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

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(Continued from p. 269.)

VARIOUS TYPES OF GRANULES.

There is, however, a further point, of great importance in the present connection, upon which I did not happen to lay any stress in my original paper, because the question of the significance of the small granules described of late was not then to the fore. That a granular condition occurs in the case of both the Negri-body and the Guarnieri-body is evident both from Negri's own accounts and also from Calkin's and Tyzzer's accounts [3]. The great mistake was that, owing to ignorance in these early days of the fact that the Romanowsky stains are not selective chromatin stains, the workers on them concluded that these various small granules contained chromatin and represented the reproductive phase of a parasite! Hence the various names attached to them, such as "elementary bodies," "initial granules." Whereas they are in reality no such thing, but merely represent further or more complete breakdown of either the globin of the haemoglobin or the nuclear material of leucocytes.

The Kurloff-body itself affords an excellent illustration in support of this condition. As is well known, the inclusions in the spherical mass of altered haemoglobin have, in this case, a most diversified form. They may appear either as narrow bacillary rods, long, thin, wavy threads, irregular little lumps and grains, or as a mass of small granules (cf. figs. 1 and 2 in text, also the other figures given by Ross [16] and Woodcock [17]). In my original paper [17], I stated that no definite sequence of change in the form and appearance of these inclusions had been determined. I did suggest, however, that the larger masses and rods, etc., were perhaps formed by coalescence of the small granules. This suggestion was undoubtedly a mistaken one. In extenuation of it, I may say that I had then the mode of origin of platelet-granules in my mind (where only residual granules are formed) and had not at that time studied, for instance, the alteration of the pigment-material in the louse. It is much more probable, on the contrary, that the finely granular condition happens to be either just one form in which the protein (or "globin") portion is separated from the iron-containing part of the hemoglobin, or that the granules result from the breaking up of the larger, more definite inclusions, in an older stage of the "body," that is, after it has been included for a longer time in a particular lymphocyte. Which variety of the process occurs depends, no doubt, upon variations in the biochemical and physical
conditions associated with the functioning of the hæmetaboly in the individual cell concerned.

Now, among the pathological conditions, we have, undoubtedly, different cases exemplifying these different variations. In the case of the Negri-body, it is probable that the characteristic "body," with its well-defined, spherical inclusions is, as a general rule, an early stage, and that the condition of a compact aggregation of granules (Negri and Levaditi, Nicolau and Schoen) is a later stage, resulting from the further comminution into small particles of the protein inclusions. The form of the characteristic "body," above indicated appears to be that most usually observed; and here, it must be noted, the matter is not complicated by an alternative origin of granular material from the breakdown of leucocytic nuclei. Nevertheless, even here, in certain conditions, notably in rabies induced by "fixed" exalted virus, only small, but "hard" and definite-looking little granules are found, which appear scattered. Incidentally, it is impossible to suppose, as do Levaditi, Nicolau and Schoen [9], that the Negri-body is the pansporoblast of a Microsporidian, and that where only the dispersed granules (spores) are found, the parasite has lost the power to produce pansporoblasts; if a species of Microsporidian has pansporoblasts in its life-cycle, it certainly cannot produce spores without them! This just shows the futility of endeavouring to explain an enigmatical "body" upon a parasitic hypothesis, without a knowledge of the biology of the type of parasite hypothesized. It must be remembered that, as I showed, the abnormal hæmetaboly may occur extracellularly, and, granted the presence of a powerful "intensified" ferment, its action on the corpuscles extracellularly may explain the scattered distribution of the granules in the cases where they are often seen outside the nerve-cells. (I would refer also to the figure I gave [17, e,
fig. 13] of an example of an elongated, typical Negri-body in a narrow segment of a capillary, abutting on two yellow, flattened corpuscles.)

