A PHAGOCYtic REACTION IN HAY FEVER

BY

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Up to the present phagocytic experiments with pollen grains and hay-fever blood have been impracticable, owing to the large size of the grains ($20\ \mu$ to $80\ \mu$ in diameter) compared with that of the leucocytes.

In the course of examining films of *Phleum pratense* pollen stained with iodine, a granular appearance of the substance of the grain was observed. This was found to be due to the grain containing large numbers of discrete granules (several hundreds per grain) held in the meshes of a reticulum. These granules are small oval and short rod-shaped bodies, and stain dark brown with iodine. They are responsible for the iodine-staining properties of the pollen grain as a whole, the remainder of the cell structure not staining to any extent. The granules, which contain a large percentage of carbohydrate, are believed to be the food supply of the generative elements of the pollen grain. Suspensions of these granules were prepared for phagocytic tests. The phagocytic index was determined in twenty-four normal and non-hay-fever patients and in twenty-five hay-fever cases. "Chiastic" counts were made in five cases.

METHODS OF INVESTIGATION

Preparation of Pollen-granule Suspension

One gram of *Phleum pratense* pollen was suspended in 5 c.cm. of saline in a stout-walled test tube, glass beads added, and the whole agitated on a mechanical shaker until microscopical examination showed rupture of the pollen grains and separation of the granules from the reticulum. The emulsion was diluted with saline to 10 c.cm. and passed through a Buchner filter containing a layer of cotton-wool over a layer of filter paper. This allowed the free granules to pass through, and retained unruptured grains, cell membranes, etc. The filtrate was yellow and cloudy. Films stained with methylene blue and followed by Gram's iodine showed the filtrate to be composed of portions of reticulum (stained blue), together with large numbers of the pollen granules. By centrifugalizing at moderate speed for three to four minutes a whitish deposit and a cloudy yellow supernatant fluid were obtained. The yellow fluid was poured off, the deposit resuspended in 5 c.cm. of saline, and centrifugalized as before. The process was repeated a third time, when a suspension of granules free from portions of the reticulum was obtained from the deposit. To harden the granules somewhat, and to assist their keeping properties and prevent contamination, they were treated with 2 per cent. formaldehyde for twelve hours, then thoroughly washed by repeated centrifugalizing and resuspension in sterile saline (5 c.cm. quantities). This suspension usually corresponded to Brown's opacity tube 8-9, which was found suitable for the phagocytic tests.

The suspension soon degenerates at room temperature (in a few days). In the refrigerator it keeps usually for ten to fourteen days, when the granules often tend to degenerate by swelling and losing their staining reaction, and small yeast-like bodies may appear, rendering the suspension unsuitable. It was found that the formaldehyde treatment did not materially affect the phagocytic counts.

Phagocytic Index Technique

The blood to be tested was withdrawn from a vein and defibrinated. Two volumes of blood and one volume of pollen-granule suspension were measured in a capillary pipette, mixed, and incubated at 37.5°C . for twenty minutes; films were made by Wright's technique.¹

"Chiastic" Technique

Washed corpuscles and serum were prepared from the case to be tested and from a normal person of the same blood group. Phagocytic mixtures of equal volumes of corpuscles, serum, and granule suspension were made of the four possible combinations of corpuscles with serum and incubated; films were made as above.²

Staining

The films were stained with methylene blue for two minutes or dilute carbol-fuchsin for thirty seconds, and washed; Gram's iodine was applied for one minute, and the films were washed and dried. The leucocytes stain blue or red, and the granules brown.

Counting

The granules in 100 or 200 leucocytes were counted and the average number per leucocyte obtained (phagocytic index). As the granule suspensions were prone to degenerate frequently, a hay-fever blood of known phagocytic index and a normal blood were included in each batch of tests. Commencing degeneration of the granules was shown by abnormally low indices, the granules becoming more easily destroyed by the blood.

RESULTS

In twenty-three out of twenty-four non-hay-fever bloods nearly all the granules were found to have been dissolved, the few remaining being recognizable inside the leucocytes. The phagocytic index was low, the average being 0.4. In twenty-two hay-fever cases the index was comparatively high, and in addition there were certain numbers of granules unphagocytosed; the average index was 2.4. In three cases (33, 35, and 42) the indices were normal or only slightly raised. The high indices were from severe hay-fever cases, but the converse did not always apply. All hay-fever cases gave positive skin tests to *Phleum*, *Dactylis*, and *Holcus*.

