might be true of other materials as well. These experiments show further that the upper and lower parts of the stalk have a relatively independent vascular supply.

The demonstration of more than one adenohypophyseal trophic factor from the hypothalamus[11-13] leads one to the question whether these materials are being released uniformly throughout the median eminence and stalk, or whether, alternatively, certain functions are located in separate areas of the stalk. One may ask further, whether the rate of release of these materials is governed completely by some functional attribute of the neuron or neuron terminals from which they are released or whether variations in the rate of blood flow through independent vascular fields contributes something to the rate of delivery of these materials into the portal trunks and thence into the adenohypophysis.

Although any conclusion on these matters at the present time would be premature, the experiments reported here demonstrate that such functional areas could exist and could be controlled in some measure by vasomotor control of blood flow into a given area. Experiments demonstrating chronic insufficiency of specific stalk-mediated functions by interrupting vessels to specific areas and maintaining the animals for long periods of time have been attempted. They have all so far failed because animals have not survived the experiment. The factors involved are not entirely clear although it does not involve the interruption of hypothalamic blood vessels or damage to the hypothalamus or to the pituitary stalk. Sham-operated animals with no manipulation of stalk vessels or hypothalamic damage also failed to survive.

The second major point which these experiments demostrate is that 'titre' or concentration of material in the circulating blood is not the only factor controlling the rate at which materials can arrive in the median eminence and stalk or in the anterior lobe.

It is obvious from these experiments that rate of capillary blood flow markedly influences the ability of the neurohypophysis to take up and concentrate the dye methylene blue. In every case where there were lighter areas of the stalk after injection of the dye there was a reduced blood flow rate. It can be concluded that there was a sufficient concentration of the dye in the blood of the stalk capillaries flowing at a rate insufficient to bring about a visible accumulation in the tissues.

In summary, these experiments with a dye which diffuses readily from primary capillaries into the neurohypophyseal tissue lends support to the following hypotheses: (1) That small regions of the pituitary stalk and median eminence are functionally separable on the basis of the arterial supply to different parts of the primary capillary system. (2) That capillary perfusion rate is an important factor in determining the amounts of materials which pass from primary capillaries to neurohypophyseal tissue. (3) That the arterial blood supply to the upper stalk and median eminence is functionally relatively independent of the lower stalk. (4) That the permeability of the primary capillaries varies with physiological state. It is possible that all these factors are involved in the fine control of pituitary function by hypothalamic neurohumours and/or materials from the general circulation.

This work was supported by U. S. Public Health Service grants *B*–3937 and *H*–4176

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A WATER-SOLUBLE POLYPEPTIDE PREPARED FROM ZYMOSAN

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ZYMOSAN, an insoluble lipoprotein-polysaccharide derived from the cell wall of yeast cells, inactivates the third component of complement[1,2], produces hyperplasia and hyperfunction of the reticuloendothelial system[3–7]. Furthermore, it increases the resistance of mice towards bacterial infections[8] and ionizing radiation[9]. It enhances the formation of antibody[5] and also prevents the development of tumours[10] in animals and is pyrogenic and antigenic in rabbit[11].

This article describes a method for producing from zymosan a water-soluble polypeptide. This material protects, after intraperitoneal injection, mice against *E. coli* infection. Furthermore, it diminishes the lesion produced by vaccinia virus in rabbit skin. It also provides some protection of chick embryo against vaccinia virus. Finally, in tissue culture it inhibits the mitotic activity of HeLa cells.

2 g of commercial zymosan (from fresh yeast, type *A*, L. Light and Co., Colnbrook, England) prepared from Fleischmann yeast was extracted at 37° C with 150 ml. of 90 per cent aqueous phenol for 2 days. Undissolved material was removed by centrifuging and the clear

supernatant was shaken with 750 ml. of ether and 500 ml. of water. The aqueous phase containing a suspension of precipitated material was re-extracted with ether to remove phenol and the precipitate subsequently brought into solution by adjusting the *p*H to 9. The solution was concentrated in vacuum to a small volume and passed through a 'Sephadex *G* 50' (40 cm high × 4 cm diam.) column previously equilibrated with 0·01 N sodium hydroxide. The high molecular fraction was collected, neutralized with 'Amberlite *IR* 120 (H)' to *p*H 7·0 and freeze-dried. 0·07 g of a white solid was obtained. To purify it further, the white solid was dissolved in water (50 ml.) and centrifuged at 20,000*g*. To the clear supernatant was added 2 ml. of 20 per cent of sulphosalicylic acid, the precipitate redissolved in water by adjusting *p*H to 7 with sodium hydroxide, dialysed at +4° C against distilled water for 24 h and freeze-dried.

