SUMMARY.

1. In the blood of the normal albino rat the blood plate count approximates 1,000,000 per c.c. and is fairly constant.

2. The total leucocyte count is variable and reacts violently to ulcerative processes.

3. The reaction of the cellular elements of the blood to the growth of transmissible sarcoma is slight and probably in no way characteristic.

4. Benzol injections produce a more rapid and proportionately greater reduction in the leucocytes of the blood than in the plates.

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Gravimetric determination of beta-oxybutyric acid.

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If beta-oxybutyric acid is oxidized with dichromate in the presence of sulfuric acid and mercuric sulfate, a precipitate of the acetone compound of mercury sulfate can be obtained in an amount proportional to the beta-oxybutyric present. Thus, if 175 c.c. of a beta-oxybutyric solution containing 9 per cent. of sulfuric acid, 2 per cent. of mercuric sulfate, and 0.25 gram of potassium dichromate are boiled under a reflex for an hour, 7.7 milligrams of mercury-acetone compound are precipitated for each milligram of beta-oxybutyric acid present. The beta-oxybutyric acid may vary from 1 to 9 mg. without affecting the ratio, if the concentrations of the other reagents are kept constant.

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Complement fixation in tuberculosis.

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In a recent communication to the New York Pathological Society, the material of which is to appear in the *American Journal*

of Medical Sciences, the writers described work on complement-fixation in tuberculosis, carried out with a very simple antigen which had yielded and is still yielding results more satisfactory than those hitherto reported by other workers who had used other antigens. The work followed a study of culture-filtrate antigens, such as those devised by Besredka and by Petroff, and the special modifications of the Besredka medium employed by Bronfen-Brenner and by Craig. These antigens did not in our hands react with the regularity which we thought should attend a reaction of specific diagnostic value. Owing to irregularities perhaps due to constituents of the media, it was thought wise to return to the bacillary substances themselves, work along this line having been attended by considerable success within recent years—notably in the hands of Radcliffe, Dudgeon, Weir, and Stimson. It should not be forgotten that the same direction of investigation was followed in the earlier work of Wassermann and Citron and in that of Calmette.

The method employed is in general identical with that which we have been using in this laboratory for the extraction of *Treponema pallidum*, typhoid bacilli and streptococci, and differs in no essential particular from the so-called "endotoxin" extraction method employed by Besredka in 1906 with organisms of the typhoid-colon group. Since we feel that the procedure at present in use in the Columbia laboratory should be thoroughly reinvestigated by other workers, we believe that it is proper to give in great detail the method by which the antigen is made.

The bacilli which, so far, have been used for the production of the antigen have been of the human type, some of them isolated by Miller, some of them obtained from Professor Theobald Smith, some from the laboratory of Professor William H. Park, and some from the laboratory of Parke Davis & Co. They have been grown mainly on the gentian-violet medium of Petroff and on Miller's modification of this medium; also on Petroff's potato broth. It is at present the impression of the writers that the medium on which the bacilli are grown plays no great part in determining the usefulness of the antigen. It seems, however, to be important that a number of different strains should be used—that is, that the antigen should be polyvalent—and the use of relatively young

cultures is advisable. So far, in most of the reactions, unheated bacteria have been used. Inasmuch as the method of production, under these circumstances, is fraught with a not inconsiderable element of danger, we have recently begun to use bacteria heated to 60° for a half hour, and, in the one series so carried out, no deteriorating effect of the heating was apparent. These problems of detail, as well as many others, are being more thoroughly investigated.

20 mgm. of the moist tubercle-bacillus mass are weighed out, placed in a conical 15 c.c. centrifuge tube, and to it are added 90 mgm. of table salt. With a glass rod, filed to roughness at the end, this paste is ground by hand for about one hour. Distilled water is then added to isotonicity; that is, 10 c.c. to the quantities above described. This is the antigen. Just before using, it is shaken up and the heavier particles are allowed to settle in the course of a few minutes. Except for the removal of these larger elements, the suspension is used as a whole without centrifugation and without filtration.

