FURTHER CONSIDERATIONS ON THE NATURE OF VIRUS AGENTS, WITH REFERENCE TO SOME RECENT WORK.

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INTRODUCTORY.

Of late, attention has again been focused upon the subject of the actual nature of the infecting agent in the virus diseases. This is apparent from the account of work done given in the latest annual report of the Medical Research Council, and also from a paper by Eagles and Ledingham [5] in the Lancet and the editorial comment upon this, and from a report of a discussion at the Royal Microscopical Society in recent numbers of the Lancet. As my view is that the causal agents of these diseases are not living organisms, but substances of the nature of enzymes or ferments, which view I originally put forward and elaborated so long ago as 1921 and 1922 [17, 18], I think it is worth while to consider the significance of recent work in this connection.

In the first place it may be readily granted that valuable work has been done upon technical methods; for example, by Barnard, on the technique of "ultra-microscopy" by dark-ground illumination with ultra-violet light, by Elford on methods of ultra-filtration, and by Ledingham on centrifugalization. All of these, in their respective ways, help in enabling the finest particles of the infective material of different viruses to be detected, and their size defined within limits. So that, as the Lancet indicates ([5], p. 843), it is becoming in a sense—a limited sense—no longer strictly accurate to designate such viruses as invisible or filterable.

Most of such workers seem now more than ever inclined to take the view that these granules are actually micro-organisms—micro-micro-organisms, they are termed—mainly, it would appear, because they are something which can be seen. Nevertheless, it is still as necessary as ever, in my opinion, to ask whether these minute granules are, indeed, independent, living units, constituting the true virus, or only the smallest ultimate (protein) particles in digested or lysed material, which, themselves inert, nevertheless cause the infection because they have adsorbed on to them the still invisible and undetected ferment which constitutes the actual agent.

To this view of mine two objections have been recently raised. It has been said that while such a view is possible, (a) it has, in the present state of knowledge, little cogency; and (b) that a similar view might be logically applied to any accepted causal bacterium on the ground that it is merely the vehicle of some lesser hypothetical agent. This latter, a priori argument, does not, it seems to me, conform to one of the essential postulates of the old logicians, namely, is it reasonable, is it likely? By a "lesser hypothetical agent" is meant, one must infer, something which is not a constituent part of the "causal bacterium," but rather something which
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has a separate existence and is not produced by the organism itself, although associated with it. I say, one must infer this, because otherwise, if by "lesser hypothetical agent" is meant the enzyme or toxin of a living bacterium, then the objection is at once non-valid; the comparison is fallacious. Because my whole point is that these granules are not living units, and hence cannot themselves produce more enzyme.

Now, a bacterium, it must be remembered, is an organism—indubitably and unmistakably so—and possesses the characteristic attributes of living matter, e.g., growth, the power of multiplication, the capacity for self-nutrition (the primary attribute), with all the cell-metabolism therewith involved. I have placed this quality last instead of first, to emphasize its importance for this argument. Because it is known that an organism possesses various ferments and can produce various toxic substances as a result of this enzyme-activity and cell-metabolism. Both these classes of substances may themselves play no small part in causing the symptoms associated with such bacterial diseases; in other words, they often supply the toxic element of the pathogenicity. Moreover, in many cases, such toxins can be isolated and separated from the bacteria and be proved to cause particular symptoms. But they cannot then produce more of themselves; they can be elaborated only by the living organism! With all these accepted facts as regards bacteria, what need then is there to hypothesize a micro-micro-organism inside a particular organism when there is not the slightest evidence of the presence of any such? It seems to me not only unnecessary, but illogical. And this conclusion is further reinforced when it is also remembered that in the particularly interesting condition in which some internal agent is known to operate pathologically upon the bacteria themselves—the case of the bacteriophages—the great majority of bacteriologists themselves decline to accept this agent as a living organism, but regard it as some form of enzyme-activity inducing autolysis!

BIological Points.