It is seen from Table 1 that the material is largely a polypeptide and contains only small amounts of carbohydrate. 'Sephadex *G* 50' excludes material with molecular weight equal or greater than 8,000–10,000. These figures could therefore represent a lower limit for the molecular weight of the polypeptide. It is precipitated on acidification and by sulphosalicylic acid, phosphotungstic acid and ammonium sulphate (completely precipitated at 50 per cent saturation).

Table 1. Analytical Results of the Material Prepared from Zymosan

	Per cent
Nitrogen*	12·69
Biuret†	92·0
Hexose content‡	1·9
Hexosamin content §	0

* Material dried at 105° C over P_2O_5 and analysed by the Dumas method. Bovine serum albumin by the same method gave 14·8 per cent (carried out by Dr. A. Schoeller, Kronach).
† The Biuret method of Gornall *et al.* (ref. 12) was used. The figure given is in percentage of the colour produced by an equal amount of serum albumin.
‡ The anthrone method of Goa (ref. 13) was used.
§ The modified Morgan–Elson method of Svennerholm (ref. 14) was used.

On electrophoresis in veronal buffer at *p*H 8·6 (ionic strength 0·1) on Whatman No. 1 followed by staining with Amido-Schwarz, the main part of the stainable material is negatively charged and moves as a homogeneous band. The distance travelled is 35 per cent of that travelled by bovine serum albumin.

Hydrolysis in 6 N hydrochloric acid at 110° for 20 h, followed by two-dimensional chromatography in *n*-butanol–acetic acid–water (200 : 48 : 200) and *n*-butanol–methylethylketone–water (200 : 200 : 1) in *cyc*lohexylamine, revealed the presence of glutamic acid, aspartic acid, arginine, lysine, histidine, glycine, serine, proline, alanine, valine, tyrosine, threonine, phenylalanine, leucine and isoleucine.

The ultra-violet absorption spectrum at *p*H 7·0 in *tris*-hydrochloric acid is seen in Fig. 1.

An aqueous solution of the material exhibits in the ultra-violet light a bluish fluorescence.

Pyrogenicity. 1 mg of the material was dissolved in 1 ml. of saline and injected intravenously into each of four rabbits and the temperature recorded as described previously[15] over a 3-h period. The maximum increase in temperature in each of the four rabbits respectively was 0·4°, 0·4°, 0·5°, 0·6° C, and shows that the material was not pyrogenic at this dose level.

Bacterial challenge. The effect of the intraperitoneal injection of 50 μg of material prior to the intraperitoneal

Table 2. Effect of the Material Isolated from Zymosan on *E. coli* Challenge in Mice

Hours between injection of material and bacterial challenge	Survivors/total mice in experiment
3	11/18
6	11/18
24	8/18
Controls	2/18

0·1 ml. pyrogen-free saline containing 50 μg of material was injected intraperitoneally into Swiss albino mice weighing about 20 g. The mice, including the controls, were challenged intraperitoneally with 0·25 ml. of a suspension of $1·25 \times 10^8$ *E. coli* grown and prepared as described previously (ref. 15).

