was advised to seek her pleasure in some less hygienically orientated *milieu*.

Summary

Nine cases are reported in which vaccination or inoculation apparently provoked the onset or exacerbation of multiple sclerosis, and the implications of these observations are discussed in relation to the aetiology and pathogenesis of the disease.

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Haemolysis in Chronic Renal Failure

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Anaemia often occurs in chronic renal failure, and is caused mainly by depression of the bone marrow but also in some patients by increased destruction of red cells. The object of this study was to determine whether increased red cell destruction is caused by the retention of products of protein breakdown or whether it is due to a reduction in renal function.

For this purpose the life span of the red cell has been measured by radioactive chromium ($T\frac{1}{2}Cr$) in a series of patients with chronic renal failure. The dissociation between renal function and the retention of products of protein catabolism has been achieved by the use of a very low protein diet. As a result of this, blood urea levels only slightly raised above normal can be found in patients with advanced renal disease (Berlyne, Shaw, and Nilwarangkur, 1965).

The results suggest that increased red cell destruction is related to retention of products of protein metabolism rather than to the extent of renal damage.

Patients and Methods

Twenty-six red cell survival times were estimated on 25 patients with moderate and severe chronic renal failure. Two estimations were made on controls.

Patients were excluded from the series if there was external blood loss, or skin-bleeding, or if they had been transfused with blood less than seven weeks previously. Occult gastrointestinal bleeding was excluded in 17 patients by the estimation of faecal radiochromium activity (Veall and Vetter, 1958). No patient was haemodialysed, but two (Cases 14 and 20B) received a peritoneal dialysis a week before labelling of their red cells and three (Cases 21, 24, and 25) were dialysed shortly after labelling. No patient had any disease other than chronic renal failure, associated with reduced red cell survival. Six of the patients with reduced red cell survival times were under treatment with methyldopa at the time of labelling (Cases 6, 14, 15, 18, 24, and 25).

Haematological Methods

Red Cell Survival Time ($T\frac{1}{2}Cr$).—The red cells were labelled with ^{51}Cr by the method described by Veall and Vetter (1958). Gamma counting was done on 5-ml. aliquots of haemolysed whole blood. All samples and the background were measured for at least 6,000 counts and at least five samples were taken at regular intervals during estimation of the $T\frac{1}{2}Cr$. The radiochromium red cell survival time was taken as the time in days for the radioactivity in the blood to decline to 50% of the radioactivity of a sample taken 15 minutes after injection of the labelled red cells. Sample readings were corrected for radioactive decay but not for chromium elution, and were plotted against time on semilogarithmic paper. In those patients whose haemoglobin level was stable the haematocrit was used to correct sample readings for fluctuations in the blood volume (Veall and Vetter, 1958).

Red cell mass was calculated as described by Veall and Vetter (1958). *Haematocrit* was measured by centrifugation for 55 minutes in Wintrobe's tubes at 1,200 *g*. The result was corrected for trapped plasma (Chaplin and Mollison, 1952). *Haemoglobin* was measured by the cyanmethaemoglobin method.

Biochemical Methods

Serum sodium and potassium were measured by flame photometer, and serum chloride, creatinine, and blood urea were measured by autoanalyser. The serum phosphorus was measured by the method of Gomori (1942) and the plasma *p*H by the micro-Astrup method, arterialized capillary blood being used. Plasma osmolality was measured on a Knauer osmometer. Clearance values were calculated from the excretion of urea and creatinine over 24 hours and the results adjusted to a body surface of 1.73 sq. m. Urea clearance was accepted as a satisfactory measure of renal function in severe renal failure (Berlyne, 1966). All values given are the mean of several estimations.

As the blood urea concentration may vary greatly during estimation of the $T\frac{1}{2}Cr$ a representative value was derived as follows. Serial urea values during the $T\frac{1}{2}Cr$ estimation were plotted graphically and the resultant line was divided into 10 segments. The readings at the beginning and end of each segment were averaged and the mean of these averages is the figure given for the blood urea. In a few patients there were sufficient readings available for a simple average of all readings to give equal accuracy.

