INVESTIGATIONS CONCERNING THE VALUE OF THE MICRO-SCOPIC EXAMINATION OF THE BLOOD FOR BACTERIA.

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The following investigation was undertaken at the suggestion of Dr. Libman, to determine, if possible, in what percentage of eases in which blood cultures were positive, bacteria could be found by direct microscopic examination, and also to determine whether organisms were present microscopically in cases in which blood cultures were negative. Altogether 250 cases were studied. The method followed was a combination of those used by previous investigators.2 We used from 1 to 2 e.e. of blood, which was obtained at the same time that the blood was drawn for cultural purposes, and poured it into 20 c.e. of sterile 1 per cent. aqueous solution of sodium eitrate, thus preventing eoagulation, The eitrate solution as well as all the stains and fluids that were used were passed through a Berkefeld filter in order to remove all contaminating baeteria and granules. The blood was then centrifuged for at least a half hour, and a few drops of the sediment were drawn up into a sterile pipette and deposited on from two to three chemically clean slides. The latter were kept in alcohol until within a few minutes before they were used. The smears were then made and fixed by heat. The blood was then laked with sterile filtered water, after which a 1 per cent. solution of acetic acid was poured over the slides to complete the laking. This was washed off and followed by a routine Gram stain. Various other stains-Giemsa, Unna's alkaline methylene blue. Loeffler's methylene blne, Jenner, fuelisin, and methyl-green-pyronin-were tried, but we found that the Grain stain gave the clearest fields for examination. From one-half to two hours were spent in examining each speeimen.

Our method gave better results than that used by Staeubli, viz., collecting the blood directly in 3 per cent. acetic acid, because the fibrin in the sediment interfered with the clearness of the microscopie pietures.

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² Staeubli, Beiträge zum Nachweis v. Parasiten in Blut, Münchener med. Wuch., 1908, vol. ii; Rosenberger, The Presence of Tubercle Bacilli in the Circulating Blood, AMER, JOUR, Mrn. Sct., February, 1909.

In cases in which the blood cultures showed the presence of Gram positive cocci, we could, with very few exceptions, and without any knowledge of the result of the blood culture, demonstrate the presence of the organisms within from one to two hours after the blood was withdrawn. By referring to the table, it will be seen that in 97 per cent. of the cases in which the blood cultures were positive, the hlood smears were also positive. Frequently the number of bacteria seen on the slide was evidently larger than the number found enlturally.

In 12 cases (see appended lists) bacteria were found on the slides although the cultures remained sterile. Of these ,5 were cases of lobar pncummia, in which one might well expect to find bacteria in the circulating blood. In these smears the cocci which were found appeared as typical lanceolate diplococci. In one case of thrombosis of the lateral sinus the culture remained sterile, but numerous Grampositive cocci were found nn the slides. We might also mention that in 2 cases of acute articular rheumatism organisms resembling streptococci were found in the smears. The only explanation we can offer for negative cultural findings is either inadequacy of methods or devitalization of the organisms due to the bactericidal power of the blood. The results in the cases of rheumatism can be accepted only if confirmed in a large series of eases.

A series of experiments was tried on rabbits in order to obtain comparative results between cultures and spreads. By inoculating rabbits intravenously with varying quantities of cocei we attempted to determine:

1. The maximum length of time after inoculation at which the organisms could still be demonstrated in the blood.

2. The relationship between the number of colonies to 1 c.c. of blood in agar plates, and the number of bacteria found in spreads microscopically.

EXPERIMENT 1. Rabbit. Intravenous injection of 2,000,000,000 Staphylococcus aureus (counted by the Wright method).

After thirty minutes, S e.c. of blood aspirated from heart, 4 c.c. plated, 2 c.c. in each agar plate, and 4 c.c. collected in sodium citrate solution. Result: Forty colonies to 1 c.c. of blood were counted in the plates after twenty-four hours, and numerous coeci were found easily in the smears.

After twenty-four hours, 5 c.e. of blood were aspirated, 3 c.e. were plated, and 2 c.e. collected in citrate. Result: 1 colony to 1 c.e. of blood on the plates, and many cocci found easily in spreads.

After forty-eight hours, 10 c.c. of blood aspirated, 5 c.c. plated, and 5 c.c. collected in citrate. Result: Plates and spreads negative.

