investigated the nature of the binding compound by using trypsin (lyophilized Trypsin 2 × crystallized, Worthington Biochemical Corporation, New Jersey).

RAS was treated with trypsin, at trypsin : RAS ratios of 1 : 1 to 1 : 50 at 37° C in a gyratory shaker for periods of 2, 6 or 12 h. Soyabean trypsin inhibitor (grade 1, Seravac Laboratories) was then added at twice the trypsin concentration (because of its relative impurity), and incubation continued for another hour. Finally, 5×10^6 retina cells were added after adjusting with Tyrode to 3 ml. and they were incubated for a further 24 h. The results show that trypsin treatment of 2 h did not greatly affect the activity, but the 6 and 12 h trypsin treatment eliminated the aggregating ability of the RAS. These results will be published elsewhere in detail. Control experiments indicated that neither trypsin nor soyabean trypsin inhibitor alone caused any cell reaggregation.

Recent examination of embryonic chick cell re-aggregates by electron microscopy after lanthanum staining[6] revealed intercellular material composed of fibrils. The results of our experiments suggest that protein is responsible for the adhesion of retina cells during the process of reaggregation and we suggest that reaggregation is caused by protein molecules in the intercellular spaces. In view of the extractability of the RAS with EDTA it is further suggested that the cell plasmalemmas are bridged to RAS by divalent cations.

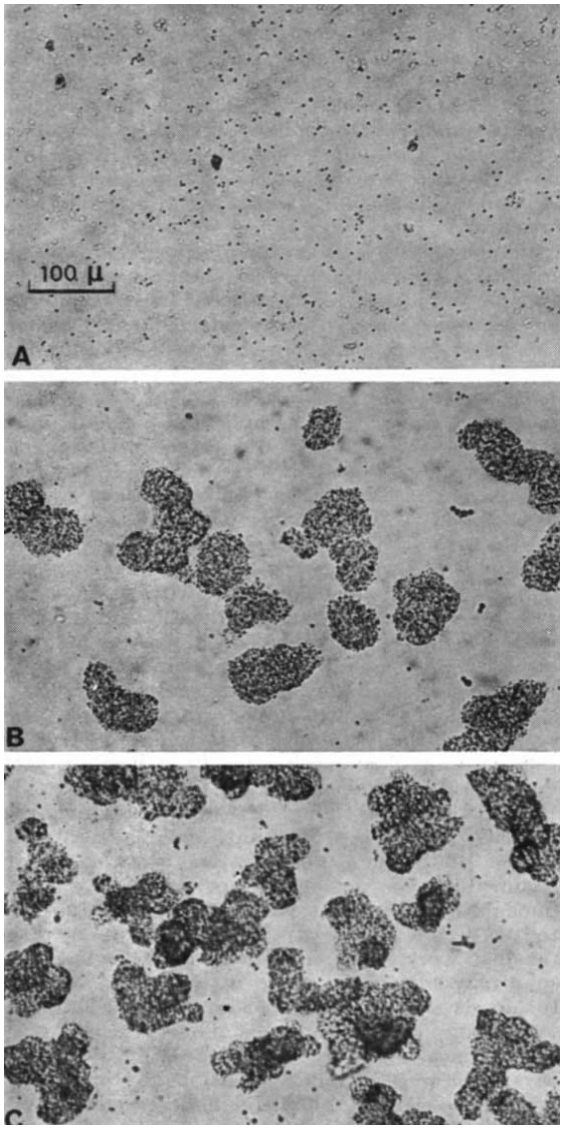

Fig. 1. The reaggregation of retina cells in: *A*, Tyrode solution; *B*, Eagle's basal medium plus 15 per cent serum; *C*, retina active substance in Tyrode salt solution (× 50).

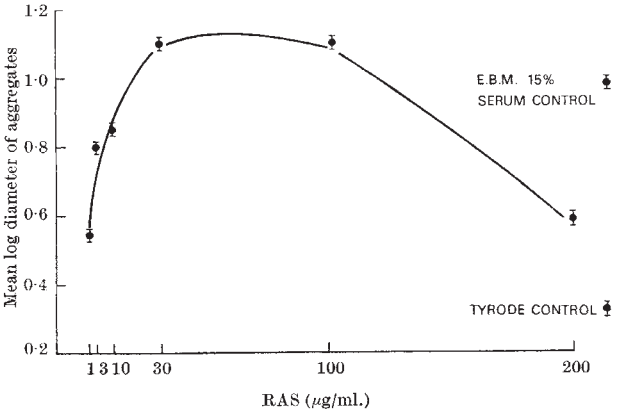

Fig. 2. Relationship between the concentration (µg/ml.) of retina active substance (RAS) and the mean log diameters of aggregates (bars denote mean and standard error).

