A Paper

ON

TISSUE CHANGES IN VIRUS DISEASES*

BY

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Since the war, investigations of virus diseases have been in many directions remarkably fruitful, and the experimental methods employed have been pursued with such intensity in particular instances that the results have illuminated many hitherto obscure problems concerning the interaction of host tissue with parasites, both visible and invisible. Intimate tissue changes have also received their full measure of attention. I emphasize this point because it has been said, with considerable truth, that the advent of the bacteriological era, with the possibility of demonstrating the causative agents of infectious disease in the tissues, has had the effect of withdrawing attention from the minute histological study of the interaction between host and parasite. The early phases of this interaction are still little understood.

On the whole, the bacteriologist has been concerned more with the demonstration in the tissue of an organism suspected to be there than with the unravelling of the tissue changes associated with its presence. Many diseases now definitely recognized as due to a virus passed through a phase of this kind when it was considered imperative to demonstrate some gross bacterial or protozoal cause. A cause of disease not demonstrable in the tissues, or, if demonstrable, not differing essentially in character from well-known aetiological agents, was unthinkable. The rapid growth of the experimental study of viruses, not only in susceptible animals but also under various *in vitro* conditions, has also reacted favourably on another side of the problem by supplying material more suitable in every way for accurate histological study than material from the spontaneous disease.

DEFINITION OF A VIRUS

That viruses are living organisms distinguished from the visible bacteria only by their small size and occupying positions on a dimensional ladder having its upper rung at or about the limit of microscopic vision is a postulate to which, presumably, a majority of workers on viruses will subscribe. I say nothing about the position of its lowest rung except that the most recent work on filtration of virus through graded collodion membranes has revealed the existence of viruses of dimensions exceeding by only very little that of a molecule of haemoglobin.

The recently ascertained fact that there exists a group of viruses—variola, vaccinia, fowl-pox, ectromelia, molluscum contagiosum, etc.—whose individual elements are just big enough to be detectable by appropriate staining methods (if not yet in the fixed tissues, at least in appropriately stained smears of the material of the lesion) will enable us to work out with greater precision than hitherto the corresponding tissue-parasite interactions. Other groups of viruses, not at present known to be associated with elementary bodies but certainly accompanied by cell inclusions of a particular type, may, it is hoped, come into line in the future, but there will remain still another group whose presence in the tissues of the infected host is not, so far as we know, characterized by any peculiar

histological picture or specific morphological cell change of the inclusion type. While cell inclusions in a virus lesion have, in certain types, greatly aided our understanding of the mode of attack of virus on tissue, no less, and possibly greater, interest attaches to the inflammations, the necroses, and the proliferations (or their combinations) which in individual virus diseases mark the essential disturbance. As yet the cultural requirements of viruses are, in all but a few instances, unknown, so that in our attempts to group apparently similar types of lesion due to different viruses we lack that valuable correlation which, among the visible bacteria, exists between the cultural and biochemical properties of a group of organisms and the type of tissue lesion. In a series of lectures Kettle has elaborated this interesting theme and shown to what an important extent the cultural characters of organisms are reflected in the lesions they produce. Unfortunately, in the virus field we can at present only hazard the suggestion that similar or cognate tissue lesions may ultimately prove a guide to relationship in cultural properties.

TYPES OF GROSS LESION IN VIRUS DISEASES

It will be convenient to consider briefly some of the more general features of systemic tissue change met with in naturally contracted virus disease before passing to the far more fascinating developments opened up by their experimental study. As in bacterial infections, so also in virus infections, we find indisputable evidence of organ and tissue affinities.

INFLAMMATORY AND DEGENERATIVE

In the great group of pock diseases, including mammalian and avian pox, we find the essential lesions in the skin and only occasional evidence of gross changes in organs; but in the skin itself there are notable differences in the mode of attack and development of the lesion. In vaccinia and small-pox the lesion is essentially an inflammatory one commencing in the vascular tissue of the dermis, which is primarily attacked with resulting oedema producing the characteristic vesicle. The adjoining epithelial cells are, from my own studies, only secondarily involved, and exhibit the swelling or ballooning and reticulation described by the older writers. As to systemic changes, it is now well recognized that ordinary vaccination may be followed by definite splenomegaly (especially in young adults), and it has been noted as a frequent occurrence in rabbits. Focal necroses in the liver have also been observed in fatal cases of small-pox. These evidences of systemic spread of virus after ordinary vaccination are of great interest as they represent, on a small scale, the miliary organic lesions obtainable with virus of artificially raised potency.

