INTERN

PDF M504438

Medische Bibliotheek, Erasmus MC Rotterdam. Tel: 010-4087782

Gegevens aanvrager / verzendadres
Ref:

W.B. van Leeuwen
MMIZ unit R&D
L253a
Erasmus MC
Dr Molewaterplein 40
3015 GD Rotterdam

Tel: 010 4633668
Fax:
E-mail: w.vanleeuwen@erasmusmc.nl
Budget: **2245**

Aanvraag kopie / Boek te leen

ISSN p/e: **0140-6736 / 0140-6736** MBSN:
Titel: Lancet
Jaar: 1932
Volume: 1
Aflevering:

Auteur: Panton P.N., Valentine F.C.O.
Artikel: Staphylococcal toxin
Pagina's: 506-508
PMID:
Opmerking: PVL

Subject: Normal electronic delivery
Date: Tue, 10 May 2005 14:17:13
From: w.vanleeuwen@erasmusmc.nl

Nota adres (indien anders dan verzendadres)
W.B. van Leeuwen Erasmus MC
MMIZ unit R&D
Dr Molewaterplein 40
3015 GD Rotterdam

Aktie: Levering uit MBTSZ collectie

A07059979

3

STAPHYLOCOCCAL TOXIN.

By P. N. Panton, M.B. Camb.,
DIRECTOR OF THE HALE CLINICAL LABORATORIES, LONDON HOSPITAL;
AND
F. C. O. Valentine, M.R.C.P. Lond.,
ASSISTANT DIRECTOR OF THE LABORATORIES.

THE hæmolytic and leucocidal actions of staphylococcal toxin have been recognised for many years. Denys and Van de Velde [1] writing in 1895 described the destructive effect upon the leucocytes in the pleural cavities of rabbits following the injection of staphylococci, as well as the appearance of anti-leucocidin in the serum of immunised rabbits. The hæmolytic action of the cocci on blood-plates was noted by Kraus [2] in 1900, and in 1922 an important paper on the subject was published by Julianelle,[3] who demonstrated that the hæmolytic titre did not correspond either to pathogenicity or to leucocidin production. More recently Burnet [4] has added to our knowledge of the toxins and in particular to their mode of production in vitro. Parker Weld and Gunther [5] have described methods of separating the different components of the toxin.

The present paper deals with an investigation, both quantitative and qualitative, of the toxins obtained from 22 strains of staphylococci derived from a variety of human lesions. Our object has been to examine the relative importance of the different toxin constituents in human pathology.

The toxic properties examined were the following: hæmolytic, leucocidal, necrosing, and lethal.

TECHNIQUE AND RESULTS.

1. *Toxin preparation.*—This was obtained by Burnet's method and as previously described by us.[6] After final sterilisation through a Seitz filter the pH was brought to 7·6–7·2, a few drops of chloroform were added, and the surface of the filtrate covered with a layer of sterile liquid paraffin.

2. *Hæmolysin tests.*—Three tubes were put up for each filtrate, giving final dilutions of 1 : 50, 1 : 200, and 1 : 800. Washed rabbit cells were used in final 1 per cent. dilution. After mixing, the tubes were left in the incubator for one hour, reshaken, left on ice over night and read next morning.

3. *Necrosis of rabbit's skin.*—Intradermal doses of 0·2 c.cm. were given. Strongly hæmolytic filtrates used in 1 : 20 dilution commonly gave an area of necrosis 1·5 to 2 cm. in diameter, and this was taken to indicate a strong necrotoxin. Feebly hæmolytic filtrates were used in 1 : 5 and 1 : 20 dilutions, the former being usually feebly positive and the latter negative, indicating a weak necrotoxin. When the 1 : 5 dilution was negative a subsequent test was made with undiluted toxin. Fresh rabbits were always taken and up to six filtrates were tested simultaneously on the same animal. We believe that the results indicate adequately whether a weak or strong necrotoxin was present.

4. *Lethal action.*—This was investigated with four toxins only, Nos. 2, 3, 4, and 6. Young rabbits each weighing 1 kg. were used and a preliminary injection of 0·5 c.cm. was made into a vein. If death did not occur within a short period, further injections were made.

