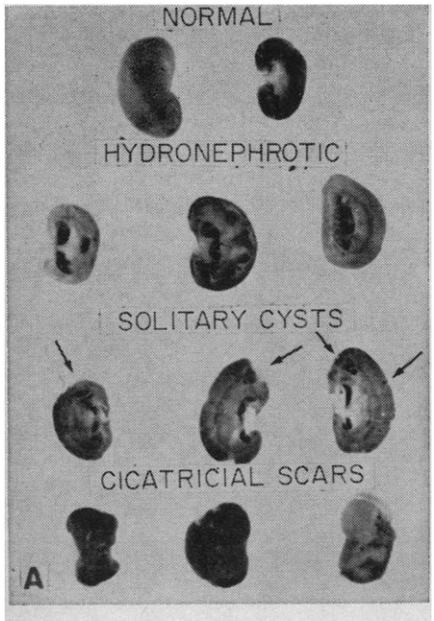

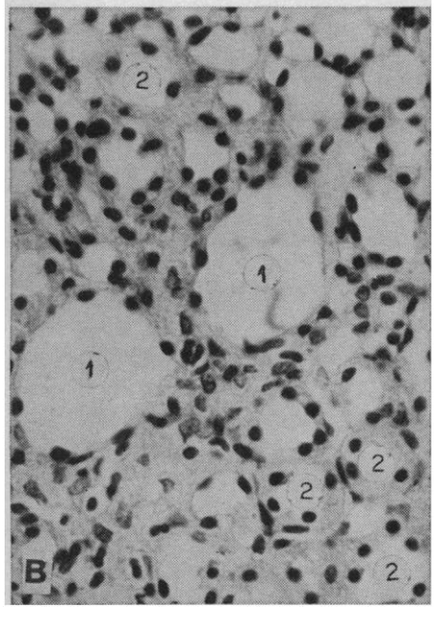

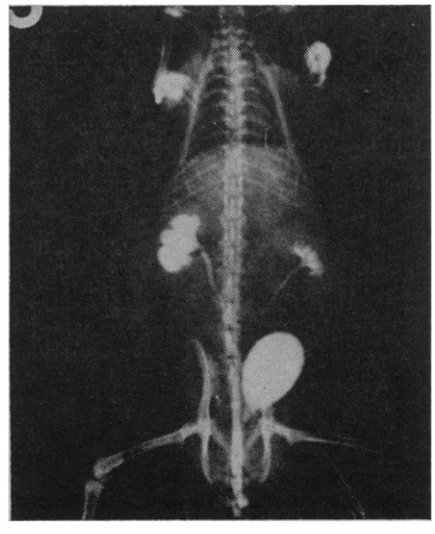

Fig. 2. (A, top left) Macroscopic lesions found in rats with hereditary renal disease. (B, center) One type of microscopic lesion in dilated (1) and normal (2) renal tubules. Periodic acid-Schiff staining × 400.

Fig. 3. (bottom left). Urogram of rat suffering from hereditary renal disease. Advanced hydronephrosis is seen in the right kidney. Normal renal pelvis and ureter are present in the left kidney.

bilirubin at the tips of the papillae (*5*). In our colony of rats this deposition of bilirubin was observed in all jaundiced rats, whether or not hydronephrosis or cysts were present. Inheritable urogenital anomalies have been described in Wistar albino rats by Hain and Robertson (*2*). Different types of renal defects, including congenital hydronephrosis, were observed in a small proportion of the animals they studied. These authors presented evidence that the renal anomalies were inherited but did not define the mode of inheritance, and no attempt was made to establish a colony of rats carrying such abnormalities. Our strain of rats has a clearly defined mode of inheritance and constitutes an experimental model with macroscopic and microscopic lesions very smilar to those observed in humans. This animal model therefore offers a unique opportunity for further studies of the embryologic defects leading to congenital hydronephrosis.

