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Rotational Frictional Coefficients of Macroscopic Models of T2 Bacteriophage*

Richard J. Douthart and Victor A. Bloomfield

ABSTRACT: We have measured rotational frictional coefficients of plastic scale models of T2 bacteriophage in order to elucidate the structures of the "fast" and "slow" forms of this virus. Results have been compared with the recent electric birefringence data of Maestre (Maestre, M. F. (1968), *Biopolymers 6*, 415). It is concluded that tail fibers cannot be extended in the fast form of T2, which has $s_{20,w} = 1000$ S and rotational diffusion coefficient $D^{\theta\theta} = 555$ sec^{-1}, and that the phage head may be somewhat larger than observed in electron micrographs. The slow form of T2, with $s_{20,w} = 700$ S and $D^{\theta\theta} = 111$ sec^{-1}, is probably formed from the fast form by extension of the tail fibers. Differences in head porosity are shown to have no effect on frictional properties, and kinking of tail fibers has little effect except when the fibers are close to the head. It is demonstrated that when the tail fibers are extended, the solvent within their domain is effectively immobilized with respect to the phage.

The existence of two distinct forms of T2 bacteriophage in solution is well documented. Fast- and slow-sedimenting forms, with sedimentation coefficients of 1000 and 700 S, respectively, were first observed by Hook *et al.* (1946) and Sharp *et al.* (1946). Recent electric birefringence studies by Maestre (1968) gave rotational diffusion coefficients of 555 ± 54 and 111 ± 22 sec^{-1} for the "fast" and "slow" forms, respectively.

The structural differences giving rise to these differences in hydrodynamic properties have been the subject of some debate. Bendet *et al.* (1957), Lauffer and Bendet (1962), and Maestre (1968) have attributed the greater frictional resistance of the slow form to a greater extension of the virus tail fibers. Cummings and Kozloff (1960, 1962) and Cummings (1963) have attributed the change to a large increase in head porosity, attendant upon a modest increase in head length, on going from the fast to the slow form. Calculations of translational (Bloomfield *et al.*, 1967b) and rotational (Filson and Bloomfield, 1967) frictional coefficients of T2-shaped structures, using the "shell model" theory developed by Bloomfield *et al.* (1967a), seemed to fix the blame for the difference in frictional properties on a large change in head size rather than tail fiber extension or head porosity.

To gain further insight into the structural changes associated with the observed hydrodynamic changes, we have measured rotational frictional coefficients of macroscopic, plastic scale models of the phage. This approach to a structural understanding of the hydrodynamic properties of viruses has precedents in the work of Haltner and Zimm (1959) and Broersma (1960). In this paper we report the effects of head size, head porosity, tail fiber orientation, and tail fiber kinks on the rotational diffusion coefficient.

Experimental Section

Models. Scale models of virus particles were machined from Plexiglass. The scale factor was 3.190 × 10^{-3} cm/Å. Figure 1 shows the shape and characteristic dimensions of the models. The head is a cylinder of length A and diameter B, capped at both ends by cones of height 230 Å. Two sets of head dimensions were used:

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TABLE I: Dimensions of T2 Bacteriophage Models.

Model	A (Å)	B (Å)
$S_E{}^a$	718	797
$F_E{}^a$	578	797
$S_c{}^b$	1938	1190
$F_c{}^b$	578	1190

[a] Cummings and Kozloff (1960). [b] Filson and Bloomfield (1967).

those measured electron microscopically for the slow, S_E, and fast, F_E, forms by Cummings and Kozloff (1960); and those calculated using the shell model procedure by Filson and Bloomfield (1967) to optimize agreement with measured sedimentation and rotational diffusion coefficients for the slow, S_C, and fast, F_C, forms. The values of A and B for these four models are tabulated in Table I. Electron microscopy indicates that the head is, in fact, a polyhedron with a hexagonal profile, perhaps a bipyramidal hexagonal prism (Kellenberger *et al.*, 1965; Moody, 1965; Bradley, 1967). Shell model calculations (Filson and Bloomfield, 1967) indicate that this type of structure should have a slightly higher rotational diffusion coefficient than that used in the models, because of its smaller volume, but the effect is not large enough to justify the more complicated machining that would be necessary to build hexagonal heads. The heads of the the model were hollow and filled with oil, to minimize buoyancy effects.

