THE BACTERICIDAL ACTION OF LYMPH TAKEN FROM THE THORACIC DUCT OF THE DOG.

By S. J. MELTZER, M. D., and CHARLES NORRIS, M. D.
(From the Physiological and Pathological Departments of the College of Physicians and Surgeons, Columbia University, New York.)

Nine years ago Nuttall* first described the bactericidal action of the blood, suggesting at the same time that the other fluids of the body may possess a similar action. He found later that the aqueous humor, pericardial fluid, and a pleuritic fluid poor in cellular elements also possessed bactericidal power. Prudden† determined that ascitic fluid, fluid taken from a hydrocele, and the liquor amnii of the pig had the same properties.

As far as we have been able to ascertain, no systematic examination of all the body fluids, in regard to this point, has ever been made.

Flexner‡ found that the blood serum of the human placenta was not distinctly germicidal for the Staphylococcus aureus, but was germicidal for the Bacillus typhosus. Caution in drawing conclusions as to the germicidal action of body fluids not hitherto examined is manifestly necessary. A controversy having lately arisen as to the channels of absorption of serous fluids, it seemed to us a matter of importance to determine whether lymph from the thoracic duct possesses bactericidal powers. The possibility was present that an exchange of the bactericidal factors between the blood and lymph—lymph from the interstitial spaces—might occur only through the walls of the blood-vessels. Lymph from the thoracic duct might not therefore possess any action. At the beginning of our work, Professor Prudden called our attention to an article by Max Neisser,§ entitled

* Nuttall, *Zeitschr. f. Hygiene*, iv, 353.

† Prudden, On the Germicidal Action of Blood Serum and other Body Fluids. *Medical Record*, Jan. 25, 1890.

‡ Flexner, *Journal of Experimental Medicine*, i, 576.

§ Neisser, *Zeitschr. f. Hygiene*, xxii, 12.

" Ueber die Durchgängigkeit der Darmwand für Bakterien." In this article Neisser devotes barely ten lines to this question, but nevertheless comes to the conclusion that lymph from the thoracic duct of the dog possesses no germicidal action.

From a scientific point of view it seems important to determine accurately whether lymph, as well as blood, is germicidal, especially as the conclusions of Neisser upon this subject are open to criticism.

The bacteria studied by Neisser with reference to this point were Bacillus prodigiosus, Staphylococcus pyogenes aureus, Bacillus pyocyaneus, and a bacillus resembling Bacillus typhosus. Nissen* furnishes a statement showing the bactericidal power of the blood on Bacillus prodigiosus. Original plate 3000 to 4000, after three hours 2800, after five hours 10,200, after twenty hours innumerable. According to this result blood can hardly be considered to have much germicidal action on this bacillus. In regard to the Staphylococcus aureus, most authors have found that its growth is but little impaired by normal blood, but Flexner† has demonstrated that normal human blood serum may exert decided germicidal action upon this organism. Buchner‡ mentions that the Bacillus pyocyaneus and a bacillus of the typhoid group cultivated from the feces are among the most resistant of the bacteria which he studied, to the action of blood serum. There is no evidence that Neisser's bacillus was identical with that of Buchner, but it certainly would have been well to determine previously the effect of blood serum upon the former. Neisser thus employed organisms over which the blood itself seems to have little or no germicidal power. In testing the germicidal action of lymph and other body fluids, the germicidal action of the blood must be taken as a standard for comparison. When fluids are found to possess no germicidal power over organisms which are likewise resistant to blood, the conclusion is not justifiable that the former fluids possess no bactericidal action. Furthermore, Neisser thus describes his primitive methods of research:§ " Sie wurden theils direct in den Chylus verimpft, theils wurde der Nährboden mit Chylus reichlich vermengt, theils wurden steril gebliebene Versuchsplatten nachträglich mit den Bakterien bestrichen, auf keine Weise aber war eine Behinderung des Bakterienwachsthums nachweisbar." With this imperfect method of experimentation the germicidal action of blood would hardly have been discovered.

* Nissen, *Zeitschr. f. Hygiene*, vi, 488.
‡ Buchner, *Centralbl. f. Bakteriol.*, v, 821.
† Flexner, op. cit., p. 574.
§ Neisser, op. cit., p. 20.