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Agglutinating Specificity for LW Factor in Guinea Pig and Rabbit Anti-Rh Serums

Abstract. *Serums produced in guinea pigs or rabbits inoculated with monkey (rhesus or baboon) or human red cells contain the same high-incidence agglutinating activity found in human anti-LW serums. After one absorption with LW-negative blood cells, anti-LW specificity was observed with stronger reactions on Rh-positive than Rh-negative cells. The 85-percent specificity was obtained after complete absorption with Rh-negative blood.*

The findings reported here show that serums of guinea pigs and rabbits injected with monkey (rhesus or baboon) or human red cells contain the same high-incidence agglutinating activity found in human anti-LW (Landsteiner-Wiener) serums (*1*, *2*). The latter serums react more strongly with Rh-positive than with Rh-negative cells, and not at all with LW-negative cells, which are very rare and consist of two types: namely, those bearing Rh antigens (*1*, *2*) and Rh_{null} bloods lacking all Rh antigens (*3*).

In the earlier studies with animal immune serums, LW-negative cells were not yet described, and hence complete absorption was carried out, as a rule, with random human Rh-negative cells which, however, are LW-positive. Thus the resulting specificity gave 85 percent of positive reactions with human red cells, as first observed by Landsteiner and Wiener (*4*). The positive and negative reactors have been called Rh positive and Rh negative and more specifically D or Rh_0 positive or negative. Later the specificity of these animal immune serums was referred to as "anti-D like" but differed from human anti-D in several respects. In the first place the D-like antigen, now known as LW (*1*), was present in Rh-negative cells as shown by their antigenicity in animals and their capacity to yield eluates of the same specificity. Second, the D-like antibody still agglutinated Rh-positive red cells which were fully coated with human incomplete antibody to D (*5*). Third, human anti-LW serums produced by Rh-positive or Rh-negative patients failed to react with selected Rh-positive or Rh-negative individuals who were LW-negative. This was observed in siblings of propositi who produced anti-LW (*1*, *2*). In still another respect, LW antigen differs from human D (Rh_0) since the latter is

Table 1. One absorption of pooled serums with LW-positive cells and with LW-negative, Rh-negative cells (readings were made after 1-hour incubation at room temperature and after light centrifugation and resuspension). Blood No. 4 was a mixture of four Rh-negative, LW-positive red cells. Test bloods No. 1 to No. 6 are group O; blood No. 7 is group B.

Antiserum		Absorbing cells		Reaction with test cells						
Series	Dilution	Type	Blood No.	Rh+ 1	Rh+ 2	Rh− 3	Rh− 4	Rh+ 5	Rh− 6	Rh− 7
				LW+	LW+	LW+	LW+	LW−	LW−	LW−
62	1:5	LW+	4	++++	++++	++	++	0	0	0
		LW−	7	++++	++++	+++	+++	0	0	0
429	1:10	LW+	4	+++	+++	+++	+++	0	0	0
		LW−	7	++++	++++	++	++	0	0	0
430	1:10	LW+	4	++++	++++	++	++	0	0	0
		LW−	7	++++	++++	++	++	0	0	0
393	1:10	LW+	4	±	+	±	+	0	0	0
		LW−	7	+	++	+	+	0	0	0
431	1:5	LW+	4	+++	+++	++	++	0	0	0
		LW−	7	+++	+++	++	+	0	0	0
432	1:5	LW+	4	0	0	0	++++	0	0	0
		LW−	7	0	++++	0	0	0	0	0
*432	1:10	LW+	4	++++	++++	+++	+++	0	0	0
		LW−	7	++++	++++	+++	+++	0	0	0

* Rabbits restimulated again 1 month later.

not present on the red cells of rhesus, baboon, and other lower monkeys (*6*).

Studies were undertaken to produce antiserums to LW in guinea pigs and rabbits injected with monkey bloods and with human Rh-positive blood. These serums were then used to carry out parallel titrations and absorptions with Rh-negative and the rare LW-negative bloods.

Six groups of animals were used in these experiments as follows: series 62, six guinea pigs injected with baboon blood; series 429, six guinea pigs injected with Rh-positive blood; series 430, five guinea pigs injected with rhesus monkey blood; series 393, five rabbits injected with baboon blood; series 431, five rabbits injected with Rh-positive blood; and series 432, five rabbits injected with rhesus monkey blood.

