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THE RESISTANCE OF FIXED TISSUE CELLS TO THE TOXIC ACTION OF CERTAIN CHEMICAL SUBSTANCES[1]

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ANY consideration of the general question of tissue resistance has of necessity to first take into account the two monuments in this division of understanding and later the particulate research which these initial contributions stimulated. First, the biological conception of Metchnikoff[2] of the protection and the resistance afforded tissues through the activity of wandering cells, and second, the chemical or humoral theory of Ehrlich[3] which postulated the production on the part of tissues reacting to injurious agents of substances highly specific in nature which had the ability to bind or destroy those agents which had incited their formation. The fundamental work of Metchnikoff has been amplified and made specific through investigations of a major character by Aschoff,[4] Mallory,[5] Maximow,[6] Sabin,[7] Gay,[8] Cunningham[9] and their pupils so that at the present time the wandering phagocytic cells of the

[1] This investigation was made possible through a grant from The Josiah Macy, Jr., Foundation. Address delivered before the General Session of The Federation of American Societies for Experimental Biology; Detroit, April 13, 1935.

[2] Metchnikoff, "L'immunitié dans les maladies infectieuses." Mason et Cie., Paris, 1901.

[3] Ehrlich, *Deut. Med. Wchnschr.*, 17: 976, 1891.

[4] Aschoff, *Ergeb. inn. Med. u. Kinderh.*, 26: 1, 1924.

[5] Mallory, *Jour. Exp. Med.*, 3: 611, 1908.

[6] Maximow, *Arch. Path.*, 4: 557, 1927; *Arch. exp. Zellforsch.*, 5: 169, 1928.

[7] Sabin, *Physiol. Rev.*, 2: 38, 1922.

[8] Gay, The Harvey Lectures, 1930–31, Williams and Wilkins, Baltimore.

[9] Cunningham, *Am. Jour. Physiol.*, 59: 1, 1922.

body and certain fixed phagocytic cells have become recognized as one of the most essential defense mechanisms which the organism utilizes against living agents of disease.

The humoral theory of Ehrlich, though at first standing apart from the earlier conception of Metchnikoff, has through continued investigation not only established itself as an entity in a chemical sense, but such studies indicate the origin of many of these bodies of a specific chemical character to be intimately associated with the specific activity of different types of cells found in the macrophage system. More and more the conception of Ehrlich takes its place as an integral and essential part of the biological defense mechanism first postulated by Metchnikoff.

As far-reaching in their applications as these two conceptions, or a combination of the two may be, there are certain types of tissue resistance which they fail to explain. The congenital and acquired resistance of certain tissues to chemical substances has no cytological or humoral explanation: the congenital tolerance of the parasympathetic endings of the rabbit for atropine when such endings have a normal degree of sensitivity to other agents acting through them; the acquired tolerance of the central nervous system to the alkaloids of opium, even though this tissue, as was shown by Rubsamen,[10] may contain a very high percentage of such bodies. The development of "dye fast" and specifically "arsenic fast" protozoa,[11] especially the trypanosomes, whose resistance to such agents may be transmitted to subsequent generations of organisms,[12] furnishes a group example of acquired tissue resistance on the part of organisms for which there is no explanation.

Tissue resistance not infrequently develops as a sequence to processes of repair. Through inflammation or chemical degeneration, cells of functional value are lost and become replaced by entirely different types of cells which either have no functional value, or their type of function is so changed that they are no longer of value to the organism in a normal, functional sense. The formation of fibrous tissue secondary to tissue destruction, the encystment of parasites or foreign bodies and the localization of an inflammatory area are familiar examples of such a process. Somewhat different, but resembling this form of repair, is the shift in cell type which was observed by Wolbach,[13, 14] and Wolbach and Howe[15] in their studies of vitamin deficiency in which normal epithelium was replaced by keratinized cells. Here again tissue resistance is acquired, but it is not acquired by the formation of cells which are of functional value. Such cell resistance developing as a result of a change in cell type is dependent upon a lack of exposure of the altered cells to the injurious agent. Tissue resistance should imply the ability of cells to function to some extent in a normal manner, to subject themselves to an injurious agent and to withstand in various degrees the action of such an agent. Related to this type of resistance dependent upon injury followed by a repair process in which fixed tissue cells undergo a metaplasia, retaining in part their function and yet becoming resistant to injury, fall the experimental observations which form the basis of this communication.

