DISTRIBUTION OF NUCLEAR INCLUSIONS IN WILD ANIMALS *

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The viruses that are most destructive receive the closest attention. But biologically the less pathogenic ones, the presence of which is not revealed by distinctive clinical symptoms, are equally important to those who wish to understand the place in nature of these elusive organisms or substances. The discovery of the latter has been largely a matter of chance. In 1920 Jackson [1] reported intranuclear inclusions, which she called protozoan parasites, in the salivary glands of guinea pigs, explaining that they were observed in examinations made for an entirely different purpose. We now know that they are caused by a virus. During the routine study of the tissues of rats in a large dietary experiment, Thompson [2] discovered somewhat similar inclusions also in the salivary glands. In the course of investigations on Rift Valley fever, Findlay [3] found intranuclear inclusions in the liver cells of mice which showed no symptoms of disease and were caused by a hitherto unrecognized mouse virus. Many other instances of chance favoring the prepared mind might be cited.

If we try to plot out the distribution of nuclear inclusions which have been proved experimentally to be of virus etiology and others under suspicion in the vertebrate series, we find them in 2 fish, 1 amphibian, no reptiles, 4 birds and at least 11 mammals. This list includes only the species in some individuals of which they naturally occur. It does not include a considerable number of other species in which inclusion formation can be induced by injection of viruses foreign to them. Impressed by the need for more data on the distribution of intranuclear inclusions, and possibly of viruses, particularly in wild animals, the Rectors [4] made a short survey of the tissues of such species in the vicinity of St. Louis and found a remarkable case of their development, without symptoms, in the salivary glands of common moles. The purpose of this paper is to report briefly a

* Aided by grants from the Rockefeller Foundation to Washington University for research in science and in virus diseases.

Received for publication August 24, 1934.

more detailed survey chiefly of the kidneys of mammals and birds from the pathological service of the Zoological Society of Philadelphia.

Material

This included 165 animals of which 78 were mammals and 87 were birds. The mammals belonged to 58 species distributed as follows: *Artiodactyla* 16, *Primates* 10, *Xenarthra* 1, *Rodentia* 8, *Carnivora* 21 and *Marsupialia* 2. The birds comprised 62 species as follows: *Passeriformes* 5, *Strigiformes* 2, *Coraciiformes* 1, *Piciformes* 1, *Psittaciformes* 20, *Columbiformes* 2, *Ralliformes* 3, *Galliformes* 4, *Accipitriformes* 9, *Anseriformes* 8, *Ardeiformes* 4, *Struthioniformes* 1, *Balearicifformes* 1 and *Phoenicopteriformes* 1. The majority, negative as far as inclusions are concerned, are listed later by themselves and the few positive are given in Table I.

Most of the tissues were fixed in formalin or Zenker's fluid and sections were colored routinely with hematoxylin and eosin. In special cases Giemsa's stain was also employed. By reference to the number accompanying each specimen complete clinical and pathological reports could be secured from the records of the Zoological Society. A large collection of specimens showing nuclear inclusions in virus diseases was available for comparison in the Anatomical Laboratory of Washington University.

Observations

Search was made for the type A and B inclusions of Cowdry.[5] Under type A are grouped those caused by the viruses of herpes, yellow fever, mad itch, fox encephalitis and so on; and under type B those in Borna disease, poliomyelitis, and so on. In both there is an increase in acidophilic material in the nucleus but there the similarity ends. Type A inclusions are formed by the accumulation in the nucleus of granular acidophilic material and the margination on the nuclear membrane of all the basophilic nuclear substance. At a certain stage in their development a clear space is interposed between the central, acidophilic and the peripheral basophilic materials. The nucleoli are altered, move to the nuclear membrane, disappear and the inclusion-laden nuclei disintegrate.

Type B inclusions, on the contrary, are usually spherical, often hyaline, droplet-like bodies which make their appearance in nuclei

that otherwise look fairly normal. The basophilic chromatin is not marginated in the same way but when the inclusions become very large, as they sometimes do, it is pushed away toward the nuclear membrane. The nucleoli persist for a longer time and nuclear disintegration is less marked.

