not to defer this examination too long, lest, later, secondary changes obscure the shadow of the tumour, making the diagnosis uncertain.

Another not uncommon mode of behaviour is abscess formation. This takes place in or around or distal to the tumour, and may occur in any part of the lung. Fig. 6 illustrates two common sites where this occurs. Another suppurative complication is bronchiectasis distal to the tumour (Fig. 7). Fig. 8 illustrates the infiltrative type of growth, which may closely resemble tuberculosis and other inflammatory conditions. The symptoms and signs may be identical, and the diagnosis only be arrived at by negative sputum examinations, and possibly bronchoscopy.

Fig. 9 illustrates another common type where there is an obvious tumour-like mass.

Fig. 10 represents a tumour near the hilum. This may be extremely difficult to interpret, and a differential diagnosis will rest between inflammatory glands, lymphadenoma, leukaemia, and malignant growth. In this diagram the right diaphragm is represented paralysed and raised. This is almost pathognomonic of malignancy, as is involvement of the recurrent laryngeal type.

Fig. 11 represents another type with early invasion of the mediastinal glands, when the symptoms may be almost entirely pressure symptoms, simulating a mediastinal tumour. Sometimes with growths in the lower lobe the x-ray appearance in the anterior view is obscure and extremely difficult to interpret, especially on the left side, because of the heart shadow (Fig. 12). In such cases the true lateral view may show quite clearly the tumour-like mass (Fig. 13).

Fig. 14 represents a similar central growth with a commencing pleural effusion, which may soon obscure all signs of growth. This effusion may become large, as in Fig. 15, displacing the heart and mediastinum to the opposite side, and the fluid may be clear and sterile, or it may be blood-stained, or it may become an empyema.

Fig. 16 illustrates a rarer type of growth, where the mass itself is so large as to displace the mediastinum to the opposite side. This is probably the giant sarcoma of the pleura or a very slowly evolving carcinoma of the lung.

E. Lenstrup (*Ugeskrift for Læger*, February 28th, 1935, p. 268) paints a rather gloomy picture of the outlook for the subjects of congenital syphilis. His conclusions are drawn from the 401 children admitted to the fifty-bed Welander Home in Denmark, and from the 135 children, born of syphilitic mothers, kept under supervision in the observation department of the same home. His analysis of the first 250 patients admitted to the home between infancy and the age of 12 shows that approximately half of those who survived were imbeciles. Yet the specific treatment they received was so effective that in no case did the disease break out again, clinically or serologically, after the first courses of treatment with mercury and other specifics had been given. Of the comparative merits of these specifics—mercury, salvarsan, bismuth, etc.—the author expresses no dogmatic opinion; alone, or in various combinations, they seemed to be effective enough, except in the matter of mental health. He traces this refractoriness of imbecility to specific treatment to the ancestry of these children, recruited from the lowest levels of society. In this connexion he notes that while acquired syphilis was reduced in 1932 in Denmark to less than a third of its incidence in 1926, the decline in the number of cases of congenital syphilis in the same period has been nothing like so dramatic. This is probably because there is a residuum of mentally inferior women who neglect to accept free specific treatment, and who continue to be a recurring source of supply for the Welander Home.