	Non-Hay-fever Cases				Phagocytic Index	Hay-fever Cases				Phagocytic Index
	1	2	3	4		25	26	27	28	
Normal	...	...	...	...	0.2	...	...	...	...	2.8
	...	...	...	...	0.8	...	...	...	...	3.2
	...	...	...	...	0.4	...	...	...	...	2.2
	...	...	...	...	0.7	...	...	...	...	4.8
	...	...	...	...	0.8	...	...	...	...	2.4
	...	...	...	...	0.2	...	...	...	...	2.5
	...	...	...	...	0.5	...	...	...	...	3.6
	...	...	...	...	0.5	...	...	...	...	2.0
	...	...	...	...	0.1	...	...	...	...	1.1
	...	...	...	...	0.3	...	...	...	...	1.4
	...	...	...	...	0.1	...	...	...	...	0.8
	...	...	...	...	0.4	...	...	...	...	4.4
Out-patients	13	Chronic bronchitis	...	...	0.1	38	...	...	...	2.0
	14	Nasal catarrh and boils	...	...	0.2	39	...	...	...	2.5
	15	Asthma	...	...	0.7	40	...	...	...	2.0
	16	"	...	...	0.3	41	...	...	...	2.5
	17	"	...	...	0.7	42	...	...	...	0.4
	18	"	...	...	0.1	43	...	...	...	2.5
	19	Dermatitis	...	...	0.2	44	...	...	...	2.3
	20	Asthma and eczema	...	...	0.1	45	...	...	...	3.9
	21	T.B. glands	...	...	0.8	46	...	...	...	2.1
	22	Eczema	...	...	0.7	47	...	...	...	2.0
	23	Asthma	...	...	0.2	48	...	...	...	1.8
	(24**)	"	...	...	3.5)	49	...	...	...	1.9
Non-hay-fever average = 0.4						Hay-fever average = 2.4				

In the chiastic tests on five hay-fever bloods the phagocytic index was high when hay-fever serum was combined with either normal or hay-fever corpuscles, and low with normal serum. The results are shown in the following table:

	Case 28	Case 31	Case 38	Case 39	Case 40
(1)	4.2	3.0	2.9	3.5	2.7
(2)	1.4	0.8	0.5	0.4	0.6
(3)	4.2	2.3	2.0	3.2	2.4
(4)	1.0	0.6	0.4	0.4	0.5

Unphagocytosed granules were present in (1) and (3), and absent in (2) and (4).

- (1) = hay-fever corpuscles + hay-fever serum.
 (2) = " " + normal serum.
 (3) = normal corpuscles + hay-fever serum.
 (4) = " " + normal serum.

SUMMARY

In twenty-two of the twenty-five hay-fever cases tested the blood was deficient in its lytic action on the granules of *Phleum pratense* pollen, as compared with normal and other non-hay-fever bloods. This lytic property originates in the serum, as the chiastic counts demonstrate deficient lysis of the granules with hay-fever serum, irrespective of the source of the leucocytes. One non-hay-fever patient (24***) was found to have a high phagocytic index—3.5. This patient presented herself for treatment of recurrent boils of the ears, and was found to be suffering from chronic nephritis, and with a history of cholecystitis. Skin tests to *Phleum*, *Dactylis*, and *Holcus* were negative. It is of interest that this patient stated she is the mother of a hay-fever sufferer; there is no evidence of hay fever or allergy in her family history or in that of her husband.

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REFERENCES

- * Wright, A. E., and Colebrook, L.: *Technique of the Teat and Capillary Glass Tube*, Chapter IX.
 * Fleming, A.: *A System of Bacteriology*, ix, 213.

RECOVERY FROM PNEUMOCOCCAL MENINGITIS

NOTES ON A CASE TREATED BY FELTON'S SERUM

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It is generally believed that recovery from pneumococcal meningitis is rare; in fact, doubt is usually thrown on the diagnosis if recovery results. Evidence, however, is now accumulating that recovery does occur in primary infections of the meninges with the pneumococcus.¹ That early diagnosis and the institution of prompt treatment are the all-important factors is well borne out by the investigations of Kolmer,² who states that "in so far as the treatment of experimental streptococcal and pneumococcal meningitis is concerned, it has been impossible for me to save any animal, when the infection has had more than a forty-eight hours' start, with diffuse purulent meningitis." Good results are reported from the use of Felton's anti-pneumococcal serum when used experimentally by Kolmer, Amano, and Rule,³ and in cases given by Stoessiger.¹ The case we mention below is the third of three cases of recovery from meningitis occurring recently in the general practice of one of us. The present case, however, seems to us to be particularly worthy of record owing to the fact that we have good evidence for considering it to be one of pneumococcal meningitis.

