of the interference with the intracellular multiplication of brucellae: 1) whether the entire, untreated or UV-treated, phage particle is phagocytosed and subsequently enters into a direct bacterium-phage interaction, 2) whether only the active protein moiety diffuses to the susceptible bacterial site, or 3) whether the phage-protein, in conjunction with monocyte constituents, can cause changes in intracellular conditions that secondarily affect bacterial multiplication. The last hypothesis seems unlikely in view of the similarity between results obtained in extra- and intracellular systems. Also, preliminary tests, in which monocytes containing many brucellae were exposed to untreated phage, showed, 24 hours later, slight but consistent increases in phage titers (3.37×10^4 per 10^6 monocytes) over those obtained from brucella-free cultures (1.06×10^4 per 10^6 monocytes) or from cultures initially containing intracellular heat-killed brucella (1.67×10^4 per 10^6 monocytes). These observations would suggest that phage is actually phagocytosed and may interact with intracellular bacteria in the same manner as with extracellular bacteria. Finally, the observation that in phage-exposed cultures there is a decreased proportion of monocytes that contain stainable brucellae suggests the possible occurrence of additive effects between phage (or phage-protein) and cellular factors that can lead to the intracellular destruction of brucellae.

Summary. Studies on the interactions between brucellaphage and *Br. abortus,* either under extracellular conditions or when the bacteria resided intracellularly within guinea pig mononuclear phagocytes maintained *in vitro,* showed: 1) inhibitory effects of untreated or UV-treated phage on the multiplication of brucellae, and 2) a lack of such effects in the case of heat-treated phage. These findings suggest an association between brucellacidal, or possibly brucellastatic, effects and phage protein. It was demonstrated that the effects on intracellular bacteria can occur even when exposure to phage is delayed until 2 hours after phagocytosis of the bacteria.

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Creatine Synthesis After Creatinine Loading and After Nephrectomy.* (26137)

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Bloch and Schoenheimer(1) established that in mammals glycine and amidine (from arginine) react to form guanidinoacetic acid (GAA) and Du Vigneaud(2) demonstrated that the latter compound is methylated to form creatine. The two enzymes necessary for this synthesis are arginine-glycine transamidinase and guanidinoacetate methylferase. It had been thought for many years that the first of these enzymes was found only in the mammalian kidney(3). However, Walker (4) has demonstrated that the pancreas contains significant amounts of transamidinase, and could be a site of GAA synthesis. We have reported(5) that we were able to recover creatine-C^{14} after injecting nephrectomized rats with glycine-2-C^{14}, indicating that there is, in fact, a non-renal route of creatine synthesis. Horner(6) has reported essentially the same observation. However, in our study

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TABLE I. Effects of Nephrectomy and Creatinine Loading on Creatine Synthesis.

	Control			Experimental			% of control
	Avg cpm	SD	n	Avg cpm	SD	n	
Nephrectomy							
Glycine 2-C^{14}	1256		7	134		5	10.7
Glycocyamine 2-C^{14}	15898	4585	5	9944	1319	5	62.5
Creatinine loading							
Glycine 2-C^{14}	909	181	3	501	127	3	55.2
Glycocyamine 2-C^{14}	10333	727	3	7095	769	3	69.1

it was observed that rats made uremic by ureteral ligation showed a depression in amount of creatine synthesis, despite the presence of the kidneys. The present study was undertaken to determine whether uremia *per se,* interferes with creatine synthesis.

Methods. Three series of experiments were performed. In the first series Long-Evans rats weighing 300-400 g were lightly anesthetized with ether and bilaterally nephrectomized in a single stage procedure. Forty-eight hours later these animals were injected in the thigh with 25 μc of GAA-2-C^{14}. Seventy-two hours after nephrectomy the animals were sacrificed and 40 g of muscle were dissected from the carcass. Creatine was obtained from this tissue as potassium creatinine picrate by the technic of Foster, Schoenheimer and Rittenberg(7). The radioactivity of this material was then determined on a flow-counter with a micromil window using an infinitely thick sample of the potassium creatinine picrate dried to a constant weight. All counts were corrected for background and coincidence. Control animals were handled in an identical manner except that nephrectomy was not performed.