HAEMOLYTIC STREPTOCOCCAL LEUCOCIDIN

PRELIMINARY REPORT *

BY

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There seems to be little doubt that the capacity for developing high virulence in strains of haemolytic streptococci is due to the elaboration by those organisms of a substance that kills or paralyzes the phagocytic cells of the body. Considerable difference of opinion exists as to the mechanism of production of such a toxin, and to its relationship to other better-known streptococcal products. Levaditi (1908) was of the opinion that leucotoxic effects were constant with all strains of haemolytic streptococci, but were not strictly related to haemolytic activities. Nayakama (1920), using the delicate but essentially artificial technique of methylene-blue reduction by normal leucocytes, considered that haemolytic streptococci secreted a filterable leucocidin, which corresponded closely in time of production in the course of culture with the filterable haemolysin. On the other hand, Todd (1927) showed that the virulence of haemolytic streptococcal strains depended upon the ability of those organisms to multiply in normal defibrinated blood, and that virulence was definitely associated with a particular type of colony formation, the matt colonial forms being virulent or capable of attaining virulence under suitable conditions, whereas the smooth or glossy forms were immutably avirulent. He also showed (1932) that antigenic haemolysin production is independent of colony formation. In other words, it is possible to obtain a strain of haemolytic streptococci which shows at the same time good haemolytic powers and a relative absence of virulence. Such a strain is in our possession: it is a Dochez strain, obtained from the National Collection of Type Cultures and subsequently artificially subcultured for more than a hundred times. There is thus some confusion of ideas upon the exact nature and relations of the streptococcal leucocidin.

In this preliminary report upon the nature of streptococcal leucocidin it is shown that the type-specific acid-soluble M protein, first isolated by Lancefield in 1928, is identical with, or very closely related to, the substance that is responsible for the death or paralysis of normal leucocytes.

Technical Details

The apparatus used in this work consisted of a small glass chamber 20 mm. in diameter and 8 mm. deep. The top edge was carefully ground. This chamber was inset to a depth of 7 mm. into a hollow rectangular copper warm stage 3 in. long, 1½ in. wide, and 1½ in. deep, provided with a glass window at the bottom and two tubular water outlets. This copper warm stage, fitting easily into the moving stage of a microscope, was connected by a siphon to a water bath, the water, after circulating through the warm stage and heating the glass chamber, running to waste. The temperature, the water-level of the bath, and the rate of flow through the siphon were very carefully controlled.

Preparations of normal leucocytes were made in the following way. A few drops of the author's blood were allowed to clot on a circular cover-slip, No. 3, 3/4 in. diameter, and were then incubated in a moist Petri dish at 37° C. for thirty minutes. The clot was then flicked off, leaving the leucocytes adhering to the cover-slip, which was then gently washed in Tyrode's solution to remove

* The cost of this research was partly defrayed by a grant from the Medical Research Council.

red cells. The cover-slip was then placed over a small test tube, $1\frac{1}{4}$ in. long by $5/8$ in. diameter, containing the test solution; the top surface of the test tube was also carefully ground to ensure perfect fit between it and the cover-slip. These were then inverted to bring the solution into contact with the leucocytes, and the whole re-incubated for a further period at 37°C . At the end of this time the cover-slip was again rinsed in Tyrode's solution, and then placed, leucocytes downwards, upon the ground glass top of the glass chamber in the copper warm stage. The glass chamber was previously filled with a 10 per cent. solution of human serum in saline. The author's serum was used; this contained fifty anti-lysin units per cubic centimetre and no bacteriotropins for the strain from which the M protein was prepared; it also failed to give a precipitate with solutions of the M protein. The temperature and rate of flow of the circulating water had also been regulated so that the resulting temperature in the glass chamber was at 37°C . with a maximum variation of $\pm 0.25^{\circ}\text{C}$. Finally a felt-lined

circular copper shield was placed around the cover-slip top and the microscope objective to minimize alterations in temperature due to air draughts from the outside. The apparatus is here shown in diagrammatic form.

Effect of Filtrates of Haemolytic Streptococcal Cultures on the Movements of Leucocytes

Filtrates were made from cultures of both matt and glossy variants of haemolytic streptococci, and were obtained from cultures at eight, ten, twelve, sixteen, twenty, and twenty-four hours from the time of inoculation. The motility of leucocytes was unimpaired when observed after exposure for five-minute periods to the action of filtrates of glossy variants taken at all stages of the growth cycle. Filtrates of early cultures of matt variants—that is, up to twelve hours' growth—also had no action upon the movements of leucocytes. After this period of cultivation filtrates of older cultures gave varying results: in some cases complete paralysis occurred, in others it merely resulted in partial inhibition. Where this leucotoxic action was noted, it was more pronounced in the twenty- and twenty-four-hour culture filtrates than in the sixteen-hour filtrates. Further incubation of the cultures did not, however, enhance this effect.

