VIII

REMARKS ON "A METHOD FOR THE QUANTITATIVE ESTIMATION OF INOCULA IN EXPERIMENTAL SYPHILIS" AND ON THE VIRULENCE VARIATION OF TREPONEMA IN GENERAL PARALYSIS *

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MORGAN and Vryonis³⁵ write that "heretofore there has been no satisfactory method for the enumeration of *Treponema pallidum* in fluid suspensions of syphilitic tissue." They add that the object of their publication is to remedy this deficiency. Their method, however, applies only to those portions of serous fluids obtained from syphilitic lesions in man and from emulsions of testicular syphilomata in the rabbit which rise to the surface after centrifugation. Thus, it is only a variation of one described by us and our collaborators in a number of publications,^{46, 24, 2, 26, 3, 4, 5, 7} to which we have given the name of "homogeneous dark-field count." Moreover, they fail to indicate that, in answer to criticisms of Levaditi and his followers,^{33, 30} we have fully established the value of our technique.²⁷

Our method has been conceived with the object of investigating the truth of the hypothesis, upheld by Levaditi,^{31, 34, 32} etc. that there exists an invisible form of *Treponema pallidum*; it was to be used particularly in the examination of organs which were either very small or very poor in treponemata, such as superficial lymph nodes or brain tissue of mice inoculated with syphilis. The results obtained with this method were published in a series of papers.^{44, 45, 47, 18, 19, 48, 23, 14, 21, 15, 6, 11, 12, 7, 8}

* See also "Revue belge des Sciences médicales," 1938, **10**, 447.

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Its direct use was to permit an approximate count of the minimum number of treponemata in certain inocula, tissues or organs. Needless to say, in these experiments we were dealing only with free treponemata and not with those which, according to Nyka,^{36, 37, 38, 39, 40, 41} are intracellular organisms. Obviously, the values recorded are only *relative*, that is, *proportional*.

The modification of our method, as worked out by Morgan and Vryonis, consists essentially in the *repeated centrifugation* of the initial emulsion and the examination of 509 *separate microscopic fields* of thin preparations * instead of considerably larger liquid discs, the volume of which is determined by a circular surface of a little over 28 sq. mm. multiplied by the height of the preparation. It is to be kept in mind that with organs which are poor in micro-organisms, it is necessary to go over 2 to 20 liquid discs, that is, about 2 to 20 times 600 microscopic fields.

At first we applied vaseline or some similar substance to the edges of our preparations ; we also used especially devised counting chambers, such as that of Oelze.⁴² But these precautions seemed to us unnecessary as soon as we found that with more experience only half an hour was necessary to study one entire disc † : in these conditions neither evaporation nor the intrinsic motility of the treponema alter the reliability of the results obtained with a well-made preparation. As stated in a previous publication,²⁷ with some experience it is possible to obtain " results which show only small variations, whether one deals with the minimum number of organisms in different areas, central or marginal, of the same preparation or of different preparations, fresh or old, of the same initial emulsion rich in spirochætes ; or with larger quantities (made up of several preparations) drawn from a primary emulsion poor in spirochætes." This uniformity is what makes our method so evidently valuable.

Regarding the centrifugations advocated by Morgan and Vryonis, we have previously indicated,^{9, 10} that

* Under a lamellar surface of 3.24 sq. cm. we spread 4 cubic mm. of the emulsion to be studied " so that it occupies the total space, free of any air bubble," between the slide and the cover slide. Morgan and Vryonis use the following figures : a surface of 5.29 sq. cm. and a volume of 2.75 cubic mm.

† Examination by means of the Morgan and Vryonis modification requires one to two hours,³⁵ no matter how experienced the observer or how favourable the conditions.

treponemata in physiological emulsion drop to the bottom of the tube, *Treponema pallidum* of syphilitic lesions in rabbit or man and *Treponema pallidoides* ²⁵ falling at a third of the speed of the treponemata in Noguchi and Aristowsky-Hoeltzer cultures. More recently, following the method of Morgan and Vryonis, we have twice in succession centrifuged fresh emulsions in physiological saline of testicular syphilomata of rabbit and of inguinal lymph nodes of hamsters with latent syphilis, the first very rich and the second ¹⁴ only relatively rich in treponemata. In both cases, after diluting to make up the initial volume, we found that the solid part contained at least as many treponemata as the liquid part of the emulsion examined after this had been made quite homogeneous.

It would be unnecessary to repeat here a number of details which have been given in our previous publication.²⁷ What has just been described shows clearly that whereas our method makes possible the approximate evaluation of the minimum number of free treponemata in various inocula * as well as the organ from which they come, the Morgan and Vryonis modification, for the same evaluation, may be used only for suspensions of organs which are large and rich in treponemes and not for the organs themselves. We see no advantage in this modification, and its practicability is doubtful.

There remains to be made a suggestion as to a possible source of error in a publication by Saunders ⁴³ which reported the results of a study of cerebral tissue taken from several individuals who had presumably died of general paralysis.

As we have previously indicated,^{4, 5, 8} Forster and Tomaszewsky,²⁹ having inoculated in the rabbit the material obtained from 53 living cases of Neisser-Pollack puncture, did not obtain specific lesions, though the material examined in dark field showed in 25 cases numerous treponemata, and in 10 cases strongly motile treponemata. Furthermore, one of us has shown that a piece of cerebral tissue, obtained by trephining a general parietic *in vivo*, and rich in motile treponemata (as revealed in the dark field) did not produce clinical signs of syphilis in the rabbit and guinea-pig; nor was

* In order to follow our method, syphilitic emulsions must be adequately diluted.

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evidence of latent infection obtained by means of transfer of lymph nodes.^{1, 4, 5, 8}

From this experiment and others¹³ we believe that these entirely negative results are explained by the hypothesis that the treponema found in cases of general paralysis is a form so well adjusted to the central nervous system of man that it has lost its usual pathogenic capacity.

The conclusions of Saunders, in so far as they relate to duly recognised general paralysis, only confirm those of Forster and Tomaszewsky and our own. Furthermore, since Saunders obtained his material three and a half, twelve and eighteen hours *post-mortem*, it is important to point out that alteration may have taken place in the treponemata concomitantly with and subsequent to *post-mortem* changes. This possibility diminishes the significance of the results he obtained by inoculation, since the vitality and virulence of *Treponema pallidum* are easily and rapidly influenced by its surroundings.^{17, 16, 18, 20, 28, 22}

CONCLUSIONS

(1) Our method of homogeneous dark-field count, the object of which is the approximate evaluation of the minimum number of free treponemata in certain inocula, tissues, or organs, is not improved by the modification advocated by Morgan and Vryonis; the method of these writers is only a restrictive of ours.

(2) The results obtained by inoculations of cerebral tissue obtained *in vivo* by Forster and Tomaszewsky and by one of us are of more significance than those obtained by Saunders in *post-mortem* conditions.

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