In experiments[16, 17] conducted many years ago, which were primarily concerned with a study of renal regeneration subsequent to an interference with the blood supply to the kidney, the observation was made that in such areas of restricted blood supply, the epithelial regeneration which occurred was of an abnormal type. The environment created experimentally in such areas was unable to maintain the physical integrity of the highly specialized type of cell which occurs in the convoluted tubules but could maintain a flattened type of cell with less specialized cytological characteristics. There was not then, and there is not now, any way to ascertain in the higher animals the functional value of such cells exclusive of other intimately related structures, the glomeruli.

In a later study[18] of the naturally acquired chronic nephropathy of the dog this same type of cell was observed as having taken the place of cells in the convoluted tubules. When such a shift in cell type had occurred as a reaction to injury during the course of a chronic nephritis it was found that such cells originating as a repair process were resistant to uranium nitrate, while the normal type of cell in this segment of the tubule had no resistance against this nephrotoxic agent. Years later than this Gil y Gil[19] observed that the kidney of the rabbit, the seat of a chronic injury from uranium, developed a certain degree of resistance against this agent. A similar observation was also made by Hunter.[20] Recent studies[21, 22, 23] from this laboratory of the acute and

[10] Rubsamen, *Arch. f. exp. Path. u. Pharm.*, 59: 227, 1908.

[11] Voegtlin, Dyer and Miller, *Jour. Pharm. Exp. Ther.*, 25: 55, 1924.

[12] Woodruff, "Organic Adaptation to Environment," Yale University Press, New Haven, 1924.

[13] Wolbach, *Jour. Exp. Med.*, 43: 753, 1925.

[14] Wolbach, *Archiv. Path.*, 5: 239, 1928.

[15] Wolbach and Howe, *Jour. Exp. Med.*, 57: 511, 1933.

[16] MacNider, *Jour. Med. Research*, 19: 425, 1911.

[17] *Ibid.*, 20: 369, 1911.

[18] *Ibid.*, 29: 177, 1916.

[19] Gil y Gil, *Beit. z. path. Anat., u. Allg. Path.*, 72: 621, 1924.

[20] Hunter, *Ann. Int. Med.*, 1: 747, 1928.

[21] MacNider, *Jour. Exp. Med.*, 49: 411, 1929.

[22] MacNider, The Harvey Lectures, 1928–29, Williams and Wilkins Company, Baltimore; *Amer. Jour. Med. Sci.*, 178: 449, 1929.

chronic nephritis induced in the dog by uranium nitrate have not only emphasized the selective affinity which uranium has for the convoluted tubule cells and the resistance which the kidney may acquire to this toxic agent, but certain of these studies have become more specific in their understanding of the nature of this resistance in that they have shown that it is dependent upon two factors: first, the severity of the epithelial injury; and second, the type of fixed tissue cell which develops as a process of repair in such injured areas.

