Table 1. Summary of Transfer Reaction Test Results

	Whole cells		Broken cells		Sediments obtained at 1,000 RCF		24,000 RCF		105,000 RCF		Cell sap†		H_2O Insol.‡	
No. times	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
React. positive	7	23	3	7	2	9	2	7	5	11	3	4	5	23
React. negative	32	16	19	15	29	22	29	24	28	22	12	11	21	3
Cont. > exp.	2		1		1		1		2		0		0	
Exp. > cont.	19		6		8		7		9		3		22	
Tie	4		1		1		0		1		1		1	
React. delayed*	14		7		8		4		9		1		5	
React. immed.*	8		4		3		4		4		2		12	

* Figures apply to both experimental and control since they were always the same.
† Supernatant after removal of 105,000 RCF sediment.
‡ Precipitated upon dialysis of 'cell sap' against cold water.

Table 2. Some Properties of the Water-Insoluble Active Fraction

Transfer reaction; mm. of induration in two diameters. Figures in parentheses are of doubtful significance. All enzymes were active under conditions used. None interfered with the transfer reaction.

Exp. No.	Untreated		Ribonuclease †		Deoxyribonuclease‡		Trypsin §	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
8	12 × 12*¶	12 × 12*¶	—	—	13 × 13*	11 × 11*	—	—
15	(7 × 7)	9 × 9	10 × 10*	21 × 23*	—	—	—	—
22	0	9 × 10*	8 × 9*	10 × 10*	—	—	—	—
31	10 × 10	11 × 11	—	—	—	—	0	0
32	0	9 × 10	0	17 × 17*	0	11 × 10	—	—
33	0	13 × 14*	(6 × 8)	15 × 16*	—	—	(7 × 8)	(7 × 7)*
34	(7 × 7)	13 × 16	(7 × 8)*	19 × 19*	—	—	0	0
35	—	—	10 × 10*	12 × 15*	—	—	—	—

* Immediate reactions. † 5 × crystallized, 0·05 mgm./ml., 37°, 4 hr. ‡ 1 × crystallized, 0·1 mgm./ml., 0·025 *M* Mg^{++}, 37°, 1·5 hr. § 'Difco', 1 : 250, 0·05 mgm./ml., *p*H 7·4, 37°, 3 hr. ¶ No enzyme, 37°, 1·5 hr.

reaction; (4) this fraction displays the greatest specificity and (5) transfer reaction usually becomes immediate when induced by the water-insoluble material.

The results in Table 2 seem to us to indicate that the active substance is unreactive to deoxyribonuclease and can be destroyed by trypsin. Its activity seems to be enhanced by the action of ribonuclease. Other experiments show it to be inactivated at 60° C. for 30 min. It is likely, therefore, that the active fraction is a protein, perhaps in combination with ribonucleic acid (RNA).

The times of development of these reactions, except for the RNase-treated material, was unpredictable. It is possible that the distinction between delayed and immediate reactions, long a classic of immunology, may have to be discarded in the study of transfer factor.

Preliminary evidence indicates that this material can passively transfer sensitivity to a skin homograft.

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Structure of Rhinoceros Horn

This investigation was designed to clarify some confusing statements concerning rhinoceros horn which appear in the literature: for example, that it appears to be composed of matted hair[1,2]; that it is formed wholly of keratin produced by stratum corneum[3]; that it is composed of coarse keratin fibres cemented together[4]. The present investigation has confirmed that rhinoceros horn has a tubular (filamentous) structure comparable with that described in other horn by Trautmann and Fiebiger[5] quoting Nickel[6]. This structure of rhinoceros horn has already been briefly recognized by Boas[7], whose term for the tubules was canals, by Makinson[8], who described the structure as cylindrical, and by Le Gros Clark[9]. But, as horn tubules (filaments) in general form over dermal papillæ, the implication of Le Gros Clark that rhinoceros horn is unique in this respect is not correct. Dove[10] said that rhinoceros horn was similar to that in cattle. During the present work it has not been possible to demonstrate horn tubules in either sheep or cattle horns, in which, according to Trautmann and Fiebiger, the tubular structure is not marked; in addition to those in rhinoceros horn, tubules have been observed in the hoof. In the present work the term filament is preferred to the term tubule (which suggests a hollow structure) and to the term fibre (which suggests equivalence to hair).