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Statistical Methods

Partial correlation and regression techniques were used to analyse the relation between three variables (Bailey, 1959). The partial correlation coefficient $r_{12.3}$ measures the correlation between two variables with the effect of a third specifically excluded. The partial regression equation, $y=a+b_1x_1+b_2x_2$, describes the independent effect of two variables on a third. Both methods were used to discover spurious relations between two variables dependent on the relation of one of them to a third.

Results

Normal Red Cell Survival.—This was 27 and 29 days in two patients without renal or haematological disease. These values lie within the accepted normal range of 25 to 32 days for this method.

Relation of Red Cell Survival ($T\frac{1}{2}Cr$) to the Blood Urea Concentration.—$T\frac{1}{2}Cr$ was normal in all 10 patients with blood urea levels below 135 mg./100 ml. (Table I, Fig. 1); mean 29.5 days, standard error of mean 0.6 day. Seven patients had blood urea levels between 135 and 200 mg./100 ml. In two the $T\frac{1}{2}Cr$ was normal, in two it was at the lower limit of normal, and in three it was slightly reduced—mean 25.9 days, standard error of mean 1.3 days. The $T\frac{1}{2}Cr$ was reduced in each of nine estimations carried out on patients with blood levels above 200 mg./100 ml.; mean 16.2 days, standard error of mean 1.6 days.

TABLE I.—*Data of 25 Patients in Chronic Renal Failure*

Case No.	Disease	Protein Intake (g./day)	Red Cell Mass (ml./kg.)	$T\frac{1}{2}Cr$ (days)	Blood Urea (mg./100 ml.)	Serum Creatinine (mg./100 ml.)	Urea Clearance (ml./min.)
1	C.P.N.	Unrestricted	21·3	27·5	130	5·7	6·8
2	,,	20	18·4	31·3	181	11·8	3·5
3	C.G.N.	Unrestricted	14·7	27·5	84	5·4	6·7
4	Polycystic	25	21·3	21	239	21·3	1·6
5	C.R.F.	25	14·1	30	63	7·6	4·2
6	C.G.N.	Unrestricted	15·2	22	150	14·3	6·3
7	Fanconi syndrome	25	18·8	30	101	10·7	6·1
8	C.P.N.	Unrestricted	19·5	30	168	9·5	4·5
9	Polycystic	20	21·4	32·5	81	8·6	6·0
10	Hypertension	20	28·2	32	123	11·0	5·1
11	C.R.F.	20	14·4	11·5	340	18·4	1·2
12	C.P.N.	14	16·7	29·5	130	14·5	2·4
13	Polycystic	Unrestricted	31·7	30	125	6·6	11·7
14	Hypertension	20	13·5	15	293	20·3	0·9
15	Polycystic	Unrestricted	16·8	23	146	7·9	6·0
16	,,	20	14·3	25·5	194	10·4	3·4
17	C.R.F.	20	17·7	8·5	300	21·2	0·7
18	Polycystic	40	23·8	24·5	181	9·5	7·3
19	C.G.N.	20	17·0	27	92	13·1	4·1
20A	Polycystic	20	15·1	22·5	205	19·9	4·3
20B	,,	20	14·8	11·5	300	18·5	1·1
21	,,	20	23·6	17·5	280	17·4	1·3
22	C.G.N.	20	13·2	25	135	13·8	3·7
23	C.P.N.	30	17·5	28·5	88	10·2	5·5
24	C.G.N.	30	16·2	19	298	23·3	2·9
25	,,	20	24·8	19	213	17·6	1·4

C.P.N. = Chronic pyelonephritis. C.G.N. = Chronic glomerulonephritis. C.R.F. = Chronic renal failure of uncertain cause. 20A and 20B = Estimations on the same patient after an interval of eight months.

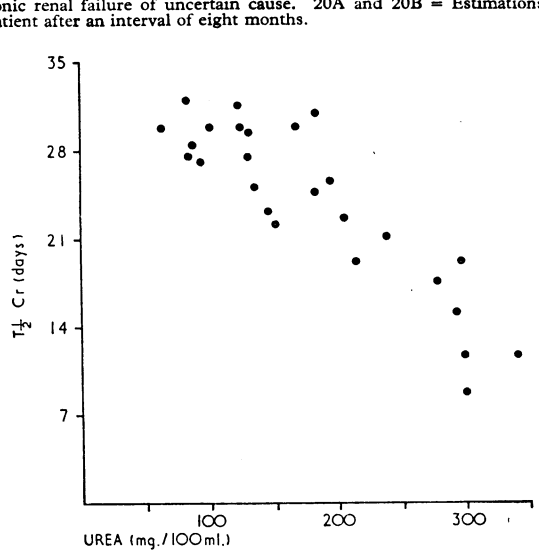

FIG. 1.—Relation between the $T\frac{1}{2}Cr$ and the blood urea level in 25 patients with chronic renal failure.