To recognize well developed inclusions of either type is not difficult. It is the border line cases that give trouble. Normally the nuclei of the renal tubules of mammals and birds are rounded bodies of fairly uniform size. One, occasionally more, nucleoli are present and are the only nuclear structures visible in them in the living unstained state. But it is with fixed tissues that we have to deal. In them the nucleoli generally color with basic dyes, but they are really amphinucleoli, for they contain some acidophilic as well as basophilic material. The association of the two components is not always the same. It is when the basophilic material is mixed with the acidophilic that the nucleolus as a whole colors blue with Giemsa or hematoxylin and eosin. Frequently the basophilic component is applied rather unevenly on the surface of acidophilic substance, which is more definitely spherical in form and acts as a kind of core (Figs. 2 and 16). More rarely it becomes detached altogether from the acidophilic mass, in which case the latter is commonly called an acidophilic nucleolus, or plasmasome, but this is not illustrated in our figures. The prefix, plasma, indicates something staining with plasmatic, acid dyes in contrast to nuclear, basic ones. In addition to the nucleolus the nuclei contain irregular masses and strands of basophilic material which are generally more marked as the basophilic nuclear membrane is approached. A small amount of acidophilic material can also be distinguished in the nucleoplasm. Sometimes it appears as a thin, more or less homogeneous deposit in which condition it is represented in gray near the nucleoli in Figures 1 and 2. It may, however, appear in the form of fairly discrete particles like those which make up inclusions caused by viruses. Its relation to the acidophilic nuclear core is not known. Microincineration shows that the latter is much richer in mineral constituents, but this difference may be only one of degree conditioned by the greater density of the nuclear core. In the nuclei of the convoluted tubules the extranucleolar acidophilic material and the nucleoli are more noticeable than in the nuclei of the collecting tubules. In the birds' kidneys they are slightly more prominent than in mammals.

But the tissues examined were not normal. They came from an extensive autopsy service and the nuclei exhibited a wide variety of modifications. We shall eliminate first those that we do not consider to have any definite bearing on our problem.

Increases in acidophilic material to the degree illustrated in gray in Figures 1 and 2 were listed as negative. As was inevitable, some of the tissues showed postmortem autolysis. In such cases the acidophilic nuclear material referred to becomes more prominent (Fig. 5) and other changes occur which will be described in detail by Lucas and Cowdry [6]; but the alteration sweeps through the tissue fairly uniformly in the case of the epithelial cells present and for this reason can easily be distinguished from the effect of virus action which is more localized, and generally, but not always, accompanied by characteristic lesions.

Hypertrophy of individual nuclei (Fig. 18) and of groups of nuclei was very commonly seen without either type A or B inclusions. It was observed in 18 species of birds and was most marked in a South American porcupine. In 5 mammalian species and in 1 bird this was accompanied by margination of basophilic chromatin. Margination alone without increase in central acidophilic material (Fig. 6) was observed in 9 mammalian species and 4 avian ones. Sometimes the nucleoli were enlarged (Fig. 16).

Though alterations such as these were noted in some of the following specimens, no definite intranuclear inclusions suggestive at all of virus action were detected. The specimens are listed because any study of distribution must take into consideration negative findings. Only the kidneys were examined, unless other organs are mentioned. The numerals in brackets indicate the number of individuals of a given species studied when this exceeded one.

Mammals

Artiodactyla
- Klipspringer
- Llama
- Indian buffalo
- Blesbok
- Himalayan thar
- White tailed gnu
- Bison (3)
- American elk
- Western water buck
- Apis deer
- Japanese sika deer
- Hog deer
- Central American deer (2)
- Virginia deer
- Red deer
- Pigmy hippopotamus

Primates
- Green monkey (2)
- Kra macaque
- Orang utan
- Hocheur monkey
- Weeper cebus (2)
- Common marmoset
- Silky marmoset

Xenarthra
- Long haired armadillo

Rodentia
- Canada porcupine (3)
- Porcupine
- Capybara
- Coypu rat
- Prairie dog
- Prevost's squirrel
- Gray squirrel (2)

Carnivora
- Sea lion
- Himalayan bear
- European brown bear
- Black bear
- European badger
- Mink (2)
- Polecat
- Otter
- White nosed coati (2)
- Ring tailed coati
- Crab eating raccoon
- Raccoon (3)
- Coyote
- Red fox
- Carpathian fox
- Jackal
- Two spotted paradoxure
- African civet
- Wild cat
- Caracal lynx
- Eyra cat (2)