Rhinoceros horn seems to be unique in the way that it frays into the tubules (filaments), in contrast to cattle and sheep horn, which, if it breaks up at all, does so into sheets. Such fraying seems to take place more readily at the base of horns removed from the animal. Rhinoceros horns which I examined were polished at the tip like cattle and sheep horns, and had frayed only at the base. This fraying is clearly

the source of the much-repeated statement that the horn is composed of matted hair, and was so marked in the piece of horn used in the present investigation that filaments could be readily split away. This horn was from the Indian rhinoceros (kindly supplied by Dr. A. S. Clarke of the Royal Scottish Museum, Edinburgh, which probably acquired the horn about a hundred years ago).

Microscopical examination of whole mounts in glycerine of individual filaments showed a central structure like the medulla of a hair. On leaving a filament overnight in 17 per cent sodium hydroxide the 'medulla' became more obvious, and vertical streaks became visible in the filament, particularly close to the 'medulla'. These gave the appearance of the cortical cells of a hair, but the lack of a surrounding cuticle indicated no further resemblance to hair. There were irregular oblong gas spaces across the 'medulla', which gave a superficial resemblance to the ladder-type medulla found in hairs from furred mammals.

Transverse sections of individual filaments showed a laminated structure, which confirmed that the filaments were not hairs. There were some 40 laminæ arranged concentrically around the 'medulla', which appeared as a clearly demarcated solid structure that had either several small gas spaces, or one large irregular one occupying almost the whole width of the 'medulla'. Boas[7] regarded the filaments as canals; but it is unlikely that they are filled, as he stated, with a horny substance from their inner walls towards their axes. The resulting thin, solid, column of horn he describes as filling the canals corresponds with the medulla-like structure described in the present study.

Transverse sections of the horn showed that the filaments were packed closely together (Fig. 1) and were some 300–500μ in diameter, as indicated by Makinson[8]. The close packing resulted in many of the filaments being triangular in transverse section; others had 4–6 sides. Intertubular (interfilamentous) horn was concentrated mainly, if not only, in the corner interstices, rather than between the flat interfaces between filaments. This is a major difference from the hoof of the horse, which had filaments only 25–50μ wide, separated by 50–100μ of interfilamentous horn in which the individual, pigmented cells were visible. The filaments in the hoof were oval in section, the long axis across the oval being 80–120μ in length. This appearance may have been accentuated by oblique sectioning. The 'medulla', too, was oval in section, as often seen in rhinoceros horn, and was of similar size, despite the much smaller size of the hoof filaments. The 'medullæ' in both were commonly about 20μ wide; some in the hoof were as narrow as 10μ wide, but the long axis of the oval in rhinoceros horn was often greater, for example, 20 × 60μ compared with 20 × 40μ in the hoof.

The lack of interfilamentous substance in the rhinoceros horn is apparently the reason why it frays so readily into the filaments. This seems to be primarily a mechanical effect, that is, it is not a question of chemical susceptibility; for example, caustic soda swells, and softens, the whole structure, and does not appear to attack the interfacial regions preferentially. The horn had often split along the interface between filaments before sectioning, and the cracks formed allowed entry of stain (Fig. 1). When a piece of horn was left overnight in picro-indigo-carmine (green) the cracks were stained green, presumably by deposition, whereas picric acid from