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Evidence against Transmission of Scrapie by Animal House Fomites

Accidental contamination is an abiding anxiety for all who work with scrapie agent and the possibility of false positive results is well recognized[1]. Few, however, have sought to evaluate this risk experimentally. In the light of the report by Morris *et al.*[2], we have examined the role of close physical contact and animal house fomites (sawdust, droppings, scrapers, and so on) in the establishment of the disease in normal animals.

Swiss mice—originally derived from the Moredun stock—were kept in totally separate "normal" and scrapie animal houses. All experiments were carried out in the latter and mouse adapted scrapie agent[3] was used. Animals were examined carefully twice a week, and reported on daily by animal house attendants. All findings were checked histologically (by Cajal staining of astrocytes) and by passage into further Swiss mice.

In contact experiments, normal mice were kept for more than two years in an animal house normally reserved for scrapie infected animals. They usually became

old and ruffled. Ordinary scrapers were used in cleaning their cages: no particular regime was instituted, but scrapers were dipped in 'Tego' disinfectant in the usual way between cages. In a second experiment, lasting 22 months, groups of six Swiss mice were inoculated intracerebrally with 0·05 ml. of a 10 per cent suspension of scrapie brain ("10^{-1}") and put either immediately or after 14 days into groups of twenty-four C57 black mice. The Swiss mice were removed as soon as their condition was severe, care being taken to prevent them dying in the cage.

In breeding-contact experiments, animals were bred from one or two scrapie infected parents. Usually two litters, occasionally three, were obtained. Sometimes they were suckled by the original mother (if normal), but most were fostered to normal animals. After being weaned, litters were kept in the scrapie animal house for 20 months. Other litters, from normal animals, were reared from birth in the scrapie animal house.

In experiments with cage refuse the faeces and contaminated sawdust from cages of mice which had received intracerebral injections with scrapie 4 weeks previously, were transferred into the cages of normal recipient mice twice a week. This was continued until the scrapie animals were far advanced but discontinued if an animal was found dead or had been cannibalized in the scrapie cage. The experiment (with successive batches of scrapie mice) has proceeded for 20 months.

Other experiments concerned inadequately autoclaved apparatus. First, a McCartney bottle in which a whole scrapie mouse brain had been stored in about 15 ml. of 10 per cent formalin was emptied and, without rinsing, a normal brain was put into it together with 15 ml. fresh formalin. After 4 weeks this normal brain was homogenized and inoculated into mice (two separate experiments carried out). Second, an MSE macerator used for making a suspension of scrapie brain was autoclaved at 1·4 atm for 30 min once (usually this is done twice). A suspension of normal mouse brain was then prepared and inoculated intracerebrally into normal mice. Third, a suspension of 10^{-1} scrapie mouse brain was autoclaved for 30 min at 0·5 atm and then inoculated intracerebrally into normal mice. Similar experiments were carried out after autoclaving at 1·2 and 2·0 atm. In all cases titrations to 10^{-6} were carried out. The original material was similarly titred.

At no time did any mouse develop scrapie through contact with infected animals. This is true of animals which shared a cage with the latter as well as of those in separate cages within the scrapie animal house. Progeny of scrapie parents were never observed to develop the disease during 22 months, with the exception of one animal from a litter of six which was bled from the tail a few weeks after birth to test for lactate dehydrogenase level in the blood.

Animals encountering soiled litter from scrapie animals took on an aged appearance—ruffled hair, slightly hunched, slow movements, rather easily pushed over—much sooner than normal. After about a year they looked twice their age. No scrapie was detectable in any animal, however, though the astrocytes were hypertrophied (especially in the vicinity of small blood vessels) in the manner common to old animals.

The use of a contaminated bottle did not result in scrapie in the recipient mice during 2 years of observation; nor did the use of an imperfectly autoclaved macerator result in the disease. When scrapie brain suspension was autoclaved at 0·5 atm for 30 min, five of six animals injected with 10^{-1} developed scrapie, the incubation periods ranging from 8–12 months. The one remaining animal is normal. At 10^{-2}, one of six animals developed the disease after 11 months. After autoclaving at 1·2 atm, inoculation at 10^{-1} produced scrapie in one of four animals after 11 months. No scrapie developed after 2·0 atm autoclaving. Control material (not autoclaved) contained scrapie down to 10^{-6}.

