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EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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NATURAL RESISTANCE TO DISEASE

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SVMMARIVM — Ex nonnullis Auctoris experimentis effectum est ut extractum substantiae haud proteicae, solubilis in alcole, quae e bovis splene vel cerebro ablata est et ab ipso Auctore specialiter tractata, valde activum evaserit contra staphylococcum aureum, streptococcum emolyticum, virus MM encaephalomyocarditis.

Physicians have long realized that the body has a defense against some common diseases, which is not the result of antibody formation. Much has been written on the subject of natural resistance — a term frequently applied to non-antigenic protection. It is widely recognized that a partial non-specific immunity to some microorganisms can result from the injection of proteins not directly related to the organism to which immunity has been developed. The resulting immunity, however, is not what is usually referred to as a natural resistance.

The type of resistance which is the subject of this paper exists even before an attack by the disease and independent of antibody formation.

It is well known, for example, that a degree of natural resistance to one of the most common infecting organisms (*Sta-*

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phylococcus aureus) exists in certain species and it is also well recognized that there is a great variation in the degree of resistance to this organism among members of the same species including human beings. Extensive researches have been conducted by FORSSMAN [1, 2], JAUBERT [3], RIGDON [4], PARKER [5], RAMON [6], RICHOU [6] and BURNET [7] to mention but a few, in attempts to develop vaccines, antitoxins or toxoid preparations against such organisms. Although minor successes have been reported with autogenous vaccines and toxoids it is generally accepted that such procedure gives very little it any protection in the majority of cases.

In a series of experiments which we presented at the Ohio Academy of Science it was demonstrated that the resistance which heretofore had been considered natural is one which can be artificially induced to a high degree. It has been further shown in work as yet unpublished that this resistance is totally independent of any type of antibody formation with which we are presently familiar.

In our first experiments [8] we cultured on nutrient agar a typical highly pathogenic strain of *Staphylococcus aureus* in the presence of a non-protein alcohol-soluble substance isolated from beef spleen. It was noted that after a 48-hour incubation period there had been a change from the typical golden colonies to white, more characteristic of *Staphylococcus albus*. Subsequent extensive laboratory tests [9] demonstrated that this white organism was biochemically vastly different from the original strain from which it had been derived. Whereas the original yellow S organism had shown + (positive) reactions to fermentation of Mannitol, liquefaction of gelatin, haemolysis, and coagulase production, the altered white organism was — (negative) in these respects.

In an effort to determine whether similar changes occurred *in vivo,* a number of mice were subjected to subcutaneous infections of a virulent strain of *Staphylococcus aureus* and treated daily by subcutaneous inoculation of a crude alcohol

extract of the beef spleen. From the time of appearance of lesions (3 days) to their complete disappearance (8 days) cultures from their surfaces were made daily. It was noted that the original yellow or orange organisms were converted to the white form preceding their ultimate disappearance and the subsequent healing of the lesions. Control animals during this period grew progressively worse.

NUTINI and LYNCH [10], of our laboratories (Institutum Divi Thomae - Cincinnati, Ohio), writing in the *Journal of Experimental Medicine,* presented extensive results on the prophylatic as well as the therapeutic effects of a spleen fraction on *Staphylococcus aureus* infections in laboratory animals.

In the combined group of experiments in which the infection was induced by either the subcutaneous, intravenous or intraperitoneal route and treated either subcutaneously or orally there was an overall mortality of 2% in the total of 444 experimental animals as against 81% in the 448 control animals. As previously stated, the extracts appeared equally effective when used therapeutically (mortality 2% of 162 experimental animals and 90% in the same number of control animals) or prophylactically (mortality 2% of 282 experimental animals and 76% in 286 control mice).

In the *Journal of Pharmacology and Experimental Therapeutics* we have reported results on penicillin-resistant strains of *Staphylococcus aureus* [11, 12]. Animals infected with 24 different resistant strains responded to the treatment equally as well as animals infected with non-resistant strains of bacteria. It is interesting to note that in the therapeutic experiments the mortality in the penicillin-treated positive control groups was higher than in the non-treated negative controls. Equally gratifying results have been obtained on strains known to be highly resistants to antibiotics other than penicillin.

Inasmuch as these spleen fractions are effective prophylactically, a series of experiments was undertaken to determine the length of time resistance resulting from their use would persist.

Mice which had been given subcutaneous injections of 50 mg. of a crude spleen fraction on 10 consecutive days were divided into groups and challenged at successive intervals over a period of 4 months with a virulent strain of *Staphylococcus aureus*. At each interval of challenge a control group of animals was similary infected. The first two groups of animals were challenged at interval of 1 week; thereafter, this was extended to 1 month. For a period of 2-1/2 months the resistance afforded by the extract was 100%; the treated animals showing no deaths on challenge, the controls 90-100% mortality. Thereafter the resistance dropped rather markedly showing only about 50% protection at the 3-month interval and none at 4 months.