Again, in the case of the Guarnieri-body, where this is formed from alteration of hæmoglobin, we have at first a very similar, well-defined “body,” the inclusions in which probably break down subsequently into small granules; thus we get many of the appearances figured by Calkins and Tyzzer (loc. cit.). These granules, like the “bodies” themselves, may be, in the case of smallpox, intranuclear, as well as intracytoplasmic. (This is clear from some of the photomicrographs of Councilman, Magrath and Brinckerhoff.) But this intranuclear absorption of hæmoglobin, in response to a powerful exciting stimulus, presents no difficulty of comprehension, when it is remembered, as I have shown, that even normally, in the rapid growth of the mammary-gland epithelium during pregnancy, hæmoglobin is absorbed actually by the nucleus [23]. It is in points like these that the varying mode of working of the normal hæmatophagic and hæmetabolic function provides such an invaluable basis for comparison with, and understanding of, these pathological conditions.

I would also take this opportunity of adding a few remarks bearing upon this point, which (a) support my interpretation of the Negri-body as altered, ingested hæmoglobin; and (b) adduce further evidence in support of the general view that tissue-cells have normally a hæmetabolic function. They are the outcome of a recent re-perusal of Acton and Harvey’s very instructive paper [1], and are very pertinent to this important question. In the first place, in my paper (b, fig. 13, loc. cit.), I figured a fairly large Negri-body (probably intracellular) in an early stage of formation, consisting of a polychromatophilic mass, with the fine, superficial granulations just appearing, a small part of which, however, is still unaltered, yellow hæmoglobin. Now, Acton and Harvey describe masses of yellow material occurring in the nerve-cells of both “normal” (i.e., non-rabid) and rabid brains. Unfortunately, they persist in regarding, without any adequate evidence, this substance as derived from the relatively small nucleolus (karyosome). Whereas, it is practically certain that when yellow-coloured material is found in a tissue-cell this is hæmoglobin or some derivative. I have on previous occasions cited numerous examples of this; for instance, in macrophages, in the intestinal cells of the rat-flea (Minchin and Thomson), in those of the ked (Anigstein), of the mites (Reichenow, Woodcock), and of the louse (Woodcock). Further, I have also found hæmoglobin (as well as “foreign” nuclear material) ingested—doubtless, to be utilized—by the eggs of the mite; and there can be little doubt that this is the main source of the material forming the so-called “yolk-nucleus” of many eggs, an example compared by Acton and Harvey in support of their view of the nucleolar origin of the Negri-bodies. The material of the “yolk-nucleus” stains intensely with iron-hæmatoxyl, because it contains the iron of the ingested hæmoglobin, and not because it is extruded nucleolar matter, which is an entirely erroneous view.
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Where leucocytes are ingested the breakdown of the nuclear material gives rise to less well-defined little clumps and small masses, and then ultimately to finer granules; compare, for instance, either cellular autolysis, such as the disintegration of the nuclei of the intestinal cells of the louse, which also results in the production of "Rickettsia" granules, or the digestion of "foreign" nuclear material ingested by macrophages (vide the excellent illustrations given by Leishman [8] and Low and Wenyon [10]). In whichever of these ways they ultimately arise, these granules in variola and vaccinia constitute undoubtedly a large proportion of the Paschen-granules which have recently caused such excitement.

On the other hand, there are certain instances of the occurrence of "bodies" in virus diseases, in which these have not, at any stage, a well-defined, characteristic "structure," but consist always of a number of more or less compact little lumps and granules, or solely of granules. This condition corresponds closely with the other extreme in the Kurloff-body, that, namely, of a mass of granules. Examples are, trachoma, molluscum contagiosum, and fowl-pox; in the last named the whole "body" or mass of granules has been termed a Bollinger-body, and the individual particles of which it is made up, Borrel-bodies. I have not studied any of these myself, but it is most instructive to compare a figure of Halberstaedter and Prowazek's [15] of a trachoma-inclusion (in an epithelial cell of an experimentally infected orang-outang) with those of a Kurloff-body, also reproduced (figs. 1 and 2, p. 344). Can there be any doubt that, in the former case as in the latter, the granules (e, fig. 3) result merely from the further breaking down of the small lumps and larger grains (p)? The authors themselves regarded the latter as being of plastin-like material. It is safe to say that nothing of an organismal nature is manifest in this illustration of their's. Just as in the case of the Kurloff-body, so the trachoma-inclusion is an instance of hæmatoboly, in this case of abnormal character. And a similar explanation will be found, I am certain, to apply to the other virus bodies and granules when they have been completely investigated.