Marsupialia
- Common opossum (6)
- Red kangaroo

Birds

Passeriformes
- Canary
- American robin
- European jay
- Wild grackle (brain only)
- Common crow (2)

Strigiformes
- Barn owl
- Spectacled owl

Coraciiformes
- Abyssinian ground hornbill

Piciformes
- Cuvier's toucan

Psittaciformes
- Undulated grass parrakeet
- Parrakeet
- Blue and yellow macaw (liver only)
- Red and blue macaw
- Red sided eclectus
- Salles amazon
- White fronted amazon (2) (also liver and lung)
- Yellow fronted amazon (3) (also liver and lung)
- Salvin's amazon
- Green cheeked amazon (2) (also liver)
- Golden naped amazon
- Festive amazon
- Levaillant's amazon (7) (also liver and lung)
- Wild parrots (6) (also brain)
- Red vented parrot
- St. Vincent's parrot
- Lesser sulphur crested cockatoo
- Sulphur crested cockatoo

Columbiformes
- Barbary turtle dove

Ralliformes
Common coot
Purple gallinule
Florida gallinule

Galliformes
Common peafowl (2)
Rain quail
Vulturine guinea fowl

Accipitriformes
Rupple's vulture
Abyssinian vulture
Cooper's hawk (2)
Red shouldered hawk
Goshawk
Marsh hawk
Red tailed hawk (2)
Batleur eagle
Bald eagle

Anseriformes
Gargany teal
Muscovy duck
Mallard duck (2)
Lesser white fronted goose
Hybrid goose
Egyptian goose

Ardeiformes
White necked stork
White stork
Scarlet ibis (2)
Straw necked ibis

Struthioniformes
Somali ostrich (2)

Balearicifformes
Demoiselle crane

Phoenicopteriformes
Ruddy flamingo

The intranuclear inclusions that we desire to report are listed in Table I.

Type A inclusions, found in the kidneys of two Guatemalan amazons (Figs. 9–15), were interesting because they exhibited points of resemblance to the inclusions caused by the yellow fever virus. Compare Figures 9 and 10 with Cowdry and Kitchen's [7] Figure 16, and Figure 11 with their Figure 24. In both, the nuclear inclusions are made up of sharply outlined particles of fairly uniform size. They appear in the form of clumps in the zone intermediate between the nucleolus and nuclear membrane. But in the Guatemalan amazon fewer nuclei show them and the condition did not lead to necrosis. In amazon No. 10602 there were approximately 1.164 inclusion-laden nuclei per oil immersion field and in No. 10240, 0.125. The sections were about 8 microns thick. A simple calculation gives the incidence of inclusions in the sections more accurately, in the first at 65.6 per sq. mm. and in the second at 7.07. In addition to the kidneys the liver, lung, testis, epididymis, striated and heart muscle of amazon No. 10602 and the liver, lung, testis, epididymis, pancreas, spleen and intestine of amazon No. 10240 were examined without finding any more type A inclusions. Type B were, however, found abundantly in the lungs of No. 10602 (Figs. 14 and 15), but were absent in the lungs of No. 10240, despite the presence in both of similar

lesions of advanced anthracosis. Nothing in the history of either animal suggests a virus infection. The autopsy reports were:

No. 10602: Injury and malnutrition; edema, congestion, hemor-

TABLE I

Animal	Type A inclusions	Type B inclusions	Figures
Mammals			
Artiodactyla — Red deer		+	
Primates — Mongoose lemur		+	3
" — Kra macaque		+	
" — Rhesus macaque		+	4
" — Lion tailed macaque		+	
Rodentia — South American porcupine			
Birds			
Piciformes — Cuvier's toucan (liver, lung, pancreas, intestine, spleen — negative)		+	7 and 8
Psittaciformes — Guatemalan amazon, 10,240 (liver, lung, pancreas, intestine, testis, epididymis, and spleen — negative)	+		9 and 10
" — Guatemalan amazon, 10,602 lung (liver, striated and cardiac muscle, testis and epididymis — negative)	+	+	11, 12 and 13
" — Black crested cockatoo	+?		17
Columbiformes — Wonga Wonga pigeon		+	18
Galliformes — Silver pheasant		+	
Accipitriformes — Marsh hawk		+	20
Anseriformes — Ceropsis goose		+	21
" — Spur winged goose		+	

rhage and anthracosis of lungs; myocardial degeneration and chronic endocarditis (?), atrophy of spleen and thyroid; atrophy and fibrosis of adrenal; pigmentation and hydropic degeneration of liver; atro-

phic enteritis, atherosclerosis (carotid and aorta); hyperplasia of bone marrow.

