γA in Exercise Proteinuria[1] (34745)

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(Introduced by J. F. Mustard)

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Normal humans excrete daily approximately 1 mg of γA in the urine (1–3). This γA is in the molecular form characteristic of the secretory γA molecule (1). It is currently thought that in most secretions where this immunoglobulin predominates, it is synthesized locally in plasma-like cells, and that an extra protein moiety separately synthesized by mucosal epithelial cells combines with the γA to form the secretory 11S molecule characteristic of external secretions (4). The secretory γA molecule thus has a molecular weight of 385,000, as opposed to its serum counterpart which has a molecular weight of 160,000 (5). The extra protein moiety, often referred to as secretory piece (SP), or transport piece, has no function clearly related to transport. It does, however, confer upon the γA molecule increased resistance to the effects of reduction (4) and proteolysis (6). Experiments designed to study the mode of interaction of SP and γA have suggested that SP labelled with ^{125}I has a special affinity for γA relative to other serum proteins (7). However, the mechanism whereby urinary secretory γA is formed is unknown. It has been suggested (8) that the γA may reach the ureter by filtration through the glomerulus, secretion by cells in the interstitial renal tissue, or selective secretion by tubular epithelium. The γA may thus complex with SP, which itself may be synthesized or secreted by tubular cells, either in the tubular cell, intercellularly as has been suggested in other organs (9), or in the tubular lumen.

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We have therefore studied the γA in urine from healthy adults following strenuous exercise to determine in what form the γA molecule is excreted in conditions where mild physiological proteinuria, presumably of glomerular origin, occurs (2). Preliminary observations had suggested that in exercise urine the γA had a greater exclusion volume on gel filtration, *i.e.*, lower molecular weight than in normal urine (10).

Methods. Urine was collected from 27 healthy but untrained male adults within 1 hr of a 3-mile cross country run for a total collection time of 2 hr. Two-hr collections of urine were also made on 16 healthy male adult sedentary workers pursuing normal daily activities. In both groups ages ranged from 20 to 36. Urine was processed as previously described (1, 2), concentrated approximately 100-fold and lyophilized.

Gel filtration, sucrose density gradient ultracentrifugation, immunodiffusion, immunoelectrophoresis, and γA quantitation were performed according to methods outlined in previous publications (1, 2).

The antisera to the human proteins used in the present study have been previously described (11) except for the antisecretory γA antiserum. This antiserum was prepared in the guinea pig by footpad immunization of a pure preparation of colostral γA emulsified in complete Freund's adjuvant. The methods of Tomasi *et al.* (4) were followed for the preparation of colostral γA, however, the γA containing material off a G200 column was recycled on Biogel A5M (Biorad Labs., Richmond, Calif.) as a final preparative step. This was found to be a useful adjunct to separation of other colostral components from the secretory γA molecule. The resultant antiserum was absorbed with cord serum to

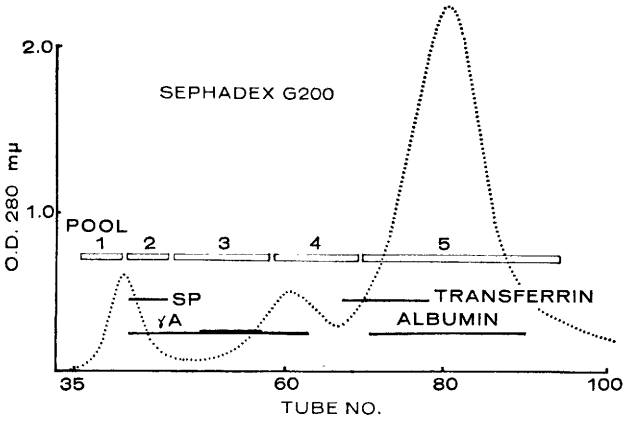

FIG. 1. Elution of exercise urine from a Sephadex G200 column with 0.85% sodium chloride. Column size 2.5 × 105 cm, flow rate 15 ml/hr, fractions 3 ml. Pools made as shown and concentrated to ⅓ the original application volume. Reactivity of pools with antisera and distribution of some proteins is indicated by horizontal black bars.

render it specific for the colostral γA molecule, and with normal human serum to make it specific for secretory piece. The antiserum did not react in immunodiffusion with lactoferrin preparations or in colostrum concentrated 15 times, with anything other than SP or secretory γA.

