may be assumed, *a priori*, that sensitization to killing by complement requires attachment of certain minimum amounts of antibody to the cell, and this in turn depends on the presence of a sufficient number of specific antigen sites, the findings reported here may serve to explain the reported successful sensitization of a heavily flagellated *Proteus* strain by anti-flagellar antibody(4), and the consistent failure to observe sensitization of the less heavily flagellated *Salmonella* strains by antibody specific for their flagellar antigens.

The apparent differences in acquired sensitivity of the various test strains to the bactericidal effect of complement and of antibody specific for adsorbed antigens need not be considered significant. In some instances, such as in the case of the mucoid *Klebsiella* strain, resistance may be inherent in the strain (9), in other cases the composition of the cell surface may limit its capacity to adsorb the particular test antigen. Optimum conditions for adsorption were not extensively investigated.

It is believed that findings presented here should be taken into consideration in the interpretation of apparently non-specific bactericidal activities of sera. Furthermore, since the bactericidal test is an extremely sensitive method for the detection of small amounts of antibody, further refinement of the technic may lead to the development of methods suitable for the detection of trace amounts of antibody against antigens that may be adsorbed to suitable bacterial strains.

Conclusion. The bactericidal activity of complement is mediated by the reaction between antibody and antigens located at the surface of bacterial cells. These surface antigens need not be constituents of the cell but may be substances which have been artificially adsorbed to its surface.

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Effects of Exposure to Cold and of Dietary Restriction upon Globulin Nephritis in Rabbits.* (19458)

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Glomerulonephritis has been induced in both rats and rabbits by a variety of experimental procedures(1-9). These have included intravenous injection of heterologous serum proteins(1-4), anti-kidney antibodies(5-7), and extracts of ground kidney and streptococci(8,9). Most attempts to increase or decrease the severity of experimental nephritis have been made with so-called "nephrotoxic" nephritis, *i.e.*, that produced by injection of an anti-kidney serum from another species. This type of nephritis has been inhibited in rabbits by preliminary general exposure of the body to x-radiation(10). In rats nephrotoxic nephritis has been prevented by restriction of dietary protein(11).

A very severe acute glomerulonephritis has been induced in rabbits by massive intravenous injections of bovine serum gamma globulin(12-14). The present report deals with the

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effects of 2 additional factors upon the nephritis produced in this way. In the first experiment, it is shown that by exposing animals to a cold environment during the experimental period, this globulin-induced nephritis is increased in severity, and possibly in incidence. A second group of experiments indicate that nephritis is completely prevented by relatively minor reduction in the animals' food and water intake.

Materials and methods. Seventy-two albino rabbits of mixed breed were used. All were maintained in separate cages, fed Ogilvie Miracle Chow and given tap water. Unilateral nephrectomy was done 3-6 weeks before further treatment. The animals of Groups I-IV inclusive were injected intravenously on 2 occasions 10 or 11 days apart with 1 g/kg body weight of purified bovine serum gamma globulin given as a 10% aqueous solution in 0.95% NaCl. The second injection was preceded by a desensitizing intravenous injection of 5-10 cc of a 1% globulin solution. During the experimental period blood was drawn at various times from animals of all groups for precipitin tests. Urine was collected daily from individual animals of all groups via a screen grid and metal funnel. Twenty-four hour urine output was measured and daily analysis for protein content was made as well as a microscopic search for red blood cells and casts. Animals were killed 6 or 7 days after the second globulin injection and autopsies performed immediately. Tissues were fixed in Zenker-formol and microscopic examination made of heart, kidney, spleen, thymus, lung, and adrenals. The animals were divided into the following groups:

Group I—Twenty-four animals were kept at room temperature and allowed *ad lib.* food and water intake. They received 2 massive globulin injections as outlined above. *Group II*—Sixteen partly shaved animals were placed in a refrigerated room where the temperature fluctuated between —9.4°C and +4.4°C. After 3 days of exposure to low temperature these animals were injected with bovine serum gamma globulin, exactly as those of Group I. Apart from continuous exposure to cold, animals of this group were treated in exactly the same manner as those of Group I. *Group III*—Six animals, kept at room temperature, were treated in the same manner as Group I, except that each was restricted to 70 g of food and 130 cc of water per day. *Group IV*—Six animals, kept in the refrigerator and on the same restricted diet as those of Group III, were injected with bovine gamma globulin in the usual way. *Groups V and VI* consisted of 10 animals each, serving as controls for Groups I and II. Group V was kept on *ad lib.* diet at room temperature and subjected to the same procedures (bleeding, urinalysis) as Group I. Group VI was handled similarly but was housed in the refrigerated room throughout the experimental period. The animals of Groups V and VI received no injections of bovine gamma globulin.