If a slight epithelial injury be induced in the convoluted tubule cells of a young dog by the subcutaneous use of 2 mgs of uranium nitrate per kilogram, there develops evidence of degeneration in these cells which is followed by a process of repair which results in the formation of cells normal cytologically for this segment of the tubule. When animals with this type of normal epithelial repair are subjected to a secondary intoxication by uranium, in the same amount per kilogram, they fail to show any evidence of resistance to this nephrotoxic agent. The epithelium undergoes a process of degeneration. During these experiments other animals were intoxicated by 4 and by 6 mgs of uranium nitrate per kilogram with the development of a more severe type of injury to the convoluted tubule cells. A large percentage of such animals failed to survive. In those animals effecting a survival a study of biopsy material has shown the convoluted tubule segment to be repaired by an entirely different type of epithelial cell. A cell metaplasia has developed with the formation of cells abnormal in their morphology for this portion of the tubule which are characterized by their flatness, an intensity of staining of both cytoplasm and nuclei, and a tendency to persist as syncytial structures. When such animals are reintoxicated by uranium in the amount employed for the first intoxication or to an amount not exceeding 8 mgs per kilogram, the atypical type of epithelial replacement fails to undergo changes of degeneration. As a reaction to a severe type of injury epithelial repair to the convoluted tubules has taken place by the formation of an abnormal type of epithelial cell which imparts to this segment of the tubule a high degree of resistance to the toxic action of this chemical substance. The functional value of this type of epithelial replacement can not be directly ascertained.

The question then arises, are such processes of repair which depend upon fixed cell metaplasia of such a nature that these cells having a functional value in turn subject themselves to the toxic action of this chemical and resist its toxic influence? In order to answer this question and for the purpose of ascertaining if the same factors influenced fixed cell resistance in other organs, the liver has been studied both functionally and structurally during phases of injury followed by periods of different types of cell repair.

The fact was established through the investigations of Whipple and Sperry[24] that when chloroform was given to dogs under standard conditions there occurred a central necrosis of the liver lobules involving one half to two thirds of their area. A technique similar to this has been employed to ascertain whether or not the livers of animals injured by uranium nitrate and subsequently repaired by different types of fixed cells has or has not acquired a resistance to chloroform when given by inhalation.[25] During these experiments the functional effectiveness of the liver has been ascertained by the use of phenyltetrachlorphthalein, according to the technique developed by Rosenthal.[26]

When young dogs are intoxicated by uranium nitrate given subcutaneously in the amount of 2 mgs per kilogram, there usually occurs by the third day evidence of hepatic dysfunction, which is indicated by an increase in the initial plasma concentration of this dye and by a delay in its removal from the plasma. Such a concentration has rarely gone above 13 per cent. and the delay in the removal of the dye has not been prolonged beyond an hour. At such a period of hepatic injury the epithelium has shown cloudy swelling, an increase in granulation and an abnormal accumulation of stainable lipoid material. Vacuolation and necrosis of the cells has usually been absent. The vascular tissue of the liver and the bile duct epithelium do not participate in the injury. Within eight to twenty days this degree of epithelial injury is repaired by the formation of a normal type of polyhedral epithelial cell and associated with such a repair, liver function as indicated by the use of phenoltetrachlorphthalein returns to its normal value. At such period of liver recuperation the animals were starved for twenty-four hours and anesthetized with chloroform for one and one-half hours. Such a procedure has caused the development of a central necrosis of the liver lobules, which is variable in its extent and which is associated with a depression in hepatic function. A slight liver injury induced by uranium is followed by a fixed cell repair to the liver resulting in a restoration of liver function which is dependent upon the formation of a normal type of liver epithelium. The liver, the seat of this type of repair process, has failed to acquire any resistance to chloroform. The repaired hepatic cells undergo necrosis in the same location in the lobules and

[23] MacNider, SCIENCE, 63: 103, 1931.

[24] Whipple and Sperry, *Bull. Johns Hopkins Hosp.*, 20: 278, 1909.

[25] MacNider, *Trans. Ass. Amer. Physicians*, 49: 14, 1934.

[26] Rosenthal, *Bull. Johns Hopkins Hosp.*, 33: 432, 1922.

in general to the same extent as occurs in the livers of normal dogs.