In natural and experimental fowl-pox the external lesions have, partly owing to their naked-eye appearance and partly owing to some misinterpretation of the histological picture, been thought to be predominantly epithelial proliferations, and it is Goodpasture's opinion that an irritative epithelial hyperplasia represents the primary lesion which later ends in regression and healing. My own studies and those of Anderson have not revealed any very obvious hyperplasia of epithelia in the comb lesion. The nodule consists of enormously swollen and obviously degenerate cells which, with the overlying purulent exudate which has passed outwards from the inflamed dermis, are confined by the resistant corneal layer. The swollen epithelia contain the well-known Bollinger bodies, shown by recent evidence to consist simply of intracellular aggregations of the elementary bodies of Borrel. These latter almost certainly represent the actual virus bodies. Lesions other than cutaneous are rarely found in the spontaneous disease. The pock viruses therefore afford excellent examples of inflammatory skin lesions with accompanying haemorrhagic necroses and degenerative changes in neighbouring epithelia, which, in vaccinia at any rate, seem to be due to interference with their nutrition by the inflammatory disturbance in the dermis below.

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DEGENERATIVE AND NECROTIC WITHOUT INFLAMMATORY
DISTURBANCE

As an example of a virus disease which apparently produces a pure necrosis of parenchymatous cells without any inflammatory change, yellow fever may be taken. Careful histological studies of yellow fever tissue by Klotz and his associates, Hudson and others, show that the lesions in this disease consist essentially of fatty degenerations, necroses and necrobioses affecting liver, kidney, heart, and spleen, with little or no trace of inflammatory response except possibly in jaundiced conditions where some hyperplasia of the Kupffer cells of the liver may be present. When recovery takes place liver necroses would appear to be replaced by regeneration of liver cells without any scarring.

CENTRAL NERVOUS SYSTEM

For purposes of grouping, based on histological findings, the viruses associated with definite lesions in the central nervous system may be divided fairly clearly into two classes, of which (a), represented by herpes and vaccinia—the latter after intracerebral inoculation only—produces meningitis with little or no encephalitis, except possibly in the immediate neighbourhood of overlying areas of intense meningitis, while (b), represented by rabies, poliomyelitis, Borna disease, and louping-ill, attack mainly the neurons. The latter group would also contain the post-infectious and, so far as we know, the post-viral encephalomyelitic conditions which are accompanied by extensive demyelination. At the moment we have no satisfactory explanation to offer of these disturbances, as, so far, it has been impossible to reproduce them experimentally. As they stand, whatever primary viral infection they follow, the lesions form a characteristic histological picture. Spontaneous disease of the central nervous system associated with demyelination has indeed been noted in dogs and monkeys but has not proved transmissible. Inflammatory changes undoubtedly accompany these degenerative lesions, but the point has been raised by neurologists like Spielmeyer whether the inflammatory conditions may be consequent on irritation due to degenerate cell products produced in the primary attack and may not themselves be primary. On the other hand the ushering in of conditions like post-vaccinal encephalitis by high and often persistent fever does undoubtedly suggest the onset of some fresh and presumably inflammatory process.

PULMONARY MANIFESTATIONS

These are of great interest in certain virus diseases which in nature are most probably contracted by the respiratory route and which can be communicated experimentally only by this channel. Thus while the involvement of the lungs in human psittacosis is the rule, these organs are involved in experimental animals such as rabbits and monkeys only when the injection of virus is intratracheal. In monkeys the characteristic picture is the thickening of the alveolar walls by cellular proliferation and infiltration, the exudate being of the mononuclear type. In human psittacosis, the pulmonary lesion differs from that in influenza in being essentially lobar, accompanied by fibrinous exudate and haemorrhages in the severely damaged areas, but unaccompanied by polymorphonuclear infiltration. So also when vaccinia is given intratracheally to rabbits (as in the experiments of Muckenfuss, McCordock, and Harter) areas of haemorrhagic and gelatinous consolidations occur and the primary response appears to be a mobilization of mononuclears within the alveoli and about the bronchi and blood vessels. As in the dermis, vascular necroses and haemorrhages are prominent. Guarnieri bodies are detected in the bronchial but not in the alveolar epithelium.

