mouths of the branching vessels, as in the thoracic region. In one of the injected rabbits and in two of the non-injected ones the changes were most profound, extending throughout the thoracic and abdominal aorta and well down into the iliacs. In these three cases large calcified bulging plaques were present, rendering the entire vessel rigid and brittle and the inner surface very uneven.

Microscopically a great variety of conditions was seen, varying on the one hand from simple degeneration of the muscle elements in small areas or bands in the media, their nuclei staining poorly or not at all, the other elements apparently being normal, to complete necrosis of all the elements, of a large portion of the media and intima with softening or calcification, at the other extreme. Apparently there results first a necrosis of the muscular elements with a tendency of the elastic elements soon to lose their normal wavy character and to appear straightened out or "stretched," as some writers have described it. Later the necrosed elements appear granular or hyaline; they may apparently undergo softening and calcification without much apparent reparative reaction, the vessel wall in this case being thinned and, if the area be of considerable size, showing

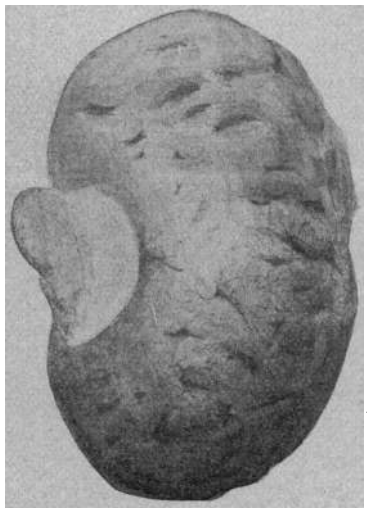

Fig. 5.—Gross drawing of arteriosclerotic atrophy of kidney, described above. Drawn by Mr. R. L. Benson; ×2.

a tendency to aneurismal bulging. Or there may occur in other cases a marked hyperplasia, resulting in a dense cellular infiltration about the necrotic area, later invading it and giving rise to new-formed connective tissue bringing about a thickening of the vessel wall. All gradations between these two extremes, and involving both the degenerative and reparative processes, were found.

The lesions apparently begin most frequently at or near the inner portion of the media, soon involving the intima, but it is by no means uncommon to see lesions in the middle or deeper portions of the media or, less frequently, involving the outer portion of the media and adventitia.[3]

ADDED NOTE BY DR. O. P. JOHNSTONE.

During the present summer quarter at the University of Chicago nine apparently normal rabbits were killed in experimental work by Dr. Wells and myself. I carefully examined the aorta of each, grossly, and three of the nine have shown decided lesions. The first one found showed many pin-point calcified lesions in the thoracic aorta, most numerous about the mouths of the branching vessels, and a few similar lesions in the abdominal aorta. All the lesions were small, raised, often somewhat angular in outline, opaque-white in color, the larger portion being calcified. The kidneys of this rabbit grossly were those of a typical arteriosclerotic atrophy (Fig. 5). On section the aorta showed lesions similar to those described by Dr. Miles, of all degrees from slight necrosis of the muscle elements to the other extreme of extensive necrosis with softening and calcification; also many lesions in which hyperplasia was the predominant feature, and others involving both processes. The kidney histologically showed the changes of an arteriosclerotic atrophy, there being very numerous areas of connective tissue hyperplasia, recent and old, and at the base of some of these areas were observed vessels with walls markedly thickened, and in one case a small vessel completely occluded by an old organized thrombus. The liver histologically showed moderate cirrhosis, although there was no gross evidence of this.

The second rabbit with lesions in the aorta showed five areas in the thoracic portion, pin-head sized, circular, raised and somewhat less translucent than the normal vessel wall. Histologically these lesions were of the hyperplastic type and involved about one-third the thickness of the media. This rabbit was about two-thirds grown.

In a third rabbit, which was about two-thirds grown, the thoracic aorta showed three lesions, the largest being two millimeters in its longest diameter by one millimeter broad, semi-translucent, raised, its surface slightly roughened and apparently of the hyperplastic type. Another lesion was circular, one millimeter in diameter, raised, surface roughened, and showed numerous opaque white pin-point sized areas as of calcification. The third lesion was a pin-point sized white, pointed elevation. They had not been examined histologically.

In a fourth rabbit no lesions were found in the aorta, but the kidneys showed the same picture grossly and microscopically as described above but not so extensive. This was also a young rabbit.

