J Clin Pathol 1988;**41**:1089–1092

Urinary excretion of albumin and retinol-binding protein during normal pregnancy

R BEETHAM,* A DAWNAY,* M MENABAWY,† A SILVER‡

*From the Departments of *Chemical Pathology, †Reproductive Physiology and ‡Nephrology, St Bartholomew's Centre for Research, St Bartholomew's Hospital, London*

SUMMARY In a cross sectional study of 88 pregnant women urinary excretion of albumin, when expressed as a ratio to creatinine concentration, was not significantly different from that in a non-pregnant control group of similar age ($p > 0.05$) and did not change significantly during pregnancy. Only when albumin excretion was expressed as a fractional clearance was the urinary excretion significantly increased in the third trimester compared with the first trimester ($p < 0.05$), although it was still not significantly different from that in the non-pregnant control group. Excretion of retinol-binding protein was significantly increased during all three trimesters of pregnancy ($p < 0.01$ in each case) and more so in the second and third trimesters than in the first. It is concluded that the increased total protein excretion that has been described during pregnancy is not explained by an increased excretion of albumin which remains essentially normal. In contrast, the tubular absorption of proteins is decreased.

There have been many studies of the quantity, nature, and selectivity of protein excretion in abnormal pregnancies[1–4] but there is little information concerning the quantity of either total or specific protein excretion in normal pregnancies. In the few studies that have been undertaken, excretion of total protein has increased as pregnancy progresses, being twice as high in the second and third trimesters as in the first[5,6]; no information was available as to which protein or proteins contributed to the increase. Of three studies on the excretion of albumin in normal pregnancy,[7–9] that of Wright *et al* is the most comprehensive.[9] Although the absolute excretion of albumin did not increase in pregnancy, when albumin excretion was expressed as a ratio to creatinine excretion or as a clearance proportional to creatinine clearance, there was a significant increase. This was considered to be consistent with the hypothesis that mean glomerular permeability to albumin rises during pregnancy. There is, however, some evidence to suggest that tubular handling of protein may also be disturbed in pregnancy,[10] and this might account for the increase in urinary excretion of albumin.

We report on the urinary excretion of both albumin and retinol-binding protein, a tubular protein, throughout normal pregnancy. We also include data on the serum concentrations of retinol-binding protein and transthyretin, which may be helpful in interpreting excretion of urinary retinol-binding protein.[11]

Accepted for publication 19 May 1988

Material and methods

Blood samples were taken from up to 88 healthy pregnant women who also provided a concurrent random daytime specimen of urine. Of the 88 women, 28, 17, and 43 were in the first, second, and third trimesters, respectively. (Differences from these numbers in tables and figures reflect lack of matched urine and serum samples or insufficient material for analysis). The mean gestations for each group were nine weeks (range 6–12), 24 weeks (14–26), and 36 weeks (28 to full-term). All subjects had normal blood pressure and urine, no symptoms of urinary tract infection, and the pregnancy was judged to be clinically normal.

Blood samples were taken and concurrent random daytime specimens of urine were obtained from 26 healthy non-pregnant women aged 18–40 years (mean 28) attending a blood donor clinic to act as a control group. They formed part of a larger reference group.[12,13] The only ones taking any drugs were 11 who were taking a variety of oral contraceptive preparations.

Blood was allowed to clot and serum assayed for creatinine and stored at −20°C until analysis for albumin, retinol-binding protein, and transthyretin. Urine was assayed for creatinine and stored at −20°C until analysis for albumin and retinol-binding protein. Serum albumin was measured by bromcresol green, and serum and urine creatinine by a Jaffe reaction, all on Technicon continuous flow equipment. Urinary