After seventy-two hours, animal killed. Cultures from liver and kidney showed Staphylococcus aureus, those from the glands and bone-marrow were negative. The sections showed multiple abscesses in the kichey with numerous Grant-positive coeci, also a moderate number of coeci in the liver capillaries.

EXPERIMENT 2. Rabbit. Intravenous injection of 1,000,000,000 staphylococci.

After ten minutes, 4 c.c. of blood uspirated from heart, 2 e.e. plated, and 2 e.e. collected in citrate. Result: 350 colonies to 1 c.e. of blood in the plates, and many coeei found easily in sprends. Animal died after aspiration. Postmortem examination negative.

EXPERIMENT 3. Rabbit. Intravenous injection of 2,500,000 streptococci.

After fifteen hours, 5 e.e. of blood aspirated from heart, 3 e.e. of blood plated, 1.5 e.e. in an agar plate, and 1.5 e.e. in a plate of 2 per cent. glucose-agar; 2. e.e. collected in citrate. Result: Plates sterile. A few diplococci found in spreads.

EXPERIMENT 4. Rabbit. Intravenous injection of 35,000,000 staphylococei.

After fifteen minutes, 4 e.c. aspirated from heart, 2 c.e. of blood plated, and 2 e.e. collected in citrate. Result: Eight colonies to 1 e.e. on plates, and cocci found easily in sprends.

From these experiments we couclude that:

1. Bacteria can be found in the spreads when the cultures remain sterile (see Experiment 3).

2. The number of bacteria seen on slides was greater than the number of colonies grown on the plates (see Experiment 1).

3. Many more bacteria circulate in the blood than can be grown culturally, or seen microscopically.

The morphology of the bacteria as seen in the spreads was not always distinctive, but, as a rule, the streptococci were smaller than the staphylococci, and were seen to occur in diplococcus forms, rarely in chains. The staphylococcus appeared singly or in clumps. Identification of the organisms was verified by careful study of the blood cultures.

					ta)	SII.	r pto	cocc	em.	α.			
Diagnosis,											No.	of cases.	Positive re-ults
pacterial endocardity	5		٠		•							5	
Sinus thrombosis											•		1
Punethronia		•	•	•	•	•	•	•	•	•	•	2	2
	•	•	•	•	٠		٠					5	5
Osteomyentis	•											5	e e
Erysipelas									-	-			
Postsborting summert:	÷			•	•	•	•		•	•	•	1	1
C in the sector of the sector of the	11114	-110	n.	٠	•				٠		•	4	4
Centurus of hand .	•	•	•									1	
Pneumonia: pericardi	tis -						-	-	•	-	•	-	
Propressingly		•	•	•	•	•	•	•	•	•	•	1	1
a Johnenmonnorax	•	•	•	•	•	٠	•	٠	·	٠	•	2	2
Total												-	-
10(a)	•	•	•	•	•	•		•		-		26	25

LIST I .- Cases with Positive Blood Cultures,

(b) Staphylococcemia.

Aleningius .	1
Osteomyelitia	
Infected hemorrhouls	-
Pyelonephritia	1
	1
Total	-
10141	7

LIST II.—Miscellancous Cases with Positive Findings in Spreads and Negative Blood Cultures.

Diagn	osis	•																2	ю.	of cases	5.
Lonar pneun	ioni	3	•																	5	
Sinus throm!	nosi														•	•	•	•	•		
Acute articul	ar i	he	ama	atisi		:	1		·	•	•	•	•	·	•	·	•	•	٠	1	
Postparture			1			•	•	•	•	•	•	•	•	•	•	•	•		•	2	
Enddoord - ba				ecu	un	٠	•	٠	٠	٠	٠	٠	٠		٠	•	•		•	2	
Chigarat and	ceas		•	•	٠	٠	٠	٠	•	٠	•	•	•	•		•				1	
inter abscess	۰.	·	٠	·	٠		٠	•	٠	٠	٠	٠	٠	•	•	•				I	
T																				-	
TOTAL .	•	٠	٠	•	٠	٠	٠	٠	٠	•	٠	٠	٠	٠						12	

LIST	III.—Cases	with	Negative	Blood	Cultures	and	Negative	Microscopic
				Findle				•