The tail, as shown in Figure 1, is a right circular cylinder of length 1000 Å and diameter 150 Å in all models. The tail, including the base plate with its spikes which has been omitted from these models for simplicity, may be as long as 1200 Å (Bradley, 1967), although the length used is within the range reported in the literature (Brenner *et al.*, 1959). It is not anticipated that these length differences could cause a variation of more than a couple of per cent in the hydrodynamic properties. Likewise, the tail diameter may be somewhat larger than the 150 Å used here, particularly if the tail fibers were retracted around it. However, shell model calculations (Filson, 1967) indicate that the tail diameter has very little effect on the frictional properties of these particles.

The models were made with snap-on tail caps with and without tail fibers attached. Copper wire of 0.025-in. diameter was used for the fibers; this corresponds to a fiber thickness of 20 Å. The fiber length was 1300 Å. In the experiments reported below, the angle, α, which the tail fibers make with the long axis of the phage was varied; and in some cases kinks were introduced in the middle of the fibers to correspond with electron microscopic observations. The relevant angles are defined in Figure 1. Although only two fibers are shown in this figure, six were actually attached; and they were either all straight or all kinked and attached at the same α.

Series of threaded holes were placed along the heads and tails of the models to accommodate the rod which suspended them in the oil bath. The center of rotational frictional resistance was located by making measurements at each hole and locating the point of minimum resistance. This minimum was usually rather broad, extending over three or four holes. All of the rotational diffusion coefficients reported here are those obtained at these minima.

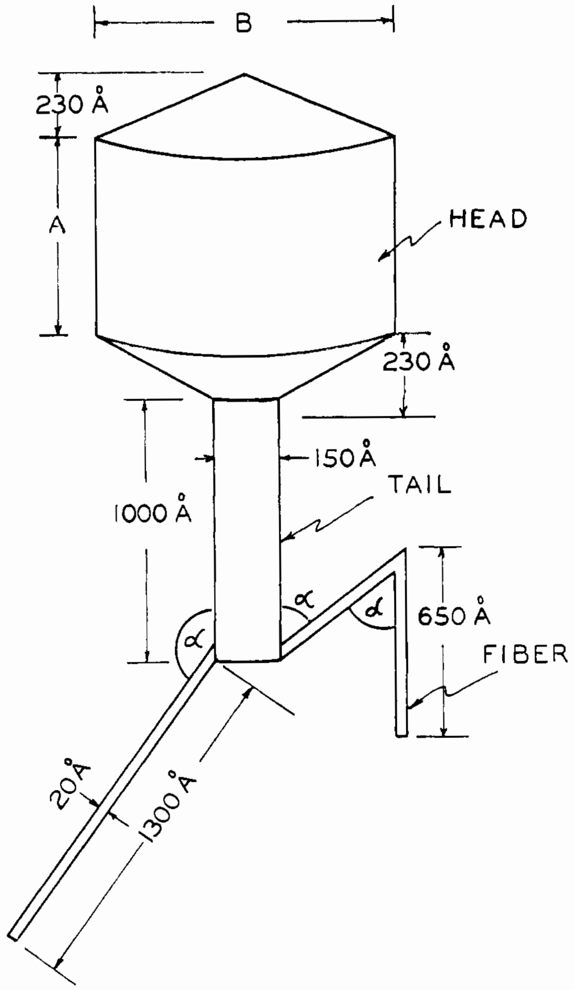

FIGURE 1: Dimensions of T2 phage models. Models were constructed to this plan with a scale factor of 3.190 × 10^{-3} cm/Å. Dimensions A and B for various models are given in Table I.

Photographs of the assembled models are shown in Figure 2.

Apparatus. The virus models were suspended from a rod of 0.050-in. diameter in a cylindrical oil bath 16 in. in diameter and 12 in. high. No variation in frictional behavior at any attachment hole was noted when the suspension rod diameter was varied between 0.028 and 0.065 in., although there was a small effect with a rod of 0.375-in. diameter when attachment was on the tail close to the head. The oil bath was filled to a depth of 9 in. with Cities Service SAE 230 "Trojan Gear Oil," which had a viscosity of 30.0 P at 25°. The models were suspended about 4-5 in. below the surface of the oil, but a variation in suspension height of ±2 in. had no measurable effect on the frictional behavior.