In a second series of experiments an intoxication was induced by the use of 4 mgs of uranium nitrate per kilogram. When the amount of uranium is increased to this extent a severer type of intoxication is established which may lead to the death of the animals. In those animals which have effected a survival the experiments have had the following course: As a result of the severe liver injury there occurs a higher initial plasma concentration of phenoltetrachlorphthalein which reaches its maximum between the fourth and sixth days of the injury. A concentration as high as 32 per cent. has been obtained at such a period. The dye is not removed from the plasma in a two-hour period. At such a stage of acute injury the hepatic epithelium has shown vacuolation with partial and in areas complete necrosis, a marked increase in stainable lipoid material in the better preserved cells with only a minor degree of invasion of such areas by polynuclear and monocytic cells.

The repair to the liver from an injury of this severity is completed between the fourth and the tenth week. The repair is accomplished by the formation of an atypical type of epithelium which is not patchy but is diffuse in its distribution. The cells and cell cords are flattened, leaving between them greatly enlarged hepatic sinuses. The cytoplasm of such epithelium has a dense appearance and stains intensely and evenly. The nuclei are large in proportion to the surrounding cytoplasm and appear hyperchromatic. In many of the liver cords the cells have not differentiated but remain as syncytial structures. Associated with this type of liver repair there has developed an improvement in hepatic function which in the case of a few animals has returned to the normal. There is evidence therefore that this type of epithelial cell is possessed of definite functional value.

At such periods of liver repair associated with a partial restoration in liver function, chloroform has been given by inhalation in order to ascertain whether or not this type of cell had acquired any degree of resistance against this toxic chemical agent. The animals were starved for twenty-four hours and deeply anesthetized for three hours, an hour and a half in excess of the time necessary to induce liver necrosis in normal dogs and in dogs in which a liver repair had occurred through the formation of a normal type of epithelial cell. In the group of animals now under consideration in which the liver repair had been effected through the formation of an atypical type of cell there was no histological evidence of liver injury. At the termination of the periods of anesthesia the functional effectiveness of the liver in so far as its ability to remove phenoltetrachlorphthalein was concerned was variable. In the larger number of animals at such a period the initial plasma concentration of the dye and the rate with which it was removed was greater than the concentration and rate of removal which the livers of such animals in a state of repair had established as their pathological normal. In a smaller percentage of animals the functional value of the liver was not interfered with. From these experiments it would appear that dependent upon the type of repair process provoked in the liver by the use of uranium, a resistance is acquired on the part of this organ to the toxic action of chloroform when it is given under standard conditions in an amount much in excess of that necessary to induce an injury to a normal type of liver cell.

The question then arises concerning the degree of resistance which has been acquired by this type of cell. Is the resistance complete, or is it relative to the amount of the toxic agent employed and the duration of the exposure of such resistant cells to it? The degree of resistance in this instance as is likely the case in all forms of tissue resistance is relative in its nature, as is shown by the fact that if animals which have acquired a liver resistance to chloroform when starved for twenty-four hours and given chloroform for three hours be again starved not for twenty-four but for forty-eight hours and given chloroform for four hours on one day or for three hours on two successive days, there then occurs a central injury to the liver lobules which, if sufficiently extensive, is expressed by a reduction in hepatic function.

During the thirteen years through which this general plan of investigation has been in effect, dogs have been studied which on account of their age may be classified as senile animals. Such animals have varied in age from eight to fourteen years, have shown the local manifestations of senility, are generally underweight for their variety, but without evidence of a definite process of disease. In twenty-one of ninety-two dogs which have fallen in this group, histological studies of the liver have shown the occurrence of an alteration in the structure of the hepatic epithelium similar to that which has been described as occurring in those animals which have survived a severe injury to the liver from uranium in which the liver repair was effected by the formation of an atypical type of hepatic epithelium. There occurs therefore in a certain number of animals, in a group which may be designated as senile, a change in the type of epithelial structure of the liver. When such animals are starved for twenty-four hours and given chloroform by inhalation for one and one-half hours, there fails to develop a central necrosis of the liver lobules. The removal of phenoltetrachlorphthalein from the plasma is not interfered with. If, however, the period of starvation be increased to forty-

eight hours and chloroform be given for four hours, a central injury to the liver lobule develops. These observations are of particular interest in that they show that a shift in cell type may develop in the liver without the use of an agent distinctly abnormal to this tissue, that the change may be acquired as a product of senility and that when it develops it imparts to the liver a degree of resistance to chloroform comparable to that induced by a process of repair following a severe hepatic injury from uranium nitrate.