Thus three out of nine apparently normal rabbits here showed decided lesions in the aorta, and in a fourth were lesions of the kidney; these if added to the forty-nine in Dr. Miles' report would make fifty-eight "normal" rabbits, of which twenty showed lesions in the aorta, or 34.48 per cent.

3. The above work was carried out under the direction of Dr. O. P. Johnstone, Professor of Pathology, University of Colorado.

THE NEPHELOMETER:

AN INSTRUMENT FOR ESTIMATING THE NUMBER OF BACTERIA IN SUSPENSIONS USED FOR CALCULATING THE OPSONIC INDEX AND FOR VACCINES.*

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Those who have used the method of Leishman or the later method of Wright for estimating the phagocytic or opsonic indexes of the blood well know that it is essential to use bacterial suspensions containing uniform numbers of bacteria in order to secure uniformity of results. If the suspension used for one estimation contains twice as many bacteria as that used at another the results will vary, though not mathematically, according to the density of suspension.

* Read in the Section on Pathology and Physiology of the American Medical Association, at the Fifty-eighth Annual Session, held at Atlantic City, June, 1907.

The method recommended by Wright is ingenious though cumbersome and depends on the actual enumeration of the bacteria contained in the suspension, and is accomplished by mixing a given volume of the bacterial suspension with an equal volume of blood, dropping a diaphragm with a small aperture into the eyepiece of the microscope, and then counting the number of blood corpuscles and bacteria in each of a number of microscopic fields. The computation that follows is a simple one. There are 5,000,000 red corpuscles in one cubic millimeter of blood, so that if the number of bacteria equal the number of corpuscles seen there are 5,000,000 per cubic millimeter; if there are two bacteria to a corpuscle, 10,000,000, etc. Proper conditions for the enumeration are secured by diluting with salt solution, so that both bacteria and erythrocytes can be conveniently counted, the proper allowances being made in the calculation.

Though this method enables one to find out how many bacteria are contained in any given suspension, it does not afford any means for preparing a suspension that shall contain any given number of bacteria. This is a matter of little importance from Wright's point of view, as by once calculating the number of bacteria, the dosage of the particular suspension when used as a vaccine can always be easily calculated, but in case one varies the technic of making the phagocytic or opsonic

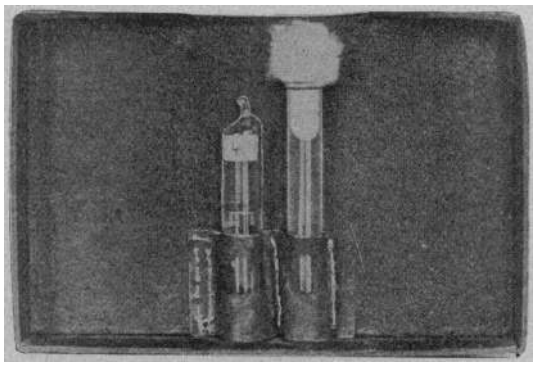

Fig. 1.—Early form of nephelometer made with a box-lid.

calculations, and especially in working with tubercle bacillus suspensions which can be kept from week to week, it seemed convenient to provide some method by which uniform suspensions could be prepared from time to time as they might be needed.

The method that suggested itself was that of using the opacity of the suspension as an index to the number of bacteria it contained and devising some standard by which this opacity could be regulated.

It was this idea that materialized in the form of the simple instrument to which the name *Nephelometer* (νεφελη the mist), has been applied.

In the simple form shown in Fig. 1 this instrument was shown at a meeting of the Society of American Bacteriologists at Ann Arbor, Mich., Dec. 28, 1905, and an account of it and its uses published[1] in 1906.

The most approved form of the instrument, and the one now in almost daily use in my laboratory, is shown in Figure 2, and is sufficiently simple to need no detailed description.

It consists essentially of a series of standardizing tubes containing a suspension of fine precipitate approximating bacterial suspensions in opacity and a holder for making their comparison easy. The standard suspensions used are precipitates of barium sulphate made as follows: Two solutions, one a 1 per cent. solution of chemically pure sulphuric acid, the other a 1 per cent. solution of chemically pure barium chlorid, are prepared, then the one added to the other so that ten standards, containing 99 c.c. of the sulphuric acid and 1 c.c. of the barium chlorid; 98 c.c. of the sulphuric acid and 2 c.c. of the barium chlorid; 97 c.c. of the sulphuric acid and 3 c.c. of the barium chlorid., etc., are prepared. According to the number of cubic centimeters of barium chlorid added, these are denominated 1, 2, 3, 4, etc. About 3 c.c. of each standard solution is sealed in a small test-tube, care being taken to use tubes of uniform diameter, and also to provide similar tubes for mixing the bacteria to be compared with the standard.

