experimentally produced cases and the finding in both of intranuclear inclusions.

The lesions are restricted to the respiratory tract and occur first and are most pronounced in the larynx and trachea. At the outset the surface epithelial cells show various forms of cellular degeneration, but inflammatory processes of various degrees in the submucosa and other parts of the mucous membrane soon follow. The destructive process in later stages is due to the combined effect of the virus and mechanical factors produced by edema, cellular infiltration, hemorrhages, and in a few instances secondarily invading bacteria. A small number of animals show bronchitis and peribronchitis, pneumonic areas, and hemorrhages in the lungs, while involvement of the nasal passages, communicating sinuses and eyes seems to be dependent upon the point of entrance of the virus.

Certain intranuclear inclusions can be demonstrated in the epithelial cells lining the mucous membrane as well as in those of the mucous glands of the larynx and trachea in many cases. These inclusions consist of round, oval or irregularly shaped, sharply outlined, homogeneous, acidophilic masses. Usually a single inclusion occurs in a nucleus. The size may be small, but it is often so large as to occupy most of the central portion of the nucleus, which then may be considerably enlarged. The nucleoli of the affected cells are commonly attached to the nuclear membrane, and the space between the inclusion and the nuclear membrane remains entirely unstained. The inclusions resist the solvent action of acetic acid, alcohol and chloroform; they do not contain fat or iron, but give the Feulgen reaction for thymonucleic acid slightly. Silver impregnation shows small argentophilic granules inside the inclusions. The inclusions described as well as other changes in the nuclei bear a close resemblance to similar structures found in such virus diseases as herpes, varicella, virus III of rabbits and submaxillary gland disease of guinea pigs.

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IONIC EQUILIBRIA IN THE SERUM IN RELATION TO THE CRITICAL TEMPERATURE

IN our systematic researches on the critical temperature of blood serum (around 56° C.) we have so far purposely neglected the part played by the salts and we have been able to account for the observed phenomena by means of simple hypotheses which explained the facts satisfactorily without necessitating any assumption concerning ionic phenomena. However, we did not overlook this factor, neither did we underestimate its importance. We were only compelled to leave it aside in order to simplify arbitrarily a problem already so complex. We have now attempted to study this side of the question, and the purpose of this paper is to report a few preliminary results.

As it was necessary to respect as much as possible the integrity of the protein molecules, we only used distilled water as a means of disturbing the normal salt equilibrium of the serum. The first step was to study quantitatively the equilibrium: albumin-globulin, when progressive amounts of pure water were added to normal serum. The method used consisted in measuring the amount of light scattered at right angles of the incident light (more accurately, the value of $\log \frac{I_o}{I}$) as a function of the dilution. As the addition of water brings forth a precipitation of the globulins, the light scattered by the solution increases. It is known that the amount of light scattered is proportional to the number of particles (Lord Rayleigh), provided the latter are small with respect to the wave length of the light used and nearly spherical in shape. It was found that the addition of 1, and even 2 cc of water to 1 cc of serum did not determine much cloudiness, while if 3 cc were added the increase in the scattered light was very important. In other words, there is, in general, a sharp break in the curve around the point corresponding to a concentration of salts equal to 33 per cent. of the normal. If we assume that the value of the ratio $\frac{I_o}{I}$ is equal to 1 for pure serum, an addition of 200 per cent. of water will bring it to

TABLE I

LIGHT SCATTERED BY NORMAL HORSE SERUM, AFTER ADDITION OF DISTILLED WATER

Cc of water added to 1 cc of serum	Relat. concent. of salts, per cent.	Readings $= \log \frac{I}{I_o}$	
		After 1 hour	After 4 hours
Ser. undiluted	1	2.18	2.23
+ 1 cc water	0.50	2.12	2.14
2	0.33	2.06	2.08
3	0.25	1.54	1.50
4	0.20	1.30	1.30
5	0.168	1.17	1.21
6	0.143	1.15	1.16
7	0.125	1.12	1.14
8	0.115	1.12	1.14
9	0.100	1.13	1.15
10	0.091	1.15	1.17
11	0.083	1.17	1.19

about 1, 3, while an addition of 300 per cent. raises it to nearly 4. Between the concentration 0.33 and 0.25 per cent. of salts we observe therefore a particular instability of the serum, and the instability was maximum, in our experiments (normal horse serum, collected without especial precautions), immediately around 0.33 per cent. Table I gives the results of one experiment.

aspect was decidedly different in the cases of unheated serum, and of serum heated above 58°. In order to obtain figures proportional to the rate of sedimentation, it was only necessary to observe the scattered light at regular intervals of time, and to take the readings. This was very easily done in the instrument previously described.[1]

Fig. 1 expresses the results of one set of experiments, made with serum diluted with 5 times its volume of distilled water.

