MULTIPLICATION OF THE ANIMAL VIRUSES

By Dr. D. J. BAUER
Wellcome Laboratories of Tropical Medicine

BACTERIA possess their own enzymes and can synthesize to a greater or less degree all the substances which are necessary for their growth; in consequence, pathogenic forms have a metabolism which is distinct from that of their host, while nearly all bacteria can be grown *in vitro* in the absence of living cells. Viruses are often considered to represent a degenerative simplification of this condition in which the enzymic equipment, although tacitly assumed to be present, is defective to such an extent that a dependence upon host metabolism has developed which deprives the virus of the capacity for independent growth. While this may perhaps be the case in the Rickettsiæ, which fall outside the scope of this article, a consideration of the available literature leaves one with the impression that there is little, if any, definite evidence that the true viruses have any enzyme activity at all, while their multiplication may well be more closely related to the mechanisms of heredity than to the growth of bacteria, being secured by some form of control over the metabolism of the host which recalls the control of the activities of the cytoplasm by genes and organisers.

Enzyme Activity of Viruses

Investigation of the biochemical activities of animal viruses is rendered difficult by the fact that pure preparations cannot be easily obtained; only a limited number of viruses are sufficiently amenable to purification procedures. These are in any event necessarily protracted, thereby affording opportunity for bacterial contamination, while viruses must usually be extracted from tissues which are rich in enzymes, which may not be completely separated from the virus bodies in the course of purification. These facts may be borne in mind when considering Table 1, in which published information concerning enzymic and related activities of viruses has been collected, together with some personal observations on the enzymic activity of influenza virus. There is good evidence that representative enzymes of glycolysis, respiration, nucleic acid synthesis and tissue invasion do not occur in the viruses studied. The negative results with influenza virus were obtained in some cases with virus concentrated a hundred-fold by red cell adsorption from fresh allantoic fluid (desoxyribonuclease, adenosine triphosphatase); fresh virus-containing allantoic fluid sometimes showed a slight phosphatase and dehydrogenase activity which was not reduced by red cell adsorption and which could not, therefore, be associated with the virus particles. The reported occurrence of certain enzymes in suspensions of vaccinia is far from satisfying. Preparation of the suspensions involves a considerable amount of manipulation, during which bacterial enzymes may gain access to the material. In the case of phosphodiesterase, the virus-substrate mixtures were incubated for up to seventy-two hours. In ordinary tissues, or with bacterial suspensions, phosphatase activity may be detected after thirty minutes incubation or less, and, even after allowance for the fact that only a small quantity of virus substance was present, it is more likely that the trace of phosphatase activity observed was due to contamination with tissue or bacterial enzymes, or even to decomposition of the substrate during prolonged incubation. Ribonuclease and desoxyribonuclease activity were detected, but only during an incubation extending for ninety-nine hours; these enzymes are usually sufficiently active to enable their presence to be detected with a 30-minute period of incubation; the substrates are, moreover, unstable and might well decompose to a certain extent under the conditions of the experiment. The reported occurrence of biotin and riboflavine in vaccinia is of interest in view of their co-enzyme nature. Riboflavine was present in a very small amount (1 mgm. per 100 gm.), and may therefore have been a contaminant; but biotin was present in comparatively large amounts and may be a genuine constituent of the elementary bodies. It has been shown[9] that vaccinia elementary bodies adsorb phosphatase, catalase and lipase, so that the enzymes can be removed from solution by the addition of elementary bodies followed by centrifugation to sediment the virus. This observation makes it all the more improbable that enzymes apparently associated with elementary bodies are genuine constituents.