No. 10240: Pigmentation, passive congestion and multiple abscesses of liver (Gram-negative bacilli in smears); hyperplasia of bone marrow; acute necrotizing enteritis; hydropic degeneration of pancreas; cloudy swelling of kidney; edema, congestion and abscess formation of lungs; pigmentation, diffuse hyperplasia and myeloid metaplasia of spleen.

The nuclear inclusions in the black crested cockatoo were less noticeable and it is doubtful whether they merit inclusion in type A, although the nucleus illustrated in Figure 17 could not itself be distinguished from one affected by the virus of herpes for example. It is the infrequency of such nuclei, coupled with the absence of tissue reaction and the unsatisfactory way that the tissue stained, which makes the diagnosis doubtful.

Nuclei containing type B inclusions were spread quite widely in the preparations among other nuclei which showed no inclusions; but in the marsh hawk the change was sharply localized, being limited to the nuclei of a single collecting tubule (Fig. 20). These type B inclusions are clearly of more frequent occurrence than those of type A and may (Figs. 4, 8 and 20) or may not (Figs. 3, 7, 14, 15 and 21) be accompanied by nuclear hypertrophy; whereas the type A inclusions in the amazon and cockatoo did not make their appearance in particularly enlarged nuclei; the largest is represented in Figure 13. No characteristic lesions, either infiltrative, proliferative or degenerative, were found with the type B inclusions, neither did the clinical history or the autopsy findings give any reason to believe that a virus was operative. Yet some of these type B inclusions were as large and conspicuous as any reported in the literature. We compared them particularly with inclusions in the kidneys of sewer rats, of which specimens were very kindly sent to us by Dr. E. Hindle.[8]

The crystalline inclusion represented in Figure 19 was unique. Others like it were not found even in the same preparation. It is quite different in appearance from the large intranuclear acidophilic crystals first reported by Szymonowicz and Macallum and found also by Cowdry and Scott [9] in the livers of dogs.

Before attempting to discuss the possible significance of these observations we wish to report the results of a search for nuclear

inclusions in kidney specimens from 1012 human autopsies courteously loaned to us by Dr. H. Gideon Wells of Chicago. No type A inclusions were found. Type B inclusions were seen in only 17 kidneys, or 1.67 per cent, as contrasted with 14 out of 163 animals, or 8.58 per cent.

But the incidence of nuclear inclusions may not be so much lower in the human series than in the wild animals as the figures indicate. If the search for them in the humans had been equally thorough a few more might have been found. The sections of the human kidneys had all been well stained with hematoxylin and eosin, which is the most satisfactory method for inclusions, because of color contrast and permanency, but some of them were rather too thick. It is more difficult to identify inclusions by focussing up and down through entire nuclei than in preparations about 5 microns thick in which many nuclei are cut in section. But none of the sections were so thick that they could not be easily studied with a 3 mm. oil immersion objective. Postmortem changes were rather more frequent in the human series. In such cases, the shrinkage of the nuclei and alteration in staining reaction would have interfered with the identification of inclusions, particularly those of type A, if not well developed.