In quite a number of well-known virus diseases such as distemper, rinderpest, fowl plague, etc., there is a strange lack of obvious tissue change in fatal cases. While the virus is distributed throughout the body it does not appear to be localized anywhere with production of gross tissue change. Accurate histological examinations in such infections are still very desirable—it should be remembered that it was not till 1914 that typhus fever was found to possess a specific histological picture. Even in such infections as rabies, a lack of correspondence between the amount of structural change

and the clinical severity has frequently been noted in experimental work with monkeys. Careful microscopical examination may be needed to detect the scanty cellular lesions present in some animals which succumb to the virus (Hurst and Pawan, 1932).

PROLIFERATIVE LESIONS

The outstanding example of an essentially proliferative lesion possessing malignant and metastatic properties is naturally the Rous sarcoma, and those other avian types which have since come to light and which are capable of being induced by cell-free material. Histologists seem to be agreed that these proliferations are genuine malignant neoplasms, but there is possibly not the same unanimity with regard to the precise mechanism involved in the transference of the disease by a cell-free filtrate. This subject is as yet too controversial for useful discussion here, but as regards the benign proliferations known as warts the evidence that these are produced by a filterable virus, both in man and in dogs, is reasonably complete. The recent work of de Monbreun and Goodpasture on infectious oral papillomatosis in dogs reveals remarkable species- and cell-specificity on the part of the virus concerned. It is capable of infecting puppies only and other animals not at all. Moreover, even in puppies it is able, unlike the human wart virus, to infect only when introduced into the buccal mucosa. Introduction of the virus into skin areas is without effect. The histology of these warts is of extreme interest from the point of view of virus action, for in the study of dog warts to which I have just referred it would appear that the essential histological feature of the wart is the layer of enlarged clear epithelial cells (the so-called wart cells), showing increase both of cytoplasm and nucleus, which overlie a completely normal basal Malpighian layer. These altered cells would correspond in position to that of the swollen epithelia in the fowl-pox lesion, but definite inclusions have not been found in them as in the latter. It is the fate of these altered cells during the stage of regression to become compressed and flattened under the overlying keratin layer and ultimately to disappear. Inflammatory disturbance in the dermis is only slight.

CYTOTROPISMS

The affinities for certain cells (or cell tracts) exhibited by certain viruses in the early, or earliest, period of their invasion of the body have given rise to considerable controversy. I have already referred to the fact that the virus of vaccinia in its invasion of the skin is now known to attack primarily the vascular endothelium of the dermis, though a strict epidermotropism had formerly been postulated. Still more recent work by Goodpasture, who has infected the chorio-allantoic membrane of the chick embryo with vaccinia virus, shows the presence of Guarnieri bodies not only in ectodermal and endodermal epithelia, but also in endothelia and fibroblasts of the mesodermal tissue. These Guarnieri bodies would, like the Bollinger inclusions, appear to consist of aggregations of the corresponding elementary bodies of Paschen.

On the other hand, so far as the presence of Bollinger bodies is concerned, fowl-pox would seem, under similar experimental conditions, to be associated only with ectodermal and endodermal structures. I am not able to subscribe to the inference drawn from this fact by Goodpasture that the Borrel bodies are not only cytotropic but cytotoxic. It is well known that the virus of fowl-pox invades the system although it localizes only in the skin. Further, impression preparations of cross-sections of fresh fowl-pox lesions in the comb have shown masses of extracellular Borrel bodies as well as the intracellular aggregations which form the Bollinger body. Moreover, inoculation of fowl-pox into the dermis of the comb causes

