

## THE MICROCOCCUS NEOFORMANS.

ITS CULTURAL CHARACTERS AND PATHOGENICITY AND THE RESULTS OF THE ESTIMATION OF THE OPSONIC AND AGGLUTINATIVE PROPERTIES OF THE SERUM OF PATIENTS SUFFERING FROM MALIGNANT DISEASE ON THIS ORGANISM AND ON THE STAPHYLOCOCCUS ALBUS.

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OWING to the somewhat contradictory results which have been obtained by various investigators as to the nature of the *Micrococcus neoformans* and the relation which this organism bears to malignant disease, we have thought it advisable to place on record our observations in a large number of cases of carcinomata, sarcomata and certain cases of anaemia which are known to run a fatal course. Doyen (1886), in a preliminary note communicated to the Academy of Sciences of Paris, stated that he had found small spherical bodies in malignant and other growths which he regarded as micro-organisms. In 1902, at the Surgical Congress, at Berlin, he stated further that he had isolated a micro-organism from simple and malignant growths which, when inoculated into animals, gave rise to neoplastic formations. To this organism he gave the name *M. neoformans*. In his opinion new growths resulted from an infection of the body with this organism. Since then he has published a number of observations showing the beneficial effects which may be obtained by the employment of a vaccine or serum against the *M. neoformans* in malignant disease.

TABLE I.  
*Cultural characters of the Micrococcus neoformans.*

Obtained from two cases of squamous-celled carcinoma and one case of osseous sarcoma, and sent to us through the kindness of Dr Doyen.

Gram	A				B				C				D			
	+				Positive				Positive				Positive			
Agar slope	Typical white glistening growth				Typical white glistening growth				Typical white glistening growth				Typical white glistening growth			
Jelly slope	Liquefied in 4 days				Liquefied in 3 days				Liquefied in 3 days				Liquefied in 3 days			
Neutral-red broth (anaerobic)	No green fluorescence				No green fluorescence				No green fluorescence				No green fluorescence			
Litmus dextrose	Acid +				Acid +				Acid +				Acid +			
Litmus lactose	Acid +				Acid +				Acid +				Acid +			
Litmus saccharose	Acid +				Acid +				Acid +				Acid +			
Litmus maltose	Acid				Acid				Acid				Acid			
Litmus arabinose	Acid				Acid				Acid				Acid			
Litmus raffinose	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus xylose	Acid				Acid				Acid				Acid			
Litmus glycerine	Acid				Acid				Acid				Acid			
Litmus erythrite	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus mannite	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus salicin	Unaffected				Unaffected				Unaffected				Unaffected			
Litmus sorbit	Acid				Acid				Acid				Acid			
Litmus milk	Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change				Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change				Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change				Acid + in 24 hrs.; solid clot and decolourised in 72 hrs. No further change			
Lead acetate	Black precipitate				Black precipitate				Black precipitate				Black precipitate			
Broth	General turbidity: diplococci and staphylococci				General turbidity: diplococci and staphylococci				General turbidity: diplococci and staphylococci				General turbidity: diplococci and staphylococci			
Nature of organism	Culture(A) from squamous-celled carcinoma, sent us iv. 1906, by Dr Doyen				Culture(B) from squamous-celled carcinoma, sent us x. 1906, by Dr Doyen				Culture(C) from a sarcoma, sent us x. 1906, by Dr Doyen				Culture (D) the same as culture (A) but grown on artificial media for several months, then inoculated intraperitoneally into a guinea-pig and recovered from the spleen			

Drs Paine and Morgan failed to obtain any improvement by injection of either vaccine or serum in nine cases of malignant disease. Lately, however, the vaccine has been given in carefully regulated doses at definite intervals determined by the opsonic power of the patients' serum with the result that some observers claim to have obtained remarkable results; almost as great an improvement has occurred as was *originally* noticed by Doyen. We do not intend, however, in this paper to discuss the treatment of malignant disease with either a vaccine or a serum against the *M. neoformans*<sup>1</sup>, but only to refer to those points which are of importance in determining the nature of this micrococcus, and its relation to other micrococci of the albus series.