Relation of Red Cell Survival ($T\frac{1}{2}Cr$) to Renal Function.—Seven patients with urea clearances of less than 5 ml./min. but blood urea levels below 200 mg./100 ml. had normal $T\frac{1}{2}Cr$. Though there are significant correlations between $T\frac{1}{2}Cr$ and the serum creatinine and the clearance of urea, the method of partial correlation shows that these correlations are false (Table II). The concentration of serum creatinine and urea clearance

TABLE II.—*Correlations of $T\frac{1}{2}Cr$*

Variables	n	r	P
$T\frac{1}{2}Cr$ and urea clearance (ml./min.)	26	+0·66	<0·001
,, ,, serum creatinine (mg./100 ml.)	26	−0·76	<0·001
,, ,, creatinine clearance (ml./min.)	25	+0·46	<0·05
,, ,, blood urea (mg./100 ml.)	26	−0·89	<0·001
,, ,, serum phosphorus (mg./100 ml.)	26	−0·67	<0·001
,, ,, ,, uric acid (mg./100 ml.)	24	−0·24	>0·1
,, ,, *p*H	24	−0·37	>0·05
,, ,, plasma osmolality	14	−0·20	>0·1
,, ,, serum potassium	22	−0·18	>0·1
,, ,, ,, sodium	22	−0·01	>0·1
,, ,, ,, chloride	21	+0·39	>0·05
,, ,, red cell mass (ml./kg.)	26	+0·31	>0·1
,, ,, haemoglobin	26	+0·44	<0·05
,, ,, haematocrit	26	+0·45	<0·05
,, ,, diastolic blood pressure (mm. Hg)	26	+0·06	>0·1

n = No. of estimations. r = Correlation coefficient.

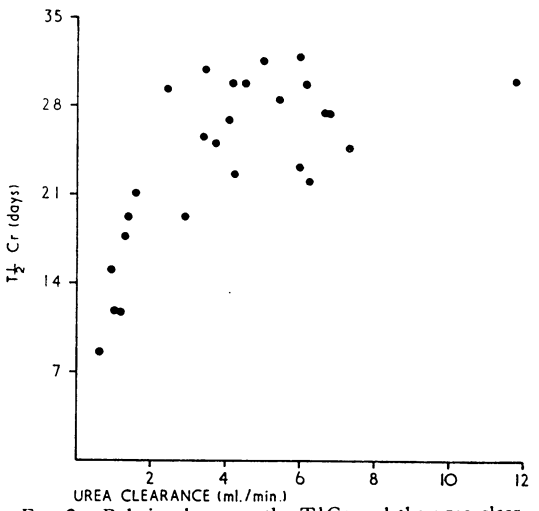

FIG. 2.—Relation between the $T\frac{1}{2}Cr$ and the urea clearance in 25 patients with chronic renal failure.

are related to the blood urea level, but it is the rise in blood urea level that is associated with a fall in $T\frac{1}{2}Cr$. The partial regression equation relating $T\frac{1}{2}Cr$ to the blood urea and serum creatinine level is:

$$y=37.6-0.065x_1-0.16x_2$$

where $y=T\frac{1}{2}Cr$ in days, x_1=urea in mg./100 ml., x_2= creatinine in mg./100 ml. The partial regression coefficient of urea is significant ($t=4.2$, $P<0.001$) but the partial regression coefficient of creatinine is not ($t=0.75$, $P>0.1$). A change in the blood urea level of 100 mg./100 ml. causes a change in the $T\frac{1}{2}Cr$ of 6.5 days, but a change in the serum creatinine level of as much as 10 mg./100 ml. causes a change in the $T\frac{1}{2}Cr$ of only 1.6 days.