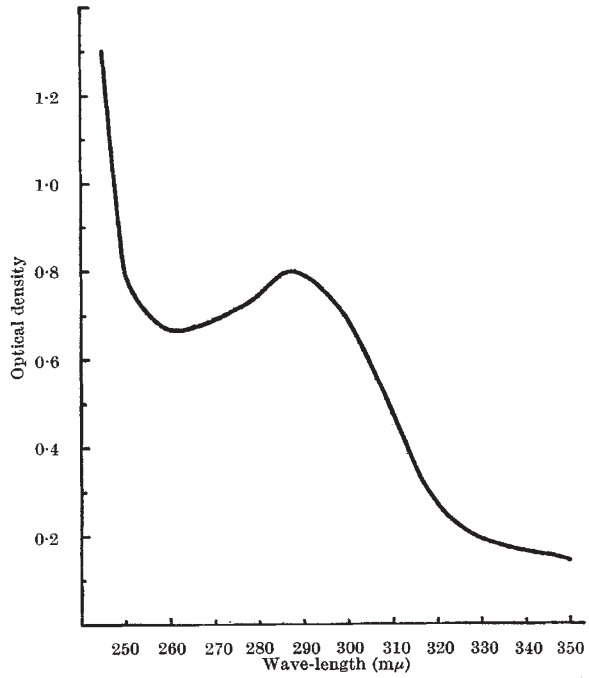

Fig. 1. The ultra-violet absorption spectrum of material prepared from zymosan in *tris*-hydrochloric acid buffer 0·1 M, *p*H 7·0 (0·33 mg/ml.)

injection of *E. coli* in mice is seen in Table 2. It is apparent that the material offers some protection against the bacterial challenge.

Vaccinia virus in rabbit skin. The effect of different quantities of material injected intracutaneously into rabbit skin prior to infection (on the same site) with vaccinia virus is seen in Table 3. It is apparent that the material reduces the development of lesions.

Vaccinia virus in chick embryo. The result of administering the material to chick embryo 24 h prior to infection with vaccinia virus is shown in Table 4. It is seen that the material provides some protection of chick embryo against vaccinia virus.

Mitotic activity in HeLa cells. The effect of the material on the mitotic activity of HeLa cells grown in the *E2a* medium of Puck, Cieciura and Fisher[16] is seen in Fig. 2. It is apparent that 0·33 mg/ml. reduces the mitotic index

Table 3. Effect of the Material Prepared from Zymosan on the Development of Vaccinia Virus in Rabbit Skin

Amount of material injected (μg)	Area free of lesions	
	Rabbit 1	Rabbit 2
400	1 cm × 0·5 cm	0·5 cm × 0·5 cm
200	0·5 cm × 0·3 cm	1 cm × 0·5 cm
100	No area free of lesions	No area free of lesions
Controls	No area free of lesions	No area free of lesions

White land rabbits weighing 3–4 kg were used. The animals were epilated with barium sulphide on the flanks. 0·2 ml. of a sterile saline solution of the material was injected intracutaneously (0·2 ml. saline as control), the area at and around the injection site scarified (ref. 15) and a solution of vaccinia virus applied to the area. The vaccinia virus used was a vaccine prepared from chick embryo infected with vaccinia virus and was free of bacteria. The results were recorded after 10 days.

Table 4. Effect of the Material Prepared from Zymosan on Chick Embryo Infected with Vaccinia Virus

Exp. No.	Amounts of material administered to each embryo (μg)	Survivors/total embryos	
1	2	23/37	
	controls	3/35	
2	10	5/5	Total 20/20
	5	5/5	
	2·5	5/5	
	1·0	5/5	
	controls	4/10	

Eggs from White Leghorn were used. After 10 days at 37° a 0·5 cm × 0·5 cm window was drilled over the chorio-allantoic membrane and the membrane dropped by suction over the air sack. 0·05 ml. of a sterile solution in saline of the material to be tested was applied to the membrane. 0·2 ml. of a vaccine (free of bacteria) prepared from a cell culture of calf embryo epithelial cells infected with vaccinia virus (ref. 15) was applied on the same spot after 24 h at 37°. The window was sealed with cellophane tape, eggs incubated for 4 days and deaths determined.