an acute necrotic and inflammatory lesion not necessarily accompanied, until late, by those characteristic changes in the epithelia which are seen after ordinary scarification with the virus. Also in the early stages of vaccinia the Paschen bodies would appear to be entirely free. In ectromelia, though the chief sites of the Marchal inclusions are the epithelia of skin, salivary glands, pancreas, and intestinal mucosa, their presence has also been noted in the mesodermal cells of the subcutaneous connective tissue. The recent experimental work of Fairbrother and Hurst, and of Hurst, has shown that the virus of poliomyelitis travels by the axis cylinders and that the primary attack is on the nerve cells, the accompanying vascular disturbances in the vicinity of the destroyed nerve cells being a secondary phenomenon. This example would constitute a genuine cytotropism, and it is of very great interest that, as Hurst, Findlay, and Brownlee and Wilson have shown, the virus of louping-ill appears to pick out for attack in the mouse, the monkey, and the sheep the Purkinje cells of the cerebellum, which undergo extensive, and sometimes complete, destruction.

EXPERIMENTAL MODIFICATIONS OF VIRUS AND TISSUE CHANGE

It is becoming increasingly evident that a change of host or a change in the method of propagation of a virus may profoundly alter the pathological picture commonly associated with that virus and may bring to light unexpected tissue affinities. This subject is really an old one, and embraces such well-established phenomena as the attenuation of variola by variolation of bovines and of the street virus of rabies by passage through rabbits. From the more modern researches on this subject I choose two instances only, one illustrating the effect of altering the method of propagation and the other the effect of change of host.

The virus of vaccinia, after serial propagation in rabbit brain or rabbit testicle, acquires a greatly enhanced power of invasion from whatever site it is introduced and is capable of producing gross lesions of characteristic type in many organs, including the lungs, liver, spleen, adrenals, and generative organs. These organ lesions, which were first described by Douglas, Smith, and Price, have, on histological examination (Ledingham and Barratt, Bijl and Frenkel, etc.), revealed the general picture of a haemorrhagic septicaemia with accompanying miliary necroses of parenchymatous cells and inflammatory response on the part of the adjacent reticulo-endothelial system precisely similar to that seen in the dermis. The lesions in their simplest form, as seen in the subpleural region of the lungs or in the ovary or under the peritoneal surface of the intestine, consist of vascular necroses with well-marked compensating hyperplasia of the overlying endothelium to form a defence plaque. These plaques resemble the well-known epithelial plaques which form in response to vaccination of the rabbit cornea. To what this increased invasive or localizing power on the part of the virus is due is not yet known. It is possible that growth of virus in testicle yields a product far richer, per unit weight of tissue, in elementary bodies than does growth in skin, but there is almost certainly some inherent change in virulence as well. Now that the elementary bodies are believed to constitute the actual virus and can be obtained in a pure state, these problems will doubtless be solved in the near future.

To illustrate the effect of change of host on tissue affinity I take the recent work on yellow fever virus. The virus of yellow fever was found by Theiler to be communicable to the mouse by intracerebral inoculation. In this animal it produces a definite encephalitis. In the monkey, on the other hand, when the virus is intra-

cerebrally introduced no encephalitis occurs, but the liver lesions are present as usual. If the virus which has been accommodated to mice by intracerebral inoculation is inoculated intracerebrally into the monkey (Sellards and Goodpasture) an acute disseminated encephalomyelitis is produced with necrosis of ganglion cells, but the meninges are not involved and focal necroses in the liver do not occur.

Such remarkable adaptations as these render dangerous the postulation of any stereotyped elective affinities of viruses for cells or tissues.

COMBINED EFFECTS OF VIRUS AND BACTERIUM

The participation of concomitant bacteria in the production of tissue changes ostensibly due to virus has always excited a good deal of attention, particularly in connexion with such controversial subjects as influenza and the common cold in man—both of which may prove to be induced, at any rate primarily, by viruses—and in conditions such as swine fever, where association of the virus with organisms of the *Salmonella* or *Pasteurella* groups is not infrequent. These latter organisms are believed by many to be responsible respectively for the characteristic diphtheroid lesions in the intestine of chronic swine fever cases and for the pneumonic lesions, but accurate data as to their relative importance in the combined lesions are still wanting.