Effect of Diet.—Two of the six patients on unrestricted protein intake had reduced cell survival and both had blood urea levels above 135 mg./100 ml. Red cell survival was normal in eight of the patients on protein restriction, and all had blood urea levels below 200 mg./100 ml. but seven had serum creatinine levels above 10 mg./100 ml.

Other Factors.—No significant relation between $T\frac{1}{2}Cr$ and other variables except the serum phosphorus emerges (Table

III). By the method of partial correlation this is shown to depend on the relation of the serum phosphorus to the blood urea level (Table IV).

TABLE III.—*Regression Equations*

Variables	Equation
T½Cr and blood urea (mg./100 ml.) (present study)	y = 34·3 − 0·078x
" " " " " (Joske *et al.*, 1956) ..	y = 29·8 − 0·049x
" " serum creatinine (mg./100 ml.)	y = 36·8 − 0·96x
" " " phosphorus (mg./100 ml.)	y = 37·8 − 1·8x

y = T½Cr. x = The variables stated in each case.

TABLE IV.—*Partial Correlations of T½Cr*

Variables Correlated	Variable Excluded	n	$r_{12.3}$	P
T½Cr and blood urea ..	Serum creatinine	26	−0·72	<0·001
" " serum creatinine	Blood urea	26	−0·13	>0·1
" " blood urea ..	Urea clearance	26	−0·80	<0·001
" " urea clearance ..	Blood urea	26	−0·15	>0·1
" " blood urea ..	Serum phosphorus	26	−0·80	<0·001
" " serum phosphorus	Blood urea	26	−0·10	>0·1

n = No. of observations. $r_{12.3}$ = Partial correlation between two variables with the effect of a third specifically excluded.

Discussion

Ragen, Hagedorn, and Owen (1960) found reduction in red cell survival to be rare in chronic renal failure whereas Desforges and Dawson (1958) found it to be common. The present study suggests that the incidence depends mainly on the blood urea level.

Chaplin and Mollison (1953) and Verel *et al.* (1959) found no relation between red cell survival and blood urea level. However, the patients of Loge, Lange, and Moore (1958) with reduced red cell survival had generally higher levels of blood non-protein nitrogen than those with normal red cell survival. The results of Joske, McAlister, and Prankerd (1956) are also similar to those of the present series. They found no definite reduction in red cell survival in patients with urea levels below 120 mg./100 ml., and the average T½Cr in patients with urea levels above 200 mg./100 ml. was 15.8 days, their normal T½Cr value being 24 days. The data of Giovannetti, Balestri, and Cioni (1965) on the analogous technique of in vitro spontaneous autohaemolysis show a correlation between percentage autohaemolysis after 48 hours' incubation and the patients' plasma ureas ($r = +0.65$, $P<0.001$).

These disagreements probably reflect the particular difficulties met in assessing red cell survival in chronic renal failure. Disturbed salt-and-water metabolism causes exaggerated fluctuations in the blood volume, thereby affecting the radioactivity of whole-blood samples. Correction for this by use of the haematocrit is not possible where the red cell mass is unstable, as is usual in severe chronic renal failure. Turnbull, Hope, and Verel (1957) approached the problem by concurrent serial measurements of the red cell mass with ^{32}P. The blood urea level to which the T½Cr is to be correlated may vary greatly during the estimation, and this problem has been approached in the present study by deriving the value of the blood urea as outlined above.

Cross-transfusion experiments (Joske *et al.*, 1956 ; Desforges and Dawson, 1958) have shown that the red cells from these patients survive normally in compatible normal recipients, whereas compatible red cells from a normal donor have a reduced survival in uraemic patients. A similar result was obtained in cross-incubation experiments on autohaemolysis by Giovannetti *et al.* (1965). It is therefore likely that plasma factors reduce red cell survival in these patients and the level of these plasma factors approximately parallels that of the blood urea. It is not suggested that urea itself has haemolytic properties.

Partial correlation and regression techniques in this study show that lack of functioning renal cortical tissue affects red cell survival only by virtue of the biochemical abnormalities which it causes. Acute renal failure experiments in dogs have given conflicting results about the protective effect of intact renal medullary tissue against haemolysis (Giovannetti *et al.*, 1963 ; Muirhead and Jones, 1963).