In our investigations the typhoid bacillus was alone tested. This bacillus had been tested by most of the observers working on the germicidal power of the blood, and our main purpose was to establish the presence or absence of any such power in the lymph. The methods employed by us were those followed by Nuttall and others and the method used by Buchner.

It was essential to obtain fluid lymph, as only in this condition can it be readily handled and measured. A sterilized glass cannula bent at right angles is firmly tied in the thoracic duct. The free end of the cannula is then inserted in the neck of a bottle containing glass beads, bottle and beads both having been previously sterilized. After collecting the lymph the bottle is closed with a sterilized glass stopper and vigorously shaken for several minutes. The lymph remains fluid, is poured into test tubes and kept ready for use in an ice chest.

We will not speak of our attempts to obtain fluid lymph by injecting peptone into the circulation or by collecting the lymph in solutions of sodium sulphate or sodium oxalate.

Tying the cannula in the duct and obtaining sterile lymph constitute by no means an easy task, and several of our attempts were unsuccessful. In all our experiments here reported the lymph was proved beyond doubt to be sterile by inoculations in nutrient gelatin and agar watched for three days. We were occasionally obliged to keep the lymph a day or two before testing it. Positive results, however, gain in importance through this circumstance. All the dogs fasted from 30 to 36 hours before being operated upon. The chyle from dogs recently fed is not suitable, as the fine fat droplets interfere in counting colonies on the plates. Artificial respiration was used to stimulate the flow of lymph. In two of the dogs normal salt solution was injected intravenously, whereby a thinner lymph was obtained. In several, small doses of curare were injected. Before and after the flowing of the lymph, specimens of blood were also collected. Unfortunately we were not always able to make parallel tests.

The following results were obtained by the method developed and first employed by Nuttall. 1 cc. of the lymph is pipetted off into a

number of test tubes. 5 cc. of sterilized normal salt solution are inoculated with several loops of a typhoid bouillon culture, thoroughly shaken to insure equal distribution of bacilli throughout the fluid. A fluid gelatin tube under 40° C. is then inoculated with two or three loops of the suspension in salt solution. The gelatin is mixed and poured, this plate serving as the control. All the lymph tubes are then inoculated with the same number of loops of the salt solution and are placed in the thermostat. At the end of the allotted time each lymph tube on removal from the thermostat receives 5 cc. of fluid gelatin (under 40° C.), is then well shaken and poured into a Petri dish. All the plates are kept on a dark shelf at room temperature and the colonies are counted after three days' growth. This method is designated as Nuttall's.

EXPERIMENT F. *Nuttall.*

	Control.	1 hr.	5 hrs.	22 hrs.
Lymph	184	382	20	0

EXPERIMENT G. *Nuttall.*

	Control.	1 hr.	17 hrs.	24 hrs.
Lymph	96	67	18	5

EXPERIMENT K. *Nuttall.*

	Control.	1¼ hrs.	4 hrs.	25 hrs.	48 hrs.	72 hrs.
Lymph	127	15	0	0	0	0

In these experiments the germicidal action of the lymph is apparent. The question presented itself, however, whether the disappearance of the bacilli could not be explained by the lack of suitable nutrient material in the lymph. It might be suggested that the lymph does not kill the bacilli, but that the latter only die from want of suitable nourishment. Although this supposition is extremely improbable, we used two further media for control, one consisting of a normal salt solution containing 1 per cent of bouillon, designated as salt bouillon, of which 0.5 cc. was always used; the other known as lymph bouillon, consisting of 1 cc. of the above salt bouillon, plus 0.5 cc. of lymph.

EXPERIMENT L. *Nuttall.*

	Control.	2 hrs.	5 hrs.	25 hrs.	48 hrs.
Lymph	4130	3339	815	5130	7727
Lymph bouillon	4130	1938	1250	8650	∞
Salt bouillon	4130	5406	25440	∞	∞

EXPERIMENT M. *Nuttall.*

	Control.	2½ hrs.	5½ hrs.	24 hrs.
Lymph	1043	334	264	0
Lymph bouillon	1043	318	215	0
Salt bouillon	1043	11720	9349*	∞
Blood (after flow of lymph)	1043	114	5	140

* Hard to count, colonies spreading on soft gelatin, probably more numerous.