Interesting in this connexion is the observation of Hare (1931), who showed that virulent haemolytic streptococci were resistant to phagocytosis by normal leucocytes during the lag and logarithmic phases of growth, and lost this property of resistance when the culture reached the decline phase. The point of time when this loss of resistance to phagocytosis occurs corresponds roughly to the time at which filtrates of the matt variant cultures begin to acquire leucotoxic properties, as is shown in the present tests. It was thought that this was due to the passage from the organisms into the surrounding culture medium at the end of the logarithmic phase of growth of some substance responsible for the resistance exerted by young virulent haemolytic streptococci. Since Todd and Lancefield (1928) have shown that a type-specific acid-soluble M protein could be extracted from the matt but not from the glossy forms of haemolytic streptococci, it was thought possible that this might be the substance responsible for their resistance to phagocytosis, and for the leucotoxic action of filtrates of older cultures of those virulent haemolytic streptococci.

Effect of the Acid-soluble M Protein on the Movements of Leucocytes

The acid-soluble M protein was extracted and purified according to the technique described by Lancefield. From 31 litres of a fifteen-hour culture a yield of 24 mg. of the pure dry M protein was obtained. Cover-slip preparations of leucocytes were exposed to the action of solutions of

different concentrations of M protein for five- and fifteen-minute periods. After rinsing in Tyrode's solution the leucocytes were examined for motility, one hundred leucocytes being observed in each case. The criterion of motility was taken as an alteration in contour, and not intracellular granular streaming. The results of these experiments, set out in Table I, show that a concentration of 1 in 50,000 of M protein completely and permanently inhibits the movements of leucocytes when exposed for five minutes; with a fifteen-minute exposure a concentration of only 1 in 100,000 is necessary. At these concentrations the leucocytes showed a well-defined clearing effect in the cytoplasm.

TABLE I

Dilution of M protein	Percentage of Leucocytes showing Motility—			
	After 5 minutes' Exposure	Control 1	After 15 minutes' Exposure	Control 2
1 in 5,000	D	92	—	—
" 10,000	0	96	D	88
" 25,000	0	89	D	91
" 50,000	0	93	0	91
" 75,000	16	92	0	90
" 100,000	28	98	0	94
" 150,000	57	94	23	97
" 200,000	60	92	40	94

D denotes complete disintegration of the leucocytes.
— denotes that the experiment was not done.

Neutralization of the Leucocidin Effect by Immune Serum

Lancefield showed that the M protein was by nature a haptene. It follows, therefore, that any attempt to neutralize the leucocidin effect must be made by the addition of an immune serum prepared by the injection into animals of whole cultures.

A goat was immunized by a series of weekly injections for a period of six months of graded doses of heat-killed whole cultures of the same strain as that from which the M protein was prepared. Serum from this animal was shown to contain bacteriotropins for that strain, since Hare (1928) has demonstrated that the addition of a bacteriotropic serum to a mixture of normal defibrinated blood and virulent streptococci enables the leucocytes to phagocytose the hitherto resistant cocci.

Preliminary Tests.—(a) A precipitin test was made upon the goat serum to demonstrate its activity towards solutions of M protein, the results of this test being shown in Table II. (b) The effects of exposure to dilutions of goat serum upon leucocytic movements was first determined. The technique adopted was as before. It was found that neat goat serum inactivated the leucocytes, but this effect disappeared when a fourfold dilution in saline was employed.

