"impurities" present, we found that the emulsion-stabilizing substances adhering to the fat globules in cow's milk apparently consist of a single globulin-like protein free from phosphorus[6] and a mixture of phosphatides of as yet undetermined nature. No glycoprotein was found, the residual traces of sugar in the protein fraction being removed by the solvents which free it from phosphorus. The phosphatides comprised by far the greater part of the total raw material in our experiments. The yield of phosphorus-free protein, which still contained several per cent of other impurities, never exceeded 15 per cent of the total raw material in the buttermilk and butter washings.

The mixture of substances, including the fat globules which resisted mechanical separation, had a gold number of 10 both before and after freeing from calcium by dialysis. In each case this value was changed to 0.5 by boiling the gold sol after the addition of the colloidal mixture and before the addition of the electrolyte. The results make it unlikely that the inorganic impurities in the buttermilk played any part in stabilizing the original cream emulsion.

The occurrence of both hydrophilic and hydrophobic colloids on the surface of the fat globules seems to offer an explanation both for the stability of the cream emulsion and for the relative ease with which the oil-in-water type of emulsion is inverted to a stable water-in-oil type of emulsion which occurs in churning cream to butter.

The detailed report of these experiments will appear later.

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Studies in tuberculosis immunity. I. Diagnostic and sensitizing properties of some new derivatives of tuberculin.

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This study, preliminary to a series of investigations in tuberculosis immunology, represents an attempt to prepare from tuberculin an active principle which may be of value in the early diagnosis of tuberculosis, particularly in connection with a wider

[6] The freeing of the protein from its phosphorus impurities was attended with great difficulty.

range of specificity for the bovine type of tubercle bacillus; secondly, it is concerned with the sensitizing properties of these derivatives.

Up to the present time no substance has been isolated or prepared from tuberculin such that it will sensitize the animal body to subsequent injections of the the same material, or to its native unaltered tuberculin. The requisite for atypical skin reaction with tuberculin is an infection with tuberculosis. Believing that the specific substance which produces a local tuberculin susceptibility is present in the tubercle bacillus and in tuberculin and is the same element in either case, I have investigated certain derivatives with a view to identify a possible antigenic "nucleus". The failure of tuberculin to develop susceptibility in the sense of a true antibody phenomenon may well be due to technical factors and to conditions related to absorption and dissemination of the material in question. Pharmacologic principles have given abundant evidence of the important rôle played by solubility, diffusibility and penetration of substances used in local and general therapy. These fundamental principles are the same for any biologic reaction between tissues and the substances injected, be these drugs, tuberculins, or proteins in general.

Landsteiner and his collaborators demonstrated some years ago the relation between serologic specificity and chemical structure of antigens. The point of departure in the present series of experiments centered on the properties of modified antigens and the possibility of extending the range of specificity through certain chemical alterations of the protein or non-protein substances in tuberculin.

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The preparation of the derivatives will be described in a complete report at a later date. These derivatives are (1) an acetyl, (2) a benzoyl and (3) an alcohol-soluble, ether-precipitable substance which I have termed "Ether-insoluble X", all of which were derived from human Old Tuberculin. I am indebted to Professor Arthur D. Hirschfelder for suggestions and advice in the preparation of these materials.

Potency of the derivatives was determined by comparing with those of untreated tuberculin the reactions which were obtained by intracutaneous injections at a stated time after inoculation of guinea pigs with human and bovine strains of tubercle bacilli. Weighed amounts of organisms were injected in definite volumes

of physiologic salt solution and the skin tests were made by injecting into the skin 0.01 to 0.02 cc. of the derivatives. In several series of animals, totalling 28, which were inoculated with both types of tubercle bacilli, and tested after 11 and 18 days, results were uniformly positive and proved that the derivatives were fully potent and unimpaired by the treatment in the course of their preparation. Uninoculated control animals gave uniformly negative results. The skin manifestations were definite for both the human and bovine types of infection and persisted for a week or more, terminating with deep-seated tissue changes, residual scarring and pigmentation. A small series of 6 tuberculous patients confirmed the results of the animal experiments. In all instances the derivatives gave positive results whenever the Old Tuberculin reacted, with the added important difference that although the tuberculin elicited no response in the animals which were inoculated with bovine tuberculosis, the derivatives produced striking reactions in such heterologous infections.