Giovannetti *et al.* (1965) have shown that spontaneous autohaemolysis is decreased after extracorporeal haemodialysis of chronic uraemic patients and also that autohaemolysis is reduced if red cells are dialysed in vitro through cellophane before incubation. Patients have been maintained for up to a year on chronic haemodialysis without blood transfusion (Comty, Baillod, and Shaldon, 1965 ; *Brit. med. J.*, 1966). If the substances which cause haemolysis had not been removed by dialysis severe haemolytic anaemia must have developed. As small molecules are dialysed preferentially through cellophane it is suggested that red cell survival is reduced in chronic renal failure by relatively small dialysable molecules. Equally, if these substances are filtered through the normal glomerulus they must have molecular weights of less than 50,000 (Hulme and Hardwicke, 1966).

It has been shown that red cell survival is related to the blood urea rather than the renal function, and the significant factor reducing the blood urea level in these patients with severely impaired renal function is the reduction in dietary protein intake. It is therefore possible that the factors which reduce red cell survival are derived from the metabolism of protein. Spontaneous autohaemolysis, however, is not reduced by low-protein diets (Giovannetti *et al.*, 1965).

In individual patients other factors may be of importance. Chaplin and Mollison (1953) and Verel *et al.* (1959) noted an association between haemolysis and malignant hypertension with papilloedema. No correlation between T½Cr and the diastolic blood pressure was found here, but as effective control of the blood pressure by salt depletion and drugs was achieved in each case before the estimation the studies are not comparable.

Of the six patients receiving methyldopa who had reduced red cell survival the Coombs test was negative in two (Cases 6 and 8) and two (Cases 14 and 25) had received the drug for less than three months. It is possible that drug therapy contributed to the reduced red cell survival in the other two (Carstairs, Breckenridge, Dollery, and Worlledge, 1966).

Summary

Twenty-six estimations of the radiochromium red cell half-life (T½Cr) were carried out on patients with chronic renal failure. The T½Cr was normal in 10 patients with blood urea levels below 135 mg./100 ml. It was reduced in nine patients with blood urea levels above 200 mg./100 ml. At intermediate urea levels reduced T½Cr was sometimes seen. The T½Cr correlated well with the blood urea level, $r = -0.89$, $P<0.001$.

Seven patients had a normal T½Cr despite serum creatinine levels above 10 mg./100 ml. The blood urea levels in all of them had been reduced below 200 mg./100 ml. by a low-protein diet. Partial correlation and regression techniques were used to show that the serum creatinine and urea clearance affected the T½Cr only by their effect on the blood urea level.

On the evidence of this and previous studies it is suggested that reduced red cell survival in chronic renal failure may be caused by dialysable molecules which accumulate in the plasma approximately parallel to the blood urea level. The molecules are possibly derived from the metabolism of protein.

I am grateful to Dr. G. M. Berlyne, Professor D. A. K. Black, and Professor S. W. Stanbury for advice and encouragement. I am also grateful to Miss J. V. Hewitt, who carried out the Astrup estimations. The patients were all under the care of Professor D. A. K. Black. The work was carried out during the tenure of a Medical Research Council junior research fellowship.

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Dissolution of Cystine Stones During D-Penicillamine Treatment of a Pregnant Patient with Cystinuria

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[WITH SPECIAL PLATE]

Brit. med. J., 1967, **2**, 216–218

Crawhall, Scowen, and Watts (1963) reported that D-penicillamine reduced the excretion of cystine in cystinuria, and that the more soluble cysteine-penicillamine disulphide was excreted in the urine. The use of D-penicillamine to prevent urolithiasis in cystinuria was suggested on the basis of these results (Crawhall *et al.*, 1963), which have now been confirmed in several laboratories (Crawhall, Scowen, and Watts, 1964; Lotz and Potts, 1964; King and Boyce, 1965; Crawhall and Thompson, 1965). The relative merits of D-penicillamine treatment and of the regimen which consists of promotion of a vigorous water diuresis throughout the 24 hours, recommended by Dent and Senior (1955) and Dent, Friedman, Green, and Watson (1965), requires long-term evaluation. There have already been reports of encouraging results in short-term studies with D-penicillamine (Crawhall, Scowen, and Watts, 1964; Lotz and Bartter, 1965; Bartter, Lotz, Thier, Rosenberg, and Potts, 1965; McDonald and Henneman, 1965).