In a series of experiments published by our colleagues of Institutum Divi Thomae in the *Journal of Experimental Medicine* it was shown that the substance is also effective when administered orally. For example, in an experiment in which the spleen fraction was administered prophylactically, control animals showed a mortality of 80% and an average healing time for the surviving animals of 18 days as against 0% mortality and an average healing time of 5 days in the experimental animals. In the therapeutic experiments at this same dosage level there was 100% mortality in the controls as against 0% in the experimentals.

After using the material successfully in the laboratory for a number of years the results began to change. The number of organisms required to kill a mouse had risen from an average of about 25,000 to 1 to 2 billion. Many strains of organisms were tested with similar results. It was thought that the organisms might have changed due to the widespread use of antibiotics and it was observed that the spleen fraction appeared less effective. Organisms were obtained from many different sources including U.S. Public Health but in no case could a strain be found capable of killing in doses in any way comparable with those effective in earlier experiments.

Throughout this period the strain of animals and the diet (as far as could be determined) had remained the same. Eventually the mystery unfolded when it was discovered that the diet had not actually remained constant even though the analyses conducted showed no changes. Final proof came when a « synthetic » diet was prepared from yellow cornmeal, casein and D-L methionine. On weaning, mice were placed on the new diet for 11 weeks and then challenged with strains of *Staphylococcus aureus* which in earlier experiments had proven to be relatively ineffective. The mice were found to have developed a considerably higher susceptibility to the organism and responded quickly to treatment with the spleen extract. It is now becoming apparent that the term « natural resistance », at least as applied to *Staphylococcus aureus,* is true only in the sense that it is natural for animals to consume foods containing these protective substances.

In unpublished results we have evidence to establish the fact that resistance induced by injecting the active principle is transmitted to a smaller degree to the first generation of offspring.

We have shown beef brain also to be a very potent source of this agent. Extensive investigations have given us little information on the identity of the active principle and would find little place in this discussion. Suffice it to say that through the use of ion exchange resins and selected solvents we have been able to produce a fraction of greatly increased potency and to show that the « immunizing » substance is not a protein or any of the substances known to elicit antibody formation.

Researches from our laboratories, published by NUTINI and KREKE [13], show that the type of resistance described herein is not limited to *Stphylococcus aureus* but can also be induced against the hemolytic streptococci. In these experiments it was noted that *in vitro* the extract was inhibitory to the growth of these organisms, and *in vivo* with the limited dosage administered, it afforded approximately 40% protection.

In a lengthy series of unpublished experiments we have been able to show that a factor present in beef brain is an effective preventive against MM virus which produces encephalo-myocarditis in mice, a condition accompanied by symptoms very closely resembling those of poliomyelitis in human subjects. Indirect evidence seems to indicate that this agent is different from the one effective in inducing resistance to *Staphylococcus aureus;* however, both factors are present in the crude alcohol-soluble fraction from the brain.

THOMAS of our laboratories, in a paper delivered before the 47th meeting of the Society of American Bacteriologists, was able to demonstrate that the alcohol-soluble fraction from beef brain had no significant effect on the tuberculous infections in mice. He was, however, able to show an effect with a similar fraction from either beef kidney or heart.

Extensive researches into the classical immune mechanisms involved in the normal defense of the body against staphylococcic infections have failed to reveal any increase in phagocytosis, opsonic index, agglutinins or precipitins following the injection of the active material from either beef brain or beef spleen. Nor has it been possible to show any conclusive relationship between the properdine level of the blood and the resistance developed as a result of the injection of brain or spleen extract.

The size of our laboratory equipment, although ample for mouse experiments, has been grossly inadequate for the production of sufficient material for human testing. However, the limited results on human infections serve to indicate that the gross findings on laboratory animals are applicable to human beings.

For example, a patient admitted to our clinic had a deep-seated staphylococcic infection (*Sycosis barbae*) covering the face and neck, confirmed by bacterial tests. The patient, forty years of age, had been under treatment for twelve years, during which period he had been subjected to X-rays, radium, plastic

surgery, sulpha drugs and penicillin with uniformly negative results. On admission to the clinic he was given two injections of 250 mgs. each of the alcohol-soluble fraction from brain twice a week for a period of six weeks, at the end of which time he returned to the clinic cleanly shaven and showing no sign of infection. As material became available other cases were treated with equally satisfactory results.

Obviously, the researches to be conducted in this field of « natural resistance » include further studies on the mechanism of action, concentration and identification of the active principles, content of the active principle in tissues (including plants) and, above all, the effect on a much broader spectrum of organisms and viruses.

Unlike the antibiotics, organisms fail to develop resistance to the drugs even when cultured in the presence of the active principle as many as fifty consecutive times.

It may be concluded that certain natural substances exist which, although having little direct inhibitory effect on pathogens, when given orally or by injection, have the power to induce extraordinary resistance (*).

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