The oil bath was mounted on a turntable which was

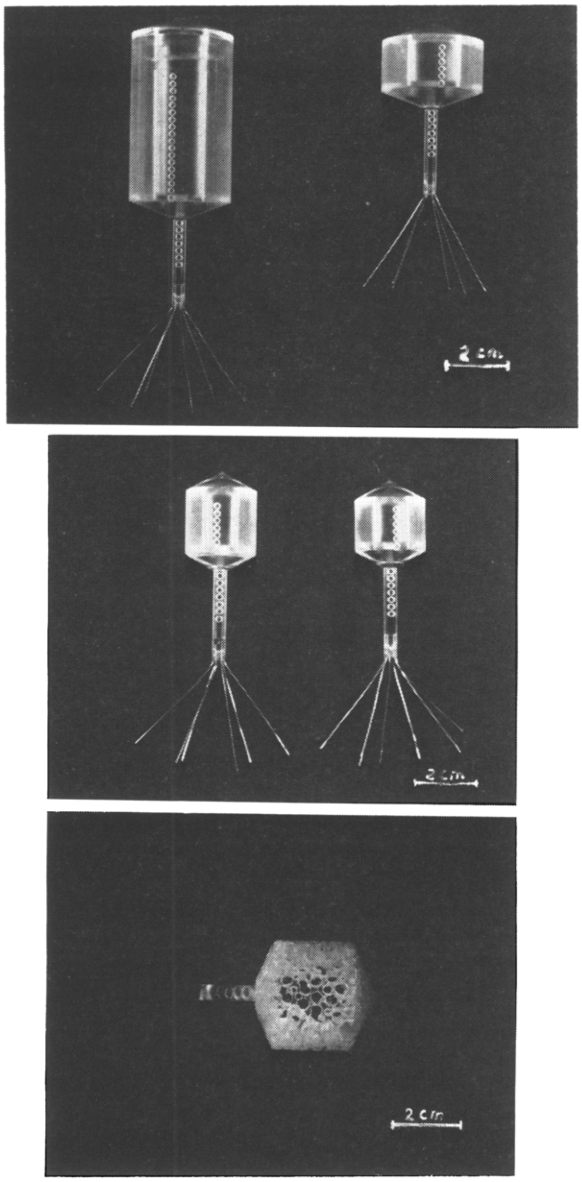

FIGURE 2: Photographs of T2 models. Upper left: models S_E and F_E; upper right: models S_C and F_C. Molecular dimensions are given in Figure 1 and Table I. Below, a phage model which has had more than 50% of its surface area removed.

fitted to a worm-gear reducer and driven by a small electric sewing machine motor. A constant angular velocity was maintained by a photoelectric feedback device fitted to the motor shaft. A wide range of turntable speed from about 10 to 400 rev per sec, with maximum variations of ± 0.2 rev/sec, was possible with this device.

The suspension rod which was connected to a 0.25-in. shaft running through an air bearing was in turn suspended from a 2-in. length of 0.010-in. diameter no. 1 spring steel music wire which was used as a torsion wire. A pointer mounted at right angles to the shaft measured displacements from the equilibrium position when the oil bath was stationary. Displacements were reproducible within 2%.

These measurements are very sensitive to temperature variations, because of the effect of temperature on the viscosity of the oil. Temperature readings were taken before and after each measurement, and runs during which a temperature fluctuation of more than 0.1° had occurred were disregarded. All data were adjusted to 25° by measuring the displacement produced by a standard sphere of radius 1.89 cm at rotation frequency of 0.02 rev/sec, over the range 24–26°. Adjustments necessary were 5% at most.

Calibration. The rotational frictional coefficient, ζ, of a sphere of radius, R, immersed in a medium of viscosity, η, is

$$\zeta = 8\pi\eta R^3 \tag{1}$$

The torque, τ, exerted on the sphere when the oil bath is rotating with angular velocity, ω, is

$$\tau = \zeta\omega \tag{2}$$

At equilibrium, this torque is balanced by that exerted by the torsion wire. The resulting displacement, D, of the pointer, which is assumed linearly proportional to τ with proportionality constant, γ, is

$$D = \gamma\tau = 8\pi\eta R^3\omega + f \tag{3}$$

f is a factor which represents the frictional resistance of the suspension rod and of the air bearing to which the rod is connected.