CONCLUSIONS

(1) The observations which have been made concerning an acquired resistance of fixed tissue cells to chemical injury should be considered of a gross order and perhaps superficial in their nature. Studies of the mitochondria, the Golgi apparatus and the chemical constitution of such resistant cells afford important suggestions for investigation.

(2) The type of fixed cell response which develops in both the kidney and liver as a reaction to injury induced by uranium nitrate depends upon the severity of the injury to the epithelial structure of these organs. If the injury which is inflicted be slight as indicated by cytological changes of degeneration and in the case of the liver by but minor interference in one manifestation of its function, the process of repair results in the formation of a normal type of epithelial cell. This type of repair process is not associated with the acquisition on the part of the kidney of any degree of protection against subsequent intoxications by uranium or in the case of the liver against the toxic action of chloroform.

(3) If the injury to the epithelium of the kidney or the liver be of a sufficiently severe order, there occurs in such animals the development of a process of epithelial repair which is atypical in character and which imparts to the kidney a relative resistance against subsequent intoxications by uranium and in the liver a similar protection against the toxic action of chloroform.

(4) This protection is not dependent upon the development during the process of repair of a type of cell which is functionally inert and therefore one which does not subject itself to the action of the toxic agents. The morphologically altered hepatic epithelium maintains its functional effectiveness, as is indicated by its ability to remove phenoltetrachlorphthalein from the plasma and furthermore when such changed fixed tissue cells in the liver are subjected to the toxic action of chloroform in a concentration and for a duration far in excess of that employed under standard conditions, they show evidence of injury and liver function becomes depressed.

(5) The observation has been made of the natural occurrence in the livers of certain senile animals of a type of morphologically altered epithelium similar in its configuration and staining reactions to the cells which may develop in the liver reacting to a severe injury from uranium nitrate. Such cells impart to the livers of senile animals an acquired resistance to the toxic action of chloroform.

(6) It would appear from these experiments that a tissue resistance to certain chemical substances may depend upon the development in tissues as a process of repair following injury of an altered type of resistant fixed tissue cell which maintains a sufficient degree of functional effectiveness to enable the organism as a whole to survive.

OBITUARY

EDWIN BRANT FROST
1866–1935

EDWIN BRANT FROST was born on July 14, 1866, at the pleasant town of Brattleboro in the southeastern corner of Vermont. It was here or in the immediate neighborhood that most of his forbears for several generations had lived and flourished. The first of the name in this country was Edmund Frost, who came to Boston in 1634, his grandchildren moving west to New Hampshire and Vermont. Edwin was the second son of Carleton Pennington Frost (1830–1896), who practiced medicine in Brattleboro and neighboring towns until 1871, when he moved with his family to Hanover, New Hampshire, there to be a professor in the Dartmouth Medical School and afterwards dean of the school and a trustee of the college.

Edwin was graduated A.B. at Dartmouth in 1886. The year following he continued postgraduate work at Dartmouth, taught school in a nearby village and at the end of the year spent a few months at Princeton, where he came under the influence of Charles Augustus Young (1834–1908), then perhaps the leading teacher of astronomy in this country. An appointment to an instructorship at Dartmouth came in 1887. At that time advanced work in astronomy and in almost every other branch of science meant a year or more in Europe. Accordingly, Frost secured a two-years leave (1890–1892) to visit most of the European observatories and to spend the second year at Potsdam in Germany, where Vogel was establishing an observatory devoted especially to the new science of astrophysics and where he had gathered around him an