It is important to note that where these various granules (as distinct from "bodies") of certain virus diseases have been carefully studied and measured in ordinary stained smears, a much greater range of variation in size is found than might be expected from the restriction of their examination to the smallest ones capable of passing a filter. This has been shown by Coles [2], with the assistance of Merlin. It may be mentioned that the author remarks that the bodies (meaning the granules) are Gram-negative and not easily stained with the customary aniline dyes, thus agreeing with the usual view. He also adds that after Giemsa, they stain practically the same tint as many of the "Rickettsia" bodies. In the case of herpes, the largest grains are as much as 0·88 μ in diameter, while the smallest are only 0·2 μ; and in the case of vaccinia, the largest are also 0·8 μ, and the most minute 0·26 to 0·21 μ—the latter size corresponding, it will be seen, fairly closely with those now distinguished as Paschen-granules. But in
both cases Coles has no hesitation in regarding them all as part and parcel of the same thing. There is no sharp separation between the extreme limits of size; all transitional stages can be met with. From what has been said in the foregoing pages, it will be clear that we have here again examples of the continued comminution or fragmentation of protein particles, down to the smallest limits of the particular substance, perfectly comparable with the manner in which the smallest “Rickettsia” particles are formed (vide also above, p. 267). In short, all the so-called multiplication of these “initial granules” and “elementary bodies” must be considered extremely suspect.

These ultimate particles, which have been discussed above, do not, it is hardly necessary to say, themselves grow into “bodies” again, when starting a fresh “infection.” What happens is that the enzyme adsorbed to such colloidal particles sets up afresh the production of the same abnormal enzyme in the “susceptible” cells, thus causing again the same pathological alteration of the blood-digestion; just as a minute quantity of “bacteriophage,” when added to a particular bacterial culture, will induce the bacteria themselves (in this case, in the course of their own multiplication) to produce more of it, in great amount. A pertinent point has been raised in friendly criticism. It has been asked why, on my view of the production of these bodies and granules by enzyme-activity, are there any solid protein masses or particles left at all, if the ferment is a proteolytic one? Would not the ultimate end-products of the hæmatoboly be relatively simple, soluble compounds, such as peptones or amines? In reply, I would say that I have never called these enzymes proteolytic; I do not know if they should be rightly so termed. I would suggest that the ferments concerned have the power of breaking down highly complex protein material (like hæmoglobin or chromatin with its nucleo-proteins) only to a certain stage or degree. Ferments with differing powers of dissociation are known to occur; thus, in the ordinary digestive processes, while pepsin converts proteins into proteoses and peptones, another ferment, either trypsin or erepsin, can carry dissociation a stage further into peptides and amino-acids. Similarly, these hæmolytic enzymes may be capable only of separating the most complicated combinations of molecule-groups into less elaborate ones, e.g., hæmoglobin into hæmatin (or some protein molecule with which is associated the iron) and “globin,” or a corresponding, but insoluble protein. Thus the non-iron-containing particles ultimately resulting in consequence of the varied physical (and, perhaps, further biochemical) action can still exist as solid material, even if, in some cases, almost colloidal in minuteness, and hence can have attached to them a minute quantity of the still invisible and undetected enzyme. That such a persistence of protein particles can occur is known from the ordinary facts of cellular autolysis, which is certainly the result of enzyme-activity.