On the other hand, what a different state of affairs is met with in the subject under review. It may be well, first, to summarize the present position, on the biological side, of these viruses and virus agents, because that is, after all, the essential question. Only two years ago, in the annual Report of the Medical Research Council, it was said (quoting from the Lancet [7]): "Whether these 'viruses' are organized as minute, ultra-microscopic bodies is still an open question; in any case they are presumably too small to have an organization similar to that of such cells as have been hitherto regarded as primary units of living matter. Nobody has yet succeeded in preparing an artificial fluid which will, by itself, provide conditions in which these viruses will reproduce themselves and multiply":

1 In a few cases claims have been made for such successful cultivation, but it has usually been realized subsequently that what had occurred was merely a persistence and carrying over of some of the original infective material for a few subcultures.
some of them, however, will grow in artificial conditions if a piece of surviving tissue, taken from a freshly killed animal, is added to a suitable medium. It seems to be characteristic of a virus that it can multiply only in the presence of living cells which it can infect." (N.B.—In this paragraph it would be more accurate to say "increase" for "multiply," since the latter term, as used in a biological sense, at once connotes a function of a living organism.)

To these points must be added others, such as the greater resistance of the viruses to heat, drying, glycerine, etc., and the extremely poor staining properties of the "bodies" and granules considered to be identical with the agent, when stained with the customary, powerful bacteriological stains. In all these important characters viruses agree in differing from bacteria.

That was still the position seven years after my first paper on this question was published (loc. cit.). Has anything more since become known about the biology of these viruses to justify assumptions that they are living organisms? The only biological points referred to in the present Report of the Council would seem, rather, to indicate the contrary. The immunological work of Gye and Purdy, for instance, on fowl-tumours, appears capable of explanation on lines similar to those on which Lumsden has already explained the results of his brilliant work on cell-toxins and antibodies in relation to malignant growths of mice and rats (vide [11]), though no comparison with this is made. In this latter case, there is certainly no question of any living organism being concerned; it is entirely a matter of cell-biology. And so it is doubtless, too, in the case of the fowl-sarcomata. And, indeed, this particular instance, as affording an example of a living "ultra-microscopic" infective agent, has been given up by practically everybody! Again, the Report points out that from Topley and Greenwood's immunological work on the new disease of mice, ectromelia, it would seem to be indicated that the process of herd-immunization in bacterial and virus diseases may be fundamentally different. It states as follows: "No opportunity has yet occurred of studying a mouse disease in which the type of immunity involved is anti-toxic rather than anti-bacterial. Experience with human diseases such as diphtheria and scarlet fever suggests that infections of this type would approximate in their behaviour to the virus diseases rather than to such bacterial infections as mouse-typhoid." Is there not here the implication of a "toxic" principle as the main factor in the virus infections?

At the present time, many workers on these viruses, having entirely failed to obtain a positive clue from experimental methods, cultivation, etc., are now tending to rely on microscopical observation in the endeavour to arrive at the real nature of the causal agents. And the determination of the very minute size of some of the particles which can convey the infection is, of itself, on biological grounds, a strong point against their being living elements. This is, indeed, recognized in the Council's Report and the possibility of at least one virus agent, that, namely, of foot-and-mouth
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disease, being of inanimate nature is admitted. Now, if this admission is made in the case of one virus-disease, it is surely only logical to admit the same possibility in the case of the others, especially when it is considered how many biological features they have in common! Yet, apart from this one instance, the Report states that "all the evidence accumulated by the use of refined new methods, such as ultra-filtration, ultra-microscopy, tends to reinforce the opinion already widely held that some, at least, of the viruses are definite, self-reproducing organisms." I venture to disagree strongly. The mere detection of the finest infective particles of these viruses does not carry us, really, any nearer towards determining their nature.

I pass now to a consideration of these granules themselves, and will endeavour to answer the objection that there is no cogency in my view as to their nature.

Cytological and Microscopical Points.

The size of these ultimate particles varies in different cases, from 0·2 μ to 0·3 μ (in the smallest "Rickettsia" bodies, e.g., of typhus) and 0·2 μ to 0·25 μ (in the case of the Paschen granules of vaccinia), downwards to 0·07 μ (in the case of the fowl-sarcoma virus), and probably still less in the case of the bacteriophage particles. It is important to remember, however, that, at any rate as regards the larger granules, this is only the "ostensible" or apparent size, as they are seen when "loaded" with Romanowsky stain, for which these protein particles have an intense affinity, and which undoubtedly enlarges them far beyond their true size. This is, indeed, admitted by Eagles and Ledingham in the case of the Paschen particles, for which they give an estimated "real" size of about 0·15 μ (according to Elford). And a similar error applies equally, it must be borne in mind, to the measurements given of the "Rickettsia" bodies, which have always been detected with readiness (because of their abundance) in ordinary stained smears.