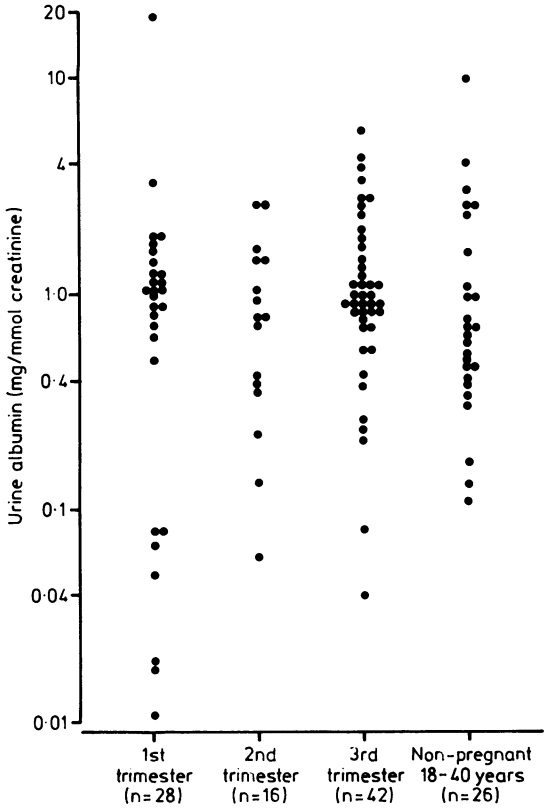

Fig 1 *Urine albumin excretion expressed as a ratio to urine creatinine in the three pregnant groups of women and non-pregnant control group.*

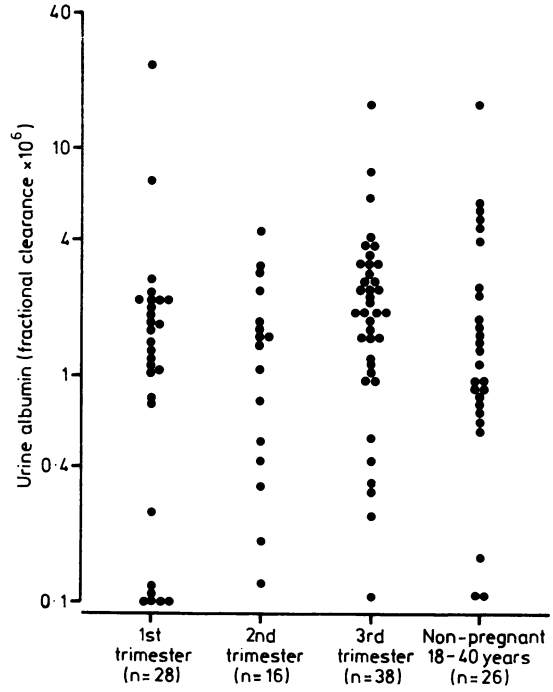

Fig 2 *Fractional albumin clearance in the three groups of pregnant women and non-pregnant control group.*

albumin,[13] and retinol-binding protein in serum and urine[12] and transthyretin in serum (unpublished data) were measured by radioimmunoassays. Urinary albumin and retinol-binding protein were expressed as ratios to creatinine to account for variations in urinary flow rate. Fractional albumin clearance was calculated as albumin clearance (V × urine albumin/serum albumin) divided by creatinine clearance (V × urine creatinine/serum creatinine) where V = volume/unit time and does not need to be measured as it cancels out. Statistical analysis was by the unpaired Student's *t* test or the Mann-Whitney U test as appropriate for distribution and variance.

Results

Urinary excretion of albumin, expressed as a ratio to urinary creatinine is shown in fig 1 for the non-pregnant control group and for the pregnant subjects according to length of gestation. There were no significant differences between albumin excretion in pregnancy as a whole compared with the control group, in any trimester compared with the control group, or between any trimester; albumin excretion did not increase during pregnancy. When albumin clearance was expressed as a fraction of creatinine clearance (fig 2), the excretion in the third trimester (median $1{\cdot}83 \times 10^{-6}$) was significantly greater than in the first trimester (median $1{\cdot}25 \times 10^{-6}$) ($p < 0{\cdot}05$), but at no time in pregnancy was it significantly different from that in the control group (median $1{\cdot}10 \times 10^{-6}$).

By contrast, urinary excretion of retinol-binding protein (fig 3) was greater in the first trimester than in the control group ($p < 0{\cdot}01$); greater in the second and third trimesters than in the first ($p < 0{\cdot}005$; $p < 0{\cdot}002$); but not greater in the third trimester than in the second.

Values for serum retinol-binding protein and transthyretin concentrations, and the retinol-binding protein:transthyretin ratio are given in the table. All were lower in pregnancy, there being a significant difference for all three measures between each trimester of pregnancy and the reference population ($p < 0{\cdot}001$ in each case), but there were no significant differences among trimesters ($p > 0{\cdot}1$ in each case). Serum albumin concentration (table) was lower in the first trimester than in the control group ($p < 0{\cdot}001$),