The second series of animals was loaded with creatinine by injecting 700 mg/kg of rat body weight intraperitoneally 2 times daily during an 8-hour period. All animals received a normal diet and water. After 3 days of such loading 50 μc of glycine-2-C^{14} were injected and the creatinine loading continued. Twenty-four hours later the animals were sacrificed and creatine-C^{14} determined as in previous experiment. Control animals were not injected with creatinine. The third series of animals was handled in an identical manner with the second, except that 10 μc of GAA-2-C^{14} were injected in place of the glycine-2-C^{14}.

Results are summarized in Table I. Nephrectomy reduced the utilization of glycine-2-C^{14} to 10.7% of control values, and was reported previously(5). Since this is the weighted average of 3 experiments, performed with modifications of dose of glycine-2-C^{14}, it is not possible to calculate a standard deviation. However, in the present series of experiments, nephrectomy reduced methylation of GAA by 37.5%, indicating that uremia not only decreases the renal component of GAA synthesis, but also impairs the non-renal methylation. The second and third phases of the study were performed to determine if the increased concentration of creatinine, inherent to renal failure, could result in reduced creatine synthesis and breakdown. It will be seen in the Table that incorporation of glycine was reduced 45% by creatinine loading, while methylation of GAA was reduced 31%.

Discussion. The original interest in this study was developed by the observation that with progressive, chronic renal failure the excretion of creatinine gradually decreased, and by extrapolation, was approximately 60% of normal when renal excretory function had decreased to zero(8). Three questions were posed: (1) Was this decrease due to an alternative pathway for creatinine excretion, (2) was it due to impaired renal synthesis of GAA, or (3) were other factors involved? Most of the alternative routes of creatinine excretion seemed to be excluded after specific examination(8). Sandburg, Hecht and Tyler(9) demonstrated that the product of the renal A-V difference in GAA concentration and the renal blood flow was inadequate to

account for more than one-third of the daily production of creatine. This value compared favorably with the observed 40% decrease in creatinine excretion in total renal failure, and suggests that the decrease is actually due to loss of the renal component. However, in acute experiments(5,6) nephrectomy reduced the synthesis of creatine 78-94% when based upon C^{14} recovery from creatinine after glycine-C^{14} injection. Indirect evidence from our experiments, the demonstration that serum creatinine concentration falls after 5 days of dialysis, also suggested that extra-renal synthesis of creatine was being impaired. Therefore, the effect of high concentrations of creatinine on synthesis of creatine was investigated. The resultant experimental data indicate that increases in creatinine do, in fact, exert a profound effect upon creatine synthesis. Nephrectomy decreased GAA methylation by 37.5%, while creatinine loading reduced it by 30.9%. These values are remarkably comparable.

The summation of the loss of renal GAA synthesis plus the effect of uremic levels of creatinine should result in production of no more than one-fourth to one-third the normal amount of creatinine rather than the 60% which is observed in chronic renal failure. Since it is now established that there is an extra-renal route of creatine synthesis, and Walker has demonstrated the presence of both of the necessary enzymes in the pancreas(4,10), it is possible that under these circumstances there is an increase in pancreatic activity. It would be of interest to observe levels of transamidinase and methyl-ferase in the pancreas and liver of animals made severely and chronically uremic as compared to normals. It is obvious that the inhibition of creatine synthesis observed in the acute nephrectomy experiments and following creatinine loading cannot operate in chronic uremia, since greater depression of creatinine excretion would result than that which is observed.