On the other hand pathological lesions were much more numerous and varied in the human than in the wild animal series. With greater frequency in functional disturbance, one would expect a somewhat higher incidence of inclusion formation quite irrespective of whether they are caused by viruses or cellular environments unusual in osmotic or other features. Indeed, the remarkable rarity of nuclear inclusions in the human series shows that, in the kidney at any rate, they are not ordinary, commonly encountered nuclear degenerations. Comparison of the 14 kidneys, in which they were seen, with the others failed to reveal any distinctive pathological process. None of the inclusions were so large and noticeable as those observed in some of the wild animals and others in preparations of frog and rat kidneys kindly sent to us by Dr. B. Lucké and Dr. E. Hindle respectively (see Lucké,[10] Figs. 14 and 15, and Hindle[8]). Neither were they so conspicuous as the inclusions reported in the kidneys of fetuses and young children by many authors (see Farber and Wolbach[11]). The kidneys that we examined were almost entirely from adults. It is possible that such inclusions, though present

earlier, had disappeared, or that a geographic factor of some kind operates, as suggested by Farber and Wolbach, and that for some unknown reason this factor is less widespread in the Middle West.

Discussion

This examination of many specimens of the kidneys of wild animals and humans has been a fatiguing but very profitable experience. It has not brought to light a single instance of inclusions that we care to attribute definitely to virus action. Those which most nearly qualify were the A inclusions in the Guatemalan amazon and the B inclusions in the marsh hawk. Neither was accompanied by an infiltrative tissue reaction. It is clear that type A are less common nuclear modifications than type B. How are we to explain their formation?

Obviously about all we can do is to mention some of the possible factors. Wells [12] states that "it is probable that chemically the differences between nucleus and cytoplasm are quantitative rather than qualitative." This view is supported by recent studies on microincineration which show that nuclei are richer in mineral constituents than was formerly supposed (Scott [13]). Chromatin holds our attention because it stains vividly and on account of its great physiological significance, but it is one of many nuclear components. Chemically, chromatin is a protein salt of nucleic acid. According to Mathews,[14] "the nucleic acid is apparently the same, or at any rate closely similar in all the different cells examined; but the protein base appears to be characteristic of the cell." The firm, jelly-like consistence of most nuclei he attributes to the gelatinizing property of solutions of nucleic acid salts. The nucleic acid is basophilic for it combines with "basic" dyes. That "basic chromatin" carries a negative electrical charge has been shown by McClendon.[15] When cells are placed in an electric current he observed the actual pushing out of the nuclear membrane toward the anode by pressure of the basic chromatin. Acidophilic material carries, on the contrary, a positive charge. The outstanding feature of the formation of type A inclusions is a change involving the entire nucleus by which the acidophilic material, perhaps the protein, protamin or histon base of the nucleic acid salt, becomes separated from the basophilic nucleic acid and accumulates in the center, whereas the acid moves to the

periphery of the nucleus. But the acidophilic substance may be only partly or not at all of chromatinic origin. Other nuclear components than chromatin may be involved and there is a possibility that some of it may enter the nucleus from the surrounding cytoplasm, for there is usually some hypertrophy which must be due to intake of extraneous material, though it may simply be water.

At first sight one gains the impression that an alteration of this sort might result from the action of a nuclease. Van Herwerden [16] was able to remove selectively basophilic components from several types of cells by allowing a nuclease to act on sections of alcohol, or hot water, fixed tissue. We experimented with a nuclease kindly prepared for us by Dr. D. J. Kooyman. This we injected into the living epidermis, because we thought that in an avascular situation we might secure a maximum change. But stained sections failed to show any sign of the development of type A inclusions. Moreover, in our studies on postmortem autolysis chromatin is broken down, presumably by nuclease, but inclusions are not formed. Lee,[17] working in this laboratory, has been more nearly successful in his investigation of osmotic factors producing acidophilic inclusions which very closely resemble those caused by viruses. The process, whatever it is, of type A inclusion formation ordinarily goes on to complete nuclear disintegration and cellular necrosis. It is generally accompanied by a local invasion of phagocytes.

Type B inclusions occurred more frequently, both in the wild animals and humans. The chromatin is not divided in quite the same way into central acidophilic and marginating basophilic fractions. When they are small the nuclear structure about them is not modified, except for the appearance of a delicate halo. When they increase greatly in size the contents of the nucleus are crowded toward the nuclear membrane. As with beginning type A inclusions, it is not possible to draw a definite line of distinction between the inclusions themselves and acidophilic material normally present in small quantities in the nuclei. The nuclear change is less radical than in type A. It has the appearance of developing more slowly and the terminal stage of necrosis is seldom seen. This may explain why type B inclusions often occur without an associated tissue reaction, toxic substances perhaps not being liberated to the same degree as in the development of A inclusions. We do not deny that type B inclusions may be the result of virus action but we think that they

may be caused directly by other factors as well, or secondarily by viruses activating or influencing these other factors. In estimating the probability that any given type B inclusions are due to virus action several considerations are important.