In both experiments growth in the salt bouillon is immediate, the plates soon becoming overcrowded, whereas in the lymph bouillon plates a steady decrease is observed. In Experiment M the 24-hour lymph bouillon and lymph plates are sterile; both series of plates are strikingly similar. In Experiment L, after 25 hours in the thermostat the bacilli begin to grow. This observation corresponds with those already made with the blood, namely, that when the germicidal factors have not been powerful enough to kill all the bacteria, the more resistant surviving forms later begin to grow, and the faster the better the nutritive media presented them. In Experiment L the growth takes place more rapidly in the lymph bouillon than in the plain lymph. The effect of the original number of bacilli on the final result should also be noted. The smaller the number of bacilli exposed to the action of the lymph the sooner are sterile plates obtained. In Experiment L, where the original showed 4130 colonies, a marked decrease alone was observed, the more resistant forms rapidly growing again.

Our next results were obtained mainly by Buchner's method. In each of three sterile test tubes 4 cc. of lymph were introduced. In order to study the effect of heat on the germicidal power of lymph we subjected one of the tubes to half an hour's heat at 56° C. All tubes were inoculated with a typhoid bouillon culture; after shaking, controls were at once made by inoculating melted gelatin tubes with three loops of the lymph. After controls from each of the lymph tubes were made, one tube was kept at room temperature, and the lymph

tube which had been heated for half an hour and the third tube were both placed in the thermostat at 37° C. From all three tubes at regular intervals gelatin tubes were inoculated with the same loop and plates poured. This method is designated as Buchner's. In most of the experiments Nuttall's method was also pursued.

EXPERIMENT N. *Buchner.*

	Control.	1 hr.	3 hrs.	5½ hrs.	24 hrs.	53 hrs.
Lymph 37°	1272	1462	445	3975	45792	∞
Lymph 22°	1481	1580	1106	636	7822	∞
Lymph (heated at 56°) 37°	973	1634	2569	7741	42930	∞
Blood serum,* ante, 37°	1284	775	362	1199	∞	
Blood serum,† post, 37°	2143	2032	1641	∞	∞	

* 4 cc. † 2 cc.

EXPERIMENT N. *Nuttall.*

	Control.	1 hr.	7 hrs.	27 hrs.	48 hrs.
Lymph* 37° C.	886	780	216	0	9471

* Seven days in ice chest before testing.

EXPERIMENT P. *Buchner.*

	Control.	1 hr.	4½ hrs.	26 hrs.
Lymph 37°	1319	550	70	78
Lymph 22°	1648	1399	455	225
Lymph (heated at 56°) 37°	2117	2308	4704	∞
Blood serum, ante, 37°	5083	2026	270	∞
Blood serum, post, 37°	2181	1461	270	∞

EXPERIMENT P. *Nuttall.*

	Control.	1 hr.	2 hrs.	5 hrs.	24 hrs.
Lymph	∞	40068	36061	5787	34344
Lymph bouillon	∞	51643	55014	27475	∞
Blood serum, ante	∞	32626	20606	18899	∞
Blood serum, post	∞	28620	21751	8295	∞

EXPERIMENT R. *Buchner.*

	Control.	1 hr.	4 hrs.	19 hrs.	24 hrs.	72 hrs.
Lymph 37°	906	340	101	196	710	∞
Lymph 22°	1484	969	466	122	89	190
Lymph (56°) 37°	975	954	1643	∞	∞	∞
Blood serum 37°	1696	1229	325	∞	∞	∞
Blood serum 22°	1240	1246	1023	405	217	∞

EXPERIMENT R. *Nuttall.*

	Control.	1 hr.	3 hrs.	5 hrs.	24 hrs.	72 hrs.
Lymph	1310	296	177	140	110	∞
Lymph bouillon	1310	334	140	66	110	6360
Salt bouillon	1310	1900	1060	7268	∞	∞

In Experiment R the lymph was obtained half an hour after the death of the dog. The lymph flowed fifty minutes with the aid of artificial respiration. The blood was obtained out of the central end of the jugular vein.

