they never grow fat like their close relatives, moose (*Alces*) or caribou (*Rangifer*); they rub off their densely furred velvet in April and rut during July and August[3].

Even if Stonehouse's assumptions are valid, his hypothesis fails to meet the following requirements.

(1) Cervids of similar body size of related lineages must have larger antlers in warm than in cold climates. If old world deer from the Mediterranean region and southern Asia are compared with those from northern Europe and Asia, by pairing off species and races of similar body size, it will be seen that all the warm climate deer of the plesiometecarpalian lineage listed in Table 1 except *Dama dama* carry antlers distinctly smaller than their cold climate counterparts. All large antlered deer come from the temperate or even arctic climates; that is, *C. elaphus, C. canadensis, Alces, Rangifer*; no deer with comparable antler size exist in hot climates.

Table 1

Warm climates	Cold climates
Cervus unicolor	*C. canadensis*
C. duvaucceli, eldi, equinus	*C. elaphus*
Axis axis	*C. nippon dybowski*
Dama dama	*C. n. dybowski*
A. porcinus	*C. n. nippon*

(2) Related deer of similar body size must have similar antler size in similar climates. The large West Siberian moose carry much smaller and less palmate antlers than their East Siberian and American counterparts. Manchurian moose carry small palmless antlers, and yet live in close proximity to the East Siberian moose which carries huge, palmed antlers. The Manchurian moose is somewhat smaller than other races, but the weights of American and West Siberian moose coincide closely[4].

(3) Cervids from warm epochs should have larger antlers than deer from cold epochs. The fossil record does not confirm this. It does show that large antlers as well as large bovid and rhinoceros horns and gigantic tusks among proboscidians are a Pleistocene phenomenon. The evolution of large horns and tusks is almost explosive in the Villafranchian and continues to be so during the ice ages. The cervids with the largest antlers (*Megaloceros, Alces, Cervalces, Rangifer, Orthogonoceros, Eucladoceros, Euctenoceros, Cervus elaphus, Parameguceros*) are all of Pleistocene age and nothing which compares with them is found in the warmer Pliocene and Miocene epochs. The same is true for bovid horns, giraffe horns, rhinoceros horns and elephant and mastodon tusks, the sole exception being the paenungulate *Arsinoitherium* from the Oligocene. The largest horns or tusks are carried by Recent and Pleistocene species. Moreover, species with giant horns, antlers or tusks are usually, but not always, confined to the periglacial areas, such exceptions being *Syncerus*, the African buffaloes, or *Bubalus*, the water buffalo. The relationship between cold climate and large horn-like structures is not causal; a theory explaining the rapid evolution of giant horn-like organs during the Pleistocene is being prepared for publication.

It is thus evident that antlers have had a parallel evolutionary history to other horn-like organs, even those for which no thermoregulatory functions are likely to be advanced; that is, rhinoceros horns and elephant tusks. This is not surprising, for behaviour studies on ungulates indicate that horns, antlers and tusks are used similarly and are analogous organs[5].

Stonehouse makes a reference to caribou (*Rangifer*) when implying that the antlered females of a high Arctic deer need growing antlers to lose excessive heat during the spring "feeding flush", because they are insulated by thick fur and fat. This suggestion is ludicrous. Female caribou in May and June have just emerged from winter, they are carrying calves or are lactating, they are thin and almost devoid of fat while their hair is shedding and they are losing their insulation. To anyone familiar with northern Canada it must be evident that the ability to regulate blood flow in growing antlers is adaptive. In the months mentioned, snow is commonly found on the ground, the lakes are still frozen or just breaking up, while snowstorms, frost and icy rain are common occurrences. Like the female caribou, the stags, as well as cow and bull moose and wapiti (*Cervus*), have lost their last fat deposits, while the old fur is dropping out. In these circumstances all heat must be conserved. As in roe deer, antlers seem to grow not because of the climate but in spite of it.