5. *Leucocidin.*—Human blood-cells obtained by venepuncture were washed in saline, a narrow centrifuge tube being used for the final washing in order to concentrate the leucocytes. The toxin, adjusted if necessary to pH 7·7·2, was diluted with saline to 1 : 2 and 1 : 8. Equal volumes of cells and filtrate were incubated for one hour in a Wright's pipette. The contents, after a thorough washing in and out of the pipette, were mixed in the proportions of 2 volumes of the toxin cell mixture with 1 volume of fresh human serum and 1 volume of a staphylococcal suspension. After 20 minutes' incubation the contents were again washed out, the drop thoroughly stirred with a platinum loop, and a film made from a loopful of the mixture and stained with Leishman. The addition of the phagocytic mixture was continued because it provided an additional functional test for the phagocytes and because it was found that this extra dilution of the cells led to more satisfactory films. The use of the platinum loop was adopted because the neutrophils tended to form small clumps, and it was felt that the loop would demonstrate the presence of these clumps more certainly than the usual method of drawing out a blood-film. With some filtrate mixtures after incubation, small masses tending to block the pipette were observed. These clots were caught whenever possible and rubbed out into films for examination.

In the control films many phagocytes were present, often in clumps. With a strong leucocidin (marked A in the Table) all the phagocytes were destroyed in both the dilutions 1 : 4 and 1 : 16 which were employed for the routine test. Well-stained lymphocytes were present. Such toxins usually produced clots, films of which showed masses of almost homogeneous basophil cell debris entangling normal lymphocytes. If this phenomenon occurs in vivo, it supplies a possible explanation of the septic emboli so commonly associated with staphylococcal septicæmia. Filtrates marked C in the Table appear to have no destructive action on the leucocytes in either dilution. B indicates a weak leucocidin with partial or almost complete destruction at 1 : 4 and very little action at 1 : 16. B filtrates sometimes produced clots which were found to contain many intact polymorphonuclears. The red cells, except in the case of one toxin, No. 7, were unaffected.

The Table indicates the amount of each type of toxin in the various filtrates: A represents a strong toxin; B a feeble one; and C almost complete absence of the toxin. In the fourth column an attempt is made to indicate in a similar manner the severity of the human lesion from which the strain of cocci was obtained: A represents a severe lesion such as osteomyelitis or large carbuncle, and A+ a case in which there was evidence of pyæmia; B indicates a small local lesion such as a boil or stye; and C a purely superficial infection such as sycosis barbæ.

Strain.	Hæmolysin.	Necrotoxin.	Leucocidin.	—	Human lesion.			—
					Sex.	Age.	D.*	
1	A	A	A	B	M.	32	—	Boil on buttock.
2	A	A	B	C	M.	30	—	Sycosis barbæ.
3	B	B	A	A	F.	46	+	Severe carbuncle of neck.
4	B	B	A	A+	F.	13	—	Fatal infection of lip.
5	C	C	C	A	M.	4	—	Osteomyelitis of tibia.
6	A	A	C	C	M.	2	—	Skin eruption on face.
7	A	A	C	B	M.	52		Prostatitis.
8	A	A	A	B	M.	49	—	Small carbuncle of wrist.
9	B	B	A	A	M.	21	—	Severe carbuncle of neck.
10b	A	A	B	C	M.	35	—	Sycosis barbæ of 15 years' history.
10r	C	C	C	C				
11	B	B	A	A+	M.	17	—	Three years' pyæmia following bone infection.
12	A	A	B	A	F.	55	+	Carbuncle; septicæmia two years before.
13	B	B	B	B	F.	19	—	Severe infected hand.
14	B	B	A	A+	F.	15	—	Osteomyelitis of humerus and empyema.
15	A	A	C	A	M.	5	—	Acute osteomyelitis with rapid improvement.
16	A	A	A	B	M.	19	+	Nasal furuncle following many small infections.
17	B	A	A	B	F.	46	+	Stye following carbuncle in Case 3.
18	A	A	C	C	F.	28	—	Sycotic condition on leg.
19	B	B	B	A	M.	13	—	Empyema following wound of thorax.
20	A	A	B	A	M.	65	+	Severe carbuncle.
21	A	A	B	A	M.	14/365	—	Fatal abscess of back, possibly after injury.
22	B	A	A	A+	F.	21	—	Fatal infection of lip.