The antigen so prepared has hardly ever been found anticomplementary in quantities as large as 1 c.c. and has given fixation with positive sera (the inactivated sera used in quantities of 0.1 c.c.) in amounts as low as 0.02 c.c. The titrations, as well as the reactions, have been done with one half the original Wassermann quantities, using a sensitization of two units of amboceptor and two units of complement. So far, we have used the anti-sheep rabbit hemolytic system. As a routine, the 37° one-hour water-bath incubation has been employed. A number of parallel series have been done by the four-hour ice-box method, but, since this seems to make little difference in the results, the time-saving 37° method was decided upon as a routine procedure. The antigen appears to be quite stable. We have used with satisfaction antigens as old as six or seven weeks, kept on ice.

We are ready to report the results of 602 cases tested. Of these 103 were negative for tuberculosis; that is to say, they represent patients in whom, clinically, tuberculosis was excluded. 226 cases were clinically diagnosed as actively tubercular. Their sera tested gave the following results:

"Stage one" cases: 32 in all with active clinical symptoms gave

positive fixations. Tubercle bacilli were not found in the sputum from 16 of these cases. 7 of these 16 were suffering from the early incipient type of the disease.

"Stage two": We tested the sera of 110 such cases. All but 12 in this series had positive bacteriological proof of infection. The fixation test was positive in all but 2 cases.

"Stage three": 84 cases tested. One very advanced case gave no fixation. The sputum was positive in all but one case. The fixation test was positive in 83 of these 84 third-stage cases.

We have, then, 226 patients suffering from clinically active tuberculosis, in whom the test was positive in 223 cases.

The reaction was done with 88 sera from so-called *healed* (arrested or inactive) cases. These were cases which, at one time or another, had suffered from active tuberculosis but which were, at present, apparently free from symptoms pointing to absorption from any active focus. 54 cases were negative for the test; 13 cases were positive, however in 8 of these tubercle bacilli were found in the sputum shortly before the test was performed; 21 cases showed weak fixation. Here we have a group of 88 healed, or better, arrested cases in which the reaction was negative in 54, weak in 21, and positive in 13, 8 of these 13 being cases with positive sputa.

Our next group consists of 140 doubtful cases where no diagnosis was established and where, obviously, no bacteriological proof was present. 32 in this group gave positive fixation; 108 negative. In some of the 32 positive cases, a definite clinical diagnosis of tuberculosis was subsequently established. In none of the 108 negative cases has there been found, thus far, any evidence of tuberculosis. This group of doubtful cases includes 84 pulmonary cases, 5 glandular cases (3 of which were diagnosed as Hodgkin's disease. In one of the Hodgkin cases, the serum report was ++++, and an excised lymph node from this patient turned out to be tubercular upon later pathological examination); also there were 21 eye cases, 1 case of sepsis of the throat, and 12 miscellaneous cases.

45 positive Wassermann sera were tested. 2 gave positive fixation with the tubercle bacillus antigen. One of these two cases was a dispensary patient who at present can not be followed

up; the other was a patient with tuberculous peritonitis who had had lues.

Almost all the healed cases had positive skin tests, yet 54 of the total series of 88 showed negative fixation; 24 of the 103 cases in which tuberculosis had been excluded gave marked intradermic reaction to tuberculin. The fixation test was negative in these 24 also.

In the foregoing communication, we have reported an antigen for complement-fixation in tuberculosis which has seemed to give us results more regular and satisfactory than those reported by other workers and which has the advantages of great simplicity. The nature of the reaction and its results incline us to believe that we are dealing with a specific reaction which depends upon the presence or absence of antibodies to the tubercle bacillus in the circulation of the patient. It is well to bear this in mind in judging the results of the reaction, since it must not be forgotten that specific complement fixation may not be a direct measure of infection, but rather indirectly it may point to the invasion of the body by a specific microörganism, by determining the presence of antibodies. Thus, it may be too much to expect, especially in a disease so chronic as tuberculosis, to find antibodies circulating in all forms and in all stages of the disease. Perhaps this will make it more easy to understand why the reaction has given positive results in active cases only, nearly always negative ones in inactive tuberculosis, and was occasionally negative when tubercle bacilli were in the sputum but the clinical condition was one of arrested disease. It is this aspect of the reaction particularly which leads us to hope that it will be of clinical value in indicating, not so much the existence of infection as of determining the activity of the focus, and, incidentally, giving us a method of studying the fluctuations of antibodies during the disease. The work will, of course, have to be continued by multiplying the number of cases already observed. We are also proceeding in our own laboratory to study the specificity of antigens made with bovine cultures and to study the relationship of this reaction to the diagnostic tuberculin tests.