

tary components, it is calculated that the rats on the "fat free" diet received daily probably less than 7 mgm. of fatty materials, other than the non-saponifiable matter from cod liver oil. At stated intervals, the rats were killed and the fat from the entire animal, with the exception of the alimentary tract, was rendered out by essentially the technic used by Anderson and Mendel,<sup>1</sup> modified slightly in order to obtain a more complete extraction of the fat. The fat yielded by the rats grown on a diet practically devoid of "true" fats, which might be designated as "physiological" or "synthetic" rat fat gave iodine values of 64 to 71 by the Hanus method. There was little difference in the iodine numbers of the fats obtained from the rats grown on the "fat free" diet and from those animals whose diets contained a small inclusion of fat.

These experiments indicate that the fat produced *de novo* in the body out of non-fat precursors has presumably a definite characteristic chemical make-up, which is not influenced materially by the inclusion of a very small amount of fat in the diet, as judged by the iodine numbers of the total body fat. An examination of the samples of fat obtained from the rats grown on the diets mentioned, for their constituent fatty acids, and the extraction of the fatty materials from the feces, is now in progress. §

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#### Studies on the Pathogenesis of Endocarditis.

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In view of the well known multiplicity of bacteria isolated from endocarditic lesions, workers in this field have always more or less distinctly felt that the essential question in the pathogenesis of endocarditis is one of bacterial *localization*. Under what conditions will bacteria localize on the endocardium? While early experimental investigators (Wyssokowitsch,<sup>1</sup> Ribbert<sup>2</sup>) succeeded in producing an endocarditis by combining the bacterial infection with a gross trauma to the heart-valves, observations on experimental endocar-

<sup>1</sup> Anderson and Mendel, *J. Biol. Chem.*, 1928, lxxvi, 729.

§ Since this report was sent to press, a paper by H. C. Eckstein, dealing with synthetic rat fat has appeared in the *J. Biol. Chem.*, lxxi, 613. The iodine values reported in the 2 papers are in general agreement.

ditis without such trauma (Lissauer,<sup>3</sup> Saltykow<sup>4</sup>) have been rare until recently. Birkhaug<sup>5</sup> produced a subcutaneous lesion with a streptococcus infected agar focus, and obtained endocarditis with regularity by intravenous reinfections with the same organism. He interprets these results in the sense of an etiological specificity of the organism employed. Siegmund<sup>6</sup> produced experimental endocarditis by daily intravenous injections of rapidly increasing doses of *B. coli* or staphylococcus. Siegmund<sup>6</sup> and Dietrich<sup>7</sup> conclude that a peculiar state of immunity, associated with an altered capacity of the endocardium to react, is the essential condition for bacterial localization on the endocardium.

In the course of observations on infectious disease processes in horse serum sensitized guinea pigs we found, that on single intraperitoneal injection of salmonella or *Staphylococcus aureus*, sensitized guinea pigs developed with considerable regularity an endocardial lesion, which was only occasionally met with in non-sensitized controls. The lesion consists of nodular monocytic proliferations—"proliferative endocarditis". Desensitization previous to the infection practically prevented the above nodular reaction of the endocard, desensitization after the infection was without detectable influence.

As a sequel of horse serum sensitization the capacity of the endocardium to react against an infection had evidently been altered. In an attempt to gain information about the possible significance of these results the above morphological findings were compared with earliest endocardial reactions in human endocarditis as well as with such reactions in an experimental rabbit's endocarditis. (Streptococcus vaccination, subsequent staphylococcus infection, after Freifeld<sup>8</sup>).

It was found that in the experimental rabbit's endocarditis as well as in human endocarditis nodular monocytic proliferations represent earliest endocardial reactions.

The close morphological analogy between the endocardial reaction of the horse serum sensitized guinea pig and early endocardial reaction in endocarditis suggested that processes somehow related to horse serum sensitization, are an essential condition for bacterial localization on the endocardium.

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<sup>1</sup> Wyssokowitsch; <sup>2</sup> Ribbert; <sup>3</sup> Lissauer; <sup>4</sup> Saltykow. Reviewed in *Henke-Lubarsch Handb. d. spez. path. Anat.*, II, 205-206.

<sup>5</sup> Birkhaug, *J. Inf. Dis.*, 1927, xl, 549.

<sup>6</sup> Siegmund, *Verhdl. d. deutsch. path. Gesellschaft*, 1923, xix, 114.

<sup>7</sup> Dietrich, *Verhdl. d. deutsch. path. Gesellschaft*, 1925, xxxvii, 188.

<sup>8</sup> Freifeld, *Klin. Wochenschrift*, 1928, vii, 1645.

In an effort to throw more light on the question under consideration, an allergic state was induced in rabbits by 5 or 10 successive daily intravenous injections of 2 cc. of 2% casein in 0.1% of sodium carbonate. It was found that after 5 to 10 casein injections 10 out of 15 animals showed a definite, 5 a less definite, *hyperergic skin reaction* to injection of casein. No morphologically detectable effects of the casein treatment, especially on the endocardium, were found in controls with the usual histological methods. On intravenous injection of 1 to 2 cc. of an 18-hour *Staphylococcus aureus* culture 5 out of 7 animals developed a definite endocarditis.

We feel justified to draw the inference that bacterial localization on the endocardium—in our experience—is due to an allergic state of the endocardium, *i. e.*, an altered capacity to react.

It is also to be inferred that a relationship between that allergic state and the subsequent infection in the sense of species-specificity is not a requirement for bacterial localization on the endocardium, and that furthermore, that allergic state may be induced by a non-bacterial foreign protein-matter. The immun-biological definition of that allergic state remains a matter of further study.

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### Adaptation of the Bloor Oxidation Procedure to the Determination of Small Quantities of Cholesterol as Digitonid.

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Micro methods for the estimation of cholesterol have hitherto been based almost entirely upon the color reaction with acetic anhydrid and concentrated sulphuric acid. The presence of interfering color producing substances in blood and tissue extracts frequently makes it highly problematical whether or not this reaction gives a true picture of the cholesterol concentration. The gravimetric estimation as the digitonid, proposed by Windaus<sup>1</sup> is impracticable where the amounts to be weighed are as small as those available in the average blood analysis.

We have, therefore, attempted to adapt the digitonin precipitation

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<sup>1</sup> Windaus, *Z. f. Physiol. Chem.*, 1910, lxx, 110.