Perhaps the most interesting recent study of this question is that of Shope (1931) in connexion with the disease known as swine influenza. The details of this study cannot be referred to here, but the author claims to have shown that the clinical syndrome of swine influenza can be experimentally induced by a true filterable virus acting in conjunction with a haemoglobinophilic organism, *H. influenzae suis*. This organism, which was regularly obtained from the respiratory tract of swine experimentally infected with material from clinical cases, proved, in pure culture, to be completely unable to induce disease in swine by intranasal inoculation. The filterable virus, on the other hand, which was obtained by trituration of diseased lungs and lymph nodes, while it could not by itself produce typical clinical swine influenza, was able to produce (in seven out of ten trials) an exceedingly mild disease, denoted the "filtrate disease," and characterized by very slight transient fever, apathy and loss of appetite for a few days, slight cough, and some leucopenia. It was also contagious. The combination of this comparatively innocent filterable virus with artificial cultures of *H. influenzae suis* was capable of reproducing with exactness the severe and typical lesions of clinical swine influenza, so that apparently the presence of the virus conferred on *H. influenzae suis* powers of invasion it did not possess in its absence.

The lung lesions met with under various experimental conditions are of interest. If normal swine were inoculated with raw material from spontaneous cases, and the animals were killed during the febrile stage, but otherwise showed no external sign of disease at time of slaughter, massive areas of atelectasis, not crepitant and not consolidated, were present in certain lobes. The adjoining portions of lungs were emphysematous. In fatal cases only those areas which would have been atelectatic in uncomplicated swine influenza were pneumonic.

If the animals were inoculated with filtrate only and then killed, the amount of pulmonary atelectasis "varied from a scant amount in one or two of the upper lobes to an amount as extensive as that shown by mild cases of swine influenza."

The pathology of the lesion after inoculation of a mixture of filtrate and *H. influenzae suis* was typical of the natural disease. Space forbids any full discussion of this interesting work, but there is no doubt that it will have to be seriously considered in connexion with further

attempts to elucidate the aetiology of epidemic human influenza. So far as it goes this work on the swine influenza virus accords well with certain experiences and impressions recorded in the early waves of the influenza pandemic of 1918, when the clinical syndrome was frequently of almost as slight a nature as was the case in Shope's experimental disease produced by the filterable virus alone.

CELL INCLUSIONS IN VIRUS INFECTIONS

I shall do no more than outline the present position with regard to these interesting structures. There can be no doubt that in the past the study of these inclusions, which are so prominent features of certain virus diseases, has withdrawn attention from the study of the more general and perhaps the more proper tissue changes associated with such viruses. So greatly has this study of cell inclusions obtruded itself that it has been thought fit to speak of certain virus diseases as "inclusion diseases" or "Einschlusskrankheiten."

In three cases only—namely, the Bollinger body in fowl-pox, the inclusion body in molluscum contagiosum, and the Marchal body in ectromelia—is the nature and composition of the inclusion understood, and it would appear almost certain—also from quite recent work—that the well-known Guarnieri body in small-pox and vaccinia is made up of elementary bodies (Paschen bodies). The effect of these demonstrations will be to stimulate investigation on similar lines, not only of other well-known intracytoplasmic bodies like the Negri body, but also of the intranuclear inclusions (as met with in varicella, herpes, virus III, yellow fever, laryngotracheitis of chickens, etc.), in the belief that they also may represent invasions of noble and other cells by elementary bodies. This belief receives some support from recent work by Pinkerton and Hass (1932), who find that the rickettsiae of Rocky Mountain spotted fever multiply in the nuclei of cells in tissue culture, forming spherical clusters sometimes resolvable into individual elements, sometimes not. In the latter state the clusters resemble the more compact nuclear inclusions of other virus diseases. It is of great interest that very similar intranuclear bodies have recently (1932) been reported in 33 per cent. of gliomatous tumours examined by Russell, who suggests that the frequency of these structures in gliomas points to a virus being the cause of the growths. In the organs (liver, kidney, salivary glands, etc.) of apparently healthy adult animals also the occasional presence of intranuclear inclusions has proved of inestimable value in drawing attention to the existence of an associated virus, especially since the work of Cole and Kuttner on the salivary gland virus in the guinea-pig. We now know that in infants, monkeys, dogs, rabbits, rats, and mice similar inclusions may be found, and it is reasonable to suppose that their presence connotes a latent virus. The fact that inflammatory lesions are not infrequent in the neighbourhood of the "infected" cells should be borne in mind. It is worth speculating, perhaps, whether this unexpected presence of latent virus without associated clinical disturbance may play some part in the causation of syndromes such as post-infectious encephalitis.