D*= diabetes.

Analysis of the Table shows that the hæmolysin and necrotoxin vary together on the whole, but that slight discrepancies may occur, as with Nos. 17 and 22. Both of these gave weak hæmolysin, but the necrotoxin had to be labelled A, though the reactions were definitely feebler than the majority in this class. These slight differences tend to support Parker Weld and Gunther in their contention that the hæmolysin and necrotoxin are distinct.

FOUR GROUPS OF STRAINS.

Comparison of the hæmolysin and leucocidin permits a division of the strains investigated into four groups:—

1. Those with high leucocidin and low hæmolysin.
2. Those with high leucocidin and high hæmolysin.
3. Those with low leucocidin and high hæmolysin.
4. Those with low leucocidin and low hæmolysin.

It is advantageous to compare these groups with the types of human lesions from which the strains were derived.

Group 1 consists of seven strains (Nos. 3, 4, 9, 11, 14, 17, 22) with strong leucocidin and weak hæmolysin. All these strains were obtained from severe lesions, the only exception being No. 17, which was grown from a small stye, following a carbuncle from which No. 3 was obtained, and it is to be presumed that the two strains were identical. All the four strains from pyæmic cases fall in this group and include two strains from rapidly fatal cases of carbuncle of the lip. The association therefore of this class of toxin with virulent disease in man is remarkably constant.

Group 2 comprises three strains (Nos. 1, 8, 16) rich both in leucocidin and hæmolysin. Two were derived from a common, small single boil, and one from a small carbuncle, of short duration, which yielded rapidly to treatment. The few strains of this group in our Table may be due to the fact that these were the only cultures examined from this common and perhaps most typical staphylococcal lesion in man.

Group 3.—Strains with weak leucocidin and strong hæmolysin (Nos. 2, 6, 7, 10D, 12, 15, 18, 20, 21). These strains were obtained from lesions of very different severity but some classification is possible. The most definite finding is that all the sycosis cases fall within this group—namely, 2, 6, 10D, 18. No. 7 may well be a secondary infection; No. 15 came from a case of acute osteomyelitis which cleared up rapidly after operation and without complication. No. 21 was obtained from a baby of 14 days with a strong suspicion of injury and presumably poor resistance. Nos. 12 and 20 both came from carbuncles in elderly diabetics.

Group 4.—Strains weak both in leucocidin and hæmolysin (Nos. 5, 10P, 15, 19) have some clinical evidence of a saprophytic origin; 10P grew in pale colonies and was isolated from strain 10D which yielded a strong hæmolysin. Nos. 15 and 19 both came from infected wounds, one of the hand, the other of the thorax. No. 5 strain is unique in our experience in that it produces no hæmolysin at all and no detectable leucocidin. It was derived from an osteomyelitis lesion in a child of 3 years. It is difficult to account for the appearance of this strain and its inclusion in this group. A streptococcus was subsequently isolated from the pus of the case, but the staphylococcus was obtained twice from earlier specimens in apparently pure culture.

The lethal action for rabbits was tested in the case of four toxins only. Nos. 6 and 2 were chosen as being strongly hæmolytic and feebly leucocidal, Nos. 4 and 3 as being of the reverse order; 0·5 c.cm. of the hæmolytic toxin No. 6 was given to two rabbits, the first died in three minutes, the second showed symptoms, but recovered and was given a further 1 c.cm. two hours later. Death was almost instantaneous. The other hæmolytic toxin No. 2 killed a rabbit in two minutes. Leucocidal toxin No. 4 was given to two rabbits in doses of 0·5 c.cm., followed two hours later by 1 c.cm. and 30 minutes later by a second 1 c.cm., without effect. Similarly toxin No. 3 showed no lethal action.

It appears therefore that the lethal action of a toxin for rabbits runs parallel with the necrosing effect on the rabbit's skin and the hæmolytic titre for rabbit red cells, but is distinct from the leucocidal action on human white cells.