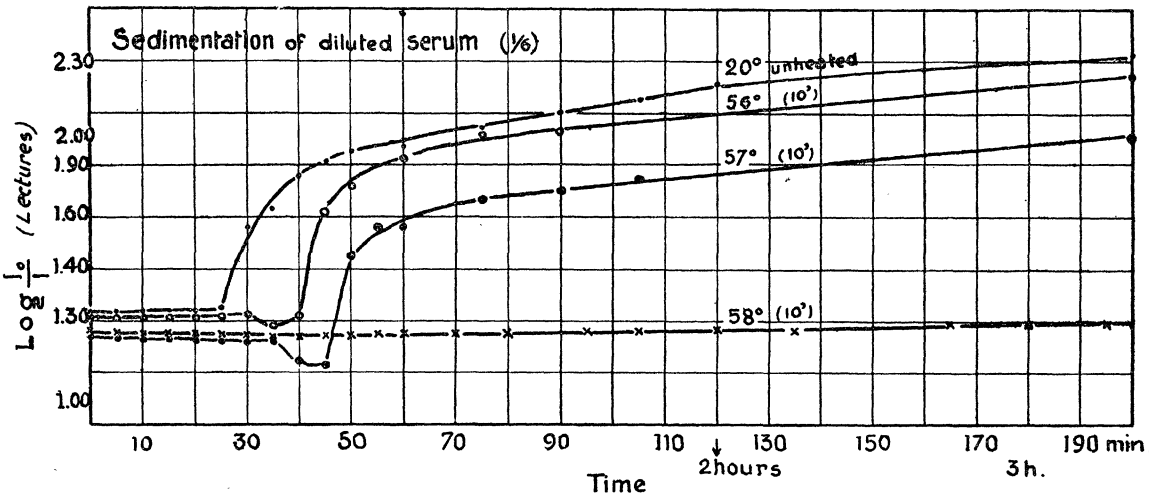

FIG. 1

When the serum is heated for 10 minutes previous to the addition of water, the fragility of the system albumin-globulin is increased, as shown by Table II. The increase in the amount of scattered light (expressed in the table by *decreasing* figures) begins sooner, in a more abrupt fashion, but only when the serum is heated above 55° C. When heated to 60° (always for ten minutes) the curves are totally different in shape.

TABLE II

LIGHT SCATTERED BY NORMAL HORSE SERUM UNHEATED AND HEATED PREVIOUS TO ADDITION OF DISTILLED WATER

Cc of water added to 1 cc of serum	Unheated	Heated at 56°	Readings = $\log \frac{I}{I_o}$	
			Heated at 60°	Heated at 64°
0	1.98	1.96	1.72	1.19
0.5	1.98	1.96	1.62	1.09
1	1.99	1.92	1.59	1.05
1.5	1.98	1.84	1.54	1.00
2	1.95	1.68	1.53	1.03
3	1.63	1.56	1.39	1.05
4	1.33	1.47	1.32	1.04
5	1.25	1.40	1.31	1.07

It was then attempted to determine the rate of sedimentation of the precipitated globulins, as their

It will be seen that the sera heated for ten minutes at 56° and 57° (in sealed tubes, of course) show a slight decrease in the value of $\log \frac{I_o}{I}$ (which correspond to a momentary *increase* in the amount of scattered light) after 35 minutes in the first case, and 40 minutes in the second case. Unheated serum shows a sudden and important *decrease* in the amount of scattered light, after 25 minutes, while the same decrease is observed after 40 minutes in the serum heated at 56° and after 45 minutes in the serum heated at 57°. The curves are practically parallel. This decrease is obviously due to the decrease in the number of scattering particles due to settling, and is a function of their number. The settling of the globulins can then be followed quantitatively. But a very important phenomenon was observed when the serum was heated for 10 minutes up to 58°, namely, the fact that *there was no decrease at all in the scattered light, no settling.* Fig. 1 shows indeed that the figures expressing $\log \frac{I_o}{I}$ are practically constant for two hours, and that even 18 hours later the change is much less marked than that which occurs in the serum kept at 57°. When the serum was heated at 60° no change whatever was observed in 22 hours.

