Colonies of monocytes have recently been found to be a better detector of certain conditions of serum than fibroblasts. We know that the rate of migration and multiplication *in vitro* of leucocytes is markedly modified by a great many substances. Moreover, their morphological appearance may change in an enormous number of ways. A monocyte readily modifies its size, shape, nucleus, neutral red vacuoles, fat granules, mitochondria, number of nuclei, undulating membrane and mode of association with other cells. As all these characters may undergo several variations, the number of the permutations of these variations is extremely large, and each monocyte and colony of monocytes is capable of having its individual aspect.

The morphological study of monocytes cultivated in fluid and gaseous media of known composition has been rendered easy by a simple technical progress. The flasks described as microflasks have lately been very much improved. The thickness of the wall is now less than 0.1 mm. Therefore, blood monocytes can be studied at a magnification of 1,000 or 1,500 diameters, while having at their disposal a large amount of nutritive fluid and gases. The leucocytes of an animal are cultivated in their own serum and in the serum of a normal or diseased animal of the same breed. The leucocytes of this second animal are also cultivated in their own serum and in the serum of the first animal. After five or six days, the cells respond to their medium by taking on an aspect that varies almost with each serum. The experiments have been made on chickens, dogs, guinea-pigs and cats. In practically every experiment, the cells acted in an identical manner. When the monocytes of an individual were grown in the serum of another individual, they assumed the appearance of the monocytes of this second individual grown in their own serum. This fact indicates that the appearance of monocytes in their own serum is a transitory character and expresses merely a certain condition of the serum. The same phenomenon occurs in a still more striking manner when the second animal is suffering from an abnormal or pathological state, such as starvation, anemia, immunization, eczema, cancer, etc. For instance, the blood monocytes of chickens inoculated in the breast with a Rous sarcoma become rounder and coarser and agglutinate in clumps when cultivated in their own serum. If placed in the serum of a normal chicken, they tend to lose their pathological character and to resemble the monocytes of this chicken. Conversely, the monocytes of the normal chicken, cultivated in the serum of the sarcomatous chicken, after a few days more or less closely resemble the monocytes of that chicken. A similar phenomenon was observed in other diseases. But the changes imposed on serum by the disease generally respect some individual characteristics. Very seldom does the blood serum of two normal chickens of the same breed, Plymouth Rock or Rhode Island Red, for instance, give an identical appearance to two cultures of the same monocytes. It is obvious that these cells are a very delicate indicator of the conditions of their medium. To summarize:

(1) The structure of blood monocytes and their mode of association depend, in a large measure, on certain qualities of blood serum.

(2) Monocytes from different sources tend to take on the same appearance in a given serum. Each serum imposes upon monocytes a definite character. Pathological monocytes become like normal monocytes in the serum of the latter. And normal monocytes assume the aspect of pathological leucocytes when cultivated in the pathological serum.

(3) The aspect of monocytes cultivated in a given serum expresses simultaneously the individual characteristics of this serum and the modifications of those characteristics under the influence of pathological agencies.

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TEMPORARY PREVENTION BY CHEMICAL MEANS OF INTRANASAL INFECTION OF MICE WITH EQUINE ENCEPHALOMYELITIS VIRUS

IN a recent report in SCIENCE[1] we described a method for active immunization of guinea pigs against experimental equine encephalomyelitis, by means of subcutaneous injections of virus adsorbed on aluminum hydroxide. Mention was made of the production by the alumina gel-virus of local indurations lasting several weeks. It was later found that in the guinea pig 0.2 per cent. tannic acid[2] (tannin) could be substituted for the aluminum compound with no increase in pathogenicity, nor decrease in immunizing power of the virus, and moreover, with no production of cutaneous induration. These methods of immunization depend, however, on the action of living although, as used here, non-infective virus. In another article all the experiments including those relating to poliomyelitis will be described in detail.

During the course of this study, tannic acid alone was dropped into the nose of white mice. We found that this simple act induced resistance against the effects of intranasal instillation of eastern and western strains of the encephalomyelitis virus, a procedure ordinarily lethal in normal animals.

