The simplicity of planting cultures using frozen-dried plasma and embryo-juice is particularly attractive when technical help is limited, since much routine of preparation can be eliminated. The only disadvantage so far experienced with the plasma is that clotting time is slightly delayed.

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## Mechanism of the Therapeutic Effect of Metrazol and Insulin Convulsions.*

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Anoxemia has been regarded as a common factor in the effects of insulin and metrazol convulsions, in that hypoglycemia diminishes the oxygen utilization of brain tissue (Holmes,[1] Wortis[2]), while the metrazol convulsions interfere with the respiratory movements (Himwich[3] and coworkers). Anoxemia may act by stimulating the sympathetic system (Gellhorn[4]), it may, however, affect the brain cells directly by increasing the permeability of the cellular surface films (Spiegel and Spiegel-Adolf[5]). The question may, therefore, be raised whether insulin and metrazol convulsions change the permeability of the cells of the central nervous system.

In 10 guinea pigs metrazol convulsions were produced (2 cc metrazol† intraperitoneally), in 10 others insulin convulsions (20-40 units of insulin). The brains of 3 guinea pigs were studied after the animals had received insulin, but before the onset of convulsions, while the measurements on 10 further animals served as normal controls. Part of the animals were killed by decapitation during the

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1 Holmes, E. G., *Biochem. J.*, 1930, **24**, 914; 1932, **26**, 2010.

2 Wortis, S. B., *New York State J. of Med.*, 1938, **38**, 1015.

3 Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., *J. Am. Med. Assn.*, 1939, **112**, 1572.

4 Gellhorn, E., *Arch. Neur. and Psych.*, 1938, **40**, 125.

5 Spiegel, E., and Spiegel-Adolf, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 799; *J. Nerv. Ment. Dis.*, 1939, **90**, 188.

† The metrazol was kindly supplied by the Bilhuber-Knoll Corp.

convulsions (minimal duration of the convulsions 5 minutes), in the remainder the convulsions or the insulin "shock" were allowed to continue until the spontaneous death of the animal. The polarizability and thus indirectly the permeability of the cell surfaces was determined 5-10 minutes postmortem by measuring the conductivity at high ($C_h$) and at low frequencies ($C_l$) on the exposed cerebral hemispheres at 37°C temperature and calculating the polarization index $\Delta = 100(C_h\text{-}C_2)/C_2$ (Spiegel and Spiegel-Adolf[5]). The frequencies used in this study were 5120 and 547 respectively. In some animals the brain was used for histological studies after the measurements; besides, the brains of animals subjected to metrazol or insulin convulsions as described above, were histologically studied without being altered by the electrical measurement. The brains were fixed in alcohol and stained with toluidine blue and hematoxylin respectively.

The normal values of the polarization index of the cerebral hemispheres varied between 11.9% and 17.8% (mean 13.75±0.37). The brains of animals subjected to metrazol convulsions showed values ranging between 9.4% and 12.9% (mean 11.44±0.22). The influence of insulin convulsions is still more marked, the Δ values after these convulsions ranging between 8.4% and 12.3% and giving a mean of 10.84%±0.24. The difference between the normal and the metrazol series is 2.31 with a probable error of ±0.43, that between the normal and the insulin series 2.91 with a probable error of ±0.44.‡ That these differences are significant could also be shown by applying the t-test (see Fisher[6]). A comparison of the means of the normal and of the metrazol series yields $t = 3.42$, comparison of the normal and insulin series $t = 4.18$. The corresponding values of P (probability of falling outside the range ±t) are below 0.01, indicating that the differences may be considered as highly significant from a statistical point of view. Control experiments in which animals received the same amount of insulin, but were killed before the onset of the convulsions, showed that this depression of the Δ did not become manifest before the convulsions developed.

Thus metrazol and insulin convulsions, the latter somewhat more than the former, distinctly lower the polarizability of the cerebral hemispheres; this finding indicates that these convulsions increase the

‡ We are indebted to Dr. S. Peller (Department of Biology, Johns Hopkins University School of Hygiene) for his help in the statistical analysis of our data.

[6] Fisher, R. A., *Statistical Methods for Research Workers*, 7th edition, Oliver & Boyd, London, 1938.

permeability of the cellular surface films. In general, the changes of Δ are the least pronounced after convulsions of short duration and the most marked after long lasting convulsions, as particularly shown by the insulin animals. It seems, however, that the duration is not the only factor determining the degree of permeability change, since some cases do not follow this rule. The histological controls may only be briefly mentioned since they chiefly confirm findings reported in the literature.[7] In agreement with the electrical measurements they showed that the nerve cells of the cerebral cortex were more affected by the insulin than by the metrazol convulsions. Particularly in the deep cortical layers cells were found with pale staining, partial (perinuclear) or total tigrolysis, occasionally cell shadows, or vacuolation of the protoplasm. Rarely some cells were more darkly stained than normally. The nuclei were usually well preserved, only occasionally homogeneous shrinking or peripheral position of the nucleus were observed. All these changes were, as mentioned, more marked in the insulin animals than in the metrazol animals. The architecture of the cortex was preserved in all animals. Hemorrhage could not be found.

The increase of the cellular permeability produced by insulin and metrazol convulsions may perhaps contribute to an understanding of the therapeutic effects of such convulsions, in that the decrease of the density of the cellular surface films facilitates the exchange of ions between the cytoplasm and its environment and the removal of products of its metabolism. It is also conceivable that repeated injuries of the cell membrane may eventually result in more severe lesions of the cells as shown by the histologic examination after prolonged convulsion treatment.

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7 Weil, A., Liebert, E., and Heilbrum, G., *Arch. Neurol. and Psychiat.*, 1938, **39**, 467; Strecker, E. A., Alpers, B. J., Flaherty, J. A., and Hughes, J., *Arch. Neurol. and Psychiat.*, 1939, **41**, 996.