Results. The total weight of lyophilized urine was 3020 mg from 27 adults following exercise. 350 mg of lyophilized material was dissolved in 3 ml of 0.85% saline, applied to a Sephadex G200 column 2.5 × 105 cm and eluted with a constant flow rate of 15 ml/hr. The protein elution pattern obtained by reading the eluant fractions at 280 mμ in a Zeiss spectrophotometer is shown in Fig. 1.

The γA reactivity extended from the second half of the first protein exclusion peak to the 7S region. Secretory piece reactivity was only found in the first protein peak. Some other proteins and their distribution in eluant fractions were plotted for convenience of reference. Pools were made as indicated, concentrated to ⅓ the original starting volume, and tested in immunodiffusion in agar against a guinea pig anticolostral γA antiserum absorbed with cord serum (Fig. 2). Colostral γA spurred over serum γA, due to the extra antigenic determinants in SP, and formed a reaction of identity with pool 2, which in turn spurred over pool 3. These relationships were confirmed in the second part of Fig. 2 developed with the same antiserum made specific for SP by absorption with normal serum. A reaction of identity appeared between pool 2 and secretory γA. Pools 3, 4, and 5 had no reactivity with the anti-SP antiserum.

Quantitation of γA by radial immunodiffusion on the G200 pools revealed a total

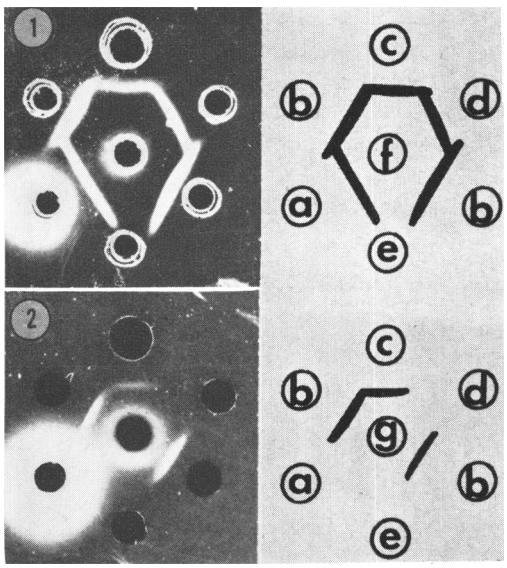

FIG. 2. Immunodiffusion in agar demonstrating relationships of G200 pools with secretory and serum γA. a, normal human serum; b, colostral γA; c; pool 2; d, pool 3; e; pool 4; f, anti-11S colostral γA antiserum; and g, anti-SP antiserum.

recoverable γA concentration of 0.99 mg as shown in Table I. The total γA applied to the column expressed as a 7S γA standard was 1.1 mg. The total recovery was 90%. Expressed as a 7S molecule, the 11S region of the column eluate (pool 2) had a total γA content of 0.14 mg which was 14.1% of the total γA recovered. Similar results were obtained on sucrose density gradient (10–40%) ultracentrifugation experiments.

Experiments were performed to confirm the presence of both secretory γA and serum type γA in exercise urine. Precipitation with 1.8 *M* ammonium sulfate was first undertaken, since immunodiffusion experiments without fractionation did not reveal this relationship. The results are shown in Fig. 3 and demonstrate the presence of both 7S and 11S γA molecules.

