95°F. Plasma volume declined 13 percent.

In the second study, housing conditions were different for birds subjected to 30°F and birds subjected to the two higher temperatures and therefore this might have been an additional factor. Blood pressure has been found to be higher in birds housed in floor pens than in those caged communally or individually (*2*). Physical activity rather than the presence of other birds may influence pressure. However, seasonal changes in blood pressure were prominent in all birds and therefore the type of housing did not seem to account for any major portion of the differences.

Our findings do not correspond to those reported for mammals, perhaps because the mechanisms bringing about circulatory adaptations to heat are different. The increase in cardiac output in man at high ambient temperatures presumably results from peripheral vasodilation and an increased vascular volume which in turn calls for a greater cardiac output to facilitate heat loss through the skin. In contrast, chickens loose heat primarily through the respiratory system instead of through the skin. Blood and plasma volume actually decreased with adaptation to heat; such a decrease would diminish venous return and lower cardiac output.

These results are from acclimatized birds and may not be comparable to most findings reported for mammals. Hyperthermia, which increases output, was not a condition in our experiments (*8*).

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Antibody to Rat Kidney: In vivo Effects of Univalent and Divalent Fragments

Abstract. *Whereas intact antibody to rat kidney (7S) produced immediate and sustained proteinuria in rats, univalent fragments (papain digests) of the antibody did not, and divalent fragments (pepsin digests) produced only transitory proteinuria. The antibody fragments differed from the intact antibody in fixing little, if any, complement in vivo which may explain why they did not cause serious renal damage.*

We have compared in rats the effects of intact antibody to rat kidney and fragments of the antibody prepared by the methods of Porter (*1*) and Nisonoff (*2*). Antikidney serum produces renal damage; the damage in rats is manifested by proteinuria which is a very satisfactory measure of the severity and persistence of the renal lesion (*3*).

Antikidney serum from rabbits that had received repeated intraperitoneal injections of rat kidney stroma without adjuvants (*3*) was fractionated on diethylaminoethylcellulose (*4*). The effluent from treatment with 0.0175*M* phosphate at *p*H 6.3 contained only 7*S* γ-globulin. This fraction produced immediate and persistent proteinuria when injected intravenously into rats (Table 1). Material eluted in a subsequent fraction containing the macroglobulin also produced proteinuria. Further resolution of this subsequent fraction on a Sephadex G-200 column showed, however, that the nephrotoxic component was in the contaminating 7*S* material rather than the macroglobulin. When it was established that the nephrotoxic component was associated only with the 7*S* antibody, sodium sulfate (*5*) was used in the later studies to separate γ-globulin.

The 7*S* γ-globulin was digested with papain (*1*), and piece III was allowed to crystallize in the cold. The supernatant solution containing 3.5*S* univalent antibody fragments I and II produced neither immediate nor delayed proteinuria (Table 1). Two exceptions were noted wherein mild and evanescent proteinuria occurred during the fourth week after injections. Piece III alone and the univalent fragments plus piece III were likewise ineffective in producing proteinuria.

A pepsin digest (*2*) containing divalent (Nisonoff) 5*S* fragments of the antikidney γ-globulin produced transient proteinuria in rats—after the usual doses the proteinuria disappeared in 48 hours (Table 1). Even after the larger doses, proteinuria disappeared within 6 days. The 5*S* fragments purified by either sodium sulfate precipitation (*6*) or Sephadex G-100 gel filtration produced the same transient proteinuria, whereas the small fragments thus removed were ineffectual. The proteinuria was not attributable to residual undigested antibody in the injected material, for no significant amount of 7*S* material was present (Fig. 1). Furthermore, reduction of the digest material with β-mercaptoethanol (*7*), which does not inactivate the antibody if present, degraded the 5*S* fragments to 3.6*S*, and the material no longer produced proteinuria.

Fragments prepared from nonspecific γ-globulin were injected into control animals. Both the univalent and divalent nonspecific fragments in the usual doses (those obtained from 2 to 4 ml of serum) failed to produce significant proteinuria. In doses obtained from 10 to 20 ml of serum, however, both types of nonspecific fragments and also the specific univalent fragments induced mild proteinuria, averaging less than 2 mg of protein per hour and limited to the first day which apparently was the result of excretion of the injected material. This proteinuria was clearly less in intensity and duration than that caused by equivalent doses of the specific divalent fragment.

Antibody fragments, both univalent

Table 1. Proteinuria produced in rats by antibody to rat kidney and its fragments. Additional dosages were used and urine was collected periodically for at least a month (*3*). The figures in parentheses indicate the number of animals tested.

Dose* (ml)	Protein (mg/hr)		
	Day 1	Day 3	Day 21
Normal controls† (*12*)			
	0.2	0.3	0.1
Antiserum to rat kidney (*9*)			
1 to 2	9.4	8.8	3.7
7S γ-globulin (*7*)			
1 to 2	8.0	7.1	5.0
Univalent pieces I and II (*7*)			
2 to 4	0.3	0.3	0.2
Divalent 5S pieces (*6*)			
2 to 4	3.2	0.3	0.1
Divalent 5S pieces (*3*)			
5 to 10	6.8	1.3	0.2

* Dose in terms of antikidney serum (adjusted to standard potency) from which the material was derived. † Similar results were obtained with rats receiving no injection and rats injected with 1 to 2 ml of normal rabbit serum. No individual protein values above 0.5 mg/hr were observed in these control rats.