The guinea pigs were injected intraperitoneally six times at intervals of 3 to 4 days with 1 ml of a 25-percent suspension of washed blood. The rabbits were injected intramuscularly according to the same schedule. The animals were bled 1 week after the last injection. The serums were separated and inactivated, and equal quantities of the individual serums of each group were pooled.

The unabsorbed pooled serums were titrated and tested with two LW-positive (one Rh-positive and one Rh-negative) and two LW-negative bloods (one Rh-positive and one Rh_{null}). Readings were made after a 1-hour incubation period at room temperature and after light centrifugation. All serums showed higher titers on the two LW-positive cells than on the two LW-negative cells, the titers varying from two- to eightfold dilutions.

The serums were diluted and absorbed in parallel with random Rh-negtive, LW-positive cells and with a selected Rh-negative, LW-negative blood. The absorptions were carried out with one-half volume of washed and packed red cell sediment for 30 minutes at room temperature, and the supernatants were tested with a panel of red cells (Table 1).

All absorbed serums except the first series of 432 contain high-incidence activity with a tendency to stronger reactions on Rh-positive bloods. The antibody activity is more pronounced in the three guinea pig reagents and in rabbit series 431. Except in the first series of 432 which contained anti-P activity, anti-M, anti-N, and anti-P were excluded.

Anti-P was found in two of the five individual serums and probably resulted from nonspecific stimulation (*7*) since specimens obtained before immunization of these two rabbits contained a weak antibody to P. The possibility that rhesus moneky cells contained the P antigen was excluded since they did not absorb human or animal anti-P. Since the rabbits in series 432 (anti-rhesus red cells) did not develop antibody to LW after the first series of inoculations, they were stimulated again 1 month later and injected intravenously five times with 2.0 ml of a 25-percent suspension of the same rhesus monkey red cells. On testing the serums again 1 week after the last injection, it was found that strong anti-LW agglutinins had been produced

Table 2. Consecutive absorptions of guinea pig anti-LW serums with Rh-positive and Rh-negative red cells.

Absorbing cell	Absorptions (No.)	Reaction with test cells				
		Rh+ 1	Rh+ 2	Rh− 3	Rh− 4	Rh− 5
		LW+	LW+	LW+	LW+	LW+
		Guinea pig antiserum to RH+, 1:10				
Rh+	1	++++	++++	+++	+++	0
Rh+	2	++++	++++	++	±±	0
Rh+	3	0	±	0	0	0
Rh+	4	0	±	0	0	0
Rh−	1	++++	++++	+++	+++	0
Rh−	2	++++	++++	++	++	0
Rh−	3	+++	+++	0	0	0
Rh−	4	++	+++	0	0	0
		Guinea pig antiserum to baboon, 1:5				
Rh+	1	++++	++++	++±	++	0
Rh+	2	++++	++++	0	0	0
Rh+	3	+±	+++	0	0	0
Rh+	4	+	++	0	0	0
Rh−	1	++++	++++	++	++	0
Rh−	2	++++	++++	0	0	0
Rh−	3	+++	++++	0	0	0
Rh−	4	++±	++++	0	0	0

Table 3. Scheme of LW.

Antigen	Anti-LW+LW_1	Incidence
LW_1	++	Rh+, ~ 85 percent
LW_2	+	Rh−, ~ 15 percent
lw	0	LW−, with or without Rh antigens (very rare)

(Table 1); anti-P disappeared and anti-M (*8*) was now evident.

It is significant that one absorption with LW-positive blood No. 4 was incomplete, but was nevertheless anti-LW specific, whereas one absorption with LW-negative blood No. 7 was complete. The apparent 85-percent specificity observed by Landsteiner and Wiener (*4*) and us (*5*) is attributable to the several treatments with Rh-negative blood required for complete absorption. If the absorption with Rh-negative blood were repeated until it became negative for itself and other Rh-negative bloods—the routine procedure carried out before our knowledge of LW specificity—the resulting supernatant would give reactions specific for random Rh-positive bloods. With this in view, repeated absorption experiments were carried out on two types of guinea pig antiserums to LW, with random Rh-positive and Rh-negative red cells. The absorptions were carried out as described for experiments listed in Table 1, except that one volume of washed, packed cells was used (Table 2).