I have laid especial stress on this origin of “elementary bodies” or “initial particles” as end-products of abnormal hæmatoboly because this
gives, almost certainly I consider, the right clue to the nature of the actual virus. But I do not mean to infer that all the huge quantity of granules which must obviously be present in virus material, as collected and used, either for examination or inoculation, is directly thus formed. Probably in few cases would there be anything like enough "bodies" or masses of granules present to account for such a quantity. The important point is this: Especially in the latter stages of such conditions, the affected and deranged cells themselves will more and more tend to undergo lysis by the action of this pathological ferment—i.e., the process will degenerate into a form of autolysis—with their resulting disintegration (particularly of the nuclear material) into many more granules, having adsorbed to them the same enzyme. Further, there is an important modification of the principle thus outlined which, as I think (vide [18] and [24]), may be in operation. In the case of certain virus diseases, these may have originated, or may originate at any time (given the necessary conditions) by the separation of a pathogenic ferment (or "toxin") from a micro-organism and its independent production and further dissemination by cell-metabolism directly. Here the question may be one, either entirely or partly, in different cases, of cyto-metabolism, or cytolysis, rather than of abnormal haemmetabolic functioning by the cells first of all. Such infections may be expected to have a relatively short incubation-period as compared with the much longer one of typical haemmetabolic virus diseases (cf., for instance, epidemic influenza, on the one hand, with chicken-pox, smallpox and hydrophobia on the other).

Finally, a few words of comment are necessary in regard to a view which has been recently put forward that the virus agents are not essentially intracellular in operation, the penetration of cells being only an "accidental event." I think that everything above detailed indicates just the contrary. Such a view certainly does not apply to the cases, for example, of rabies and poliomyelitis. During the discussion on virus diseases at the recent meeting of the British Medical Association, both Galloway and Fairbrother had some instructive remarks to make bearing upon this point (vide Lancet, September 3, 1932, p. 518). From what they said, it is clear that the virus in both these instances has a very elective affinity for the neurones—a fact which has been, indeed, long known in the case of hydrophobia. When the path of tetanus toxin in the body is remembered, does not the parallel behaviour in these cases irresistibly suggest also a toxic principle as the cause? Moreover, no one, let it be remembered, has ever found specific organisms, bodies or granules at the site of a rabid bite, for instance! In my opinion, the pathological change in rabies is due to the nerve-cells being no longer able to metabolize ingested red corpuscles in a normal manner, so that a portion of the haemoglobin containing the iron is set apart in the form of Nissl bodies or substance; the haemoglobin is merely altered, by the action of the abnormal enzyme, into the form of Negri bodies—entirely comparable with Guarnieri and Kurloff-bodies.
SUMMARY.

To sum up. The above analysis of my view of the nature of these various "bodies" and related granules does not differ in any essential respect from that which I originally outlined in my first papers ten years and more ago. Having ascertained, by careful observation, the nature and mode of origin of certain of these abnormal "bodies" and granules, when I learnt about the bacteriolysin and the explanation of its method of transmission which was suggested to me by Ledingham, I applied a similar interpretation to the virus diseases and concluded that the causal agent of such conditions must be also a pathogenic enzyme, capable of transmission in a similar manner (vide especially [18], p. 256).

Now I have noticed that in one or two cases, e.g., [6] and [13], where reference has been made to this view of the causation of virus diseases by particular ferments, it has been ascribed particularly to a German worker, Doerr. I would point out the following facts: The first similar suggestion or expression of opinion on the part of this author, of which I am aware, was made in a paper by Doerr and Zdansky, when reviewing the state of our knowledge regarding herpes and encephalitis lethargica, which was published in April, 1924 (vide 22). Doerr and Zdansky based their suggestion, not upon any positive findings of their own (unlike my original view, which was founded upon the definite occurrence of abnormal hæmetaboly), but upon certain experimental work, chiefly biochemical and cytotoxicological, by other German investigators, which they considered afforded a starting point in this direction.