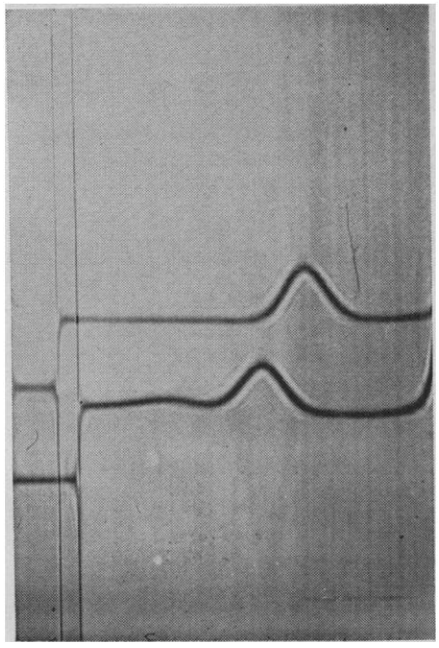

Fig. 1. Simultaneous ultracentrifugal analysis of undigested "7S" γ-globulin (top) and the γ-globulin digested with pepsin (bottom). The top pattern shows a single component 6.6S, uncorrected, whereas the bottom pattern shows a major component 5.0S and trailing material of approximately 2.5S.

(*1, 2, 8*) and divalent (*2*), retain the ability to combine with the appropriate antigen. Stelos *et al.* (*9*) demonstrated that univalent fragments prepared from antikidney γ-globulin and labeled with I^{131} were selectively although transiently fixed in the kidney in vivo and bound by kidney sediment in vitro. Thus, the antibody fragments might be expected to inhibit competitively the reaction between antikidney antibody and kidney antigen and prevent production of renal damage by the antibody. In this study, univalent fragments did not provide protection in vivo against intact antibody injected simultaneously or 5 to 15 minutes later. Probably the quantity of univalent fragments was inadequate to protect all of the kidney antigenic sites (*10*) from antibody attachment. Next, by adding univalent antibody fragments, we attempted to inhibit in vitro the reaction between a large dose of antibody and its neutralizing dose of solubilized kidney antigen (*11*). After addition of the fragments in amounts up to 10 to 20 times that of the antibody, there was sometimes enough free antibody in the mixture to produce proteinuria in a rat, but more often the result was negative. Combination of the antibody and antigen seemed to be more effectively blocked by addition of divalent fragments, although potentiation of residual free antibody by these fragments was not completely ruled out as a possible alternative explanation. The experiments are complex and cannot now be fully interpreted.

Since Stelos *et al.* (*9*) noted that univalent fragments in contrast to the intact antibody were largely eliminated from the kidney tissue in 3 days, possibly the failure of fragments to induce late or delayed proteinuria is explained by their rapid elimination. However, Stelos *et al.* showed roughly comparable binding of the intact antibody and the univalent pieces 1 day after the injections; hence the marked difference in proteinuria, caused by intact antibody and its fragments, presumably must be explained on a basis other than differences in their kidney localization.

The titers of complement in serum decreased to about 25 percent of normal after the usual doses of antiserum. The extent to which the changes in complement were caused by the same antibody (or antibodies) which causes the proteinuria is not known; however, others have demonstrated complement in the renal lesions (*12*). Relatively little change in complement titers occurred, on the other hand, after injections of univalent or divalent antibody fragments, confirming in vitro studies (*13*) and in vivo studies (*14*) with other antibodies. Therefore, the observations reported here are consistent with the hypothesis that fixation of complement is required for the production of serious damage by antikidney antibody (*15*). However, the complement studies, possibly because of their limitations, did not disclose why divalent fragments produce transitory proteinuria and univalent fragments do not. Alternatively, the failure of antibody fragments to produce severe renal damage may conceivably result from one of a number of other differences (*1, 2, 9, 16*) between intact antibody and the fragments.

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Haploids: High-Frequency Production from Single-Embryo Seeds in a Line of Pima Cotton

Abstract. *Progenies of a doubled haploid from Pima S-1, a commercial variety of* Gossypium barbadense *L., contained a very high frequency of haploid plants. The haploid plants, in contrast with those previously reported in cotton, originated from single- rather than twin-embryo seeds. Apparently the haploid-producing ability of this line of cotton is inherited.*

Occasionally haploid plants with a somatic chromosome number of 26 are found in cotton species (*Gossypium hirsutum* L. and *G. barbadense* L.) cultivated in the New World. In field plantings in Arizona, haploids are rarely found in *G. hirsutum* or Upland cotton, but in the Pima varieties of *G. barbadense*, a frequency of one to three haploids per 25,000 plants is not uncommon. Haploids are distinguished from diploid plants (*1*) by their lack of pollen shedding, by smaller plant parts, and sometimes by greater height because of their lack of fruitfulness (*2*).

Haploids occur as one or both members of twin-embryo seeds (*3–6*). In *G. hirsutum*, Roux found a frequency of one twin embryo in 20,000 to 25,000 seeds (*5*); Kimber reported two twins in 127,500 seeds (*4*), and Blank and Allison reported frequencies from one twin in 20,511 seeds to one twin in 2639 seeds (*7*). An increase in the incidence of twin embryos in a line of *G. hirsutum* was accomplished by selection pressure for this characteristic (*7*).

In *G. barbadense* the frequency of