In order to be certain that the lack of detection of free SP in G200 fractions was not simply due to lack of sufficient normal excretion of SP in the 2-hr collection period, we examined this problem in normal healthy adults. Urine was collected from 16 adults at work but not under conditions of strenuous exercise. The total lyophilized material from this collection weighed 128 mg. Since 350 mg of exercise urine used in the gel filtration experiment represented 3 times the average 2-hr protein excretion per adult, an equivalent quantity of normal urine, *i.e.*, 24 mg, was subjected to gel filtration on the same calibrated column described above. The fractions were collected and concentrated to ⅓ the original starting volume as before (0.3 ml) by Aquicide II (Calbiochem, Los Angeles, Calif.). Immunodiffusion experiments on the concentrated pools now revealed SP reactivity in both pool 2 and pool 4. As previously reported (1) only pool 2 showed reactivity with the anti-γA antiserum. Although no γA was found in other pools and 100% of the γA recovered was in pool 2, considering the experimental errors inherent in the methods used, these results were considered to be confirmatory of the results presented elsewhere (1) *i.e.*, that 90% of normal urinary γA is in the secretory form.

TABLE I.

		Total γA (mg; expressed as 7S γA)
Applied to column		1.1
G200 Pool	1	0
	2	0.14
	3	0.61
	4	0.24
	5	0
	6	0
Total recovered		0.99
11S region (pool 2) as % of total recovered		14.1

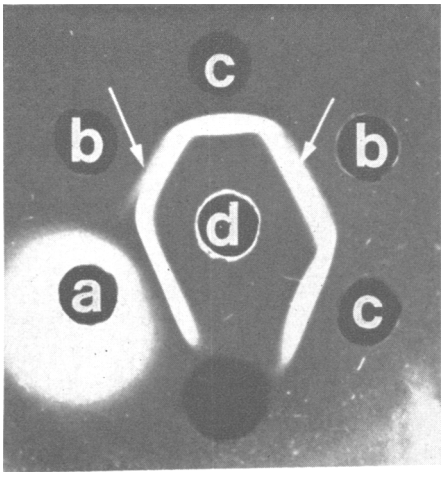

FIG. 3. Reactions in agar between normal human serum γA (a); 1.8 *M* ammonium sulfate precipitate of exercise urine (b); and 11S colostral γA (c); revealed by an antiserum to 11S colostral γA (d). Arrows point to 11S γA.

Discussion. In exercise urine a minimum of 14% of the γA excreted is in the 11S secretory form as opposed to normal urine, where over 90% is excreted as 11S γA and free SP is also always found (1). No free SP was found in exercise urine either at concentrations of up to 350 mg of protein/ml, or in individual pools following gel filtration concentrated to ⅓ the original starting volume.

It is not known whether the immunofluorescent demonstration of SP in renal tubular cells (9) represents synthesis, absorption, or secretion. Although the bladder appears capable of synthesizing some secreto-

ry γA (12), the amounts involved appear insignificant relative to that coming from the kidney.

Previous attempts to demonstrate, by *in vitro* methods, combination of unlabeled SP, isolated from urine from agammaglobulinemic patients, with serum γA, have proved fruitless (1). It is possible, however, that in normal and agammaglobulinemic urine, the free SP is present as a nonreactive dimer, or complexed to other protein moieties since the molecular exclusion volume on G200 corresponds to molecules with a molecular weight of about 100,000 (1). However, free SP obtained from colostral γA has a molecular weight of 58,000 (5) and a molecular exclusion volume which corresponds to molecules with molecular weights of 76,000 (13). Similar findings have been reported for free SP in human colostrum (13) and milk (14). The presence in exercise urine of easily detectable but minor amounts of 11S γA and major amounts of 7S γA in the absence of free SP would suggest to us that the combining sites of available free SP had been saturated by an excess of tubular 7S γA. An alternative explanation for this finding might be that following exercise increased proteolytic activity in the urine with selective proteolysis of free SP might have occurred.

It is apparent from this study, at least with regard to the γA, that the proteinuria of exercise is not simply an exaggeration of the normal pattern of physiological proteinuria. The results obtained could be interpreted as due either to an excess of γA passing into the glomerular filtrate, or a partial or complete block of γA reabsorption by the tubules, in the presence of a normal secretion or synthetic rate for SP.

Summary. Examination of urine from patients who underwent severe exercise has demonstrated that approximately 14% of the γA is present in secretory form whereas the remainder is excreted as 7S serum type γA. In normal urine at least 90% of the γA is excreted as the secretory molecule. No evidence was found for the presence in exercise urine of free secretory piece, in contrast to normal urine which contains free secretory piece unassociated with γA.

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