CONCLUDING REMARKS

The types of tissue change met with in various virus infections do not differ in essence from those encountered in infections due to the visible bacteria. Where they seem to differ, as in those virus infections associated with characteristic cell inclusions, whether intracytoplasmic or intranuclear, the difference is probably not really of a fundamental nature, or one bearing the implication that viruses are essentially intracellular parasites. It is more probable that the penetration of epithelial cells,

which we know to take place in fowl-pox, ectromelia, and vaccinia, is an accidental occurrence in the course of massive proliferation of the virus or of elementary bodies in their neighbourhood, and to which the invaded cell cytoplasm or cell nucleus reacts to its own or the parasite's advantage, or to the advantage of both. In any case a temporary symbiosis would appear to be established. It would be difficult to differentiate a process such as this from that occurring in leprosy and kala-azar, the parasites of which can establish themselves inside macrophages of the reticulo-endothelial system, or from that occurring in tularaemia, where the minute organisms of the disease invade the parenchymatous cells of the liver. The only difference between the two sets of phenomena is that in the former elaborate histological and microchemical techniques have usually failed to unravel the complex of invading parasite and cell constituents, owing to the smallness of the invading parasite, while in the latter demonstration of the invader is, as a rule, relatively easy. Further light on this problem will doubtless come from further attempts to cultivate viruses in the presence or in the absence of living cells. I may say here that the demonstration by Eagles and McClean of multiplication of vaccinia virus in cell-free medium makes it unsafe to postulate intracellular proliferation as a necessary attribute of viruses. Where living cells are present we know that characteristic inclusions appear and are valuable evidence of growth having taken place, but what we have not yet learnt from these studies is a knowledge of the early phases, the presumably free proliferative phases, which precede the obvious invasion of the cell. It will be the task of the future—certainly no simple task—to penetrate more deeply than has been hitherto possible into those earliest reactions between the host tissue cell and the invading virus or bacterium, and towards this end it is essential that the closest co-ordination be established between the experimental pathologist and the expert morphologist.

RECENT LITERATURE

General

Kettle: *Lancet*, 1927, i, 1169, 1225; *System of Bacteriology* (Medical Research Council), vol. vii (Virus Diseases).
Rivers: *Filterable Viruses*, Baltimore, 1928.

Histology of Vaccinia and Fowl-pox

Goodpasture, Woodruff, and Buddingh: *Amer. Journ. Path.*, 1932, viii, 271.
Muckenfuss, McCordock, and Harter: *Ibid.*, 1932, viii, 63.
Goodpasture and Woodruff: *Ibid.*, 1931, vii, 1.
Woodruff and Goodpasture: *Ibid.*, 1929, v, 1; *ibid.*, 1930, vi, 713.
Goodpasture and King: *Ibid.*, 1927, iii, 385.
Woodruff, A. M., and Goodpasture: *Ibid.*, 1931, vii, 209.
Ledingham: *Brit. Journ. Exp. Path.*, 1924, v, 332.
Idem: *Journ. State Med.*, 1926, xxxiv, 125.
Ledingham and McClean: *Brit. Journ. Exp. Path.*, 1928, ix, 216.
Ledingham and Barratt: *Lancet*, 1929, ii, 515.
Douglas, Smith, and Price: *Journ. Path. and Bact.*, 1929, xxxii, 99.
Bijl and Frenkel: *Zentralbl. Bakt.*, 1929, cxii, 412.
Andervont: *Amer. Journ. Hyg.*, 1926, vi, 719.
Walshard: *Zeit. Hyg.*, 1927, cvii, 221.
Marsden and Hurst: *Brain*, 1932, lv, 181.