EXPERIMENT O. *Buchner.*

	Control.	1 hr.	3 hrs.	6 hrs.	26 hrs.
Lymph 37°	1368	1163	3048	16854	∞
Lymph 22°	998	1188	786	1812	∞
Lymph (56°) 37°	1717	1807	2862	∞	∞

EXPERIMENT O. *Nuttall.*

	Control.	1 hr.	3 hrs.	6 hrs.	25 hrs.
Lymph	2353	4293	∞	∞	∞
Lymph bouillon	2353	7504	∞	∞	∞
Salt bouillon	2353	2925	4897	5533	1106

EXPERIMENT O. *Buchner.*

	Control.	1 hr.	3 hrs.	26 hrs.
Blood serum 37°	909	1507	1596	∞
Blood serum 22°	1125	858	975	∞

In Experiment O the blood and lymph remained nineteen days in the ice chest before being tested.

Our results, we believe, justify the conclusion that lymph from the thoracic duct of the dog possesses marked germicidal action on the typhoid bacillus. A noticeable decrease in the number of colonies in the lymph plates is observed, the decrease being the more marked the longer the exposure to the action of the lymph.

The decrease cannot be accounted for simply on account of lack of suitable nutrient material in the lymph, and for the following reasons: The lymph bouillon plates show the same immediate decrease in the number of colonies as do the pure lymph plates, whereas the bouillon salt solution at once shows a marked increase in growth. The addition of lymph produces a direct inhibitory action on the growth of the typhoid bacillus. The germicidal action of lymph is destroyed by too long standing (nineteen days in Experiment O) and by heating for half an hour at 56° C. The lymph after such treatment furnishes a good medium. The action of the lymph is most apparent when the number of bacilli inoculated in the lymph is relatively small. (Compare Experiments F and P: in Experiment F, starting with 184, after twenty-two hours the plate is sterile; in Experiment P (Nuttall) with an

overcrowded original plate a temporary relative diminution alone is observed, growth soon beginning anew.) The quantity of the lymph must also be considered. The greater the quantity of lymph with the same inoculation the more apparent does the action of the lymph become. The bactericidal power of the lymph was rendered much more apparent by Nuttall's method than when Buchner's method was employed. In Buchner's method only two or three loops of lymph are transferred to the gelatin, and this small quantity can hardly continue to exercise any further action, whereas by Nuttall's method the dilution is only one-fifth or one-tenth of gelatin and the action of the lymph in such dilution may very well continue to retard the growth in the Petri dish.

The lymph possesses not merely an inhibitory action on the growth of the typhoid bacillus, but is definitely germicidal. We convinced ourselves of this fact by placing several of the sterile lymph plates in the thermostat. No growth was observed on any of the plates. On the other hand, we have seen lymph plates, which after 3 days' growth at room temperature were sterile, several days later develop a few small colonies, the control plates usually obtaining their full growth in 48 hours. The 5-hour plate in Experiment F furnishes an example. After 53 hours' growth it was sterile, 3 days later 20 colonies appeared. On the whole, a retardation in the appearance of the colonies on the lymph plates as compared with the control plate was always noticeable. The more resistant forms of the bacilli, *i. e.* those not destroyed by the lymph, were nevertheless inhibited in their growth. We believe, however, that the difference between partial and complete inhibition of growth is only a quantitative and not an essential one.

We do not feel justified in forming a positive opinion as to the comparative bactericidal powers of blood serum and lymph, as the number of our successful parallel experiments with lymph and blood serum has not been sufficiently numerous. The experiments here cited, as well as others not published because the lymph or blood serum was not obtained sterile, justify us in believing that the germicidal power of lymph is not much less than that of blood serum.

In one respect a difference between lymph and blood serum was

present in our experiments. The bactericidal action of blood serum diminished and disappeared sooner than did that of lymph. Perhaps blood serum furnishes a better medium for growth, and when the bactericidal property is exhausted the nutritive factors are enabled to gain the upper hand and thus determine a more rapid growth than with lymph.

During the course of our experiments the effect of room temperatures on the bactericidal action of lymph and blood serum was also noticed. Nuttall says "the temperature at which blood specimens after inoculation are kept appears, at least with rabbit's blood, between the limits of 19° and 38° C. to have little effect on the germicidal action." We have, however, obtained a different result. This is evident in a comparison of the development of the germicidal action of lymph and blood in Experiment R, where blood serum or lymph kept at room temperature developed its germicidal power more slowly but retained it longer than blood or lymph kept at 37° C.