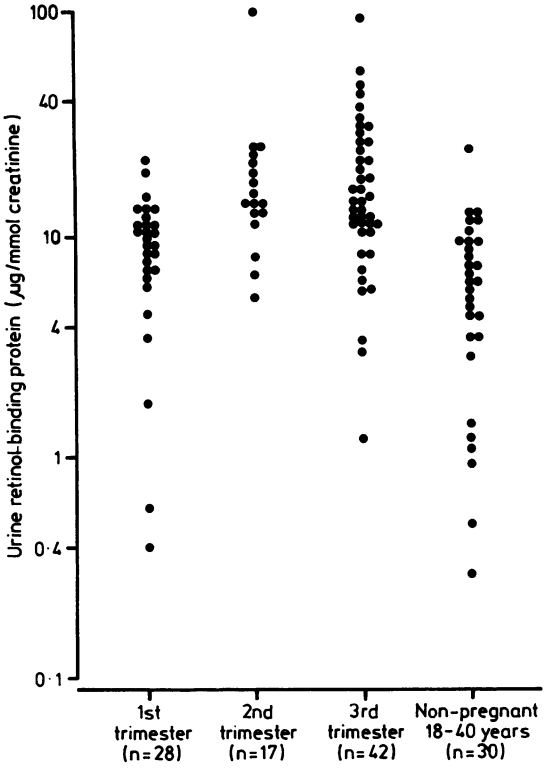

Fig 3 *Urine retinol binding protein excretion, expressed as a ratio to urine creatinine, in the groups of pregnant women and non-pregnant control group.*

and lower in the second and third trimesters than in the first trimester ($p < 0.001$).

Discussion

Our results have shown that urinary albumin excretion, expressed as a concentration ratio to creatinine, is not significantly different in pregnant women and non-pregnant women. Wright *et al*[9] found no significant difference in the rate of albumin excretion between pregnant and non-pregnant women, but a significant difference emerged when albumin excretion was expressed as a concentration ratio to creatinine. Incomplete urine collection from pregnant women was the reason given for the lack of difference in rates of excretion between the pregnant and the non-pregnant women. In a smaller study it was found that rates of albumin excretion increased slightly during pregnancy although they still remained within the non-pregnant reference range.[8] To see whether the amount of albumin excreted was normal with respect to its decreased serum concentration, we also calculated the clearance of albumin and related it to that of creatinine to take account of the known increase of glomerular filtration rate in pregnancy. There was a significant increase from the first to the third trimester, as also reported by Wright *et al*,[9] though at no stage was the ratio significantly different from that found in non-pregnant women. Irgens-Moller *et al*[7] found the clearance ratio in the latter half of pregnancy to be double that of non-pregnant women. Taken together the data suggest that although there is some tendency for the amount of albumin excreted to be inappropriately high for the filtered load, the absolute amount excreted in pregnancy is not abnormal.

The amount of protein in the urine is the result of two processes—glomerular filtration and tubular absorption. No other study of albumin excretion in pregnancy has simultaneously assessed tubular absorption. Our finding of an increased excretion of retinol-binding protein, which substantiates that in a recently published report,[10] provides evidence that tubular absorption of protein is impaired in normal pregnancy. Although this could occur as a consequence of an increased filtered load of retinol-binding protein, this is an unlikely explanation. Retinol-binding protein in serum is predominantly bound to transthyretin and it is not possible to measure the free fraction available for filtration easily. The large decrease in serum retinol-binding protein and only marginal decrease in the retinol-binding protein:transthyretin ratio, however, argue against any significant increase in the concentration of free retinol binding

Table *Serum concentrations of retinol-binding protein and transthyretin, the retinol-binding protein:transthyretin ratio and albumin concentration in pregnant and non-pregnant groups*

	Women in first trimester	*Women in second trimester*	*Women in third trimester*	*Non-pregnant subjects*
Mean (SD) retinol-binding protein concentration (mg/l)	33 (6·7)	33 (9·7)	34 (7·2)	49 (10·2)
Mean (SD) transthyretin concentration (mg/l)	186 (24)	185 (36)	192 (30)	231 (26)
Mean (SD) retinol-binding protein: transthyretin ratio	0·18 (0·03)	0·18 (0·03)	0·18 (0·02)	0·21 (0·03)
Median (range) albumin concentration (g/l)	41 (37–46)	35 (31–43)	35 (31–38)	46 (41–50)

protein. Even with the known increase in glomerular filtration rate during pregnancy, it is unlikely that the filtered load of retinol-binding protein is increased sufficiently to account for its increased excretion. As proteins absorbed by the tubule are catabolised within the cells and not returned to the circulation, increased excretion due to impaired tubular absorption has no influence on serum concentrations. The marginal increase in albumin clearance in pregnancy could thus be accounted for by diminished tubular absorption and cannot be regarded as firm evidence of increased glomerular permeability as argued previously.[9] The reasons for the decrease in tubular protein absorption, which becomes more pronounced during pregnancy, are not known.