Cultural characters.—The separation of 22 strains of staphylococci into four groups on the basis of toxin production suggested the possibility that cultural differences might be detected for the groups. The full cultural characters of 16 strains were investigated. In the fermentation reactions of the 13 carbohydrates selected all showed complete uniformity. A few strains acidified but failed to clot milk, and three strains (Nos. 7, 8, and 18) failed to liquefy gelatin. There was thus no association between cultural characters and toxin production. The peculiar strain No. 5 which yielded no toxin gave typical cultural reactions throughout.

DISCUSSION.

Antitoxin production.—In a recent paper with Mr. V. W. Dix the use of an antitoxic serum prepared for us by Dr. G. F. Petrie was described. This serum was derived from a horse inoculated with a single strain toxin of high hæmolytic titre. It was shown to have the power of neutralising both the hæmolytic and necrosing properties of the toxin. The toxin was that derived from strain No. 1 in the Table, and was subsequently found to have a high leucocidal titre also. Titration of the concentrated antiserum against normal horse serum showed an anti-leucotoxic power of 20 to 1. The selection of this toxin for immunisation was thus, in part, a matter of chance. It is, at least, suggested that the most important toxic fraction in severe human lesions is the leucocidal, and it is possible that a more potent serum might be obtained from the use of a Group 1 rather than a Group 2 toxin.

In our description of the toxic action upon the leucocytes we have suggested that the tough macroscopic clots found in vitro may be a source of pyæmic emboli in vivo. Dr. Petrie's antitoxic serum seemed most efficacious in the pyæmic cases and we noted at the time the complete cessation of metastatic abscess in all the cases so treated.

Constancy of toxin production.—The 22 staphylococcal strains have been divided into groups on the basis of the proportional production of hæmolysin, with which are associated necrotoxin and lethal toxin, and of leucocidin. Only four main divisions are possible and all are represented in our strains. It can be said therefore that the staphylococcus may put out much or little hæmolysin or leucotoxin or both, and any classification on these lines would be without meaning unless these outputs are constant for the strain. We believe that toxin production by the strain is constant within such limits as we have been able to examine them. The majority of these strains have been isolated for some months, from many of them fresh toxins have been periodically prepared on different occasions and no serious alteration in the

relative proportion of hæmolysin to leucocidin has ever been proved. The peculiar strain, No. 5, which grows in unusually small colonies on agar (though in its general cultural properties identical with the other strains) was twice isolated from the same patient. The Group 1 strain No. 3 isolated from a carbuncle was again obtained from a stye in the same patient four months later. The association of certain classes of toxin producers with certain types of human lesions is, we think, as close as could reasonably be expected in view of the comparative crudity of our measurements and of variations in the resistance of the individuals affected, and such association supports the belief that toxin production is a constant, at any rate for the period of infection. Clinical instances also occur to us of the accidental transfer of similar staphylococcal lesions from one person to another, for example, a nursemaid with a stye transferring to a child an identical lesion or a small superficial boil.

Toxin and cell species.—A survey of the literature shows that, in estimating toxic action, the animal species from which the test cell was derived seems to have been considered a matter of indifference. Rabbit red and white cells were employed by Neisser [7] and Wechsberg, and subsequently by others, while Julianelle [3] compares the lethal action on mice or rabbits, the hæmolytic titre for horse red cells, and the leucocidal action upon guinea-pig cells. We find that toxins with a high titre for rabbit cells commonly have a lower titre for sheep cells, and may have no action upon human cells. The titre for rabbit cells often reaches 1 in 800, that for human cells rarely attains 1 in 5. Previous saturation of a toxin strongly hæmolytic for rabbit and inactive for human red cells with human cells does not reduce the rabbit cell titre. It is true that a clear zone often appears around staphylococcal colonies on human blood-agar plates, but only one of our 22 strains yielded a titre for human cells of any strength. This toxin (No. 7) gave incomplete hæmolysis throughout with a trace up to 1 in 800 and saturation of this toxin with human cells did not seem to affect the subsequent titre for rabbit red cells. It appears that human and rabbit red cell hæmolysins are distinct, and that staphylococcal toxin rarely acts upon the human red cell in vitro. The leucocidin was tested against human cells, but in some instances was tried against rabbit white cells also. It is difficult to compare these results; the human cell experiments were clear-cut, the rabbit cell results may have been prejudiced by the hæmolysis which was nearly always present, and by what appeared to be the necrosing action of the toxin imposed upon the leucocidal action. It appeared that toxin leucocidal for human cells destroyed rabbit cells also, but toxins with low titres for human cells had considerable action upon rabbit cells. There was some histological suggestion that the main effect of the latter toxins was rather upon the cell membrane than a disruptive action upon both nucleus and cytoplasm as would seem to be the case with the Group 1 toxins.