TABLE II

Antigen		Serum	Saline	Precipitin Test	
Dilution	Amount			2 Hours at 37°C .	Overnight in Icebox
1 in 3,000	0.4 c.cm.	0.1 c.cm.	—	+	+
" 6,000	"	"	—	++	++
" 12,500	"	"	—	++	++
" 25,000	"	"	—	++	++
" 50,000	"	"	—	++	++
" 75,000	"	"	—	+	++
" 100,000	"	"	—	±	+
" 200,000	"	"	—	—	+
" 400,000	"	"	—	—	—
Control A	"	—	0.1 c.cm.	—	—
Control B	—	0.1 c.cm.	0.4 c.cm.	—	—

Leucocidin Neutralization Tests.—Mixtures of equal parts of 50 per cent. serum in saline and dilutions of M protein were incubated in a waterbath for thirty minutes at 37° C. At the end of that period cover-slip preparations of leucocytes were exposed for five minutes and then examined as before. The results of these tests, given in Table III, show that the leucocidin activity of M protein is inhibited by the previous admixture of immune serum, although the neutralizing properties of the latter are not so marked as its precipitating power.

TABLE III

Dilution of M protein	Percentage of Leucocytes showing Motility after Exposure to—		
	M only	M and 50 per cent. Immune Serum	M and 50 per cent. Normal Serum
1 in 12,500	0	0	0
„ 25,000	0	4	0
„ 50,000	0	92	0
„ 75,000	18	96	22
„ 100,000	30	91	37
„ 200,000	69	94	73

Conclusions

In this preliminary report it has been shown that the type-specific acid-soluble M protein obtained from matt variants of haemolytic streptococci exerts a marked inhibitory action on the motility of normal leucocytes. This action can be neutralized by the admixture, before exposure of the leucocytes, of immune serum. This immune serum gave a positive precipitin reaction with solutions of the M protein, and also contained bacteriotropins for the strain of haemolytic streptococci from which the M protein was prepared. Attention has been drawn to the close similarity that exists between the resistance to phagocytosis exerted by young virulent cultures of matt variants of haemolytic streptococci and the leucocidin action of the acid-soluble M protein that can be obtained from the organisms of such a culture.

BIBLIOGRAPHY

- Hare, R.: *Brit. Journ. Exper. Path.*, 1928, ix, 337.
Idem: *Ibid.*, 1931, xii, 261.
Lancefield, R.: *Journ. Exper. Med.*, 1928, xlviii, 469.
Levaditi, C.: *C. R. Soc. de Biol. (Paris)*, 1918, lxxxi, 406.
Nayakama, Y.: *Journ. Infect. Dis.*, 1920, xxvii, 86.
Todd, E. W.: *Brit. Journ. Exper. Path.*, 1927, viii, 289.
Idem: *Journ. Exper. Med.*, 1932, lv, 267.
Todd, E. W., and Lancefield, R.: *Ibid.*, 1928, xlviii, 751.

The following medical promotions in, and appointments to, the Venerable Order of the Hospital of St. John of Jerusalem are announced in the *London Gazette* of June 25th: As Knights of Grace—Lieut.-Colonel Sir Louis Edward Barnett, C.M.G., M.B., F.R.C.S., I. G. Modlin, O.B.E., M.D., Major John Orton, M.D.; as Commanders—W. G. Rogers, M.B., Surgeon Lieut.-Colonel E. J. H. Luxmoore, M.C., M.R.C.S., Lieut.-Colonel G. E. Peacock, M.R.C.P., S. S. Pringle, O.B.E., P.R.C.S.I., I. Fraser, M.D., F.R.C.S.; as Officers—C. H. Perran, M.D., H. L. Garson, O.B.E., M.C., M.B., R. H. Read, M.R.C.S., W. O. Gliddon, M.D., W. C. Winfrey, M.D., J. J. Danby, M.D., D. Samuel, M.R.C.S. (since deceased), Major S. R. Gibbs, M.C., T.D., M.R.C.S., R. V. de A. Redwood, F.R.C.S.Ed., Colonel C. R. White, D.S.O., T.D., M.B., Mrs. Janet W. MacInnes, M.B.; as Serving Brothers—L. H. Wharton, M.R.C.S., Major S. Copley, F.R.C.S., C. H. Upham, M.R.C.S., P. C. Davie, M.B., F.R.C.S., A. E. A. Palmer, M.B., H. Riddell, M.B., Captain R. H. Hadfield, R.A.M.C., Colonel H. H. E. Russell, O.B.E., M.D., F.R.C.P., O. H. Hudson, M.R.C.S., A. J. Hopper, L.M.S.S.A., W. A. Jones, M.D., F.R.C.P., S. C. Betten-court-Gomes, M.B., F. F. A. Ulrich, M.B., J. A. Carman, M.D., J. E. Brereton, L.R.C.P., H. B. Pierce, M.B.