Moreover, there are two very important points in connection with this red or reddish-lilac staining of particles with Romanowsky stains to which reference must be made—even again, for I have already dealt with this point (vide [19]). But, as was indicated there, these points are still being continually overlooked, and no real progress is possible in this granule question until they are driven home to all workers upon this subject (loc. cit. p. 76). Now that, as mentioned above, bacteriologists are finding themselves almost driven, as it were, to microscopy for the determination of the nature of these virus agents, it is more than ever requisite that the expert knowledge of the microbiologist, with his biological outlook and true cytological methods, should be properly appreciated. There is, first, the colour which these granules stain, which, as indicated, is quite different from the intense purple-blue, almost blue-black colour of bacteria when so stained. Even Rocha-Lima, an upholder of the organismal nature of "Rickettsia" bodies
was considerably exercised in mind by this very difference. Secondly, there is the well-known fact that the Romanowsky stains are not selective for chromatin. Granules of protein substances which are not chromatin often show an intense affinity for these stains. For example, the nucleus (trophonucleus) of a trypanosome, when stained with Giemsa, appears to consist of a mass of small granules. But these do not constitute the chromatinic elements of the nucleus, the true structure of which is, indeed, as different as possible from the false impression thus created. All the chromatin is contained in a central karyosome, surrounded by nuclear sap. This latter shows no granules when stained by cytological methods; nevertheless, the very fine "ultra-microscopic" colloid, protein particles in this sap, when "loaded" with the Romanowsky stain, appear as a number of definite grains, and were regarded as the nuclear chromatin until this peculiarity of this staining method became realized. Again, metaplastic granules, the by-products of cell-metabolism, also have a strong affinity for these stains. As a very pertinent instance, the so-called chromatoid granules of a trypanosome may be cited. Because, in the case of one particular, admitted, "Rickettsia," that, namely, of the sheep-kid, I have shown that, to a very large extent, this is merely the masses of these granules liberated by the disintegration of dead criithidal forms of the sheep-trypanosome which occur in vast numbers in the intestine [20].

Next, as regards the question of their "behaviour" in an organismal sense, i.e., as regards multiplication (division) and so on. The Council's Report states that in the case of the minute particles representing the infective agent of the mouse disease, ectromelia (which are estimated to be only about 0.1 μ to 0.15 μ in diameter), "some forms of these submicroscopic cocci have been seen which suggest the manner of multiplication by fission." It is true this is a very modest statement when compared, for instance, with the elaborate life-cycle originally described by Gye and Barnard for their cancer organism. But how much credence can really be given to it; to how much does it really amount? The multiplication or division of a living organism is a definite, positive act, a normal sequel to growth and increase of size. Now trituration, comminution or fragmentation—whatever it may be called—is a well-known phenomenon, induced by outside conditions or circumstances, of frequent occurrence in the case of globules or spherules of liquid or semi-solid, non-living matter. This is of course a "negative," or non-voluntary act. Mercury may be cited as a common example. And I have found, beyond any reasonable doubt, that this is what occurs in the course of the production of the fine "Rickettsia" granules in the intestine of the louse. Discrete spherules, 1 μ or 2 μ in diameter, of pigment, are abundant. They have been moulded into this form, simulating cocci, by the breakdown of the amorphous haemoglobin masses, as these become altered and of thicker consistency in the course of the digestion. These pigmentiferous spherules continue to "divide," becoming smaller and smaller as the physical and chemical conditions of
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the medium alter and the contents of the intestine undergo continual churning and friction. Concurrently, in the later stages of the digestion, owing to the pathological action of the abnormal hæmatabolic enzyme, a further change takes place in the constitution of these small comminuted pigment-granules, the iron-containing moiety being separated and lost and the residual protein material, now staining reddish, or reddish-lilac with Giemsa, being left as minute "Rickettsia" granules, the ultimate product of the pathological hæmataboly. All this process has been described in detail in my paper on the subject [21].

VARIOUS TYPES OF "BODIES."