The present paper records briefly the case of a patient whose stones had disappeared completely and asymptomatically after 18 months' continuous D-penicillamine treatment, during which time she became pregnant and gave birth to a normal child.

Case Report

The patient's first urinary symptoms were an attack of right loin pain and increased frequency of micturition when she was 12 years old. These symptoms subsided spontaneously after a few days, and she remained well until August 1963, when, at the age of 18, she had a sudden attack of severe pain in the right loin, associated with increased frequency of micturition and macroscopic haematuria, which lasted for about one day. Attacks of similar pain recurred, and she was referred to St. Bartholomew's Hospital in September 1963. She also gave a history that her only sib (a brother aged 21) had had an attack of pain similar to renal colic, and he has been shown to be a homozygous cystinuric (Patient No. 5, Crawhall, Saunders, and Thompson, 1966).

There were no abnormal findings on physical examination; blood-pressure was 105/85, height 59 in. (150 cm.), weight 7 stone 12 lb. (50 kg.). The urinary centrifuged deposit contained erythrocytes, pus cells, and cystine crystals; urine culture gave a scanty growth of *Escherichia coli*. The cyanide-nitroprusside test (Lewis, 1933) for excessive urinary cystine was positive, and increased amounts of lysine, ornithine, and arginine were also present. The right kidney contained multiple radio-opaque calculi (Special Plate, Fig. 1), it was hydronephrotic, and its ability to excrete sodium diatrizoate was impaired.

The patient was admitted for further study in December 1963, when the following additional observations were made: haemoglobin 13 g./100 ml.; leucocyte count 4,000/c.mm., with normal differential count; blood urea 34 mg./100 ml.; quantitative urine culture 100,000 organisms/ml. Mean urinary cystine excretion determined gravimetrically (Crawhall *et al.*, 1963) 719 mg. (S.D. = 120; 8 observations)/24 hr. or 664 mg. (S.D. = 156; 8 observations)/g. creatinine; and 733 mg. (S.D. = 113; 9 observations)/24 hr. or 666 mg. (S.D. = 108; 9 observations)/g. creatinine when determined by the 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) colour reaction (Crawhall, Saunders, and Thompson, 1964, 1966). The validity of the results obtained on representative specimens was also confirmed by quantitative ion-exchange chromatography with the Technicon automatic amino-acid analyser.

D-Penicillamine hydrochloride (450 mg. eight-hourly) lowered the cystine excretion to less than 100 mg./24 hr., determined gravimetrically (the colorimetric method of Crawhall, Saunders, and Thompson (1964, 1966) cannot be used during treatment with D-penicillamine). A diffuse morbilliform rash developed acutely on the eighth day of treatment; this was accompanied by fever (101.2° F.; 38.4° C.) and tachycardia, but there was no lymphadenopathy or splenomegaly. The drug was stopped and prednisolone was given orally (an initial dose of 15 mg. followed by 10 mg. every eight hours for two days and then 5 mg. eight-hourly for two days). D-Penicillamine was resumed after a total lapse of 10 days, and the dose increased in increments of 300 mg./24 hr. on successive days. The rash recurred on the third day after prednisolone was stopped. It involved the face and feet only, and was accompanied by a tachycardia but no fever. The administration of D-penicillamine was continued 300 mg. every 12 hours, and prednisolone, 10 mg. every eight hours, suppressed the rash in 24 hours; the dose of the steroid was gradually diminished and stopped completely after seven days. The rash again recurred on the forearms on the third day after the cessation of prednisolone therapy; when this was reinstituted for a further five days the eruption disappeared within 12 hours. The rash recurred for the third time after two days without steroid cover, and disappeared within 24 hours; pyridoxine (200 mg. stat. and two 50-mg. doses at eight-hourly intervals) was given on this occasion. The patient was discharged from hospital taking 300 mg. of D-penicillamine hydrochloride every 12 hours. She was not given any more steroids or pyridoxine, and the rash did not recur. The dose of D-penicillamine was gradually increased to 1,050 mg./24 hr. over the course of one month.

The patient conceived in September 1964. Except for some vomiting in the early months the pregnancy proceeded normally to term; delivery was complicated by transverse arrest of the head, but was effected without undue difficulty. The puerperium was normal. D-Penicillamine (1,050 mg./24 hr.) was continued for the whole

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