A CONFUSIONAL STATE ASSOCIATED WITH INFECTIVE ENDOCARDITIS

BY

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This case is interesting as showing the development of a confusional state associated with septic endocarditis. In the light of the post-mortem findings, the extensive area of ulcerative endocarditis, the damage to the brain, and the widespread area of infarction, it appears highly probable that the patient's dulled mental state, emotional instability, and incontinence were due to repeated small embolic haemorrhages in the frontal lobes and hemispheres generally and possibly in the thalamus. It suggests, too, that the confusional states which sometimes occur in advanced cases of endocarditis (apart from septic endocarditis) might be explained on this physical basis of repeated embolic haemorrhages in the brain, not large enough or situated in the necessary position to cause the usual sudden marked physical lesions of hemiplegia, etc., but sufficiently destructive to damage the associational paths subserving the higher centres of cerebration.

Clinical History

Mrs. X., a widow aged 28, was recently admitted to Graylingwell Hospital as a voluntary patient. The family history, as far as could be made out, was negative. She was described as a cheerful, steady girl, who had married at 21, but had lost her husband some few months after marriage. More recently she appeared to be somewhat unstable emotionally, and, in spite of serious organic disorder, had been energetic and overactive in her pleasures and amusements, and had taken alcohol freely. There was no previous history of "nervous breakdown," fits, or paralysis. Eighteen months ago she had a severe attack of rheumatic fever with endocarditis, and about six weeks before admission had an attack of "influenza."

Present Illness.—Her present illness commenced, according to her parents, some six weeks before admission, and was attributed by them to the attack of influenza and also to domestic worries. She gradually became weak, dull, and drowsy, persistently complained of headaches, and had lost weight considerably. Insomnia and constipation were other marked features during this time. More recently, however, mental symptoms had been more in evidence. She had become more confused and disorientated, and was generally dull, drowsy, and semi-stuporose, although her state of consciousness and alertness seems to have fluctuated very much from time to time. Often she would appear brighter mentally, but she was facile and emotional, and would talk freely about her symptoms, describing peculiar pains which seemed to rush from the head and focus themselves on the root of the nose, "like a ball of fire," which dispersed in flames, giving her instant relief from her pain.

Physical State on Admission.—She was a tall and very thin woman, with poor, flabby musculature and a sallow complexion—in fact, almost café-au-lait. Her temperature was 100.4°, pulse 92, and respirations 22. She looked "toxic" and dehydrated. There were sordes about the gums, and her tongue was dry and red. There was a diffuse purpuric rash on the trunk and limbs, particularly marked on the forearms, which had almost a flea-bitten appearance. In addition, there was a small bruised area on the sacrum and some discoloration over both buttocks. A cough was present: the chest moved fairly well; the note was good, but scattered rhonchi were audible in the left lung. The apex beat was in the sixth intercostal space, about one inch outside the mid-clavicular line, the cardiac impulse diffuse and forcible. There was a loud blowing systolic murmur over the mitral area conducted outwards for a short distance. The pulse was rapid, regular, and raised in tension. The liver and spleen were not palpable. The urine contained a heavy cloud of albumin, but no sugar or blood. The pupils were dilated and sluggish; there was asymmetry of the face; the left side was paretic, and occasional irregular twitchings of the right facial muscles,