The hypothesis that antlers evolved as thermoregulatory organs does not meet the necessary requirements and fails to predict correctly. Behaviour studies of ruminants have shown that horn-like organs can function as weapons, as display organs or rank symbols[6], as shields to catch and neutralize attacks of conspecifics[5] and as determinants of dominance rank[7], and as locks to hold the heads of opponents together during pushing and wrestling[8]. Hence they evolved as social adaptations and are of great importance to the reproductive success of the individual[9].

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18?

Using the Rat as a Cholera "Model"

AFTER the discovery of the *Vibrio cholerae* by Koch in 1883, many investigators attempted to develop animal models in a wide variety of species[1]. Results were erratic and it was not until 1953 that De and Chatterje[2] developed the ileal loop in adult rabbits as a model which gave consistent results. Shortly afterwards, Dutta and Habbu[3] developed the "suckling bunny" model which also gave consistent results. Since then, these two models have been widely used to study the effects of vibrios and their products on fluid accumulation into the gut lumen. Recently, Carpenter and his colleagues[4] developed an animal model using dogs with chronic Thiry-Vella loops in the jejunum and ileum. We report now that the rat can also be used as a cholera model.

The fluid accumulation factor (FAF) in *Vibrio cholerae* culture filtrates rapidly loses its effectiveness even if the filtrates are stored at 4° C. Van Heyningen has suggested (personal communication) that stabilization might be achieved by the addition of glycerine to the filtrate in a ratio of 2 : 1. Twelve hour culture filtrates of *Vibrio cholerae* Inaba 569B grown in 2 per cent peptone and 1 per cent NaCl were centrifuged, pooled and sterilized on 'Millipore' filters. This crude filtrate was concentrated 100-fold by ultrafiltration through dialysing tubes. Glycerine was added to this concentrated product in a 2 : 1 ratio. This preparation has been stored in the refrigerator at 4° C for 4 months without measurable loss of FAF potency. On the day of an experiment one volume of the concentrated, glycerinated filtrate was diluted 100-fold with a gel borate buffer (*p*H 7·5) before injection into the rat or rabbit loop.

The initial results of rat loop experiments were erratic until it was noted that both succus entericus and faeces would nullify FAF activity. Finally, the following procedure was developed. Forty day old rats were given water and Nabisco biscuits for 72 h. During the next 72 h they were given freely a 5 per cent solution of glucose in water. Laparotomy was then performed under ether anaesthesia and the lumen of the small intestine was washed thoroughly with 20 ml. of normal saline. Two loops measuring approximately 10 cm were then prepared, starting about 2 cm above the caecum, with a 1–2 cm interloop between the study loops. The diluted glycerinated filtrate or the diluent was then injected into one end of the loop in a volume of 0·5 ml. and the injected site was ligatured. The intestine was replaced in the peritoneal cavity, the wound was closed with sutures and the animal was allowed to recover from the anaesthetic. Water was supplied freely during the postoperative period. After 8 h the animal was killed by excessive ether anaesthesia. The peritoneal cavity was opened, the loops were examined, and the fluid accumulated in the loops and the loop lengths were measured. Table 1 shows that of ninety-six loops in forty-eight rats only one loop recorded a false positive and there were no false negatives. An average of 3 ml. of fluid accumulated in an average loop 12·8 cm long which had received the equivalent of 0·05 ml. of the original unconcentrated filtrate.

Table 1. RAT ILEAL LOOP MODEL FOR THE DETECTION OF FAF IN CHOLERA CULTURE FILTRATE

Rat No.	Buffer* Average loop length (cm)	Buffer* Average fluid volume (ml.)	Cholera culture filtrate† Average loop length (cm)	Cholera culture filtrate† Average fluid volume (ml.)
1–8	9·8	0	11·8	2·8
9–16	10·0	0·1	11·5	2·5
17–24	10·4	0	13·7	3·1
25–32	9·6	0	14·0	3·0
33–40	10·5	0	11·6	2·8
41–48	10·5	0	14·2	3·0
Average of 48	10·1	0	12·8	3·0

* The quantity used was 0·5 ml.
† The quantities used were 0·05 ml. of culture filtrate diluted to 0·5 ml. with buffer (see text).

