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Specific Soluble Substances of the Pneumococcus in the Blood in Pneumonia.

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Avery and Dochez[1] detected the specific soluble substances of the pneumococcus in the urine of patients with pneumonia, and on the basis of their observations the determination of the type of pneumococcus causing the lesion in the lung may often be accomplished early in the course of the disease.

There is every reason to believe that the specific substances are brought to the kidney by the blood and concentrated in the urine. It is believed by some that the precipitin occurs in the urine only in cases with organisms in the blood. Our own observations have shown that at least the soluble antigen may be detected even where the blood culture is negative. It is of course possible that, as many believe, the pneumococci were present in the blood very early in the course of the disease and could not be found by blood culture when the patient came into the hospital. Since the specific soluble substances are so readily diffusible, it is not necessary to presuppose that organisms must be present in the blood when the soluble substances are detectable in the urine.

Since the soluble substances give a precipitate under appropriate conditions in high dilution, it might be possible to detect them early in the course of the disease and thus determine the type for serum treatment when specimens of sputum and urine are not obtained. But Blake,[2] using the untreated serum from patients with pneumonia, obtained precipitation by adding type specific antipneumococcus serum, only relatively late in the disease, on the sixth day. Out of 17 cases, he found the soluble substances in only 2 (one was a type II and the other Friedlander's bacillus infection).

By concentrating the ultra filtrate or the filtrate from coagulated patient's serum, the specific soluble substances in all 3 types have been found, and in one as early as the thirteenth hour after the initial chill.

The two methods in use at present are as follows:

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[1] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26**, 477.

[2] Blake, F. G., *Arch. Int. Med.*, 1918, **21**, 779.

Method A. Twenty cc. of the patient's blood are defibrinated by shaking with glass beads in an Erlenmeyer flask and centrifuged. The serum is placed in a Coor's filter impregnated with celloidin and centrifuged at high speed for 5 minutes (Toth's method[3]). Two cc. of sterile distilled water are added to the inside of the filter and the tube centrifuged again to wash through the filtrate which might be held in the filter. The collected filtrate is transferred to a test tube which is set in the steam bath and a bent glass tube connected to a vacuum pump is placed loosely in the test tube so that the open end of the bent tube does not quite touch the liquid. Rapid concentration of the filtrate to 3 cc. volume is accomplished in a few minutes. 0.25 cc. of the clear concentrated filtrate is added to each of 4 small tubes and 0.25 cc. of the type specific antipneumococcus serum is added. To the fourth tube containing filtrate, 0.24 cc. of salt solution is added for control. The tubes are placed in the 37° C. water bath and read in a beam of light at 5 minute intervals. In several instances precipitation has been observed after 5 minutes incubation.

The remainder of the filtrate (about 2 cc.) is used in a longer method of checking. To it 8 volumes of 95% alcohol are added and after mixing set in the ice-box over night. The flocculent precipitate is collected by centrifuging, discarding the supernatant fluid and drying the residue by heat, under a stream of air. 2 cc. of salt solution are added and after shaking is distributed to tubes for type differentiation.

Method B. To the serum collected as in Method A, are added 10 cc. of distilled water and 5 cc. of M/5 sodium acetate-acetic acid buffer pH 4.6 and the mixture boiled until coagulation is complete. The paper filtrate is evaporated to dryness over a free flame and heated carefully until the odor of acetic acid is no longer present. To the residue is added 5 cc. of sterile distilled water for extraction. The solution is either centrifuged or passed through a small paper filter and tested against the type specific sera as in Method A.

The results in 2 cases of type I, 4 cases of type II, and 1 case of type III have been checked either by sputum or by blood culture. In 6 cases the specific soluble substances were detected in blood when the blood culture remained negative. The method requires less time than the sputum culture or mouse method but has no advantage over the sputum extract method. It is useful for typing in cases in which neither sputum nor urine can be obtained.

The occurrence of specific soluble substances in the blood in cases

[3] Toth, A., *Biochem. Z.*, 1927, **191**, 355.

in which the blood cultures were negative is consistent with the fact that these substances are readily diffusible. Determinations of the specific soluble substances may have application in determining the amount of serum necessary and in following more closely the results of serum therapy.

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Effect of Tar on Experimental Teratoids in the Rat.

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Carrel,[1] White,[2] Fisher,[3] and others obtained metastasising sarcomata by injecting into fowls a mixture of embryo pulp and dilute arsenious acid or embryo in combination with tar. In a previous publication,[4] it was found that rat embryo combined with arsenic did not produce malignant growths in the rat, and so far as determined, the arsenic did not influence favorably the development of teratoids. In the present experiments, coal tar in various dilutions was combined with embryo pulp to determine its effect on teratoid growth.

From a thick ether extract of coal tar a 1-1000 stock dilution was made in a 5% gelatin and from this the various higher dilutions were made by mixing with one per cent gelatin. Usually about one-half an embryo was drawn directly into a syringe fitted with a needle having a 1-mm. lumen and in the syringe mixed with an equal volume of the tar dilution. The injections into the subcutaneous groin tissue followed immediately. The rats averaged three-quarters grown.

Several factors influence the development of teratoids. The genetic relationship of the animals no doubt, is an important variant in an in-bred strain of animals, such as the one used in these experiments. However, when a 1-500,000 strength of tar was used (experiment 9) all rats showed distinct teratoid growth. When the embryo pulp was mixed with 1-200,000 tar, the teratoid growth took place irregularly and often failed. With tar concentrations of

[1] Carrel, A., *Compt. rend. Soc. de Biol.*, 1925, **93**, 1083.

[2] White, A. W. M., *J. Cancer Res.*, 1927, **11**, iii.

[3] Fisher, A., *Compt. rend. Soc. de Biol.*, 1926, **94**, 1217.

[4] McJunkin, F. A., and Cikrit, M. F., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 179.