[1] P. L. du Noüy, *Ann,* Inst. Pasteur, 45: 251, 1930.

The globulins do not settle, and remain in suspension for many days. The pH of the solution plays an important part in the phenomenon; but this side of the question will be taken up in another paper. Suffice it to say at present that the solution must be slightly acid in order to observe an immediate difference in the rate of sedimentation. With less acidity, the phenomenon is only postponed.

In the serum unheated, or heated below 57°, the phenomenon of sedimentation starts very abruptly, and is very rapid at first, as in a given volume the number of scattering particles is more than halved in 5 minutes. However, as Rayleigh's formula only holds for particles which are small with respect to the wave-length of light, and as we have at present no information concerning their size, we can not as yet interpret the figures satisfactorily.

Nevertheless, a new phenomenon can be added to the four which, as we have already shown, characterize the critical temperature of serum, namely, viscosity, rotatory power, scattered light, depolarization factor. But this last one brings forth something more, as it applies to the behavior of the globulins alone, when the total serum was heated at or above 58°. Without drawing any hasty conclusions, one can not help wondering if the destruction of the complement might not be connected primarily with the globulin fraction of the serum.

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THE POSSIBLE RÔLE OF MICRO-ORGANISMS IN THE PRECIPITATION OF CALCIUM CARBONATE IN TROPICAL SEAS[1]

FOLLOWING the same procedure carried out in the investigation of marine "calcium bacteria" by Drew, Kellermann and Smith, Lipman and others, extensive personal observations were made in the region of Andros Island, while the experimental studies were carried out in the laboratories of soil microbiology at the New Jersey Agricultural Experiment Station, at New Brunswick, New Jersey. Only a brief summary of these experiments may be presented here. The results so far obtained make it appear that the question concerning the rôle of micro-organisms in the formation of sediments in the ocean, especially their activity in the precipitation of calcium carbonate, may be reopened again.

In the course of a trip of three weeks' duration during the months of March and April, 1930, to the Great Bahama Banks, to Andros Island and to a smaller island off the west coast of Andros, or Williams Island, various samples of mud were collected with special instruments, at several carefully selected locations and from different depths, in order to carry out the investigations in the laboratory named above; likewise direct microscopic observations of the microflora of the material collected were made immediately, soon after the samples were taken.

At the laboratory in New Brunswick, experiments were begun first by counting the numbers of micro-organisms in the mud, since to date there is no sufficiently definite information dealing with the number or with the quantitative distribution of organisms which might possibly take a direct or indirect part in the process of calcium carbonate precipitation. These experiments led to the discovery of certain interesting and characteristic differences in the different regions of the ocean.

In agreement with the observations of the earlier investigators, it was discovered—if we disregard the water of the high seas with its well-known low bacterial population—that relatively few bacteria occur in the white calcium carbonate mud of the Bahama Banks and off the west coast of Andros; the numbers of bacteria in the different layers of this mud, as determined by the plate method, vary from a few cells to 5,000, 10,000 and 100,000 cells per gram of moist mud. At other locations, however, as on the coast of Williams Island, and especially in the mangrove swamps situated farther inland, the number of bacteria increase considerably. The surface layers of the mangrove swamp of Williams Island, for example, contain over sixteen million bacteria per gram and, even at a depth of four feet, over two million bacteria could be found in one gram of material.

Among the organisms which have been isolated by special culture methods from the samples, there were many forms which have been for the first time found to exist on the coast of this interesting island. The existence of a typical microbial population was found both by direct examination and by laboratory studies.

Of this population, sulphur bacteria, Oscillatoria, certain diatoms and Protozoa play the chief part. It was possible to identify several colorless and red colored Thiobacteria and it was further possible to

[1] This is one of a number of papers resulting from The International Expedition to the Bahamas in 1930. The object of the expedition was to collect all possible data concerning the relation of the stability of the Bahama "Block" to the origin, migration and alteration of the sediments which mantle its surface. A further paper "On the Decomposition of Agar-agar by an Aerobic Bacterium," by Dr. Selman A. Waksman and Dr. Bavendamm, is being published. Dr. Bavendamm was appointed to the staff of the expedition by the Deutsche Forschungsgemeinschaft. All the bacteriological work was done with the advice and under the direction of Dr. Waksman at the New Jersey Agricultural Experiment Station.—Richard M. Field.