Table 2. FAF INHIBITION BY RAT INTESTINAL WASH FLUID (HEATED AND UNHEATED)

Rabbit No.	Cholera culture filtrate	Filtrate and RIW	Filtrate and heated* RIW	Control
1	1·37†	0	0	0
2	1·00	0·20	0·14	0·1
3	0·68	0	0	0
4	0·54	0	0	0

* Subjected to 56° C for 30 min.
† Fluid accumulated in ml. divided by ileal loop length².
Cholera culture filtrate (0·02 ml.) was in 2 ml. of buffer in all cases, except the control which used 2 ml. of buffer.

Table 3. FAF INHIBITION BY CENTRIFUGED AND 'MILLIPORE' STERILIZED RAT INTESTINAL WASH FLUID

Rabbit No.	Filtrate	Filtrate and RIWM†	Filtrate and RIWM† frozen and thawed	Control
1	0·00*	0	0	0
2	1·3	0	0	0
3	1·0	0	0	0
4	1·08	0	0	0

Filtrate (0·02 ml.) was used in 2 ml. of buffer in all cases, except the control which used 2 ml. of buffer.
* Compare Table 2.
† Rat intestinal wash passed through a 'Millipore' filter, gauge 220 mμ.

It was also established that rat faeces or saline washings of the small bowel of the rat (RIW) would render filtrates containing FAF inactive. Studies were made in rabbit ileal loops using the technique of Burrows and Musteikis[5] except that the loops were collected after 8 instead of 18 h. A sample (1 ml.) of a suitable dilution of the conventional glycerinated filtrate in buffer was mixed with 1 ml. of buffer, or 1 ml. of unheated RIW, or 1 ml. of RIW which had been heated for 30 min at 56° C. Table 2 shows that both heated and unheated RIW are effective in nullifying FAF activity. Table 3 shows that inhibitory activity is retained by RIW after 'Millipore' filtration, freezing and thawing.

We have shown that a rat, after suitable pre-treatment, can be used as a model for the study of FAF in cholera culture filtrates. We are now investigating the nature of the inhibitory material in the intestine of the rat.

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Structure of the Reaggregated Protein Shells of Two Spherical Viruses

After the reconstitution of the rod shaped tobacco mosaic virus (TMV) from its protein and RNA components[1], several spherical viruses have now been reconstituted[2–7]. In the case of TMV, the protein component alone can, in suitable conditions, be repolymerized into a form in which the arrangement of subunits is closely similar to that in the original virus[8,9]. The reaggregation of the protein of certain spherical viruses into shells similar in size to the original virus particles has been reported[10,11], they have not been shown to have the same arrangement of subunits. Naturally occurring empty protein shells ("top component") devoid of RNA are found associated with virus particles in many different systems, and artificial top components can be made in some cases, for example from turnip yellow mosaic virus[12], by removal of RNA from the virus particles. Both natural and artificial top components of turnip yellow mosaic virus are structurally very similar to the intact virus particles[13,14]. In this communication we show that the structures of shells formed by the reaggregation of the protein subunits of two spherical viruses are identical or very similar to those of the original RNA-containing virus particles.

For both viruses considered here, the protein subunits lie in quasi-equivalent environments, in one of the icosahedral surface lattices derived by Caspar and Klug[15] in their theory for the formation of spherical shells from large numbers of identical subunits. On the basis of this theory Caspar and Klug suggested that the formation of such a shell would be possible without the need for another directing component, just as for simple structures built of exactly equivalent subunits, provided that this aggregate represents a state of minimum energy for the subunits alone. This seems to be so in the work presented here.

Low molecular weight protein isolated from the small isometric plant viruses, cowpea chlorotic mottle (CCMV) and brome mosaic (BMV), reaggregates in certain conditions to form spherical shells similar in size to the respective original virus particles[11], and called "pseudo-top component". While the surface charges of BMV and its top component are about the same, there is a marked difference between those of CCMV and its top component. Such a difference could be accounted for by a slight change in the packing of the subunits in the two shells or by the exposure of different groups by the absence