

Blood cultures were made on 50 rachitic rats which had not been infected, and only 2 of these showed *S. murriotitis*. Of 84 fecal cultures on similar rats 3 yielded *S. murriotitis*. In 9 rats the fecal cultures were repeated 3 times at intervals of a few days and were uniformly negative. A series of rachitic rats (McCollum's diet 3143⁴ for 4 weeks) were fed ½ cc. of the *S. murriotitis* culture and killed at daily intervals. The organism was recovered from the mesenteric glands, liver and spleen in 48 hours and from the blood stream in 5 days.

Using the results in Tables I and II, it appears that the difference in the survival rate between the Vita and the plain glass rats in each experiment corresponds roughly with the difference in the bone ash between the corresponding rats. The difference in the bone ash indicates the difference in the degree of rickets. In other words, it seems probable that the degree of resistance varies approximately with the degree of rickets present.

Summary.—Of 41 rats fed a rachitic diet and exposed to sunshine through Vita glass, 61% survived a *per os* enteriditis infection, as compared with 28% of 32 similar rats exposed to sunshine through plain or ordinary glass.

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Effects of Ethyl Alcohol on Red Blood Cells.

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

From the Department of Pathology, Tulane University, New Orleans, La.

The observations present the microscopic changes occurring in human red blood cells when subjected to different dilutions of alcohol in saline. Approximately 50 specimens of blood from various patients were studied and the results obtained conform as a whole. Studies were made by the hanging drop method. Cells deposited in the serum of clotted blood were examined within 4 hours after withdrawal and whole blood was examined immediately following withdrawal. The red cells were diluted so that the suspension contained approximately 4000 per cu. mm.

When the red blood cells were found to be in rouleux formation the alcohol dilutions overcame this formation more quickly than

⁴ McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, **47**, 507.

when normal saline was added, and the rapidity of this change in all cases depended upon the concentration of the alcohol.

In one series, various iso-osmotic solutions of alcohol and sodium chloride were employed. In 0.1% alcohol the cells were more rounded and regular and this was more apparent in 0.5% alcohol. In the 1% alcohol, granules were extruded from the cells, coincidentally they lost their pigment, became swollen and faded away as shadows.

In the other series, alcohol was diluted with normal saline. In 0.2% (approximately $1.0016 \times$ iso-osmotic) alcohol a cupped appearance was noted and a few of the cells buckled in and bent upon themselves; the inner edges of the cups showed in a few instances serrations and the margins of the cups were more refractile and apparent and the centers clearer. In 0.5% (approximately $1.003 \times$ iso-osmotic) alcohol the irregularity of the cells was more marked. In this concentration numerous cells possessed very clear centers shaped like triangles and slits, while a few appeared small and round and no longer presented the cup effect. In 1% concentration (approximately $1.008 \times$ iso-osmotic), many cells gave the impression of rubber tires with a clear refractile rim, and many appeared larger than the circular cells in normal saline. In 5% alcohol (approximately $4 \times$ iso-osmotic) the cells were largely circular while in 10% concentration (approximately $7.3 \times$ iso-osmotic) they became smaller, crenated, the cup effect disappeared and the cells appeared irregular and shrunken. In the 15% concentration (approximately $19.54 \times$ iso-osmotic) most of the cells were irregular and crenated, but a few were small, uniform in consistency and clear. In 20% alcohol (approximately $13.8 \times$ iso-osmotic) they were finely granular with a fuzzy periphery but uniform in size, some extruded their granules and became clear and pale and in some cases larger. In the 25 to 30% concentrations (approximately 17 and $20 \times$ iso-osmotic) the cells lost their granules, swelling in most cases, and faded away as shadows. When the majority of the cells examined were already crenated, there was a tendency for their granules to become smaller and for the cells to become more regular in the 0.2%, 0.5% and 1% alcohol concentrations. In 5 and 10% alcohol they were irregular and crenated but apparently clearer than those in saline. In the 20% concentration the cells began to clear and become round, and in the 30% they faded away as shadows.

When 95% alcohol was added directly to the red cells, many, maintaining their cup effect, faded away as shadows, while others maintained their original size or became even larger, faded away,

resulting in clumps of granules. This granular residue collected in masses which in many cases seemed located in the framework of the original cells.

In the test tube, complete hemolysis was effected in each case in a 30% concentration of alcohol in normal saline. The red cells showed no greater fragility to 20% alcohol in hypotonic solutions of sodium chlorides of varying concentrations than to hypotonic solutions of sodium chloride in varying concentrations.

This work was originally intended to correlate the microscopic changes in red blood cells with the percentages of alcohol present in blood of man coincident with the feeling of well being and the condition of stupor. However, the microscopic changes which take place in these percentages are not sufficiently marked to assume that they might not take place physiologically as a result of physico-chemical changes occurring normally in circulating blood. The results noted in iso-osmotic solutions of alcohol and sodium chloride indicate that tonicity of solution is not the only factor which plays a part in the case of alcohol. In the solutions studied at least 2 forms of crenation were noted, the one in the solution of iso-osmotic normal saline, and the other in the dilutions of higher tonicity as, for example, the 10% alcoholic solution. In the first type the cells were irregular in outline, coarsely granular and often larger than the unaffected cells and their periphery was studded with projecting knobs. In the second type the cells were shrunken, finely granular, uniform in size and shape and their periphery was studded with fine spicules. Whether this later type of crenated cell is characteristic of hypertonic solutions cannot as yet be definitely stated. The disappearance of the cup effect in the red cell is followed in the hypertonic solutions by this crenated form, and it might be assumed that the shape of the crenated cell is the result of the filling of the cup with the cellular contents normally present at the rim. It is hoped that further work will clear up these details.*

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