The conclusion that influenza virus contains mucinase is based on the fact that the virus agglutinates the red cells of a number of animal species, that the cells can be rendered insusceptible to the action of the virus by previous treatment with mucinase of bacterial origin and that a given amount of virus is apparently capable of reacting with an unlimited

Table 1

Virus	Reference	Absent	Present
Vaccinia	1	Zymohexase Enolase Phosphoglucomutase Adenosine nucleosidase Peptidase Triosephosphate dehydrogenase	Phosphodiesterase Ribonuclease Desoxyribonuclease Phosphomonoesterase
	2	Cytochrome oxidase Cytochrome C	Riboflavine Copper
	3		Biotin
	4	Hyaluronidase	
Influenza	5, 6		Mucinase
	7	Mucinase	
		Desoxyribonuclease Phosphatase Xanthine oxidase Adenosine triphosphatase Succinic dehydrogenase	
Lansing	8	Hyaluronidase	
MM	8	Hyaluronidase	

number of cells, in the manner of an enzyme activating an unlimited amount of substrate. It has been claimed that influenza virus will bring about a reduction in the viscosity of a solution of mucin[6]; but the evidence is unconvincing to those with experience of viscosimetry in biological work. It has recently been denied that influenza virus has any action on mucin[7]; but the lack of agreement between these results may be due to differences in the composition of the substrates employed. The mucinase activity of influenza virus needs to be put upon a firmer footing by characterization of the enzyme and its substrate; if it be accepted that this is a true enzyme action, it stands in remarkable contrast to the lack of other enzyme activities shown by influenza and vaccinia viruses. It may act only as a means of attaching virus to the surface of those cells which form a suitable site for its proliferation, without playing any part in virus metabolism.

Relation of Viruses to the Enzyme-containing Structures of the Host Cell

From a consideration of the foregoing it seems improbable that viruses are generally endowed with enzymic activity, at least so far as one can generalize from the limited information available. If this be the case, the metabolic activities necessary for the synthesis of new virus material must be contributed by the host cell; this implies that the virus must exert some sort of control over the enzymes of the host. These are not distributed at random throughout the cytoplasm but are located for the most part in discrete bodies known as chromidia[10]. These are round, with an average diameter of 50–300 mμ, and are thus of the same order of size as the virus body. They contain ribonucleic acid, calcium and magnesium, phosphatides and lipides, and respiratory and hydrolysing enzymes. They are linked together in chains by fused strands of polypeptides which form filaments known as interchromidia which are about 50 mμ in thickness. The ribonucleic acid is probably concerned with protein synthesis and is activated and inhibited by the magnesium and calcium ions; the lipides are considered to form a screen between the enzymes and their potential substrates so as to prevent the dissolution of the whole structure. After entering the host cell the virus must act upon the chromidia, and new virus material must be laid down in their neighbourhood. There is a certain amount of evidence available which could be interpreted as showing that virus bodies have an affinity for the chromidia and develop in contact with them. The course of development of psittacosis virus in the chorioallantoic membrane has been studied by electron microscopy[11]. The membranes were prepared for examination by disruption by sonic vibration. Many round bodies attached to fibres were seen, both in infected and in uninfected control membranes; although no interpretation of these structures was given, they were recognized to be normal cell constituents; they must, in fact, be the chromidia and interchromidia. Virus-like bodies were seen attached to the presumed chromidia, although the appearance was interpreted as division of a virus body. Many examples were also seen of virus-like bodies attached to fibres which were presumably interchromidia. These observations suggest very strongly that the virus develops in close association with the enzyme-containing structures of the cytoplasm.

A direct relation between virus infection and enzyme activity has also been demonstrated[12,13]. In mouse brain infected with the viruses of neurotropic yellow fever, lymphocytic choriomeningitis and lymphogranuloma inguinale, a considerable increase of xanthine oxidase activity occurs; a similar occurrence is seen in chick embryos infected with the 17*D* strain of yellow fever virus[14]. In the latter case there is some evidence that the increased enzyme activity represents a synthesis of new enzyme in response to the virus infection. Whether this be the case or not, it seems likely that the virus, after gaining entry to a cell, exerts some sort of control over the cell metabolism, presumably by directly affecting the chromidia. An affinity between virus and enzyme has been demonstrated by the work on vaccinia already described[9], in which it was shown that vaccinia elementary bodies would adsorb phosphatase, catalase and lipase from solution. A certain specificity was also observed, since a plant enzyme, urease, was not adsorbed by the virus. One might speculate that the virus has an affinity only for those enzymes which are required for the synthesis of new virus material.