Elementary Bodies

Ledingham: *Lancet*, 1931, ii, 525.
Idem: *Journ. Path. and Bact.*, 1932, xxxv, 140.
Eagles and Ledingham: *Lancet*, 1932, i, 823.
Nauck and Paschen: *Zentralbl. Bakt.*, 1932, ccxiv, 91.

Yellow Fever

Sellards: *Proc. Nat. Acad. Sci.*, 1931, xvii, 339.
Klotz and Belt: *Amer. Journ. Path.*, 1930, vi, 663, 655, 689.
Klotz and Simpson: *Amer. Journ. Trop. Med.*, 1927, vii, 271.
Magalhaes: *Arch. Path.*, 1931, ii, 561.
Lloyd: *Amer. Heart Journ.*, 1931, iv, 483, 504.

Intranuclear Inclusions Apparently Unassociated with Disease

Covell: *Amer. Journ. Path.*, 1932, viii, 151.
Hindle and Stevenson: *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1930, xxiii, 327.
Findlay: *Brit. Journ. Exp. Path.*, 1932, xiii, 223.
Thompson: *Journ. Infec. Dis.*, 1932, l, 162.
Hudson and Markham: *Journ. Exper. Med.*, 1932, lv, 405.
Farber and Wolbach: *Amer. Journ. Path.*, 1932, viii, 123.

Russell, Dorothy: *Journ. Path. and Bact.*, 1932, xxxv, 625.
 von Glahn and Pappenheimer: *Amer. Journ. Path.*, 1925, i, 445.
 Cole and Kuttner: *Journ. Exper. Med.*, 1926, xlv, 855.
 Cowdry and Scott: *Arch. Path.*, 1930, ix, 1184.
 Pinkerton and Hass: *Journ. Exper. Med.*, 1932, lvi, 151.

Swine Influenza

Shope: *Journ. Exper. Med.*, 1931, liv, 349, 373.
 Lewis and Shope: *Ibid.*, 1931, liv, 361.

Dog Warts

de Monbreun and Goodpasture: *Amer. Journ. Path.*, 1932, viii, 43.

Histology of Psittacosis

Rivers and Berry: *Journ. Exper. Med.*, 1931, liv, 105, 119, 129.
 Rivers and Sprunt: *Ibid.*, 1931, liv, 91.
 Sturdee and Scott: Reports on Public Health and Medical Subjects, No. 61, 1930. H.M. Stationery Office.
 Elkeles and Barros: *Weichardt's Ergebnisse*, 1931, xii, 529.

Brazilian Parrot Disease

Pacheco and Bier: *C. R. Soc. de Biol.*, 1930, cv, 109.
 Rivers and Schwentker: *Journ. Exper. Med.*, 1932, lv, 911.

Poliomyelitis and Rabies

Fairbrother and Hurst: *Journ. Path. and Bact.*, 1930, xxxiii, 17.
 Hurst: *Ibid.*, 1930, xxxiii, 1133.
 Idem: *Ibid.*, 1931, xxxiv, 331.
 Idem: *Ibid.*, 1932, xxxv, 41.

Louping-ill

Hurst: *Journ. Comp. Path. and Therap.*, 1931, xlv, 231.
 Findlay: *Brit. Journ. Exper. Path.*, 1932, xiii, 230.
 Brownlee and Wilson: *Journ. Comp. Path. and Therap.*, 1932, xlv, 67.

POCK DISEASES *

BY

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The aetiological agents of the pock diseases are the so-called elementary bodies, first discovered by Borrel in fowl-pox and sheep-pox (Borrel bodies), and by myself in variola vaccine (Paschen bodies). Morphologically they are identical. We have here to do with minute, coccoid, sharp, round corpuscles, which divide into two dumb-bell shaped parts, and are always to be found in enormous quantities. They pass through the Berkefeld filter V, and can readily be deposited from the filtrate by high-speed centrifugalization—namely, 10,000 revolutions or more per minute.