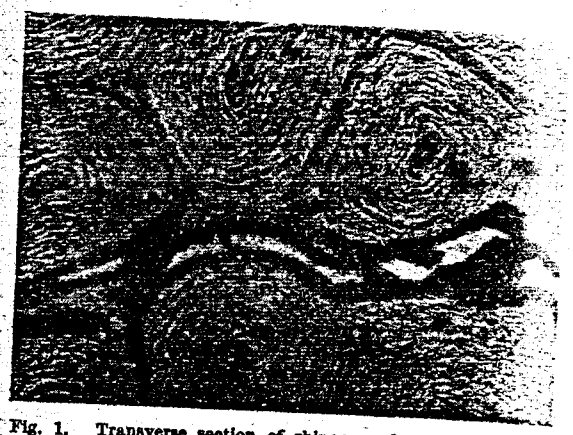

Fig. 1. Transverse section of rhinoceros horn showing horn filaments closely packed together. The dark areas indicate stain that entered a crack prior to sectioning. (× c. 220)

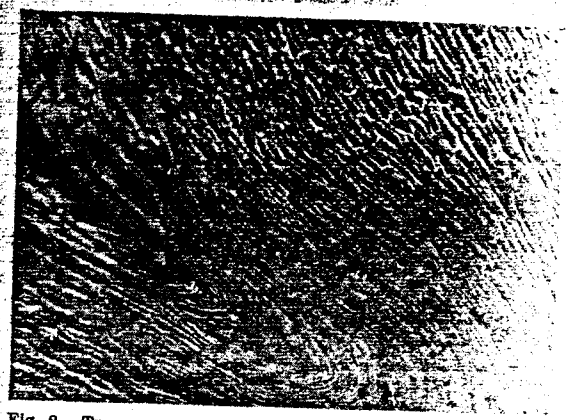

Fig. 2. Transverse section of horn filament showing laminated structure in which the individual cell outlines have been revealed with *o*-chlorophenol. (× c. 800)

the mixture had diffused varying distances into the filaments staining them yellow. When mercury orange was used to detect thiol (SH)-groups only the 'medulla' gave reaction, as in hair[11], indicating a further resemblance to the medulla of hair.

When sections of rhinoceros horn were mounted in *o*-chlorophenol[12], the structure swelled, and the cell outlines became visible (Fig. 2). The cells were more obvious in the outer part of the filaments, and it appeared that the laminæ were only one cell thick. The cells appeared flattened, and were arranged transversely around the filament. No evidence was seen of the alternate horizontal and transverse spiralling strata of cells depicted by Trautmann and Fiebiger[5] in the hoof. This structure is, however, claimed to be a specialization in the hoof to provide elasticity. An individual filament mounted in *o*-chlorophenol showed vertical cell outlines. The cells therefore appeared elongated in both planes, and were thus plate-like and roughly rectangular in shape.

In the present work both cattle and sheep horns showed a dense laminated structure when cut either transversely or longitudinally. The laminations were wavy, and were more dense in cow horn. Sheep horn (Merino ram) differed from cow horn in having elliptical holes orientated parallel with the laminæ. Their hollow nature, and the lack of any concentric laminæ around them, showed that they were not the centres of filaments. The development of filaments in hooves and in rhinoceros horn, and the lack of, or at

least poor development of, filaments in other horns may be associated with the need to provide rigidity and strength in hooves and the rhinoceros horn. The latter contrasts with other horns in being composed entirely of horn[3], and having only a very short and rudimentary bone support.

Although I have avoided the view that horn filaments are homologous to hairs, one cannot escape their similarity. This is partly due to the similarity of the dermal papillæ with those of hair follicles, and there is no doubt an evolutionary association between them.

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A Possible Role of the Boundary Layer in Insect Flight

It has been recently proposed by Greenewalt[1] that the observed wing-beat frequency of insects and birds is equivalent to the characteristic frequency of the flight system as a mechanical oscillator. Evidence for this has come primarily from work on those higher orders of insects in which direct neural control of frequency is absent ('asynchronous' type of Roeder[2]). From this point of view the inverse variation of wing-beat frequency with air density reported in some species by Chadwick and Williams[3] and Sotavalta[4] appears anomalous. Evidence is given here which shows that such an anomaly may be due to a factor hitherto not considered in investigations of the flight of small insects: a variation of effective wing inertia with air density due to a functionally significant additional mass corresponding to a volume of air 'attached' to the wings.