The first is the extent of deviation from normal. When the inclusions are of small size, consisting only of droplets of acidophilic material in nuclei whose cells otherwise look as usual, one is inclined to dismiss them as of little if any significance from the point of view of viruses. Another feature that leads to scepticism is uniformity in distribution of the inclusions in practically all cells of the same sort in a preparation; for, as already stated, the action of viruses is generally limited to relatively small foci and unequal in degree, some cells being altered slightly and others more severely, as if they had been attacked one after another.

Inclusions of this type B are only exceptionally marked by a tissue reaction detectable by routine techniques, by which we mean necrosis of cells, infiltration by phagocytes, fibrosis and so on. It is possible that the application of special cytological and microchemical methods might reveal alterations in the neighboring epithelial cells and we exclude arbitrarily, in specifying the tissue reaction, hypertrophy and other modifications in the inclusion-laden cells themselves. A tissue reaction of this kind was not a noticeable accompaniment of type B inclusions in either our wild animal or human series. It may have occurred to a mild degree and have disappeared, or the inclusion formation in any particular locality may perhaps be secondary to considerable increase in virus elsewhere and transport of the virus to the area by the blood stream. This may apply also to certain type A inclusions. A dog which had been inoculated with fox encephalitis virus showed in its kidney (given to us by Dr. R. G. Green [18]) inclusions limited to the renal glomeruli and there was no tissue reaction. But the affinity of this virus is primarily for endothelium. Moreover, the inclusions in fetuses and young children are often devoid of tissue reaction, as noted by Farber and Wolbach.[11]

Early type B inclusions may be of the same size as nucleoli or considerably smaller. One or more of the following distinguishing features can generally be made out. (1) Clear halos common about the inclusions are rare or absent about the nucleoli. (2) The inclusions are not definitely placed in the nuclei and of limited number like the nucleoli. (3) When several inclusions occur in a single nu-

cleus, they often exhibit a gradation in size from bodies much smaller than nucleoli to considerably larger ones. (4) They are usually wholly acidophilic, whereas the nucleoli may color with both acid and basic dyes or consist of an acidophilic core on which basophilic material is plastered.

Further investigations may bring to light other differences. Nucleoli differ from type A inclusions in the possession of large amounts of mineral.[19, 20] We have incinerated the type B inclusions in Hindle's sections of rat's kidneys and wish to report very little mineral, but the specimens were not fixed by the best method for microincineration. We have no data for other type B inclusions but we think it likely that this difference from nucleoli will hold. Nucleoli are known to be very compact structures. In the nerve cells of some lower forms they settle in the lowest parts of the nuclei by their own weight and most histologists have seen instances of nucleoli carried right out of the nuclei by the microtome knife. A careful comparison of both types of inclusions with nucleoli and basophilic chromatin in centrifuged cells is indicated.

When, on the other hand, type B inclusions are localized in distribution, are very large, and the containing nuclei are much distended, we entertain the possibility of virus action and await experimental proof. This is the status of the inclusions in the kidney of the marsh hawk and in the kidneys of sewer rats of Hindle. Bodies difficult to distinguish morphologically from inclusions of type B apparently occur normally in the cells of the vesicula semenalis of certain mammals and humans, [21-24] and in those of the nucleus supraopticus and paraventricularis of the midbrain of many vertebrates and humans.[25-28] In both localities they are interpreted as phases in the elaboration of nuclear secretions, for which, however, the evidence, in our opinion, is insufficient. But their similarity to inclusions in other tissues, which some have attributed to virus action, inspires caution.

How type B inclusions are formed remains to be determined. Splitting of the nucleic acid salt of protein is not a conspicuous feature. When first they begin to grow, the internal organization of the nuclei is not lost. They seem to be passive accumulations of substance, the nature of which awaits chemical analysis. All we know at present is that, like the type A inclusions, they contain little or no thymonucleic acid and are not of fatty nature. They may conceivably be produced by faulty elimination of nuclear material or by the entry of extraneous material in unusual amounts. Either might be

occasioned by a modification in the permeability of the nuclear membrane, which in turn might result directly or indirectly from the operation of a virus or simply from a functional derangement of common occurrence in some tissues and very rarely seen in others, depending upon their intrinsic attributes.

