blood sugar disturbances did not produce any effect on the α-cells and caused only a slight reaction in the β-cells, from what can be judged from the karyometric results.

There would thus seem to be some reason for assuming that the hyperglycemia has some extra-insular source. Disappearance of the blood sugar elevation has also been observed after cobaltous chloride administration in experimental animals with excised adrenal glands[8,9], or after preceding treatment with dihydroergotamine[10], and it therefore seems probable that in rats also the effect produced by cobaltous chloride might be in some way connected with the adrenal glands.

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Résumé

L'effet de l'administration de chlorure de cobalt chez le rat normal est une hyperglycémie prononcée dans l'espace de quelques heures après l'injection. Il n'est pas possible de déceler des changements dans les cellules α des îlots de Langerhans. Les noyaux des cellules β ne montrent qu'une diminution faible après 24 h de l'injection.

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Dehydrogenase Activity of *Borrelia recurrentis*

So far, little is known about the metabolism of pathogenic spirochetes, since their mass cultivation *in vitro* in a defined medium has only been partially successful[1,2]. Recently, BUCCA *et al.*[3] and BARBAN[4] reported about certain metabolic observations; however, they used in their studies the non-pathogenic Reiter strain of Treponema for testing.

In the present work, the dehydrogenase activity of a known pathogen, *Borrelia recurrentis*, was investigated in a fairly well defined medium, using Tetrazolium reduction as an indicator.

Suspensions of washed *Borrelia recurrentis*[5], containing 10^5–10^7 spirochetes per mm^3, were prepared as follows: Mice infected with the organisms were exsanguinated and the blood placed in an anticoagulant fluid (1 p sodium citrate (3%), 1 p saline (0·85%) and 2 p tryptose-phosphate broth (Difco)). The spirochetes were sedimented by spinning and resuspended in fresh fluid (2 p saline and 2 p tryptose-phosphate broth). Several washings were then made, to remove all extraneous materials. The washed spirochetes remained viable for at least 12–15 h in the wash fluid without added nutrients.

The various dehydrogenase systems to be tested were made up in test tubes containing (1) 0·5 ml of the spirochete suspension; (2) 0·1 ml of a 1% solution of one of a number of substrates (glucose, pyruvate, succinate, fumarate, lactate, formate, glutamate, and asparagine); (3) 0·1 ml of a 0·05% solution of either cysteine, cystine, oxidized or reduced glutathione, or ascorbate; (4) 1 ml of freshly prepared 1:10 dilution made from a 1% stock solution of 2, 3, 5 triphenyltetrazolium chloride in phosphate-saline buffer, pH 6·85 (TTC); and (5) saline (0·85%) to bring the total volume up to 2 ml. In the controls, either the borrelia suspension or one of the substrate components was replaced by buffered saline. All tubes were layered with petrolatum and incubated in a water bath for periods up to 6 h at 30°C. Higher incubation temperatures tended to have detrimental effects on the spirochetes. It was also observed that a pH of 6·85 eliminated the troublesome auto-reduction of the TTC, which frequently occurred at a pH of 7, or above.

Results.—On preliminary examination, the dehydrogenase systems concerned with oxidation of all substrates were found either equivocal or negative. After ruling out the possibility that inadequacy of pH was responsible for these results, a deficiency of appropriate coenzymes was considered. It has already been noted that addition of fresh serum somewhat enhanced activity of the borrelias. However, addition of DPN (0·1%) only slightly improved the results. Apparently, during preparation of the microbial suspension, sufficient coenzymes were carried over so that additional DPN had but little influence.

Since it is known that SH-compounds have the ability to activate several dehydrogenase systems, compounds such as cysteine, cystine, reduced and oxidized glutathione were added to the various test systems. The reaction which was previously weak and unduly protracted (requiring overnight incubation) became accelerated in presence of the reduced agents (less than 6 h) but remained inactive with the oxidized form of compounds. Under the experimental conditions employed, the most active enzyme was one attacking the formate; but following it, the other substrates were also oxidized after variable lapse of time.

In the controls, in the presence of heat-killed organisms, no reduction occurred. Similarly, omission of either of the substrates or of the borrelias failed to reduce the dye.

It remained to be seen whether the SH-compounds acted specifically or not. Using ascorbate as an alternate reducing agent, the very same results were obtained as with SH-compounds. Hence there can be little doubt that the catalytic effect is not specific and is not limited to SH-compounds alone.

The activation phenomenon may be explained by assuming that the enzymes became inactivated in the course of preparation (due to oxidation or perhaps due to trace metal poisoning), and reactivated by adequate reduction or neutralization of the metal poisons.

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Zusammenfassung

Es wurde gezeigt, dass *Bor. recurrentis* unter üblichen Bedingungen nur eine schwache Fähigkeit besitzt, verschiedene Substrate zu dehydrogenieren. In Gegenwart von reduzierenden Agentien (wie SH-Verbindungen oder Ascorbinsäure) wird diese Aktivität hingegen bedeutend verstärkt.

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