Development of the Virus Colony

In the work on psittacosis virus already quoted[11], it was noted that virus could not be found in the chorioallantoic membrane six hours after infection had occurred, and that while some virus bodies were seen after twelve hours they did not become numerous until twenty-four hours or so after infection. The development of psittacosis virus has also been studied by dark-ground photography of cells in tissue culture over prolonged periods[15]. For eight hours or so after infection has occurred nothing can be seen in the cell; then some time between eight and twenty-four hours a homogeneous plaque appears, 5–10 μ in diameter. This event seems to occur quite suddenly, within an hour or so. The plaques enlarge and can be seen to be composed of comparatively large round bodies; as the colony enlarges, the round bodies decrease in size and become elementary bodies, which are liberated by rupture of the cell. In certain cases it was possible to trace the origin of a colony backwards beyond its time of appearance as a visible plaque, as it could be distinguished as an area of cytoplasm which, although of normal appearance, showed no Brownian movement. This suggests that the colony is formed out of normal cytoplasm which is smoothly transformed into virus material; moreover, large colonies may increase in size so as to fill the whole cell, suggesting a successive systematic conversion of cytoplasmic components into virus material.

These observations can scarcely be reconciled with the prevalent concepts that viruses multiply, like bacteria, by a process of binary or plural fission. A somewhat less detailed study has been made of the development of the colonies of lymphogranuloma[16] in mouse brain. Here the earliest appearance was that of a mass of large bodies, containing from twenty up to several hundreds, which eventually condense into the smaller elementary bodies. Here again there is a suggestion, not of the production of a colony as the end result of a continuing process of fission, but rather of the sudden appearance of a preformed mass of large bodies, as in psittacosis. Elementary bodies were often seen occurring in pairs or in short chains. This appearance

was interpreted as indicating multiplication of the elementary bodies by fission; but such an arrangement would be expected if virus bodies are laid down in association with chromidia, which are themselves arranged in chains. The large body of psittacosis and lymphogranuloma may represent a diffuse deposition of new virus material adjacent to a chromidium, which condenses in the process of organisation into a smaller elementary body. This would account for the observed occurrence of large bodies in young colonies and their virtual disappearance from mature colonies. The development of lymphogranuloma virus has also been studied in infections of the yolk-sac[17]. Observation of stained sections showed that virus material first appeared, again rather suddenly, twelve hours after infection, in the form of initial bodies of diameter 1 μ. These increased in size to form plaques of diameter up to 7 μ, in which elementary bodies then appeared. During this cycle the yolk-sac is not infective; the titre of virus in the yolk-sac rises when the elementary bodies appear, but then falls again. This fall was interpreted as due to a loss of virus into the yolk, but it could be that the virus is not infective in the early stages of cellular re-invasion. Essentially similar appearances have been described for other viruses of the psittacosis–lymphogranuloma group[18].

Of viruses not belonging to this group, vaccinia and ectromelia have been studied by dark-ground observation of infected chorioallantoic membranes *in vivo*[19]. In ectromelia infections nothing abnormal can be seen for twenty-four hours; inclusion bodies of diameter 1 μ–2 μ then appear, and increase in size up to 8 μ. The smaller bodies are granular; the granules are embedded in a matrix and arranged in chains. This arrangement again suggests association with chromidia; the presence of a matrix also indicates that the future virus bodies arise in centres having an appreciable spatial separation, which would not be the case if reproduction were by fission. As the virus inclusions enlarge, the remaining cytoplasm does not appear to be displaced; there is rather the same suggestion of a gradually extending conversion of cytoplasm into virus which has been noted in the case of psittacosis. In the case of vaccinia, new virus material is not seen until the second day after infection. A layer of particulate material then appears beneath the cell surface, often extending to surround the whole cell. There is no sharp line of demarcation from the surrounding cytoplasm, again suggesting that virus material arises by an extending process of cytoplasmic transformation; the cytoplasm may eventually become complete y replaced by virus material apparently without undergoing any process of compression or degeneration.