Recently Walker found that feeding 3% creatinine to rats reduced kidney transamidinase activity 12%, while feeding 3% creatine reduced transamidinase activity 66-68%(11). However, creatine feeding did not depress chick liver methyl-ferase activity, although creatine (but not creatinine) depressed chick liver transamidinase activity(12). Interestingly, one day of starvation or inadequate diet depressed the latter enzyme. Fitch, Hsu and Dinning(13) also report that creatine, but not creatinine, feeding reduces the activity of rat kidney transamidinase, with the maximum effect reached 2.5 to 4.5 days after institution of the test diet. Several possible explanations can be considered for the decreased creatine synthesis noted in our study. (1) Poor dietary intake may have depressed the methyl-ferase, as it does the transamidinase. Although originally matched in weight, creatinine-loaded animals averaged about 12 g less than controls at time of sacrifice. (2) The observed level of enzyme activity may not indicate the actual rate of reaction. (3) The rapid clearance of creatinine by intact kidneys may have prevented development of high levels of serum creatinine if the rate of intestinal absorption was slow. Intraperitoneal injection of 700 mg/kg body weight of creatinine caused serum creatinine to rise to approximately 40 mg%, with a rapid fall to normal levels (1 mg%) within 8 hours. However, total creatinine intake on a diet containing 2.5 to 3.0% (11,13) should be comparable to the amount in the daily intraperitoneal injections.

It seems probable that in chronic renal failure the rate of creatine synthesis is reduced by the amount of renal GAA component. In acute renal failure, and following loading with large amounts of creatinine, there is inhibition of both GAA formation and its subsequent methylation. This apparently occurs without depression of either of the essential enzymes. In chronic renal failure this inhibition seems to be overcome. It would be interesting to determine if there is an actual increase in either enzyme necessary for this response.

Summary. Previously, it was demonstrated that nephrectomy causes a 90% reduction in creatine synthesis in rats. In the present study, nephrectomy was found to cause a 37.5% decrease in methylation of guanidinoacetic acid (GAA)-2-C^{14}. Rats loaded with creatinine by intraperitoneal injection dem-

onstrated a 30% reduction in GAA methylation and a 45% reduction in total synthesis of creatine from glycine. It is concluded that chronic renal disease causes reduced creatine (and creatinine) synthesis both by loss of the renal mechanism and by interference with methylation. It is suggested that extra-renal (pancreatic?) synthesis may be relatively increased to partially compensate for these 2 inhibitory factors.

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Separation of Plasma Thromboplastin Antecedent (PTA) and Hageman Factor (HF) from Human Plasma.* (26138)

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Plasma acquires increased coagulability after contact with glass or certain other charged surfaces such as kaolin or celite. Following the discovery of patients deficient in plasma thromboplastin antecedent (PTA) (1) or Hageman Factor (HF)(2), these plasma clotting factors were shown to be involved in development of this increased coagulability. It has been postulated(3) that the glass surface "activates" HF enabling it to react with PTA and so initiate coagulation.

Waaler(4) found that clotting time of plasma lengthens markedly when it is treated with glass powder, the powder removed, and the plasma allowed to stand for several hours at 37°C. He called the product exhausted plasma (EP) and believed it was depleted of HF and PTA.

Others have attempted to purify HF and PTA, but these factors have not been separated. This communication describes the separation of HF and PTA by ion exchange chromatography. As shown below, each separated factor corrects the defect of its corresponding deficiency plasma. However, the combined purified factors fail to shorten clotting time of EP unless another as yet unidentified component of plasma is also present.

Methods. Our assays were based upon the kaolin clotting time technic of Margolis(5). The clotting system consisted of 0.1 ml of a suspension of cephalin-kaolin, 0.1 ml of a substrate plasma, 0.1 ml of the test substance, and 0.1 ml of 30 mM $CaCl_2$. The cephalin-kaolin suspension was prepared by combining equal parts of a 1/35 (v/v) suspension of cephalin in veronal buffer(6) and a 40 mg/ml kaolin suspension in physiological saline. The first 3 reagents were incubated together for 3 minutes at 37°C before the pre-warmed calcium was added and clotting time determined. The following substrate plasmas were used: PTA deficiency plasma for assay of PTA, HF deficiency plasma for the assay HF, or EP for assay of the com-

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