Results. Diffuse glomerulonephritis developed in globulin-treated animals on *ad lib.* diet, whether kept at room temperature or in the cold (Groups I and II). The severity of the disease was graded roughly as zero to 4 plus (13). The incidence of lesions of the various grades in these 2 groups of animals is shown in Table I. It is seen that whereas 75% of the animals of Group I developed diffuse nephritis, similar renal damage was found in *all* animals treated in the cold (Group II). It was further found that severe (+++ to ++++) nephritis occurred in 29% of Group I animals, while 56% of animals of Group II had nephritis of this degree.

There was no difference in the gross or microscopic features of the renal lesions in these 2 groups. The remaining kidney was usually larger in nephritic animals than in controls kept at the same temperature. The mean kidney weight for room temperature control animals (Group V) was 12.4 g, the largest being 16.0 g, compared to an average of 13.8 g in cold room controls (Group VI). Group I animals had an average kidney weight of 14.3 g, whereas those of Group II had kidneys averaging 17.8 g. Kidneys in which severe nephritis was present were usually distinctly yellower and paler than normal, but not hemorrhagic. Microscopically these kidneys showed diffuse damage to all glomeruli similar to that reported previously(13). The lesions corresponded in appearance to those of

TABLE I. Effect of Exposure to Cold on Incidence and Severity of Experimental Glomerulonephritis.

Group	No. of animals	Severity of nephritis: 0	+	2+	3+	4+	Incidence: % with nephritis	Incidence: % with severe nephritis
I. Room temp.	24	6	4	7	4	3	75 (60.2–86.3)*	29 (17–44.1)
II. Refrigerated	16	0	2	5	7	2	100 (86.6–100)	56 (37.5–73.7)

* Figures in parentheses represent confidence limits (%) at probability of .10.

the extra-capillary glomerulonephritis of Fahr (15), or Type I nephritis of Ellis(16).

Urinary changes of acute nephritis (proteinuria, cylindruria, hematuria) generally became manifest between 7 and 10 days after the initial globulin injection. The urinary changes in the animals treated at room temperature were less severe after the 10th day, but in animals treated in the cold, there was a secondary exacerbation between the third and sixth days after the second massive injection with globulin. Uremia developed in 3 of the 40 treated animals and one died in uremia.

Serum protein estimations on several of the animals showed a rise in globulin, and fall in albumin, coincident with the development of nephritis, and also at about the time when antibodies to the injected globulin became demonstrable in the sera. The details of these findings, together with more extensive studies in similar experiments will be published(14).

The findings in animals of Groups III and IV were in striking contrast to those in the first 2 groups. In none of these animals was there any morphological evidence of glomerulonephritis, nor did any of them show persistent proteinuria during life.

Table II shows relative daily weight gain in the various groups. It is evident that animals of Group III gained only about half as much per day as animals on *ad lib.* diet, while those of Group IV registered only very slight weight gain.

The results of ring tests to demonstrate precipitins to bovine gamma globulin are shown in Table III. It is evident that all globulin-treated animals on *ad lib.* diet had precipitins on the 10th day, whereas only 5 of the 6 in Group III and one of the 6 in Group IV gave positive reactions at this time.

Discussion. There seems little doubt that the diffuse glomerulonephritis which appeared in animals of Groups I and II was in fact produced by the bovine gamma globulin injections they received. Similar lesions were not found in any of the unilaterally nephrectomized control animals (Groups V and VI), kept under identical conditions, nor were such lesions found in any of the kidneys surgically removed before treatment. The numbers of animals in the first 2 groups are too small for the difference in incidence of nephritis to be significant. However, within confidence limits of 0.005, the increased percentage of refrigerated animals showing severe nephritis is statistically significant.

It should be emphasized that grading of incidence and severity was done before any

TABLE II. Effect of Dietary Restriction on Rate of Weight Gain and Development of Glomerulonephritis.

Group	No. of animals	Avg wt when killed, kg	Avg daily wt gain, g	No. with glomerulonephritis
I	6	2.66	25.2	4
II	15	2.33	25.2	15
III	6	2.12	13.6	0
IV	6	1.74	2.5	0
V	10	2.81	25.8	0
VI	10	2.60	26.2	0

TABLE III. Effect of Dietary Restriction on Antibody Production. 6 animals in each group.

Group	Day 0	Day 10	Glomerulonephritis
I	0*	6*	4
II	0	6	6
III	0	5	0
IV	0	1	0

* Figures indicate number of animals showing precipitins to bovine serum gamma globulin.

statistical calculations had been made. Furthermore, in the grading criteria employed, the difference between severe (+++ and ++++) and mild (+ and ++) lesions was so striking as to render incorrect classification in these categories very unlikely.

The findings in animals of Groups III and IV indicate complete inhibition of experimental globulin nephritis, apparently produced by minor general dietary restriction. Since all animals were otherwise treated in exactly the same manner, this suggests that inhibition was in fact the result of dietary restriction.