Soluble Phase of Viruses

These observations suggest that the entry of a virus particle into a cell is followed by the sudden transformation into virus material of a considerable volume of cytoplasm, represented by an initial body of 1 μ diameter or so, and amounting in the case of psittacosis to a plaque of 5 μ–10 μ in diameter, about 10^4 times the volume of the original virus particle. In vaccinia the first appearance is the sudden transformation of a considerable area of cortical cytoplasm. In consideration of the various mechanisms by which these changes might be brought about, the most probable seems the dissolution of the virus body into a soluble phase, which then diffuses through a considerable volume of cytoplasm and organises it into virus material by an action upon the chromidia and their enzymes. Such a soluble phase has already been postulated for influenza virus[20]. It has been shown in the case of infection of the chorioallantoic membrane by influenza virus that for six hours after infection the membrane apparently contains no virus; that is, the virus is present in some form which is non-infective. This is presumed to be the soluble complement-fixing antigen. In the disappearance of the infective phase after infection has occurred, influenza virus resembles psittacosis and lymphogranuloma.

The early stages of development of those viruses which are large enough to be examined by direct microscopy seem to be invisible as well as non-infective, so that there is a latent period between the initiation of infection and the first appearance of visible virus material. The available information concerning this latent period is summarized in Table 2; it ranges from 6 to 24 hours and seems to be of general occurrence. Its existence can be explained by the assumption that the infecting particle dissociates into an invisible phase which diffuses through the cytoplasm. By the end of the latent period sufficient new material will have been formed under the influence of the soluble phase to form a visible initial body or plaque; the size of this body would represent the extent of diffusion which had already occurred, while its subsequent enlargement might be brought about by an extension of the process of diffusion and organisation. The new virus material appears as a matrix in which centres of condensation appear, perhaps in association with chromidia, which represent the future elementary bodies. A connexion with chromidia is suggested by the spatial separation of the elementary bodies from the moment of their origin, and by their tendency to occur in chains and pairs.

Table 2

Virus	Tissue	Latent period in hours	Reference
Ectromelia	Allantoic membrane	24	19
Vaccinia	,,	24	19
Lymphogranuloma	Yolk sac	12	17
,,	Mouse brain	present	16
Psittacosis	Tissue culture	8–24	15
,,	Allantoic membrane	6–12	11
Mouse pneumonitis	Mouse lung	6	18
Feline pneumonitis	,, ,,	12–18	18
Meningo-pneumonitis	,, ,,	18	18

Soluble phases ('soluble antigens') have been found associated with certain other viruses, such as mumps[21] and vaccinia[22]. While there is no evidence which might suggest that these represent an intracellular developmental phase, the fact of their existence is at least suggestive. The soluble antigens are, moreover, of smaller size than the viruses concerned, which is a necessary condition for the hypothetical soluble phase. A considerable amount of information is available concerning the properties of the soluble (*LS*) antigen of vaccinia[23]. It is a protein of molecular weight 214,000; the molecule is elongated, with an axis ratio of 1/20. From these figures the size of the molecule may be estimated as 2 × 60 mμ[24]. The size of the vaccinia elementary body is 222 × 284 mμ[25], and it contains 89 per cent of protein; the protein