In a second experiment, scrapie material (one-tenth suspension) was autoclaved for 1·3 atm at 30 min. It produced scrapie in one out of four mice after 13 months' incubation. The others are well after two years. Passage of the brain of the positive mouse produced full blown disease in 6 months. When the scrapie material was autoclaved a second time in the same conditions, it produced scrapie in four of five mice, 13, 14, 15 and 18 months after intracerebral inoculation. Again further passage of these animals resulted in the disease in 6 months. Experiments are in progress with the same material subjected to a third autoclaving process.

It is a common fear that scrapie may contaminate normal mice or experimental mice when attempts are made to establish other putative slow infections. Indeed, Morris *et al.*[2] reported the occurrence of seven cases of scrapie among two hundred normal mice which had been housed in the same room as affected mice, the disease making its appearance among the normals 18 or 19 months later. Morris *et al.* thought it likely "that virus might have been carried from cage to cage by forceps, scattered bedding, or unwashed hands, mixed water bottles or cages, or even insufficiently sterilized cages in the long period of over one year of daily handling and feeding". The experiments described here, in which healthy animals were exposed to contaminated litter, do not support this suggestion. The observations of Morris *et al.* are of the greatest importance in assessing transmission attempts so it is important to consider them critically. Unfortunately not all their "scrapie" mice were fully examined histologically, and even more important—for histological diagnosis of scrapie in old mice can be difficult or even impossible—passage was not carried out. Some doubt must accordingly attach to their results.

Fitzsimmons and Pattison[4] have reported unsuccessful attempts to transmit scrapie to sheep, goats, mice or rats by nematode parasites. Pattison[5] found that scrapie did not occur in scrapie susceptible (Cheviot) sheep or goats kept indoors in contact with a succession of affected animals; nor was he able to transmit the disease with faeces or saliva—findings which fit in well with our observations. Only when scrapie and normal animals inflicted injury in fighting did cross-infection occur —apparently from ingestion of infected fragments of tissue. Chelle[6], on the other hand, reported a case of scrapie in a goat which had been in continuous contact from birth with scrapie affected sheep. More recently, Brotherston *et al.*[7] found scrapie in ten out of seventeen goats kept for a long time in contact with sheep suffering from natural scrapie, but contact infection (in a different institute) did not occur when sheep with experimental scrapie were used as a source of infection.. Indeed, as long ago as 1954, Sigurdsson[8] presented evidence that the agent of rida (the Icelandic form of scrapie) might survive outside the host on farms where it had been prevalent and invoked an intermediate host or vector. Zlotnik[9] reported contact spread in four out of forty-two mice in 15–16 months, though the normal animals might have licked some of the inoculum leaking from the injection site. But in our study no spread was seen following either immediate contact or contact delayed for 14 days after inoculation. Contact within a cage has also been reported to result in spread[10], and Dickinson and Stamp (unpublished work cited in ref. 7) believe that lateral contagion may sometimes occur in the field.

Only Morris *et al.*[2] have reported the development of scrapie among normal animals housed with scrapie infected animals, and our work—specially designed to test for it in the worst possible conditions of contamination —has not revealed it. Animals exposed to contaminated cage litter seem to "age" more rapidly than usual but no case of scrapie has been proven histologically or by passage. We conclude that the dangers (in this particular animal house) are very small. Transmission experiments

nevertheless have always been carried out in a separate animal house.

There are, however, two sources of error which may well affect long-term experiments. The possibility cannot be entirely excluded that an occasional escaped animal is replaced (instead of being killed). A wrongly labelled bottle may also be a source of error. Occasionally the disease may be inadvertently transmitted by ear-punching normal animals with an instrument used for scrapie animals[11] and infection might be carried over during withdrawal of blood by tail snipping with contaminated scissors. Handling of normal material with contaminated instruments cannot be ruled out, though direct experiment suggests that carelessness would have to be gross. On the other hand, very low scrapie titres may lead to greatly prolonged incubation periods. Recently a mouse injected with erythrocytes taken from an animal inoculated with scrapie intracerebrally 2 h previously, developed the disease after 17 months. Incubation periods beyond 6 or 7 months were found by Pattison and Jones[12], who reported that scrapie occasionally resulted from inoculation of preparations from non-scrapie brain or tumour material.

