as well as the black color, of cell masses of *C. diphtheriae* grown on potassium tellurite chocolate agar disappear upon the addition of small amounts of bromine water. It is inferred, therefore, that the black color is due to the tellurium metal which occurs in the form of needles. (4) It is to be further inferred that the tellurite ion is able to diffuse through the cell wall and is reduced with the precipitation of tellurium metal within the cell boundaries. (5) With the aid of the electron microscope it is now possible to obtain pictorial records of the location of sites of certain chemical reactions incident to the metabolism of the bacterial cell.

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Inactivation of Estrone by Liver After Exclusion of Reticuloendothelial System.*

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It has previously been established[1] that estrone injected in rats disappears rapidly from the organism without being excreted in the urine and feces. To recover the estrone injected the animals were minced, and from the resulting pap the hormone was extracted by organic solvents. Three hours after the injection less than 5% of the estrone could be extracted, after 20 hours, only 0.2%. Hydrolysis permitted the extraction of as much as 20%. No transformation of the estrone into the less active estriol takes place, the hormone being inactivated in the organism. As has been demonstrated by experiments *in vitro*, this inactivation takes place mainly in the liver, and to a small extent in the spleen. Other organs have no inactivating effect. From the following it was concluded that the inactivation in the liver is a fermentative process (estrinase):

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1 Zondek, B., *Lancet*, 1934, **227**, 356; *Skand. Arch. Physiol.*, 1934, **70**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd edition, Springer, Vienna, 1935, p. 124.

(a) The inactivating property of the liver is destroyed at a temperature of over 70°C. (b) A cell-free liver extract still retains the inactivating properties of the liver. (c) The estrone inactivated by liver-pap cannot be reactivated by hydrolysis. That estrogenic hormone is being inactivated by the liver has in the meantime been confirmed by several authors.[2]

The fact that estrone is inactivated by the liver and partly by the spleen, and not by other organs, led to the possibility that the reticuloendothelial system is responsible for this inactivation. We have tried, therefore, to eliminate the function of the reticuloendothelial cells by blocking them. Partial or complete exclusion of this system is possible by means of different substances; coloring agents like methylene-blue or trypan blue, cholesterol, and best by means of heavy metals (copper, iron, gold, etc.). A criterion of the completeness of the "blockade" is the decrease of storing capacity[3] of the reticuloendothelial system. Moreover, it is possible to ascertain the effect of this blocking histochemically.

According to Jansco,[3] the electrocolloidal copper solution (Heyden) given intravenously blocks the reticuloendothelial cells, and in appropriate dosage is able to destroy the whole Kupffer cell system of the liver as well as the reticuloendothelial cells of the spleen and bone-marrow. 0.025 cc of colloidal copper solution (equal to 0.015 mg of copper) are sufficient to eliminate the storing capacity of the Kupffer cells in the mouse. This effect, which begins 10 minutes after injection, reaches its peak in 6 hours. On the basis of these results, we have employed the electrocolloidal copper solution (Heyden) containing 0 06% of copper and a protective colloid. We found that 0.4 cc (0.24 mg of copper) of this solution, injected intracardially in rats weighing 50 g produced death in 48 hours in most cases. This dosage was chosen for our experiment.

Experiments. Three infantile rats, weighing 50 g each, received 0.4 cc of copper solution intracardially. Half an hour later the rats received 0.25 mg of estrone each in oily solution, subcutaneously. Control animals not treated with copper, received the same quantity of estrone. Four hours after the administration of the estrone, the animals were sacrificed and reduced to a pap. This, covered with 5 times its amount of acetone, was left for several days in room

[2] Dingemanse, E., and Laqueur, E., *Am. J. Obst. and Gynec.*, 1937, **33**, 1000; Israel, Meranze, and Johnston, A., *J. Med. Sci.*, 1937, **194**, 835; Golden, J. B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1939, **39**, 361; Talbot, N. B., *Endocrinology*, 1939, **25**, 601; Heller, C. G., *Endocrinology*, 1940, **26**, 619.

[3] Jansco, N., *Klin. Wschr.*, 1931, **12**, 537.

temperature. The acetone was replaced several times. The acetone removed was evaporized and the sediment (A) was used later. The dry powder left after the removal of acetone was extracted for 24 hours with alcohol in a Soxleth. The alcohol was evaporized, and the remainder (B) added to A, cleaned with alcohol, and the alcohol soluble part dissolved in oil. The experiments revealed that in each of the 3 blocked rats, only 0.005 mg of estrone was to be regained, which means that 98% of the estrone administered was inactivated. It was possible to extract the same amount from the non-blocked control rats. It therefore follows that the exclusion of the reticuloendothelial cells does not affect the inactivation of the estrone in the organism.

In a second series the duration of experiment was shortened because the exclusion of the reticuloendothelial cells by electrocolloidal copper solution reaches its peak 6 hours after injection, subsequently decreasing.[4] Three rats weighing 50 g received intracardially 0.4 cc (0.24 mg Cu) of colloidal copper solution, and 4 hours later 0.25 mg estrone, subcutaneously in oily solution. Two hours later, *i. e.*, at the peak of "blockade" the animals were sacrificed, and treated in the manner described above. Two control animals received only estrone. These experiments have also led to clearcut results: An average quantity of 0.025 mg of estrone was extracted from each animal in the control as well as the blocked series, *i. e.*, only 10% of the introduced estrone was detected, 90% having been inactivated.

These experiments show that the liver as well as other organs is able to inactivate estrone after exclusion of the reticuloendothelial cells. This fact, together with the above mentioned experiments *in vitro*, shows that the liver cell itself must contain the inactivating factor (ferment) and that the reticuloendothelial cells have no part in the inactivation.

Summary. The influence of the reticuloendothelial system on estrone inactivation in the organism has been investigated. It is concluded that the reticuloendothelial cells have no part in this process and that the liver cell in itself is responsible for this inactivation, *i. e.*, contains the inactivating factor (enzyme).

[4] Letterer, E., *Klin. Wschr.*, 1933, **15**, 597.