INCLUSION BODIES: SIZE, STAINING METHODS, AND DISTRIBUTION

I have fixed their size in the coloured preparation at from 0.2 to 0.25 μ . In tests based upon mathematical and physical calculations Bechhold arrived at the value of 0.21 to 0.23 μ , while filtration experiments carried out by Elford and Andrews suggested that vaccinia virus particles have a diameter of 0.125 to 0.175 μ —that is, considerably smaller than those of Bechhold and Schlesinger.

Ledingham judged correctly that the actual diameter of the naked Paschen bodies is smaller than 0.2 μ . The stained bodies which he obtained in pure suspension in formalized saline solution are obviously smaller than they would be in their natural element, where their size is increased by the addition of colouring matter and the presence of serum protein in the lymph.

In 1911 Jacobsthal and I showed that the elementary bodies of infant lymph are specifically agglutinated by immune serum. Ledingham, by an improved method for obtaining pure suspensions of elementary bodies, was able to follow up the agglutination in the hanging drop—the stained clumps consisted entirely of elementary bodies.

Presumably, the Paschen bodies possess a lipid mem-

brane, and are thus very resistant to the process of drying. They cannot be shown with the usual dyes, but only by using hot Löffler mordant, followed by heated acid carbolic or aniline fuchsin, gentian, or methyl-violet. The methods of Fontana-Tribandau, Morosow, and Giemsa also render them visible. A slight trick is, however, first necessary—namely, to minimize the layer of serum surrounding the elementary body. The dry smears are, before staining, placed perpendicularly in saline solution made with distilled water (I prefer Ringer solution) for several minutes. After they have dried they are fixed in methyl-alcohol, and are then ready to be stained.

The Paschen bodies can be regularly demonstrated in large numbers in infant and cow-pox lymph, also in the rabbit virus lymph—especially in the two-day virus, which is almost a pure culture. They are also found in the content of variola pustules—that is, in variola vera, varioloid, and alastrim. The same bodies were found by me in appropriate material from various countries—Germany, Switzerland, England, Portugal, Haiti, Togo—and in the primary, secondary, and early throat lesions in inoculated pox (Togo). When the cornea of the rabbit is vaccinated with variola vaccine material, besides the Guarnieri bodies the Paschen bodies can always be demonstrated when using the "Klatsch" method. When vaccinating with neurolapine (neurovirus) the Paschen bodies are always found in great quantities in the metastatic lesions of the skin and internal organs (lungs, spleen, liver, adrenal glands, testis), in the smears as well as in the sections, when the method of Levaditi is used.

Are they Causative Agents?

In the case of fowl-pox, according to the method of Goodpasture and Woodruff, I believe it would be possible to isolate separate Guarnieri bodies from the pustules of vaccinated infants and to infect with them, if this should be actually desired as a crucial experiment, to prove the nature of the agents concerned. Actually, this proof has already been given by the morphological demonstration of the Paschen body in the tissue culture (Nauck and Paschen) and in the vaccine culture in a medium free from cells (Eagles and Ledingham). In vaccination with cultures originating from an infant lymph they always, in spite of high potency, produced the same type of reaction as the usual calf lymph. On the other hand, the typical reactions of neurolapine, with accompanying necrosis and haemorrhages, were obtained with cultures derived from testis vaccine, kindly sent me three years ago by Dr. Eagles. E. Gildemeister made similar observations with his cultures (as he informed me personally), which also started from testis vaccine. Thus we see that a quality acquired by a virus through organ passage is maintained in artificial cultures *in vitro*. Considering the results I have obtained, I believe myself justified in declaring that the so-called Paschen bodies, discovered by me, are the aetiological agents of the variola vaccine. I owe special thanks to Professor Ledingham for having given credit to the Paschen bodies by the excellent work which he has now been carrying on for many years.

INTERRELATION OF POCK DISEASES

Starting from a common source—the original pock (probably the human pock)—and resulting from the continuous passages through one and the same host (that is, the same species of animal), three different strains have developed. These are: human pox, fowl-pox, and sheep-pox. It is true that in a great number of animals the pock virus is only primarily present, and develops later into ephemeral pock eruptions. As a rule, the illness soon wanes, cannot be carried on in passage, and leads to no epidemics. This process can be experimentally

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