Summary

Type A inclusions resemble those of herpes and usually: (1) exhibit a change which sweeps through the entire nucleus whereby acidophilic material accumulates in the center to form the inclusion and basophilic material marginates on the nuclear membrane; (2) lead to complete disintegration of the injured cells, and (3) are accompanied by a noticeable tissue reaction. Inclusions of this kind were seen only in the kidneys of 2 Guatemalan amazons. In so far that each inclusion was made up of clumps of discrete acidophilic particles and the nucleoli were not displaced early in the process, they look quite like the intranuclear inclusions caused by the yellow fever virus, but they were rather evenly distributed in the tissue and were not accompanied by distinctive lesions.

Type B inclusions resemble those in Borna disease and usually: (1) develop as local accumulations of acidophilic material in nuclei, the remaining parts of which at first show no other modifications; (2) crowd all other nuclear materials toward the nuclear membrane as they increase in size; (3) do not lead to extensive nuclear disintegration or to severe accompanying tissue reactions. These were more widely distributed. They were found in the lungs of one of the Guatemalan amazons and in the kidneys of 7 out of 62 species of birds, 6 out of 58 species of mammals and 17 out of 1012 individual humans.

Acidophilic nuclear material is normally present in small amounts in the nuclei of renal epithelial cells. There is no sharp line of distinction between it and inclusions of either type. It is the degree of change that raises the question of virus etiology. When the inclusions are (1) so conspicuous that they can be seen without the aid of oil immersion objectives, (2) localized in distribution, and (3) related to marked lesions, one looks for a virus. But no type A inclusions, and especially no type B ones, which are so much more common, should be regarded as pathognomonic of virus action without experimental proof.

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DESCRIPTION OF PLATE

PLATE 31

The drawings of selected nuclei were made with camera lucida at a magnification of 2400 diameters. Nuclear material, which was acidophilic and colored red in the original stained preparations, is represented in shades of gray and basophilic material, which was blue, in black or very dark gray.

FIG. 1. *Canada porcupine.* Convoluted tubule. A little acidophilic material is shown near the nucleolus.

FIG. 2. *Straw necked ibis.* Convoluted tubule. Slightly more acidophilic material. Nucleolus with an acidophilic core.

FIG. 3. *Mongoose lemur.* Convoluted tubule. Type B inclusion.

FIG. 4. *Rhesus macaque.* Convoluted tubule. Type B inclusion; basophilic chromatin not marginated, just pushed aside; nuclear hypertrophy.

FIG. 5. *Prairie dog.* Convoluted tubule. Marginal clumping of chromatin with central basophilic granules.

FIG. 6. *Red kangaroo.* Convoluted tubule. Margination of basophilic chromatin alone without inclusion formation.

FIG. 7. *Cuvier's toucan.* Convoluted tubule. Type B inclusion surrounded by a distinct halo.

FIG. 8. *Cuvier's toucan.* Convoluted tubule. Small type B inclusion with marked nuclear hypertrophy.

FIGS. 9–10. *Guatemalan amazon.* (*No. 10240*). Convoluted tubule. Two stages in development of type A inclusions leading to complete margination of basophilic chromatin.

FIGS. 11–13. *Guatemalan amazon* (*No. 10602*). Convoluted tubule. A further stage in inclusion formation (11). Coalescence of acidophilic inclusion particles in a shrunken and a hypertrophied nucleus (12 and 13).

FIGS. 14 and 15. Same. Type B inclusions in epithelial cells of the lung.

FIG. 16. *Yellow fronted amazon.* Convoluted tubule. Nucleolar hypertrophy.