For example, 0.05 cc of 0.5 or 1 per cent. tannic

[1] H. R. Cox and P. K. Olitsky, SCIENCE, 79: 459, 1934.
[2] Mallinckrodt's Acid Tannic, Analytical Reagent.

acid[3] in distilled water was dropped into the noses of 114 mice, three times a day for three successive days, and 0.05 cc of 1:250 dilution of virus-infected mouse brain, or at least 1,000 minimal lethal doses, on the fourth day. Only six of the mice succumbed to experimental encephalomyelitis; whereas of 153 untreated, control animals that received the same virus at the same time, 142 died. The results are more remarkable when considered in contrast with the general ineffectiveness, in 76 mice, of other substances substituted for tannic acid. They include normal saline solution, normal and antivirus rabbit serum, merthiolate (1:5000), hexylresorcinol (in S. T. 37, 1:3), ephedrine (1:100), and formalin (1:50). The formalin was administered to induce especially increased nasal secretion; it is noteworthy that the excessive mucoid discharge so produced did not hinder local infection with the virus. On the contrary, the tannic acid solution caused no grossly visible damage to the nasal mucosa.[4]

The chemical effects of the acid on tissue, as they are now known, are dehydration, precipitation of the soluble proteins of superficial cells and secretions and combination of all proteins with it to form a material which, among other properties, shows a greater degree of resistance to the destructive action of ordinary bacteria and enzymes.[4, 5, 6, 7] Histological studies of nasal membranes of tannin-treated mice reveal either no effect or slight shrinkage of the lining cells, accompanied by a deposition of more or less uniform precipitated material covering the ciliary surfaces. The open nasal spaces contain a slightly increased cellular exudate. By following the method of Clark,[8] who reported the transit, within 3 hours, of Prussian blue particles from the nasal cavity to the brain of the rabbit, by way of the olfactory nerve, we found that in normal mice there occurred within this time a generalized dispersion of the dye throughout the epithelium and invasion of the nerve by finer blue granules. In tannin-treated animals, however, the dye was less evident in the epithelial layers and not visible in the olfactory rootlets and nerve.

The preventive action of tannic acid is exerted locally: Tannin-treated mice are as susceptible to lethal infection after cerebral inoculation of virus as are normal animals. Another point showing that a barrier, as it were, may be set up against the infectious agent at a portal of its entry is that virus, if dropped into the nose first and tannin later, even in so short a time as 15 minutes, loses none of its activity.

It appears, besides, that a sufficient amount of tannic acid is necessary to insure its action in warding off virus infection. The method as described above was shown to yield protection in 94.7 per cent. of the treated mice, but when 0.25 instead of 0.5 or 1 per cent. of the chemical was employed, resistance was induced in 75 per cent. of the animals. Again, when the acid was administered in the usual dose of 0.5 or 1 per cent. but instilled on one or two, instead of three successive days, 39 and 62 per cent., respectively, of 23 mice in each instance, were found to resist the virus given intranasally on the day following the last instillation of the chemical.

The duration of protection afforded by tannin is, however, transient; the nasal passages approach their normal condition of sensitiveness to virus infection on the eleventh day after the beginning of the tannic acid instillations. Thus, when virus was dropped into the nose of mice on the fourth day, 94.7 per cent. of the animals were protected; on the fifth, 87.5; sixth, 83; seventh, 58.8; eighth, 30 per cent. and on the ninth and tenth days, only an occasional mouse was found to be refractory to infection. Thereafter all tannin-treated mice were normally reactive to the virus. Nevertheless, experimental results indicate that the treatment can be repeated in the same animal so as to bring about a similar temporary virus-resistant state.

To conclude, it has been found possible to induce a transient resistance in mice to intranasal infection with two strains of equine encephalomyelitis virus by prior applications of tannic acid to the nasal mucosa.

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[3] An unknown quantity is lost at each instillation through insufficient sniffing by the mouse, for the method consists in depositing the fluid employed drop by drop at the nasal orifices by means of a 27 gauge needle. The animal then inhales the material, requiring 2 minutes for the procedure. By this method injury to the nasal mucosa is avoided. However, for reasons as yet unknown, the same technique, when applied to guinea pigs, has thus far failed to protect these animals against intranasal infection with the same virus.

[4] For the innocuousness of tannic acid, especially in the amounts given, see W. A. Bastedo, "Materia Medica," etc., W. B. Saunders Co., Philadelphia, 1933, 3rd edit.

[5] H. P. Kruyt, "Colloids," John Wiley and Sons, New York, 1930.

[6] H. Gnamm, "Die Gerbstoffe und Gerbmittel," Wiss. Verlags., M.B.H., Stuttgart, 1933.

[7] M. Bergmann, personal communication.

[8] W. E. Le G. Clark, "Reports on Public Health and Medical Subjects," Ministry of Health, London, 1929, pp. 1–27.

X-RAY DIFFRACTION STUDIES ON NERVE

ANALYSIS of x-ray diffraction photographs of nerve made by us during the past few months indicates what appears to be a fundamental similarity between the fine structure of the axis cylinder of nerve and that of other animal fibers, such as hair, tendon and