In guinea pig antiserum to Rh-positive red cells, the high-incidence anti-LW specificity is still evident after two absorptions. A third absorption with Rh-positive blood removes almost all activity, whereas third and fourth absorptions with Rh-negative blood show 85 percent specificity.

In the guinea pig antiserum to baboon erythrocytes, one absorption with either Rh-positive or Rh-negative blood cells revealed anti-LW specificity. After the second, third, and fourth absorptions with both Rh-positive and Rh-negative blood, 85 percent specificity is observed, thus confirming Landsteiner and Wiener's original findings (*4*). Absorption with Rh-negative blood results in stronger reactions than absorption with Rh-positive blood cells.

In all instances in which anti-LW specificity is evident, reactions are stronger with Rh-positive than with Rh-negative blood, as in the case of human anti-LW serums (*1, 2*). In general, the loss of reactivity is greater on repeated absorption with Rh-positive blood. These observations suggest that Rh-positive red cells may have more LW sites than Rh-negative red cells.

The findings presented indicate that the antibody for the high-incidence LW factor is readily revealed in immunized guinea pigs and rabbits if their serums are properly absorbed and tested with a panel of red cells, including the rare LW-negative cells. The LW system shows remarkable parallels with A_1A_2O and P_1P_2p systems.

Thus anti-LW acts on LW_1 (about 85 percent Rh-positive) and LW_2 (about 15 percent Rh-negative). Anti-LW fails to react with the very rare LW negatives which may be called lw. Just as anti-A reacts more strongly with A_1 than A_2, so anti-LW reacts more strongly with LW_1 than with LW_2. Also absorption of anti-LW with LW_2 leaves behind a fraction active for LW_1. Thus anti-LW may be represented as anti-LW+LW_1. The analogies extend also to the P_1P_2p system (*9*).

Schematically these facts may be represented as shown in Table 3, insofar as direct reactions are concerned.

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Ring D Chromosome: A Second Case Associated with Anomalous Haptoglobin Inheritance

Abstract. *A second child with a ring D chromosome and anomalous inheritance of haptoglobin has been identified. Autoradiographic studies of peripheral lymphocytes from this child and of those from the previously described patient indicate that the ring in each is derived from chromosome No. 13. These findings are evidence that the locus for the haptoglobin alpha-chain is situated on one end of chromosome No. 13.*

A mentally retarded patient with multiple minor congenital anomalies and anomalous inheritance of haptoglobin has been described by Gerald *et al.* (*1*). Cytogenetic studies of that child revealed 46 chromosomes with a ring chromosome replacing one of the members of the D (13 to 15) group. The ring chromosome was presumably formed by breaks occurring at both ends of the D chromosome, with reunion of the broken ends and loss of the distal fragments. Typing of haptoglobin from family members indicated that the child had failed to inherit the paternal gene for haptoglobin. Since typing of the remaining blood group systems gave indirect confirmation of paternity, it was hypothesized that the paternal gene for haptoglobin was lost during formation of the ring and that the structural locus for the alpha-chain of haptoglobin (*2*) is situated on one end of a D chromosome.

A second and unrelated child with 46 chromosomes including a ring D, who also exhibits anomalous inheritance of haptoglobin, has now been studied. The clinical findings in this patient have been briefly reported (*3*). The results of haptoglobin typing in this second family are again most simply interpreted if it is assumed that the locus for the alpha-chain of haptoglobin is situated on one end of a D chromosome and that it has been lost during formation of the ring. We now report results of these studies and of the autoradiographic studies of both patients with ring D chromosomes.

Routine chromosomal analyses were performed by a modification of the method of Moorhead *et al.* (*4*). Studies of DNA replication with tritiated thymidine were done by a modification of previously described methods (*5, 6*). Typing of haptoglobin was performed by starch-gel electrophoresis of the hemoglobin-haptoglobin complexes. Subtyping of the alpha-chains of haptoglobin was done according to the method of Smithies (*7*). Quantitative determinations of haptoglobin were