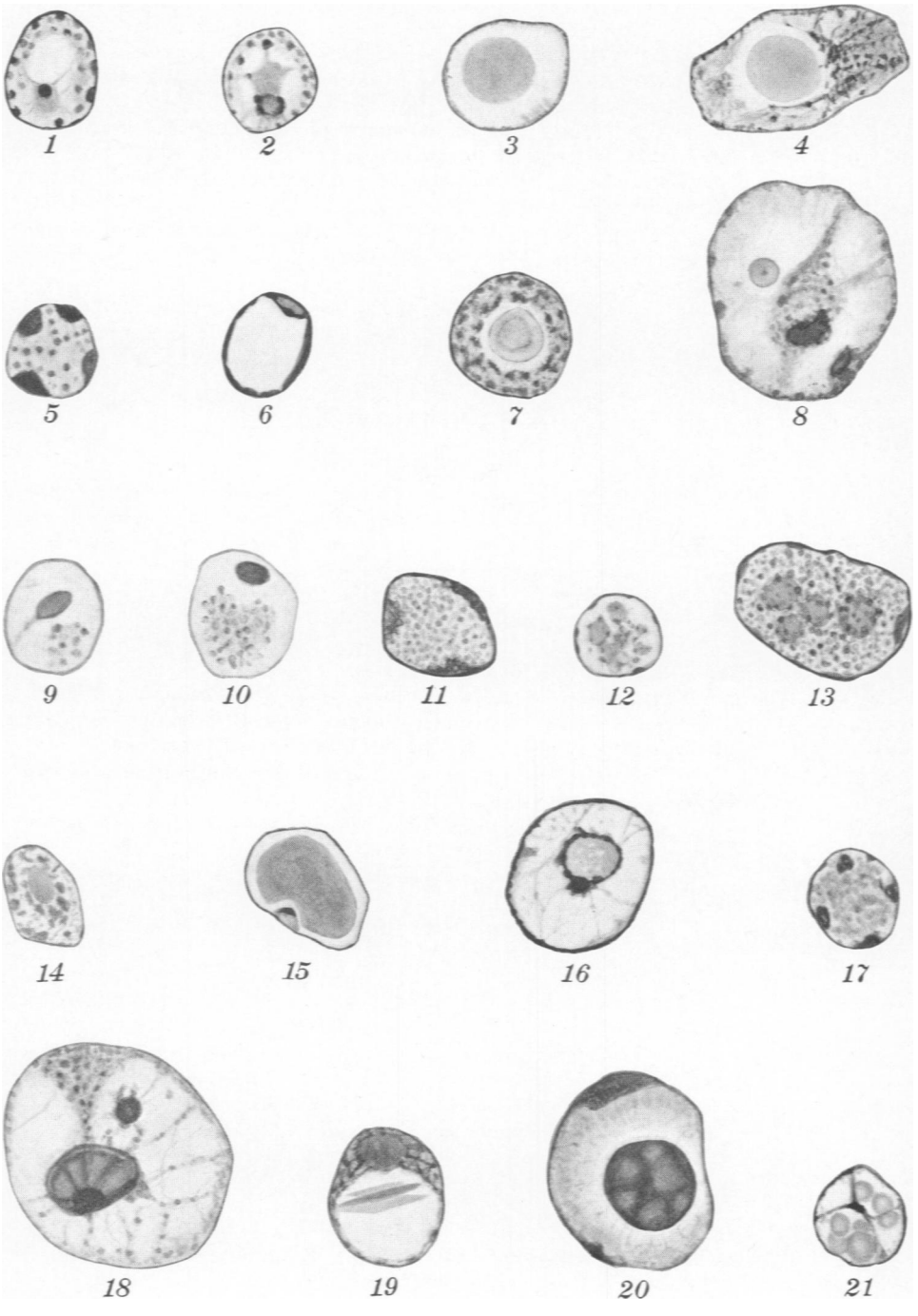
FIG. 17. *Black crested cockatoo.* Convoluted tubule. Type A inclusion with margination of basophilic nuclear chromatin.

FIG. 18. *Wonga Wonga pigeon.* Convoluted tubule. Nuclear hypertrophy.

FIG. 19. Same. Convoluted tubule. Nucleolar hypertrophy. Atypical crystalline acidophilic inclusion in a large clear area.

FIG. 20. *Marsh hawk.* Collecting tubule. Large type B inclusion staining also with basic dyes. Nuclear hypertrophy.

FIG. 21. *Ceropsis goose.* Convoluted tubule. Several types of B inclusions.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