component would thus be sufficient in amount to form four thousand particles of soluble antigen. Even assuming that only a small part of the protein is transformable into *LS* antigen a large number of soluble particles could still be generated. Each of these might perhaps associate with one chromidium and organise the development of one virus body. Thus, although there is no evidence that the *LS* antigen constitutes such a phase in the reproduction of the virus, there is nothing in the available information which is inconsistent with such a view. It has, moreover, been established that the *LS* antigen is liberated from the elementary bodies *in vitro*[26] and is therefore not a by-product of infection of cellular origin. It might be argued that the absence of nucleic acid from the soluble antigen makes it unlikely that it is concerned in a process, such as virus multiplication, which involves the synthesis of protein. The soluble phase could, however, stimulate the synthesis of protein by acting upon the nucleic acid which is present in the chromidia, and would not necessarily contain nucleic acid itself. A more serious objection is the existence of other constituents in the vaccinia elementary body, such as nucleic acid and lipoids, which presumably have some function which is distinct from that of the soluble antigen and which is presumably essential for the perpetuation of virus material. There is, however, the possibility that these other constituents act as a framework on which soluble antigen is deposited, or even represent inclusions of material derived from the dissolution of cytoplasm, so that they would not participate directly in the reproductive process. There is evidence which favours the latter supposition[34]; it has been shown by means of precipitin tests that purified influenza virus derived from allantoic fluid contains an antigen which is present in uninfected allantoic fluid. This antigen constitutes up to 30 per cent of the virus particle, and is therefore a real constituent and not an impurity. Similarly, virus purified from infected mouse lungs contains an antigen which is present in normal mouse lung. It has also been shown[35] that equine encephalomyelitis virus purified from infected chick embryos contains up to 70 per cent of an antigen which is present in normal embryo tissues. These observations suggest that virus is formed by the conversion of normal cell constituents, during the course of which an appreciable amount of normal material is incorporated into the virus body in an unchanged condition. The virus body might, therefore, consist of a soluble phase carrying the power of infectivity deposited upon a framework derived from the residue of normal cell components. The influenza mucinase might represent such a residuum.

The ultimate simplification of the concept of the soluble phase is the soluble crystallizable protein of the plant viruses, where the absence of a particulate phase may perhaps be correlated with adaptation to specific methods of infection. The case of tumours which are not transmissible by filtrates may perhaps represent a condition in which a presumptive infection is prevented from leaving the cell by the absence of a particulate phase and cannot, therefore, be demonstrated by transmission experiments. If the existence of the soluble phase can be established in fact and shown to be a general phenomenon, it would unify the heterogeneity of the animal viruses among themselves and form a link with the plant viruses, which have hitherto been considered to be entirely dissimilar.

Cycles and Titres

In addition to the developmental cycles which have been directly observed in the cases of psittacosis, vaccinia and other viruses, a growth-cycle has been demonstrated for influenza virus[36,20]. When allantoic fluid is removed and titred at intervals after infection, the amount of virus in the fluid rises after six hours in the case of the *PR8* strain, or after 9–10 hours with the Lee strain. This is brought about by the sudden simultaneous liberation of virus into the allantoic fluid If reinfection of the membrane is prevented by injecting a large amount of irradiated heterologous virus, the titre of virus in the fluid remains constant after the rise, and a one-step growth curve can be obtained. A succession of such steps at six-hour intervals has been observed[20], suggesting a series of intracellular growth cycles. The amount of virus which is formed in one cycle can be estimated by means of a comparison of the titres of virus in the allantoic fluid before and after the occurrence of a step; a yield of sixty-three ID_{50} is found by this means for the *PR8* strain, and thirty-six ID_{50} for the Lee strain[36]. If one assumes in the absence of precise information that the cells of the chorioallantoic membrane are cubes of side 10 μ, sixty-three virus particles will have a total volume which is only $1{\cdot}5 \times 10^{-5}$ of the cell volume. If the virus is scattered throughout the cytoplasm, or aggregated into a small colony, it is difficult to see how the presence of such a small volume of foreign material could lead to the death of the cell. It seems more likely that the virus multiplies in the nucleus, or within the cell surface, where localized damage could lead to disruption of the cell and liberation of the virus. If the virus grows within one surface of a cell of side 10 μ, sixty-three infective particles will represent approximately 1/200 of the cell surface, and their presence there might conceivably lead to sufficient damage to the cell membrane. In this connexion it is possible that the mucin affinity of influenza virus is a means of bringing the virus to a suitable tissue and anchoring it within the cell surface.