We have suggested elsewhere(14) that the development of experimental glomerulonephritis may be a function of quantitative antibody response, *i.e.*, the greater the amount of antibody produced, or the faster the rate of its production, the greater the liability to nephritis. The present findings are in agreement with this suggestion. While accurate measurements of food intake were not made on rabbits of Group II, it was noted that larger quantities of food were required to maintain their rate of weight gain. Furthermore, refrigerated animals on restricted diet gained much less weight than animals maintained at room temperature on the same diet. These findings seem to indicate a higher rate of metabolic turnover in the refrigerated animals. Other investigators(17) have found that antibody protein participates in the general metabolic ebb-and-flow to the same extent, and at about the same rate, as do other tissue and body proteins. It has also been reported that antibody production is increased in animals exposed to cold(18,19). It is possible, therefore, that the increased severity of nephritis in animals exposed to cold was related to a general increase in metabolic turnover, in which increased antibody production was the determining factor. On the other hand, prevention of nephritis in animals on restricted diet appears to have been accompanied by delayed antibody production. This is particularly noticeable in the refrigerated animals (Group IV).

It is clear that further investigation will be required before much more can be said of the mechanism of the dietary inhibition of nephritis. The fact that antibodies did appear in some of the animals on restricted diet is in keeping with the suggestion that the development of nephritis may be related to the potency of the antibody reaction rather than to its presence or absence.

It should be emphasized that any experimental procedure which impaired the animals' appetites might produce a similar result and receive unjustified credit as a preventive. Indeed, certain experimental results reported in the past might profitably be reassessed in the light of this finding.

Summary. 1. Eighteen (75%) of 24 unilaterally nephrectomized albino rabbits given 2 massive injections of bovine serum gamma globulin at 10 to 12 day intervals, developed lesions of acute diffuse extra-capillary glomerulonephritis. The disease was severe in 7 (29%). In 16 animals similarly treated, while continuously exposed to a cold environment, nephritis developed in 100%, and was severe in 56%. It is concluded that these differences in severity are probably significant, and may be related to a general increase in metabolic activity, in which increased antibody production is the important factor. 2. None of 12 animals similarly treated while under mild starvation contracted nephritis whether maintained at room temperature or exposed to cold. Inhibition of nephritis appeared to be accompanied by impairment of antibody production. 3. In view of these results it is suggested that the dietary status of animals in similar experiments must be evaluated before credit can be given any other therapeutic agent for prevention or inhibition of experimental nephritis.

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Experiments with C^{14}-Menadione (Vitamin K_3)* (19459)

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Dam(1) established that hemorrhage in chickens on certain diets was due to the absence of a new accessory food factor, vit. K. Schonheyder(2) established that the hemorrhagic condition was due to a decrease in plasma prothrombin, and Greaves(3), following up the work of Hawkins and Brinkhous (4), established that the prothrombin deficiency in obstructive jaundice was due to failure to absorb vit. K, the presence of bile in the intestine being necessary for this. Extensive experimental and clinical observations (5-7) have been made connecting a decrease in plasma prothrombin concentration with interference with liver function. By inference, it has been assumed that vit. K acts through the liver, although little evidence has been obtained for this. Likewise little information is available regarding distribution of the vitamin in tissues. The senior authors have previously reported the results of studies of dicumarol labelled with C^{14}(8). As this substance and vit. K appear to be related as antivitamin and vitamin, radioactive K has been synthesized as 2:C^{14}-methyl-1:4-naphthoquinone(radioactive menadione) and administered to mice and dogs. The present communication reports the results of these experiments.

Methods. Experimental procedure. 2-methyl-1:4-naphthoquinone with C^{14} in the methyl group was synthesized by Mr. R. V. Phillips, as described elsewhere(9). The specific activity was 4.10 x 10^4 counts per minute per mg (c.p.m./mg). The material was dissolved in corn oil and injected intramuscularly into dogs and mice. The mice were sacrificed at different time intervals and the activity determined on aliquots of the dried tissues. To determine the activity of the dog tissues, the organs were boiled in dilute alkali and an aliquot counted. Radioactivity measurements were made using a gas-flow type of Geiger-Müller Counter. The efficiency of the counting system was checked frequently with a sample of C^{14}-dicumarol used as a standard. Counts were corrected for background, efficiency change with time, self-absorption, resolving time and geometry. Twice the standard deviation of the background was 9.6

TABLE I. Test of Radioactivity of Expired CO_2 of Mouse after Receiving C^{14}-Menadione.

Sample hr	Wt of $BaCO_3$ counted, mg	Observed activity −bkgd. c.p.m.	Total activity
0-11	11	2.6	N.A.
11-17	36	7	
17-24	60	3.8	

N.A. = No significant activity.

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