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A Preliminary Contribution from the Laboratory of Hygiene of Michigan University.

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IN studying the subject of immunity we have been led to test the action of certain constituents of the cell upon the life and growth of microörganisms, because it has seemed to us that the cells of the animal body must be concerned in the production of immunity, and in a way not explained by the phagocytic theory of Metschnikoff.

Immunity may be natural or acquired. Natural immunity may be peculiar to the race or to the individual. An example of racial immunity is that of the domestic fowl to anthrax. The chick, even at the moment when it comes from the egg, is immune to the most virulent cultures of the bacillus anthracis. It is true that this animal may be made susceptible to this disease, but this is an artificially induced susceptibility, and the immunity is natural to every period of life. Another example of racial immunity is that of the frog to the same disease, and here again an artificial susceptibility may be induced. Racial immunity must be inherent in the parent cell.

The natural immunity which is peculiar to the individual usually comes with adult life. The young are susceptible to a given disease, but adults of the same species lose this susceptibility, and become immune. Examples of this are common. The young rat is susceptible to anthrax, while the adult is naturally immune, but can be rendered susceptible by reducing the vital strength. The child is highly susceptible to scarlet-fever and diphtheria, while the adult, though not wholly immune to these diseases, loses much in susceptibility, and is likely to become infected only when much reduced in vitality, or when exposed to the prolonged influence of the infecting agent. The only reasonable explanation of this immunity is that it is inherent in the parent cell, and comes as naturally as the changes in form and voice at puberty, or as the growth of the beard in early manhood. The evolution of the condition of immunity in these cases is due to natural development of the functions of certain cells of the body.

In selecting the constituent of the cell upon which we could begin our studies, the nucleins, constituting as they do the most complex part and possessing marked physiologic properties, naturally suggested themselves. We will not in this paper discuss the chemistry of the nucleins any farther than to say that they consist of nucleic acids combined with a complex proteid base. It is more than probable that nucleins from diverse sources differ in both their acids and bases. The nucleins are not digested by hydrochloric acid and pepsin, and this affords in many cases a means for their isolation.

Nuclein from dog's testes. The testicles of two small dogs were stripped of their investing membranes, and were digested for some days (until the supernatant fluid failed to respond to the biuret test for peptones) at 40° C., with pepsin and 0.2 per cent. hydrochloric acid. The undigested portion was collected on filter-paper, and washed first with dilute hydrochloric acid, then with alcohol. Finally, it was dissolved in a 0.5 per cent. solution of potassium hydrate, and filtered through a Chamberland filter without pressure.

This solution is clear, golden-yellow, and feebly alkaline. On the addition of a drop of nitric acid a white precipitate forms, and dissolves colorless in the cold on the further addition of the acid. The nuclein solution does not give the biuret reaction, but does respond to the Millon test.

With this solution the following experiments have been made in order to test its germicidal effect.

The solution of testicular nuclein was diluted with seven volumes of physiologic salt-solution. This reduced the alkali to 0.0625 per cent., an amount too small to have itself any germicidal effect.

One tube of this solution was inoculated with a loop of a beef-tea culture of the staphylococcus pyogenes aureus, and plates were made after 5 min., 4 hrs., 6 hrs., 23 hrs., and 54 hrs. The number of colonies on each plate was as follows:

Time	5 min.	4 hrs.	6 hrs.	23 hrs.	54 hrs.
No. of colonies	10	0	0	0	0

Another tube was inoculated with a loop of a beef-tea culture of bacillus venenosus, and plates were made, with the following results:

Time	5 min.	4 hrs.	6 hrs.
No. of colonies	210	8	9

Another portion of the testicular nuclein solution was diluted with four parts of the physiologic salt-solution. This reduced the alkalinity to 0.10 per cent. (water containing 0.5 per cent. of potassium hydrate failed to destroy this culture of the aureus after twenty-four hours' exposure). To a tube of this a loop of a beef-tea culture of the aureus was added. The plates showed the following:

Time	5 min.	1 hr.	2 hrs.	14 hrs.	23 hrs.
No. of colonies	250	0	0	0	0

In another, in which the same dilution and the same germ were used, the number of colonies was as follows:

Time . Immediately.	20 min.	1 hr.	2 hrs.	17 hrs.	24 hrs.
No. of colonies 680	0	0	0	0	0

In still another experiment, in which the same dilution of the testicular nuclein was used with the anthrax-bacillus (without spores), the colonies numbered as follows:

Time . Immediately.	20 min.	1 hr.	2 hrs.	17 hrs.	24 hrs.
No. of colonies 45	0	0	0	0	0

With nuclein obtained from the testes of a rat and diluted with physiologic salt-solution until the amount of alkali was reduced to 0.06 per cent., one experiment with aureus gave the following results:

Time . . Immediately.	5 min.	1 hr.	2 hrs.	27 hrs.
No. of colonies 1110	0	0	0	0

Nuclein from thyroid glana. We took the thyroid gland of a rabbit, killed by drawing the blood from the carotid, cut the gland into fine pieces, extracted

with alcohol and ether, then placed the extract in 0.2 per cent. hydrochloric acid with pepsin, and kept it in the incubator at 40 C. for two days, having decanted and renewed the digestive fluid several times. The slight glanular residue which remained undigested was collected upon a filter, and washed with 0.2 per cent. hydrochloric acid until the washings failed to give the biuret reaction. After this treatment there appeared on the filter glistening scales which, under the microscope, showed bundles of radiating needles. These proved to be fat, and were dissolved by washing with alcohol and ether. The residue now on the paper was exceedingly small. This was dissolved in 5 c.c. of a 0.25 per cent. potassium hydrate solution, diluted with an equal volume of physiologic salt-solution, and with this the experiments were made.

This solution gave a faint opalescence on the addition of nitric acid. It did not color on heating with nitric acid, but did become markedly yellow on the further addition of ammonia. It failed to respond to the biuret test.

This solution was inoculated with a loop of a beef-tea culture of the aureus, and the plates showed the following number of colonies:

Time . . .	Immediately.	10 min.	1 hr.	18 hrs.
No. of colonies	805	830	256	0

Nuclein from yeast-cells. Yeast-cells, after having been washed with water by decantation, were extracted with dilute alkali. The alkaline solution was precipitated with dilute acid, and this process repeated a number of times. The solution, as kept for use, was made by dissolving the nuclein in 0.25 per cent. alkali. That the nuclein in this solution

is not free from albuminous bodies is shown by the fact that it responds promptly to the biuret, xantho-proteic, and Millon tests. However, this solution has stood in an ordinary glass-stoppered bottle, which is frequently opened for nearly five months, and remains germ-free.

The amount of nuclein (impure) in this solution is nine milligrams per cubic centimeter. With this solution the following experiments were made :

Two parts of the yeast nuclein solution were diluted with three parts of the physiologic salt-solution, and this was inoculated with a loop of a beef-tea solution of the aureus. Plates made showed the following results :

Time	5 min.	1 hr.	2 hrs.	14 hrs.	23 hrs.
No. of colonies	1110	0	0	0	0

The same experiment repeated gave the following :

Time	5 min.	1 hr.	2 hrs.	14 hrs.	23 hrs.
No. of colonies	1490	20	0	0	0

In one, with the staphylococcus pyogenes albus, the following are the figures :

Time . .	Immediately.	20 min.	1 hr.	2 hrs.	17 hrs.	24 hrs.
No. of colonies	680	0	0	0	0	0

In one, with the bacillus anthracis :

Time .	Immediately.	20 min.	1 hr.	2 hrs.	17 hrs.	24 hrs.
No. of colonies	45	0	0	0	0	0

During the past year we have also tested the effects of injections of nuclein upon the progress of certain infectious diseases. Moreover, we have some evidence that the germicidal constituent of blood-serum belongs to the nucleins. This work, however, we wish to confirm before publishing.