TREATMENT.

The association of certain combinations of toxins with certain types of staphylococcal infection suggests differences in the specific treatment of these conditions. We have found the employment of an antiserum to be chiefly efficacious against pyæmic infections and have already indicated our belief that the important antibody in the serum is probably the anti-leucocidin. The sycosis cases were, on the contrary, all associated with a strong hæmolysin and a weak leucocidin. These cases notoriously respond badly to vaccine treatment and are sometimes made worse by it. There is also some suggestion that they do worse with an autogenous than with a stock vaccine. In these infections the hæmolysin may be associated with the sensitising factor to which we have previously drawn attention.[8] It appears to us that treatment with toxoid in these and possibly in other staphylococcal lesions is more rational than treatment by vaccine, and that in the sycosis cases the toxoid should contain leucocidin and possibly the minimum amount of hæmolysin. We have treated only two such cases but both were carefully selected as examples of extreme chronicity, widespread lesions, and resistance to every form of treatment. The result in both cases has been sufficiently encouraging to test the method further. Immunisation by toxin or toxoid is, in theory, preferable to vaccine therapy. The vaccine dose comprises a roughly calculated number of dead bacteria, and a very uncertain amount of toxins, mixed in quite unknown proportions. The dose of toxoid can be accurately measured and by a choice of toxin the relative proportions of the different constituents can be chosen at will. The comparative values of toxin and vaccine treatment can only be determined by thorough clinical trial.

SUMMARY.

(1) Toxic filtrates have been prepared from 22 strains of *Staphylococcus aureus*. Their capacity to hæmolyse rabbit red cells, necrose rabbit's skin, and destroy the leucocytes in human blood has been estimated. (2) The results confirm the findings of others, that the hæmolysin and necrotoxin vary closely together with no relation to the leucocidin. (3) The lethal action for rabbits, when investigated, was found to vary with the hæmolysin content of the filtrate. (4) Comparison with the human lesions indicates that strains yielding strong leucocidin and weak hæmolysin are commonly associated with acute severe infections and the reverse combination with long-standing superficial infection of the sycotic type. (5) Up to the present no evidence of variation in the qualitative production of toxins by the strains has been obtained. (6) Suggestions as to possible lines of treatment are discussed.

REFERENCES.

1. Denys, J., and Van de Velde, H.: 1895, Cellule XI., Fasc. 2, 350.
2. Kraus, R.: Wien. Klin. Woch., 1900, xiii., 49.
3. Julianelle, L. A.: Jour. Infect. Dis., 1922, xxxi., 256.
4. Burnet, F. M.: Jour. Path. and Bact., 1930, xxxiii., 1.
5. Weld, J. T. Parker, and Gunther, A.: Jour. Exp. Med., 1931, liv., 315.
6. Panton, P. N., Valentine, F. C. O., and Dix, V. W.: THE LANCET, 1931, ii., 1180.
7. Neisser, M., and Wechsberg, F.: Zeit. f. Hyg. u. Infectionskrankh., 1901, xxxvi., 299.
8. Panton and Valentine: Brit. Jour. Exp. Path., 1929, x., 257.

LUNACY IN THE IRISH FREE STATE.—According to the inspection report submitted to the Grangegorman Mental Hospital committee there is a continuous increase in the number of persons in the Free State of unsound mind; the two institutions of Grangegorman and Portrane contain 3750 patients from the counties of Dublin, Louth, and Wicklow. The report continues: "Statistics for the Free State show that one in every 200 of the population is a patient of a public mental asylum. In addition, there are a large number in private asylums, workhouses, and hospitals, and a large number are hidden away in private homes. Add to this the number of nurses, attendants, and other officials engaged in the work of these asylums, and you will approach closely to a figure of one in every 100 of the population not only dead to the State, but actually a burden to the State. A figure of one in every 300 would be a more normal level." Bad housing is suggested as one of the causes of mental disorder.