There is, further, one other point which has, throughout, struck me as remarkable. The great majority of bacteriologists freely admit the operation of this principle—that is, of a transmissible lytic agent, not of living nature—in explanation of the case of the bacteriolysin. But, with rare exceptions, they seem most reluctant to admit the operation of a similar principle in the case of virus diseases, where typical cells are affected, and, in consequence, strain against facts and reasonableness in the endeavour to show that a living organism is concerned in such cases. Now this is both illogical and non-biological, because, in both sets of cases, living protoplasm is concerned, and the basic attributes and modes of functioning of living matter are universal! Hence, if this remarkable principle does operate in the case of bacteria, there is no reason why it should not do so in the case of tissue-cells.

I would like to refer briefly to one or two of the exceptions mentioned, that is, to writers who have expressed themselves as not satisfied that the granules which have recently aroused such enthusiasm are indeed living organisms and the actual causal agents of virus diseases. Rivers, as editor of a general compilation upon this subject of filterable viruses [15a], remarks as follows: "No virus has been obtained in an absolutely pure state. Not even the washed granules of vaccine-virus can be accepted as representing only virus. Therefore it is impossible to say that virus alone is
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being filtered rather than virus attached to aggregates of protein or particles of degraded cells.” Later on, he says: “It is impossible at present to say whether the viruses are animate or inanimate.” Again, Carrel, in his account of tissue-cultures in the same work (i.e.), says that “some viruses may be non-living substances manufactured by tissues or blood-cells, and not ultra-microscopic organisms.”

The fundamental arguments in favour of my view are these: (1) The undoubted occurrence of abnormal hæmatoboly (and of cytomataboloy or cytolysis), implying the action of an abnormal enzyme, is the characteristic, diagnostic pathological feature of most of these conditions; (2) no living organism—or micro-micro-organism!—has been detected as a causative agent; and (3) if, as seems most probable, the actual virus is something, as yet undetected, adsorbed on to these particles and granules, to whatever degree of minuteness they may become comminuted, the “units” of which this substance is constituted must be so extremely minute that it is, biologically, most unlikely that it can have the properties of living matter.

On the other hand, also from the biological aspect, it is certainly a remarkable and, at first sight, astounding quality of behaviour of living protoplasm that the influence of a particular substance, of enzyme-nature, should induce the cell to produce—with harmful effects upon its own metabolism and, ultimately, on its life—more of this same ferment. There are, however, certain analogous conditions known which may have a bearing upon, or be an indication in favour of, such a mode of behaviour as being possible. One is the action of hormones in causing functional activity on the part of other cells. Where such activity takes the form of secretion, this certainly means that ferment-action is excited in the particular type of cell concerned. It is, of course, by no means a parallel case, because the enzyme whose production is stimulated is a normal enzyme of that particular cell-type and not the same substance (or enzyme?) as the exciting hormone. The other analogy is afforded by certain interesting experiments by Frey [5a], referred to by Doerr and Zdansky, on the contagion, or “infection,” of healthy red corpuscles through or by means of others, which have been damaged by being subjected to the action of piperidin or of boiled extracts of organs. In these experiments, direct intervention of the primary toxin or poison was excluded. Frey considers that the primary poison induces the formation of cell-products in the damaged corpuscles, which, in their turn, can affect

1 According to Rivers, Sanfelice, working with fowl-pox (contagious epithelioma of chickens) in 1914, found that the virus was not inactivated by one per cent caustic soda, and because of this he was led to think of it as an inanimate poison, capable of attacking normal cells and producing within them a poison of a similar nature, which in turn could attack other normal cells. Thus he described his idea of how a lifeless agent might be passed in series reproducing itself indefinitely. Therefore this, of which I was hitherto unaware, seems to have been the first time it was actually suggested that an animal virus disease might be due to a non-living agent (the reference given is Zs. Hyg., 76, 1914, p. 257).
fresh, healthy ones. The great difference in this case is that, although the same toxic substance is apparently produced afresh, red corpuscles, though of highly-organized material, are not themselves any longer living elements. And this is as far as biology can yet take us in regard to this most important and intriguing question.

REFERENCES.

[13] Nature (reprint of address to Physiological Section, British Association), 1921, cxxvii, p. 599.
[22] Idem. T. c., p. 469.