TABLE II.
Pre-Inoculation Basophil Values and Events in the Course of the Syphilitic Infection.

Events	19 animals with basophils above 972 per cmm.	20 animals with basophils below 972 per cmm.
	%	%
Primary orchitis	100.0	100.0
Delayed incubation period	21.0	5.0
Edema in inoculated testicle	47.0	20.0
Metastatic orchitis	65.0	95.0
Generalized lesions	47.0	79.0
Bone lesions	26.0	79.0
Skin lesions	37.0	32.0
Number of generalized lesions per animal	1.46	4.47

the pre-inoculation basophil count might be used as a rough index of the potential reaction of the rabbit to syphilis. It has been found, however, that there are striking exceptions to this rule when applied to individual animals, and at present, the extent to which such exceptions may affect group results is not entirely clear. An analysis of the material from the standpoint of the relation of basophils to other cells of the blood may clarify this situation.

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Diagnostic Significance of Precipitin Tests With Anderson Phosphatide Fractions from Human, Bovine, and Avian Tubercle Bacilli.

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Working in cooperation with the Research Committee of the National Tuberculosis Association, Drs. F. R. Sabin, C. E. Forkner, and the writer, in this laboratory, have been assaying biologically during the past 3 years the various chemical fractions obtained from the tubercle bacillus by Drs. T. B. Johnson and R. J. Anderson of the Sterling Chemistry Laboratory, Yale University. The investigation includes a parallel study of the chemistry and biology of several members of the acid-fast group of bacteria grown in large quantities by Mulford and Parke, Davis under standard conditions on Long's synthetic media. It has been found that the phosphatide fraction isolated from the tubercle bacillus by Anderson[1] contains an active principle capable of stimulating and maturing monocytes and

[1] Anderson, R. J., *J. Biol. Chem.*, 1927, lxxiv, 537.

epithelioid cells.[2] This phosphatide fraction from the human tubercle bacillus H-37 has been shown by Pinner[3] to act as a complete antigen when given intravenously in animals, as proven by precipitin and complement fixation tests. We have confirmed this finding with reference to the precipitin reaction. Readings are made after 5 minutes at room temperature, after 2 hours at 38° C., and after 24 hours in the ice-box. Pseudo-positive reactions are thus avoided. Considerable experience in reading the tests is necessary for accurate determinations. Variations in hydrogen ion concentration, pH 2.5 to 6.02, have had no influence on the reaction.

The sera of normal rabbits have been found to give a positive precipitin test in low dilutions only, 1-20 with dilutions of a 1% antigen, or with a fixed antigen with only the undiluted serum. The exceptional rabbit direct from stock showing higher titres is now discarded for all experimental work in fraction testing and other studies on tuberculosis in this laboratory. There is some evidence that small variations in the phosphatide precipitin titre may accompany a non-tuberculous monocytosis in blood and connective tissues.

In 36 rabbits given from 3 to 8 antigenic doses of 10 to 25 mgm. each of phosphatide A-3 from H-37 human strain of tubercle bacilli, either alone or combined with horse serum, the rabbit sera on the 12th day after the last dose have shown precipitin titres with diluted antigen of from 1-160 to 1-450. Dilutions of sera against a fixed antigen (1-10) have been positive in 1-30 to 1-100. The higher titres have been obtained when the phosphatide was given in combination with horse serum and in the larger dosage. Specific absorption with the homologous antigen has rendered these sera inactive.

The sera from tuberculous rabbits have been positive in degree directly proportional to the dosage of organisms and extent of lesions (0.01 to 2 mgm. B-1 intravenously). An increase in the titre of the blood serum may be noted as early as the third day after inoculation when a dosage of 0.5 mgm. or greater has been given. During the first 2 weeks particularly, testing for antigen with anti-phosphatide rabbit sera gives positive precipitin tests with the sera from tuberculous animals diluted up to 1-40. The anti-phosphatide serum is kept in sterile sealed ampoules and used as needed. In rabbits with chronic generalized tuberculosis and involvement of the central nervous system both blood serum and ventricular fluid show strongly positive precipitin tests. Although the phosphatides from human, bovine and avian bacilli all react with bovine tubercu-

[2] Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, xlvi, 645.