The most striking feature in the above table is the remarkable similarity of the three strains of the *M. neoformans* which we received from Dr Doyen. The results are almost identical in each case although so many media were employed. The first culture which we examined was subcultured for some months on artificial media, and then passed through a guinea-pig, yet, when recovered from the spleen of the animal, the cultural characters were practically identical with those of the original coccus and also with two strains obtained from two other cases of malignant growths. It is difficult to understand how this organism was recognised from other white micrococci by those who have previously studied its cultural characters, as there is nothing in the morphology, staining properties, or in the appearance of the growth on the ordinary laboratory media by which it could be identified. On the other hand, Dr Doyen has sent us an organism obtained from three distinct cases of malignant disease, the three strains of which agree in almost every particular. It occurred to us that if we employed an elaborate series of tests based on the valuable work of Mervyn Gordon, that we should be able to show that this organism might really represent various strains of the *Staphylococcus albus*, but our investigation gives no support to this view. One of us (L. S. D.) has examined large numbers of strains of staphylococci obtained from every possible source and has occasionally met with staphylococci which are identical in their appearance with the *M. neoformans*. These results will be published in detail at some future date.

The *Staphylococcus pyogenes albus* (obtained from Král), which Dr Gordon examined by his ten tests, gave identical results to those which we have obtained with the *M. neoformans*, except that it acidified mannite, but possibly this may be an important difference. It is

<sup>1</sup> This will be dealt with at a later date.

unnecessary in this communication to refer more fully to these points beyond mentioning that the morphology and cultural properties of this organism appear to be constant and that a similar organism is only occasionally met with in simple inflammatory affections.

*Agglutination Reactions.*

TABLE II.

*To illustrate the number of cases in which Agglutination occurred with the Patients' serum and with the M. neoformans and Staph. albus.*

Total number of cases tested = 67.

Operative or ulcerative cases = 47.

Non-operative or non-ulcerative cases = 20.

The serum of 22	ulcerative	cases	agglutinated	<i>M. neoformans</i> .		
"	"	37	"	"	<i>Staph. albus</i> .	
"	"	12	non-ulcerative	"	"	<i>M. neoformans</i> .
"	"	14	"	"	"	<i>Staph. albus</i> .

N.B. The word ulceration is used merely as a convenient term to denote either an open operation wound or an ulcerating surface formed by the new growth.

*Nature of the Agglutination Reaction.*

With *M. neoformans* :—

	Serum dilution
25 cases gave a reaction with	1 : 50.
7 " " "	{ 1 : 50, and 1 : 100.
2 " " "	{ 1 : 50, 1 : 100, and 1 : 500.

With *Staph. albus* :—

36 cases gave a reaction with	1 : 50.
10 " " "	{ 1 : 50, and 1 : 100.
5 " " "	{ 1 : 50, 1 : 100, and 1 : 500.

In 80 cases no agglutination reaction occurred with either organism :

- 38 ulcerative cases of carcinoma.
- 2 ulcerative sarcomatous cases.
- 22 non-ulcerative cases of carcinoma.
- 3 non-ulcerative cases of sarcoma.
- 4 cases of primary anaemia.
- 11 various cases.

Both the microscopical and macroscopical methods were employed for the determination of the agglutination reaction.

In every instance young agar cultures were used about twenty-four hours old, and emulsions were made with sterile normal saline. It was only occasionally that a positive reaction was observed, such as is seen in the case of typhoid fever.

It will be seen from studying the results of the agglutination reaction that the serum of patients suffering from all forms of malignant disease reacts more frequently to a standard laboratory culture of the *Staphylococcus albus*<sup>1</sup> than to the *M. neoformans*, and that by far the majority of cases only react with a dilution of 1 in 50. It was only in exceptional circumstances that a reaction was obtained with a dilution of 1 in 500.

These observations were made with great care and were carefully controlled. Another point of equal importance is that the agglutination reaction is much more frequently met with in those instances in which the new growth had undergone ulceration than in the non-ulcerative cases, and here also a positive reaction occurred more frequently with the *Staph. albus* than with the *M. neoformans*.