Table 3

Titre	Tissue	Virus
10^6–10^7	Mouse liver	Ectromelia, rift valley fever, lymphocytic choriomeningitis
	Mouse brain	Yellow fever, Theiler's mouse encephalomyelitis[28], louping ill[27], lymphocytic choriomeningitis, rabies[29]
10^9	Mouse brain	Equine encephalomyelitis[30], SK[31], St. Louis encephalitis[30], Japanese *B* encephalitis[30], encephalomyocarditis[32]
10^{11}	Mouse brain	*MM*[33]

A certain amount of information concerning growth cycles can be deduced for those viruses which are less amenable to experimental handling by a consideration of the maximum infectivity titres which they attain. A selection of representative titres is given in Table 3; where no reference is given, the figures are based on personal observation. The titres fall roughly into three groups: 10^6–10^7, 10^9 and 10^{11}. The minimum number of infective particles formed in one cell can be estimated from the titre and the average cell size. A calculation of this type is no

possible in the case of brain infections, where only certain specialized cells are infected, the total volume of which is unknown. In the case of mouse liver, assuming as a reasonable approximation that it consists entirely of epithelial cells of side 25 μ, a titre of 10^6, as with ectromelia or rift valley fever, gives a figure of 10^8 infective particles per gram of tissue, or a yield of between one and two particles per cell if the distribution of virus is assumed to be uniform. This latter cannot be the case, as it is unlikely that the presence of so few particles could cause any damage to the cell. If one then assumes a more reasonable figure for the yield, say a hundred, it follows that only 1 per cent of the cells would contain virus. It is extremely improbable that destruction of 1 per cent of the cells of the liver would lead to symptoms sufficiently severe to cause death. It is more likely that most of the cells of the organ are damaged, in which case they must necessarily contain virus in some form which cannot be detected by infectivity titration. Thus the occurrence of a non-infective phase, such as has already been demonstrated for influenza virus[20], can be inferred to hold also for those viruses which multiply in mouse liver. The presence of virus in maximum titre is not in itself sufficient to produce symptoms, since maximum titres can be observed some hours before the onset of symptoms in the case of infections of mouse brain with the viruses of yellow fever and lymphocytic choriomeningitis. Symptoms presumably do not appear until a large number of new cells have become occupied by the non-infective phase of the last cycle, the completion of which is interrupted by the death of the host. The exceptionally high titre attained by *MM* virus suggests that it forms a special case. A titre of 10^{11} indicates that the whole brain contains 10^{13} infective particles. Assuming as an approximation that the brain consists entirely of cells of average size 10 μ, one cell would then contain $2{\cdot}5 \times 10^3$ infective particles. While this figure is necessarily grossly inaccurate, one can at least infer that the virus must be widespread and not confined only to specialized cells, and that the final cycle is infectious and is therefore detected by the infectivity titration.

Conclusion

Future work on viruses must be directed towards a search for further examples of the existence of a non-infectious phase, and to establishing whether such a non-infectious phase can be isolated from infected tissues in soluble form. Evidence must also be sought for the existence of interactions between a virus, or its postulated soluble form, and enzymes, or enzyme-substrate reactions. It might be possible to demonstrate some sort of control by viruses over enzymes *in vitro*. It has already been found that tobacco-mosaic virus will inactivate crystalline ribonuclease by forming a complex with it[37]. This is a further example of enzyme-virus affinity, of the kind which has already been mentioned for vaccinia, and further work might reveal that enzyme affinity is a general property of viruses which is of fundamental importance in their reproduction.

Although most of what has been said in the preceding sections is entirely speculative, no kind of support has emerged for the concept that viruses are degenerated bacteria; there is much more reason for believing in the alternative view that viruses resemble genes. One is also reminded of the role of chemical organisers in embryonic induction by the way in which the virus appears to organise the cytoplasm and induce the production of new virus material. The problem of antiviral chemotherapy should be reviewed in the light of these concepts. Work up to the present has been based upon the implied assumption that viruses, like bacteria, possess a metabolism which is distinct from that of the host, and can therefore be selectively interfered with by chemotherapy. It seems more probable that the metabolism of the virus is actually that of the host itself, so that no selective interference can be possible; the problem is therefore transformed into the entirely different one of trying to interfere with the action of something analogous to a chemical organiser. The fact that viruses of the psittacosis group respond to penicillin and sulphonamides suggests that the problem in relation to viruses as a whole is not insoluble. The property of response to chemotherapy, together with certain other peculiarities, has given rise to the belief that the psittacosis viruses are distinct from other viruses, being more closely related to bacteria. Study of their development, however, leads to the conclusion that they are essentially viral in nature, have no affinities with bacteria, and are by no means sharply differentiated from the other animal viruses; one has therefore some cause for hoping that chemotherapeutic methods may be successful in virus infections in general.