A series of calibration spheres ranging from 0.32 to 5.08 cm radius were run at 25° at rotational frequencies ranging from 0.100 to 0.004 sec^{-1}. It was found that the plots of D *vs.* ω for each sphere were accurately linear and passed through the origin. Thus f in eq 3 is zero, indicating that the effects of the suspension rod and air bearing are negligible. Likewise, plots of D *vs.* R^3 at constant ω were accurately linear, indicating that the torsion wire was responding linearly and that wall effects in the oil bath were unimportant.

Results

Rotational diffusion coefficients, $D^{\theta\theta}$, for the various T2 phage models were obtained as follows. The center of frictional resistance (hole giving minimum displacement of the pointer) was located and the displacement was measured at a particular ω. Interpolation on a standard graph of D *vs.* R^3 enabled the assignment of an effective hydrodynamic radius, R_e, for the model. This was adjusted to molecular dimensions by the scale factor of 3.190×10^{-3} cm/Å mentioned above. R_e is related to ζ by eq 1, and ζ to $D^{\theta\theta}$ by the well-known relation

$$D^{\theta\theta} = kT/\zeta \tag{4}$$

The numerical relation between $D^{\theta\theta}$, which is the quantity measured by Maestre (1968) and calculated by Filson and Bloomfield (1967), and R_e for the macroscopic model (measured in centimeters) is, in these experiments, $D^{\theta\theta}_{20,\mathrm{w}} = 5225/R_e^3$.

Results of these measurements are given in Table II.

TABLE II: Rotational Diffusion Coefficient, $D^{\theta\theta}_{20,w}$ (in sec^{-1}) for T2 Virus Models.[a]

Model:	F_E		S_E		F_C		S_C	
	Exptl	Calcd[b]	Exptl	Calcd[b]	Exptl	Calcd[b]	Exptl	Calcd[b]
No tail fibers	626	698	556	552	468	504	131	138
Straight fibers								
Angle α								
$\pi/4$		579	350	496				
$\pi/2$		476	187	412				
$3\pi/4$	143	392	130	345	123		50.8	115
Kinked fibers								
Angle α								
$\pi/4$	277		243					
$\pi/2$	185		176					
$3\pi/4$	151		139					

[a] Experimental values from electric birefringence (Maestre, 1968) are 555 ± 55 sec^{-1} for fast, F, form and 111 ± 22 sec^{-1} for the slow, S, form. [b] From Filson (1967) and Filson and Bloomfield (1967).

Comparison is made with the shell model calculations (Filson and Bloomfield, 1967; Filson, 1967) where possible.

It is immediately evident that, in the absence of tail fibers, the shell model calculations are in good agreement (within 10%) with the model experiments. Furthermore, if only a head size change occurred in the fast–slow transition, it would have to be of the magnitude indicated by the difference in size between models F_C and S_C.

Moreover, it was possible to show that changes in head porosity cannot be responsible for the large change in frictional properties. More than 50% of the surface area of a hollow phage model was removed by drilling holes in it. A photograph of this mutilated model is shown in Figure 2. There was no measurable difference in frictional resistance of the model before and after ventilation. This result is consistent with experiments on cylinders (Broersma, 1960) and with shell model calculations for translational (Bloomfield *et al.*, 1967a) and rotational (Filson and Bloomfield, 1967) motion.

However, it is clear from Table II that the shell model calculations substantially underestimate the effect of tail fibers in lowering $D^{\theta\theta}_{20,w}$. This has the consequence, on the one hand, that it appears impossible to obtain sufficiently high values of $D^{\theta\theta}$ for the fast form if the fibers project at all; and, on the other hand, that the $D^{\theta\theta}$ measured for the slow form can be achieved by tail fiber extension and only moderate changes in head size.

Table II also indicates that kinking of tail fibers does not greatly affect the frictional behavior of the phage, save for small α when the fibers are drawn up near the head.