In well over half the cases no agglutination reaction occurred (i.e. with a dilution of 1 in 50) with either organism, while of these cases exactly half the number were examples of ulcerative growths. Therefore, in many cases of malignant disease there is complete absence of any agglutination reaction with the high dilutions which we employed in this investigation.

#### *Opsonic Actions.*

It is important to draw attention to the fact that 19 out of the 25 cases of carcinoma, of which the *M. neoformans* opsonic index was determined, had undergone ulceration, and 17 of the 23 cases of carcinoma of which the *Staphylococcus albus* index was made presented similar changes.

Drs Bulloch and Western have shown<sup>2</sup> that there are specific opsonins present in both normal and immune sera. The contact of normal serum with the *Staphylococcus aureus* leaves the opsonic action of the serum for *B. pyocyaneus* unchanged, while the specific opsonins for the *Staph. aureus* were practically removed. We undertook some experiments, therefore, for the purpose of determining whether there

<sup>1</sup> This culture was isolated from the blood during life from a case of acute endocarditis. The same culture was used throughout this investigation.

<sup>2</sup> Bulloch, W., and Western, G. T. "The Specificity of the Opsonic Substances in the Blood Serum," *Proc. Roy. Soc. B.* LXXVII. 1906.

was a specific opsonin in immune sera for the micrococci referred to in this paper.

TABLE III.

*Showing the Opsonic Index in 23 cases of Carcinomata, 4 of Sarcomata, and in various Diseases, also in 5 healthy medical men.*

No. of case		Opsonic Index	
		<i>M. neoformans</i>	<i>Staph. albus</i>
1.	Ulcerating carcinoma of tongue	0·4	—
2.	Carcinoma of liver	0·5	—
3.	„ pancreas	0·2	1
4.	Ulcerating carcinoma of glands of neck	0·65	0·5
5.	„ „ colon	0·78	0·9
6.	Suppurating carcinoma of ovary	0·57	1·1
7.	„ „ penis	0·9	0·9
8.	Carcinoma of pancreas	0·8	1·1
9.	Ulcerating carcinoma of cervix	1·1	1
10.	„ „ glands of neck	0·8	1
11.	„ „ penis	1·1	1·2
12.	„ „ breast	0·7	1·4
13.	Carcinoma of breast	0·6	1·2
14.	Recurrent carcinoma of breast	1·2	1·1
15.	Ulcerating carcinoma of cervix	0·7	1·2
16.	„ „ rectum	1	0·9
17.	„ „ lip	0·5	1
18.	„ „ oesophagus	1	1·1
19.	„ „ lip	0·8	0·9
20.	Duct carcinoma of breast	0·9	1
21.	Ulcerating carcinoma of oesophagus	0·6	0·7
22.	„ „ cervix	0·5	0·8
23.	„ „ colon	0·8	0·9
24.	Malignant disease of peritoneum (?)	1	0·5
25.	Ulcerating carcinoma of oesophagus	0·9	0·8
26.	Sarcoma of scapula	0·6	1·2
27.	Ulcerating sarcoma of tonsil	0·9	0·7
28.	Suppurating sarcoma of parotid gland	0·9	0·9
29.	Sarcoma of meninges (operation)	1	0·9
30.	Pernicious anaemia	0·5	—
31.	Myelaemia	0·9	0·9
32.	„	1	0·4
33.	Adenoma of buttock	1	0·9
34.	A. Healthy medical man	0·9	0·8
35.	B. „ „	1	0·9
36.	C. „ „	0·9	0·75
37.	D. „ „	1·1	0·9
38.	E. „ „	0·4	0·9

*Average opsonic index for each group in the foregoing table.*

With *Staph. albus* :—

	Opsonic index
23 cases of carcinomata	0.96
4 „ sarcomata	0.92
5 „ healthy medical men	0.85

With *M. neoformans* :—

25 cases of carcinomata	0.75
4 „ sarcomata	0.85
5 „ healthy medical men	0.86

The serum from a case of ulcerating carcinoma of the colon (case 5) was employed for this purpose (serum 141). Serum 141 + equal parts of the *M. neoformans* was digested for one hour at 37° C. and then centrifugalised; in this way a deposit and a supernatant liquid (serum A) were obtained. The opsonic content of the patient's serum for the *M. neoformans* and *Staph. albus* was as follows :—