Experience has shown that Standard 3 is most appropriate for staphylococci; and that the average phagocytosis for normal human blood when the corpuscles are mixed with this suspension and incubated 30 minutes at 37 C. is 15 bacteria per polymorphonuclear leucocyte. For the tubercle bacillus the most appropriate density is No. 5, and the average normal phagocytosis 3 bacteria per cell.

If the colorless barium sulphate suspension is difficult to compare with the yellowish or brownish bacterial suspensions this difficulty may be overcome by placing a

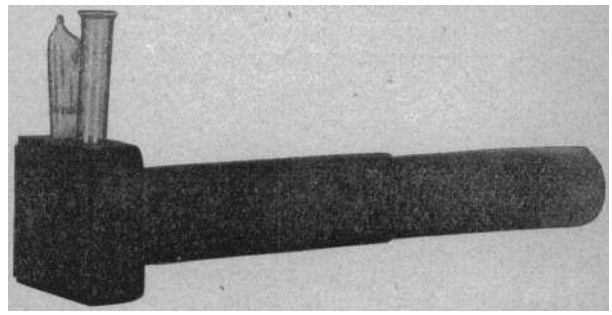

Fig. 2.—Improved form of nephelometer made with a label box and a thermometer case.

ground glass screen before the tubes and comparing the suspensions by the use of an artificial (candle) light.

The modus operandi of using the instrument is very simple. The standard tube appropriate to the experiment is selected, well shaken, stood in the left hand pocket of the instrument and a tube containing sterile salt solution stood in the other. The surface growth of the bacterium to be suspended is taken up with a platinum loop and stirred in, rubbing the bacterial mass on the side of the tube just above the surface of the liquid and gradually adding the fluid to it by tilting the tube so as to avoid suspending masses of the culture. When the mixture appears homogeneous a comparison is made by looking through the instrument against the sky. If the density be insufficient more bacteria are added; if it be too dense, fluid can be added drop by drop from a medicine dropper or, what is better, the tube can be given a few turns in the centrifuge, by which the larger clumps of bacteria are thrown down and compared again. One should not be content with the comparison until the suspension be tried with others of the standard tubes than that which one intends to imitate. It is surprising at times to see how what is thought to be an exact equivalent will turn out to be 1 per cent. more or less dense.

1. Observations on the Phagocytic Power of the Blood of Supposedly Normal Human Beings, Medico-Chirurgical College Bulletin, i, 3, 5. January, 1906, and Medicine, xii, 4, 247, April, 1906.

When the density of a ready-prepared vaccine is to be determined the procedure is varied by making the tube containing the vaccine the standard of comparison and varying the standardizing tubes until its density is fully determined.

Those who are constantly working in this field will do well to determine once for all the number of bacteria represented by each standard of density and to make a little table for future use.

A modification that is sometimes to be preferred to the fixed standards is that of comparing the new suspension with the previously used suspension until exact uniformity is secured. The disadvantage of this method is that slight variations may pass undetected unless always compared with the fixed standards, and that, if the density of the suspensions used are not measured by some fixed standards, exact repetition either by the experimenter himself or by those who try to repeat his work will be impossible.

THE SPECIFIC NATURE OF OPSONINS.*

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AND
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In the course of certain experiments it became important for us to inform ourselves as accurately as possible as to the absolute or relative specificity of opsonins, the close resemblance in nature between opsonins, precipitins, agglutinins, etc., preparing us for the statement that they are specific.

This is not, however, as yet entirely proved. Wright and Douglas and Bullock[1] seem satisfied about it, and Bullock made experiments as follows: Serum was digested with staphylococci at 37° C. (98.6° F.) and then freed of the organisms by centrifugalization, after which it was found to have lost all opsonic power for staphylococci, though it largely retained that for tubercle bacilli. Conversely, when digested at 37° C. (98.6° F.) with tubercle bacilli and then centrifugalized, it had lost the opsonin for the tubercle bacillus, though it retained it for the staphylococcus.