D ¹											· .									
inagn	0515.																		cn.	of cases
Local infectio	ns																	•	••••	70
Appendicitis						÷.		-			•	•	•	•		•	•	•	•	
Mastoiditis	÷.				•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	8
Sinns through		·	•	· · ·			•	•	•	•	•	•	•	•	•	٠	•	٠	٠	8
C the theothe	~ 253		щ	2.40	otat	(13	٠	٠	•	•	•		•	•						2
Utitis media		•																		1
Puerperal inf	ectio	n											•	•	•	•	•	•	•	1.
Peritonitis			2				•	•	•	•	•	•	•	•	•	•	•	•	•	10
Liver absense	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	٠	٠	٠	٠	2
ALL ADACCES		•	•	•	•	•	•	•	•	•	•	•	•	•	•					5
Cholecystitis	•	•	•	•																3
Pyelitis .			•												-			•	·	
Erysipelas									-				•	•	•	•	•	•	•	:
Chronin and		1.1.					•	•	•	•	•	•	•	•	•	•	٠	•	٠	
11-100 11		uus		•	•	•	٠	•	•	•		•	•	•		•		•	•	25
Durit & mechanic		•	•	٠	•	٠	•													5
Influenza (un	relia	ible	ofi	tare	ou	11 0	t Gi	sm	-ne	cati	ve i	nd -	a –					•		
Acute articul	ar rl	ieu	hia	tiar	n								~	•	•	•	•	•	•	
Gonorrheal a	rthr	1114			-	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	4
1 million in			•	•	•	•	•	•	•	•	•	•	٠	٠	•	٠	•	٠	٠	2
izukenna .	•	•	•	•	٠	•	•	•	•	•	•	•	٠	٠	•	•	-			2
-																				
Total .	٠	•	•	·	٠	٠	٠	٠	•	٠	•	•	•		•	•	•			120

Besides the cases already discussed, we studied 53 cases of typhoid fever. The method which we employed was found to be useless for this purpose, as Gram negative rods were found not only in the blood of eases of typhoid fever, but also in a large majority of spreads made from the blood of culturally positive streptococcic and staphylococcic eases, and also from the spreads made from many of the eases in which there was no bacterienia. We can offer no explanation for the presence of these rods, nuless possibly that they are hemokonia. We therefore tried to find typhoid bacilli with the ultramicroscope by examining a drop of the sedimented blood, proved to be impracticable on account of the difficulty of distinguishing the bacteria from hemokonia.

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In some cases we poured the greater part of the sediment into a tube of bouillon, and placed it in the thermostat for from five to seven hours. The bouillon culture was then centrifuged, and slides were stained for microscopie examination. The number of Gramnegative bacilli in these cases, which were positive culturally, was so large that one could not mistake them for the Gram negative rods seen in small numbers in cases that were not instances of typhoid fever.

In this connection it is evident that because of the presence of Gram-negative rods the method used by us would be impracticable in examining the blood of any case in which the presence of Gramnegative bacilli was suspected. This would, of course, include cases of influenza, and, in view of our findings, we are unable to accept the results published by Canon.

CONCLUSIONS. The studies which we have here outlined will be contioued in the laboratory to see if further data can be obtained. At the present time we can draw the following conclusions:

1. Bacteria can be found microscopically in the blood of the large majority (97 per cent.) of cases in which their presence has been demonstrated by blood culture.

2. In a number of the cases (12 in 132, about 0 per cent.)³ bacteria were demonstrated in the blood which had been found sterile by means of blood eultures taken according to the methods now in vogue in our laboratory. Further studies must be made to determine whether there is any error in our findings.

3. It is probable that in cases of sinus thrombosis and in cases of osteomyelitis, microscopie examination of the blood may be of service in coming to a more rapid conclusion as to the presence of a bacteriemia.

4. Unless all possible precautions are followed in regard to asepsis, and unless all fluids and stains are filtered, the method is unreliable.

5. Microscopic examination of the blood with all the methods that have so far been used is not of service in cases in which the presence of Gram-negative organisms is suspected.

6. Mieroscopie examination of sedimented bouillon eultures may be of value in making an earlier diagnosis in some eases of typhoid fever.

* This includes all the cases with negative blood cultures except the cases of typhoid fever.