[1] MacFarlane, M. G., and Dolby, D. E., *Brit. J. Exp. Path.*, **2**, 219 (1940).
[2] Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. M., *J. Exp. Med.*, **74**, 69 (1941).
[3] Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. M., *Proc. Soc. Exp. Biol., N.Y.*, **45**, 669 (1940).
[4] Duran-Reynals, F., *Bact. Rev.*, **6**, 197 (1942).
[5] Burnet, F. M., *Aust. J. Sci.*, **10**, 21 (1947).
[6] Woolley, D. W., *J. Exp. Med.*, **89**, 11 (1949).
[7] Briody, B. A., and Hanig, M., *Proc. Soc. Exp. Biol., N.Y.*, **67**, 485 (1948).
[8] Glick, D., and Gollen, F., *J. Infect. Dis.*, **83**, 200 (1948).
[9] Hoagland, C. L., Ward, S. M., Smadel, J. E., and Rivers, T. M., *J. Exp. Med.*, **76**, 163 (1942).
[10] Monné, L., "Advances in Enzymology", **8**, 1 (1948).
[11] Heinmets, F., and Golub, O. J., *J. Bact.*, **56**, 509 (1948).
[12] Bauer, D. J., *Nature*, **159**, 438 (1947).
[13] Bauer, D. J., *Brit. J. Exp. Path.*, **28**, 440 (1947).
[14] Bauer, D. J., *Nature*, **161**, 852 (1948).
[15] Bland, J. O. W., and Canti, R. G., *J. Path. Bact.*, **40**, 231 (1935).
[16] Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., *Trans. Roy. Soc. Trop. Med. Hyg.*, **32**, 183 (1938).
[17] Rake, G., and Junes, H. P., *J. Exp. Med.*, **75**, 323 (1942).
[18] Weiss, E., *J. Infect. Dis.*, **84**, 125 (1949).
[19] Himmelweit, F., *Brit. J. Exp. Path.*, **19**, 108 (1938).
[20] Hoyle, L., *Brit. J. Exp. Path.*, **29**, 390 (1948).
[21] Henle, G., Henle, W., and Harris, S., *Proc. Soc. Exp. Biol., N.Y.*, **64**, 290 (1947).
[22] Craigie, J., and Wishart, F. O., *Brit. J. Exp. Path.*, **15**, 390 (1934).
[23] Smadel, J. E., Hoagland, C. E., and Shedlovsky, T., *J. Exp. Med.*, **77**, 165 (1943).
[24] Gorvin, J. H., personal communication.
[25] Beard, J. W., *Physiol. Rev.*, **28**, 349 (1948).
[26] Craigie, J., and Wishart, F. O., *J. Exp. Med.*, **64**, 803 (1936).
[27] Edward, D. G. ff., *Brit. J. Exp. Path.*, **28**, 368 (1947).
[28] Theiler, M., and Gard, S., *J. Exp. Med.*, **72**, 49 (1940).
[29] Levinson, S. O., Milzer, A., Shaughnessy, H. J., Neal, J. L., and Oppenheimer, F., *J. Immunol.*, **50**, 317 (1945).
[30] DeBoer, C. J., and Cox, H. R., *J. Immunol.*, **55**, 193 (1947).
[31] Jungeblut, C. W., Saunders, M., and Feiner, R. R., *J. Exp. Med.*, **75**, 611 (1942).
[32] Warren, J., and Smadel, J. E., *J. Bact.*, **51**, 615 (1946).
[33] Jungeblut, C. W., and Dalldorf, G., *Amer. J. Pub. Health*, **33**, 169, (1943).
[34] Knight, C. A., *J. Exp. Med.*, **83**, 281 (1946).
[35] Engel, L. L., and Randall, R., *J. Immunol.*, **55**, 331 (1947).
[36] Henle, W., Henle, G., and Rosenberg, E. B., *J. Exp. Med.*, **66**, 423 (1947).
[37] Loring, H. S., *J. Gen. Physiol.*, **25**, 497 (1941).