

The publication of this paper was immediately followed by an extensive trial being given to the new substance, both in Germany and England. Doubtless many cases were subjected to treatment which were quite unsuitable and hopeless from the first, and it was not long before cases were recorded of diseases which from being chronic became acute, and cases of localized diseases which became acutely disseminated as a sequel to the injections. The reaction of opinion was so great that by the end of 1891 practically all those who had taken up the new treatment had lost faith in it and abandoned it.

The fluid to which the name of tuberculin (now known as old tuberculin) was given was prepared as follows:—

Bacilli were grown from six to eight weeks in a slightly alkaline veal broth, containing 1% of peptone, and from 4 to 5% of glycerine. The liquid was then evaporated to 1-10 of its bulk and filtered through porcelain. This filtrate constituted the old tuberculin. It will be seen, therefore, that this fluid contained only such toxic products of the tubercle bacillus as were soluble, without disintegrating the micro-organisms, and therefore could not give rise to an active bactericidal immunity. Koch and others were now, however, working to separate from this tuberculin the active immunizing principle, and also to free it from the noxious constituents.

In 1897 Koch announced his discovery of a further tuberculin, known as the new tuberculin, or T.R. (Tuberculin Rest) which is now in common use. Its preparation is as follows:—

Cultures, grown as for old tuberculin, are ground up in distilled water in agate mortars. The first quantity of the resulting fluid is decanted and constitutes tuberculin oberer (T.O.). Fresh quantities of water are added, and the trituration is repeated until no solid residue remains. The suspension of the comminuted bacilli is known as New Tuberculin—the T.R. or Tuberculin Rest.—and 40% of glycerine is added until 1cc of the fluid contains 10 milligrammes of the dried powder. The tuberculin, therefore, in so far as it contains all the constituents of the bacilli with the exception of the soluble exo-toxins eliminated in the T.O., should on injection give rise to the formation of active bactericidal substances. The dose of this tuberculin, which was recommended, varied from  $\frac{1}{4}$  to  $\frac{1}{2}$  milligramme. A fair trial was given to this