CASE RECORD

The patient, a male aged 27, came under observation on October 21st, 1932, having felt unwell for about five days previously. He complained of pain in the lumbar region. His previous history was negative, except for a football injury to the sternum at the age of 16 years. His family history was good. He stated that his trouble began with backache, which was worse in bed, and he was sleeping badly, but he continued his usual duties up to the date of first seeking medical advice. Examination revealed tenderness of the muscles of the lumbar region and tenderness in the muscles of both thighs. The tongue was furred and the patient was constipated. The temperature and pulse rate were normal.

His condition remained much the same until October 25th, when he complained of weakness. His face was slightly swollen, his tongue was furred, and he complained of a slight headache. Temperature and pulse were still normal. He was sleeping badly, but there were no other symptoms. During all this period he was not confined to bed. On October 26th there was a striking change in his condition. He was lying helpless in bed, the face was swollen, the head markedly retracted. He was drowsy, with a thick and sluggish speech, and complained of pains down the spine and in his legs. He had double vision, severe headaches, sickness, and vomiting. There was some conjunctivitis and tache cérébrale was present. The abdomen was retracted and abdominal reflexes were absent. Knee-jerks were absent and plantar reflexes could not be elicited. Kernig's and Brudzinski's signs were strongly positive. Temperature and pulse were normal. There was no evidence of a lesion of the ears, nose, or throat, nor of any infection of the lungs.

A lumbar puncture was performed and a specimen of cerebro-spinal fluid, which was under considerable pressure, was sent by messenger to Dr. Cronin Lowe of Southport for his opinion. The remainder was retained and tested by one of us immediately. The following is Dr. Cronin Lowe's report.

Cells, 18 per c.mm. Polymorphs, 82 per cent.; small lymphocytes, 12 per cent.; large lymphocytes, 6 per cent. Cultures sterile. Smear: Gram-positive diplococci (probably pneumococci).

Two or three contaminatory organisms of coliform type were reported present, but these were also found by us in this and in subsequent specimens, in addition to Gram-positive diplococci. Great care was observed to avoid contamination.

On October 27th the patient's condition was unaltered except that now the disks were injected. Treatment with Felton's anti-pneumococcal serum, Types I and II, was commenced. A first injection of 10,000 units was given intravenously. Two hours later 10,000 units were injected and followed by 20,000 units at eight-hour intervals. As there was no improvement in the general symptoms on October 28th the serum was continued until 140,000 units in all had been given in a period of fifty hours. The first improvement was noticed on October 29th. The patient seemed more alert to his surroundings and the double vision had disappeared. The cerebro-spinal fluid still contained polymorphs, Gram-positive diplococci, and Gram-negative bacilli. The urine showed a heavy infection with coliform bacilli. White cells amounted to 7,600 per c.mm., 75 per cent. being polymorphonuclear. On October 30th the improvement was maintained, except for retention of urine, ptosis of the right eyelid, and slurring speech.

On November 1st Dr. Cronin Lowe's report on the cerebro-spinal fluid was as follows.

Protein, 200 mg. per cent.; globulin, ++; sugar, 0.072 per cent.; non-protein nitrogen, 25 mg. per cent.; total cells, 3 per c.mm. Cultures remained sterile.

On November 2nd improvement was more noticeable. The patient could see more clearly, there was no headache, and the pains in the legs had disappeared. The optic disks were now clearer. By November 20th the symptoms of meningitis had completely subsided and the patient appeared to be well on the road to recovery.

DISCUSSION

We are here dealing with a case of meningitis which could, from the clinical findings, fall under one of the three following types: (1) serous meningitis; (2) meningococcal meningitis; (3) pneumococcal meningitis.

With regard to the first type, our case differs markedly from those described by Braithwaite and Mitchell Innes.⁴ There was no evidence of concomitant influenza or bronchopneumonia, and lumbar puncture failed to relieve the symptoms. Furthermore, the cerebro-spinal fluid was of entirely different composition. The opinion of the bacteriologist weighs heavily against our case being of the meningococcal type—no Gram-negative diplococci were found on repeated search. On the other hand, we have here an infective meningitis, presenting early severe symptoms, in which organisms resembling pneumococci were found by two observers, and we believe that we have correctly placed our case in the latter class, even though