It is apparent from these and previous results that as albumin excretion is not increased during normal pregnancy it cannot account for the increase in total protein excretion of 100 mg that has been described.[5,6] Is there an alternative explanation for this increase? Low molecular weight proteins account for only a few mg of normal urine protein. Our data suggest that retinol-binding protein output may be increased by three or four times in pregnancy. If this is representative of low molecular weight proteins in general, then this will not account for the increased total protein excretion that has been described. Tamm-Horsfall glycoprotein contributes about 50 mg to normal urinary output of protein[14] but concentrations in pregnancy are not known.

Proteinuria during pregnancy is sought as one indication of a pathological process that may have a deleterious effect on the wellbeing of both mother and fetus. Screening for proteinuria is usually done with reagent strips that have a lower detection limit of 150 mg/l or more.[15] There is thus a gap of at least 100 mg/l between the amount of albumin excreted in normal pregnancy and the lower limit of sensitivity of reagent strips. Albumin excretion could therefore be pathologically increased and yet remain undetected by this method of screening. More sensitive techniques for measuring low concentrations of albumin should be applied in a prospective study in pregnancy to determine if they offer an advantage over standard procedures. Such attempts have been made[7,8] with somewhat conflicting results and need to be repeated. With tubular handling under the stress of pregnancy, it may be that certain pathological processes are more readily reflected in tubular dysfunction. Measurement of the excretion of low molecular weight proteins such as retinol-binding protein should also be included in such studies.

We thank the staff of the antenatal clinic at St Bartholomew's Hospital for their help in collecting samples.

References

1 Simanowitz MD, MacGregor WG, Hobbs JR. Proteinuria in pre-eclampsia. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1973;**80**:103–8.
2 Kelly AM, McEwan HP. Aetiology and effects of proteinuria in pregnancy. *Contrib Nephrol* 1981;**24**:134–8.
3 Kaltenbach RJ, Boesken WH, Wilhelm C, Ziupa J, Toussaint MN, Quaas L. Urinary protein patterns and preeclampsia. *Clinical and Experimental Hypertension* 1983;**2**:133–44.
4 Clark PMS, Evans SE, Weaver JB, Flora PS, Hughes SH, Whitehead TP. The investigation of proteinuria in pregnancy using iso-dalt analysis. *Br J Obstet Gynaecol* 1984;**91**:979–85.
5 Wearing MP. Protein in the urine during pregnancy. *Obstet Gynecol* 1957;**9**:549–53.
6 Davison JM. The effect of pregnancy on kidney function in renal allograft recipients. *Kidney Int* 1985;**27**:74–9.
7 Irgens-Moller L, Hemmingsen L, Holm J. Diagnostic value of microalbuminuria in preeclampsia. *Clin Chim Acta* 1986;**157**:295–8.
8 Lopez-Espinoza I, Dhar H, Humphreys S, Redman CWG. Urinary albumin excretion in pregnancy. *Br J Obstet Gynaecol* 1986;**93**:176–81.
9 Wright A, Steele P, Bennett JR, Watts G, Polak A. The urinary excretion of albumin in normal pregnancy. *Br J Obstet Gynaecol* 1987;**94**:408–12.
10 Gero G, Anthony F, Rowe DJF, Dennis KJ. Increased urinary excretion of retinol-binding protein during normal pregnancies. *Clin Chem* 1986;**32**:916–17.
11 Fex G, Felding P. Factors affecting the concentration of free holo retinol-binding protein in human plasma. *Eur J Clin Invest* 1984;**14**:146–9.
12 Beetham R, Dawnay A, Landon J, Cattell WR. A radioimmunoassay for retinol-binding protein in serum and urine. *Clin Chem* 1985;**31**:1364–7.
13 Silver A, Dawnay A, Landon J, Cattell WR. Immunoassays for low concentrations of albumin in urine. *Clin Chem* 1986;**32**:1303–6.
14 Dawnay A, Thornley C, Cattell WR. An improved radioimmunoassay for urinary Tamm-Horsfall glycoprotein. *Biochem J* 1982;**206**:461–5.
15 Viberti GC. Early functional and morphological changes in diabetic nephropathy. *Clin Nephrol* 1979;**12**:47–53.

Requests for reprints to: Dr R Beetham, Department of Chemical Immunology, Westminster Hospital, London SW1P 2AR England.