[3] Pinner, M., *Am. Rev. Tuber.*, 1928, xviii, 497.

lous rabbit sera, it has been our experience thus far that the bovine phosphatide tends to give a more massive precipitate in corresponding dilutions and to react in slightly higher dilutions than do the other 2, the differential reaction being quantitative rather than qualitative. Lecithin, phospholipin from brain tissue (Dr. P. A. Levene), and resorcinol, the substance used in the Verne flocculation test, give positive readings with tuberculous rabbit sera but when run in dilutions parallelling the phosphatides from the tubercle bacillus, they usually cease to react in relatively low dilutions. Old Tuberculin, protein, 304-B from the bacillus (Dr. T. B. Johnson), 304-F from the filtrates (Long and Seibert), and the polysaccharide (Anderson) from H-37 human tubercle bacilli always give precipitin reactions in well advanced stages of the disease, but in rather lower titre than the phosphatide and with less consistency. It may be that when similar fractions are available from the bovine organism more consistent results will be obtained in the bovine tuberculosis of rabbits.

The blood sera from chicken and guinea pig with avian tuberculosis (Dr. L'Esperance) have shown positive reactions with the homologous antigen in titre of 1-320, while the titre with human and bovine phosphatide has been 1-160.

We have received from Drs. Cotton and Woolfolk of the Bureau of Animal Industry, U. S. Dept. of Agriculture, blood sera from 10 tuberculous cows, condemned tuberculin reactors with lesions proved at post mortem. These sera have reacted positively with the precipitin test, bovine phosphatide being used as antigen, in titres ranging from 1-640 to 1-10,000. The human and avian phosphatides have given less massive precipitates and in somewhat less dilution. The sera from 10 non-tuberculous cows were furnished by Dr. Little, of the Princeton branch of the Rockefeller Institute, none of which showed a titre above 1-80. It has been noted that certain of the tuberculous sera show a faint cloud at the juncture between serum and fresh glass distilled water at the 5 minute reading, which has not been seen with non-tuberculous sera. This reaction later, however, is minimal and permits differentiation between positive and pseudo-positive readings.

Absorption experiments with equivalent weights of the various phosphatides and the tuberculous cattle sera showed a distinctly larger quantity of precipitate when the bovine material was used than with either human or avian, and subsequent precipitin tests showed corresponding degrees of absorption. Human tuberculous blood sera and pleural effusions giving strongly positive reactions

with the phosphatide fractions have been rendered non-reactive after absorption with the homologous phosphatide.

Through the interest and courtesy of Dr. E. A. Park of the Harriet Lane Home, Johns Hopkins Hospital, Baltimore, the beginning of a clinical study with these chemical fractions was suggested and has been made possible. Because of the many disappointing failures in the past with precipitin tests, a considerable experience with the newer fractions now available must accumulate before critical judgments may be formed. It has seemed worth while to repeat some of the earlier work along these lines both because of the standard bacteriological and chemical technique that is now being used in these cooperative studies and because of the eventual availability of parallel fractions from different members of the acid-fast group of bacteria for comparison and contrast. We are greatly indebted to a number of clinics and sanatoria for the fluids tested and the clinical data.*

We have tested thus far blood sera, spinal fluids, pleural effusions, ascitic fluids, or joint fluids from 134 individuals. Of 12 cases of tuberculous meningitis in infants, only one has failed to give precipitin tests either with the phosphatide, or with the anti-phosphatide rabbit sera. The latter has given promise of being the most sensitive test in early cases and in those apparently unable to react to the antigen of the bacillus. Whereas normal rabbit serum is entirely inert, the sera from rabbits which have reacted to the phosphatide in antigenic doses give precipitin reactions with dilutions of the tuberculous fluids as high as 1-5000. The "zone phenomenon" is strikingly brought out in these tests. For example, in one tuberculous spinal fluid 1-0, 1-5, and 1-10 dilutions gave ± readings, with maximum precipitate ++++ at 1-40 and 1-80, and final positive + at 1-5000. In one case in which repeated studies of the spinal fluid were made during the course of tuberculous meningitis, the precipitin titre for antibody starting at a positive of 1-20 increased 12 hours later to 1-40 and 4 hours later to 1-160. Sixteen control spinal fluids from infants representing mumps meningitis, influenza, pneumococcus III and IV, and meningococcus meningitis, congenital lues, pertussis, middle ear infection with convul-

