pickless, was employed as in previous research studies.

In the operative conditions of the inhibition test (2 volumes of undiluted serum, 1 volume of fluid under test or of control saline, 1 volume of 10% suspension of red cells) the titer was 1:4.

The indicator cells for all tests were from a single donor and gave a good reaction with anti-Sd^a.

The inhibition test was performed according Macvie [1] using undiluted collected samples or, if possible, their serial, 2-fold dilutions with saline.

Other details are more conveniently described in the text.

Results

Besides urine and saliva of tested Sd(a+) people, also tears (emitted either naturally or after stimulation), sweat and nasal secretion (obtained either by touching or by washing) showed Sd^a activity.

Likewise it was present in cerebrospinal fluid from leukaemic young patients without meningopathy.

In samples obtained postmortem from ten human adults, Sd(a+) at the urinary test, Sid substance was detectable in aqueous humor and tracheobronchial secretions while it was always found absent in bile. In the only Sd(a-) corpse, all these fluids showed no inhibition.

With regard to the anti-Sd^a inhibitory power, the lowest concentrations were observed in cerebrospinal fluid, aqueous humor, and in tracheobronchial mucus, with complete inhibition for only undiluted material or material diluted 1:2. For nasal secretion, the inhibition titer refers to the liquid from squeezing the saline-soaked cotton swabs soon after collection of the mucus and it ranged between 1:2 and 1:4.

The results of estimations made on specimens from corpses are shown in Table 1.

Table 1. Presence and inhibition titers of Sd^a in fluids obtained postmortem, as maximum dilution showing complete inhibition of anti-Sd^a

		Urine	Aqueous humor	Tracheo- bronchial mucus	Bile
Adults Sd(a+)	1	8	2 (')	2	0
	2	32	2 ('')	2 ('')	0
	3	8	1	1	0
	4	8	1	2	0
	5	16	1	1	0
	6	8	1	2	0
	7	16	1	2	0
	8	8	2	2	0
	9	32	2	2	0
	10	32	2	2	0
	11	8	1	2	0
	12	16	1	1	0
Adults Sd(a-)	1	0	0	0	0

Collected 2h (') and 4h (") after death

Table 2. Comparison of the inhibition power in fluids of seven adults Sd(a+) and one Sd(a-) as maximum dilution showing complete inhibition of anti-Sd^a

		Urine	Saliva	Tears	Sweat
Adults Sd(a+)	1	32	4	4	8
	2	32	4	2	4
	3	8	8	2	4
	4	16	4	4	4
	5	32	8	4	8
	6	32	8	4	4
	7	32	4	8	8
Adults Sd(a-)	1	0	0	0	0

In seven living subjects for whom a comparison of the inhibition titer of anti-Sd^a by urines, saliva, tears, and sweat was possible (Table 2), the range of inhibition power was 1:2–1:8 for tears and 1:4–1:8 for sweat against 1:4–1:32 of corresponding urine, saliva concentration ranged between 1:4 and 1:8, mostly lower, in one case equal to the urinary one.

Discussion

The observations reported here expand the range of human biologic fluids where Sd^a activity is present: particularly Sd^a has been demonstrated to occur in tears, sweat, cerebrospinal fluid, and nasal mucus in living beings; in tracheobronchial secretion and in aqueous humor in corpses. Its recovery in specimens drawn a few hours after death and the recurrence of the same positive results suggest that also in these latter fluids Sd^a activity is a real data.

No activity Sd^a could be found in bile and this agrees with the reports of Morton et al. [3] who proved its absence in liver. It is relevant that Sd^a activity is present in cerebrospinal fluid where, according to Putkonen [10], AB0 substances are absent and, moreover, that it is absent in bile where AB0 substances are present.

Positive findings in tears and sweat (resulting to contain about the same amount of Sd^a as the saliva), in nasal secretion, and also in aqueous humor and tracheobronchial mucus although feebly are added to the already known presence of Sd^a substance in urine, serum, saliva, milk, gastric juice, meconium (particularly of the lower intestine and colon), feces until 10 months of age¹, vaginal secretion, and semen.

The biologic meaning of this diffuse secretion of Sd^a (with the exception of the bile) is not yet clear. However, with regard to the medico-legal implications, the presence of Sid substance in sweat, tears, and nasal mucus is very interesting since these fluids can be questioned as stains in criminal investigations. In other words,

¹ Pickles and Morton [11] found that Sd^a substance lacks in the feces of children over 10 months of age and in adults. They suggested that this is probably due to the breakdown by bacterial enzymes. The same authors reported also the absence of Sid substance in ovarian cyst fluid

the occurrence of employing Sid substance in forensic laboratories must be put in evidence because whenever the nature of such stains are proved and substrata controlled, this mucopolysaccharide is a reliable individual time-persisting marker.

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