Potter, Ditman and Bradley,[2] however, came to different experimental results by employing a different technic. They used a pool of four normal bloods, which was divided into three parts and placed in tubes. In one tube staphylococci were thoroughly mixed with the serum; in another, colon bacilli were mixed with the serum, and the third tube was used as a control. The tubes were placed in an incubator at 37.5 C. (99.6 F.) for forty-five minutes and at the end of that time were subjected to centrifugalization at high speed for thirty to forty minutes. The serum digested with staphylococci showed an opsonic index (the control being taken as 1.0) against the staphylococcus of 0.55, against the colon bacillus of 0.12. The serum digested with the colon bacilli gave an index against the staphylococcus of 0.26, but against the colon bacillus of 1.4. From this they conclude that staphylococci take from the serum not only the opsonin for the staphylococcus but also that for the colon bacillus and vice versa.

The behavior of hemopsonins seems to be confirmatory of the specific nature of these bodies. Savtchenko,[3] Levaditi[4] and Gruber[5] all observed that the injection into the peritoneal cavity of guinea-pigs of the serum of rabbits immunized with guinea-pig blood is followed by a marked erythro-phagocytosis not only in the abdomen but also in the blood-making organs, especially the spleen, and even in the circulating blood.

Ruziczka[6] noticed the occurrence of phagocytosis *in vitro* of red corpuscles in the presence of corresponding immune serum.

Neufeld and Töpfer[7] found that the blood of rabbits immunized with goat's blood contains a substance that, after absorption by them, renders goat corpuscles subject to phagocytosis by guinea-pig leucocytes *in vitro*. This substance, which they designated as hemotropic, has no direct action on the leucocytes.

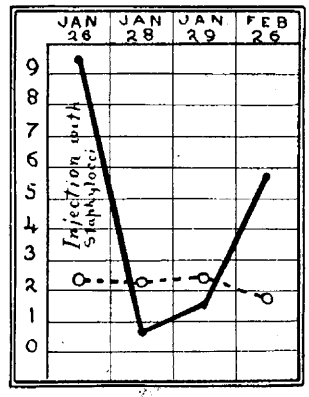

Fig. 1.—Chart showing result of former experiment. Solid line, index to staphylococcus; broken line, index to tubercle bacillus.

Hektoen[8] seems to have been the first to identify this hemotropic substance with opsonin. Barrett, Neufeld and Töpfer, and Keith[9] found the hemopsonins distinct from the hemolytic amboceptors.

The fact that it took some time to differentiate these hemopsonins from the hemolytic amboceptors shows that there are close resemblances between them, of which specificity of action is one. Hektoen[10] found that "immune hemopsonic serum may contain common or non-specific hemopsonins as well as hemopsonins directed particularly against the corpuscles employed for the injection."

We attacked this problem differently. We first determined the phagocytic (i. e., opsonic) power of a rabbit's blood against staphylococci and tubercle bacilli

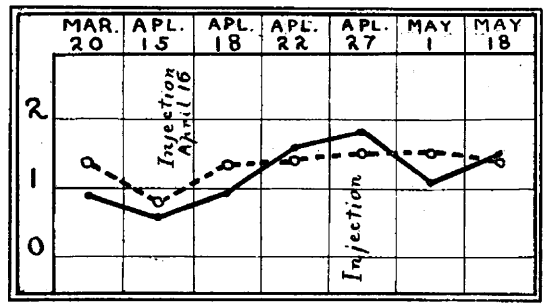

Fig. 2.—Chart showing index of both yeast rabbit and blood rabbit to yeast. Solid line, yeast rabbit; broken line, blood rabbit.

respectively and then vaccinated the rabbit with a large dose of dead staphylococci. If the result was, as we expected, a negative followed by a positive phase of opsonic disturbance for the staphylococci, without any corresponding effect on the tubercle bacilli, it would be in favor of the specific nature of the reaction. The following results were obtained:

* Read in the Section on Pathology and Physiology of the American Medical Association, at the Fifty-eighth Annual Session, held at Atlantic City, June, 1907.

1. Lancet, Dec. 2, 1905, 1603.
2. THE JOURNAL A. M. A., Nov. 24, 1906, and Dec. 1, 1906.
3. Ann. de l'Inst. Pasteur, 1902, p. 106.
4. Ann. de l'Inst. Pasteur, 1902, p. 233.
5. Wien. klin. Wochschr., 1903, xvi, 1907.
6. Quoted by Gruber.
7. Centrbl. f. Bact., 1905, xxxvii, 456.
8. THE JOURNAL A. M. A., 1906, xlvi, 1407.
9. Proc. Royal Soc. of London, 1906, lxxvii, 537.
10. Jour. Infect. Dis., Oct. 30, 1906, iii, 721.