	Cocci contained in 50 phagocytes
Serum 141 + <i>M. neoformans</i> (epithelioma) + leucocytes	155
„ 141 + <i>Staph. albus</i> + leucocytes	197
„ 141 + <i>M. neoformans</i> (sarcoma) + leucocytes	145
„ A + <i>M. neoformans</i> (epithelioma) + leucocytes	21
„ A + <i>Staph. albus</i> + leucocytes	29
„ A + <i>M. neoformans</i> (sarcoma) + leucocytes	12

These experiments serve to show that there is no specific opsonic substance in immune serum for either the *M. neoformans* or for the *Staph. albus*. This is what we were led to expect from other experiments made during this investigation.

The average *Staph. albus* opsonic index in cases of carcinoma and sarcoma appears, from our investigations, to be higher than in healthy men, although in all instances the average is below 1, but within the normal limits. On the other hand, the *M. neoformans* opsonic index in cases of carcinoma is much lower than in the cases of the *Staph. albus* index. The one case among the "healthy" medical men who had a low *M. neoformans* opsonic index (0.4), was afterwards found to be suffering from furunculosis on the back. This fact is of great interest, considering that the *Staph. albus* opsonic index was within the normal limit, and also in view of the important part which the organism has been considered to play in malignant disease. In the large majority of cases in which the opsonic index was determined, ulceration of the neoplasm was found to have occurred.

*Inoculation Experiments.*

We have made very few inoculation experiments, as this branch of the subject has been so fully dealt with by Paine and Morgan.

*Experiment 1.* 1 c.c. of a 24 hours' agar growth (emulsified in sterile normal saline) was injected into the peritoneal cavity of a guinea-pig. The animal was ill for a few days, but complete recovery followed. A second inoculation was made three weeks later, a similar result ensued.

The animal was killed at the end of one month after the date of the first inoculation. At the post-mortem examination, the animal's body was quite healthy. There were no nodules on the peritoneum or elsewhere. A bacteriological examination was made of the heart-blood and peritoneal fluid. All the cultures were found to be sterile. Film preparations made with the peritoneal fluid showed a few cells which were almost entirely macrophages.

*Experiment 2.* A guinea-pig was inoculated in a similar manner as in the above experiment. The animal was killed three weeks later. At the post-mortem examination nothing abnormal was detected.

*Experiment 3.* Three mice received intraperitoneal injections of 2 c.c. of the *M. neoformans*. Three weeks later, they received a second inoculation and only showed a slight illness for the first twenty-four hours. All three animals were killed at the end of seven weeks from the date of the first inoculation. At the post-mortem examination, they were found to be quite healthy.

Paine and Morgan inoculated 200 animals (110 mice and 90 rats) intraperitoneally. They failed to find any evidence of simple or malignant tumours as the result of these inoculations, although many of the animals were kept alive for three months and some for six months.

Our inoculation experiments are so few that the results cannot be compared with those obtained by either Doyen himself or by Paine and Morgan, but they are similar to those of the latter observers.

*Conclusions.*

1. Our results appear to bear out the conclusion that the *Micrococcus neoformans* is an organism which is only occasionally met with in simple inflammatory affections. Although not identical with the so-called *Staphylococcus pyogenes albus* or the *Staphylococcus epidermidis albus* (Welch), it is closely related to these organisms.

2. The cultural properties of the organism obtained from various sources are identical. (This applies to three cases.)

3. The serum of patients suffering from malignant disease, although the neoplasm may have ulcerated, does not develop any very marked agglutinative property for the *M. neoformans*, in fact it is less than that which is formed for the *Staph. albus*.

4. There are no specific opsonins for *M. neoformans* and the *Staph. albus*. These cocci appear to have about an equal power for removing the opsonic substance in immune sera which is present for them.

5. *M. neoformans* is an organism of very low pathogenicity.