Thus we conclude that when large numbers of animals become ill with scrapie within 7 or 8 months of inoculation of a material there can be little danger in accepting the findings as valid[13]. Very long incubation may be associated with low titre scrapie and such conditions have yielded some of the most interesting findings[12]. Further contamination experiments—especially with imperfectly sterilized instruments and glassware—are therefore necessary.

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Gigantopithecus and the Mountain Gorilla

Pilbeam[1] has recently described the peculiarities of the extinct pongid genus *Gigantopithecus*, and commented on the striking parallelisms between its functional morphology and that of both *Theropithecus* (following Jolly[2]) and the early Hominidae. I hope to show here that a similar functional complex has evolved in a living pongid, the rare mountain gorilla (*Gorilla gorilla beringei*).

Mountain gorillas live in the Virunga Volcanoes of the Great Lakes region of Central Africa, and on nearby Mt Kahuzi. To the west, in the Congo lowlands and in the less elevated ranges of Itombwe and Tshiaberimu, lives the eastern lowland gorilla (*G. g. graueri*); while 700 miles to the west of this subspecies occurs the western lowland gorilla (*G. g. gorilla*), living in the region between Nigeria and the lower Congo.

Table 1

	G. g. gorilla (Cameroun) ♂ Skull measurements (mm)	Sample size	*G. g. graueri* (Utu) ♂ Skull measurements (mm)	Sample size	*G. g. beringei* ♂ Skull measurements (mm)	Sample size
Cranial length	198·5	112	186·5	12	200·2	13
Maxillary toothrow	66·3	112	71·3	12	73·5	13
Palate length	117·1	112	126·3	12	134·8	13
Ascending ramus ht.	127·1	98	124·1	11	133·7	
Bigonial breadth	128·4	98	128·1	11	144·6	14
Mandibular length	204·0	98	205·6	11	206·9	14

The differences between the three subspecies concern skull characters, limb proportions, coloration and so on, and are described in a forthcoming paper[3]. The mountain gorilla, while not of much greater size than the other forms, has much larger teeth, and correspondingly more developed jaw musculature, than the western gorilla; the eastern lowland form is intermediate. A good idea of the differences can be gained from the average values[4] for male skulls (measurements in mm) in Table 1. It can be seen that *beringei*, the mountain gorilla, has larger teeth, a longer palate, higher ascending ramus and more widely flaring jaw angles than a lowland gorilla of the same

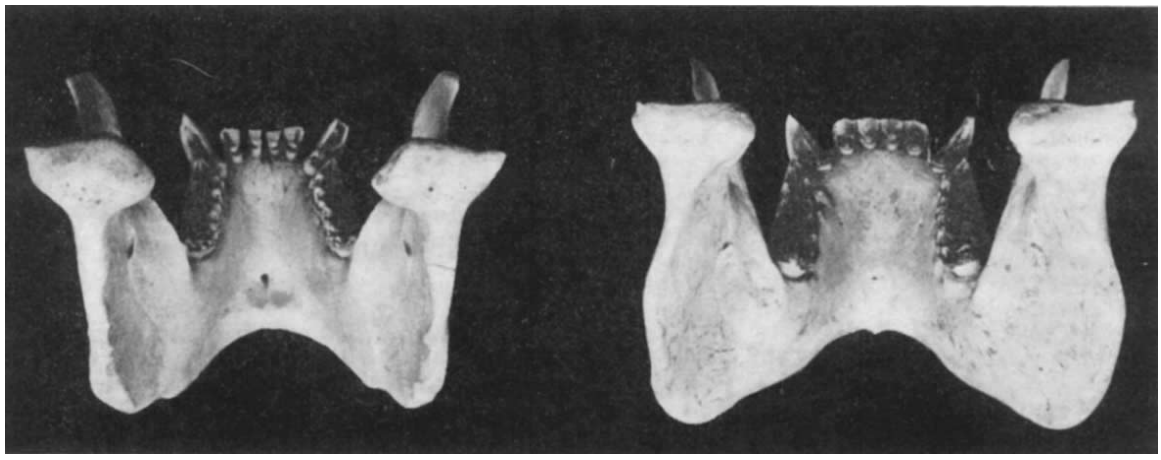

Fig. 1. Mandibles of *G. g. gorilla* (left) and *G. g. beringei* (right). Note the higher ascending ramus and more flared jaw angles of the latter. (Photo by Uno Samuelsson, Naturhistoriska Riksmuseet, Stockholm.)