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sions, post-influenzal hemorrhagic encephalitis, and lethargic encephalitis have all been negative with the exception of one case. With a positive precipitin reaction of 1-160 in blood and spinal fluid this case has shown negative tuberculin tests and is running the course of an epidemic encephalitis. Eight spinal fluids from tuberculous individuals not showing involvement of the central nervous system have been negative, 24 normal spinal fluids from adults, and 3 four plus Wassermann spinal fluids were negative. The spinal fluids from 3 non-tuberculous monkeys with poliomyelitis (Dr. C. P. Rhoads) did not react with the phosphatides. The blood sera from all tuberculous infants have been strongly positive, the sera from young non-tuberculous children seldom giving titres above 1-80. Tuberculous joint fluid has given strongly positive precipitin reactions in one case, in contrast to fluids from joints in acute rheumatic fever. In infants and children with primary infections it may be expected that such precipitin tests as these, if found dependable, will prove of greatest value.

In adults the application of the principle to serous exudates and fluids of questionable etiology may prove of considerable aid to early diagnosis. The 14 pleural effusions from known tuberculous cases have proven to be strongly positive when tested with phosphatide and have reacted with the protein 304 and the polysaccharide in some instances in comparable titre. A proper evaluation of these findings must await a larger series of cases with non-tuberculous controls. The blood sera from normal adults, 2 with known positive tuberculin skin tests, and from cases of lymphosarcoma, lymphatic leukemia, lethargic encephalitis, gastric carcinoma and pyogenic infections have all given readings under 1-40 or 1-80. Three non-tuberculous ascitic transudates have been negative. In certain suspected cases still being studied and in all well advanced cases of pulmonary tuberculosis the precipitin titre in the blood against a fixed antigen has been 1-80 to 1-240 and with antigenic dilutions 1-400 and above. Tentatively, a titre of 1-160 to 1-320, antigenic dilution, or 1-20 to 1-40 serum dilution may be taken as suggestive, and titres above these as usually indicative of an active process. As in most tests for tuberculosis positive findings are more significant than negative.

Through the cooperation of Dr. Fred W. Stewart the blood sera from 9 cases of Hodgkins disease have been studied. All have given positive precipitin titres of from 1-320 to 1-3000. Further comparative studies are in progress with the above mentioned and other fractions from the various acid fast organisms.

Differential counts of the living supravitally stained cells from freshly drawn fluids other than blood were made in selected cases at the Harriet Lane Home, Johns Hopkins Hospital, Baltimore, through the courtesy of Dr. E. A. Park. For example, complete cellular studies of blood and spinal fluid were made in one infant under 1 year with generalized tuberculosis and meningitis. In the peripheral blood there were 47% neutrophilic leucocytes, 11% lymphocytes, 40% monocytes and 2% epithelioid cells. The ventricular fluid was clear with 450 cells and the centrifuged sediment showed 10% neutrophilic leucocytes, 46% small lymphocytes, 2% intermediate lymphocytes, 6% young monocytes, 20% monocytes, 5% epithelioids, 2% Langhans giant cells, 3% clasmatocytes, 2% serosal cells, 1% neutrophilic myelocytes, and 3% degenerating cells. The 2 giant cells had 5 and 7 nuclei respectively arranged peripherally around a central rosette of neutral red bodies. Other tuberculous spinal fluids have shown similar trends with epithelioid cells present, in contrast to the cellular findings in meningeal irritations of other etiology, as for example pneumococcus meningitis. It is essential that the studies be made within 20 minutes after withdrawal of the fluid to be examined as degenerative changes occur very rapidly rendering identification of individual cells impossible. These observations suggest the importance and possible value of careful studies of the cells in serous exudates and fluids from cases with questionable or obscure diagnoses.

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Is the Sensitizing Effect of Cholesterol for the Antigen Used in the Complement-fixation Test for Syphilis Due to the Contaminating Sterol, Ergosterol?

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Cholesterol as ordinarily prepared is now known to be contaminated with another sterol of an unsaturated and labile type of which ergosterol is the only known representative. Moreover, it is because of this contaminating sterol, ergosterol, that after irradiation with ultra-violet light, cholesterol acquires antirachitic properties. After removal of ergosterol, cholesterol is inert, following irradiation.

Professor Knudson of the Albany Medical College very kindly