Experiments were devised to study the diagnostic range of tuberculin derivatives with the following results in a series of 22 guinea pigs: (a) Positive skin reactions were obtained with all derivatives regardless of the type of infection present. (b) Reactions could be elicited as early as 3 days after experimental infection with bovine or human tubercle bacilli, and appeared to vary in intensity with the stage of infection, since most pronounced reactions were obtained 7 or more days after initial infection. In these very early stages tuberculin failed to produce a local response, yet the derivatives, without exception, gave marked reactions. (c) "Ether-insoluble X" gave the best reactions and proved to be a very potent preparation with diagnostic possibilities. A series of 6 guinea pigs which were tested by intracutaneous injections after 4, 8, 15, 29 and 152 days gave pronounced reactions for human and bovine infections. The local response could be elicited repeatedly in the course of the disease. Control animals were uniformly negative. (d) Typical reactions which followed intracutaneous injections of acetyl and benzoyl derivatives did not interfere with the development of an intense local reaction to "Ether-insoluble X". This observation is significant because the reverse was found to be the case in animals which were uninoculated and treated with the same derivatives prior to intracutaneous tests with the "Ether-insoluble X".

Sensitizing properties of tuberculin derivatives were studied in several series of guinea pigs which were divided into groups of healthy and infected animals. In the former group, numbering 8, the animals were given repeated subcutaneous injections of the different derivatives, using one preparation at a time, and then tested intracutaneously with each of the homologous and heterologous derivatives and also with untreated Old Tuberculin. Intense reactions developed in these animals after 24 hours, reached a maximum after 48 to 72 hours and persisted for 5 to 21 days. The lesions were of variable extent but were typical of true skin reactions. Four control animals which were tested with one each of the derivatives and Old Tuberculin, gave negative results. The "Ether-insoluble X", injected intracutaneously 55 to 66 days after an initial injection of the other two derivatives, failed to elicit a local response in three guinea pigs which were so tested in this series. An explanation for this interesting observation may be found in the fact that these animals had by this time received numerous injections of the derivatives, with the result that a state of resistance or immunity had been developed from an initial state of hypersusceptibility. A striking demonstration of sensitization was observed in another group of 5 animals which showed marked symptoms of protein intoxication after subcutaneous injections of acetylated derivatives in the course of systematic treatment over a period of several weeks. Within a few minutes after the injection, dyspnea and weakness were noted and the animals became stuporous. Recovery took place within 12 hours. A loss of weight occurred regularly in these animals, until a tolerance to the substance had been developed. Infected guinea pigs presented a somewhat different picture. In a series of ten animals with bovine and human tubercle infection, acetylated derivative was injected subcutaneously in one cc. doses at intervals of one week. The first dose was given 11 to 21 days after inoculation. In the course of this treatment it was observed that the animals developed a diffuse reddening over the entire abdomen and showed in addition a flare-up of old intracutaneous sites of injection. In some cases where cold abscesses were present, an intense reddening of these areas was observed. With each subsequent injection of the derivative, the animals showed similar local reactions. After the fourth and last injection in this series, all of the animals developed local

areas of infiltration and edema varying in size from 1.0 x 2.0 cm. to 3.0 x 3.5 cm., with central purplish zone of about 1.0 to 1.5 cm. in diameter. These reactions persisted for several days and subsided with residual pigmentation. One of the guinea pigs manifested a typical anaphylactic reaction with dyspnea weakness, involuntary micturition and defacation, and distress lasting 30 minutes, during which time it lay on its side. These final injections were made 36 to 44 days after initial inoculation with tuberculosis of human and bovine origin. The series of five guinea pigs with bovine infection gave results somewhat different. In general, the reactions were not so intense and the size of the lesion was rarely over 1.0 cm. in extent. In one animal local necrosis developed with some erythematous changes in the remainder local infiltration and edema were observed.

These derivatives are being studied in a large series of tuberculous patients, adults and children. There is evidence at hand to show that sensitization can be demonstrated even in terminal cases. Furthermore, typical skin reactions can be elicited in children who have failed to react to human tuberculin while reacting to bovine, and vice versa.

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Observations on the mechanism of the tyrosine-tyrosinase reaction.

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Raper and Wormall[1] in a recent paper conclude as follows:

"Tyrosinase first produces from tyrosine a red substance. This process requires the presence of the enzyme and only takes place in the presence of oxygen. This red substance becomes colorless spontaneously and is then oxidized to form melanin. These

[1] Raper, H. S., and Wormall, A., The tyrosine-tyrosinase reaction, *Biochem. J.*, 1923, xvii, 454.