Now, with this actual example before us, what reason is there to suppose that a comparable disintegration and comminution of other virus "bodies" and granules does not occur? In the present enthusiasm for these fine, ultimate particles, there is a tendency to forget the known, conspicuous, diagnostic "bodies," which have long been associated with certain virus diseases. But these are still there and have to be taken into account! I will refer especially to two, the Negri-body (occurring in hydrophobia) and the Guarnieri-body (occurring in smallpox and vaccinia), both of which are now generally admitted to be, not parasites or living organisms of any kind, but cell inclusions. With these may be included the Kurloff-body, which, though not pathological, is a characteristic formation found regularly in the guinea-pig, occurring more or less frequently in the lymphocytes. In the case of the Negri-body and the Kurloff-body, I have shown [17] that so far as microscopical evidence can be certain, both these are the result of hæmataboly, pathological in the one case, normal, but of an exceptionally unusual type in the other; that is to say, these peculiar bodies are formed by the alteration or incomplete digestion of hæmoglobin by some pathogenic or unusual enzyme. They are essentially similar formations, consisting of inclusions (or "inner formations"), most probably representing the "globin" portion of the hæmoglobin, which are enclosed or contained in a mass which consists of the iron-containing portion. This mass, constituting the whole "body," is either of a fairly solid consistency (as in the Negri-body), or else liquid (as in the case of the Kurloff-body, when it is dissipated in the course of the preparation of a dry smear, leaving behind the inclusions apparently in a vacuole). The size of the body, which is variable, depends, in either case, entirely upon how much corpuscular material has been included in the particular little mass.

Now further, as regards the Guarnieri-bodies, it is apparent beyond doubt, from certain of the excellent microphotographs given long ago by Councilman, Magrath and Brinckerhoff, in their collected studies of variola and vaccinia [3], that many of the Guarnieri-bodies are also the same type of formation. It will be noticed that I do not say that all of these bodies

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1 This must be altered in some way which renders it insoluble, at any rate in the various media used in making preparations.
are formed by this characteristic splitting up and alteration of haemoglobin. Because in the case of the ectodermic epithelium in these conditions, the cells also ingest and abnormally metabolize leucocytes, the resulting pictures of the "bodies" then being not only more variable, but less well defined. Thus both Ewing and Salmon were, in a measure, right in the view they took of the nature of these formations. Nevertheless, in the earlier stages of the derangement, the epithelial cells most probably ingest and alter red blood-corpuscles before they are stimulated to attack leucocytes; just as in the case of the normal skin, hremetabolie is mainly of the corpuscles (with pigment production) and only very occasionally are leucocytes taken in.

I have recently come across an account of certain early observations of von Prowazek, which were made upon fresh preparations of rabbit epithelium, containing Guarnieri-bodies, and these are so suggestive from the above point of view, that I propose to extract the following sentences from his paper [14]: "Im frischen Präparat besitzen die jüngsten Guarnierischen Körper ein opakes Aussehen und liegen oft dicht am Kern an, wobei sie nicht etwa eine Tropfenform annehmen, sondern sich halbkugelförmiig der Kernmembran anschmiegen, eine Tatsache die nicht für eine vollkommen leichtflüssige Beschaffenheit der fraglichen Gepilde sprechen würde. Später runden sie sich mehr ab und man kann an ihnen nun eine dichtere, etwas lichtbrechendere periphere Kontur, der nach innen zu auch verschiedene Granulationen anliegen, und einen mehr flüssigen Inhalt feststellen." (The italics are mine.) "In vielen Körpern beobachtet man . . . 1-2 und mehr meist hantelförmig oder stäbchenartig gestaltete Initialkörper, die keine deutliche Struktur erkennen lassen und ein lichtbrechenderes, sehr leicht grünlich schimmerndes Aussehen besitzen."

Bearing in mind that Prowazek was preoccupied with the idea that a living, parasitic organism was concerned, and making allowance for this, is it not nevertheless clear that he was observing actually a very similar formation to the Kurloff-body? In the above instance, he was observing the "youngest stage," that is to say, a single included corpuscle (this is evident, indeed, from the figure accompanying his description). The "body" is evidently for the most part liquid or slightly viscous in consistency (as is the principal mass of a Kurloff-body), and surrounded by a membrane, the corpuscular "skin." For this reason it does not always have the form of a spherical "drop" (although, in Prowazek's figure referred to, it actually has), but may be flattened to one side, where it abuts on the cell nucleus (cf. my fig. 11, loc. cit. of a Kurloff-body). The "Initialkörper" are the inclusions, one, two or more, which appear in the altering haemoglobin as the protein elements become separated from the liquid, iron-containing part. The superficial granulations mentioned are a feature which are also to be observed very commonly in the Negri-body; and, under certain circumstances, I have observed them in the Kurloff-body. So much, then, for the ordinary Guarnieri-body.

(To be continued.)