blood similar to those noted when the cure is effected by cod liver oil. This is of interest as affording testimony that the curative process occasioned by these divergent therapeutic agents is fundamentally the same. These observations establish a chemical basis for heliotherapy in rickets. They furnish also, as far as we know, the first definite evidence of metabolic change in the animal body brought about by the solar rays.

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Dissociation of microbic species.

II. Mutation in pure-line strains of the bacillus of rabbit septicemia.

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The coexistence of two distinctly different types of microbe in cultures of the rabbit septicemia bacillus has been reported in a previous paper.[1] These varieties, once separated, appear to breed true to type for many passages. The organisms have been designated as types D and G. Type D is very virulent for rabbits, grows diffusely in liquid media, and yields highly fluorescent, rather opaque colonies on serum agar. Type G is of extremely low virulence, exhibits a granular sedimenting growth in fluid media, and grows in the form of translucent, non-fluorescing colonies on serum agar. The two types show no noticeable differences in morphology or in fermentation reactions. Immunization and agglutination reactions indicate their antigenic community.

It seemed necessary to determine whether the two varieties coexist in cultures isolated from infected rabbits or whether one variety arises from the other. Type D (virulent) is the microbe invariably obtained from the naturally infected rabbit. Type G has only been found after artificial cultivation has been carried on for some time. But since the primary isolations were made from colonies which conceivably might arise from two or more

[1] *Jour. Exper. Med.*, 1921, xxxiii, 773.

organisms, it would be unjustifiable to conclude that the original type D had changed into the microbe of the G variety. Consequently, 8 pure-line strains were isolated from a D culture by the Barber method. Single cells, removed from three-hour cultures, were planted in undiluted rabbit serum. The percentage of positive cultures obtained by performing the entire operation in serum was much higher than when broth was employed.

The resulting pure-line strains were planted daily in undiluted rabbit serum. Tests were carried out to determine the conditions under which the low-virulent type G makes its appearance. The method of detection of this type consisted in streaking the test material upon the surface of 10 per cent. rabbit serum agar. The G type colonies can be readily distinguished from those of the strongly fluorescent type D. With proper attention to the technique of streaking, quantitative estimates of the proportionality between the D and G varieties can be made.

In undiluted rabbit serum, with daily transplant, type D breeds true for long periods of time. The G variety has seldom been observed to arise under these conditions. In plain broth, transplanted daily, a few G colonies have been detected after 25 passages. On the other hand, when 3 or 4 days are allowed to elapse between transplants in this medium, many colonies of this type make their appearance on the serum agar sub-plates.

This observation led to the following experiment. 0.05 c.c. of a pure-line strain, type D, was seeded into tubes of plain broth and of undiluted rabbit serum. The tubes were placed at 37° C. and a loopful of the material from each tube was streaked at 12-hour intervals on rabbit serum agar plates. In the sub-plates from undiluted rabbit serum no G colonies were detected during incubation for 109 hours at 37° C. In the plain broth, G colonies began to appear at 48 hours, and had reached a concentration of 50 per cent. of the total organisms in 109 hours. These G colonies, fished from serum agar plates, remained true to type for over 50 passages, showing no tendency to revert to the parent D form, even when returned to undiluted rabbit serum. All of the pure-line strains under study have been found to undergo this mutation when allowed to stand in plain broth, but do so with varying degrees of rapidity and completeness.

It was considered probable that filtrates from D cultures might hasten the D ⇀ G transformation. Accordingly, cultures of 6, 24, 48, and 72 hours were filtered through Berkefeld candles. After sterility had been proved, 0.05 c.c. of pure-line strain B–D_2 was seeded into 10 c.c. of each of the above filtrates, into controls of sterile broth, and into undiluted rabbit serum. The tubes were incubated at 37° C. and streaked on serum agar plates at intervals up to 176 hours. Contrary to expectation, the number of G colonies arising in the 6- and 24-hour filtrates was extremely small, and comparatively few appeared in that of 48 hours. In the 72-hour filtrate G colonies appeared at a rate and in a concentration approximately parallel to that of the control broth. The mutation had reached 50 per cent. in 176 hours. In the undiluted rabbit serum no G colonies appeared at any time during the experiment. It would seem, then, that early filtrates from D cultures are antagonistic to the D ⇀ G mutation.

The C_{H+} of the broth seems, within limits, to have no effect upon the rapidity of the mutation. If anything, an acidity $> P_H = 7.0$ retards the process. Tests were made down to $P_H = 6.0$, beyond which point it is difficult to obtain growth.

An effort was made to discover the constituents of plain broth that encourage the tendency of type D to change to the G variety. Pure-line strains of the former were planted in beef infusion, and in various concentrations of peptone (Fairchild). The P_H of all the media was adjusted to 7.4. It was found that little or no mutation occurred in the beef infusion up to 200 hours at 37° C. In 0.5 to 1.0 per cent. concentrations of peptone some D ⇀ G change was noted. But when higher concentrations, up to 20 per cent., were employed, a very rapid mutation set in, reaching 90 per cent. of the total organisms in 96 hours. This was true even when the peptone solutions were made up to volume with beef infusion. Control tubes of undiluted rabbit serum and of beef infusion showed one or two G colonies at 120 hours, but none after 144 hours or after 8 days. This experiment indicates that peptone in suitable concentrations accelerates the D ⇀ G process.

The G colonies arising in these experiments, and sub-cultured to undiluted rabbit serum, were frequently tested for their distinguishing characters, *i.e.*, low virulence and granular growth in

fluid media. They were found in every case to satisfy these criteria. What is more, the acid agglutination point is distinctly different to that of the D variety. It is in the nature of a physical constant for each type, and is an important differential criterion. All of these characters persist throughout many passages in undiluted serum, a medium markedly antagonistic to the original change. It cannot be said that the presence of the peptone causes the mutation D → G, since the change occasionally occurs, though very rarely and in small amount, in undiluted rabbit serum. On the other hand, the presence of peptone in suitable concentration greatly accelerates a reaction toward which a tendency already exists. It is of interest to note that four pure-line strains, kept on ice in undiluted rabbit serum for three months without passage, showed no evidence of the appearance of G colonies.

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Dissociation of microbic species.

III. Differentiation of microbes D and G by acid agglutination.

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Granular sedimenting growth in liquid medium is one of the principal characters differentiating microbe G (bacillus of rabbit septicemia) from its parent D form. Type G exhibits the granular appearance not only in plain broth, but in serum broth and in undiluted serum as well. This fact led to the examination of the comparative acid flocculation points of the two types. The method used was that of Michaelis, later described in full by Beniasch.

The suspensions of types G and D were prepared by washing the sediments from 5 per cent. serum broth cultures in large volumes of distilled water. After this procedure had been repeated four times, the final suspensions were carefully brought to equal turbidity. Prepared in this way, the G type suspension shows a stability equal to that of D.

The tests for acid agglutinability were carried out with mix-