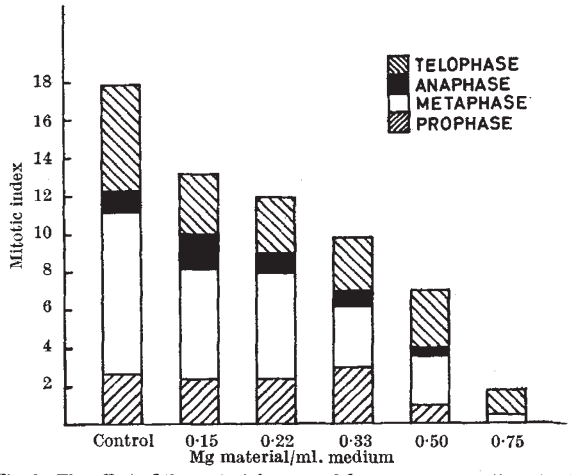

Fig. 2. The effect of the material prepared from zymosan on the mitotic index of HeLa cells
HeLa cells strain *S3* were grown in tubes as a monolayer on glass plates in *E2a* medium at 37°. The control is the average of the results from 10 tubes and the experiments the average of results from five tubes. The mitotic index was determined after 24 h exposure to the material. For further details of the experimental technique see Oftebro, Laland, Dedichen, Laland and Thorsdalen (ref. 17)

of the HeLa cells with 50 per cent. Furthermore, the results suggest that the material interferes with some processes in the interphase. No chromosomal abnormalities were seen.

The experiments described here demonstrate that a high molecular polypeptide can be extracted from zymosan. This is rather surprising since the preparation of zymosan involves exhaustive digestion with trypsin[1].

The polypeptide material prepared from zymosan resembles in its biological properties material isolated from liver and blood and described previously[15]. These latter materials are, as the zymosan material, essentially polypeptides[16]. However, they differ from the zymosan material in being more soluble at acid *p*H. They are similar so far as they all exhibit in aqueous solution a bluish fluorescence in ultra-violet light. It is tempting to speculate that the fluorescent substance is required for biological activity and that this substance is the same or a closely related substance to those present in the materials prepared from liver and blood.

The reported biological properties of the polypeptide material prepared from zymosan indicate that it increases the resistance of the animal both against bacterial and viral challenge. It is thought that this might be caused by a general cellular effect. The effect on the mitotic activity of HeLa cells provides an example of the cellular effect of the material on one particular kind of cells.

The substance in zymosan responsible for its biological properties is still a question of disagreement. Heller[19] reported to have isolated from zymosan a non-toxic lipid which stimulated the reticuloendothelial system whereas Riggi and di Luzio[20] claim that the glucan part of zymosan is responsible for the stimulation of the reticuloendothelial system. Whether or not the water-soluble polypeptide prepared from zymosan and described in this paper is responsible for all the biological properties of zymosan cannot be concluded from the present experiment.

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ELECTRON-DONOR OR ELECTRON-ACCEPTOR PROPERTIES AND CARCINOGENIC ACTIVITY OF ORGANIC MOLECULES

THE possible correlation between the electron-donor or -acceptor capacities of aromatic molecules (as exemplified by their ionization or reduction potentials) and their carcinogenic activity was examined for the first time in 1955 [1]. We concluded that no significant correlation existed between these two types of property although it was pointed out that some restricted correlations between the electron-donor capacity and the ability to produce tumours could be observed in some limited series of active molecules. Recently the problem of the possible involvement of the electron-donor or -acceptor properties of molecules in promoting carcinogenic activity has been raised again by some authors[2–5], and we have already discussed some of these contributions[6,7]. This type of approach seems to have culminated in the very recent paper by Allison and Nash[8], who postulate that "carcinogenicity arises as a result of a suitable combination of *both* electron-donating and electron-accepting properties in a compound". The authors explicitly recognize that neither of these properties alone is sufficient by itself for carcinogenesis, because as they say "many drugs which are apparently not carcinogenic are either donors or acceptors of electrons". They consider that the combination of both electron-donating and electron-accepting properties in the same molecules is, however, rare, and that it is a particular characteristic of carcinogenic compounds.

The erroneous nature of this proposition may be demonstrated in a number of ways. Thus let us consider the aromatic polybenzenoid hydrocarbons, which not only represent one of the most important and largest group of compounds investigated for carcinogenic activity but which are also the most advantageous example, from the point of view of Allison and Nash, because, as is well known from general quantum chemistry, the electron-donor and electron-acceptor properties of these molecules run parallel, so that in this series the same molecules combine these two properties. This situation already destroys one of the arguments of Allison and Nash. In these molecules, at least, the properties of being a good donor and good acceptor always go together and are in no way an exceptional situation. Moreover, it results from this fact that if there is no correlation in this series of molecules between their carcinogenic activity and their electron-donor or their electron-acceptor capacity (and