

degree of inhibition varied greatly with the different acids. In a general way it can be stated that the carboxylic acids of benzene and of other cyclic compounds acted more strongly than fatty acids, and that stronger inhibition was caused by the meta and para substituted acids than by the ortho substituted. The tests were made with solutions neutral to litmus, and also in the presence of buffers. Most of the substances acted similarly on mushroom extract and on potato extract, but in some instances differences were observed.

A part of the results is presented in Table I, the figures indicating the degree of oxidation as judged from the intensity of the color.

Somewhat analogous phenomena were noticed when in place of ferment, iron salts and H_2O_2 were used for the oxidation of tyrosin.

A similar difference in the action of ortho, meta and para substituted aromatic acids was also seen in their inhibiting effect upon the color reaction of sodiumsalicylate with ferric chloride. Apparently this was due to the formation of iron compounds which separated in the form of precipitates, but the effect was the same when the precipitation was prevented by addition of a solution of gelatin.

This is a preliminary report.

¹ Landsteiner, K., *Biochem. Z.*, 1920, civ, 280.

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Further Studies on the Heterogenetic Haptene.

K. LANDSTEINER AND P. A. LEVENE.

From the Laboratories of the Rockefeller Institute for Medical Research.

In continuation of the studies recorded previously¹ the following observations were made.

The crude material obtained from horse kidney by extraction with alcohol, and freed from ether soluble substances in the manner described previously, was extracted with boiling benzene. From the solution on standing a precipitate separated out which was removed by centrifugalization. The solution was concentrated. On addition of an equal volume of alcohol a precipitate formed. This was dissolved in benzene and precipitated with alcohol, and the procedure was repeated many times until there appeared in the benzene solution a substance giving a distinct copper orcinol reaction. The

final precipitate was dissolved in water and precipitated from the solution with two volumes alcohol, and was then extracted thoroughly with methyl alcohol and with chloroform. The fraction soluble in methyl alcohol (A) was water soluble and gave a strong orcinol copper test. The insoluble part (B) did not give this reaction and was practically insoluble in organic solvents. With hot water it yielded a viscous pseudosolution. This solution gave a flocculent precipitate on addition of Fehling's reagent. On hydrolysis of "B" with 3 per cent H_2SO_4 , after about 30 minutes, an insoluble substance settled out and the solution reduced Fehling's reagent and gave a crystallized osazone. After hydrolysis for 12 hours the quantity of sugar amounted to about 35 per cent, calculated as glucose. After precipitation with Fehling's solution substance B contained neither P nor S. The material was active in the complement fixation test with heterogenetic immune serum and produced heterogenetic hemolysins in rabbits when several injections of 1 mg. each of the substance mixed with pig serum were given. Even quantities as small as 0.2 mg. had still noticeably immunizing properties. Fraction "A" was distinctly less active in the immunization experiment, though similar in activity to "B" when tested *in vitro*. It should be mentioned, however, that it is apparently difficult to prepare suitable solutions of substance "B".

In a simpler way a product apparently identical to "B" could be prepared by treating the crude material, mentioned in the beginning, with hot pyridine. The crude material is dissolved in 20 parts of hot pyridine and allowed to stand in the refrigerator for 24 hours. A voluminous sediment is then formed. The filtrate contains the principal part of the substance similar to "B."

For the preparation of substance "B" the filtrate is concentrated under reduced pressure to nearly dryness. The residue is taken up in hot methyl alcohol and poured into acetone. A precipitate is formed. This is removed by filtration and dried. The dry material is extracted with 10 parts of a mixture of equal parts of chloroform and of methyl alcohol. The insoluble part is again treated in the same manner. The residue is dissolved in 100 parts of hot water. The solution is allowed to cool and half the volume of Fehling's solution is added. A flocculent precipitate is formed. It is washed with a small volume of water. It is then suspended in water and acidulated with hydrochloric acid. To the opalescent solution alcohol and ether, or acetone is added to precipitate the material. The individual samples of the substance still varied in their elementary composition. The yield of substance "B"

was about 1.5 gr. per 100 gr. of crude material when 2 extractions with pyridine were made.

From the pyridineinsoluble material a water soluble fraction similar to "A" was prepared, giving strong orcinol copper reaction and only weak complement fixation with heterogenetic antiserum. Substances of similar chemical properties as "A" could be obtained from beef kidney and beef brain.

This is a preliminary report.

¹ PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 343-344; *J. Immunol.*, 1925, x, 731-733.

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Mononuclear Cells of Blood in Relation to Clinical Findings in Human Tuberculosis.*

R. S. CUNNINGHAM AND EDNA H. TOMPKINS.

From the Department of Anatomy, Vanderbilt University Medical School and the Medical Service of the Vanderbilt University Hospital.

In 1925 a new hypothesis in regard to the propagation and resistance of tuberculosis was presented by Cunningham, Sabin, Sugiyama and Kindwall.¹ The data for their conclusions were obtained from a study of rabbits injected with viable bovine tubercle bacilli; differential blood studies were made throughout the course of the infection by the supra-vital technique of Sabin, and freshly autopsied material was examined by the same technique. They found that, during the course of a tubercular infection, the so-called "reticular cells" are stimulated to an increased production of monocytes and these in turn are transformed into typical epitheloid and giant cells. From a study of the appearance and number of the bacilli, which were found in the monocytes and in the epitheloid cells, they concluded that these cells phagocytize organisms but are unable to destroy them, and hence act virtually as culture tubes. The concept was thus advanced that the monocytes are stimulated, in tubercular infections in rabbits, to such an increase in number and in phagocytic capacity, that, inasmuch as they seem unable to destroy the bacilli, they aid in increasing and disseminating the infection. Furthermore, the authors showed that these changes became more

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