The inertial contribution of the apparent additional air mass has been evaluated as follows: For an undamped oscillator in which the total stiffness of the system is constant, variations of the reciprocal of frequency squared should be directly proportional to changes in the moment of inertia. Considering the total moment of inertia of the flight system as three components: the internal thoracic inertia, I_t, the moment of inertia of the wing proper, I_w, and that of the apparent additional mass, I_a:

$$1/f^2 = K(I_t + I_w + I_a)$$
$$\Delta\, 1/f^2 = K\, \Delta\, I$$

f is wing-beat frequency.

For *Drosophila virilis* (1) I_w was estimated as 0·015 mgm.mm.^{2} by summing the weights of transversely cut wing segments multiplied by the square of their approximate radii of gyration. (2) $1/f^2$ when $I_a = 0$ was estimated by extrapolation of the data of Chadwick and Williams[3] to an air density of 0 as $1{\cdot}66 \times 10^{-5}$ sec.^{2}. (3) $1/f^2$ when $I_a + I_w$ approaches insignificance averages $0{\cdot}625 \times 10^{-5}$ sec.^{2} in measurements of peak frequencies of the very short 'flights' of tethered specimens with the distal 3/4 of the wings removed. Thus $\Delta 1/f^2 = 1{\cdot}035 \times 10^{-5}$ sec.^{2} for a ΔI of 0·015 mgm.mm.^{2}, and $K = 6{\cdot}9 \times 10^{-4}$ sec.^{2}/mgm.-mm.^{2}. $1/f^2$ under normal conditions of tethered flight is about $2{\cdot}25 \times 10^{-5}$ sec.^{2}. This corresponds to $\Delta I = I_a = 0{\cdot}0085$ mgm.mm.^{2}, equivalent to a layer 0·3 mm. thick distributed evenly on both surfaces of a wing of area 3·4 mm.^{2} at one atmosphere and room temperature

Prandtl[5] gives the following formula for the boundary layer in flow parallel to a thin, flat plate:

$$\delta = 3{\cdot}4\sqrt{\frac{x\mu}{u\rho}}$$

where δ is boundary layer thickness, x is distance from leading edge, u is free stream velocity, ρ is density of medium, μ is viscosity of medium. With data from sequential flight photographs of *D. melanogaster* for total angular distance per stroke and an average over the stroke for velocity, integration of this equation gives a value for the moment of inertia of the boundary layer, I_b, of 0·0080 mgm.mm.^{2} using the wing dimensions of *D. virilis*. Thus the effective additional inertia, I_a, needed to explain the observed effects is similar to the inertia, I_b, of the boundary layer as the latter is conventionally described. The boundary layer, representing a velocity profile, should not be entirely inertial in effect, so the closeness of the two figures is somewhat surprising; moreover, inertial contributions of the wake have been neglected; and Prandtl's formula may not hold strictly for this dimensional range (Reynolds's number about 10^2).

Further evidence that the occurrence of inverse frequency changes with air density may be considered a boundary layer effect comes from the work of Chadwick and Williams[3] and Chadwick[6]. These workers give extensive data on the wing-beat frequency and amplitude of *D. repleta* and *D. virilis* in media varying from 0·07 to 5 times normal atmospheric density. Their experimental runs give linear plots of $1/f^2$ versus I_b if, from Prandtl's formula:

$$\delta \propto \sqrt{\frac{1}{u\rho}}$$
$$u \propto \beta f$$

where β is the amplitude;

and thus:

$$I_b \propto \rho^{1/2}\beta^{-1/2}f^{-1/2}$$

In addition, while the work of Sotavalta[4] shows that insects which show an appreciable shift in frequency with density are not distinguished on simple dimensional considerations from forms in which the effect is absent, consideration of the relative magnitude of wing inertia and inertia of the boundary layer leads to a clear separation of the two groups. Again using Prandtl's formula, at constant pressure and temperature, and assuming constancy of all flight parameters besides frequency along with dimensional similarity of the various species