In order to investigate further the nature of solvent flow in the cone outlined by the tail fibers, we attached a thin rod, capped by a ball of diameter 0.6 cm, coaxially to the end of the tail of model F_C. The rotational properties of this assembly were studied in the absence of tail fibers, and with fibers attached at an angle α of $3\pi/4$. The results are given in Table III. The length in molecular units of the height of the cone defined by the tail fibers is 919 Å. Even when the added rod and ball extend to 896 Å (2.86 cm), there is no appreciable change of frictional coefficient in the presence of tail fibers. This is striking evidence for the immobilization of solvent inside the tail fiber region, and thus of the lack of free draining.

Discussion

The results reported above have important implications for the structures of the fast and slow forms of T2 phage in solution and for the structural change sattendant on the transition between them. It appears that, contrary to the electron microscope results of Cummings and Kozloff (1960), but in accord with the results of Bendet *et al.* (1957) and Lauffer and Bendet (1962), tail fibers must not be extended in the "fast" form of the virus. In all the cases listed in Table I, the presence of tail fibers makes $D^{\theta\theta}$ much too low to be consistent with the electric birefringence result of 555 sec^{-1} obtained by Maestre (1968). On the other hand, if tail fibers were not

TABLE III: Effect of Thin Rod and Ball (0.6-cm Diameter) Extending from Tail into the Tail Fiber Region.

Distance from Tip of Tail to End of Ball (cm)	$D^{\theta\theta}_{20,w}$ (sec^{-1})	
	No Fibers	Straight Fibers at $\alpha = 3\pi/4$
	468	123
0.60	374	122
0.73	360	122
1.62	311	122
2.86	236	120

present at all, or were very compactly wrapped about the tail, $D^{\theta\theta}$ as measured for the model F_C which is constructed using the electron microscopically determined head dimensions (Cummings and Kozloff, 1960) would be substantially too large (626 sec^{-1}). The real discrepancy is even larger, since the head with hexagonal cross section has a D^{θ} 12% larger than the cylindrical head models used here (Filson and Bloomfield, 1967). Whether the tail fibers project slightly out into solution away from the body of the phage, or whether the actual head dimensions in solution are more like those of models S_E or F_C than F_E, must remain a moot point at present.

If there were no tail fibers, the greatly expanded head of model S_C would provide a good account of the frictional properties of "slow" T2. However, all workers have agreed that extended tail fibers are seen in electron micrographs of this form of the virus. Therefore, models such as S_E or F_C with tail fibers extended at $\alpha = 3\pi/4$ or greater must be regarded as giving best agreement with all the evidence.

However, electron micrographs rarely show all six fibers extended, and extension is usually not symmetrical. Some experiments were performed, which need not be described here in detail, in which $D^{\theta\theta}$ for the S_C model with less than six fibers extended were measured. With every other fiber removed, and the remaining three kinked up rather close to the head ($\alpha = \pi/4$), we obtained a value for $D^{\theta\theta}$ of 90 sec^{-1}, barely within the lower limit of the Maestre (1968) value of 111 $\pm$ 22 sec^{-1}. It is clear that with a head slightly smaller than that of S_C, and judicious choice of tail fiber disposition, configurations could be found that would attribute most of the change in the fast–slow transition to head expansion. However, there seems no justification for such arbitrary variation of parameters. If the tail fibers were rather flexible, their contribution to the frictional resistance of the phage would be less, thus increasing the importance of head size changes; but tail fibers appear in electron micrographs to be quite rigid save at the kink.

It therefore appears probable that the structural change occurring in the transition between the fast and slow forms of T2 in an extension of tail fibers, as proposed by Bendet *et al.* (1957) and Lauffer and Bendet (1962), from a configuration in which they are very closely wrapped around the phage to one in which they trail out far behind the virus. There may, in addition, be a modest change in head size as observed in electron micrographs by Cummings and Kozloff (1960). There is no convincing evidence for massive changes in head size, and significant effects due to head porosity appear to be ruled out.

The work reported here would seem to bear on the interpretation of experiments on phage T2H in the presence of indole (Kanner and Kozloff, 1964) and on phages T4B and T4B$_{0_1}$ in the presence of L-tryptophan (Cummings, 1964). In the former case it was found that indole fixed the phage in an "inactive state," in which the sedimentation coefficient $s_{20,w}$ is approximately 1025 S; while the "active state" has $s_{20,w} \simeq 900$ S. In the latter case $s_{20,w}$ is 1000 S in the absence, and 900 S in the presence, of L-tryptophan for both phages. In both cases it was assumed that the faster sedimenting form had fibers retracted, and the lower sedimenting form had fibers extended. However, it appears from the present study that the form with fibers extended far back from the head probably corresponds to the "slow," 700S form of Hook *et al.* (1946) and Sharp *et al.* (1946). This dilemma may speculatively be resolved by the proposal of Kellenberger *et al.* (1965) that in T4 there is a dynamic equilibrium between an extended and nonextended state of the fibers. If the establishment of this equilibrium is rapid relative to the sedimentation rate, then a single species sedimenting with an average sedimentation coefficient about midway between 700 and 1000 S would be seen. This is consistent with the observations.

The "shell model" method of calculating hydrodynamic properties of subunit structures (Bloomfield *et al.*, 1967a,b; Filson and Bloomfield, 1967) has proved to be fairly accurate for T2 phage models not possessing tail fibers. However, for models with tail fibers it has given results seriously in error. More specifically, it seriously underestimates the frictional resistance due to the fibers, although it predicts quite accurately the lack of influence of head porosity on hydrodynamic properties (Bloomfield *et al.*, 1967a; Filson and Bloomfield, 1967), which presents a similar physical situation. In both cases, it appears that the frictional behavior of an assembly of subunits will be close to that of the volume outlined by the subunits, although the density of occupation of that volume by subunits may be quite small. This conclusion is strikingly illustrated by the results reported in Table III. The reason for the failure of the shell model method in this case, given its considerable success in other applications, is not clear. Several other paradoxes resulting from the use of the basic theory which underlies the shell model method have been noted recently by Zwanzig and coworkers (Zwanzig, 1966; Zwanzig *et al.*, 1968). Until the theoretical situation is clarified, it seems appropriate to view the results of such calculations with caution when the structure under consideration deviates markedly from spherical symmetry.

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Kidney Antihemophilic Factor. Partial Purification and Some Properties*

Emily M. Barrow and John B. Graham

ABSTRACT: Antihemophilic factor activity has been isolated from leucine aminopeptidase preparations of porcine, human, and canine kidneys. The antihemophilic factor activity can be separated from the leucine aminopeptidase activity by Sephadex G-200 gel filtration. Removal of contaminating proteins by this single step has resulted in a large increase in antihemophilic factor activity, the specific activity increasing more than 800 times. No other clotting factors have been detected in the purified preparations of kidney antihemophilic factor. $MgCl_2$ is required for preservation of the antihemophilic factor activity during purification, and maximum activation of antihemophilic factor before assay requires incubation at 40° in the presence of $MnCl_2$. The molecular weight of kidney antihemophilic factor, estimated by gel filtration methods, is consistent with that of proteins with molecular weights between 25,000 and 28,000. This is in contrast to plasma antihemophilic factor which is excluded from G-200 with the proteins of molecular weights exceeding 200,000. Kidney antihemophilic factor is greatly retarded by DEAE-Sephadex, is eluted under starting conditions from CM-Sephadex, and moves as a prealbumin on polyacrylamide and agar gels. A specific neutralizing antibody against kidney antihemophilic factor has been prepared by injecting the purified kidney antihemophilic factor into rabbits. The antibody which neutralizes kidney antihemophilic factor activity does not influence the hydrolysis of L-leucinamide by crude kidney preparations.

The differences in molecular weight of plasma antihemophilic factor and kidney antihemophilic factor suggest that kidney antihemophilic factor may be the monomeric form or one of the subunits of plasma antihemophilic factor.

We reported earlier that the antihemophilic factor activity of normal plasma could be protected from proteolytic destruction by trypsin and thrombin if the plasma were pretreated with $MnCl_2$, and that this antihemophilic factor was not different from the antihemophilic factor of untreated plasma by several criteria (Barrow *et al.*, 1966a,b). The unsuspected relationship between Mn^{2+} and antihemophilic factor suggested that antihemophilic factor might be a Mn^{2+}-dependent enzyme which would be expected to be present in mammalian tissues and susceptible of purification. In searching for a tissue enzyme which requires Mn^{2+} for stabilization or activation and which might be identical with antihemophilic factor, we have examined brain, kidney, and red cells of man, dog, and hog.

Antihemophilic factor activity has been found in leucine aminopeptidase preparations of kidney of all three species. Most of our experiments have been performed

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